

Jyotsna Dhakal

Biology Major

First Place, Oral Presentation Category at the 2014 Pennsylvania Academy of Science



Dhakal, Jyotsna*, Brad E. Engle and M. Dana Harriger. Wilson College, Chambersburg, PA 17201. *Determining the Effect of Vitamin D Supplementation on Bacillus Calmette-Guérin Vaccine-Induced Immune Response in Guinea Pigs* – The reduced efficacy of the Bacillus Calmette-Guérin (BCG) vaccine at close proximity to the equator is not fully understood. Ultraviolet radiation exposure a few days before or after BCG vaccination impairs BCG-induced resistance against tuberculosis in guinea pigs. A possible explanation for this could be the greater production of vitamin D due to enhanced sunlight exposure near the equator. Clinical studies suggest that vitamin D enhances immunity against mycobacteria, and that vitamin D deficiency is associated with susceptibility to active tuberculosis. Furthermore, vitamin D suppresses BCG proliferation *in vitro*. Paradoxically, *in vitro* studies indicate that vitamin D inhibits Th1 mediated immunity, a crucial component of the immune response against mycobacteria. These properties suggest that vitamin D could impair the efficacy of BCG either through antimicrobial activity against BCG or by restricting the immune response produced following vaccination. The aim of this study was to investigate whether vitamin D impairs the protective efficacy of BCG. Guinea pigs were given weekly doses of 1000 IU or 2000 IU vitamin D supplementation orally for 12 weeks. On the fifth week of vitamin D administration, animals were vaccinated with BCG. Seven weeks after BCG vaccination, a whole blood assay using *Mycobacterium tuberculosis* purified protein derivative was employed to generate IFN- γ responses. IFN- γ levels in the culture supernatants were quantified by ELISA. Preliminary analysis indicated that the samples from BCG-vaccinated groups that were given vitamin D had lower levels of IFN- γ than the BCG-vaccinated group that was not given any vitamin D, suggesting that vitamin D impaired the efficacy of BCG. Results of this study may provide evidence that vitamin D is a factor contributing to the variability of BCG efficacy with global latitude.

Meghan Stine

Biology Major

Outstanding Research Grant from the Pennsylvania Academy of Science

John D. Rose Award

Second Place, Oral Presentation Category at the 2014 Pennsylvania Academy of Science



Stine, Meghan*, Laura F. Altfeld, and Deborah S. Austin. Wilson College, Chambersburg, PA 17201. *Habitat effects on captive elephant reproduction: A study of size and quality of captive elephant habitats for both Asian and African elephants.* Acyclicity, or abnormal reproductive cycle, is a problem in captive elephants. The reason for this is unknown; however, if it is not addressed, captive elephant populations may die out. It is possible that acyclicity is due to environmental stress, including insufficient space in zoos and small group size of the captive elephants, leading researchers to ask whether it is more important to have a large quantity of space or a higher quality of space. This research is designed to address the effects of the size of captive environment on the reproductive status of female elephants. Surveys were sent to 66 zoos in Europe and the United States, asking for information regarding number, type, and sex of elephants, reproductive status, and total area of habitat. Behavioral observations were then conducted at five of the participating zoos within the United States. Biotic and abiotic variables were correlated with reproductive status as reported in surveys. Ethogram data from behavioral observations were quantitatively analyzed using cluster analysis on three categories of behavior: basic, social, and contact. The results indicate that specific size of the habitat does not have a direct effect on the reproductive status, but species and age of elephant did show differences in reproductive status. Social structure and behavior, however, varied based on the area of the captive environment. The results suggest that social structure and behavior of captive elephants can be manipulated through maintenance of the captive environment, and may lead to differences in the reproductive status of those females within the habitat.

Monica Drummond

Biochemistry Major



Drummond, Monica*, Deborah S. Austin, and Rebecca M. Smith. Wilson College, Chambersburg, PA 17201. *Effects of Holy Basil, St. John's Wort, and German Chamomile on Fecal Corticosterone Levels in a Rat Model*- Experiencing high levels of the stress hormones cortisol or corticosterone, particularly for extended periods of time, can cause numerous health issues in an organism. Many herbs are thought to have the ability to reduce the levels of stress hormones. This study focuses on three herbs *Ocimum sanctum* (holy basil), *Hypericum perforatum* (St. John's wort), and *Matricaria chamomilla* (German chamomile). Two different types of herbal extracts, commercial supplements and freshly prepared ethanolic extracts, were used to study the effects of the three herbs on rat corticosterone levels. Each herbal extract was given orally, via a micropipette, to female Sprague Dawley rats (n=20), who subsequently received subcutaneous corticosterone injections. The fecal matter from each rat was collected, corticosterone extraction was performed, and competitive enzyme-linked immunosorbent assay (ELISA) was used to quantify the corticosterone levels. The ELISA analysis showed that corticosterone was present in all of the fecal samples; however, preliminary analysis does not reveal any trends which demonstrated that the herbs have a reductive effect on corticosterone levels. Future research, such as studying the effects of long term administration of the herbs, could help support the hypothesis that holy basil, St. John's wort, and German chamomile can reduce the levels of stress hormones.

