Consequences of stress and diet on the immune and endocrine systems of cattle and their effects on the seasonal shedding of *Escherichia coli* O157:H7

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**Background**

The purpose of this study is identifying the mechanism responsible for increased shedding of *E. coli* O157:H7, growth, and virulence factor expression. The hypothesis for this study, is that changes in diet do influence the expression of NE levels by the endocrine system, and the activation of the immune system in cattle during stressful versus non-stressful conditions, during various seasons, which does lead to increased expression of virulence factors and shedding of *E. coli* during the peaks seasons.

Studies have demonstrated that catecholamines, such as norepinephrine (NE) are able to stimulate bacterial growth in the gut environment of mammals. Catecholamines may be an important factor related to the shedding of *E. coli* O157 in cattle and may be impacted either by the diet of the animal or by the stresses that the animal encounters. NE is able to sequester iron, which both pathogenic and non-pathogenic *E. coli* has evolved to utilize. Recent studies conducted in cattle have demonstrated an increase in K99 pilus expression, growth, iron acquisition, motility, and the expression of intimin from *E. coli* O157 when the cattle are stressed or exposed to various conditions. Additionally, research has demonstrated that certain feedstuffs can have higher concentrations of catecholamines; indicating that dietary factors may have an impact on catecholamines and subsequently on shedding of *E. coli* O157. Variations in the concentration of these compounds in the diet of mammals could influence the homeostasis of the gut environment. Studies conducted in humans have demonstrated that increased levels of epinephrine act as autoinducers which increase the growth of *Enterohemorrhagic E. coli* (EHEC) and *Clostridium perfringens*. Similar events may occur in cattle that are exposed to stress related conditions or from changes in diet. These elements may induce an increase in *Enterohemorrhagic E. coli* (EHEC) O157:H7 in cattle based on nutritional and environmental changes over the course of seasonal variations. Research efforts have been made to identify the cause of increased shedding of *E. coli* O157:H7 during the spring to summer seasons by cattle, however direct correlations have not been identified with diet or environmental stresses alone. In addition to seasonal differences that are observed in shedding of *E. coli* O157 in cattle, there appears to be variation in shedding related to the types of diets the animals have been fed (grass vs. concentrate) and the time in the feedlot. In general, when cattle enter the feedlot the prevalence is lower than the prevalence observed mid-feeding and then the prevalence begins to drop in the populations as the animals go to slaughter. The events that lead to peak shedding conditions may be due to a combination of events, such as stresses, which may suppress the immune system, in addition to rapid changes in diet, and heat, leading to fluctuations in the activity of the endocrine system which could all disrupt the homeostasis of the gut flora leading to the increased growth of *E. coli* O157:H7 during the spring to summer seasons. Identification of the possible elements that lead to increases in *E. coli* O157:H7 may allow for methods to decrease environmental or physiological factors that influence the increase in *E. coli* O157:H7 shedding in cattle, thus increasing the safety of the beef products distributed by the beef industry.

The stated objectives for this work were:

Evaluate the influence of diet and stress conditions on the homeostasis of the immune and endocrine system of cattle. Second, to determine the influence of these factors on the shedding of *E. coli* O157:H7, over the course of four seasons (winter, spring, summer, and fall).
Methodology

Fecal samples will be collected from these cattle at the same time points serum samples are collected to evaluate levels of *E. coli* O157:H7 being shed by the cattle. In addition, fecal samples that are culture positive for O157 will be used to isolate *E. coli* O157 samples for further analysis of virulence factor expression and growth in supplemented minimal media.

**Cattle feed collection.** Feed samples will be collected from 3 different farms and feedlots. Samples will be homogenized and sterilized prior to addition into SAPI minimal salts medium. In vitro fermentation culture techniques will be utilized to mimic events similar to ruminal digestion as described by Galyean et al [19]. Samples of the feed will be further analyzed for concentrations of monoamines using high performance liquid chromatography (HPLC) methods to quantitate the concentrations of these compounds in the feed from various seasons. Samples will be compared to control standards for verification.

**Serum sample collection.** Serum samples from 400 cattle will be collected from the jugular vein using current established protocols. Blood will be collected into vacutainer test tubes with 15% (K3) EDTA solution. Samples will be centrifuged for 5 minutes at 1500rpm at room temperature (25°C). These samples will be collected during the winter, spring, summer, and fall seasons- 100 per season. The sera will be obtained from cattle at re-implant in the feedyard 25 cattle will be sampled each sampling period resulting in 4 sampling intervals/season. Serum samples will also be collected from cattle prior to transport to feedlots, and after arrival at the feedlots. Serum samples will be kept at -80°C in 1.5ml cryogenic tubes during storage and 4°C during experimental examination.

Samples of the sera will be analyzed for concentrations of monoamines and catecholamines, such as NE, using ELISA methods to quantitate the concentrations of these compounds. Samples will be compared to control standards for verification. In addition, sera will be utilized to supplement a minimal nutrient media (SAPI) minimal salts medium containing 30% serum, for the evaluation of *E. coli* growth and virulence factor expression [17].

**Fecal sample collection.** In addition to the serum samples, fecal samples will be collected from cattle and evaluated for *E. coli* O157:H7. Sampling methods will be conducted according to established protocols. Isolates of *E. coli* O157:H7 from fecal samples will be used further experiments in this study.

**E. coli virulence factor expression.** The expression of Shiga toxin 2 levels will be quantitated using ELISA methods established by the manufacturer. In addition, Shiga toxin 2 RNA, quantity will be evaluated using, Real Time PCR methods (RT-PCR).

**Statistical Analysis.** The statistical software program that will be used to evaluate the results of these experiments is produced by Jandel Scientific, SigmaStat. Results will be graphically represented using SigmaPlot, also by Jandel Scientific. Analysis of variance (ANOVA) will be the test preformed for all of the data collected, with a significance level alpha set to $\alpha = 0.05$.

Findings

These results demonstrated, that the growth rates of *E. coli* O157:H7 and *Salmonella* species increased significantly in conditions containing, a minimal medium (SAPI), feed, cattle serum and NE compared to growth conditions containing either only SAPI, SAPI and serum, or SAPI with serum and feed. When growth rates were evaluated with Shiga toxin 2 concentrations, preliminary RT-PCR results demonstrated that there was a significant increase in the production of Shiga toxin 2 by *E. coli* O157:H7 strains, grown in the presence of a collective condition containing the following: 70% roughage, serum, SAPI, and NE, compared to samples grown in the presence of serum, or with serum and NE. ELISA results for NE, Epi for pooled- serum collected from cattle and feed results indicated the presence of NE in all of the cattle tested over the course of 8 weeks.
Implications

These results demonstrate, that in the presence of feed components, NE (a stress related hormone), cattle serum (containing NE), there is an increase in the growth rate and concentration of pathogenic \textit{E. coli 0157:H7}. In addition there are detectable increases in the production of virulence factors associated to \textit{E. coli 0157:H7} such as Shiga toxin 2 in similar conditions.

These results suggest that stress-associated conditions in cattle and diet may induce increased proliferation and virulence factor expression by \textit{E. coli 0157:H7}.

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