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

A.D. Rowson, *DVM;* **Y. Wang,** *PhD;* **N.E. Forsberg,** *PhD;* **S.B. Puntenney,** *PhD*
OmniGen Research LLC, Corvallis, OR 52068

© Copyright American Association of Bovine Practitioners; open access distribution.

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.