

# EFFECT OF TRACE MINERAL SUPPLEMENTATION ON FECAL SHEDDING OF *E. COLI* O157:H7 IN CALVES.

K. D. Skinner<sup>1</sup>, J. A. Paterson<sup>1</sup>, T. T. Standley<sup>1</sup>, B. M. Rainey<sup>1</sup>, K. Hager<sup>2</sup>

<sup>1</sup>Montana State University, Bozeman, MT USA, <sup>2</sup>CHS Nutrition, Billings, MT USA

**ABSTRACT:** Twenty-four early weaned heifers (avg. wt. 217 kg) were used in a 60 d experiment to determine if trace mineral supplementation would alter the rate of shedding of *E. coli* O157:H7. Calves (12/treatment) were allotted to either a control or supplemented diet based on initial liver Cu concentrations. All heifers were individually fed a fixed amount of DM daily. The basal diet was composed of wheat middlings, soybean hulls, and corn grain (15% CP and 79% TDN). The supplemented treatment was formulated to provide an additional 399 mg Cu, 1001 mg Zn, and 707 mg Mn/d. The trace minerals were complexed minerals from Zinpro Inc. (Availa-4<sup>®</sup>). The control diet had no supplemental trace minerals added. All heifers were inoculated with an oral dose of *E. coli* O157:H7. Fecal samples were collected every 18 h for the first three days after dosing and then once daily every three d thereafter to determine *E. coli* O157:H7 concentration. On d 21, liver tissue and venous blood for IBR titers were collected. Trace mineral supplementation did not increase IBR titers (P=0.50) but did increase (P<0.005) Cu concentration in the liver. There were no differences in the rate of fecal shedding of *E. coli* O157:H7 between control and supplemented treatments over the initial 21 d. But, the SEM between treatments often were as great as the mean values. There was a numerical trend for *E. coli* O157:H7 to decrease in concentration during the first 21 d. However, after d 21, fecal *E. coli* O157:H7 concentration increased to a level almost as great as was measured during the first three d after dosing. The results of this study suggest that supplemental trace minerals did not influence the rate of *E. coli* O157:H7 shedding. This may be due to a lack of nutritional stress on the animals, (no differences in IBR titers) or because the control diet provided adequate trace minerals. The study did not explain why fecal shedding rates increased dramatically after d 7 and again at d 21. Further research is needed to look at long term shedding patterns of *E. coli* O157:H7.

Key Words: *E. coli* O157:H7, Beef cattle, Immunity

## Introduction

*Escherichia coli* O157:H7 is a food-borne pathogen that can cause significant health risk to consumers. Cattle are a major reservoir of *E. coli* O157:H7 and beef is contaminated during harvesting procedures (Elder et al., 2000; Barkocy-Gallagher et al., 2001; Rivera-Betancourt et al., 2003). Current post-harvest methods have proven effective in reducing *E. coli* contamination on carcasses (Elder et al., 2000; Barkocy-Gallagher., 2003; Rivera-Betancourt et al., 2003), through a multiple-hurdle intervention system. This system needs to be expanded to decrease the amount of *E. coli* O157:H7 contaminated cattle during the pre-harvest stage.

*E.coli* O157:H7 is ubiquitous from the farm to the packing plant (Hancock et al., 1997; Kudva et al., 1997., Rivera-Betancourt et al., 2003). Rice et al. (2003) and Mcgee et al. (2004) found that the introduction of one animal which was shedding at high rates quickly infected other cohorts. Furthermore, Bach et al. (2004) indicated that stress likely increased susceptibility to shedding.

Preharvest nutrition of cattle has been implicated as a preharvest tool that may decrease *E.coli* O157:H7 shedding (Kudva et al., 1997; Berg et al., 2004). Trace mineral and vitamin supplementation play a critical nutritional role by optimizing the immune status of beef cattle. A functional immune system is necessary for an animal to immunologically respond to foreign antigens (Greene et al., 1998). Trace mineral supplementation has increased humoral and cellular immune response in cattle (Ansotegui et al., 1994; Clark et al., 1995).

Results from our laboratory (Choat et al., 2005) showed decreased *E. coli* shedding in MT feeder cattle compared to cattle from other parts of the U.S. The only common management procedure among different groups of cattle was supplementation with increased levels of trace minerals and vitamins prior to shipment to Midwestern feedlots. The objective of this study was to compare fecal shedding of calves dosed with *E. coli* O157:H7 which were either supplemented with trace minerals and vitamins or not supplemented.

