Impact of mash feeding versus pellets on propionic/butyric acid levels and on total *Escherichia coli* load in the gastrointestinal tract of growing pigs[1]

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ABSTRACT: Feed characteristics may influence the bacterial community composition and metabolic activities in the pig gastrointestinal tract, known to be associated with positive effects on the gut. Use of mash feed is associated with reduced *Salmonella* excretion, but little is known of its effect on the *Escherichia coli* population or of the mechanism of action. Our objectives were to assess the effect of feed texture combined with feed particle size on VFA profiles and levels, total *E. coli* count, and the presence of genes encoding virulence factors of pathogenic *E. coli* strains in the digestive tract along with their impact on pig performance of fattening pigs. Pigs ($n = 840$) on a commercial farm received mash or pellet diets of different particle sizes during the fattening period. Caecal and colon contents from 164 pigs were sampled at the slaughterhouse for enumeration of *E. coli* by quantitative PCR (qPCR) and for VFA quantification by capillary gas chromatography. The *yccT* gene was used to enumerate total *E. coli*. Improved pig performances associated with pellet texture and a 500-μm size were observed. Caecal ($P = 0.02$) and colon ($P < 0.01$) propionic acid concentrations were lower for pigs receiving pellet rather than mash feed. Similarly, caecal ($P = 0.01$) and colon ($P < 0.001$) butyric acid concentrations were also lower for pigs receiving pellet rather than mash feed, as determined by capillary gas chromatography. Moreover, caecal ($P = 0.03$) and colon ($P < 0.001$) butyric acid concentrations were higher for pigs receiving a feed with a 1,250-μm particle size rather than a 500-μm particle size. On the other hand, total caecal and colon *E. coli* levels were higher for pigs receiving pellet feed than for those receiving mash feed. For total *E. coli* enumeration, caecal ($P < 0.01$) and colon ($P < 0.01$) *yccT* gene copies were higher for pigs receiving pellet rather than mash feed. No effect of particle size on fatty acid concentrations or on *E. coli* numbers was observed. Virulence gene quantification revealed no trend. Taken together, results showed that mash feed is associated with lower growth performance but with favorable intestinal changes linked to VFA levels and *E. coli* reduction in the intestine.

Key words: *Escherichia coli*, feed particle size, feed texture, pigs, pig performance, volatile fatty acid

INTRODUCTION

Foodborne diseases are still a major public health concern, as many etiological agents may be zoonotic and their control on farms can be quite challenging. Moreover, antimicrobials are commonly used to control bacterial infections at the farm level, generating public health concerns. Changes in feed characteristics, such as the use of nonpelleted rather than pelleted feed or different particle sizes, may enhance pig gastrointestinal tract health, reduce *Salmonella* spp. prevalence, and influence pig performance (Lo Fo Wong et al., 2004; O'Connor et al., 2008). However, this has not yet been clearly confirmed in field conditions, and the mechanism of action is still unknown. In addition, no data are available regarding the effect of feed characteristics on *Escherichia coli* populations. For instance, dietary modifications resulting in a modulation of the bioregulation of VFA can have a potential impact on the intestinal microflora (Knudsen et al., 2003). It has been recognized for some time that VFA, such as acetic, propionic, butyric, valeric, and lactic acids, have antibacterial activity. Propionic and butyric acids are particularly important metabolites because of their specific inhibition of enteric bacteria such as *Salmonella* spp. and because of their general antibacterial effects on *E. coli* in pigs (Hedemann et al., 2005; Stecher and Hardt, 2011). The aim of this study was to investigate the effect of feed texture (mash versus pellet) combined with particle size (500 μm vs. 750 μm vs. 1,250 μm) on VFA profiles and levels, total *E. coli*, and genes encoding virulence factors of pathogenic *E. coli* in the digestive tract. As a secondary objective, the performance of fattening pigs was also evaluated with respect to the different diets.

