

## Fermentation of oats (*Avena sativa*) by the faecal microbiota using an *in vitro* colonic fermentor system

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Diets high in whole grain cereals may reduce the risk of cardiovascular disease and colorectal cancer<sup>1-2</sup>. The mixed link  $\beta$ -glucans found within oats (*Avena sativa*) could be partly responsible for this. The cholesterol lowering effect of  $\beta$ -glucans are well documented and currently has several endorsed health claims<sup>3-4</sup>. Little is known about how oat consumption influences the gut microbiota. The development of culture independent techniques using 16S rRNA-targeting arrays have demonstrated the importance of bacterial community composition, and bacterial metabolites, in health and disease. Prebiotics, non-digestible plant derived carbohydrates, act as fermentation substrates stimulating preferential growth and activity of potentially beneficial microbes. *In vitro* models may provide a useful insight into how these substrates might influence microbial composition *in vivo*<sup>5</sup>.

Here we investigated the effects of a pre-digested oat sample on microbial activity and composition using a continuous flow anaerobic fermentor model with both a luminal and mucosal environment. Firstly the oat sample underwent a pre-digestion with stomach and intestinal enzymes to reduce starch, free sugars, protein, and fat content. Fermentors were then inoculated with faecal samples obtained from four different volunteers and then run for 98 h on a growth medium before switching to oats. Fermentors were run for a total of 264 h including the initial 98 h growth phase, at pH 5.8 and 6.5 with half the mucosal environment replaced daily. An additional fermentor was run using the initial growth medium as a comparison. Samples were taken daily for short chain fatty acids (SCFA) and at 0, 98, 168, and 264 h for microbial composition analysis of both the luminal and mucosal environment.

An analysis within volunteers comparing oats pH 6.5, and 5.8 to growth pH 6.5 was performed using an ANOVA. Total SCFA analysis indicated the oats were more fermentable at pH 6.5 compared to pH 5.8 ( $p < 0.05$ ), and that acetate was the most abundant SCFA followed by propionate. Fermentation was significantly higher with the growth medium compared to TDS ( $p < 0.05$ ).

Fermentor Short Chain Fatty Acids at 264 h

	Concentrations mmol/l				Ratio of Ace : Pro : But		
	Acetate	Propionate	Butyrate	Total SCFA	Acetate	Propionate	Butyrate
Oats pH 5.8	5.85 <sup>a</sup>	0.42 <sup>a</sup>	0.03 <sup>a</sup>	6.02 <sup>a</sup>	93	7	0
Oats pH 6.5	11.61 <sup>b</sup>	2.53 <sup>b</sup>	1.11 <sup>a</sup>	15.06 <sup>b</sup>	76	17	7
Growth pH 5.8	30.71	24.25	10.06	56.26	49	36	15
Growth pH 6.5	29.80 <sup>c</sup>	19.50 <sup>c</sup>	4.81 <sup>a</sup>	54.25 <sup>c</sup>	55	36	9

Growth was a carbohydrate rich mixed medium

Different letters within columns denote significant difference ( $p < 0.05$ ).

Microbial analysis of the oat fermentors by fluorescence in situ hybridisation found a shift towards the *Bacteroides* genus in the luminal compared to the mucosal environment. Eubacterium *rectale*, Lactobacillus and Desulfovibrio group all had a preference for the mucosal environment ( $p < 0.05$ ). A trend was also noted indicating the Atopobium may have a preference for the growth medium compared to the oats ( $p = 0.08$ ). The total bacterial counts were greater for the control and oats at pH 6.5 compared to oats pH 5.8, indicative of the challenging growth conditions ( $p < 0.05$ ). Bias towards the *Bacteroides* groups in fermentor experiments has been reported previously<sup>6</sup>, incorporation of the mucosal environment into our model reduced this bias. Further refinement of the model however may be required to identify microbes that ferment oat fibre *in vitro*.

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