DISSERTATION

VACCINATION AS AN INTERVENTION AGAINST *ESCHERICHIA COLI* O157:H7 IN CATTLE FECES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY WILLIAM TRAVIS CHOAT ENTITLED VACCINATION AS AN INTERVENTION AGAINST ESCHERICHIA COLI O157:H7 IN CATTLE FECES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

VACCINATION AS AN INTERVENTION AGAINST *ESCHERICHIA COLI* O157:H7

Three hundred sixty-seven recently weaned steers were used in a growing (45 d) and finishing (189 d) experiment to determine if vaccination with an experimental *E. coli* O157:H7 vaccine would reduce fecal shedding and elevate antibody titers. Treatments (TRT) compared were: Group 1) received two doses of the *E. coli* vaccine in the growing phase only; Group 2) received two doses of the *E. coli* vaccine during the growing phase and a third dose on d 100 of the finishing phase; Group 3) received two doses of the *E. coli* vaccine; one on d 21 of the finishing phase and a second dose on d 100 of the finishing phase. Treatment group 4 served as the control (no vaccination). On d 0 and 45 of the growing phase and d 21, 100 and 162 of the finishing phase, fecal grab and venous blood samples were collected. Fecal samples were analyzed for *E. coli* O157:H7 by Food Safety Net Services. Blood samples were sent to Fort Dodge Laboratories for analysis of antibody titers against *E. coli* O157:H7. Prevalence of fecal *E. coli* O157:H7 was not different ($P > 0.05$) at any sampling period among treatment groups. The initial and final prevalence rates during the feedlot phase for TRT 1 were one and 10%; TRT 2 were 3 and 8%; TRT 3 were 3 and 17%, and TRT 4 (Control) were 5 and 8%, respectively. Serum titers showed elevated immune response, during the 45 d growing phase, vaccine increased ($P < 0.001$) blood titers from an average of 62, to 2,217. During the finishing period, vaccinated calves had higher titers compared to Control calves (avg. of Trt 1, 2, 3 vs. TRT 4 ($P < 0.05$). Vaccination during the 45 d growing phase, and then again on d 100 of the finishing phase (TRT 2), resulted in the greatest ($P = 0.012$) pre-slaughter titer

level, but did not affect ($P > 0.05$) *E. coli* O157:H7 prevalence. At harvest, TRT 1 steers' titer levels had returned to near pre-vaccination levels, indicating that immune levels declined over time. Although an immune response was generated by this vaccine, the limited number of animals shedding *E. coli* O157:H7 warrants additional research in calves with higher levels of shedding.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

OBJECTIVE OF DISSERTATION

The objective of this dissertation was:

(1) To evaluate the effectiveness of vaccinating live cattle, before harvest to reduce pre-harvest prevalence of *Escherichia coli* O157:H7 in the feces.

CHAPTER II

OVERVIEW OF *ESCHERICHIA COLI* IN LIVE CATTLE

INTRODUCTION

Escherichia coli O157:H7 that produce shiga-like toxin cause an estimated 62,458 human cases of foodborne illness in the United States each year resulting in 1,843 hospitalizations and 52 deaths (Mead et al., 1999). This pathogen was first associated with beef in 1982 when it was responsible for outbreaks of foodborne illness in Michigan and Oregon where undercooking of ground beef was identified as the cause (Riley et al., 1983). Following this initial outbreak, the monitoring and reporting of *E. coli* O157:H7 has increased, resulting in an increased number of outbreaks reported since that time (USDA:APHIS:VS, 1997). One such outbreak was reported in the spring of 1993 (CDC, 1993). This outbreak again resulted from undercooked ground beef and involved a quick-service restaurant; the outbreak caused more than 500 illnesses and four deaths in four states. Additionally, this severe, multi-state outbreak was responsible for increased consumer awareness and concerns among health authorities, the industry, and government regulators regarding sanitary conditions and pathogen control in beef slaughter and processing facilities (Sofos and Smith, 1998). One immediate action taken by the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) was to implement the 'Cattle Clean Meat Program' or the 'Zero Tolerance Program' of 1993 which required that all feces, ingesta, and milk be removed from carcasses by knife trimming before washing (Sofos and Smith, 1998; FSIS-USDA,

1993). The 1993 Zero Tolerance rule was then followed in 1996 by the 'Mega Reg' or the Pathogen Reduction, Hazard Analysis Critical Control Point (HACCP) system final rule for meat and poultry inspection, which included microbiological performance criteria (FSIS-USDA, 1996).

In an effort to comply with the zero tolerance rule and the microbial performance criteria, industry and university research focused on the development of in-plant interventions. These interventions focused on known weaknesses of target bacteria *Escherichia coli* and *Salmonella* spp. -- mainly temperature and pH. Some of the specific interventions included steam/hot water vacuuming for spot decontamination, carcass washing with steam and/or hot water at differing pressures for differing amounts of time, both pre- and post-evisceration carcass rinsing with organic acids and/or with certain other chemical solutions (Castillo et al., 1998; Dorsa, 1997; Hardin et al., 1995; Phebus et al., 1997; Sofos and Smith, 1998). Today, these interventions have become refined into what is called a 'multiple hurdle approach.' The multiple hurdle approach is predicated upon the idea that many sequentially applied interventions are more effective then any one alone. The design of the multiple-hurdle intervention systems in most large beef processing plants in the U.S. begins with a dressing best practice, perquisite program of some kind in order to minimize the transfer of bacteria from the hide to the carcass; this would be followed by the first intervention; a pre-evisceration wash including an organic acid rinse, and the carcass would then be eviscerated and split. The split carcass would then undergo trimming, final inspection and hot water or steam pasteurization. The final step in supporting a good multiple hurdle intervention system is proper carcass chilling and maintenance of proper holding temperature. While some plants may have more and

some less, the described system has proven very effective (Bacon et al. 2000). However, the multiple hurdle approach, as described, has its limitations. Depending upon the transfer rate of bacteria, the system will fail some percentage of the time when pathogens entering the system overwhelm the capabilities of interventions, resulting in contaminated finished product. This fact is the primary reason for investigations into pre-harvest pathogen reductions.

PRE-HARVEST

Cattle as a reservoir of E. coli O157:H7

Beef was first implicated as a source of *E. coli* O157:H7 infection in 1982 (Riley et al., 1983) although early investigations revealed that prevalence of *E. coli* O157:H7 in the U.S. cattle population was low (Hancock et al., 1994; NAHMS., 1995). Specifically, Hancock et al. (1994) found *E. coli* O157:H7 in 10 of 3,570 (0.28%) fecal samples from dairy cattle in 5 of 60 (0.83%) herds, and in 10 of 1,412 (0.71%) fecal samples from pastured beef cattle in 4 of 25 (16%) herds. Near that same time, Chapman et al. (1993) reported a 4% *E. coli* O157:H7 positive rate in beef cattle in Europe. These two early studies resulted in somewhat different findings, which may have been attributed to differences in geographic location; but nevertheless, both experiments revealed a relatively low prevalence of the pathogen that was not overly alarming at the time.

It is important to note that similar culture methods were used by both Chapman et al. (1993) and Hancock et al. (1994); both included broth enrichment followed by direct plating on Sorbital McConky agar. The key problem with this culture method -- especially when used on fecal samples -- was its lack of selectivity/sensitivity.

Specifically, samples with large populations of background bacteria made it difficult to detect target pathogens.

More recent studies have addressed sensitivity problems by using immunomagnetic bead separation techniques (Dynal® [Invitrogen Corporation, Oslo, Norway]) which concentrate pathogens by incorporating antibodies specific to *E. coli* O157:H7 which allow beads to attach to the pathogen and inturn allow for the pathogen to be segregated from non-target bacteria by way of a magnet and washing procedure. This immunomagnetic separation technique has increased the sensitivity of detection of *E. coli* O157:H7 by 10- to 100- fold (Chapman et al., 1994), and more recent experiments using the more advanced culture methods revealed a much higher *E. coli* O157:H7 prevalence rate: NAHMS (2001) revealed a 11% positive pen rate for *E. coli* O157:H7 in U.S. feedlots with greater than 1,000 head; Elder et al. (2000) reported that 28% of fecal samples and 11% of hide samples from single source cattle presented for slaughter in mid-western packing plants were positive for *E. coli* O157:H7; and Smith et al. (2001) tested feces of 3,162 cattle from 29 pens of five mid western feedlots, observing and observed a 23% individual point positive rate and a 100% pen point positive rate (at least one animal from each pen tested positive). Another survey conducted in the U.S. reported that *E. coli* O157:H7 was present in 52% of 711 mid-western feedlot pens and in 95% of 73 mid western feedlots (Sargaunt et al., 2003) indicating that the pathogen was widespread.

