Feed size and texture influence propionic and butyric acid concentrations and *Escherichia coli* populations in the pig gastrointestinal tract

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Abstract

Natural approaches are now being considered to replace antimicrobials to reduce the risk of antimicrobial resistance development. This has put new emphasis on using diet to control bacterial infections in pigs, some of which having recently demonstrated a zoonotic disease potential. Moreover, dietary modifications can lead to a modulation of the bioregulation of volatile fatty acids (VFA). Our objective was to assess the effect of feed size and texture on intestinal VFA profiles and concentrations, *E. coli* populations, and on growth performance. Fattening pigs (n=840) received one of six different diets (mash feed 500, 750 and 1250 µm and pellet feed 500, 750 and 1250 µm). Weight gain of pigs was monitored for each diet formulation over the fattening period. At the slaughterhouse, caecal and colon contents from 165 pigs were sampled for enumeration of *E. coli* by quantitative PCR (qPCR) and for VFA quantification. Acetic, propionic, and butyric acids were quantified by capillary gas chromatography. The *yccT* gene was used to enumerate total *E. coli*. A decrease in feed conversion associated with pellet texture and/or 500 µm particle size was observed for each diet formulation (p<0,05). In addition, caecal (p=0,0271) and colon (p=0,0012) propionic acid concentrations were lower for pigs receiving pellet rather than mash feed. Similarly, caecal (p=0,0167) and colon (p=0,0008) butyric acid concentrations were also lower for pigs receiving pellet rather than mash feed. Moreover, caecal (p=0,0208) and colon (p=0,0006) butyric acid concentrations were higher for pigs receiving a feed with a 1250 µm rather than 500 µm particle size. For total *E. coli* enumeration, caecal (p=0,01) and colon (p=0,04) *yccT* gene copy numbers were higher for pigs receiving pellet rather than mash feed. Taken together, results showed that mash feed is associated with favourable intestinal changes (VFA levels) and with a reduction of *E.coli* in the pig.

Introduction

Certain *Escherichia coli* are associated with postweaning diarrhoea and oedema disease in pigs, others are important zoonotic pathogens in the food chain. Antibiotics are commonly used to control this pathogen at the farm level, but natural approaches, such as feed strategies, are now being considered to increase the gut health and reduce the use of antimicrobials as growth promoters, and then, reduce the risk of antimicrobial resistance development. This has put new emphasis on using diet to control bacterial infections in pigs. It is now shown that the porcine intestinal health and intestinal microbiota composition can be modified by feed strategies, specifically by feed texture and feed particles size changes of the pig's diet (1). Moreover, dietary modifications can lead to a modulation of the bioregulation of volatile fatty acids. Particularly, propionic and butyric acids are important metabolites because of their bactericidal potential (2, 3). The aim of this study was to better understand the effect of feed particle size and texture on intestinal VFA profiles and concentrations, *E. coli* populations, and on growth performance of fattening pigs.

Material and Methods

A total of 840 crossbred Yorkshire-Landrace fattening pigs received one of six different diets (mash feed 500, 750 or 1250 µm or pellet feed 500, 750 or 1250 µm). Pigs were assigned a diet randomly and were fattened for a total of 120 days. Weight gain of pigs was monitored for each diet formulation over the fattening period. At the slaughterhouse, ileal, caecal and colon contents from 165 pigs were sampled individually. Contents were collected using conical 15 ml plastic tubes. The tubes were filled and stored at -20°C for analysis of volatile fatty acids (VFA). VFA concentrations were measured with a Perkin-Elmer gas chromatograph model 8310 (Perkin-Elmer, Waltham, Mass.), equipped with a DB-FFAP high resolution column. Caecal contents were also sampled for multiplex PCR. A group of 12 virulence genes reported in the literature to be associated with different *E. coli* pathotypes, were selected to be used in our study (4). A series of 4 multiplex PCR procedures were performed according to a protocol of the Reference Laboratory for Escherichia coli (EcL – Faculty of Veterinary

Medicine from the Université de Montréal) available at http://www.apzec.ca/en/APZEC/Protocols/APZEC_PCR_en.aspx. Also, caecal and colon contents were sampled individually and stored at -80°C for bacterial DNA analysis. . Total DNA was extracted from caecal and colon contents of pigs by use of a physical-chemical method with phenol-chloroform essentially as previously described. Quantitative PCR was performed on a Eco Real-Time PCR System (Illumina, San Diego, CA) using the Eco Software (version 4.1). All standard curves were constructed using PCR products and each reaction was run in triplicate. Volatile fatty acids and qPCR data were analysed according to multiple linear regression of the Statistical Analysis System version 9.3 (SAS Institute Inc., Cary, NC).