## **Materials and Methods**

Twenty-four crossbred heifers (avg. wt. 217 kg) were weaned at approximately 165 d of age. Calves originated from the Montana State University beef herd and had limited access to mineral supplementation prior to weaning. The protocol for the experiment is described in Figure 1. At weaning, heifers were weighed and had fecal, hide, and liver samples collected to determine baseline levels of *E. coli* O157:H7 shedding and liver trace mineral status. Calves were allotted to either a control treatment (no additional trace minerals) or to a supplemented treatment (diet fortified with trace minerals) based on initial liver Cu concentrations (Table 1). The supplemented diet provided an additional 176mg/d of Cu, 587 mg/d of Zn and 388 mg/d of Mn. The control diet did not have additional trace minerals added. Diet samples were analyzed, according to AOAC, (2000) procedures for protein and energy and by induction coupled argon plasma methods (Fassell, 1978) for trace minerals.

Heifers were weighed and placed in six pens of four heifers, with three pens per treatment. Heifers were fed to gain 0.68 kg/d and were fed at 0800 daily using individual feeding gates (American Calan). They were placed on their respective diets 30 d prior to dosing with *E.coli* O157:H7 cocktail.

Heifers were inoculated with *E.coli* O157:H7 using the following protocol. Inoculums were prepared by culturing each of five strains of *E.coli* O157:H7 (55AC1, 114AC1, 131AC1, 237AC1, 299AB3) in separate flasks of Luria-Bertani (LB) broth. The cultures were grown for 18 h at 37 C with agitation until culture densities reached  $10^9$  CFU of *E.coli* O157:H7/ml. Viable cell counts were estimated by spread plate culture of six serial dilutions on LB agar (Brown et al., 1997; Kudva et al., 1997). The five strains were subsequently mixed and cells

were obtained by centrifugation and resuspended at  $10^9$  CFU/ml in sterile saline (PBS). Inoculum was pipetted onto 0.45 kg of ground corn grain and then offered to each heifer at 1200.

Fecal samples were collected at time of dosing and again at 18 h, 32 h, and 50 h post-inoculation. Fecal samples were then collected every three d thereafter until d 21 to measure presence and concentration of fecal shedding of *E.coli* O157:H7. Heifers were weighed off trial on d 21. *E. coli* O157:H7 prevalence in feces was determined by collecting a rectal fecal sample from each heifer followed by shipment in insulated containers containing ice packs for next day delivery to a commercial laboratory (Food Safety Net, San Antonio, TX) for analysis of prevalence and most probable number of *E. coli* O157:H7.

Fecal samples were evaluated for prevalence of *E. coli* O157 following enrichment, use of immunomagnetic separation, and plating on ctSMAC and Rainbow agars (Barkocy-Gallagher et al., 2002). Morphologically typical colonies were tested for latex agglutination. Samples positive for latex agglutination were then subjected to most probable number analysis.

On d 7 of the experiment liver tissue and venous blood samples were collected. After this, heifers were injected with a modified live vaccine for infectious bovine rhinotracheitis (IBR) to determine humoral immune response (Ansotegui et al., 1994) due to trace mineral supplementation. Subsequent venous blood samples for IBR titers were collected on d 21 to determine if trace mineral supplementation affected primary and secondary IBR antibody titers.

Blood samples were placed on ice and were centrifuged (2,000 rpm for 20 min) and the serum separated for analysis. The analysis of serum for IBR antibody titers was conducted at Colorado State University Diagnostic Laboratory, Fort Collins, CO. Liver biopsy samples were frozen and shipped on dry ice to Michigan State University Diagnostic Lab for Cu, Zn, Mn, and Co analysis by inductively coupled plasma atomic emission spectroscopy techniques.

*Statistical Analysis* Data were analyzed using the GLM procedures for SAS (SAS Inst. Inc., Cary, NC). Changes in *E.coli* O157:H7 CFU/g of feces from d 0 to d 21 were analyzed by repeated-measures in time. Differences were determined using the LSD procedures of SAS (SAS Inst. , 2003). Changes in liver Cu concentration and IBR titers were analyzed by a simple ANOVA.

## **Results and Discussion**

No differences ( $P>0.05$ ) in initial body weights, liver Cu concentrations (Figure 2) or age was measured at the initiation of the experiment. All heifers were tested for *E.coli* O157:H7 shedding and were negative at d -30 and d 0 of the experiment. Similarly *E.coli* O157:H7 prevalence was not detected on any of the hide swab samples at d 0.

