

# INFLAMMATION, IMMUNE FUNCTION, AND THE TRANSITION COW

B. J. Bradford  
Department of Animal Sciences and Industry  
Kansas State University

## INTRODUCTION

Dairy producers and those who advise them are well aware of the challenges that face cows during the transition to lactation. The 2-3 week period after calving typically accounts for 25 – 50% of health problems on a dairy, and high cull rates in early lactation are a costly problem for many farms. Research conducted over the past 2 decades has revealed strong evidence that part of the problem during this time is suppression of the immune system during the transition period. Both innate immunity (responsible for quick, general responses to pathogens) and acquired immunity (dependent on immune “memory”) are impaired during this period of time. This diminished immune function occurs at a time when nearly all cows experience some degree of inflammation, which is somewhat counter-intuitive given that inflammation is a critical tool for immune system activation. Furthermore, measures of immune function and inflammation are predictive of disease incidence in dairy cows, suggesting that changes to support immune function and to limit inflammation may improve the well-being and productivity of cows.

## IMMUNITY IN TRANSITION COWS

During infections such as mastitis or metritis, immune cells in the body recognize invading pathogens and become activated. The non-specific, rapid response arm of the immune system is called innate immunity, and is triggered by general pathogen-associated molecules, such as endotoxin. The pathogen-specific arm that provides a potent “memory” based targeted response is called adaptive immunity; this is the arm that is targeted by vaccination. Even though these aspects of immunity are often discussed separately, in reality these systems communicate constantly.

The activation of local and systemic host defense mechanisms requires cross-talk between numerous types of immune cells, and one component of this response is inflammation. The host of signaling molecules released by activated immune cells includes inflammatory mediators such as nitric oxide, prostaglandins, and cytokines. Whereas many of these molecules promote local inflammation and increased blood flow to the infected tissue, inflammatory cytokines play a key role in stimulating systemic inflammatory responses, including increased body temperature, increased heart rate, and decreased feed intake. One effect of cytokines is to activate production of acute phase proteins. Primarily produced by the liver, proteins that participate in the acute phase response to infection are generally found in very low abundance in the bloodstream, but are greatly elevated during periods of systemic inflammation.

Why is the immune system of transition cows suppressed? The exact reasons for decrease 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.

Infections are obviously caused by a pathogen, but this new information suggests that the response of the cow to the pathogen should receive as much focus as the pathogen load when trouble-shooting disease problems on a farm. Data currently available suggests that cows have improved postpartum 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) postpartum total serum calcium concentrations are maintained near 9 mg/dL, and 5) postpartum blood BHBA and NEFA concentrations stay below 1 mM. 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.

## INFLAMMATION DURING THE TRANSITION PERIOD AND ASSOCIATED OUTCOMES

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 postpartum cows than is currently recognized. Whatever the explanation, the prevalence of postpartum inflammation raises important questions about the implications of this inflammation 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

