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Short communication: Effects of an immunomodulatory feed additive on phagocytic capacity of neutrophils and relative gene expression in circulating white blood cells of transition Holstein cows

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ABSTRACT

High-producing dairy cows typically experience immunosuppression with dysregulated neutrophil function (e.g., compromised phagocytosis) during the transition period (3 wk before to 3 wk after parturition), which is causally associated with increased risk of infections. Enhanced neutrophil immune competence has significant bearing with the wellbeing of transition dairy cattle. In the current study, we investigated the effect of OmniGen-AF (OG; Phibro Animal Health, Quincy, IL) and its dose in modulation of neutrophil function of transition cows. Forty-eight multiparous cows were stratified by parity, somatic cell count, and expected calving date and randomly assigned to 3 treatments: OG fed at 0 g/head per day (CON), 60 g/head per day (OG60; recommended dose), and 90 g/head per day (OG90; 1.5× recommended dose). The OG was added from dry off (61.8 ± 1.69 d before parturition) to 28 d in milk (DIM), and removed from all treatment groups at 29 to 35 DIM (the last week of the experimental period). Neutrophil phagocytic ability against *Staphylococcus aureus* and *Escherichia coli* was improved and tended to be improved, respectively, by OG from d 28 before parturition to 28 DIM. Cows in OG60 had higher neutrophil phagocytic ability against *S. aureus* and *E. coli* compared with CON cows from d 28 before parturition to 28 DIM. Neutrophil phagocytosis of *S. aureus* and *E. coli* was higher and tended to be higher for OG60 compared with CON on 35 DIM. The relative gene expression of *CXCL8* and *SELL* were upregulated and tended to be upregulated by OG from 60 d before parturition to 28 DIM; this was due to cows in OG60 having greater *SELL* and *CXCL8* gene expression than CON. Expression of *SELL* in circulating white blood cells of OG60-treated cows was greater than OG90 and the relative expression of *CXCL8* gene tended to be greater for OG60 compared with CON on 35 DIM. In conclusion, feeding OG at the recommended dose of 60 g/head per day from dry off was effective in maintaining peripheral blood neutrophil function in transition dairy cows, and it is not necessary to feed OG beyond the recommended dose.

Key words: OmniGen-AF, neutrophil phagocytosis, *CXCL8*, *SELL*

Short Communication

It is common for high-producing dairy cows to experience immunosuppression during the transition period (3 wk before to 3 wk after parturition), which makes them more susceptible to opportunistic infections. As the first line of defense, neutrophils are crucial effector cells in immune surveillance and protection against invading pathogens (Burton and Erskine, 2003). Neutrophil trafficking, phagocytosis, and killing abilities are impaired by many of the physiological changes associated with parturition, which contributes to immunosuppression (Kulberg et al., 2002; Yuan et al., 2014). Diminished immunocompetence not only increases the number of new infections leading to disease, but may easily result in a subclinical disease developing into a clinical one (Kimura et al., 1999). Increased incidence of disease can be associated with reduction in milk yield and quality (Goff and Horst, 1997; Drackley, 1999).

Neutrophil phagocytic activity is crucial in prevention of animal diseases (Moya et al., 2008). To accomplish its mandate, the expression of adhesion molecules and cytokines is necessary. Whenever bacteria infect the body, the neutrophils need to egress the blood system and enter the infection sites with the aid of adhesion molecules and cytokines (Kimura et al., 1999). This is achieved by adhesion molecules binding and rolling along the endothelial cell surface of the vascular system (Kimura et al., 1999).

According to the manufacturer, OmniGen-AF (**OG**; Phibro Animal Health, Quincy, IL) contains mainly

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a mixture active dried *Saccharomyces cerevisiae* and B-complex vitamins and other trace compounds, such as plant proteins, diatomaceous earth, rice hulls, and mineral oil (Ryman et al., 2013; Brandão et al., 2016). OmniGen-AF is a widely used proprietary immunomodulator that has been proven to improve the neutrophil function in immunosuppressed sheep (Wang et al., 2007). A study on growing heifers also reported positive effect of OG on leukocyte function and gene expression (Ryman et al., 2013; Nace et al., 2014). Moreover, the study of Brandão et al. (2016) demonstrated that OG enhanced innate immunity parameters in an in vivo LPS challenge model. To the best of our knowledge, effects of OG dose on peripheral blood neutrophil phagocytosis and gene expression of multiparous transition Holstein cows has not been reported. We hypothesized that feeding OG beyond the normal recommended dose of 60 g/head per day would offer additional benefits to high-producing Holstein cows during the transition period by investigating its effect on the neutrophil phagocytic ability against *Escherichia coli* and *Staphylococcus aureus* and the gene expression of the adhesion molecule *SELL* and the cytokine *CXCL8*.

