

**EVALUATION OF THE RELATIONSHIP BETWEEN STRESS RESPONSE
AND THE FECAL SHEDDING OF *ESCHERICHIA COLI* O157:H7**

A Thesis

by

CELESTE ELAINE SCHUEHLE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Jeffrey W. Savell
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ABSTRACT

Evaluation of the Relationship between Stress Response and Fecal Shedding of

Escherichia coli O157:H7. (August 2005)

Celeste Elaine Schuehle, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jeffrey W. Savell

This study was conducted to determine if a relationship exists between temperament, stress response, and the shedding of *Escherichia coli* O157:H7. Cattle (n = 150) were evaluated for disposition and stress response before shipping to the feeding operation, upon arrival at the feedlot, at approximately 70d on feed, and prior to transport to the harvesting facility. Chute and pen scores, as well as serum cortisol concentrations, were measured in order to assess individual temperament and stress response. A temperament index was created to classify cattle as Excitable, Intermediate, or Calm. The presence of *E. coli* O157:H7 was determined by rectal swabs on the live cattle and swabs of colons collected postmortem at the processing facility. As expected, variables for pre-shipment temperament index, exit velocity, pen score, arrival and mid-point exit velocity, and mid-point cortisol concentrations differed ($P < 0.05$) greatly between temperament groups. However, pre-shipment chute scores and cortisol concentration, as well as arrival and final cortisol concentrations differed ($P < 0.05$) only for Excitable cattle compared to both Calm and Intermediate groups. The percentage of cattle shedding the pathogen at arrival was approximately equal between temperament groups. When sampled before shipment to the processing facility, a higher proportion ($P = 0.03$) of cattle displaying Calm temperaments shed *E. coli* O157:H7 than the other

groups. Results from postmortem colon samples exhibited a similar trend. When the results from all four sampling periods were pooled, the Calm cattle had a greater numerical percentage test positive for *E. coli* O157:H7. However, the pooled frequency distribution is largely dictated by the results of the final sampling time. Based on these results, it appears that Excitable cattle are not more likely to shed *E. coli* O157:H7. In fact, it seems that Calm cattle may be equally or more susceptible to shed at later points in the feeding period. However, it is important to note that a relatively small number of the samples tested positive for *E. coli* O157:H7, thus, potentially causing dramatic changes in the distributions.

DEDICATION

I dedicate this work to my husband, Kyle, and my family.

ACKNOWLEDGEMENTS

This project was funded by beef and veal producers and importers through their \$1-per-head check off through the Cattlemen's Beef Board and state beef councils by the National Cattlemen's Beef Association.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Foodborne pathogens have been estimated to cause 6 million illnesses and approximately 9,000 deaths each year (Mead et al., 1999). *Escherichia coli* O157:H7 was first associated with human disease during two investigations of hemorrhagic colitis in 1982 (Wells et al., 1983). Since then, *E. coli* O157:H7 has been the cause of numerous foodborne disease outbreaks in the United States and continued public attention has contributed to its emergence as a pathogen of significant public health concern. Several *E. coli* O157:H7 outbreaks have been linked to beef products, particularly the consumption of raw or undercooked contaminated ground beef.

Knowledge contributing to the control of this pathogen continues to be a priority for the beef industry. The industry has been successful at identifying post-harvest methods to reduce the prevalence of this pathogen on carcasses. Various treatments have been designed to decontaminate carcasses, including the use of sanitizing agents such as hot water sprays, organic acid sprays, or combinations of these treatments (Castillo et al., 1998). Also, steam vacuuming has been shown to be effective in reducing the numbers of pathogens on carcasses (Kochevar et al., 1997).

Additionally, several packers are implementing innovative hide intervention technologies such as chemical dehairing, cetylpyridinium chloride (CPC), on-line hide wash cabinets, and ozonated and electrolyzed oxidizing (EO) waters. Nou et al. (2003)

demonstrated that chemical dehairing effectively reduced the incidence of hide-to-carcass contamination with *E. coli* O157:H7. Bosilevac et al. (2004) tested the efficacy of a combined water wash and CPC treatment under conditions simulating a hide wash cabinet and concluded that the prevalence of *E. coli* O157:H7 was greatly reduced, resulting in near elimination of the pathogen prior to evisceration. On-line hide wash cabinets have been shown to reduce the prevalence of *E. coli* O157:H7 on hides, as well as pre-evisceration carcasses (Bosilevac et al., 2005a). Most recently, ozonated and electrolyzed oxidizing waters evaluated as a hide washing system were found to be effective methods of reducing the prevalence of *E. coli* O157:H7 on hides and carcasses (Bosilevac et al., 2005b).

Currently, the industry is making vast progress in the development of pre-harvest pathogen intervention strategies as well. Many of these technologies include vaccines, antibiotics, and feed additives administered to the animals at the feedlot. Ransom and Belk (2003) investigated several on-farm management practices as pre-harvest beef safety microbiological interventions, including a microbial feed additive, an antimicrobial feed additive, and a vaccine. They reported that all three interventions produced effective reductions of *E. coli* O157:H7 prevalence on hides and in feces.

