Effects of Feeding OmniGen-AF® on Phagocytic Ability of Neutrophils Isolated from Dairy Cattle, Rats and Mice

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Introduction

In recent studies (Wang et al., 2007, 2009), we determined that feeding OmniGen-AF® (Prince Agri Products, Quincy, IL) to ruminant animals alters expression of markers of immunity. In a study with immunosuppressed sheep (Wang et al., 2007), feeding OmniGen-AF® increased concentrations of neutrophil L-selectin and interleukin-1 beta. A subsequent study with dairy cattle (Wang et al., 2009) indicated that the product altered expression of a broad spectrum of neutrophil genes including interleukin-8 receptor and interleukin converting enzyme. While changes in gene expression are of interest academically, one might question whether this regulation results in a change in the physiology of neutrophils. As a result, the goal of this study was to assess effects of feeding OmniGen-AF® on the phagocytic ability of neutrophils. Ability of OmniGen-to elicit these changes was examined in three species (bovine, rat, mouse) against three bovine pathogens (Streptococcus uberis, E. coli, and Arcanobacterium pyogenes).

Materials and Methods

Bovine isolates of S. uberis, E. coli, and A. pyogenes were recovered as field isolates from bovine clinical cases. S. uberis and E. coli were cultured and identified following cases of bovine mastitis. A. pyogenes was isolated and identified following a case of bovine metritis. S. uberis and E. coli were grown in Luria Bertani broth. A. pyogenes was grown in brain-heart broth. Bacteria were grown to mid-log density then diluted and combined with neutrophils for neutrophil killing (phagocytosis) assays (CellTiter 96 Non-radioactive cell proliferation assay, Promega, Madison, WI) as per manufacturer’s directions at a 30:1 ratio (bacteria:neutrophil). To carry out phagocytosis assays, neutrophils were isolated from control-fed and OmniGen-AF®-fed dairy cattle, mice, and rats. Neutrophils were purified via Percoll gradient centrifugation (Wang et al., 2007, 2009). Dairy cattle (n = 15 per treatment) were provided with OmniGen-AF® for 60 days prior to recovery of neutrophils. Ability of cow neutrophils to phagocytose S. uberis was then assessed. Neutrophils were also isolated from control and OmniGen-AF®-fed mice (n = 8 animals per treatment). Mice were fed for 17 days prior to assessment of phagocytosis of E. coli. Finally, rats (n = 6 per treatment) were assigned to control or OmniGen-AF® diets for 14 days prior to recovery of their neutrophils and assessment of A. pyogenes phagocytosis. Neutrophils were incubated with pathogen for two hours, after which ability of bacteria to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was assessed as a marker of pathogen killing ability (phagocytosis; 655 nm). Differences between treatments were assessed using a Student’s t-test.

Results

Ability of neutrophils to kill all three pathogens used in this study (S. uberis, E. coli, and A. pyogenes) was enhanced (P < 0.05) by the pre-feeding of OmniGen-AF®. This effect was observed in both ruminant animals (dairy cattle) and non-ruminants (rats and mice). Feeding dairy cattle OmniGen-AF® for 60 days prior to recovery of neutrophils enhanced their killing of S. uberis by 40% (P < 0.05). Feeding rats OmniGen-AF® for 14 days prior to recovery of neutrophils enhanced their killing of A. pyogenes by 50% (P < 0.05). Feeding mice OmniGen-AF® for 17 days prior to recovery of neutrophils enhanced their killing of E. coli by 44% (P < 0.05).

Significance

Previous studies have shown that feeding OmniGen-AF® altered expression of neutrophil genes isolated from peri-parturient dairy cattle and sheep (Wang et al., 2007, 2009). Others have recently reported that feeding OmniGen-AF® reduces incidence of mastitis in dairy cattle (Wada et al., 2008). The goal of this study was to determine whether we might identify a physiologic consequence and basis for these observations at the molecular level and at the farm level, respectively. Results indicated that one mechanism by which OmniGen-AF® may benefit herd health is via increased ability to phagocytose (kill) pathogens.