PRE-HARVEST INTERVENTIONS FOR REDUCING *ESCHERICHIA COLI* O157:H7 ASSOCIATED WITH MARKET READY BEEF FEEDLOT CATTLE

By

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PRE-HARVEST INTERVENTIONS FOR REDUCING *ESCHERICHIA COLI* O157:H7 ASSOCIATED WITH MARKET READY BEEF FEEDLOT CATTLE

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Escherichia coli O157:H7 is an important foodborne pathogen and beef cattle represent important reservoirs. *E. coli* O157:H7 has caused important economic losses to the beef industry and can cause severe illness and death in humans. Pre-harvest interventions have been suggested as a tool to help reduce the occurrence of *E. coli* O157:H7 associated with beef feedlot cattle. Five clinical trials were conducted to evaluate the effects of *Lactobacillus acidophilus* strain NPC 747 or an experimental vaccine for reducing the occurrence of *E. coli* O157:H7 associated with market ready beef feedlot cattle. Feedlot cattle supplemented with 10^9 CFU daily of NPC 747 were 35% less likely to test positive for *E. coli* O157:H7 in feces. In an evaluation of dose response and herd immunity to a vaccine, cattle receiving 1, 2, or 3 doses of the vaccine were 68, 67, and 73% less likely to test positive for *E. coli* O157:H7 in feces compared with external controls. Additionally, vaccinated cattle may have conferred protection to unvaccinated cattle within the same pen, providing evidence for a herd immunity effect. In a second vaccine study, vaccine efficacy for cattle receiving 3 doses of the vaccine was 98.5% when evaluating colonization of *E. coli* O157:H7 in terminal rectum mucosa (TRM)

samples. In two large-scale clinical trials pens of vaccinated cattle were less likely to test positive for *E. coli* O157:H7 using a novel sampling device (ROPES) and vaccine efficacy was calculated at 76.0% when evaluating colonization of *E. coli* O157:H7 in TRM samples. NPC 747 and vaccination had no adverse effects on the important feedlot performance outcomes of average daily gain, daily dry matter intake, and feed efficiency. These data support the use of pre-harvest interventions for controlling *E. coli* O157:H7 in market ready beef feedlot cattle.

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CHAPTER I

INTRODUCTION

Pre-Harvest Control of *Escherichia coli* O157:H7 Associated With Market Ready Feedlot Cattle

Americans expect and demand a safe food supply; yet each year, more than 76 million American citizens become ill from consuming foods contaminated with pathogenic bacteria (Mead et al., 1999). The bacterial group most commonly associated with human illness is *Escherichia coli* O157:H7 and cattle represent a significant reservoir (Hussein et al., 2001). More specifically, the consumption of ground beef is frequently associated with outbreaks of human illness (Peterson and James, 1998; Slutsker et al., 1998; Sparling, 1998).

Hazard Analysis-Critical Control Point (HACCP) plans are being used to decrease the risk of foodborne illness by intervening at stages of processing that pose a plausible risk of carcass contamination (Elder et al., 2000). In fact, the most recent report from the Centers for Disease Control (CDC) suggests that HACCP plans implemented in major commercial beef processing plants have improved the safety of the American beef supply, and reduced the incidence of foodborne illnesses due to *E. coli* O157:H7 contamination. For example, during 1996-2003, the estimated incidence of *E. coli* O157 infection decreased 42% (95% CI = 58% to 19% decrease), with the primary decrease in *E. coli* O157 infections (35%) occurring during 2002-2003 (MMWR, 2004). The most

recent reductions coincide nicely with the adoption of interventions implemented in the packing segment of the beef industry in response to protocols based on HACCP plans. However, the incidence of *E. coli* O157 infection was 4.5/100,000 for young children, compared with 0.9/100,000 for other persons in 2003. Clearly, there is still opportunity for improving the safety of the American beef supply and reducing the incidence of *E. coli* O157 infection not only in children, but all Americans.