The beef industry also is focusing greater attention on management practices that may contribute to controlling *E. coli* O157:H7. Through increased producer education, the industry has provided producers with best practice information that has amplified awareness of the implications their management practices have on the survival of this pathogen. Furthermore, the industry has identified the pre-harvest/harvest interface as a

vital interval in the beef chain where many cattle may become contaminated with *E. coli* O157:H7. This interface is defined as the period when cattle leave the feedlot to the point of hide removal at the packing plant. The industry has recognized transport and commingling at the packing plant as ample opportunities for cross-contamination of *E. coli* O157:H7. Guidance documents focusing on the cleanliness of cattle trailers and the maintenance of holding pens at packing plants is one of the methods the industry is utilizing to enhance knowledge and awareness of the pre-harvest/harvest interface.

The industry's shift in focus can be attributed to the knowledge that a strong relationship exists between the presence of this pathogen on live animals entering a harvest facility and the presence of this pathogen on carcasses. Bacon et al. (2000) and Elder et al. (2000) concluded that reducing the incidence of *E. coli* O157:H7 in fecal material and on hides of animals entering a processing facility should reduce the numbers of this microorganism on carcasses. Since 2001, USDA: FSIS sampling results have indicated a significant decline in the number of ground beef samples positive for *E. coli* O157:H7 (USDA:FSIS, 2005). The most recent Centers for Disease Control and Prevention (CDC) report on the sources and incidence of foodborne disease has shown a dramatic decrease in *E. coli* O157:H7 infections (CDC, 2004).

These data indicate the industry's determination and focus on employing more strategies to control and eliminate this pathogen within the beef chain have been successful. However, the existing knowledge of the factors affecting the transmission, colonization, and shedding of *E. coli* O157:H7 is insufficient to eliminate this pathogen as a food safety risk factor.

Evidence suggests that *E. coli* O157:H7 commonly occurs throughout all segments of beef production (Sargeant et al., 2000, 2003; Smith et al., 2001).

Interestingly, even when cattle are known to have environmental exposure to *E. coli* O157:H7, not all cattle will shed the microorganism (Sargeant et al., 2000).

A factor that may affect the colonization and shedding of pathogens in cattle is stress response. Stress is linked to reduced immune capacity and is understood to have a negative influence on growth and production efficiency in livestock. Exposure to environmental stressors can adversely affect fertility, immunocompetency, and growth efficiency of livestock (Carroll et al., 1996). Gibbs and Vale (1982) concluded that the perception of a stressor causes neurotransmitters in the brain to stimulate the synthesis and release of the hypothalamic neurohormones corticotrophin-releasing hormone (CRH) and vasopressin (VP). Both CRH and VP stimulate the release of adrenocorticotropin (ACTH) from the anterior pituitary gland, which in turn stimulates the synthesis and secretion of progesterone and cortisol (Verkerk et al., 1994). Curley et al. (2004) indicated strong relationships between animal temperament and stress responsiveness. Additionally, the stress hormone epinephrine has been linked to the expression of bacterial genes essential for colonization of the host intestine (Sperandio et al., 2003).

Experimentally inoculated piglets have been reported to have increased antibody titer and shedding of *E. coli* in response to weaning, commingling, and cold stress (Jones et al., 2001). Additionally, Cray et al. (1998) reported that fecal shedding of *E. coli* O157:H7 increased when inoculated calves were stressed by fasting. In contrast,

Barham et al. (2002) reported that *E. coli* O157:H7 incidence did not increase after transport to the abattoir, however, an increase was observed in the shedding of *Salmonella*. Furthermore, stress factors such as distance of transport, administration of antibiotics, colic, gastrointestinal tract surgery, and duration of hospitalization have all been identified as significant factors affecting the shedding of *Salmonella* in horses evaluated at a veterinary hospital (Alinovi et al., 2003). Brown et al. (1996) concluded that withholding feed from inoculated calves increased fecal shedding of *E. coli* O157:H7 by 1 to 2 log₁₀/g in three of four calves previously shedding small populations of the pathogen. Kudva et al. (1997) evaluated dietary influences on *E. coli* O157:H7 in sheep and recognized an increase in the number of culture-positive animals after an abrupt diet change. Byrne et al. (2003) compared the prevalence of *E. coli* O157:H7 in healthy cattle and downer cattle. Downer cattle were defined as those cattle suffering from assorted maladies, such as mastitis, calving paralysis, milk fever, and/or injuries incurred during transport rendering them immobile. Interestingly, data from this study indicated a 3.3-fold-higher prevalence of *E. coli* O157:H7 in downer cattle compared to healthy cattle.

The fact that some animals do not shed *E. coli* O157:H7 despite being exposed to stressful stimuli might be due to variation in stress response among individuals. Research on human subjects suggests that individuals neurologically respond to stressful situations with different regions of the prefrontal cortex (Rosenkranz et al., 2003). The neurological response seemed to be related to variation in the activation of the adrenal

axis, which subsequently adversely affected the production of antibodies in response to an immune challenge.

This study was designed to examine the relationship between stress response and the fecal shedding of *E. coli* O157:H7. Much of the current research evaluating this relationship involves experimental inoculation of animals, along with the application of a specific stressor such as dietary or temperature changes. These types of parameters have been successful experimental techniques; however, these extremes do not sufficiently represent “real world” circumstances. To adequately represent “real world” conditions, steers and heifers, typical of those entering a commercial feeding operation, were utilized in this experiment, and these animals were handled according to common industry management practices.

