EFFECTS OF SUPPLEMENTAL PROTEIN REMOVAL ON TOTAL AND ACID-RESISTANT *E. COLI,* TOTAL COLIFORMS, AND PERFORMANCE IN FINISHING STEERS

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Summary

Fifty-four crossbred finishing steers were used to measure the effects of reducing supplemental protein (nitrogen) on feedlot performance and fecal shedding of acid-resistant *Escherichia coli* and total coliform bacteria. A control diet (15.0% crude protein; high protein) was compared to a low protein diet (8.9% crude protein; low protein) from which supplemental nitrogen sources (urea and soybean meal) were removed for the last 8 days of the feeding period. Fecal *E. coli* and coliform populations were measured prior to harvest. Removal of supplemental nitrogen from feedlot cattle diets did not substantially reduce populations of acid-resistant fecal *E. coli* and coliforms. Fecal pH tended to be lower ($P=0.11$) and the molar percentage of fecal isobutyrate and valerate were lower ($P<0.05$) for steers receiving low protein diets, but total fecal volatile fatty acid concentrations were not affected by dietary treatment. Dry matter intake tended to be lower ($P<0.10$) for steers fed low protein diets, whereas daily gains, feed:gain, final weights, dressing percentages, and carcass characteristics were similar for cattle fed low and high protein diets.

Introduction

The Centers for Disease Control estimates that about 74,000 cases of illness due to enterohemorrhagic *E. coli* O157:H7 occur annually in the United States. Cattle are a principal reservoir of *E. coli*, and ground beef is the major source of transmission. The disease can cause diarrhea, hemorrhagic colitis, and/or kidney damage that eventually may result in hemolytic uremic syndrome. *E. coli* O157:H7 is known to resist the human gastric barrier and proliferate in the lower gastrointestinal tract. The survival of *E. coli* in the human gastrointestinal tract may be enhanced by preconditioning bacteria to acidic conditions prior to infection. Development of acid resistance by *E. coli* may be induced by protein(s) that exist in high grain diets of ruminants. It has been documented that the presence of protein in culture medium confers acid resistance to *E coli*. Decarboxylation of protein elevates pH in the micro-environment surrounding the organism, thus enabling it to survive in harsh acidic environments. Increases in local pH may reduce the susceptibility of *E. coli* to the acidity of the human gastric defense system. Previous research at Kansas State University indicated a tendency for lower populations of fecal *E. coli* and coliforms capable of surviving a pH 2 acid shock when supplemental nitrogen was removed from the diet for 48 hours. In commercial cattle feeding operations, precise slaughter dates can be difficult to predict, making short-term reductions in pathogen shedding management intensive. Extending that time by reducing protein levels in finishing cattle diets one or two weeks prior to shipping, without sacrificing performance, could help reduce the risks of *E. coli* contamination prior to and during harvest. This approach would be more manageable for commercial operations, allowing for greater flexibility in marketing of fed cattle. In addition, lower feed costs can be expected with the reduction of dietary protein during this period. The objective of this experiment

was to measure the effects of removing supplemental nitrogen from finishing diets on generic *E. coli* and coliform populations 8 days prior to harvest while examining resulting cattle performance and carcass characteristics.

Experimental Procedures

Fifty-four crossbred steers were used in this experiment. Steers were housed in open lot, dirt-floor pens with fence-line waters that were cleaned twice weekly. Steers were adapted to a common high-concentrate, flaked corn-based finishing diet and allowed *ad libitum* access to feed for 12 days. On day 13, fecal grab samples were collected to establish baseline *E. coli* and coliform populations. Then, steers were provided diets (Table 1) with 15.0% crude protein (high protein) or 8.9% crude protein (low protein). High protein diets contained supplemental urea and soybean meal, whereas urea and soybean meal were replaced with steam-flaked corn in the low protein diets. On day 21, final fecal *E. coli* and coliform populations were measured for both groups of cattle. Upon arrival at the laboratory, fecal samples were combined with a citrate buffer (pH 7 or 2) for determination of total and acid-resistant *E. coli* and coliforms. Samples were serially diluted, plated onto *E. coli*/coliform Petrifilm™, incubated at 35°C for 24 hours, and enumerated. Steers were harvested on day 22. Animal performance and carcass traits were evaluated in each group to quantify effects of short-term removal of supplemental protein. Five steers of different breeds and similar finish were selected from the high protein treatment to participate in a market steer judging event after the final fecal sampling period. Bacterial counts from these five animals were used in the final analysis of the data, but unfortunately performance and carcass characteristics were not obtained from these animals.