Historically, little emphasis has been placed on the development of intervention strategies in the live animal prior to harvest, however, more recently this has changed with an increased emphasis on pre-harvest intervention strategies (Callaway et al., 2002). The concept of controlling foodborne disease agents, such as *Escherichia coli* O157:H7, in the livestock reservoir may seem foreign to many research and health communities (Hancock et al., 2001). Traditionally, much of the research effort aimed at improving the safety of meat products has focused on post-harvest sanitation (Callaway et al., 2002). However, given the non-hygienic environment that beef cattle are finished in it seems critical to identify pre-harvest controls aimed at reducing the carriage and shedding of *E. coli* O157:H7 by feedlot cattle. In an attempt to illustrate this, the 2000 National Beef Quality Audit (NBQA) found greater than 18% of beef steers/heifers harvested in 2000 had a least a small amount of visible mud/manure on their hides (McKenna et al., 2002). Furthermore, research has shown (Elder et al., 2000) a significant positive correlation between *E. coli* O157:H7 prevalence in feces and hides, and prevalence of carcass contamination within lots. Other recent research has supported this claim. Arthur et al. (2004) reported a significant positive correlation of the prevalence of *E. coli* O157:H7 on hides of beef cattle and the prevalence of *E. coli* O157:H7 on pre-evisceration carcasses

($R^2 = 0.68$; $P < 0.05$). Nou et al. (2004) compared conventional hide removal versus chemical dehairing, which has the effect of sanitizing the hide, on prevalence of *E. coli* O157:H7 on carcasses. Prevalence of *E. coli* O157:H7 was lower ($P < 0.05$) on treated than on control pre-evisceration carcasses (1% versus 50%; Nou et al., 2004). The results from these studies (Arthur et al., 2004; Nou et al., 2004) suggest pre-harvest intervention strategies aimed at reducing carriage and shedding of *E. coli* O157:H7 in the feces of feedlot cattle could further reduce the incidence of carcasses contaminated with the food-borne pathogen *E. coli* O157:H7.

Although the concept of controlling *E. coli* O157:H7 at the production segment of the beef industry is a relatively new concept, beef industry leaders have recognized the importance of developing pre-harvest interventions. For example, the Executive Summary of the Beef Industry *E. coli* Summit (2003) stated a need for the development of effective intervention strategies aimed at reducing the prevalence of *E. coli* O157:H7 associated with market ready beef cattle. In response to this need, concerns from other industry groups, and the overall impact *E. coli* O157:H7 has had on the beef industry; research was conducted to evaluate two pre-harvest interventions aimed at reducing the carriage and shedding of *E. coli* O157:H7 associated beef feedlot cattle. The two interventions evaluated herein include a direct-fed microbial product, *Lactobacillus acidophilus* (NPC 747), and a vaccine, designed to protect against *E. coli* O157:H7 type III secreted proteins. This research was conducted as a cooperative effort between Nutrition Physiology Corp., Bioniche Life Sciences, the State of Nebraska, the United States Department of Agriculture, and a number of privately owned beef feedlots in Nebraska. The objectives of the research contained herein are as follows:

1) Evaluate the effects of feeding a *Lactobacillus acidophilus* (NPC 747) direct-fed microbial product on shedding of *E. coli* O157:H7 in the feces and feedlot performance of beef feedlot cattle in a research feedlot.

2) Evaluate the effects of varying the number of doses and potential herd immunity benefits of an experimental vaccine on shedding of *E. coli* O157:H7 in the feces beef feedlot cattle in a research feedlot.

3) Evaluate the effects of three-dose vaccination regimen on shedding of *E. coli* O157:H7 in feces of feedlot steers, the proportion of the same steers colonized by this organism at harvest in the terminal rectum, and feedlot performance in a research beef feedlot

4) Evaluate the effects of a two-dose vaccination regimen on the environmental load of *E. coli* O157:H7 within commercial feedlots using a novel sampling device (ROPES) and the proportion of commercial feedlot cattle colonized by this organism in the terminal rectum.