CHAPTER II

MATERIALS AND METHODS

Animal Selection and Handling

Previously weaned steers and heifers (n = 150), typical of those entering a commercial cattle feeding operation, were selected to evaluate the relationship between animal temperament and stress responsiveness to the fecal shedding of *E. coli* O157:H7. The cattle utilized in this study were fed at two feeding operations in the Southern region of the United States. The cattle were divided into three groups to facilitate data collection. Depending on the group, cattle were on feed from the month of June until the month of February.

Measures of disposition and stress response were obtained when the cattle were weighed before shipment, upon arrival at the feedlot, at approximately 70d on feed, and prior to transport to the processing facility. Upon arrival at the feedlot, the cattle were implanted, vaccinated, and placed on feed according to the standard practices of the facility.

Assessment of Temperament

All of the calves used in this study were obtained from herds previously used in disposition and stress studies. Earlier calf crops from these calves had produced a wide range in temperament scores. Therefore, the calves used in this study were expected to provide sufficient variation in disposition and stress response to test the stated hypothesis.

The disposition of the animals was evaluated by measuring exit velocity prior to shipping the cattle, upon arrival at the feedlot, and at approximately 70d on feed (mid-point). Exit velocity (Burrow et al., 1988) was determined by the rate at which an animal exited the working chute and traveled a fixed distance of 1.83 m. The rate at which the animal exited the chute was measured using infrared sensors connected to an electronic timing unit (FarmTek Inc., North Wylie, TX).

During the pre-shipment data collection, each animal was evaluated visually and assigned a chute score (Grandin, 1993) while it was confined, but not restrained, in a working chute. Chute scores consisted of a scale from 1 to 5, with 1 equaling a completely calm animal, and 5 equaling an extremely excited animal (Table 1). Small groups of animals were visually appraised by evaluators as they were confined to a pen and assigned a pen score (Hammond et al., 1996). These scores were based on the individual animal's response to the assessors as they approached the animal. Pen scores also were based on a 1 to 5 scale, where 1 was a completely calm animal, and 5 was an extremely excited animal (Table 2).

Based on measurements prior to shipping the cattle to the feedlot, a temperament index $\{(exit\ velocity + pen\ score)/2\}$ was created to classify cattle as Excitable, Intermediate, and Calm. Temperament groups were segmented based on mean \pm standard deviation values of the temperament index. Index values greater than one standard deviation of the mean were classified as Excitable, whereas more than one standard deviation below the mean identified Calm cattle. Temperament index values within one standard deviation of either side of the mean incorporated Intermediate cattle.

Exit velocity was used to classify cattle as Excitable, Intermediate, or Calm at the arrival sampling period. Temperament groups were sorted based on mean \pm standard deviation values of the exit velocity. Exit velocity values greater than one standard deviation above the mean were classified as Excitable, while values less than one standard

Table 1. Observations associated with the individual categories of chute scores to evaluate animal temperament (Grandin, 1993).

Chute score	Description
1	calm – no movement
2	slightly restless
3	squirring, occasionally shaking the chute
4	continuous, vigorous movement and shaking of the chute
5	rearing, twisting, and struggling violently

Table 2. Observations associated with the individual categories of pen scores to evaluate animal temperament (Hammond et al., 1996).

Pen score	Description
1	not excited by humans
2	stands in corner if humans stay away
3	runs along fences, head up, stops before hitting gates and fences, avoids humans
4	stays in back of the group, head high, and very aware of humans
5	excited, runs over anything in its path

deviation below the mean categorized Calm cattle. Values equal to the mean were classified as Intermediate. The use of exit velocity as the method of classification was repeated for the mid-point sampling.

Blood Sample Collection

Blood samples were collected via coccygeal venipuncture prior to transporting the cattle to the feeding facility, upon arrival at the feeding facility, at approximately 70d on feed, and prior to transporting the cattle to the harvesting facility. Serum samples were collected in an evacuated blood collection tube with no added anti-clotting agents and stored on ice, allowing the sample to coagulate. Within 3h of collection, the tubes were rimmed with a wooden applicator to ensure the clotted blood was not attached to the tube and then centrifuged to harvest serum. The serum was aspirated into 12 × 75 mm plastic storage tubes and frozen (-20°C) until the cortisol concentrations were measured.

Serum Cortisol Concentration Measurement

Serum concentration of cortisol was determined on duplicate aliquots of sera samples using a single antibody radioimmunoassay procedure that was adapted from Carroll et al. (1996) and utilized: rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards made by serial dilution (8000 pg/100 µl) of 4-pregnen-11 β ,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000); and radio-labeled cortisol: ³H-Hydrocortisone (1,2-³H, NEN, Boston, MA, Cat. #NET-185). Unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and counts per minute (cpm)

obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500). Cortisol antiserum cross-relatives were with: corticosterone, 60%; deoxycorticosterone, 48%; progesterone, 0.01%; and estradiol, 0.01% (determined by Pantex).

Rectal and Colon Sampling Procedures

Fecal samples were obtained rectally upon arrival at the feedlot, at approximately 70d on feed, and before transport to the harvesting facility. Cotton swabs were inserted into the animal's anus and twisted, contacting the sides of the rectum. The swabs were placed in Cary Blair medium and placed on ice packs for transport to the laboratory.

