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Food, Pro and Prebiotics: Effects Beyond the Gut

Effect of diet on *E. coli* populations in the faeces of cattle

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Background - A study on enterohaemorrhagic *Escherichia coli* (EHEC) contamination of beef carcasses at slaughter concluded that faecal and carcase levels of EHEC are positively correlated and that there was a role for control of EHEC in live cattle.[1] In this current study we examined the effect of dietary inclusion of molasses (simple sugars), grain (starch) and roughage (structural carbohydrate) on the shedding of *E. coli* in cattle faeces. Enterohaemorrhagic *E. coli* (EHEC) virulence factors [shiga toxin genes, stx_1 and stx_2; accessory virulence factors, intimin (*eaeA*) and plasmid-encoded enterohemolysin (*hlyA*)] in cattle faeces were also investigated.

Objective - To determine firstly, whether roughage and/or molasses based diets reduce the population of *E. coli* and EHEC virulence factors compared with grain based feedlot diets, and secondly, if commercial lairage management practices promote or diminish these responses.

Design - Thirty Brahman cross steers (mean LW ± sem) 329±3.2kg, were initially fed a high grain (80%) diet. The cattle were then allocated into 3 groups of 10 animals and fed *ad libitum* (a) 50% molasses, 28% Rhodes grass (*Chloris gayana)* hay, 15.0% whole cotton seed, 4.5% cotton seed meal, 1.5% urea and 1% mineral/vitamin premix (M+R); (b) 80% sorghum, 5% peanut shells, 5.5% cotton seed meal (G); and (c) Rhodes grass plus 20g urea/kg DM (R). A fresh faecal sample (100g) was collected from each animal on the baseline grain diet, on 2 separate days during the final week of each dietary treatment (PL), and just prior to slaughter at lairage (L). A multiplex PCR method was used to quantify the virulence genes stx_1 and stx_2, *eaeA* and *hlyA* in faeces.[2]

Outcomes - Prior to lairage, faecal E. coli numbers were two logs lower (8.1 vs 5.6 $\log_{10}$/g digesta) in the R and R+M diets compared with G fed animals and this difference increased to 2.5 logs at lairage. Analysis of the concentration of EHEC virulence factors in faeces indicated a marked decrease in hlyA, eaeA and stx_1 genes in the R and R+M diets and this trend remained at lairage. VFA patterns were similar in the roughage and molasses diets whereas increased E. coli numbers, decreased pH and enhanced butyrate and lactate fermentation pathways were associated with the grain diet. This would indicate a shift in the microbial population of the hindgut. Cluster analysis of predominant E. coli serotypes isolated from faeces from each of the three dietary treatment groups showed that the R and R+M groups were similar, but quite distinctive from populations isolated from grain fed animals.

	Diet			
	R	M+R	G	SEM
Faecal pH	7.4^a	7.3^a	6.3^b	0.04
Volatile fatty acids (mg/ml)				
Total	0.42^a	0.89^a	3.91^b	0.31
Acetate	0.32^a	0.69^b	1.96^c	0.14
Propionate	0.06^a	0.13^a	0.39^b	0.03
Butyrate	0.021^a	0.05^a	1.43^b	0.13
Acetate: Butyrate	15.3^a	13.8^a	1.4^b	1.8
Acetate: Propionate	5.3^a	5.3^a	$5..0^a$	0.2
Lactic acid (µg/ml)	21.3^a	34.7^a	122.7^b	9.0

Values in rows that do not have a common superscript letter are significantly different ($P < 0.05$).

Conclusions - This study indicates that the type of dietary carbohydrate has a significant effect on the *E. coli* community structure and therefore may determine the level of pathogenic serotypes. Future work is focussed on developing detection methods for quantification of putative EHEC populations in response to diet. These detection methods will be used to determine whether diets based on R or R+M combinations, which have low fermentable carbohydrate reaching the hindgut, have the potential to reduce EHEC populations.

1. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher G, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Nat Acad Sci 2000; 97: 2999-3003.
2. Paton AW, Paton JW. Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA,* rfb_{O111} and rfb_{O157}. J Clin Microbiol 1998; 36:598-602.

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