Animal care and use were in accordance with practices outlined in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010). Forty-eight multiparous Holstein cows were stratified by parity, SCC, and expected calving date and assigned into 3 treatments (16 cows for each group: 9 cows in the third parity and 7 cows in the first parity): fed at 0 g/head per day (**CON**; SCC = 173.13 ± 128.57 × 1,000/mL, BW = 723.87 ± 86.51 kg, BCS = 3.43 ± 0.26), 60 g/head per day (recommended dose, **OG60**; SCC = 173.50 ± 126.93 × 1,000/mL, BW = 726.43 ± 72.73 kg, BCS = 3.46 ± 0.29), and 90 g/head per day (1.5× recommended dose, **OG90**; SCC = 174.56 ± 139.16 × 1,000/mL, BW = 729.07 ± 67.84 kg, BCS = 3.50 ± 0.28). Cows were transferred to an experimental barn 74 d before expected parturition and dried off 2 wk later (61.8 ± 1.69 d before parturition). The experimental barn was equipped with a delivery room, 48 feeding bunks, and the Roughage Intake Control System (Insentec B.V., Marknesse, the Netherlands). All the cows were trained to use the bunks before the start of the experiment. The experiment was conducted from dry off to 35 DIM. All cows were fed diets as a TMR, offered once (at 1600 h) daily prepartum and twice (at 0730 and 1430 h) postpartum. All the cows were milked 3 times daily (at 0700, 1400, and 2030 h). The OG was mixed with corn meal before being added into a TMR mixer; the additive was added from dry off to 28 DIM.

Blood samples were collected (at 0800 h) on d −60, −28, −14, −7, 1, 7, 14, 28, 32, and 35, by puncturing the coccygeal vein with 20 gauge × 2.5 cm needles (all the collection days were relative to calving date). Blood sample (10 mL) used for neutrophil isolation was collected in 1 K2-EDTA vacuum tube (Shandong Aosaite Medical Devices Co. Ltd., Shandong, China) and a 3-mL blood sample used for RNA isolation was collected in 1 Tempus Blood RNA tube (Life Technologies, Foster City, CA) per cow. The Tempus Blood RNA tube was hand-shaken vigorously for 10 s after collection and stored at −20°C until RNA isolation. The blood sample in K2-EDTA tube was sent to the laboratory for neutrophil isolation and phagocytosis ability (against *S. aureus* and *E. coli*) test.

Neutrophils were isolated from EDTA anticoagulated whole blood within 3 h of blood sampling using the Bovine Peripheral Blood Neutrophils Isolation Kit (Tianjin Haoyang Biological Manufacture Co. Ltd., Tianjin, China; catalog number: LZS1094). The density gradient separation method was used to isolate neutrophils from whole blood, as per Oh et al. (2008), with few modifications. A total of 9 mL of whole blood was blended with the same volume of blood diluent in the process of neutrophil isolation. After washing with Hank's Balanced Salt Solution (Yocon Biology Co. Ltd., Beijing, China) without Ca^{2+}/Mg^{2+}, the isolated neutrophil pellet was dissolved with 500 μL of RPMI1640 media (HyClone, Logan, UT) in a 1.5-mL centrifuge tube. A subsample (10 μL) was taken and diluted with 90 μL of PBS in a new 1.5-mL centrifuge tube for cell counting. The final concentration of the suspension was adjusted to 3×10^{E6} cells/mL with RPMI-1640 media. Phagocytic activity was measured with pHrodo Green labeled *E. coli* or *S. aureus* BioParticles (Life Technologies). In brief, 2 mL of PBS was pipetted into a vial containing pHrodo Green labeled *E. coli* or *S. aureus* BioParticles. The solution was then briefly vortexed to completely suspend the particles. One milligram per milliliter (approximately 3×10^{E8} *E. coli* or *S. aureus* particles per milliliter) of pHrodo Green *E. coli* or *S. aureus* BioParticles was obtained. Suspensions of PMNL (200 μL) were placed into a 1.5-mL sterile centrifuge tube with 10 μL of 1 mg/mL pHrodo Green labeled *E. coli* or *S. aureus* BioParticles. The solution was briefly vortexed to mix the cells with BioParticles. The mixture was incubated for 30 min at 37°C and 100% humidity in a 5% CO_2 incubator. Two hundred microliters of FACS Fix (freshly made 4% paraformaldehyde in 1 × PBS) was then added and placed at 4°C for 30 min. The solution was centrifuged at 135 × *g* at 4°C for 5 min. The supernatant was removed and the cell pellet was resuspended in 50 μL of 1 × PBS. Finally, the phagocytosis ability was assessed by flow cytometry (488 nm of excitation with a 530/30-nm bandpass filter; BD LSRFortessa SORP, San Jose, CA). The rate of phagocytosis was calculated as the percentage of cells