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# Comparison of immune responses in calves fed heat-treated or unheated colostrum

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## ABSTRACT

Understanding the mechanisms that underlie neonatal immune function is important for appropriately treating and preventing disease. Cytokines provided in colostrum may affect immune development and function, but data describing cytokine absorption in calves and the effects of colostrum heat treatment on absorption are limited. The objectives of this experiment were to characterize immune responses in calves that received heat-treated (HT) or unheated (UH) colostrum (in terms of growth, rectal temperature, and blood cytokine and IgG concentrations) and to determine calves' ability to absorb IFNγ and IL1β from HT and UH colostrum. A single large batch of colostrum was divided to create treatments. The HT colostrum was heated to 60°C for 60 min. Both treatments were frozen until needed and warmed immediately before feeding. Bull calves (n = 26) were randomly assigned to receive 8% of their birth weight in colostrum from 1 treatment at birth. Blood was collected at 0 and 24 to 48 h after birth for IL1β, IFNγ, and IgG analyses. Subcutaneous injections of ovalbumin (5.0 mg/mL) were given at 14 and 35 ± 3 d of age. Rectal temperature and growth were monitored for 10 d following each injection. Plasma samples were collected at 0, 4, 8, and 12 h post-injection and daily for the subsequent 10 d to measure IL1β, IFNγ, and IgG concentrations. Colostrum heat treatment failed to increase blood IgG concentrations or the apparent efficiency of IgG absorption. Serum IL1β concentrations were higher in UH calves 24 to 48 h after birth and remained higher than those in HT calves through 15 d of age. Both IFNγ and IgG increased in response to ovalbumin injection; we observed no differences between treatments. Rectal temperature increased and peaked 12 h after injection at 14 and 35 d. Growth rate was reduced by exposure to the foreign antigen. Interactions of calf age and colostrum treatment with time post-injection indicate that calves tended to show greater loss in average daily gain at 35 d than at 14 d, and UH calves tended to recover greater rates of growth 6 to 10 d after receiving ovalbumin injection. Thus, feeding HT colostrum did not inhibit neonatal immune response, but it may have affected recovery from exogenous antigen challenge.
**Key words:** calf, immune response, cytokine, colostrum heat treatment

## INTRODUCTION

Illnesses in calves and heifers can hinder them from achieving their genetic potential as mature cows (Heinrichs and Heinrichs, 2011). Lost production, combined with the costs associated with disease treatment, can limit dairy farm profitability. In addition, disease treatment and prevention using antibiotics faces increasing scrutiny in all species of livestock. A clear understanding of neonatal immune function and the external changes observed during an immune response may provide insights into possible means of disease intervention and times when intervention may be necessary.

One important factor that affects bovine neonatal immune function is the first feeding of colostrum (Nonnecke et al., 2012). Colostrum is a vital source of energy, minerals, and bioactive proteins and peptides for neonatal mammals (Kehoe et al., 2007). The majority of research investigating the role of colostrum on bovine neonatal immune function has focused on Ig absorption and "maternal interference," where absorbed Ig interact to prevent vaccine response (Windeyer et al., 2012; Yang et al., 2015). These data have great value, but with the development of new methods for assessing immune function, we need to evaluate aspects of colostrum and immune response in addition to Ig concentration. One factor that can provide some insight into innate and T-cell function is cytokine production. Hagiwara et al. (2000) described high concentrations of IL1β, IFNγ, tumor necrosis factor α (**TNFα**), and IL6 in bovine colostrum, and decreases in each with subsequent milkings, similar to IgG (Elizondo-Salazar and Heinrichs, 2009). In mature animals, these cytokines are important for activating immune cells and

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stimulating proinflammatory responses (Kushibiki et al., 2003; Dinarello, 2009); they may also be involved in activating the neonatal immune response. Some cytokines, including IL1β, IFNγ, and IL6, are absorbed by neonatal piglets and can affect piglet immune response, whereas others, including TNFα, do not appear to be absorbed, implying selective absorption (Nguyen et al., 2007). Calves appear to be able to absorb IL1β (Goto et al., 1997), but to our knowledge no studies have investigated calves' ability to absorb other cytokines or the possible effects of cytokines on immune response.