The many prevalence and longitudinal studies conducted between 1997 and 2003 (Hancock et al., 1997[b]; Lagreid et al., 1999; Elder et al., 2000; Keen and Elder., 2002; NAHMS, 2001; Smith et al., 2001; Van Donkersgoed et al., 2001; Sargaunt et al., 2003)

and the many healthy cattle that tested positive for *E. coli* O157:H7 in those studies demonstrated that cattle are asymptomatic carriers of *E. coli* O157:H7. Therefore, presence of the pathogen in a given animal can only be determined by testing. The task of identifying affected animals has been further complicated by the fact that cattle can shed it intermittently, carry more than one strain, or become re infected (Zhao et al., 1995). However, the difficulty by which an animal can be diagnosed as a host has not limited the amount of research occurring that investigated the factors that may be associated with shedding or prevalence.

Factors influencing the prevalence and persistence of E. coli O157:H7

Numerous sources of *E. coli* O157:H7 prevalence variability have been detected and/or hypothesized including, but not limited to, feed, water, manure, soil, transportation, geographic location, health status and gastrointestinal location of colonization.

Feed and Water

Many studies have evaluated the prevalence of *E. coli* O157:H7 in feed and water and its possible associations or correlations with *E. coli* O157:H7 prevalence in cattle. Initially, in order to understand the potential of water to spread *E. coli* O157:H7 infection, its survivability needed to be known. Hence, several experiments have evaluated the survivability of *E. coli* O157:H7 in water. The first (Rice et al., 1992) was associated with a waterborne outbreak. In this experiment, 3 strains, 2 of which were *E. coli* O157:H7, were used to inoculate well water and then the water was housed at 5°C and 20°C. Results indicated no real reduction in bacteria counts until after day 7, but interestingly, the bacteria in water held at 20°C died off by approximately day 50 while the bacteria held at 5°C were recorded at viable levels until day 70 -- which was the last

day of sampling. Other experiments have supported these findings. Rice and Johnson (2000), whose experiment was much shorter, found survival of *E. coli* O157:H7 throughout the entire experiment and also observed a similar response to temperature. McGee et al. (2002) found survival at 31 days post-inoculation and Wang and Doyle (1998) reported survival for 12 weeks, but to a lesser extent in "dirty" water (lake water) compared with water initially sterile.

Given that there is little doubt that water has the potential to proliferate spread of *E. coli* O157:H7, Smith et al. (2001) included water as one of the factors that may affect shedding and reported that prevalence of cattle in pens shedding *E. coli* O157:H7 was not associated with recovery of the agent from water ($P = 0.15$). Additionally, they stated that prevalence was not correlated with the temperature, pH or cleanliness of the water. In a similar survey, water prevalence was found to be correlated with cattle prevalence (Sargeant et al., 2003). To date, the relationship between water and cattle prevalence of *E. coli* O157:H7, if existent, has not been completely defined, but given the consistently long life of *E. coli* O157:H7 in water, it is probable that it does play some role in proliferating infection; especially at cattle-concentration points such as feedlots.

The survivability of *E. coli* O157:H7 in feeds has not been studied as extensively as it has been in water, but evidence suggests an important role of feed in the spread of *E. coli* O157:H7. First, shedding of *E. coli* O157:H7 by cattle has been reported to occur in short periods separated by periods of low or undetectable shedding (Hancock et al., 1997[a]) which was related to the patterns observed in outbreaks of human foodborne illness (Lynn et al., 1998). Secondly, distribution of shedding *E. coli* O157:H7 is seasonal (Hancock et al., 1997[a]; Van Donkersgoed et al., 2001), occurring mostly in

warm months -- suggesting that the pathogen must replicate or sustain itself in the environment in which feed is a part. Thirdly, indistinguishable subtypes of *E. coli* O157:H7 have been identified (PFGE) at distant locations; feed is largely centralized and distribution may play a role in spreading the pathogen from one farm to another.

Lynn et al. (1998) demonstrated that *E. coli* could survive in a total mixed ration (TMR) for up to 48 h, and that Lasalocid (an ionophore) had no effect on *E. coli* growth in feeds. Other scientists have reported that specific types or kinds of feedstuffs may be related to cattle shedding *E. coli* O157:H7. Tkalcic et al. (2000) demonstrated that high-concentrate diets promote acid resistance of *E. coli* O157:H7 in the rumen and that this may provide a mechanism by which the pathogen can bypass the abomasum (true stomach) and colonize the colon. These conclusions were not completely in accord with findings of Kudva et al. (1997) and Van Baale et al. (2004) both of whom observed increased shedding when cattle were fed a forage-based diet (which created a less acidic rumen environment). Two trials reported by Harmon et al. (1999) and Jordan and McEwen (1998) examined the effects of fasting on *E. coli* O157:H7 shedding and neither reported any effect.

Along with feed effects on persistence, it also is important to note research findings relative to GI tract colonization with *E. coli* O157:H7. As previously stated, Tkalcic et al. (2000) demonstrated that high-concentrate diets promote acid resistance of *E. coli* O157:H7 in the rumen, and this may provide a mechanism by which the pathogen can bypass the abomasum (true stomach) and colonize the colon. This finding was significant in that the abomasum in cattle is equivalent to the gastric stomach in humans, and the gastric environment serves as a major defense mechanism for humans against

pathogens. In support, several epidemiological studies of human foodborne illness outbreaks have identified use of antacids as a major cause of increased susceptibility. Acid resistance in the rumen indirectly allows bacteria to colonize the colon, and in turn, contaminate the feces. Several studies support the colon as the major site of E. coli O157:H7 colonization. Grauke et al. (2002) studied the gastrointestinal tract location of *E. coli* O157:H7 in experimentally infected sheep and observed that *E. coli* O157:H7 was rarely recovered from the rumen and was most prevalent in the lower GI tract. Additionally, Laven et al. (2003) studied the GI tract location of *E. coli* O157:H7 in naturally infected cattle and reported greater prevalence of *E. coli* O157:H7 in the colon than in the rumen. Moreover, these two experiments indicated that the pathogen was more persistent in GI tract contents than inactual tissue. In contrast, a more detailed examination of the GI tract by Naylor et al. (2003) reported that in both, naturally and experimentally infected cattle, *E. coli* O157:H7 was more adherent to the tissue of the colon as opposed to its lumen contents, and even more specifically, the tissue-adherent bacteria were adhered to mucosal epithelium within a defined region extending up to 5 cm proximally from the recto-anal junction. Given these results and those of other experiments, it is generally recognized that the colon is the major site of colonization in cattle; however, more information is needed to properly determine if the content, the tissue or some combination of both is primarily related to cattle shedding *E. coli* O157:H7.

Mud and Manure

In addition to feed and water, mud and manure may play an important role in the proliferation and shedding of *E. coli* O157:H7. Kudva et al. (1998) reported survival of

E. coli O157:H7 in feces for up to 21 months. Kudva et al. (1998) reported findings similar to those of Rice et al. (1992) in terms of effects of temperature on survival of *E. coli* O157:H7 in water (i.e., *E. coli* O157:H7 preferred a temperature <23°C) and also that *E. coli* O157:H7 survival was longer when manure was not aerated. "Both of the latter findings may be of benefit in the development of best practices." In an earlier experiment, Wang et al. (1996) reported that *E. coli* O157:H7 survived in bovine feces kept at 37°C for 42 and 49 days with low and high inoculation rates, respectively.

Feces or mud score also has been correlated to *E. coli* O157:H7 contamination. Ridell and Korkeala (1993) in a large survey, reported that excess 'dunginess' (mud or tag) resulted in increased microbial contamination. In addition, McEvoy et al. (2000) visually scored cattle based on external contamination and reported that total viable counts from carcasses of dirty cattle were significantly higher compared with total viable counts of carcasses from cleaner cattle.

Transportation and Health Status of Calves

Few studies have examined the association of animal health and transportation with *E. coli* O157:H7 shedding. Recently , Bach et al. (2004) evaluated the effects of pre-conditioning and transportation distance on *E. coli* and *E. coli* O157:H7 shedding by 174 steer calves – none of which was positive before transport. Results showed that the calves most susceptible to *E. coli* infection (as evidenced by shedding) were those transported a long distance (15 h) without prior pre-conditioning Bach et al., 2004). The results of the latter experiment were substantiated by those of LeJeune et al. (2004) and Stanford et al. (2005) both of whom (in large longitudinal studies) observed highest prevalence by calves early in the finishing phase. Although the transportation and health

status of those with the highest prevalence of *E. coli* O157:H7 was not stated, previous research has shown increased health problems among newly received calves (Galyean et al., 1999) regardless of transportation status. A new environment, dietary changes and commingling are all stresses associated with newly received calves thatmay be associated with increases in *E. coli* O157:H7 shedding.

