EFFECT OF PRESLAUGHTER FEEDING SYSTEM ON GUT BACTERIA IN CATTLE

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Background

The New Zealand meat processing industry is putting greater effort into ensuring that meat is *E. coli*-free. There have been two reasons for the move towards zero tolerance for this group of bacteria. Firstly, the presence of *E. coli* in foods of animal origin indicates that there is a moderate to high risk that the food has been contaminated at some stage with faeces, non-potable water or diluted faecal matter. Whether the *E. coli* that are detected could give rise to gastro-intestinal problems in consumers, depends on the type and number of *E. coli* that are present. Secondly, certain types of *E. coli*, and in particular *E. coli* 0157:H7, have emerged as serious pathogens in children and convalescent subjects, and it has been decided that one way of ensuring that this particular organism is not likely to be present in a food, is to control or exclude all *E. coli*, using a zero tolerance policy. Live cattle have been implicated as the main reservoir for foodborne *E. coli* 0157:H7, and the main sites where they proliferate and survive are the rumen, reticulum and omasum (Brown et al. 1997; Chapman et al. 1997).

It is well-recognised that the counts of *E. coli* in faeces at the time of slaughter can be highly variable (Davidson and Taylor 1978). For example, in some cattle there is less than 10^2 counts g^{-1} faeces whereas others have more than 10^7 g^{-1}. One reason for this sizeable variation is difference in the time off feed. Fasting before slaughter encourages the growth of *E. coli* in the rumen, and this could lead to more *E. coli* in the faeces (Jordan and McEwen 1998). In New Zealand, when cattle are despatched for slaughter, they are usually loaded onto the truck directly from pasture. Less commonly the cattle may be fasted for up to 4 hours before transport. Little is known about the effect that these preslaughter feeding schedules have on gut microflora.

Objectives

This study aimed at comparing transport directly off pasture with either feeding hay for two days before transport, or fasting for 24 hours before transport. Comparisons were made in terms of liveweight loss, surface soiling of the skin, gut microflora and digesta consistency.

Methods

Forty five Angus steers were divided into three preslaughter treatments: hay feeding for 48 hours before transport to the processing plant (48h hay), fasting for the last day before transport (Fasted), and allowing to feed on pasture up to the time of transport to the processing plant (Pasture). Transport lasted 2 hours. The cattle were then held overnight at the processing plant for 16 hours before slaughter. They were not fed during this period but had free access to water. Just before slaughter, they were scored for surface soiling with fresh faeces. The alimentary tract with its contents were collected at evisceration. The rumen with reticulum, omasum plus abomasum, small intestine, caecum and large intestine were tied off to ensure that there was no mixing between these regions or loss of digesta. Digesta samples were taken from the rumen, duodenum, distal ileum, caecum, and distal colon from each set of viscera within 1 hour of slaughter. These samples were transferred directly to the microbiology laboratory for estimation of bacteria numbers, and they were also used for dry matter, and physical consistency assessment. Each gut region was weighed separately before and after emptying its digesta. At 24 hours after slaughter, pH and stickiness were measured in the *longissimus dorsi* muscle. Microbiological measurements made on the digesta and faeces included the number of *E. coli*, *Enterobacteriaceae*, *Enterococci* and facultative anaerobes. Runniness of the faeces or digesta was measured using a splatter test with planimetry. The physical phases of the digesta or faeces were assessed using a press test. In this test, the digesta or faeces separated into three distinct phases or rings. The innermost ring consisted of coarse fibrous matter (fibre ring). The next ring surrounding the fibre ring consisted of a dark opaque fluid (sludge ring), and the outermost ring was an almost translucent watery ring (free-water ring).

Results and Discussion

The effect of three of the preslaughter feeding systems on the counts of *E. coli* g^{-1} of faeces are shown in Table 1. The corresponding total counts of *E. coli* in the entire gastrointestinal tract are shown in the lower half of Table 1. The fasted group had significantly higher concentrations and total burden of *E. coli* than the pasture-fed and the 48h hay-fed animals ($p<0.001$). The total burden of *E. coli* in the large intestine and the counts g^{-1} of *E. coli* in the faeces were lowest in the 48h hay treatment. The difference in faecal *E. coli* counts g^{-1} between the fasted and the 48h hay fed treatments was substantial: about 10^3.

