

**Department of Agriculture, Trade and Consumer Protection
Division of Agricultural Development
Agricultural Development & Diversification Program (ADD)
Grant Project Final Report**

Contract Number: 23023

Amount of Funding Awarded: \$32500

Report Submitted on: 2-17-10

Grant Project Title:

Efficacy of an Immuno-Modulating Agent in Dairy Cattle

Name of Principal Contact Person:

Mark Burton

Immuno-Dynamics, Inc.

Location: Fennimore

Email Address: mburton@immunodynamics.com

WEB Address: www.immunodynamics.com

The Safety and Efficacy of ID-1, an Injectable Immunomodulating Agent, and Vitapak, an Immunomodulating Dietary Supplement, Individually and in Combination for the Amelioration of Mastitis Induced in the Lactating Cow by *Staphylococcus aureus* (*S. aureus*)

The present research has examined the efficacy and effectiveness of ID-1 and Vitapak, alone and in combination, on components of innate and acquired immunity *in vitro* and *in vivo* in relation to bovine *S. aureus* mastitis.

This study was basically designed to determine if the colostral products, Vitapak, an immunomodulating dried dietary supplement given orally, and/or ID-1, an immunomodulating sterile liquid product injected parenterally or administered into the mammary gland via the streak canal, together or separately, were beneficial in reducing experimental mastitis in dairy cows infected with *Staphylococcus aureus*.

As expected from previous studies, both the Vitapak and the ID-1 were very safe when administered according to manufacturer's directions. The product was also found to be safe when infused into the mammary gland at volumes as high as 10 ml per quarter, even when given in all four quarters. The ID-1 did cause a significant transient increase in the SCC of the treated quarter that persisted for several days.

The following *in vitro* and *in vivo* assay were performed as described:

- I. *In vitro* Bioassays
 - a. Immunomodulatory components in ID-1
 - b. Effects on selected innate and acquired immunity assays
- II. *In vivo S. aureus* Mammary Gland Challenge Study
 - a. CFU/mL and percentage positive culture results among the 4 test groups of cows
 - b. *S. aureus* growth inhibition assay using treated vs. non-treated animal sera and quarter milk samples
 - c. SCC analysis by test group from selected pre and post challenge days
 - d. Milk conductivity analysis
- III. Conclusions

I. *In vitro* Bioassays

a. Immunomodulatory components in ID-1

A number of *in vitro* bioassays were used to determine the presence of ID-1 immunomodulatory components. Both *S. aureus* antibody (Ab) ELISA and IgG radial immunodiffusion (RID) results displayed high levels of antibody to *S. aureus* when compared to sera from known *S. aureus* positive bovine samples. ID-1 also demonstrated high antibody titers to BVDV I, BVDV II, and IBR and high levels of IgG by RID. IL-8 and IFN- γ cytokines were not detected with their respective ELISAs. No hemolytic complement activity was observed with the total hemolytic complement assays when varying ID-1 dilutions (1:5 to 1:1000). Lastly, a low cortisol concentration, 3nmol/L, was determined through a baseline cortisol assay (performed at Michigan State's Diagnostic Center for Population and Animal Health [reference range: 8-55nmol/L].)

III. Conclusion

- 1) Antibody activity to *S. aureus* was present in ID-1 at high levels, but no activity was present in infected or control quarters post treatment, even in the Group D cattle directly infused with ID-1. This would not be unexpected, as high dilutions of the product occur very quickly, irrespective of route of inoculation.
- 2) High concentrations of ID-1 displayed the following *in vitro* activities: lymphocyte suppression in LBTs and inhibition of *S. aureus* growth in bacterial killing assays.
- 3) No significant biological differences were seen among the four treatment groups with regard to *S. aureus* bacterial culture results based on number of days positive or CFU/sample.
- 4) Inhibition of *S. aureus* growth wasn't seen *in vitro* when sera or individual quarter milk samples of treated animals were mixed *in vitro* with bacteria. All samples had CFU of bacteria equal to or greater than the media control.
- 5) Results of milk conductivity meter readings didn't correlate with infection status of the animals in this study!

IV. Future Test Analysis

Future analysis will include the following: *S. aureus* Ab ELISA and IgG single radial immunodiffusion (RID) on quarter milk and sera samples from select pre and post *in vivo* study dates. Also, PhagoTest (ORPEGEN, Pharma) analysis of ID-1 dilutions (1:5, 1:10, 1:50, 1:100, 1:1000) to determine the influence on leukocyte phagocytic activity against FITC-labeled *E. coli* (nonspecific enhancement).

A weakness of the current *in vivo* study was the small number of animals in each of the four treatment groups. However, if the treatment was 50% or greater effective in reducing the severity of the mastitis, it should have been observed, and it was not, based on positive bacterial cultures and SCC, the two most common criteria for diagnosis of bacterial mastitis. Future *in vivo* studies to show an effect for treatments with Vitapak and/or ID-1 should be done in herds with a high incidence of *Staphylococcus aureus* mastitis or mastitis caused by any organism. One half of the animals would be given the treatment of choice and the other half of the herd treated with placebo in a double blind trial. Over a period of 6 months up to 1 year, the number of confirmed mastitis cases and severity would be compared between the treated and placebo group. SCC, bacterial cultures, clinical disease signs, and culling rates of affected cows, days in milk and production and reproduction records would all be used to compare treated cows and untreated cows.