CHAPTER II

REVIEW OF LITERATURE

Pre-Harvest Control of *Escherichia coli* O157:H7 Associated With Market Ready Feedlot Cattle

Approximately two decades ago *Escherichia coli* O157:H7 was recognized as a pathogen following two outbreaks of severe bloody diarrhea associated with a fast-food restaurant chain in the USA (Riley et al., 1983; Wells et al., 1983). Cattle feces are recognized as a major source of *E. coli* O157:H7 and these human pathogens are considered widespread on cattle operations (Hancock et al., 2001; Rasmussen and Casey, 2001). In 1997, it was suggested that the widespread presence of *E. coli* O157:H7 in cattle feces may represent a serious public health risk through direct transmission to people by fecal contamination of food, water, and/or the environment (Altekruse et al., 1997). Although the food supply of the United States is indeed the safest in the history of the world (Callaway et al., 2004), some researchers estimate that more than 76 million citizens become ill from consuming foods contaminated with pathogenic bacteria (Mead et al., 1999). Therefore, tremendous resources have been devoted to determining the ecology and epidemiology of these bacteria in cattle and in cattle production environments so that they might be controlled on cattle farms or operations (Renter and Sargeant, 2002). This review will focus not only on the economic and social impacts this bacteria has had on the American beef industry and population, but also the ecology and

epidemiology of *E. coli* O157:H7 in beef cattle. Additionally, this review will evaluate potential interventions and discuss how and why pre-harvest intervention might ellicit reductions in the incidence of *E. coli* O157:H7 infection in the American population.

Economic and Social Impacts

One of America's best-known pathogens that cause disease in humans is *E. coli* O157:H7. In 2003, the CDC estimated approximately 0.9/100,000 adults and 4.5/100,000 young children became ill due to infection with *E. coli* O157:H7 (MMWR, 2004). Compared with other causes of sickness in the American population, relatively few cases of sickness and death can be attributed to *E. coli* O157:H7. However, because *E. coli* O157:H7 clinical cases are five times higher in young children than in adults, the importance of *E. coli* O157:H7 as a foodborne pathogen becomes personal for many Americans. Additionally, one source (Meat and Poultry, 2003) reported between 1993 and 2002 the impact of *E. coli* O157:H7 outbreaks associated with beef product has cost the beef industry in excess of $2.7 billion. Because of the implications with human health, as well as the economic impact on the beef industry, *E. coli* O157:H7 has driven change. For example, commercial beef packers now use organic acid rinses to reduce or eliminate bacteria from the surfaces of harvested cattle (NCBA, 2004). In addition, there has been a widespread adoption of hot water or steam vacuuming at specific points in the harvest process and on areas on the carcass that are at risk to carcass contamination (NCBA, 2004). Although the beef industry has made considerable advancements in controlling the occurrence of *E. coli* O157:H7 contamination in beef product, these

advancements have come at a cost to the beef industry. This section of the review will discuss the economic impact *E. coli* O157:H7 has had on the beef industry as well as the impacts this foodborne pathogen has had on the health of the American population.

Cost to the Industry

E. coli O157:H7 has cost the industry in numerous ways. For example, beef producers and major beef packing plants have invested millions of dollars in beef safety research (NCBA, 2004), and loss in consumer confidence due to beef recalls associated with foodborne pathogen contamination has been shown to have a negative impact on demand (Schroeder et al., 2000). The cost each segment of the beef industry has incurred due to improving production practices and implementing intervention strategies has been evaluated (Meat and Poultry, 2003). Additionally, Schroeder et al. (2000) did an economic assessment of the economic impact the loss in consumer confidence in beef products has on beef demand. These two reports will form the foundation of the discussion of *E. coli* O157:H7's cost to the beef cattle industry.