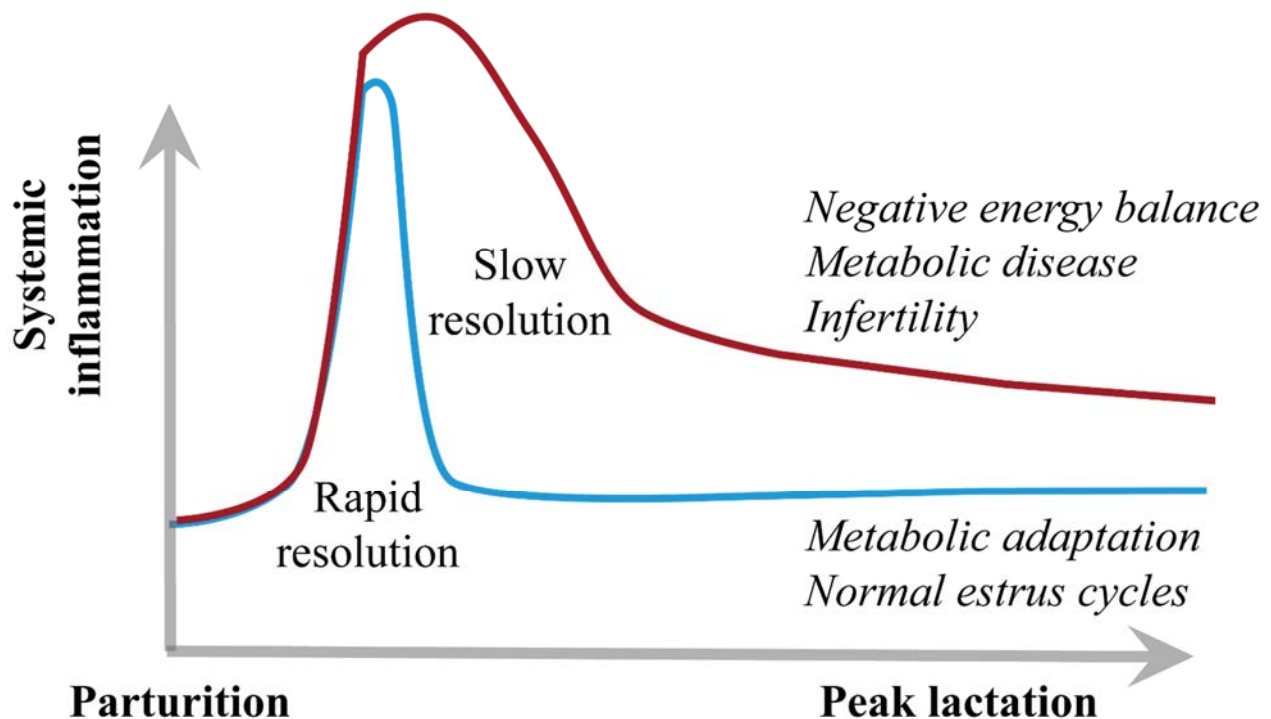
inflammation. Cows in the highest quartile had significantly lower milk yields than those in the lowest quartile throughout the first month of lactation, differing by 20% on day 28 of lactation (Bertoni et al., 2008). One metric that has been used in this respect is paraoxanase, a plasma biomarker that 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.

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 aid in physiological adaptations to lactation and the end of pregnancy. 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.

## RESPONSES TO ADDED INFLAMMATORY STRESS

We recently reported on a study where we administered a very low dose of the inflammatory cytokine tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) for the first 7 days of lactation to assess the impact of a subtle increase in postpartum inflammation (Yuan et al., 2013). Although our treatment did not induce any of the classical physiological signs of acute inflammation, we did observe significant increases in circulating mediators of inflammation, validating our approach to enhancing sub-acute inflammation. During the week of treatment, TNF $\alpha$  decreased feed intake by 18% and energy-corrected milk yield by 17%, with no change in energy balance. Furthermore, in the highest TNF $\alpha$  treatment group, 7 of 11 cows were diagnosed with at least one subclinical transition disorder, compared to just 2 of 11 in the control group. Along with a few other studies that have directly induced inflammation in the transition period (Trevisi et al., 2009), these findings demonstrate a causative role of inflammation in at least some common problems in early lactation.

**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*.



#### IMPACT OF POSTPARTUM ANTI-INFLAMMATORY TREATMENTS

##### Milk Production Responses

To address whether endogenous inflammation is also a problem, a variety of labs have used non-steroidal anti-inflammatory drugs (NSAID) to treat postpartum inflammation. In an preliminary study, Bertoni et al. (2004) treated 11 cows/treatment with lysine acetyl-salicylate (aspirin) or placebo for the first 5 days postpartum and monitored milk production through day 126 of lactation. Peak milk yield tended to increase with aspirin treatment ( $P < 0.10$ ). The same group subsequently conducted a similar study with 23 cows/treatment and found that aspirin treatment over the first 5 days of lactation increased milk yield through day 60 of lactation ( $P < 0.05$ ), with a 13% increase in peak milk yield (Trevisi and Bertoni, 2008).