Results and Discussion

Removal of supplemental protein from finishing cattle diets did not significantly affect populations of total and acid-resistant fecal *E. coli* and coliforms (Table 2). Previous research at Kansas State University indicated that when supplemental nitrogen was removed from finishing cattle diets, populations of fecal *E. coli* and coliforms dropped within the initial two days after removal, but tended to be higher at later sampling times. The data suggests that the microflora inhabiting the lower gastrointestinal tract of cattle may adapt to the nitrogen deficit over time. In the present study, the relatively small change in bacterial populations (3.06 to 2.75 log colony forming units/gram wet feces for acid-resistant *E. coli* and 3.18 to 2.84 colony forming units/gram wet feces for acid-resistant coliforms) following removal of supplemental protein may indicate that protein (nitrogen) is either nonessential as a mechanism for development of acid resistance, or that the microflora were able to adapt to this change within the 8-day period prior to the final sampling. Eliminating or reducing the adaptation of the bacteria may aid in the management of *E. coli*.

Fecal pH tended to be slightly lower for steers fed low protein diets (Table 3; $P = 0.11$). The molar percentages of fecal isobutyrate and valerate were lower ($P < 0.05$) for steers receiving low protein diets, but total fecal volatile fatty acid concentrations were not affected by dietary treatment. Dry matter intake also tended to be lower ($P<0.10$) for steers fed low protein diets, but average daily gain, feed:gain, final weights, dressing percentages, and carcass characteristics were unaffected by diet (Tables 4 and 5). Because performance was not affected by dietary treatment, the data suggests that there may be additional economic incentives to removing supplemental nitrogen in finishing diets to reduce feed costs. Additional large scale

studies analyzing cost of gain and performance are warranted.

Populations of fecal *E. coli* and coliforms, performance, and carcass characteristics were not significantly affected by removal of supplemental nitrogen from finishing cattle diets 8 days prior to harvest.

Table 1. Composition of Experimental Diets (% of Dry Matter)

Item	High Protein[a]	Low Protein[b]
Steam-flaked corn	75.67	81.08
Alfalfa hay	5.78	5.77
Cane molasses	4.88	4.89
Soybean meal, 52% crude protein	4.37	-
Tallow	4.11	4.11
RT premix[c]	2.27	2.26
Urea	1.22	-
Limestone	1.34	1.38
Sodium chloride	0.30	0.30
Potassium chloride	0.02	0.18
Vitamin/trace mineral premix[d]	0.04	0.04
Crude protein, analyzed	15.0	8.9

[a]High Protein = 15.0% crude protein.
[b]Low Protein = 8.9% crude protein.
[c]RT premix = provided 33.3 grams/ton Rumensin® and 10 grams/ton Tylan® in a ground corn carrier.
[d]Formulated to provide (total diet dry matter): 1,200 IU/lb vitamin A, 0.1 ppm cobalt, 0.5 ppm iodine, 50 ppm manganese, 0.3 ppm selenium, 50 ppm zinc, and 8.3 ppm copper.

Table 2. Effect of Diet and Buffer Treatment on Fecal *E. coli* and Coliforms

Item	Treatment		SEM
	High Protein[1]	Low Protein[2]	
Fecal *E. coli*[a]	------log_{10} colony forming units/gram wet feces-----		
Buffer treatment			
pH 2	3.06	2.75	0.37
pH 7	5.93	5.87	0.36
Fecal Total Coliforms[a]			
Buffer treatment			
pH 2	3.18	2.84	0.37
pH 7	5.95	5.95	0.37

[1]High Protein = 15.0% crude protein.
[2]Low Protein = 8.9% crude protein.
[a]Detection limit = 1.18 log_{10} colony forming units/gram wet feces.