Heifers gained 0.61 kg/d for 50d while on experiment with no differences ( $P> 0.10$ ) measured between treatments. Supplementation with Cu did increase ( $P< 0.05$ ) liver Cu concentration (198 vs. 131 ppm for supplemented and control treatments, respectively; Figure 2). Unexpectedly, the control heifers consumed 223 mg/d of Cu provided in the non-supplemented

diet which was approximately four times their requirement. The source of this mineral contamination was not determined but was provided by one of the feedstuffs.

Supplementation did not ( $P=0.50$ ) increase IBR antibody titers compared to the control treatment. This may be due to the level of trace mineral in the control diet which did not cause nutritional stress/deficiency on the heifers.

All heifers were shedding *E.coli* O157:H7 within 18 h of dosing and there was no morbidity observed in any of the heifers post-inoculation. This agrees with other research since cattle are typically asymptomatic to *E.coli* O157:H7 infection (Cray and Moon 1995; Zhao et al., 2003).

Trace mineral supplementation did not ( $P=0.71$ ) cause differences in the rate of *E.coli* O157:H7 shedding. The SEM were often larger than the mean values. Because no differences in shedding were measured, average values were pooled and are presented in Figure 3 to show the pattern of shedding. Following inoculation there was a peak in *E.coli* O157:H7 by 18 h and agrees with research of McGhee et al. (2004) and Cray and Moon (1995). Although fecal shedding declined by days 2 and 3 post inoculation, there was another peak in shedding at d 7. Following d 7 there was a reduction in fecal *E.coli* O157:H7 concentration for the next two wks. This agrees with research reported by Brown et al. (1997), who showed a decrease in the fecal concentration of *E.coli* O157:H7 the first two wks after inoculation. There was another smaller increase in *E.coli* O157:H7 shedding at d 21. These periods of high *E.coli* O157:H7 fecal excretion could indicate the colonization of *E.coli* in the lower gut and more specifically at the recto-anal junction (Grauke et al., 2002; Naylor et al., 2004).

### **Implications**

Copper levels in the liver were increased by supplementation with Cu.. However, in this study, trace mineral supplementation did not change *E. coli* O157:H7 shedding or increase antibody titers for IBR. Further research is needed to determine long term fecal shedding patterns of *E.coli* O157:H7 since there was not a sustained reduction over 21 d.

### **Literature Cited**

- Ansotegui, R.P., C.K. Clark, E.J. Swensson, T.J. Milner, K.S. Bryan, and J.A. Paterson. 1994. Effects of chemical form and intake of mineral supplement on blood profiles and inflammatory reaction to phytohemagglutinin (PHAP) in pregnant heifers. *Proc. Am. Sec. Soc. Anim. Sci.* 45:222.
- AOAC.2000. Official Methods of Analysis. 17<sup>th</sup> ed. Assoc. of Official Analytical Chemists, Washington, DC.
- Bach, S. J., T. A. McAllister, G. J. Mears, and K. S. Schwartzkopf-Genswen. 2004. Long-haul transport and lack of preconditioning increases fecal shedding of *Escherichia coli* and *Escherichia coli* O157:H7 by calves. *J. Food Prot.* 67:672-678.
- Barkocy-Gallagher, G. A., E.D. Berry, M. Rivera-Betancourt, T.M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7

and Salmonella from beef carcass sponge samples and bovine fecal and hide samples. J. Food Prot. 65:1527-1534.