E. coli O157:H7 may have cost the beef industry more than $2 billion over the past 10 years in lost demand, lower prices, and expenditures on food safety intervention technology (Meat and Poultry, 2003). Other than costs due to lost demand and lower prices, beef processors have incurred much of the cost associated with *E. coli* O157:H7. Meat and Poultry (2003) reported the largest beef packers have spent a combined $750 million to reduce the incidence of *E. coli* O157:H7 in ground beef. These costs have occurred in three primary areas: capital expenditures on food safety intervention technologies, additional operating costs (such as testing, added personnel, and slower

chain speeds), and in the actual cost of beef recalls (Meat and Poultry, 2003). In the survey conducted by *Cattle Buyers Weekly*, which is the basis for this discussion and reported in Meat and Poultry (2003), the top-ten beef packers spent $400 million during the past ten years on capital expenditures relating to food safety. It is estimated that smaller beef packers spent an additional $100 million on food safety associated costs (NCBA, 2004). Furthermore, those same companies were reported to have spent another $250 million in added operating costs (NCBA, 2004). Clearly, the beef processing sector of the beef industry has invested heavily in research and technology aimed at reducing the occurrence of *E. coli* O157:H7 associated with beef product. Although an estimated $750 million is a significant cost, the majority of the costs associated with *E. coli* O157:H7 appear to be attributable to loss in product demand.

Between November 1992 and February 1993, a large outbreak of *E. coli* O157:H7 infections occurred in the western US and those infections were associated with eating ground beef patties at restaurants of one fast-food chain (Tuttle et al., 1999). *E. coli* O157:H7 has seriously hurt beef demand since this tragedy (Meat and Poultry, 2003). Ted Schroeder, economist at Kansas State University, explains the impact by saying "It takes much longer to regain a consumer's trust in a product than it does to lose it" (Meat and Poultry, 2003). Schroeder et al. (2000) reported that recalls in 1993 caused a 1.6 percent decline in demand. An impact of 1.6% in lost demand per year may have cost the industry as much as $1.6 billion between 1991 and 1999 (Schroeder et al., 2000). In a separate study, McKenzie and Thomsen (2001) studied 25 *E. coli*-related recalls between October 1994 and October 2000. Results of this study consistently showed that a large drop in boneless beef price occurs on the day of a recall, and on average, boneless beef

prices declined by 2 to 2.5% in the five days after each recall (McKenzie and Thomsen, 2001). Although McKenzie and Thomsen (2001) did not quantify the economic impact of their reported decline in price, other reports using their calculations (Meat and Poultry, 2003) suggest the cost may be more than $3 million immediately after a mid-size to large recall. Nevertheless, the cost of *E. coli* O157:H7 to the beef industry in terms of lost demand and depressed markets is substantial.

Clearly, *E. coli* O157:H7 has cost the beef industry dearly. As indicated above, these costs come from a loss in consumer confidence in beef, investments in research and technology, and ground beef recalls. Although not all beef recalls are associated with *E. coli* O157:H7, many of the advancements in quality control have been driven by this organism. This begs the question as to why one pathogenic bacterial strain can cause such economic impacts and influence such dramatic change in quality control processes. The answer to this question lies in the social impact of *E. coli* O157:H7. More specifically, *E. coli* O157:H7 has been implicated with human health tragedies in the American population.

Human Health Consideration

Human exposure to the pathogen *E. coli* O157:H7 can cause severe diarrhea (hemorrhagic colitis), and in a small percentage of cases, hemolytic-uremic syndrome (HUS). Preliminary FoodNet data for 2003 indicates the incidence of *E. coli* O157:H7 infection was 0.9 per 100,000 adults and 4.5 per 100,000 young children (MMWR, 2004). According to the 2000 U.S. Census, it was projected that in 2003 children under the age of 5 would represent approximately 20 million citizens, or 6.9% of the