TABLE 1. Effect of feed texture and feed particle size on volatile fatty acid concentrations (mmol/l)

	Feed texture			Feed particle size			
VFA	Mash	Pellet	*P* value	500 µm	750 µm	1250 µm	*P* value
Ileum							
Acetic	20,3±3,0	23,8±2,9	0,09	22,4±3,7	23,0±3,6	20,7±3,5	0,65
Propionic	2,9±0,9	3,4±0,8	0,44	3,7±1,1	3,0±1,0	2,8±1,0	0,47
Butyric	3,02±0,6	3,50±0,6	0,26	3,46±0,7	3,14±0,7	3,19±0,7	0,81
Caecum							
Acetic	85,9±4,6	82,6±4,5	0,31	85,1±4,1	85,8±5,5	81,9±5,4	0,56
Propionic	36,8±2,1	33,4±2,1	0,03	34,8±2,7	33,4±2,5	37,1±2,5	0,12
Butyric	16,3±1,2	14,3±1,2	0,02	14,3±1,5	14,9±1,4	16,7±1,4	0,05
Colon							
Acetic	83,4±4,0	83,4±3,9	0,99	84,5±5,0	85,9±4,8	79,7±4,7	0,16
Propionic	37,1±2,1	32,2±2,0	<0,01	33,6±2,6	33,7±2,5	36,6±2,4	0,16
Butyric	20,7±1,3	17,4±1,3	<0,01	17,5±1,7	18,1±1,6	21,6±1,6	<0,01

Results

Ileal, caecal and colon volatile fatty acid production is shown in Table 1. Caecal (p=0,03) and colon (p<0,01) propionic acid concentrations were higher for mash than for pellet fed animals. Similarly, caecal (p=0,02) and colon (p<0,01) butyric acid concentrations were also higher for mash than pellet fed animals. With respect to the feed particle size, caecal (p=0,05) and colon (p<0,01) butyric acid concentrations were higher for the 1250 µm diet than for that of 500 µm diet. Ileal acid production was similar for all feed textures and feed particle sizes.

Caecal *E. coli* virulence factors detection by multiplex PCR showed interesting results, especially concerning the F4 fimbriae (Table 2). Presence of the F4 fimbriae gene was detected only in pellet fed animals. Moreover, the genes for two virulence factors, F18 and Stx1, were not detected in any of the samples.

TABLE 2. Number of positive caecal samples (%) for *E. coli* virulence factor genes

Feed texture	N	Virulence factor									
		LT	STa	STb	F4	Stx2	EAE	CNF	P	Aero	Tsh
Mash	81	43(53)	69(85)	21(26)	0(0)	20(25)	17(21)	56(69)	30(37)	58(72)	75(93)
Pellet	84	42(50)	59(70)	23(27)	6(7)	16(19)	14(17)	58(69)	32(38)	63(75)	73(87)

For total *E. coli* enumeration by real-time PCR (Table 3), caecal (p=0,01) and colon (p<0,01) *yccT* gene copy numbers were higher for pellet than mash fed animals. On the other hand, the enumeration of the genes *faeG, estB* and *cnf1*, amplified for the quantification of virulent *E. coli* populations, showed no differences between the pellet and the mash fed animals.

In addition, a decrease in feed conversion associated with pellet texture and/or 500 µm particle size was observed for each diet formulation (p<0,05).

Discussion

In the present study, we demonstrated that administration of mash rather than pellet feed was related to higher intestinal propionic and butyric acid levels. Furthermore, a 1250 µm diet is also associated with higher intestinal butyric acid production. Mash feeding may possibly be associated with an increase of bacteria producing volatile fatty acids and contribute to gut health by preventing the proliferation of injurious bacteria (5). Total *E. coli* enumeration was lower in mash fed animals. This could result in minimizing the contamination of the carcass with E. coli at the slaughterhouse. However, there was no evidence that mash feed affected the virulent *E. coli* populations. Consistent effects of feed texture and feed particles size were not observed for *cnf1, faeG* and *estB* gene copies in caecal and colon contents. Surprisingly, a feed texture effect was observed on the F4 fimbriae (*faeG* gene) caecal prevalence by multiplex PCR. In addition, feed conversion rates associated with mash feed were higher to those associated with pellet feed. As already demonstrated, administration of

pellet feed results in improved feed conversion but not necessarily average daily gain for pigs of all ages (6).

TABLE 3. Effect of feed texture and feed particle size on *Escherichia coli* populations (log gene copies/g faeces)

		Feed texture			Feed particle size			
Gene	N*	Mash	Pellet	*P* value	500 µm	750 µm	1250 µm	*P* value
Caecum								
yccT	111	14,45±0,6	15,53±0,6	0,01	15,67±0,8	14,54±0,7	14,87±0,7	0,07
cnf1	87	10,54±1,0	11,58±0,8	0,11	10,95±1,0	11,13±1,2	11,10±1,1	0,97
faeG	1	10,11	-	-	-	10,11	-	-
estB	105	7,97±0,7	8,08±0,7	0,81	8,59±0,8	7,68±0,8	7,81±0,9	0,24
Colon								
yccT	131	12,66±0,5	13,64±0,5	<0,01	13,70±0,6	12,80±0,6	12,97±0,6	0,10
cnf1	118	8,50±0,7	8,61±0,7	0,83	8,61±0,9	8,68±0,9	8,37±0,9	0,88
faeG	18	8,59±1,8	8,39±1,9	0,87	7,79±2,3	8,01±2,3	9,66±2,3	0,41
estB	119	7,07±0,5	7,12±0,5	0,88	7,27±0,6	6,72±0,6	7,31±0,6	0,28

*Positive samples by real-time PCR

Conclusion

Mash feed diet is associated with higher propionic and butyric acid levels and a reduction of total *E. coli* numbers in the digestive tract of pigs. Moreover, economic disadvantages of mash feeding can be countered by optimizing strategies, such as the use of mash feed for curative purposes or in the maternity to reduce the piglets exposure to potential pathogens and during stressful periods associated with greater vulnerability of animals. Thus, such strategies provide interesting alternatives to antibiotic use.

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