

RESEARCH PROJECTS COMPLETED - 2005	Date Completed
<p>Dr. C. Annett University of Saskatchewan</p> <p>‘Prevalence of <i>Clostridium perfringens</i> type A in broiler chickens in Saskatchewan and its effects on flock performance and condemnations at slaughter’.</p> <p><i>Clostridium perfringens</i> (CP) is the bacterium that causes necrotic enteritis in broiler chickens. The consequences of necrotic enteritis include increased mortality, reduced average daily gain, decreased feed efficiency and increased condemnations at slaughter. Dr. Annett’s objectives were to i) identify the prevalence of <i>Clostridium perfringens</i> in Saskatchewan broiler flocks ii) characterize and quantify production losses due to this bacterium and iii) identify management practices that effect the incidence of CP infections. The study involved visiting 23 farms and obtaining fecal samples from 41 barns. In two rotations Dr. Annett determined that <i>Clostridium perfringens</i> was isolated from 12% of birds in barns and 18% of birds at the processing plant. <i>Clostridium perfringens</i> were present in 32 of the 38 flocks in the first rotation and 33 of the 37 flocks sampled during the second rotation. Anti-alpha toxin antibody levels were present in both chicks at hatch and broilers at slaughter, however, <i>Clostridium perfringens</i> could not be isolated from newly hatched chicks. All flocks had birds infected with coccidial organisms and it was hypothesized that feed could represent a means for a continual source of <i>Clostridium perfringens</i>.</p> <p>Data collected from the study could not confirm that <i>Clostridium perfringens</i> had an effect on condemnations, birds arriving dead at the plant, total mortality, weight gain or feed conversion. Factors that affect the incidence of <i>Clostridium perfringens</i> in broiler flocks are relative humidity and CO₂, feed form, antibiotic type, floor type and barn level on which the birds are raised.</p> <p>The project was approved in July, 2002 at a total cost of \$94,500 over three years.</p>	2005/03/01
<p>Dr. S. Gomis University of Saskatchewan</p> <p>‘Is Inclusion Body Hepatitis a Primary or Secondary Disease in Broilers in Saskatchewan’</p> <p>In recent years, inclusion body hepatitis has become an economically important disease in Western Canada. Dr. Gomis’ objective was to determine if outbreaks of inclusion body hepatitis in broiler flocks in Saskatchewan were caused by other primary diseases, in particular chicken infectious anemia or infectious bursal disease that compromise the immune system or whether inclusion body hepatitis is itself a primary disease.</p> <p>Birds for the study were selected from 17 broiler breeder flocks from 8 of the 9 producers in the province and placed in 31 flocks owned by 11 broiler producers. Inclusion body hepatitis was present in 21 of the 31 flocks and accounted for more than 10% of total mortality in 7 of these 21 flocks. Five source flocks were associated with higher incidences if inclusion body hepatitis, however, Dr. Gomis concluded that management practices were not responsible for these higher incidences. Antibody levels for IBD and CIA were measured in the 17 breeder flocks, in 20 bird samples of day old chicks and at slaughter. The high levels of antibodies in breeders and day old chicks indicated that inclusion body hepatitis and chicken infectious anemia were under control in the breeder flocks and that mothers passed immunity to their chicks. Since maternal antibodies dissipate after about 21 days, the lower levels of antibodies at slaughter indicated that the birds had not been exposed to infectious bursal disease or chicken infectious anemia during grow-out. Necropsies conducted when broilers were two, three and four weeks of age and at slaughter found no relationship between inclusion body hepatitis or chicken infectious anemia. Thus the results suggest that inclusion body hepatitis in Saskatchewan is not associated with infectious bursal disease or chicken infectious anemia and is itself a primary disease.</p>	2005/10/12

The project was approved in July, 2004 at a total cost of \$35,000.