Emma Echanis

Biology Major

Outstanding Research Grant from the Pennsylvania Academy of Science



Echanis, Emma*, M. Dana Harriger and Brad E. Engle. Wilson College, Chambersburg, PA 17201. *The Effects of Sonic Hedgehog and Overexpression of the DCDC2 Dyslexia Associated Gene on Growth of Primary Cilia in Developmental Cortical Neurons* – Abnormal primary cilia (Pc) growth in cortical neurons during development has been linked to many neurologic disorders, such as Dyslexia and Attention Deficit Hyperactivity Disorder. Increased expression of *DCDC2*, Dyslexia associated gene, has been shown to stimulate elongation of Pc in developmental cortical neurons and increase extracellular concentrations of Sonic Hedgehog (Shh). To determine whether increased extracellular concentration of Shh and/or the overexpression of the *DCDC2* gene causes abnormal neural outgrowth, an *in vitro* study using E17 rat cortical neurons and cell culture techniques was performed. Half of the cell cultures were transfected with cDNA containing a *DCDC2*-GFP construct, and half were transfected with a control GFP plasmid. Then, half of each transfection group was placed in unaltered cell culture media, and the remaining groups were placed in media supplemented with Shh. After 3 days, the cells were fixed and stored at 4°C. Immunofluorescence targeting of MAP2 and gamma-tubulin was used for visualization and z-axis imaging of the neuronal processes. Data from the images will be evaluated for Pc growth and general cellular outgrowth. If extracellular Shh is linked to abnormal neural outgrowth, then cell cultures containing Shh, overexpressed *DCDC2* or both should exhibit abnormal neural outgrowth. However, if Shh is not linked to abnormal neural outgrowth, then only cultures with overexpressed *DCDC2* will exhibit abnormal neural outgrowth. Results from this study may help to elucidate the mechanisms related to abnormal neural migration, and assist in future efforts to identify and treat the disorders that may be linked to these abnormalities.

Cortney Roper

Biology and Veterinary Medical Technology Major
Outstanding Research Grant from the Pennsylvania Academy of Science



Roper, Cortney*, Brad E. Engle and Laura F. Altfeld. Wilson College, Chambersburg, PA 17201. *Chemotactic Factors Involved in the Migration and Homing of Canine Adipose Tissue-Derived Mesenchymal Stem Cells* - Canine adipose-tissue derived mesenchymal stem cells (cAdMSCs) have the capability of differentiating into osteocytes to form new bone and can be transplanted into an area of injury to elicit healing. However, the signaling molecules and homing mechanisms involved with cAdMSC migration to damaged sites in bone are poorly understood. The objective of this study was to test the effectiveness of several growth factors, transforming growth factor-beta (TGF- β), bone morphogenetic protein (BMP), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), on the migration of cAdMSCs. Adipose tissue was collected from canines that underwent ovariohysterectomies and harvested for mesenchymal stem cell (MSC) isolation. The MSCs were allowed to expand and maintained in cell culture (37°C; 5% CO₂). The chemotaxis of cAdMSCs toward the growth factors was observed using an *in vitro*, transwell, cell migration assay. Following a 48 hour incubation period, the migratory cells were fixed, stained and counted using a digital imaging system. Preliminary results showed that PDGF and BMP elicited the greatest migratory effect, whereas VEGF and TGF- β had a more limited effect. Furthermore, a combination of PDGF and BMP produced an even greater migratory capacity, possibly indicating a synergistic effect. Results from this study may lead to a better understanding of the signaling molecules and homing mechanisms responsible for cAdMSC migration to damaged sites in bones and/or joints, and ultimately may help in the development of more effective regenerative therapies using cAdMSCs.