Surveys of colostrum bacterial concentration on dairy farms report values as high as 7.0 log cfu/mL and 6.6 log cfu/mL of total and coliform bacteria, respectively, due to post-harvest contamination (Houser et al., 2008). Heat treatment of colostrum is becoming common practice on dairy farms as a means of reducing bacterial infection in neonatal calves and increasing IgG absorption. Most recent data indicate that approximately 20% of dairy heifers in the United States receive heat-treated colostrum (NAHMS, 2016). Recommended heat-treatment protocols require that colostrum be heated to 60°C and maintained at that temperature for 30 to 60 min, based on data that IgG concentration and colostrum viscosity are not largely affected by this temperature–time combination (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009). Colostrum heat treatment does reduce concentrations of IGF-1 and lactoferrin and may cause losses of other biologically active proteins, including cytokines (Gu et al., 1991; Abd El-Fattah et al., 2014). Therefore, we hypothesized that calves would be able to absorb cytokines from colostrum, but that absorption would be lower in calves that received heat-treated colostrum. Further, we expected that reduced cytokine absorption would affect neonatal immune development. With the growing popularity of heat treatment, data are needed to determine any indirect effects on calf development. The objectives of this experiment were to determine calves' ability to absorb IFNγ, TNFα, and IL1β from heat-treated and unheated colostrum, and to characterize calves' growth and immune responses to an ovalbumin challenge.

## MATERIALS AND METHODS

### Colostrum Treatments

First-milking colostrum was collected from individual cows at the Pennsylvania State University dairy farm into clean 1.89 L containers and stored at −20°C until treatment preparation. Approximately 114 L of colostrum was thawed at 4°C, pooled, and divided into 2 equal batches. Half of the colostrum was poured into sterile 1.89 L containers and stored at −20°C until needed for feeding to calves (unheated; **UH**). The other half was heated in stainless steel containers (28 L each) in a commercial steam vat pasteurizer (Girton Manufacturing Co., Millville, PA; Elizondo-Salazar and Heinrichs, 2009). All containers were fitted with agitators to ensure even heating of the colostrum. Colostrum temperature was monitored throughout heat treatment and never exceeded 60°C. Colostrum was heated to 60°C, maintained at that temperature for 60 min, and then rapidly cooled, bottled in sterile 1.89 L containers, and stored at −20°C until needed for feeding to calves (heat-treated; **HT**). Aliquots from both treatments were collected at the time of preparation and plated for total bacteria count. The remaining samples were stored at −20°C for IgG analysis.

### Calves and Sampling

All animal procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC #46062). Twenty-six Holstein bull calves (n = 13 per treatment), sourced from a large local dairy farm, were enrolled in the experiment. Sample size was dictated by housing availability and determined to be sufficient based on the final sample size reported from a study that used a similar immune challenge (Ballou and DePeters, 2008). To be included in the experiment, calves must have had an unassisted birth observed by the lead author and a birth weight of 34 to 50 kg. Calves were born onto a nylon tarp, immediately removed from the calving pen, and towel-dried to minimize bacterial exposure and prevent disease. Navels were coated with a 7% iodine tincture, and blood was collected from the jugular vein into serum separator tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for analysis of IgG, IFNγ, IL1β, and TNFα. Colostrum treatments were randomly assigned at birth using a random number generator, and each calf received a volume equal to 8% of its birth weight in a single feeding. Colostrum was thawed in a warm-water bath (38 to 43°C) and fed to calves using an esophageal feeder to ensure the treatment was completely consumed. Calves received colostrum within 4.5 h of birth and were transported to the Almquist Research Center at the Pennsylvania State University within 12 h. All equipment for transporting and feeding calves was cleaned and sanitized between each calf or group of calves, as was the case of the truck for transport to the research center. A second blood sample was collected into a serum separator tube 24 to 48 h after birth. Blood samples were allowed to clot, then cen-