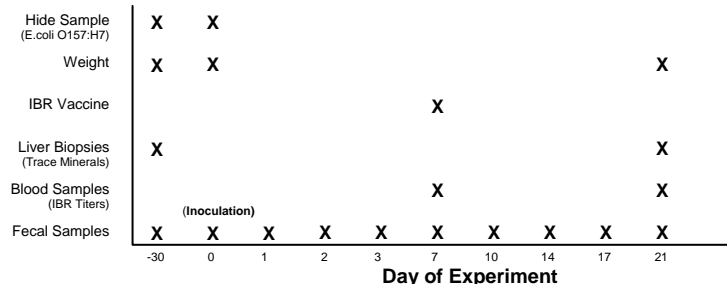
- Barkocy-Gallagher, G.A., T.M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analysis of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. Appl. Environ. Microbiol. 67:3810-3818.
- Barkocy-Gallagher, G.A., T.M. Arthur, M. Rivera-Betancourt, X. Nou, S.D. Shackelford, T.L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157:H7 Serotypes, and Salmonella in commercial beef processing plants. J. Food Prot. 66:1978-1986.
- Berg, J., T. A. McAllister, S. J. Bach, R. Stilborn, D. D. Hancock, and J. LeJeune. 2004. *Escherichia coli* O157:H7 excretion by commercial feedlot cattle fed either barley- or corn-based finishing diets. J. Food Prot. 67:666-671.
- Brown, C.A., B.G. Harmon, T. Zhao, M.P. Doyle. 1997. Experimental *Escherichia coli* O157:H7 carriage in calves. Appl. Environ. Microbiol. 63:27-32.
- Choat, T., J. Paterson, B. Rainey, G. Smith, K. Belk, R. White. 2005. Vaccination as an intervention strategy for reduction of *Escherichia coli* O157:H7 in cattle feces. Proc. Am. Sec. Soc. Anim. Sci. 56: .
- Clark, C.K. R.P. Ansotegui, J.A. Paterson, E.J. Swensson, A.G. Swenson, and A.B. Johnson. 1995. Effects of chemical form of mineral supplementation on cellular and humoral immune responses of yearling beef heifers. Proc. West. Sec. Am. Soc. Anim. Sci. 46:495-498.
- Cray, W. C., Jr., and H. W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 61:1586-1590.
- Elder, R.O., J.E. Keen, G.R. Siragusa, G.A. Barkocy-Gallagher, M. Koohmaraie and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides and carcasses of beef cattle during processing. Proceedings of the National Academy of Sciences, USA. 97(7):2999-3003.
- Fassell, V.A. 1978. Quantitative elemental analyses by plasma emission spectroscopy. Science. 202:183
- Grauke, L. J., I. T. Kudva, J. Won Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. Appl. Environ. Microbiol. 68:2269-2277.
- Greene, W.L., B.A. Johnson, J.A. Paterson, R.P. Ansotegui. 1998. The Role of Trace Minerals in the Cow-Calf Life Cycle. Purina Consultants Report.
- Kudva, I.T., C.W. Hunt, C.J. Williams, U.M. Nance, and C.J. Hovde. 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ. Microbiol. 63:3878-3886.
- Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. Epidemiol. Infect. 118:193-195.

- McGee, P., L. Scott, J. J. Sheridan, B. Earley, and N. Leonard. 2004. Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. *J. Food Prot.* 67:2651-2656.
- Naylor, S. W., J. Christopher Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. Smith, and D. L. Gally. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.* 71:1505-1512.
- Rice, D. H., H. Q. Sheng, S. A. Wynia, and C. J. Hovde. 2003. Recto anal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. *J. Clin. Microbiol.* 41:4924-4929.
- Rivera-Betancourt, M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. Rossman, J.O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.* 67:295-302.
- Zhao, T., S. Tkalcic, M. P. Doyle, B. G. Harmon, C. A. Brown, and P. Zhao. 2003. Pathogenicity of enterohemorrhagic *Escherichia coli* in neonatal calves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli*. *J. Food. Prot.* 66:924-930.

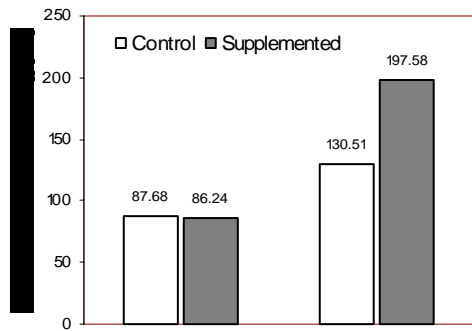
**Table 1. Ingredient, nutrient composition and calculated trace mineral intakes of diets fed to heifers**

Item	Supplemented	Control
Ingredient, % DM basis		
Wheat Middlings	23.21	23.21
Corn Grain	46.12	46.12
Dried Distillers Grain	8.91	8.91
Corn Cob	8.81	8.81
Soybean Hulls	3.71	3.71
Canola Meal (34%)	2.40	2.85
Nutrient analysis		
CP%	15.0	15.0
TDN%	79	79
NE <sub>m</sub> , Mcal·kg <sup>-1</sup>	1.38	1.38
NE <sub>g</sub> , Mca·kg <sup>-1</sup>	0.95	0.95
Calculated consumption		
Cu, mg/day	399	223
Zn, mg/day	1001	414
Mn, mg/day	707	319

**Figure 1. Timeline for experiment in which heifers were supplemented with trace minerals and dosed with E. coli O157:H7**



**Figure 2. Initial and final liver copper concentrations for heifers supplemented with 176 mg/d copper for 50d**



Means differ ( $P < 0.005$ ) for final Cu

**Figure 3. Change in average fecal excretion pattern for calves dosed with E. coli O157:H7 with standard errors for each sampling date**

