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Dietary supplementation with chitosan at high and low crude protein concentrations promotes Enterobacteriaceae in the caecum and colon and increases manure odour emissions from finisher boars☆

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ABSTRACT

The hypothesis of this study is that supplementation of a high crude protein (CP), wheat-based diet with chitosan may increase protein-fermenting bacteria in the large intestine at the expense of carbohydrate-fermenting bacteria, resulting in increased manure odour emissions. A 2×2 factorial experiment was conducted to investigate the effect of dietary chitosan inclusion (0 vs 20 g/kg) and CP concentration (200 vs 150 g/kg) on intestinal microflora, volatile fatty acid concentrations (VFA) and manure odour from finisher boars. The inclusion of chitosan decreased Lactobacilli and increased Enterobacteriaceae in the caecum ($P<0.05$) and colon ($P<0.001$) compared with pigs offered unsupplemented diets. Dietary chitosan decreased the molar proportion of butyric acid and increased valeric acid in the caecum ($P<0.05$) and colon ($P<0.001$) compared with unsupplemented diets. Dietary chitosan increased manure odour emissions ($P<0.05$) at 72 h post excretion. In conclusion, dietary chitosan decreased Lactobacilli and increased Enterobacteriaceae in the hind gut and subsequently increased manure odour emissions.

1. Introduction

Bacterial fermentation of undigested crude protein (CP) contributes to odour from intensive pig production, however lowering dietary CP has not been accompanied by consistent reductions in odour emissions (Lynch et al., 2008). Dietary fermentable carbohydrates have demonstrated reduced manure odour emissions (Garry et al., 2007), attributed to promoting a commensal gastrointestinal microbiota (Lynch et al., 2007). Chitosan, a derivative of chitin has been demonstrated to inhibit Lactobacilli *in vitro* (No et al., 2003). Therefore, in a luminal environment harbouring a suppressed population of Lactobacilli, proliferation of Enterobacteriaceae may emerge, increasing proteolysis and contributing to manure odour development. This positive control model may provide a basis by which the effect of Lactobacilli in moderating manure odour may be examined. The hypothesis of the current experiment is that dietary chitosan may increase Enterobacteriaceae, resulting in heightened manure odour emissions from finisher boars.

2. Materials and methods

2.1. Experimental diets

The experiment was designed as a 2×2 factorial comprising four dietary treatments: (1) 200 g/kg CP; (2) 200 g/kg CP + 20 g/kg chitosan (A&Z Food additives Co., LTD Hangzhou, Zhejiang); (3) 150 g/kg CP; (4) 150 g/kg CP + 20 g/kg chitosan. Diets were formulated to contain similar concentrations of net energy (10.3 MJ/kg) and digestible

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Table 1
Composition and analysis of experimental diets (as fed basis).

Dietary treatments	1	2	3	4
Crude protein (g/kg)	200		150	
Chitosan	No	Yes	No	Yes
Ingredients (g/kg)				
Chitosan	0	20	0	20
Starch	20	0	20	0
Wheat	660	660	805	805
Soyabean meal	265	265	112.5	112.5
Soya oil	30	30	30	30
LysineHCL	0	0	4.9	4.9
Methionine	0	0	0.5	0.5
Threonine	0	0	2.1	2.1
Dicalcium phosphate	7.5	7.5	7.5	7.5
Salt	5	5	5	5
Limestone	10	10	10	10
Minerals and vitamins	2.5	2.5	2.5	2.5
Analysed composition (g/kg)				
Dry matter	867.1	878.3	863.8	859.7
Crude protein	199.4	200.9	152.9	158.9
Lysine	9.7	9.7	9.7	9.7
Methionine and cysteine	6.3	6.3	5.4	5.4
Threonine	7.0	7.0	6.7	6.7
Tryptophan	2.5	2.5	1.7	1.7
Neutral detergent fibre	112.7	126.4	94.5	130.6
Ash	47.1	46.4	38.4	40.5

lysine (8.5 g/kg) (Sauvant et al., 2004) and offered in meal form. Dietary composition and analysis are presented in Table 1.