At the harvesting facility, a 25.4 cm section of the distal colon from each animal was removed. The colon sections were packaged individually in sealed, plastic bags, and placed on ice packs for transport to the laboratory. Upon arrival at the laboratory, the colons were opened longitudinally, and a 25-cm² area was swabbed. The rectal swabs and the swabs obtained from the distal portion of the colon were placed in a tube containing 10 mL of m-EC broth supplemented with 20 mg/L novobiocin and incubated for 16h at 42°C.

Enzyme Linked Immunosorbent Assay

An indirect enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies specific for O157 lipopolysaccharide and H7 flagellar antigen was used as a screening test to identify positive samples. A 1 mL sample of each pre-enriched culture was transferred to a tube containing 50 µL of the sample additive and heated in boiling water for 15 min. After the heated samples cooled to room temperature, 200 µL aliquots

of the positive and negative controls and the samples were transferred into individual wells of the test kit (TECRA® E. COLI O157 VISUAL IMMUNOASSAY™, Dynex Laboratories, Inc.). The wells were covered with plastic film and incubated for 30 min at 37°C. After incubation the wells were emptied and rinsed three times using the prepared wash solution that was provided with the test kit. Then 200 µL of the conjugate was added to each well. Again, the wells were covered with plastic film and incubated for 30 min at 37°C. The wells were emptied and rinsed four times according to the procedures described previously, and 200 µL of the substrate was added to each well. The wells were incubated at room temperature for 15 min and the results of the test were read visually using the color card provided with the test kit.

Immunomagnetic Separation

Presumptive positive samples from the ELISA screening test were subjected to immunomagnetic separation. A 1 mL sample of the pre-enriched solution was added to a tube containing 20 µL of Dynabeads® anti-O157:H7 (DynaL Biotech ASA, Oslo, Norway) and agitated for 10 min at room temperature. The tubes were placed in the Dynal MPC-S (DynaL Biotech ASA, Oslo, Norway) and a magnetic plate was inserted into the rack. The rack was inverted for 1 min, concentrating the beads into a pellet on the side of the tube. Following a 3 min recovery period, the sample supernatant was aspirated and discarded. The magnetic plate was removed from the Dynal MPC-S and the beads were washed with 1 mL of physiological buffered saline with 0.05% Tween-20. The inversion, recovery, and wash steps were repeated two more times before resuspending the Dynabeads-bacteria complex in 50 µL of the wash buffer. Then 25 µL

of the bead-bacteria mixture was streaked onto Dynal® CT-SMAC (Dynal A.S., Oslo, Norway) plates containing 0.05 mg/mL cefixime and 2.5 mg/L tellurite. The remaining 25 µL bead-bacteria mixture was streaked onto CHROMagar™ O157 (CHROMagar Microbiology, Paris, France) plates supplemented with 2.5 mg/L potassium tellurite. The plates were incubated for a minimum of 16h at 37°C.

Selection of Typical Colonies

Following incubation, typical O157:H7 colonies were selected and streaked onto blood agar plates. When possible, a minimum of three colonies from each plate containing typical O157:H7 growth were selected and streaked individually. Typical O157:H7 colonies selected from the CT-SMAC plates appeared clear or colorless. Colonies selected from the CHROMagar™ plates were a mauve color. The blood agar plates were incubated for a minimum of 16h at 37°C.

Gram Stain

Using a bacteriological loop, each suspect colony was transferred and fixed onto a slide. The slides were coated for 30 sec with a crystal violet stain. Following a 5 sec rinse using distilled water, the slides were covered with Gram's iodine mordant for 1 min and rinsed again. The stains were decolorized using 99% isopropyl alcohol and acetone until the crystal violet stain failed to wash and then rinsed with distilled water. Safranin was applied to counter stain the slide. The slide was rinsed with distilled water, blotted dry using bibulous paper, and examined microscopically under an oil immersion. When examined, O157:H7 colonies appeared red in color and rod-shaped.

Cytochrome Oxidase Test

The Pathotec® Cytochrome Oxidase (Remel, Lenexa, Kansas) test strips were placed on a paper towel and an isolated colony was selected for transfer from each blood agar plate. Using a bacteriological loop, each inoculum was transferred and spread onto the grey reagent band of a separate test strip. The test strips were allowed to stand for 30 sec before the color of the inoculated area was observed. A blue color indicated a positive reaction, while the absence of blue color indicated a negative reaction.

Latex Agglutination

Colonies from the blood agar plates were subjected to latex agglutination using the *RIM*® *E. coli* O157:H7 Latex Test (Remel, Lenexa, Kansas). The reagents included in the kit were allowed to warm to room temperature and were thoroughly suspended by gentle agitation. Holding the reagent vials vertically, one drop of each reagent was dispensed onto one separate well of the test slide. Using a plastic stick provided in the kit, portions of the suspect colonies were emulsified into each reagent. The test slide was rotated for 1 min using complete circular motions or until agglutination was evident.

Biochemical Confirmation

Isolates that were presumptive for *E. coli* O157:H7 were confirmed biochemically using a Vitek system.

Statistical Analysis

Temperament data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC), whereas the Chi-square option in the PROC FREQ procedure of SAS was used to analyze fecal shedding of *E. coli* O157:H7.