Potential Mechanisms of Reduction

Probiotics

Ruminants and non-ruminants -- develop a protective intestinal microflora soon after birth. These microflora are important for good GI tract health and diet digestion. However, this microflora is subject to influence by numerous factors including disease, diet changes, transportation and environmental changes (Bach et al., 2002). Fuller (1989) defined probiotics as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance". The concept of administering probiotics or direct-fed microbials to animals involves the hypothesis that these beneficial microorganisms will combat the effects of stress and prevent undesirable microorganisms from establishing themselves in the intestine. The mode of action for probiotics can include production of an antimicrobial compound, competition for nutrients or adhesion sites, or changes in metabolism by affecting enzyme activity (Fuller, 1989).

Two probiotics have been tested for their ability to reduce *E. coli* O157:H7 shedding -- each with a different mode of action. The first, a proprietary strain of *Lactobacillus acidophilus* is hypothesized to reduce *E. coli* O157:H7 shedding by competing for nutrients and binding sites within the lower GI tract. Several studies

(Younts-Dahl et al., 2004; Younts-Dahl et al., 2005; Peterson et al., 2005) have evaluated effectiveness of this intervention. Younts-Dahl et al. (2004) reported that supplementing NP51 at 10^9 CFU per steer daily for ~ 200 d reduced fecal shedding of *E. coli* O157:H7 by 57% compared with controls (OR = 0.42; P = 0.007). Similar results were reported in a second study (Younts-Dahl et al. 2005) where three levels of NP 51 (low, medium and high) all reduced shedding compared to controls, including a linear response with an increased dose of NP 51. In further support, Peterson et al. (2005[a]) observed that steers supplemented with *Lactobacillus acidophilus* (NPC 747) were 35% less likely to shed *E. coli* O157:H7 as compared to controls.

The second, a proprietary non-pathogenic strain(s) of colicin producing *E. coli* isolated from cattle has been evaluated in vitro (Zhao et al., 1998) and determined to inhibit the growth of *E. coli* O157:H7. Zhao et al. (1998) further tested this probiotic by inoculating 9 calves with 10^{10} CFU of *E. coli* O157:H7 as controls and comparing shedding with 6 calves treated with 10^{10} CFU of the probiotic followed 2 d later by 10^{10} CFU of *E. coli* O157:H7. These researchersobserved that control calves shed continuously throughout the experiment (28 d) while probiotic-treated calves only shed for an average of 18 d (Zhao et al., 1998). In additional experimentation with the strain used by Zhao et al., 1998; Tkalcic et al. (2003) experimentally inoculated 12 calves with *E. coli* O157:H7 and then treated half, 72 h later, with the experimental probiotic *E. coli*. The probiotic treated calves shed less ($P < 0.05$) *E. coli* O157:H7 on days 8, 12, 14, 16, 20, 22, 28, and 30 compared with non-treated calves. Additionally, *E. coli* O157:H7 was isolated from five of six untreated calves at necropsy (study conclusion) and from two of six probiotic-treated calves (Tkalcic et al., 2003).

Vaccine

An *E. coli*O157 vaccine currently is not available commercially for use in cattle. However, a great deal of research has been conducted to characterize the mechanism of attachment by *E. coli* O157:H7 to the gut wall, as well as to asses the immune response that has been shown to prevent such attachment. One of the best accounts of the attachment of *E. coli* to the gut wall was provided by Goosney et al. (2000). In general, *E. coli* O157:H7 temporarily attach to the host plasma membrane via a series of secreted proteins, including *E. coli* secreted proteins A, B, and D (EspA, B, and D). These secreted proteins enable the bacteria to secrete what is called 'Translocated Intimin Receptor' into the host cell cytosol where it is then linked with Intimin from the bacteria's outer membrane. This final attachment completes the invasion of the bacteria, allowing it to inject virulence factors (Figure 2.1). It is this attachment mechanism that is the target of the vaccine (Goosney et al., 2000).. Dean-Nystrom et al. (1998) first demonstrated that intimin was required for colonization of new-born calves by inoculating them with intimin-positive or intimin-negative strains of *E. coli* O157:H7. From results of the latter study, Dean-Nystrom et al. (1998)postulated that studies to determine if intimin based vaccines reduce *E. coli* O157:H7 levels in cattle were warranted. A second experiment by the same group(Dean -Nystrom et al., 2002) determined that passive immunization of neonatal piglets (a surrogate model for cattle) with $Intimin_{O157}$ protected them from EHEC O157:H7 colonization and intestinal damage as compared to controls. The first experiment to use cattle as the model (Cornick et al., 2002) confirmed that intimin was required for colonization by comparing fecal shedding

of cattle orally inoculated with either a wild type strain of *E. coli* O157:H7 or with an intimin-free mutant strain.

Following these initial experiments, two commercial vaccines were developed for clinical and field experimentation. Potter et al. (2004) conducted the first series of challenge and clinical trials using one of the two commercial vaccines. The vaccine used in those experiments was based on type III secreted proteins. The first challenge experiment used 8 vaccinated and 8 placebo cattle both initially free of existing titres to EHEC O157. After treatment, all calves were dosed with 10^8 CFU of *E. coli* O157:H7 and fecal shedding was monitored for 14 days. The group receiving EHEC vaccine showed a 13-fold increase in specific antibody titre to type III secreted proteins after a single immunization and on each of the post challenge days, fewer EHEC-vaccinated animals shed *E. coli* O157:H7 compared to the placebo group. A second challenge study observed similar results (greater shedding by placebo treated cattle; P = 0.0002, Kruskal-Wallis ANOVA) in yearling cattle that were dosed with either with a supernatant of secreted proteins, a Tir mutant, or placebo and then dosed two weeks later with *E. coli* O157:H7 as in the previous experiment (Potter et al. 2004).

Potter et al. (2004) vaccinated cattle with either the EHEC vaccine or placebo on d 0, 21 and 42 and reported that cattle in EHEC vaccinated pens only had an average prevalence of 8.8%, compared with a 21.3% prevalence in placebo vaccinated pens. Peterson et al. (2005[b]) reported that cattle in pens receiving one, two, or three doses of vaccine were less likely to shed *E. coli* O157:H7 than cattle in pens not receiving vaccine (OR=0.33; P=0.0008) – a vaccine efficacy of 59%.

Ransom and Belk. (2003) administered a second commercial vaccine to finishing cattle, twice, thirty days apart, and observed a 67.9% reduction in fecal shedding compared with controls. Additionally, Standley et al. (2005) used passive immunization by vaccinating cows in the last trimester of pregnancy and observed no vaccination effect due to low prevalence of *E. coli* O157:H7 in calves. However, Initial titre levels of passively immunized calves showed a ten-fold increase compared with control calves.

Antibiotics

Currently, no antibiotics are licensed by FDA for control of human pathogens in animals. However, one antibiotic 'Neomycin sulfate' [NEOMIX® AG 325 Medicated Premix] has been experimentally tested for this purpose. Ransom and Belk (2003) reported that cattle dosed with Neomycin sulfate at slightly less than manufacturer recommended levels for 3 d prior to slaughter resulted in a 100% reduction in fecal shedding compared with controls and a 78.9% reduction in hide contamination. It should be noted, however, thatuse of antibiotics as a method of reducing *E. coli* O157:H7 in live cattle has come under scrutiny due to the possibility of the pathogen gaining resistance to the antibiotic.

Sodium Chlorate

E. coli O157:H7, along with some *Salmonella* spp., possess an enzyme called nitrate reductase. This enzyme, usually responsible for the normal metabolic conversion of nitrate to nitrite, also converts chlorate to chlorite resulting in cell death (Anderson et al., 2000). Callaway et al. (2002) supplemented cattle with sodium chlorate via drinking water and observed reduced populations of *E. coli* O157:H7 in treated cattle with no adverse side effects to normal GI tract microflora. Sodium chlorate is not currently

licensed for use as an intervention but, its ability to kill only bacteria containing the nitrate reductase enzyme make it a viable alternative in a multiple hurdle intervention program to reduce E. coli O157:H7 in live cattle.

Multiple Hurdle Approach + Good Agricultural Practices

Given the science presented, our goals for pre-harvest food safety should not be to eradicate pathogens but, simply to reduce the amount of variation in hide contamination so that animal-concentration points (e.g., packing plants) can fine-tune their decontamination intervention systems in order to eliminate carcass contamination. With this goal in mind, we should look to the success of the multiple hurdle intervention systems currently being utilized in U.S. packing plants. It is apparent, based on the literature that no one live-animal decontamination intervention is suited to reach this pre-harvest goal; conversely,a combination of several decontamination interventions that are complimentary is a more rational approach to lowering pathogen prevalence on, and in, harvest cattle. Industry information suggestthat cattle infected with high levels of *E. coli* O157:H7 can overwhelm the current multiple hurdle intervention systems in packing plants and result in adulterated beef.