Journal Articles:

Avian Diseases

Inclusion Body Hepatitis as a Primary Disease in Broilers in Saskatchewan, Canada
50:550-555, 2006

RESEARCH PROJECTS COMPLETED 2006	Date Completed
<p>Dr. G. Fasenko University of Alberta</p> <p>‘A microbiological assessment of cleaning methods and disinfectants for broiler barns’</p> <p>Consumer concerns over food safety have increased over the past several years and poultry producers have developed on-farm food safety programs in part to address these concerns. However, very little is known about the effectiveness of current cleaning and disinfection procedures in broiler barns. The objectives of this study were to: (i) test the effectiveness of standard cleaning procedures in lowering overall and pathogenic bacteria counts and (ii) examine the effectiveness of two widely used disinfectants. These objectives were achieved in four experiments. In June 2004 the SCIDF Board approved \$8,900 in funding for this project. The Alberta Chicken Producers, the Alberta Livestock Industry Development Fund and the University of Alberta also provided funding for this project. The major findings of the study were as follows;</p> <ul style="list-style-type: none"> - there were more bacteria in barn corners than in other locations even after all cleaning and disinfection was finished illustrating that special attention should be given to particular barn locations. - the litter removal and washing steps resulted in lower counts of the group of bacteria that cause food poisoning. - disinfectants are more effective when litter removal and cleaning is performed prior to their application. - Proquat® and Virkon® were equal in their ability to reduce the number of bacteria on metal and cement but Proquat® performed better on wood surfaces. - Both cement and metal surfaces had lower numbers of bacteria than wood surfaces. <p>Two peer reviewed publications are expected from this research and the findings were presented at an international conference by the student who worked on the project.</p> <p><i>Journal Articles:</i></p> <p><u>Journal of Applied Poultry Research</u>, 2006, 15:326-332 A Microbiological Assessment of On-Farm Food Safety Cleaning Methods in Broiler Barns</p>	2006/10/31
<p>Dr. S. Sharif University of Guelph</p> <p>‘Gut microflora manipulation for control of food-borne pathogens in chickens’</p> <p>Antibiotics are frequently used in commercial poultry production to prevent bacteria infections and to promote growth and performance. This practice could result in the development of resistance to antibiotics in bacteria including Salmonella and Campylobacter residing in the gut. These two pathogens account for over 90% of all food poisoning cases worldwide. The main objective of the study was to determine if the chicken gut microflora could be manipulated with commercially available prebiotics/probiotics to effectively control and reduce Salmonella burden in the gut and determine if there are any effects on the immune system. The project was approved in July 2004 for \$28,200 over three calendar years. Dr. Sharif also received funding from the Ontario Ministry of Agriculture and Food, the Poultry Industry Council, NSERC and Agriculture and Agri-Food Canada for this project.</p> <p>The study determined that probiotic products could significantly reduce salmonella in the cecum, that the prebiotics used in the study when used alone or in combination with probiotics were not effective in reducing salmonella and that one probiotic product significantly enhanced systemic antibody response but had no effect on antigen-specific antibodies in the gut. The same probiotic product significantly enhanced natural antibodies in serum and intestinal contents. The study resulted in published articles in two refereed journals and several presentations at conferences and industry meetings.</p>	2006/11/07