Table 3. Effects of Supplemental Nitrogen 8 Days Prior to Slaughter on Fecal pH and Volatile Fatty Acid Proportions

Item	Treatment		SEM
	High Protein[1]	Low Protein[2]	
Fecal pH	6.81	6.66	0.08
Total volatile fatty acids, m*M*	78.7	75.8	5.51
Acetate:propionate	3.96	3.57	0.26
	---------- m*M* ----------		
Acetate	53.3	49.9	3.61
Propionate	14.2	15.1	1.75
Butyrate	7.3	7.6	0.81
Isobutyrate	0.87[a]	0.45[b]	0.16
Valerate	0.95[a]	0.74[b]	0.08
Isovalerate	2.21	1.93	0.17

[1]High Protein = 15.0% crude protein.
[2]Low Protein = 8.9% crude protein.
[a,b]Means with different superscript differ ($P<0.05$).

Table 4. Effects of Removing Supplemental Protein 8 Days Prior to Slaughter on Performance in Finishing Steers

Item	Treatment[1]		SEM
	High Protein[2]	Low Protein[3]	
No. of steers	22	27	
Live weight			
day 1	1092	1122	39
day 13	1145	1177	40
day 21	1163	1188	42
Carcass adjusted live weight [a]			
day 21	1161	1192	46
Dry matter intake, lbs/day			
day 1 to 13	19.78	19.94	0.63
day 13 to 21	20.75[d]	19.31[e]	0.76
Gain, lbs/day			
day 1 to 13	4.42	4.58	0.32
day 13 to 21	2.25	1.38	0.31
Carcass adjusted gain, lbs/day [b]			
day 13 to 21	2.00	1.88	0.68
Feed:gain			
day 1 to 13	4.48	4.35	0.47
day 13 to 21	9.2	14.0	4.1
Carcass adjusted feed:gain [c]			
day 13 to 21	10.4	10.3	9.6

[1]Steers were placed onto a common diet on day 1. Starting on day 13, initial baseline *E coli*/coliform populations were obtained. Low protein steers were switched to a diet containing no supplemental protein (soybean meal or urea) or non-protein nitrogen; High protein cattle were left on the initial diet. Steers were fed for eight days, final *E. coli*/coliform populations were obtained, then harvested on day 22.
[2]High protein = 15.0% crude protein
[3]Low protein = 8.9% crude protein
[a]Carcass adjusted live weight = carcass weight ÷ common dressing percent of 61.74%.
[b]Carcass adjusted gain = (adjusted live weight – initial weight) ÷ days on feed.
[c]Carcass adjusted gain:feed = adjusted daily gain ÷ dry matter intake.
[d,e]Means with different superscript differ ($P<0.10$).

Table 5. Effects of Removing Supplemental Protein 8 Days Prior to Slaughter on Carcass Traits of Finishing Steers

	Treatment		
Item	High Protein[1]	Low Protein[2]	SEM
No. of steers	22[a]	27	
Hot carcass weight, lb	717	736	28
Dressing percent, %[b]	61.6	61.9	0.66
Yield grade	2.52	2.30	0.17
Back fat, inches	0.41	0.36	0.04
Kidney, pelvic & heart fat, %	2.06	2.18	0.08
Ribeye area, square inches	11.61	12.40	0.55
USDA quality grade, %			
Choice or Prime	22.2	37.0	11.4
Select	64.8	59.3	5.3
Standard	13.0	3.7	7.6

[1]High Protein = 15.0% crude protein.
[2]Low Protein = 8.9% crude protein.
[a]Five steers were removed after day 21 prior to harvest.
[b]Dressing percent = hot carcass weight ÷ live weight before shrink.