CHAPTER III

RESULTS AND DISCUSSION

Classification by Temperament Index

Least-squares means for temperament indicating traits for cattle segmented into groups according to pre-shipment temperament index are reported in Table 3. As expected, pre-shipment temperament index, exit velocity, and pen score, as well as arrival and mid-point exit velocity differed ($P < 0.05$) greatly between temperament groups (Table 3). Pre-shipment exit velocity and pen score values are similar in magnitude to the findings of Curley et al. (2004). Pre-shipment chute scores for Excitable cattle were higher ($P < 0.05$) than the Calm and Intermediate groups. Serum cortisol measurements at the pre-shipment and arrival samplings were higher ($P < 0.05$) in the Excitable cattle than the other two groups. Contrary to expectations, the cortisol concentrations of the Calm and Intermediate groups did not differ at these samplings. It is unclear why the Calm and Intermediate groups differed at the mid-point sampling, but not at the earlier sampling periods.

Table 3. Least-squares means for temperament indicating traits of yearling-fed cattle segmented into groups according to temperament traits measured before shipment to the feeding facility.

Trait	Temperament Classification			RMSE ^a	<i>P</i> > <i>F</i>
	Calm	Intermediate	Excitable		
n	25	93	33		
Pre-shipment temperament index ^b	1.63 ^c	2.85 ^b	4.30 ^a	0.22	<0.001
Pre-shipment exit velocity (m/s)	1.37 ^c	2.27 ^b	3.60 ^a	0.40	<0.001
Pre-shipment pen score ^c	1.24 ^c	2.40 ^b	3.63 ^a	0.58	<0.001
Pre-shipment chute score ^d	1.32 ^b	1.52 ^b	1.94 ^a	0.51	<0.01
Pre-shipment cortisol (ng/mL)	10.29 ^b	10.87 ^b	15.20 ^a	27.85	<0.001
Arrival exit velocity	1.57 ^c	2.16 ^b	2.99 ^a	0.65	<0.001
Arrival cortisol (ng/mL)	10.23 ^b	11.74 ^b	16.83 ^a	55.20	<0.001
Mid-point exit velocity	1.54 ^c	1.99 ^b	2.76 ^a	0.52	0.001
Mid-point cortisol (ng/mL)	10.44 ^c	13.21 ^b	16.37 ^a	37.35	0.001
Final cortisol (ng/mL)	12.15 ^b	12.89 ^b	16.42 ^a	39.82	0.01

^aRoot Mean Square Error from analysis of variance

^bTemperament index = (exit velocity + pen score)/2

^c1 = not excited by humans; 2 = stands in corner if humans stay away; 3 = runs along fences, head up, stops before hitting gates and fences, avoids humans; 4 = stays in back of the group, head high, and very aware of humans; 5 = excited, runs over anything in its path (Hammond et al., 1996)

^d1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the chute; 4 = continuous, vigorous movement and shaking of the chute; 5 = rearing, twisting, and struggling violently (Grandin, 1993)

Least-squares means within a row with different letters (a-c) differ (*P* < 0.05)

Frequency diagrams for the fecal shedding of *E. coli* O157:H7 in cattle sorted according to temperament index are shown in Figure 1.

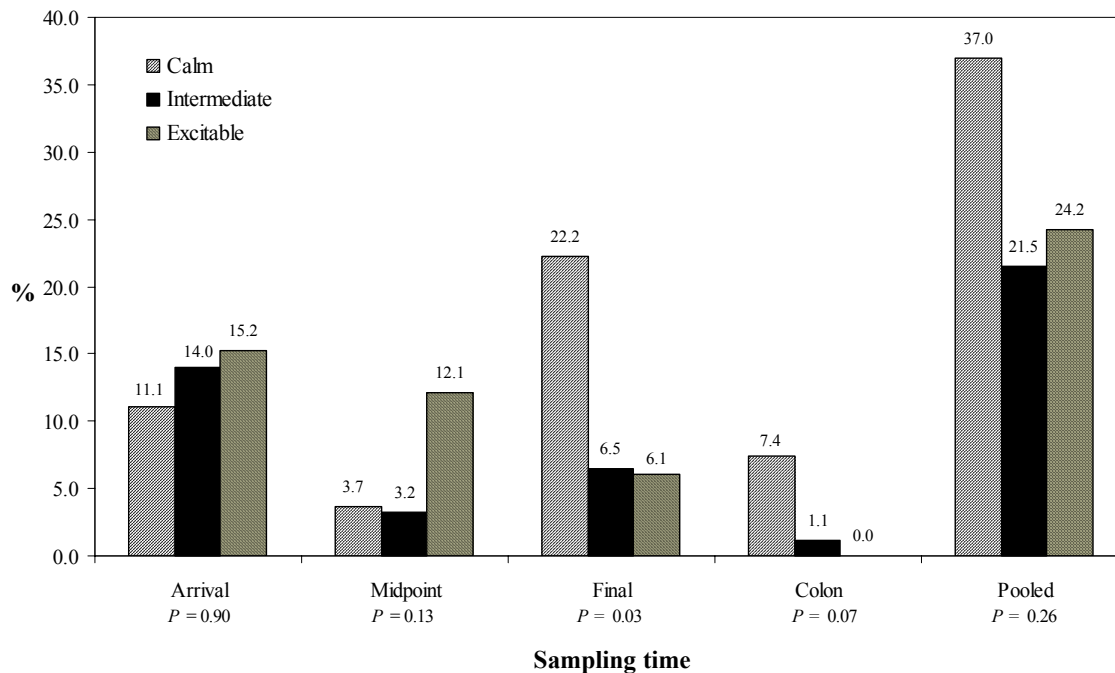


Figure 1. Fecal shedding of *Escherichia coli* O157:H7 at three different sampling times in feedlot cattle segmented into temperament groups before shipment to the feeding facility. (P-values are for chi-square tests.)