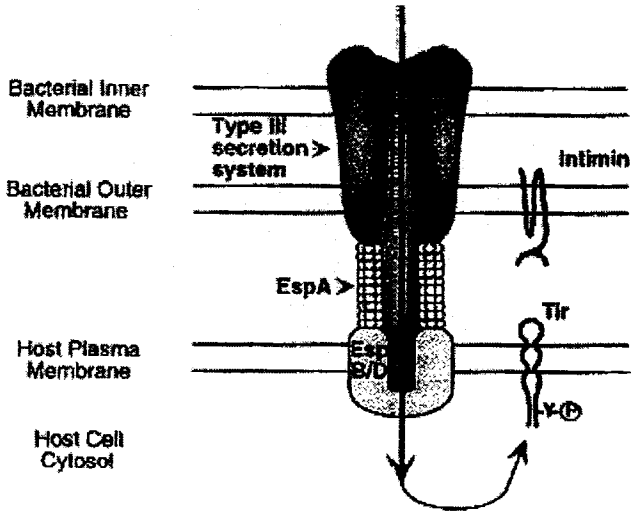

Figure 2.1. The type III secretion system of EPEC used to deliver virulence factors, including Tir, into the host cell cytosol or membrane. Several gram-negative pathogens use this conserved secretion system to deliver diverse effectors into host cells to mediate several different effects within mammalian and even plant cells (Goosney et al., 2000).

CHAPTER III

VACCINATION AS AN INTERVENTION STRATEGY FOR REDUCTION OF *ESCHERICHIA COLI* 0157:H7 IN CATTLE FECES

INTRODUCTION

During the past few years, significant investment dollars have been allocated towards research investigating intervention strategies to reduce contamination of beef carcasses with *E. coli* O157:H7. Both pre- and post-harvest methods have been evaluated. Post-harvest interventions have dealt with controlling foodborne pathogens in a "Multiple Hurdle Approach" including washing cattle before harvest followed by pre-evisceration wash with water and an organic acid, steam vacuuming and hot water wash. Recently, more emphasis has been placed on attacking pathogens at their source. Possible "pre-harvest" methods for controlling pathogens have included dietary changes, direct fed microbials, water treatments, and the addition of antibiotics to diets before slaughter. Direct fed microbials are currently the only products commercially available claiming to reduce *E. coli* O157:H7 in live cattle. Many of the attempted pre-harvest interventions however, have met with challenges including side effects, low overall effectiveness and/or production feasibility.

Several studies have prposed the possibility of vaccination against Enterohemorrhagic *E. coli* (McKee et al., 1995, Dean Nystrom et al., 1998 Cornick et al., 2002). These experiments revealed the basic mechanisms by which pathogenic *E. coli* attach to the epithelial cells of the gastrointestinal tract. Immunity was first used to

protect livestock (pigs; Dean-Nystrom et al., 2002) from asymptomatic infection with Enterohemorrhagic *E. coli*. Two vaccines have been developed and are currently awaiting government approval for use in beef cattle. One of these vaccines has been successfully tested (Potter et al. 2004; Peterson et al. 2005).

The objective of this study was to evaluate the effectiveness of vaccinating live cattle prior to harvest, as a method of pre-harvest reduction in the shedding of *E. coli* O157:H7.

MATERIALS AND METHODS

Three hundred sixty-seven steers were transferred from a 45 d pre-conditioning experiment where 183 of the 367 steers had received vaccination against *E. coli* O157:H7 on d 0 and 21 of the 45 d pre-conditioning period.

Pre-conditioning Experiment Background. Three hundred eighty-nine heifers and three hundred sixty-seven steers were selected from a single herd in central MT. The selected herd was composed of Angus and Simmental genetics and the calves were born primarily in February and March. The calves selected were removed randomly from their mothers (weaned) over a three-week period in October of 2003 with approximately one-third of the calves being weaned each week. On the day of weaning, calves were transported from pasture to a receiving yard consisting of six, 150-hd pens and an appropriate cattle handling facility. Upon arrival at the receiving yard, calves were sorted by gender (steers vs. heifers), placed in holding pens, and then processed separately. At processing, all calves received an individual electronic identification tag, an individual panel tag (each with a unique number) and a single dose of: 1) Nasalgen™ IP (Schering Plough); 2) Pyramid 4 + Presponse™ (Fort Dodge Animal Health); 3) Vision 7 +

Somnus™ (Intervet); and 4) Cydectin pour-on™ (Fort Dodge Animal Health). The Pyramid 4 + Presponse™ and Vision 7 + Somnus™ were administered subcutaneously on the right side of the animal in the lateral neck and boosted on d 21.

A systematic randomization scheme was utilized so that every other animal through the chute would be given a dose of the experimental *Escherichia coli* vaccine (E-vac) designed to prevent the attachment of *Escherichia coli* O157:H7 to the intestinal wall of cattle. The *E. coli* vaccine was administered subcutaneously in the left side of the neck at a dose of 2 ml / head and was boosted with a second dose on d 21. Following processing on d 0, calves were systematically allocated to pens as they exited the chute; resulting in pens with a final allocation of approximately 50% E-vac and 50% control within gender. Calves were fed a grass hay-based diet combined with a protein supplement that contained additional minerals and vitamins. The response variables measured were initial weight (d 0), final weight (d 45), average daily weight gain, blood antibody titers (d 0 and 45) and individual fecal prevalence of *E. coli* O157:H7 (d 0 and 45).

Finishing Phase Experimental Design. Only steers (n = 367) from the pre conditioning experiment were utilized in the finishing experiment. Before shipping from the preconditioning facility to the feedlot, steers were individually weighed (feedlot initial weight), treated again for internal and external parasites with Cydectin pour-on™, boosted with Pyramid 4 + Presponse®, and randomly sorted four ways. Randomization was conducted by sorting steers only (in a database) by previous vaccinated vs. previous controls. All sorted steers were then assigned a random number using Microsoft Excel's random number generator. Steers which were previously controls were sorted by random

number in ascending order, and previously vaccinated steers were sorted by random number in ascending order. The first 92 steers from the previously-vaccinated group were assigned to finishing group 1; the second 92 steers from the previously-vaccinated group were assigned to finishing group 2; the first 91 steers from the previously-control group were assigned to finishing group 3 and; the remaining 92 steers from the previously-control group were assigned to finishing group 4. Four feedlot pens were utilized and pens were assigned by alternating pen numbers 1 through 4 throughout the list of steers in each finishing group until all steers were assigned to a pen. Pen allotment was designed to provide for equal finishing group representation in each pen.

When shipped to the feedlot, each of the four sort-groups of cattle were placed on a separate truck, and upon feedlot arrival, the sorted calves from each truck were placed in a separate pen as was previously assigned in the randomization. All steers were fed a similar high-energy diet(9.92% corn silage; 64.6% corn grain; 20.8% wheat midds and 4.7% protein supplement, dry basis) throughout the experiment, were individually weighed on d 0, 21, 100 and before harvest on d 162, and were given a Synovex Choice™ implant on d 21 and 100.

Treatments were assigned to each finishing group as follows (Table 3.1): Group 1 received two doses of the *E. coli* vaccine in the pre-conditioning phase only; Group 2 received two doses of the *E. coli* vaccine during the pre-conditioning phase, and a third dose on d 100 of the finishing phase; Group 3 received two doses of the *E. coli* vaccine -- one on d 21 and one on d 100 of the finishing phase; and Group 4 received no *E. coli* vaccine (Control).

Sample Collection. On d 0 and 45 of the preconditioning experiment, and on d 21, 100 and 162 of the finishing phase fecal grab and venous blood samples were collected from all calves. Fecal samples were collected aseptically via rectal palpation using a new obstetrics glove for each animal and then placing approximately 50 g of feces into a new screw top cup (Fisher Scientific). Cups were labeled with barcodes and then placed in shipping containers with ice packs to prevent temperature abuse. Samples were shipped overnight to Food Safety Net Services (San Antonio, TX) for analysis.

Recovery of E. coli O157:H7. Samples were analyzed for the presence of *E. coli* O157 using the "MARC MRU" method as described by Barkocy-Gallagher et al. (2002). Briefly, the procedure called for enrichment in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.), immunomagnetic separation, and selective plating.