2.2. Animals and management — manure odour and ammonia emission study

Thirty-two finishing boars (Large White × Landrace) (60.3 kg s.d. 2.1) were blocked on the basis of live-weight and allocated a 14-day dietary adaptation period. Sixteen boars (four per dietary treatment) of a uniform weight were selected and transferred to individual metabolism crates. Manure collections and the ammonia emissions study were performed as described by O'Connell et al. (2005). Air samples were used to measure the odour threshold concentration of the manure and analysed as described by Lynch et al. (2008) Odour emissions are expressed in European odour units per cubic metre air (OU_E/m^3).

2.3. Laboratory analysis of samples

Proximate analysis of diets for dry matter and ash was carried out according to the AOAC (1995). The neutral detergent fibre fraction of diets was analysed using a Fibertec extraction unit (van Soest et al., 1991). The nitrogen content of diets was determined using a LECO FP 528 instrument according to the Dumas method (Etheridge et al., 1998). Digesta samples were recovered from the caecum and colon of each animal immediately post slaughter (n = 8). Selected microbial populations were isolated as described by O'Connell et al. (2005). Digesta pH from the caecum and proximal colon was determined using a Mettler Toledo MP 220 pH meter. Digesta VFA composition recovered from the caecum and the proximal colon was analysed as described by Porter and Murray (2001) using a modified method (Lynch et al., 2008). The dietary concentrations of lysine, threonine, tryptophan, methionine and cysteine were determined by high-performance liquid chromatography (Iwaki et al., 1987).

2.4. Statistical analysis

Experimental data were analysed as a 2 × 2 factorial using the GLM procedure of the SAS Institute (1985). The statistical model investigated the main effects of dietary CP concentration, chitosan inclusion and the associated two-way interactions. All data in the tables are presented as least-square means (LSM ± sem).

3. Results

There was no effect of dietary chitosan on nutrient digestibility or nitrogen balance compared with unsupplemented diets (data not shown).

Dietary chitosan decreased Lactobacilli in the caecum ($P<0.05$) and colon ($P<0.01$) and increased in Enterobacteriaceae the caecum ($P<0.01$) and colon ($P<0.001$) and caecal pH ($P<0.001$) compared with unsupplemented diets.

There was an interaction between dietary CP concentration and chitosan inclusion on the pH of the colon ($P<0.05$). The low CP diet containing chitosan increased colonic pH compared with the unsupplemented low CP diet. However, there was no effect of chitosan inclusion on colonic pH at high CP concentration (Table 2).

Table 2
Effect of dietary CP concentration and chitosan inclusion on intestinal microflora and pH (LSM ± sem).

	CP (g/kg)			Chitosan			Significance	
	200	150	sem	No	Yes	sem	CP	Chitosan
n	8	8		8	8			
Caecal bacterial populations (log 10 cfu/g digesta)								
Lactobacilli spp.	6.51	6.46	0.220	6.88	6.09	0.211	ns	*
Enterobacteria	3.91	3.90	0.280	3.24	4.56	0.278	ns	**
Caecal pH	5.56	5.69	0.041	5.42	5.83	0.041	*	***
Colonic bacterial populations (log 10 cfu/g digesta)								
Lactobacilli spp.	7.30	7.30	0.190	7.80	6.80	0.194	ns	**
Enterobacteria	4.67	5.05	0.280	3.88	5.84	0.279	ns	***
Colonic pH [a]	6.09	6.13	0.057	5.92	6.30	0.057	ns	***

Probability of significance; * = ($P<0.05$); ** = ($P<0.01$);*** = ($P<0.001$). ns = not significant.
[a] There was an interaction between dietary CP concentration and chitosan inclusion on the pH of the colon ($P<0.05$).