Janelle Wills

Biology Major

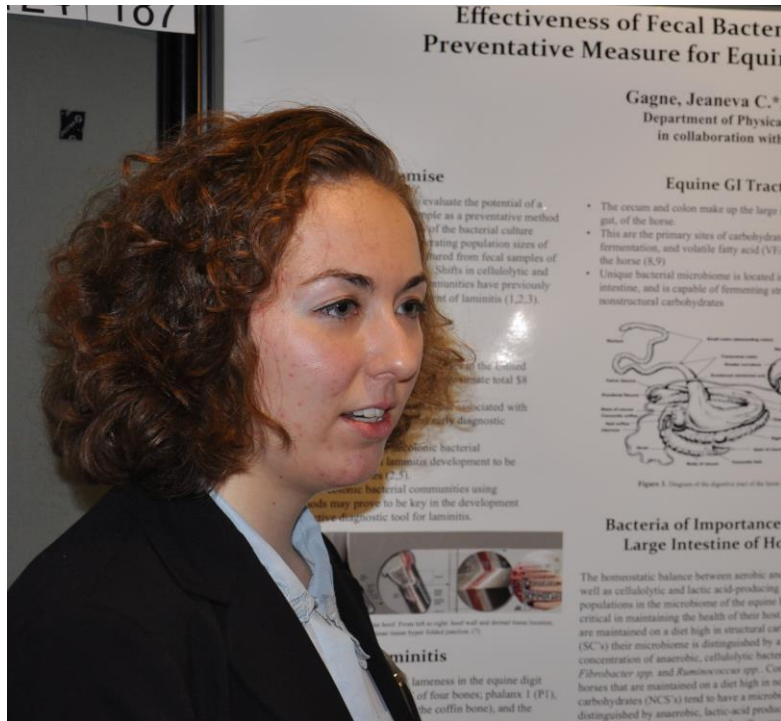
E. Grace White Summer Research Scholarship



Wills, Janelle S.*, Deborah S. Austin and M. Dana Harriger. Wilson College, Chambersburg, PA 17201. *Quantification of Estradiol in Bovine Milk from Cattle Fed Diets Supplemented with *Salvia hispanica* and *Linum usitatissimum** - Each year, 300,000 people in the United States are diagnosed with breast cancer and 44,000 die from the disease (American Cancer Society, 2013.) It is the leading cause of cancer deaths among American women aged forty to fifty-five years. One key factor in the development of breast cancer is elevated levels of estrogen. Studies have indicated that levels of hormonal estrogen can be lowered by phytoestrogens. Chia (*Salvia hispanica*) and flax (*Linum usitatissimum*) contain high levels of phytoestrogens in their seed embryo. In this study, Holstein dairy cows were given chia or flax seed to supplement their standard diet for 30 days. Whole milk samples were collected daily during this period. The milk samples were aliquoted and frozen at -80°C until analysis. Samples were then thawed and an ELISA was conducted to quantify the estradiol concentration levels. The data were then analyzed using ANOVA at significance level of .05 to determine which diet more effectively decreased the amount of estradiol. No observable trends were seen throughout the data; however, less variation in estradiol concentration was seen within the chia group compared to that of the flax and control groups. Future research may include feed additives using an animal model and their relationship to improving human health. This could provide consumers an option, when purchasing milk, to lower their estradiol levels and decrease their risk for cancer naturally.

Jeaneva Gagne

Biology Major



Gagne, Jeaneva^{*a}, M. Dana Harriger^a, Laura F. Altfeld^a, Kurt D. Hankenson^b, and Julie Engiles^b.

^aWilson College, Chambersburg, PA 17201 and ^bNew Bolton Center, School of Veterinary Medicine University of Pennsylvania, Kennett Square, PA 19348. *Effectiveness of Fecal Bacterial Population Enumeration and Analysis as a Preventative Method for Equine Endocrinopathic and Inflammatory Laminitis*- Laminitis is a condition of the equine digit that is characterized by inflammation of the laminae tissue and rotation of the third phalanx; resulting in lameness and permanent hoof alteration. Diets high in non-structural carbohydrates have been identified as an initiating factor in the development of laminitis and are also associated with an alteration in the microbiome of the equine hindgut, from a largely cellulolytic population to one that is predominately lactic-acid producing. The objective of this study was to utilize bacterial culture techniques both *in vitro* and *in vivo* to determine if alterations of carbohydrate sources in the equine hindgut result in a change in population of *Fibrobacter intestanillis*, *Ruminococcus flavefaciens*, *Lactobacillus mucosae*, and *Streptococcus bovis* and if this trend could be observed using fecal samples from both healthy and laminitic horses. The findings from this study have the potential to contribute to the development of a laminitis preventative tool. Such a tool would essentially monitor the microbiome of an animal by regularly culturing fecal samples, the results of which could detect the early onset of laminitis before resulting in permanent damage.