Antibody Titer Analysis. Ten milliliters of whole blood were collected from the jugular vein or from the tail vein. Blood samples were placed on ice and transported to the Montana State nutrition laboratory where they were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. Analysis of serum for antibody titers of *E. coli* O157:H7 were conducted by Fort Dodge Animal Health Laboratories, Overland Park, Kansas. Serum titers against *E. coli* O157:H7 were analyzed using an ELISA (Widiasih et al., 2004). Samples were dissolved at 1mg/ml in 1XPBS (phosphate buffered saline) solution and diluted to 1:100 with the 1XPBS for a concentration of 10μg/ml (Cray and Moon. 1995). Microtiter plates were coated with diluted LPS solution. The samples were placed through a series of similar steps of washing and coating the plates. Results were then read by a spectrophotometer at 405 nm, approximately 10 to 30 min after the addition of a substrate solution. The results are

expressed according to the final dilution factor on the plate (Fort Dodge Animal Health, 2004).

Statistical Analysis. The odds of a vaccinated animal shedding *E. coli* O157:H7 was compared to the odds that an that of unvaccinated control animal would shed the pathogen, accounting for repeated measures and pen using the GENMOD procedure of SAS (SAS Inst., Inc., Cary, NC). Odds ratios (ORs) and their 95% confidence limits are reported. Antibody titer data was evaluated using the MIXED procedure of SAS accounting for repeated measures and pen. Feedlot performance was evaluated using the MIXED procedure of SAS accounting for pen.

RESULTS AND DISCUSSION

E. coli O157:H7. In total, *E. coli* O157:H7 was recovered from 101 of 1,835 (5.5%) fecal samples. The ORs, the 95% confidence limits and the probability that differences were due to vaccination are presented in Table 3.1. The fifth sampling period was the only period used in the overall analysis because it was the only period in which allvaccination s were given according to the original design. Results presented in Figure 3.1 show the prevalence of cattle shedding *E. coli* O157:H7 by treatment group and sampling period. The levels of shedding at the initial sampling period while not statistically different were greater for the control group as compared with the vaccinated groups indicating an unequal initial prevalence level. Sampling periods 2 through 4 (point in time estimates) cluster at or below 5% shedding prevalence for each treatment group and test period 5, following all vaccinations, shows a slight spike in shedding by all groups with no effect of vaccination.

During the design phase of this experiment, power tests were conducted, indicating that with an overall shedding prevalence of 45% and a 50% reduction in shedding by vaccination – approximately 90 calves were required per treatment group in order to have a 90% chance of detecting a difference. However, with the low level of shedding prevalence actually observed (5.5%) observed, we required nearly 8 times as many calves per treatment group to detect a difference.

There are a number of possible reasons for the low prevalence observed in this experiment. Animal health, the environmental impact, and the fact that point in time estimates of cattle shedding *E. coli* O157:H7 may misrepresent the true prevalence of *E. coli* O157:H7 associated with cattle (Smith et al., 2005). Additionally, in a similar experimental design where 75% of cattle in a pen received vaccination, Peterson et al. (2005) reported that unvaccinated cattle commingled with vaccinated cattle were less likely to shed *E. coli* O157:H7 than cattle in pens where no vaccine was administered (external controls). Therefore, the low probability to detect *E. coli* O157:H7 in the current experiment may have resulted from development of herd immunity. Nevertheless, the low shedding prevalence observed in this experiment lowered the probability of detecting a difference due to vaccination to ~ 20%.

Antibody Titers. Venous blood samples were collected on d 0 and 45 of the pre-conditioning experiment, and on d 21, 100 and 162 of the finishing phase. Blood titer levels were elevated after vaccination ($P < 0.0001$; Figure 3.2). There were no differences in blood titer levels ($P = 0.25$) of cattle prior to any vaccination (d 0). Vaccination during the pre-conditioning phase (groups 1 and 2) increased blood titers ($P < 0.0001$) compared to those for controls (groups 3 and 4). Blood titer levels of steers

: vaccinated during the pre-conditioning phase only (group 1) returned to
iately pre-vaccination levels by the fourth sampling period, indicating that
f vaccination are not sustained by the immune system, supporting continued
accinations over time in order to maintain immunity. The group that maintained
st overall immune response was cattle vaccinated during pre-conditioning and
d 100 of the finishing phase (Group 2). The steady decline of titer levels in
from Groups 1 and 2 following test period 2, indicate that the optimum time
r booster vaccination is before d 100. The fact that delaying booster
ons until d 100 of the finishing phase lowers the overall immune response, if
ated by additional studies, could prove beneficial to the overall acceptance of the
n production systems, especially if vaccination during pre-conditioning and
on feedlot entry proves to be the most efficacious. The steers in Group 3 were
cinated on d 21 and 100 of the finishing phase and blood samples from these
d lower titer levels as compared with titer levels of blood samples from steers
ed during the pre-conditioning phase. At harvest, all vaccinated groups still had
≤ 0.02) titer levels compared with controls.

eedlot Performance. Least squares means for finishing performance measures
nted in Table 3.2. There were no differences ($P > 0.10$) in measures of
nce during finishing for steers reeiving the *E. coli* vaccine in three different
These data suggest that vaccinating cattle against Type III secretory proteins of
morrhagic *Escherichia coli* will not affect performance of cattle during the
period.

IMPLICATIONS

The sample size in this experiment was too small for the level of *Escherichia coli* shedding and, concluding that the vaccine was ineffective based upon the shedding prevalence results may be incorrect due to the high probability of encountering type II statistical error in the current study. However, elevated blood titers against *Escherichia coli* O157:H7 in samples from vaccinated steers indicate that an immune response was generated. The limited number of animals found positive for *Escherichia coli* O157:H7 in this experiment coupled with limited post-vaccination test periods and an elevated immune system warrant additional research on the effectiveness of this vaccine. The immune response observed in the control and vaccinated steers across the pre-conditioning and finishing periods may be beneficial in the development of further experiments targeting practical use of this vaccine.

Table 3.1. Experimental design and odds of detecting *E. coli* O157:H7 in cattle feces at harvest with 95% confidence limits by group.

Day of Trial / Phase	Treatment Groups 1	2	3	4
- 45 / Pre-Conditioning	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control	Sampled / Control
- 24 / Pre-Conditioning	Vaccinated	Vaccinated	Control	Control
0 / Finishing	Sampled	Sampled	Sampled	Sampled
		Feedlot Arrival		
21 / Finishing	Sampled / Control	Sampled / Control	Sampled / Vaccinated	Sampled / Control
100 / Finishing	Sampled / Control	Sampled / Vaccinated	Sampled / Vaccinated	Sampled / Control
183 / Finishing	Sampled	Sampled	Sampled	Sampled
		Harvest		

Treatment Group	Odds Ratio	95% Confidence Limits Lower	Upper	P-value
1	1.29	0.46	3.61	0.2742
2	1.02	0.34	3.05	
3	2.37	0.92	6.13	
4	1.00	1.00	1.00	

Table 3.2. Effects of vaccination against *E. coli* O157:H7 on two measures of finishing performance of steers

	Treatment Group[a]					
Item	1	2	3	4	SEM[b]	VAC[c]
Number of steers	92	92	91	92		
Initial BW, kg	274	264	270	273	3.28	0.16
Final BW, kg	525	511	512	520	5.38	0.19
Daily gain, kg	1.55	1.52	1.49	1.53	0.02	0.48

[a]1=vaccination during pre-conditioning only; 2=vaccination during pre-conditioning and on d 100 of finishing; 3=vaccination on d 21 and 100 of the finishing phase; 4=no vaccination (control)
[b]Standard error of least squares means
[c]Main effect of vaccination treatment

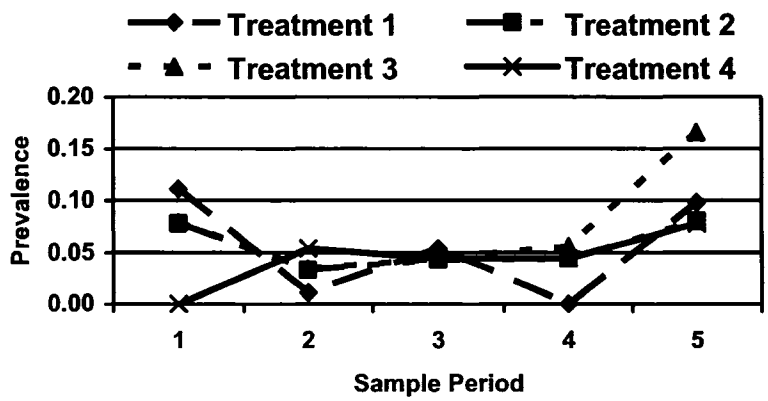

Figure 3.1. The proportion of steers shedding *E. coli* O157:H7 stratified by sample period and treatment group. Where: Treatment 1 = vaccination during preconditioning only, Treatment 2 = vaccination during preconditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