Motivated by these promising findings, 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. At first the results did not look very promising – salicylate decreased blood glucose and increased ketone concentrations in early lactation, with no increase in early milk yield (Farney et al., 2013a). 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 (Farney et al., 2013b). 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., 2016) across 153 cows. Despite this very limited treatment window, cows treated with either NSAID produced 7 – 9% more milk over the whole lactation compared to placebo. Unfortunately, such treatments are not currently approved for commercial use.

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 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.

## Health Impacts

Several NSAIDs have been used effectively in treating mastitis. In one study, carprofen had limited ability to suppress inflammation, but was shown to partially alleviate the decrease in ruminal contractions during induced mastitis in early-lactation cows (Vangroenweghe et al., 2005), which could help prevent a subsequent displaced abomasum. In another study, meloxicam treatment lowered somatic cell count and a reduced the number of cows removed (culled) from the herd after mastitis; most cows on the study were in early lactation (McDougall et al., 2009).

A few studies have evaluated health impacts following blanket treatment with NSAID after parturition. In general, there has been little evidence of overall improvements in health or decreased risk of culling following early lactation NSAID treatment (Farney et al., 2013b, Meier et al., 2014). In fact, one small study suggested possible increased risk of infections after NSAID treatment (Bertoni et al., 2004). However, our commercial farm study that generated some intriguing results (Carpenter et al., 2016). Over the 365 days following treatment, meloxicam tended to delay removal from the herd based on survival analysis ( $P = 0.06$ ; 30, 35, and 38 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. More research is necessary to determine whether blanket treatment of postpartum cows can really decrease culling risk.

## Reproductive Responses

The links between inflammation, reproductive tract infections, and infertility have motivated numerous groups to evaluate impacts of anti-inflammatory treatments on reproductive outcomes. Five studies that have utilized NSAID treatments in the first 2 weeks postpartum and reported reproductive outcomes have been published, adding up to over 1,500 cows. Overall, these studies have shown little promise for decreasing the time to pregnancy after calving. However, Amiridis et al. (2001) found that adding flunixin meglumine to the antibiotic regimen used for treatment of puerperal metritis resulted in more rapid uterine involution and return to estrus. The unique benefit in this study suggests, not surprisingly, that NSAID treatment may have a greater impact on cows with an abnormal degree of uterine inflammation. Readers should note that many other studies have investigated various NSAID treatments near the time of breeding, outside of the transition focus of this paper.

In addition to NSAID treatments, diets with elevated concentrations of omega-3 fatty acids (protected in part from ruminal biohydrogenation) can be used to mildly decrease inflammatory status of postpartum cows. However, interpretation of most such studies is complicated by differences in not only inflammation but also in supply of multiple fatty acids that serve as precursors for prostaglandins and other reproductive hormones. Indeed, a recent study demonstrated that supplementing either omega-3 or omega-6 fatty acids during early lactation increased peak progesterone concentrations during the estrus cycle (Dirandeh et al., 2013), suggesting that essential fatty acid deficiency may be a separate issue worth considering.

## CONCLUSIONS

Growing interest in postpartum immunity and inflammation has led to a large number of basic and applied dairy cattle studies in the past decade. We now have a handful of nutritional and environmental risk factors that are known to contribute to poor immune function, adding additional motivation to address these problems. Health and productivity responses to anti-inflammatory treatment have been inconsistent, despite fairly consistent evidence that cows with heightened inflammation in the days after calving produce less milk and suffer from more disease. Ongoing research will hopefully bring additional clarity about the benefits and potential risks of several postpartum anti-inflammatory strategies. In the meantime, reducing subclinical and clinical infection incidence can provide the additional benefit of decreasing pro-inflammatory signaling, improving expected production and health outcomes across the lactation.

## REFERENCES

Amiridis, G. S., L. Leontides, E. Tassos, P. Kostoulas, and G. C. Fthenakis. 2001. Flunixin meglumine accelerates uterine involution and shortens the calving-to-first-oestrus interval in cows with puerperal metritis. *Journal of Veterinary Pharmacology and Therapeutics* 24:365-367.

- Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. *J. Dairy Sci.* 91:3300-3310.
- Bertoni, G., E. Trevisi, and F. Piccioli-Cappelli. 2004. Effects of acetyl-salicylate used in post-calving of dairy cows. *Veterinary Research Communications* 28:217-219.
- Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. *J. Dairy Sci.* 90:1740-1750.
- Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* DOI:10.3168/jds.2015-9683.
- Carpenter, A.J., C.M. Ylloja, C.F. Vargas, L.K. Mamedova, L.G. Mendonca, J.F. Coetzee, L.C. Hollis, R. Gehring, and B.J. Bradford. 2016. Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. *J. Dairy Sci.* 99:672–679. DOI:10.3168/jds.2015-10048.
- Dirandeh, E., A. Towhidi, Z. A. Pirsaraei, F. A. Hashemi, M. Ganjkanlou, S. Zeinoaldini, A. R. Roodbari, T. Saberifar, and H. V. Petit. 2013. Plasma concentrations of PGFM and uterine and ovarian responses in early lactation dairy cows fed omega-3 and omega-6 fatty acids. *Theriogenology* 80:131-137.
- Duffield, T. F., H. Putnam-Dingwell, D. Weary, A. Skidmore, L. Neuder, W. Raphael, S. Millman, N. Newby, and K. E. Leslie. 2009. Effect of flunixin meglumine treatment following parturition on cow health and milk production. *J. Dairy Sci.* 92 (E-Suppl. 1):117 (Abstr.).
- Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013a. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. *Am J Physiol Regul Integr Comp Physiol* 305:R110-R117.
- Farney, J. K., L. K. Mamedova, J. F. Coetzee, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013b. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. *J. Dairy Sci.* 96:7709-7718.
- Huzzey, J. M., S. Mann, D. V. Nydam, R. J. Grant, and T. R. Overton. 2015. Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. *Prev Vet Med* 120:291-297.
- McDougall, S., M. A. Bryan, and R. M. Tiddy. 2009. Effect of treatment with the nonsteroidal antiinflammatory meloxicam on milk production, somatic cell count, probability of re-treatment, and culling of dairy cows with mild clinical mastitis. *J. Dairy Sci.* 92:4421-4431.
- Meier, S., N. V. Priest, C. R. Burke, J. K. Kay, S. McDougall, M. D. Mitchell, C. G. Walker, A. Heiser, J. J. Looor, and J. R. Roche. 2014. Treatment with a nonsteroidal antiinflammatory drug after calving did not improve milk production, health, or reproduction parameters in pasture-grazed dairy cows. *J. Dairy Sci.* 97:2932-2943.
- Nonnecke, B.J., K. Kimura, J.P. Goff, and M.E. Kehrl. 2003. Effects of the mammary gland on functional capacities of blood mononuclear leukocyte populations from periparturient cows. *J. Dairy Sci.* 86:2359–2368.

- Trevisi, E., M. Amadori, A. M. Bakudila, and G. Bertoni. 2009. Metabolic changes in dairy cows induced by oral, low-dose interferon-alpha treatment. *J. Anim Sci.* 87:3020-3029.
- Trevisi, E. and G. Bertoni. 2008. Attenuation with acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. Pages 22-37 in *Aspirin and Health Research Progress*. P. I. Quinn, ed. Nova Science Publishers, Hauppauge, NY.
- Vangroenweghe, F., L. Duchateau, P. Boutet, P. Lekeux, P. Rainard, M. J. Paape, and C. Burvenich. 2005. Effect of carprofen treatment following experimentally induced *Escherichia coli* mastitis in primiparous cows. *J. Dairy Sci.* 88:2361-2376.
- Waelchli, R. O., R. Thun, and H. Stocker. 1999. Effect of flunixin meglumine on placental expulsion in dairy cattle after a caesarean. *Veterinary Record* 144:702-703.
- Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNF $\alpha$  altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. *PLoS ONE* 8:e80316.