MATERIALS AND METHODS

Animals and Diets

This study was conducted with an integrated pig production company owning feed mills, farms, a slaughterhouse, and animal transportation trucks. A total of 840 crossbred Duroc-Yorkshire-Landrace fattening pigs from an all-in all-out commercial herd in Québec, Canada, were distributed in 84 pens (5 females and 5 males per pen). Each pen received 1 of 6 different diets (mash feed 500, 750, or 1,250 μm and pellet feed 500, 750, or 1,250 μm), resulting in 14 pens (140 pigs) allocated to each diet. Diets were assigned randomly for each pen at the beginning of the assay (55 d of age) and were maintained until the end of the fattening period (120 d later) in a commercial production unit. Each pig had 2.29 m^2 of space. Formulation of each of the 6 different diets was the same (Appendix). Only the feed texture and/or the

feed particle size were variable. Particle size of the diets was determined by using a Tyler Ro-Tap shaker machine with sieve stack (W.S. Tyler, Mentor, OH) \as recommended by the American Society of Agricultural and Biological Engineers (**ASABE**; Standard, 2003). Four diet formulations were given over the fattening period: the prestarter formulation was fed from d 1 to 21, the starter was fed from d 22 to 46, the grower was fed from d 47 to 88, and the finisher was fed from d 89 to the end (d 120) according to common commercial feeding practices. All pigs were weighed at each change of formulation, and the feed consumption data were collected. Feed consumption was estimated by the amount of feed used divided by the number of pigs per pen. All procedures were approved by the ethics committee on animal use of the Université de Montréal based on the guidance of the Canadian Council on Animal Care (**CCAC**).

Sample Collection

After a transport to the slaughterhouse early in the morning and a lairage of 2 h in cleaned pens, the pigs were slaughtered as the first batch of the day. Ileal, cecal, and colon contents from 164 pigs were sampled individually, with pigs being selected from each pen and followed until the end of the assay. More specifically, 2 pigs (1 male and 1 female) were selected per pen. However, some pigs could not be sampled because they had died previously at the farm or because they were condemned by an inspector at the slaughterhouse. Intestinal contents were collected using 2 conical 15-mL plastic tubes. For each sample, 1 tube was filled and stored at −20°C for analysis of VFA and the other tube was stored at −80°C for subsequent DNA extraction for quantitative PCR (**qPCR**) analyses of individual samples of cecal and colon contents. Following the period of fasting, the gastrointestinal tract of the pigs was almost empty, especially the ileum. It was decided to use the small amount of ileum content for the VFA quantification. In addition, cecum and colon are associated with an increase risk of contamination at the slaughterhouse during evisceration.

Volatile Fatty Acids Analysis

Samples of ileal, caecal, and colon contents from 164 pigs were thawed at 4°C overnight, and 4 g of each sample was weighed into a centrifuge tube containing 6 g of deionized water. Tubes were centrifuged at $38,724 \times g$ for 30 min. Then, 0.25 mL of sulfuric acid 0.5 M was added to 1.30 g of supernatant. The suspension was vortexed and centrifuged at $13,793 \times g$ for 15 min. The supernatants (0.5 mL) were separated and mixed with 0.5 mL of an internal standard (2-ethylbutyric). Resin Dowex 50WX8 from Sigma-Aldrich (0.2 g;