Chi-square analysis showed that the percentage of cattle shedding the pathogen at arrival was approximately equal between temperament groups. At mid-point sampling, cattle with Excitable temperament index values tended ($P = 0.13$) to have a greater proportion of animals shedding than cattle within the other two groups. When sampled before shipment to the processing facility, a higher proportion ($P = 0.03$) of cattle displaying Calm temperaments shed *E. coli* O157:H7. Results for postmortem colon samples had a similar trend. However, the disparity in the results of these two sets of

tests is troubling particularly because swabbing the excised colon was expected to be more sensitive than the fecal swab. Ransom et al. (2001) reported colonic swabbing to be a more effective method for detecting *E. coli* O157:H7 in feces, however, this was in comparison to a rectal palpation sampling method, not rectal swabbing. When the results from all four sampling periods were pooled, the Calm cattle had a greater numerical percentage of cattle test positive for *E. coli* O157:H7. However, the pooled frequency distribution is largely dictated by the final sampling time. It is also important to note a relatively low incidence of the pathogen, thus this may dramatically influence the frequency distributions.

Classification by Arrival Exit Velocity

Least-squares means for temperament indicating traits for cattle segmented into groups according to their exit velocity upon arrival at the feedlot are reported in Table 4.

Table 4. Least-squares means for temperament indicating traits of yearling-fed cattle segmented into groups according to temperament traits measured upon arrival at the feeding facility.

Trait	Temperament Classification			RMSE ^a	P > F
	Calm	Intermediate	Excitable		
n	20	113	19		
Arrival exit velocity	0.97 ^c	2.11 ^b	3.89 ^a	0.28	<0.001
Arrival cortisol (ng/mL)	8.95 ^b	12.17 ^b	18.57 ^a	54.03	<0.001
Mid-point exit velocity	1.32 ^c	2.06 ^b	2.88 ^a	0.52	<0.001
Mid-point cortisol (ng/mL)	12.55 ^a	13.34 ^a	14.36 ^a	40.76	0.68
Final cortisol (ng/mL)	10.87 ^b	13.40 ^b	16.57 ^a	40.17	0.02

^aRoot Mean Square Error from analysis of variance

Least-squares means within a row with different letters (a-c) differ ($P < 0.05$)

As expected, arrival and mid-point exit velocities differed ($P < 0.05$) between temperament groups. Arrival and final cortisol concentrations for Excitable cattle differed ($P = 0.05$) from the other two groups. Unusually, mid-point cortisol concentrations between groups did not differ.

Frequency diagrams for the fecal shedding *E. coli* O157:H7 in cattle segmented based on arrival exit velocity are reported in Figure 2.

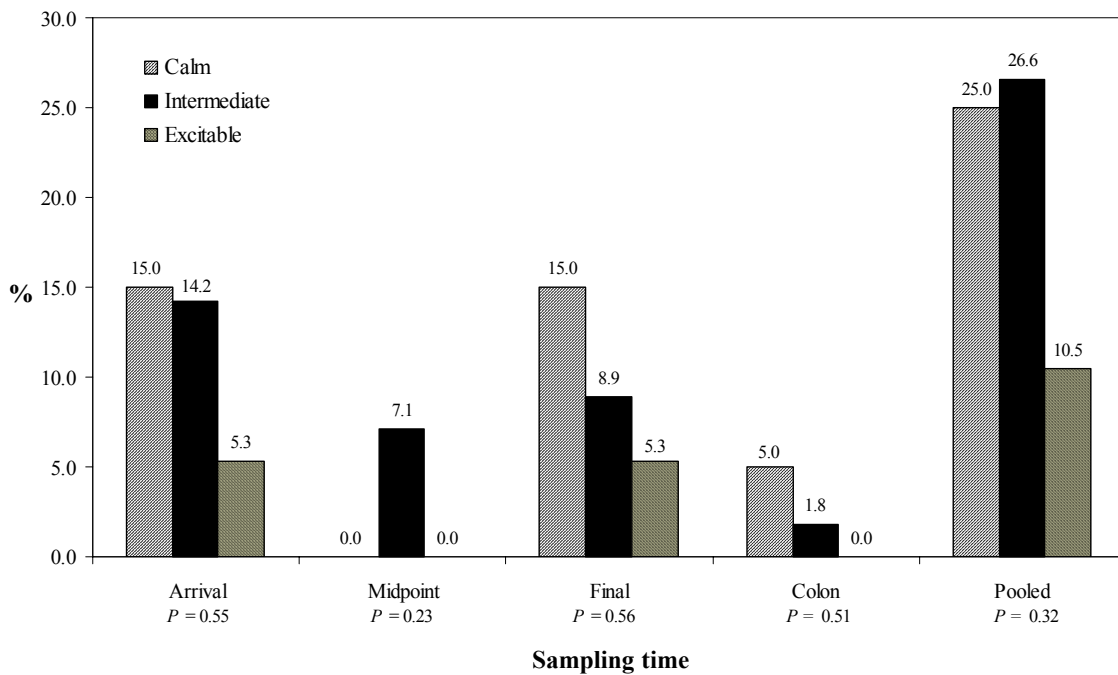


Figure 2. Fecal shedding of *Escherichia coli* O157:H7 at two different sampling times in feedlot cattle segmented into temperament groups based on exit velocity at arrival sampling. (P-values are for chi-square tests.)