The number of facultative anaerobes g^{-1} of faeces was higher in the fasted animals in comparison with the pasture and 48h hay-fed steers. The total burden of facultative anaerobes in the entire alimentary tract was higher in the fasted steers in comparison with the pasture-fed animals ($p<0.05$).

In general, the differences between the treatments in faecal *E. coli* counts were present along the whole length of the gut except for the duodenum. Within the rumen, it appeared that moist conditions and a relatively high pH favoured the growth and survival of *E. coli*, as counts of *E. coli* in the rumen digesta were negatively correlated with rumen digesta dry matter percent ($r=-0.75$, $p<0.001$), and were positively correlated with rumen contents pH ($r=0.52$, $p<0.001$). *Enterococci* and *E. coli* counts g^{-1} were negatively correlated in the rumen ($r=-0.47$, $p<0.001$)

Faeces which were runny, as measured by the splatter test, had a low dry matter ($r=-0.82$, $p<0.001$) and a relatively large sludge phase ($r=0.86$, $p<0.001$). This association was particularly noticeable for the pasture-fed steers which had a larger sludge phase and runnier faeces than the other groups ($p<0.05$). In many respects, the preslaughter feeding treatment differences in the sludge phase of the faeces were also present for the digesta in the caecum and the rumen, which suggests that the preslaughter feeding treatment influenced this aspect of digesta consistency throughout the digestive tract. Whereas, this did not apply to runniness. The fasted group had the runniest rumen and caecal contents, whereas the pasture group had the runniest faeces. The prevalence of live animal surface soiling in the pasture-fed steers after overnight holding at the processing plant was 24% whereas in the other treatments it was less than 7% ($p<0.05$).

An important implication from the findings in this study is that differences in *E. coli* counts between animals in the rumen will probably persist further down the tract. The pattern in the treatment differences in *E. coli* numbers persisted along the gut after the digesta had passed from the rumen and through the duodenum. This could indicate that the entry rate of *E. coli* from the rumen into the small intestine was an important factor influencing subsequent *E. coli* numbers. It implies that manipulating the growth and survival of *E. coli* in the rumen before transport could be an effective way of influencing the counts of *E. coli* in the faeces during the critical preslaughter period. Several factors could be influencing the numbers of *E. coli* in the rumen. A low dry matter material with a near-neutral pH favoured *E. coli*. Fasting the animals before transport favoured the formation of a high pH-low dry matter rumen environment. In practical terms, the most effective way of manipulating gastro-intestinal counts of *E. coli* was to feed hay. However, hay feeding did not have much effect on rumen contents pH and dry matter, and so it is suspected that some other factors mediated the *E. coli*-suppressing effect provided by the hay. These could include the presence of inhibitory compounds that may have been present in the hay, but to a lesser extent in the pasture. Duncan et al. (1998) observed that the coumarins, esculetin, umbelliferone and scopoletin, which are normal constituents in some swards, inhibited the growth of *E. coli* 0157 *in vitro*. These components were not monitored in the present study, but the hay that was used did include species such as *Anthoxanthum odoratum, Trifolium repens, Trifolium pratense, Polygonum aviculare* and *Taraxacum officinale* which normally contain some of those compounds (Murray et al. 1982). Other possible inhibitors of *E. coli*, that could have been present in hay but not in fresh pasture, include products from fungi that could have been growing in the hay. An alternative explanation is that the lower counts of *E. coli* in the hay-fed animals could have been due to greater competition from other bacteria in the gastro-intestinal tract. The hay-fed animals had more *Enterococci* in their alimentary tracts, and there was a negative relationship between *Enterococci* and *E. coli* in the rumen. Some *Enterococci* are known to inhibit the growth of *E. coli, Salmonella, Shigella* and *Enterobacter* species, and have been used in other species as probiotics because of their beneficial effect on the gut microflora (Franz et al. 1999).