<p><i>Journal Articles:</i></p> <p><u>Clinical and Diagnostic Laboratory Immunology</u> Modulation of Antibody-Mediated Immune Response by Probiotics in Chickens http://dx.doi.org/10.1128/CDLI.12.12.1387-1392.2005</p> <p><u>Clinical and Vaccine Immunology</u> Probiotics Stimulate Production of Natural Antibodies in Chickens http://dx.doi.org/10.1128/CVI.00161-06</p>	
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<p>Dr. S. Sharif University of Guelph</p> <p>‘The impact of <i>in ovo</i> vaccination on vaccine efficacy and development of the chicken immune system’</p> <p><i>In ovo</i> vaccination is used throughout the world for the delivery of vaccines especially Marek’s disease yet there is little scientific information available on the ways that <i>in ovo</i> vaccines could confer immunity. <i>In ovo</i> vaccines are usually delivered at day 18 of embryonation when the chick’s immune system is not able to fully respond to foreign antigens, thus there is also the possibility that the vaccine could have a negative impact on the chick’s immune system after hatch. The primary objective of the study was to study the impact of <i>in ovo</i> vaccination on the chick’s immune system. The second objective of the study was to determine the appropriate dose of Marek’s disease vaccines since there is no information available on the efficacy of reduced doses of the vaccine and it is apparently common practice to dilute Marek’s disease vaccines. The project was approved in July 2004 in the amount of \$20,000 over three calendar years. Dr. Sharif also received funding from the Ontario Ministry of Agriculture and Food and the Poultry Industry Council for this project. The study had the following major findings;</p> <ul style="list-style-type: none"> - <i>in ovo</i> vaccination for Marek’s disease does not have a negative impact on the chick’s immune system. - Marek’s disease vaccine doses below the recommended dose may lead to the breakdown of immunity against the disease. - all chicks in a flock should be vaccinated against Marek’s disease because the vaccines are not effective in containing the transmission of the virulent Marek’s disease virus. <p>During the course of the study, several immunological correlates of immunity against Marek’s disease that may result in the development of more efficacious vaccines were identified. The results of this study were presented at three scientific conferences and resulted in two journal articles in refereed journals.</p> <p><i>Journal Articles:</i></p> <p><u>Journal of Virological Methods</u> 133:34-40 Development of a real-time PCR assay using SYBR Green chemistry for monitoring Marek’s Disease Virus genome load in feather tips. M.F. Abdul-Careem, B.D. Hunter, É. Nagy, L.R. Read, B. Sanei, J.L. Spencer and S. Sharif (2006). http://dx.doi.org/10.1016/j.jviromet.2005.10.018</p> <p><u>Vaccine</u> 25(3):424-32 Cytokine gene expression patterns associated with immunization against Marek’s disease in chickens. M.F. Abdul-Careem, B.D. Hunter, P. Parvizi, H.R. Haghghi, N. Thantrige-Don and S. Sharif (2007). http://dx.doi.org/10.1016/j.vaccine.2006.08.006</p>	<p>2006/11/17</p>
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RESEARCH PROJECTS COMPLETED 2007	Date Completed
<p>Dr. S. Gomis University of Saskatchewan</p> <p>‘Formulation and Delivery of CpG-ODN against Bacterial Infections in Chickens’</p> <p>The emergence of antibiotic resistant bacteria has focused attention on immune based methods of disease protection. Vaccines are a well known method of directing the immune system to improve disease protection but they are not available for some diseases and are not effective when an immediate protective effect is required. . Dr. Gomis is attempting to develop DNA based immune system stimulants (CpG-ODN motifs) as alternatives to antibiotics against bacterial infections in chickens.</p> <p>In August, 2003, the Board approved funding of \$75,000 over three years for this project. Dr. Gomis was able to secure significant additional funding from other sources to enable the project to be expanded. Dr. Gomis demonstrated that CpG-ODN stimulates the innate immune system of broiler chicks against lethal bacterial infections following <i>in ovo</i> administration. He further demonstrated that CpG-ODN is an effective vaccine adjuvant against bacterial diseases. In the final year of the study, Dr. Gomis demonstrated that CpG-ODN formulated with different delivery systems enhanced systemic protection in broiler chicks against <i>E. Coli</i> infections. Presentations of the project’s findings were made at several national and international conferences.</p> <p><i>Journal Articles</i></p> <p><u>Avian Disease</u> Protection of chickens against a lethal challenge of <i>Escherichia coli</i> by a vaccine containing CpG oligodeoxynucleotides (CpG-ODN) as an adjuvant. Avian Diseases 51:78-83 2007</p> <p><u>Developmental and comparative immunology</u> (submitted) Oligodeoxynucleotides containing CpG motifs (CpG-ODN) induced cytokine and chemokine gene expression in neonatal chickens.</p> <p><u>Infection and Immunity</u> (in preparation) Delivery of CpG-ODN in embryonating eggs to protect neonatal chickens against <i>Salmonella typhimurium</i> infection.</p> <p><u>Curr. Drug Deliv.</u> (in preparation) Formulation of CpG-ODN with polyphosphazines for <i>in ovo</i> delivery and enhancement of immunoprotective effect against <i>Escherichia coli</i> septicemia in neonatal chickens.</p>	2007/02/25
<p>Dr. J. Prescott University of Guelph</p> <p>‘Vaccine Based Control of Necrotic Enteritis’</p> <p>Necrotic enteritis is an important disease of broiler chickens which is currently controlled by antimicrobial drugs. If drugs are banned for this purpose in Canada, as they have been in Europe, alternative strategies to control the disease will need to be developed. One approach is by immunization and this project, which was approved in August 2003 for \$51,000 over three years, provided three years of stipend funding for a PhD student to develop a vaccine against necrotic enteritis. The approach was to (i) identify the differences in genes between strains of the causative agent, <i>Clostridium perfringens</i>; virulent (disease causing) strains which cause the chicken to become immune to the disease and non-virulent strains</p>	2007/02/18