When segmented according to arrival exit velocity, shedding at all sampling periods does not differ. Interestingly, the percentage of Calm and Excitable cattle shedding at the mid-point, final, and pooled samplings in Figure 2 are numerically less

than those in Figure 1, thus the percentage of Intermediate cattle shedding at these sampling periods was higher. This suggests that the re-classification based on arrival exit velocity affected the distributions.

Classification by Mid-point Exit Velocity

Least-squares means for temperament indicating traits for cattle classified based on exit velocity at mid-point sampling are detailed in Table 5.

Table 5. Least-squares means for temperament indicating traits of yearling-fed cattle segmented into groups according to temperament traits measured at approximately 70d on feed.

Trait	Temperament Classification			RMSE ^a	P > F
	Calm	Intermediate	Excitable		
n	22	109	21		
Mid-point exit velocity	1.05 ^c	1.97 ^b	3.58 ^a	0.20	<0.001
Mid-point cortisol (ng/mL)	11.82 ^b	12.96 ^b	17.22 ^a	38.23	0.01
Final cortisol (ng/mL)	13.40 ^b	12.24 ^b	20.29 ^a	34.43	<0.001

^aRoot Mean Square Error from analysis of variance

Least-squares means within a row with different letters (a-c) differ ($P < 0.05$)

Mid-point exit velocity differed ($P < 0.05$) for all groups of cattle. Mid-point and final cortisol concentrations for Excitable cattle differed ($P < 0.05$) from the other two groups.

Frequency distributions for the fecal shedding of *E. coli* O157:H7 in cattle segmented according to mid-point exit velocity are presented in Figure 3.

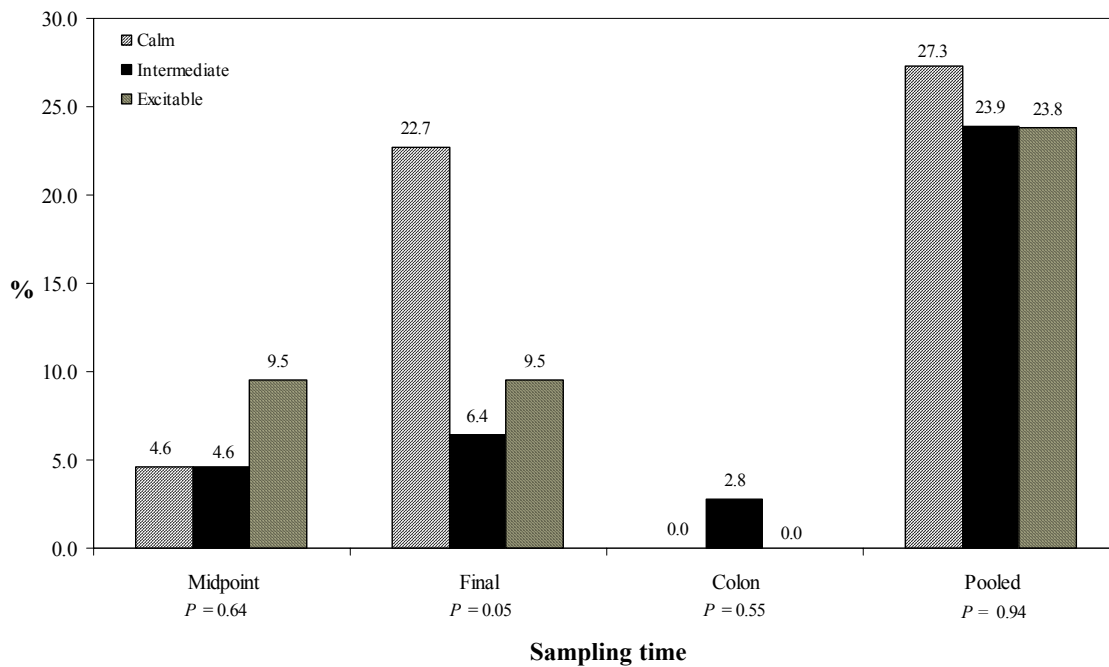


Figure 3. Fecal shedding of *Escherichia coli* O157:H7 at the final sampling period in feedlot cattle segmented into temperament groups based on exit velocity at mid-point sampling. (P-values are for chi-square tests.)

Chi-square analysis of cattle classified into temperament groups according to mid-point exit velocity indicated that a higher percentage ($P = 0.05$) of Calm cattle shed *E. coli* O157:H7 at the final sampling than the other groups of cattle. When results were pooled, the percentage of cattle shedding the pathogen was approximately equal between temperament groups.