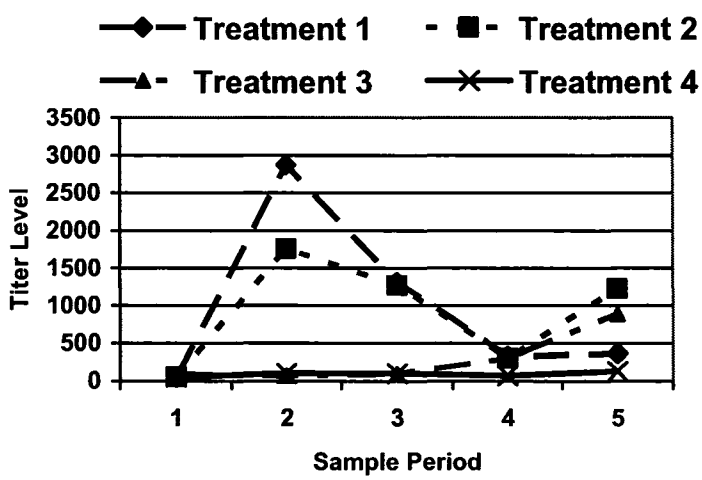

Figure 3.2. *E. coli* O157:H7 antibody titer levels stratified by sample period and treatment group (Vaccination effect - P = 0.0001; Interaction effect – P = 0.0001). Where: Treatment 1 = vaccination during preconditioning only, Treatment 2 = vaccination during preconditioning and on d 100 of the finishing phase, Treatment 3 = vaccination on d 21 and 100 of the finishing phase and Treatment 4 = controls (no vaccination).

APPENDIX

THE EFFECTS OF CATTLE GENDER ON CARCASS CHARACTERISTICS AND LONGISSIMUS MUSCLE PALATABILITY

INTRODUCTION

The success of branded-beef programs that guarantee tenderness have strengthened the belief that consumers are willing to pay higher prices for greater tenderness. Unfortunately, a significant portion of beef cuts are "unacceptable" in tenderness (Morgan et al., 1991). Several antemortem factors, such as genetics (Wulf et al., 1996; O'Connor et al., 1997), growth promoting implants (Platter et al., 2003a) and maturity (Field et al., 1997) have been shown to affect beef tenderness. Moreover, many studies have evaluated different postmortem strategies for improving beef tenderness problems that developed antemortem. Strategies such as postmortem aging (Smith et al., 1978) and electrical stimulation (McKeith et al., 1981) have been shown to have positive effects on beef tenderness. By understanding antemortem factors thatdetract from beef tenderness, as well as strategies available to offset some of these problems, it is possible to minimize the number of beef cuts which are unacceptable in tenderness. However, not all antemortem factors have been clearly evaluated for their effects on tenderness. One such factor is gender. Some studies have reported no difference in tenderness between steaks from steers and heifers (Gracia et al., 1970; Prost et al., 1975; Zinn et al., 1970),

but too little background information on the cattle used in each experiment was provided. Therefore, the objective of these two experiments was to test the hypothesis that strip-loin steaks from non-implanted steers and heifers from similar genetic backgrounds are not different in tenderness and to define benchmark differences and/or similarities in performance and carcass characteristics of steers and heifers.

MATERIALS AND METHODS

Experiment 1

Animals, Experimental Treatments and Cattle Management. Ninety-nine steers and 97 heifers managed in a similar manner from birth to slaughter were used to evaluate the effects of gender on carcass characteristics and cooked beef steak palatability measurements. Steers and heifers were randomly selected from a single Angus-based cowherd in northwestern Montana which employed two sire breeds (Angus, steers = 50, heifers = 43; Simmental, steers = 49, heifers = 54). Calves were weaned in October, 2001, backgrounded (MBQA. 2004) for 45 d and transported to a commercial feedlot in south-central Montana. Upon arrival at the feedyard, 19 Angus and 27 Simmental heifers were spayed (intra-vaginal method) by a practicing veterinarian.

Following a short growing phase on a corn silage-based starter ration, intact and spayed heifers were placed on a gradual adaptation program using three adaptation diets each fed for eight d, while steers were rapidly adapted to the finishing diet (9.92% corn silage; 64.6% corn grain; 20.8% wheat midds and 4.7% protein supplement, dry matter basis) using diets similar to those used to adapt heifers, each fed for four d. Steers were more aggressively fed in order to increase the likelihood that steers and heifers would

have similar levels of subcutaneous fat at comparable slaughter ages. Steers and heifers were group-fed for 161 d in two pens, and steers were fed separately from heifers. Growth-promoting implants were never administered and heifers were not fed melengestrol acetate to suppress estrus. Steers and heifers were harvested when the average fat thickness of the 196 cattle reached 10 mm over the longissimus muscle at the 12^{th} rib (measured using real-time ultrasound).

Carcass Data Collection and Sampling Methods. At shipping, steers and heifers were weighed in separate groups on a platform scale, transported approximately 617 km to a commercial beef packing plant, and harvested using conventional procedures. Carcasses were chilled in a cooler with an air temperature of 2°C for 36 h, and sprayed intermittently (2 min on, 8 min off) with a fine mist of 2°C water for the first 8 h of the chilling period. Following carcass-chilling, a commercial carcass data collection service evaluated and recorded measurements/assessments of hot carcass weight, USDA Quality Grade, USDA Yield Grade, fat thickness, longissimus muscle area, percentage of kidney/pelvic/heart fat, and marbling score for each carcass.

Strip loins (IMPS 180; USDA, 1988) from the right sides of each of the 196 carcasses were collected after fabrication and transported immediately to the Colorado State University Meat Laboratory. There, each strip loin was fabricated into three sections (anterior, middle, and posterior). The anterior, middle, and posterior sections were each placed in a vacuum-sealed bag and aged at 2°C for 7, 14, or 21 d postmortem, respectively. After reaching the appropriate length of aging time, sections were frozen and stored at -20°C. Frozen sections were then fabricated into steaks (2.54 cm) using an AEW-Thurne band saw (model 400 m, AEW Engineering Co. ltd., Norwich, England).

The anterior section (aged 7-d) was cut to provide two steaks for shear force and fatty acid analysis (data not presented here), the middle section (aged 14-d) was cut to provide three steaks for shear force and sensory panel analysis, and the posterior section (aged 21-d) was cut to provide one steak for shear force analysis. Upon completion of fabrication and cutting, each steak was identified, placed in a vacuum-sealed bag, sorted for intended use, and returned to frozen storage (-20°C). Samples used for sensory analysis were stored for approximately 135 d at frozen temperatures.

Tenderness Measurements. For shear force determination, frozen steaks were thawed at approximately 2°C for 24 hr and cooked using a belt grill (model TBG-60, Magikitch'n, Quakertown, PA) set to cook steaks to an endpoint internal temperature of 70°C (settings: top heat = 177°C, bottom heat = 177°C, preheat = disconnected, height = 1.85 cm, cook time = 6.35 min). Final endpoint internal temperatures were monitored using a handheld thermometer (model HH21; Omega Engineering, Inc., Stamford, CT). Cooked steaks were allowed to equilibrate to room temperature (22 – 25°C) and six to eight cores (1.27 cm in diameter) were removed from each steak parallel to the muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, using an Instron load frame (model 4443, Instron Corporation, Canton, MA) fitted with a Warner-Bratzler Shear Head (settings: crosshead-speed = 200 mm/min, load cell capacity = 100 kgf) . A single peak shear force was calculated using series IX software (Instron Corporation, Canton, MA) for each core. Individual-core, peak shear force values were averaged to assign a mean peak shear force value to each steak.

Trained Sensory Evaluation. Two steaks from each carcass, aged 14 d postmortem, were used for sensory evaluation. Individual steaks were thawed and cooked using the

same procedures used in preparing samples for shear force measurements. Warm samples (1.3 x 1.3 x 2.5 cm) from each steak were evaluated by an eight-member, trained sensory panel. Panelists were trained for 2 wk according to procedures outlined by AMSA (1995). Panelists assigned scores to each steak for juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness, and flavor intensity using 8-point, structured rating scales (AMSA, 1995).

Statistical Analysis. Analyses of live-animal performance traits was negated due to a lack of pen replication and due to spayed and intact heifers being fed in the same pen. Descriptive statistics of animal performance are reported in Table 4.1. Analysis of carcass characteristics, cooked beef steak palatability measurements and predicted consumer acceptance were conducted using the least squares, mixed model procedure of SAS (SAS Inst., Inc., Cary, NC) and individual animal was used as the experimental unit. The statistical model included treatment (gender) as the independent fixed effect, and sire-ID nested in sire-breed was added to the ANOVA as a random effect. Marbling score was added as a covariate in certain analysis of tenderness measurements because beef is purchased on a common quality grade basis.

When *F*-tests were significant ($P < 0.05$), least squares analysis with Tukey's adjustment was used to separate least squares means.