derived from the parent that do not cause the chicken to become immune. (ii) clone and purify the different genes identified and confirm through challenge studies, their role in immunity. (iii) produce a live oral vaccine either by cloning or mutation. The first two objectives have been met and a number of proteins that could be used to immunize birds have been identified. Funding to continue the project for a fourth year and beyond has been obtained from the Canadian Poultry Research Council, matched by NSERC and Agriculture Canada. Although a vaccine was not produced in the three year time frame, it appears that there is a good possibility that the research funded by SCIDF will eventually lead to the development of one. The research resulted in the publication of two articles in refereed journals and three presentations at international conferences.

Journal Articles:

Veterinary Microbiology (in press)

Live attenuated vaccine-based control of necrotic enteritis in broiler chickens

<http://dx.doi.org/10.1016/j.vetmic.2005.10.015>

Clinical and Vaccine Immunology

Clostridium perfringens proteins recognized by broiler chickens immune to necrotic enteritis.

December 2006, p. 1358-1362, Vol. 13, No. 12

Dr. C. Ruiz-Feria
McGill University

2007/07/31

'Plant Phenolics and Prebiotics as Alternatives to Antibiotics in Poultry Production'

The project intended to determine whether lignin could be used as an alternative to antibiotic growth promoters or a commercially available prebiotic, and was approved in February, 2005 for \$30,000 over two years. The effects of a purified form of lignin, a compound found naturally in plant cell walls and available as a by- product of paper manufacturing, was evaluated on: (i) growth performance (ii) gut development and microbial populations in gut and feces (iii) susceptibility to *E. coli* and *Salmonella* invasion and (iv) cellulites. For each on these parameters, the effectiveness of lignin was to be compared with an antibiotic growth promoter and a commercially available prebiotic. The general conclusion was that the prebiotic and to a lesser extent low levels of lignin could potentially replace antibiotic growth promoters since: broiler growth was not significantly affected, the prebiotic increased the concentration of beneficial bacteria in the cecum and reduced *E. coli* load in the cecum and in the litter. The research demonstrated the superiority of the prebiotic and resulted in a second study to add to the understanding of the effects of the prebiotic. The project resulted in two journal articles

Journal Articles:

Poultry Science

Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. B. Baurhoo, L. Phillip, C. A. Ruiz-Feria.
2007 86:1070-1078

Poultry Science

Cecal populations of *Lactobacili* and *Bifidobacterial* and *Escherichia coli* Populations after *in vivo Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. B. Baurhoo, A. Letellier, X. Zhao, C. A. Ruiz-Feria.
2007 86: 2509-2516