Animal Temperament and *Escherichia coli* O157:H7

The temperament data from all three types of classifications, temperament index, arrival exit velocity, and mid-point exit velocity suggests that the Calm and Intermediate cattle were much more similar in disposition than either group compared to the Excitable cattle. It also seems that the distribution of the cattle was largely dictated by the method

of classification, also implying that there were not great differences between the Calm and Intermediate groups of cattle.

It appears that Excitable cattle are not more likely to shed *E. coli* O157:H7. In fact, it seems that Calm cattle may be equally or more susceptible to shedding.

However, it is important to note that a relatively small number of the samples tested positive for *E. coli* O157:H7 (Figure 4). Therefore, a relatively small number of positive samples could potentially cause dramatic changes in these distributions.

It is difficult to compare these distributions to those of similar studies examining the relationship between stress and the shedding of *E. coli* O157:H7 because these studies utilized inoculated cattle. However, comparisons can be made to studies that established overall prevalence levels of the pathogen. Low et al. (2004) investigated the rectal carriage of *E. coli* O157:H7 in slaughtered cattle and reported 35 of 267 animals (13.0%) to be positive for the pathogen. Ransom et al. (2001) indicated that 4 of 60 (6.7%) fecal samples from cattle at slaughter were positive for *E. coli* O157:H7. These results are similar to the percentage of positive animals at slaughter (9.2%) in this study. Contrastingly, Elder et al. (2000) found a prevalence of 28% for *E. coli* O157:H7 in the feces of cattle at processing plants.

Interestingly, the greatest numerical percentage of cattle shedding *E. coli* O157:H7 was at the arrival sampling time (Table 6). Additionally, the percentage of cattle shedding the pathogen at arrival was approximately equal between temperament groups. This could potentially be attributed to the stress caused by transport, although, stress indicating traits, exit velocity and cortisol concentration, at arrival do not appear to

be numerically different from those at other sampling times. However, an increase in the opportunity for cross-contamination of the pathogen while animals are confined on the truck may be a factor influencing the percentage of positive cattle at arrival.

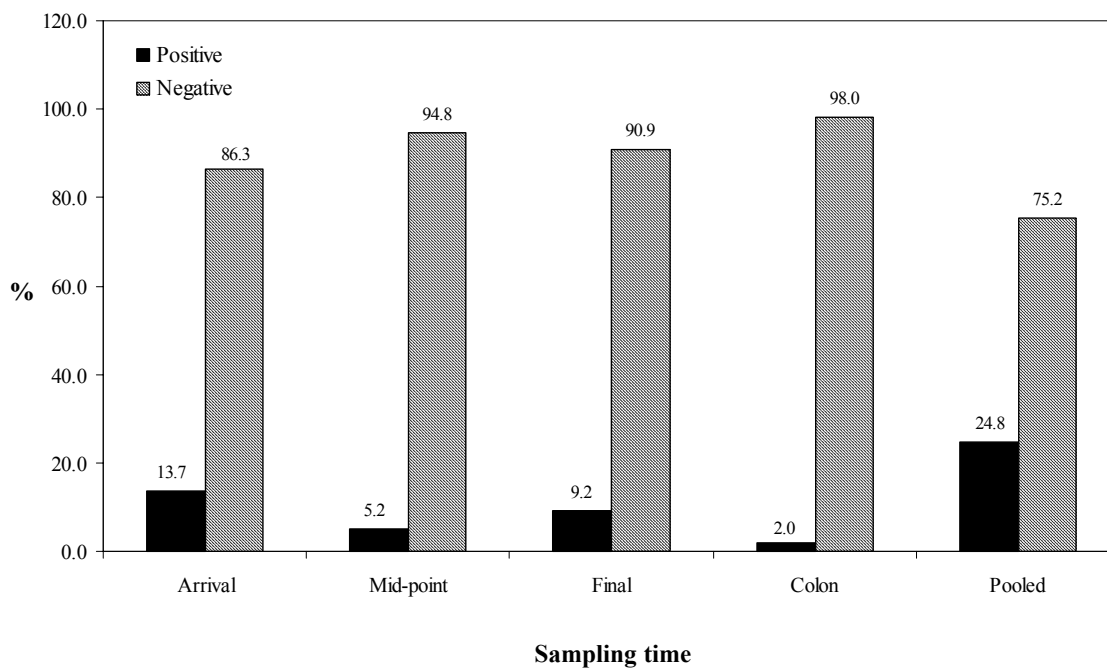


Figure 4. Percentage of positive and negative *Escherichia coli* O157:H7 tests at each sampling period.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Variables used for estimating animal temperament appear consistent throughout the trial groups of Excitable, Intermediate, and Calm cattle, however, cattle tended to change temperament groups throughout the feeding period. This implies that there were not great disposition differences, especially between the Calm and Intermediate cattle.

It appears that Excitable cattle are not more likely to shed *E. coli* O157:H7. According to the prevalence data, it seems greater fecal shedding occurs in Calm cattle at later points in the feeding period. However, the greatest percentage of shedding occurred at the arrival sampling compared to the other sampling times. It would be interesting to further examine the potential for cross-contamination of *E. coli* O157:H7 during transport.

Based on these data, it is difficult to determine whether or not a relationship exists between stress response and the fecal shedding of *E. coli* O157:H7 because of potential bias attributable to a relatively low occurrence of the pathogen. This research may serve as a helpful resource to others attempting to design a study investigating the relationship between stress and the shedding of *E. coli* O157:H7.

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