Experiment 2

Animals, Experimental Treatments and Cattle Management. Sixty steers and 60 intact heifers from the same ranch source used in experiment 1 were fed in two locations (sites 1 and 2) in order to compare the effects of gender and transportation distance to the packing plant (data not presented here) on carcass characteristics and tenderness. Thirty

steers and 30 heifers (site 1) and 30 steers and 30 heifers (site 2) were fed a similar finishing diet (9.92% corn silage; 64.6% corn grain; 20.8% wheat midds and 4.7% protein supplement, dry matter basis) for 206 ± 11 d. As in experiment 1, steers were scheduled to be more aggressively adapted to the finishing diet; however, the advanced adaptation was hindered by digestive upset problems in steers, resulting in equal adaptations to the finishing diet for steers and heifers. No implants were administered, but heifers were fed melengestrol acetate to suppress estrus. Steers and heifers were harvested when they attained an average of 10 mm of fat thickness over the longissimus muscle at the 12th rib (measured using real-time ultrasound).

Carcass Data Collection and Sampling Methods. Equal numbers of steers and heifers from each site were harvested using conventional procedures on two separate dates. Steers and heifers fed at site 1 were transported 617 km, while steers and heifers from site 2 were transported approximately 30 km. Carcasses were chilled in a manner comparable to that for carcasses in experiment 1. Following carcass-chilling, two expert graders evaluated and recorded measurements/assessments of hot carcass weight, USDA Quality Grade, USDA Yield Grade, fat thickness, longissimus muscle area, percentage of kidney/pelvic/heart fat, skeletal maturity, lean maturity and marbling score for each carcass.

Strip loins (IMPS 180; USDA, 1988) from the right sides of each of the 120 carcasses were collected after fabrication and transported immediately to the Colorado State University Meat Laboratory. There, each strip loin was placed in a vacuum-sealed bag and aged at 2°C for 14 d. After reaching the appropriate length of aging time, strip loins were frozen and stored at -20°C. From each frozen strip loin, one steak (2.54 cm)

was removed from the anterior end using an AEW-Thurne band saw (model 400 m, AEW Engineering Co. ltd., Norwich, England) for subsequent Warner-Bratzler shear force determination.

Warner-Bratzler Shear Force. All procedures used for determination of Warner-Bratzler shear force were identical to those used in experiment 1.

Statistical Analysis. Analyses of live-animal performance traits was negated due to a lack of pen replication and due to spayed and intact heifers being fed in common pens. Descriptive statistics of animal performance are reported in Table 4.6. Analysis of carcass characteristics, cooked beef steak shear force values and predicted consumer acceptance were conducted using the least squares, mixed model procedure of SAS; individual animal was used as the experimental unit. For analysis of carcass traits, harvest date X gender was added along with sire-ID (sire-breed) as random effects because not all animals were harvested on the same day.

When *F*- tests were significant ($P < 0.05$), least squares analysis with Tukey's adjustment was used to separate least squares means.

RESULTS

Experiment 1

Carcass Characteristics. Steers, compared to intact heifers; had ($P < 0.05$) heavier carcasses, with smaller longissimus muscle areas per cwt, and less desirable marbling scores and quality grades. Steers, compared to spayed heifers had ($P < 0.05$) larger actual longissimus muscle surface areas, less desirable marbling scores and quality grades.

Intact heifers, compared to spayed heifers; (a) had larger actual and carcass-weight adjusted longissimus muscle surface areas, and (b) more desirable yield grades.

Shear force and trained sensory panel ratings at a common level of carcass fat thickness. At a common level of carcass fat thickness (1.24 cm), steaks from steers had lower ($P = 0.04$) shear force values at 7 d postmortem than did steaks from intact heifers, but were not different from steaks from spayed heifers. Gender had no effect on shear force values at 14 and 21 d postmortem. Gender also had no effect on sensory panel ratings for juiciness, muscle fiber tenderness, overall tenderness or flavor. However, steers had more favorable ratings for connective tissue amount when compared to intact heifers ($P = 0.01$).

Shear force and trained sensory panel ratings at a common marbling score. Steaks from steers, compared to those from intact heifers; (a) had ($P < 0.05$) lower shear force values at 7 or 14 d postmortem, and (b) received ($P < 0.05$) more desirable ratings for muscle fiber tenderness, connective tissue amount and overall tenderness. Steaks from steers, compared to those from spayed heifers: (a) had ($P < 0.05$) lower shear force values at 7 or 14 d postmortem, and (b) received ($P < 0.05$) more desirable flavor ratings. There were no differences between steaks from intact vs. spayed heifers in any of the cooked beef steak palatability measurements at a common marbling score.

Predicted probability of consumer acceptance of steaks based on shear force and marbling score. The predicted probability of consumer acceptance of steaks from steers and intact or spayed heifers, based on individual marbling score and shear force value, were calculated using equations developed by Platter et al. (2003b). Predicted consumer acceptance rates based on marbling score were lower ($P < 0.0001$) for steaks from steers

than for steaks from intact or spayed heifers. Predicted acceptance rates based on shear force values were not different for steaks from cattle of the three gender classes.

Experiment 2

Carcass Characteristics and Warner-Bratzler Shear Force. Steers, compared to intact heifers; (a) had ($P < 0.05$) less fat thickness at the 12th rib, and (b) produced cooked steaks that required ($P < 0.05$) less shear force to sever sample cores.

Predicted probability of consumer acceptance of steaks based on shear force and marbling score. The predicted probability of consumer acceptance of steaks from steers and intact heifers, based on individual marbling score and shear force value, were calculated using equations developed by Platter et al. (2003^{b}). Predicted consumer acceptance rates based on marbling score or shear force values were not different for steaks from steers or intact heifers.

DISCUSSION

Carcass Characteristics. Part of the goal of these experiments was to define benchmark differences and/or similarities in carcass characteristics between steers and heifers managed without using growth promotants. In exp. 1, steers had heavier carcass weights compared with intact and spayed heifers which were similar. Similar to results of exp. 1, Zinn et al. (1970) reported greater carcass weights for steers compared to heifers, which had numerically lower gains across the feeding period.

Carcass fatness measured at the 12th rib was similar between steers and heifers in exp. 1; however, heifers had greater 12th rib fat thickness compared with steers in exp. 2 which may have resulted from the failure to properly adapt steers to the finishing diet in exp. 2. Kidney/pelvic/heart fat responded similarly in both experiments, as carcasses

from steers and heifers had comparable percentages of kidney/pelvic/heart fat as was previously reported by Marchello et al. (1970). Marchello et al. (1970) also reported that spaying reduced longissimus muscle area, which was consistent with the findings in exp. 1 and may coincide with reduced estradiol production. Results of gender on quality grade in exp. 1 contradicted findings of Zinn et al. (1970) and Marchello et al. (1970) as heifers produced carcasses with higher quality grades compared with carcasses from steers.

Tenderness and Consumer Acceptability. Current findings relative to effects of gender on cooked beef steak tenderness were inconsistent with previously reported results of Gracia et al. (1970) and Prost et al. (1975) who concluded that gender had no effect on cooked beef steak tenderness. However, current findings agreed with those of Wulf et al. (1996) and O'Connor et al. (1997) who did observe differences in tenderness between steers and heifers. Both Wulf et al. (1996) and O'Connor et al. (1997) though confounded gender by implant regimen. The tenderness advantages observed for steaks from steer carcasses compared to steaks from heifer carcasses in the present study were difficult to explain. Differences in tenderness may have resulted from ovarian function of intact heifers, and the fact that no differences were observed between intact and spayed heifers may have been attributed to the chronological age at which ovaries were removed from spayed heifers. However, the latter hypothesis would not be supported by the findings of Field et al. (1996), who also observed no difference in tenderness of steaks from spayed vs. intact heifers. Initially, it was hypothesized that differences in tenderness observed in exp. 1 were due to differences in maturity. However, this

hypothesis was not supported by results of exp. 2 inasmuch as there were no differences in carcass bone maturity, lean maturity, or overall maturity between steers and heifers.

Equations developed by Platter et al. (2003b) were used to predict the possible differences in consumer acceptability of steaks from steer and heifer carcasses. However, in this particular case, the low shear force values observed in steaks from steer and heifer carcasses resulted in similar estimates of consumer acceptability and the ultimate effect of gender on consumer acceptability of beef based on results of the current experiments would be expected to be minimal.

IMPLICATIONS

Findings of the present study suggest that, in branded-beef programs that guarantee tenderness or palatability and do not allow added growth hormones, constraints on minimum marbling levels should be higher for heifer beef than for steer beef to assure comparable eating satisfaction—across gender—among beef steaks.

