FINAL REPORT
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DETERMINATION OF PROTEIN CONTENT AND POTENTIAL USES OF BOVINE AND SWINE MAMMARY GLAND TISSUE HOMOGENATES

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Objective (s): Assess the content and potential uses of extracted mammary tissue proteins from processed dry dairy cows and culled sows.

Project Overview: This project was proposed last year as a study to determine if mammary tissue, a by-product of meat processing, could be used as a protein source, especially for use as a serum replacement in cell culture medium. To date, the research has involved the collection of udders from both dairy and beef cows processed at a local plant in the Upstate. Diary and beef udders were collected at the plant within minutes of euthanasia. The udders were packed in ice and transported to the Clemson University Meats lab were the udders were further processed for tissue separation. Due to the abundance of milk and blood in the udders, it was determined that the whole udder tissue would be adulterated with these fluids and would not allow for accurate determinations of udder proteins only. However, it was determined that each udder had attached to it a pair of super mammary lymph nodes near the surface of the expose udder tissue. These apparent rich sources of protein containing specific growth and differentiation factors became the tissue of interest for the project. One super mammary lymph node from each dairy and beef udder were cut away and cleared of adherent adipose tissue. The lymph nodes were packed in sealed plastic bags and stored at –20 C until homogenized for protein recovery.

The frozen lymph nodes were thawed and 2.5gram pieces of each node were homogenized in a blender with 25 mL of phosphate buffered saline (PBS). Following homogenization, the tissue slurry was transferred to a 50 mL centrifuge tube and spun at >13,000 x *g* for 30 minutes. The resulting supernatant was recovered from the tissue pellet and clarified by passage through a 0.2-micron filter. The clarified supernatant was then used in a protein assay to determine protein concentrations of dairy verses beef lymph nodes. The average protein concentration for dairy and beef lymph node extracts were 8.87 and 8.43 mg/mL, respectively. Upon comparison of the protein concentrations from both sources, it was concluded that there was not a difference in protein concentrations. Therefore, the use of either dairy or beef super mammary lymph nodes will provide high concentrations of protein.

At present, our laboratory group is culturing a bovine mammary epithelial cell line, MAC-T. This cell line has been used in previous studies with udder extracts and insulin-like growth factor-1 (IGF-1) to determine if these can support the growth of MAC-T cells. We have determined that MAC-T cells growth well in the presence of 10% bovine growth serum. We will next conduct a MAC-T cell starvation/refeeding experiment to determine the percent of lymph node supernatant that can effectively support the growth of these cells equivalent to 10% BGS.

Impacts and Significance: At present, udders are discarded at the processing plant and must be rendered. The udder itself may still be a useful source of proteins; however, more research will be required to make that determination. Presently, we are focusing on the super mammary lymph nodes as a source of proteins and factors that could be useful for cell culture supplementation. Additionally, the proteins and factors recovered from the lymph nodes could be beneficial for the development of biomedical and veterinary products. Based on the ease of recovery of the lymph nodes, a small departure from removal and discard of the udder at the processing plant could be added post-euthanasia for the quick recovery of the nodes. This would then provide a rich source of proteins that can be processed on site or shipped to another facility for homogenization and clarification. Ultimately, biomedical and veterinary products could be

harvested that would be profitable on a scale of hundreds to thousands of dollars per milligram of protein. Comparatively, FBS or BGS cost $37-72/100 mL from various sources. Additionally, bioactive proteins and factors that support cell growth and differentiation often cost hundreds to even thousands of dollars on a milligram or microgram basis. Therefore, efficient recovery and isolation of super mammary lymph node proteins could prove to be profitable for the processing/rendering industry as value added products.

Publications: None at this time, but one manuscript is anticipated from the initial work to be done with the MAC-T cells.

Future Work: Our lab group will complete the comparison of dairy and beef lymph node proteins on growth of MAC-T cells. We will then incorporate additional cell lines (e.g., cancer cell lines, hybridomas, etc) in order to determine the overall usefulness of the proteins for serum replacement in cell culture. Eventually, we will determine the different proteins in the clarified supernatants with the use of proteomics analysis by mass spectrometry. The latter will allow us to isolate specific proteins that will be of most interest for biomedical and veterinary product development. A Masters level graduate student will begin work on this project in August 2005.

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