The cattle that were pasture-fed up to the time of trucking had more runny faeces. The faeces from the pasture-fed cattle contained a higher proportion of the sludge phase, relative to the fibre and free-water phases. In addition, the pasture-fed animals were dirtier, in terms of fresh faecal soiling on the hide. In summary, feeding pasture on the farm up to the time of transport has only a small effect on the composition of gut contents but it has an adverse affect on faeces consistency and stock cleanliness.

Conclusions. Pasture-fed cattle were carrying approximately 75 billion aerobic bacteria and 86 billion facultative anaerobes in their gastro-intestinal tracts when they were slaughtered. The number and type of bacteria were strongly influenced by the preslaughter feeding system. Fasted animals had less acidic and more moist rumen contents, and they had more *E. coli*, *Enterobacter* and facultative anaerobes throughout the gut. Cattle fed hay for 48 hours before transport to slaughter had higher numbers of *Enterococci* and fewer *E. coli*. The pasture groups had runnier faeces, and were dirtier after overnight holding at the processing plant. It was concluded that feeding cattle hay for 48 hours before despatch for slaughter provided several advantages over other preslaughter feeding systems.

Pertinent Literature

Brown, C. A.; Harmon, B. G.; Zhao, T.; Doyle, M. P. 1997. Experimental *Escherichia coli* 0157:H7 carriage in calves. *Applied and Environmental Microbiology 63*: 27-32.

Chapman, P. A.; Siddons, C. A.; Cerdan Malo, A. T.; Harkin, M. A. 1997. A 1-year study of *Escherichia coli* in cattle, sheep, pigs and poultry. *Epidemiology and Infection 119*: 245-250.

Davidson, C. M.; Taylor, M. 1978. Variability of E.coli levels in bovine feces and its implications on guidelines for ground beef. *Canadian Institute of Food Science and Technology Journal 11*(1):53

Duncan, S. H.; Flint, H. J.; Stewart, C. S. 1998. Inhibitory activity of gut bacteria against *Escherichia coli* 0157 mediated by dietary plant metabolites. *FEMS Microbiology Letters 164*: 283-288.

Franz, C. M. A. P.; Holzapfel, W. H.; Stiles, M. E. 1999. Enterococci at the crossroads of food safety ? *International Journal of Food Microbiology 47*: 1-24.

Jordan, D.; McEwen, S. A. 1998. Effect of duration of fasting and a short-term high-roughage ration on the concentration of *Escherichia coli* biotype 1 in cattle feces. *Journal of Food Protection 61*(5):531-534.

Murray, R. D. H.; Mendez, J.; Brown, S. A. 1982. The natural coumarins: occurrence, chemistry and biochemistry. Chichester, John Wiley and Sons Ltd. 702 p.

Table 1. Effect of preslaughter feeding system on bacteria in the faeces and the entire alimentary tract in cattle. No SEDs are given for *E. coli* numbers as non-parametric Kruskal Wallis one-way ANOVA on ranks was used for analysis due to unequal variance of the treatments. Means in a row without a common superscript letter were significantly different at $P < 0.05$.

	48h Hay	Pasture	Fasted	SED
Counts (log_{10}) g^{-1} of faeces				
Aerobic plate count	6.109 [a]	6.274 [a]	7.358 [b]	0.173
E. coli	3.716 [a]	5.002 [b]	6.650 [c]	-
Enterobacteriaceae	4.634 [a]	5.163 [a]	6.968 [b]	0.385
Enterococci	5.001	4.600	4.382	0.348
Anaerobes	6.016 [a]	6.410 [a]	7.439 [b]	0.226
Total burden in the alimentary tract, log_{10}				
Aerobic plate count	11.889 [a]	10.875 [b]	10.255 [b]	0.111
E. coli	7.609 [a]	8.779 [a]	10.480 [b]	-
Enterobacteriaceae	8.142 [a]	9.161 [a]	10.700 [b]	0.288
Enterococci	9.901	9.383	8.982	0.329
Anaerobes	11.867 [a]	10.932 [b]	11.189 [b]	0.157
Liveweight loss before slaughter, g kg^{-1} empty body weight				
During transport	22 [a]	23 [a]	12 [b]	2
Up to slaughter	77	95	102	8