Table 4.1. Summary statistics of feedlot performance for steers and heifers (exp. 1)

Treatment	Variable	Mean	Std Dev	Std Error	Min	Max
Steers	Weaning wt, kg	229	21.8	2.26	173	272
	Final wt, kg	492	34.0	3.42	395	580
	Daily gain, kg	1.62	0.16	0.02	1.21	2.09
Intact Heifers	Weaning wt, kg	219	18.8	2.66	182	250
	Final wt, kg	457	36.7	5.14	355	529
	Daily gain, kg	1.48	0.17	0.02	1.04	1.85
Spayed Heifers	Weaning wt, kg	221	23.4	3.49	182	284
	Final wt, kg	450	42.3	6.23	357	554
	Daily gain, kg	1.42	0.21	0.03	0.95	1.76

Table 4.2. Carcass characteristics of steers and heifers (exp. 1)

	Treatments				
		Heifers			
Item	**Steers**	**Intact**	**Spayed**	**SE[a]**	***Pr > F***
Carcasses	N=99	N=51	N=46		
HCW, kg	310[h]	289[i]	285[i]	3.7	< 0.0001
12[th] rib fat thickness, cm	1.21	1.23	1.29	0.06	0.387
KPH fat, %[b]	1.97	1.96	1.95	0.02	0.6433
LMA, sq cm[bc]	76.5[h]	77.4[h]	72.8[i]	1.1	0.0004
LMA /cwt, sq cm[bd]	24.8[i]	26.9[h]	25.7[i]	0.4	< 0.0001
Yield grade[be]	2.89[hi]	2.69[i]	2.94[h]	0.08	0.0242
USDA quality grade[bf]	483[i]	537[h]	549[h]	20.0	0.0004
Marbling score[g]	531[i]	598[h]	619[h]	20.8	< 0.0001

[a]Standard error of least squares means
[b]Gender x sire breed interaction (P < 0.05)
[c]LMA = longissimus muscle area
[d]LMA /cwt = longissimus muscle area per 100 kg carcass weight
[e]Yield Grade = 2.5 + (2.5*fat-thickness, in) + (0.2*KPH) – (0.32*LMA, in^2) + (0.0038*HCW, lb)
[f]USDA Quality Grade; Choice$^-$ = 400; Choice^0 = 500; Choice$^+$ = 600; Prime$^-$ = 700
[g]Marbling score; 400 = Small 00; 500 = Modest 00; 600 = Moderate 00
[h,i]Means within a row lacking a common superscript differ (P < 0.05).

Table 4.3. Effects of gender on Warner-Bratzler shear force and trained sensory panel ratings of beef palatability at a common level of carcass fat-thickness (1.24 cm); (exp. 1)

	Treatments				
		Heifers			
Item	Steers	Intact	Spayed	SE[a]	*Pr > F*
	N=96	N=51	N=46		
7-d aging					
Shear force, kg	3.40[d]	3.70[c]	3.45[cd]	0.13	0.0418
14-d aging					
Shear force, kg	3.37	3.58	3.46	0.11	0.1005
21-d aging					
Shear force, kg	3.30	3.42	3.38	0.13	0.6396
Sensory panel ratings[c]					
Juiciness	5.89	5.95	5.85	0.07	0.5165
Muscle fiber tenderness	6.56	6.44	6.51	0.1	0.4033
Connective tissue amount	6.73[d]	6.53[c]	6.65[cd]	0.06	0.0115
Overall Tenderness	6.52	6.34	6.48	0.1	0.1117
Flavor	5.81	5.73	5.72	0.05	0.1833

[a]Standard error of least squares means.
[b]Panel ratings based on a structured scale of 1 to 8, with 8 being most desirable and 1 being least desirable.
[c,d]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 4.4. Effects of gender on Warner-Bratzler shear force and trained sensory panel ratings of beef palatability at a common marbling score (583); (exp. 1)

	Treatments				
		Heifers			
Item	Steers	Intact	Spayed	SE[a]	*Pr > F*
	N=96	N=51	N=46		
7-d aging					
Shear force, kg	3.33[d]	3.76[c]	3.56[c]	0.12	0.001
14-d aging					
Shear force, kg	3.31[d]	3.62[c]	3.52[c]	0.1	0.006
21-d aging					
Shear force, kg	3.23	3.48	3.47	0.12	0.13
Sensory panel ratings[c]					
Juiciness	5.95	5.91	5.77	0.06	0.1165
Muscle fiber tenderness	6.64[c]	6.40[d]	6.42[c,d]	0.08	0.014
Connective tissue amount	6.77[c]	6.51[d]	6.61[c,d]	0.06	0.0005
Overall tenderness	6.58[c]	6.31[d]	6.41[c,d]	0.08	0.0041
Flavor	5.84[c]	5.71[c,d]	5.67[d]	0.05	0.0109

[a]Standard error of least squares means.
[b]Panel scores based on a structured rating scale of 1 to 8, with 8 being most desirable and 1 being least desirable.
[c,d]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 4.5. Effect of gender on predicted probability of consumer acceptance of steaks using individual marbling scores and shear force values from steaks aged 14-d postmortem (exp. 1).

	Treatments				
		Heifers			
Item	Steers	Intact	Spayed	SE[a]	*Pr > F*
Acceptance rate based on marbling scores, %[b]	59.1[e]	64.9[d]	66.7[d]	1.8	< 0.0001
Acceptance rate based on shear force values, %[c]	74.0	69.9	72.3	2.3	0.1438

[b]Mean predicted probability of consumer acceptance of steaks by individual marbling score, calculated using equations developed by Platter et al. (2003b).
[c]Mean predicted probability of consumer acceptance of steaks by individual shear force values from steaks aged 14 d postmortem, calculated using equations developed by Platter et al. (2003b).
[d,e]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 4.6. Summary statistics of feedlot performance for steers and heifers (exp. 2)

Treatment	Variable	Mean	Std Dev	Std Error	Min	Max
Steers	Weaning wt, kg	217	28.1	3.65	154	277
	Final wt, kg	478	45.6	5.94	349	584
	Daily gain, kg	1.04	0.16	0.02	0.50	1.34
Heifers	Weaning wt, kg	223	31.1	3.75	154	304
	Final wt, kg	488	42.8	5.15	390	615
	Daily gain, kg	1.05	0.14	0.02	0.63	1.38

Table 4.7. Effects of gender on carcass characteristics and Warner-Bratzler shear force (exp. 2).

Item	Gender		Std Err[a]	P > F
	Steers	Intact Heifers		
Carcass weight, kg	301	307	3.6	0.1957
12th rib fat thickness, cm	1.21[j]	1.50[k]	0.04	<0.0001
KPH fat, %	2.11	2.18	0.04	0.1448
LMA, sq cm[b]	78.17	80.55	1.26	0.1606
LMA /cwt, sq cm[c]	25.81	26.27	0.37	0.3634
Yield Grade[d]	2.80	2.99	0.08	0.0892
Bone maturity	A[50]	A[52]	1.19	0.2527
Lean maturity	A[55]	A[54]	1.66	0.7293
Overall maturity	A[52]	A[53]	1.07	0.4504
Marbling score[f]	541	566	16.0	0.2532
USDA Quality Grade[g]	436	474	16.0	0.0847
Shear force, kg[h]	3.12	3.35	0.09	0.0620
Shear force, kg[i]	3.11[j]	3.36[k]	0.08	0.0310

[a]Standard error of least squares means.
[b]LMA = Longissimus muscle area.
[c]LMA /cwt = Longissimus muscle area per 100 kg carcass weight.
[d]Yield Grade = 2.5 + (2.5*fat-thickness, in) + (0.2*KPH) – (0.32*LMA, in^2) + (0.0038*HCW, lb).
[e]Bone maturity, lean maturity, and overall maturity; A= 100.
[f]Marbling score; 400 = Small 00; 500 = Modest 00; 600 = Moderate 00.
[g]USDA Quality Grade; Choice$^-$ = 400; Choice^0 = 500; Choice$^+$ = 600; Prime$^-$ = 700.
[h]Shear force = Warner-Bratzler shear force values of steaks aged 14 d postmortem at a common level of carcass fat thickness (1.36 cm).
[i]Shear force = Warner-Bratzler shear force values of steaks aged 14 d postmortem at a common level of marbling (554).
[j,k]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 4.8. Effect of gender on predicted probability of consumer acceptance of steaks using individual marbling scores and shear force values from steaks aged 14-d postmortem (exp. 2).

	Gender			
Item	Steers	Intact Heifers	Std Err	Pr > F
Acceptance rate based on marbling score, %[b]	59.8	62.1	1.4	0.2452
Acceptance rate based on shear force values, %[c]	77.9	74.6	1.6	0.1422

[b]Mean predicted probability of consumer acceptance of steaks by individual marbling score, calculated using equations developed by Platter et al. (2003b).

[c]Mean predicted probability of consumer acceptance of steaks by individual shear force values from steaks aged 14 d postmortem, calculated using equations developed by Platter et al. (2003b).

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