Table 1. Oligonucleotide primers used for multiplex PCR

Virulence factor	Gene	Accession no.	Orientation[1]	Primer sequence (5′–3′)	Size of PCR product, bp	Annealing temperature, °C	Reference
LT	*eltB*	J01646	F	TTACGGCGTTACTATCCTCTCTA	275	60	Furrer et al., 1990
			R	GGTCTCGGTCAGATATGTGATTC			
STa	*estA*	M58746	F	TCCCCTCTTTTAGTCAGTCAACTG	163	60	Ojeniyi et al., 1994
			R	GCACAGGCAGGATTACAACAAAGT			
STb	*estB*	M35586	F	GCAATAAGGTTGAGGTGAT	368	60	Lortie et al., 1991
			R	GCCTGCAGTGAGAAATGGAC			
F4	*faeG*	M29374	F	ATCGGTGGTAGTATCACTGC	601	60	Ojeniyi et al., 1994
			R	AACCTGCGACGTCAACAAGA			
Stx1	*stxA*	M19437	F	TTAGACTTCTCGACTGCAAAG	531	60	Woodward et al., 1992
			R	TGTTGTACGAAATCCCCTCTG			
Stx2	*Stx2A*	X07865	F	TTATATCTGCGCCGGGTCTG	327	60	Woodward et al., 1992
			R	AGACGAAGATGGTCAAAACG			
EAE	*eae*	U66102	F	CATTATGGAACGGCAGAGGT	791	60	Beaudry et al., 1996
			R	ATCTTCTGCGTACTGCGTTCA			
CNF1/2	*cnf*	U42629	F	TCGTTATAAAATCAAACAGTG	446	55	Ewers et al., 2007
			R	CTTTACAATATTGACATGCTG			
P	*papC*	M30806	F	TGATATCACGCAGTCAGTAGC	501	55	Ewers et al., 2007
			R	CCGGCCATATTCACATAAC			
Aerobactin	*iucD*	M18968	F	AAGTGTCGATTTTATTGGTGTA	778	60	Herrero et al., 1988
			R	CCATCCGATGTCAGTTTTCTG			
Tsh	*tsh*	L27423	F	GGTGGTGCACTGGAGTGG	640	55	Dozois et al., 2000
			R	AGTCCAGCGTGATAGTGG			
F18	*F18*	M61713	F	GTGAAAAGACTAGTGTTTATTTC	510	60	Ngeleka et al., 2003
			R	CTTGTAAGTAACCGCGTAAGC			

[1] F = forward; R = reverse.

Oakville, Ontario Canada) was added to each sample. Tubes were then gently vortexed, and the suspension was filtered using a BD Luer-Lok 3-mL syringe with a 25-mm Syringe Filter with 0.45 μm cellulose (VWR International, Mississauga, ON). All of the vials were kept at 4°C until they were analyzed with the chromatograph. The VFA (acetic, propionic, butyric, isobutyric, isovaleric, and valeric) concentrations were measured using a PerkinElmer gas chromatograph model 8,310 (PerkinElmer, Waltham, MA) equipped with a DB-FFAP high resolution column.

Multiplex PCR Analysis

Samples from cecal and colon contents were enriched in Luria-Bertani broth overnight at 37°C, and DNA templates were prepared from the resulting enrichments by heat lysis. Twelve virulence genes (Table 1) defining the *E. coli* pathotypes found in animals were selected for use in our study (Herrero et al., 1988; Furrer et al., 1990; Lortie et al., 1991; Woodward et al., 1992; Ojeniyi et al., 1994; Beaudry et al., 1996; Dozois et al., 2000; Ngeleka et al., 2003; Ewers et al., 2007). Four 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 with slight modifications (Maluta et al., 2014).

Quantitative PCR

For qPCR, total DNA was extracted from cecal and colon contents of pigs by the use of a physical-chemical method with phenol-chloroform, essentially as previously described with a few modifications (Juteau et al., 2004). Briefly, cells were disrupted in FastPrep Lysing Matrix tubes using the FastPrep-24 Instrument (MP Biomedicals, Solon, OH). The DNA was purified by phenol-chloroform-isoamyl alcohol extraction and precipitated with ethanol. DNA was quantified using a NanoDrop ND-1000 Spectrophotometer (ND1000 V3.1.2; NanoDrop products, Wilmington, DE). The qPCR was performed on an Eco Real-Time PCR system (Illumina, San Diego, CA) using the Eco Software (version 4.1). Each reaction was run in triplicate in a volume of 20 μL. The reaction mixture consisted of 4 μL of MBI EVOlution EvaGreen qPCR master mixes (Montreal Biotech Inc., Dorval, Canada), 1 μL (10 m*M*) of primers (Table 2), 9 μL of sterilized water, and 5 μL (10 ng/μL) of DNA of cecal or colon contents. Amplification involved 1 cycle at 95°C for 10 min

Table 2. Oligonucleotide primers used for quantitative PCR

Targeted bacterial species or virulence factor	Gene	Accession no.	Orientation[1]	Primer sequence (5'-3')	Size of PCR product, bp	Annealing temperature, °C	Reference
E. coli	*yccT*	EG13722[2]	F	GCATCGTGACCACCTTGA	59	56	Clifford et al., 2012
			R	CAGCGTGGTGGCAAAA			
CNF1	*cnf1*	U42629	F	TTAAGGGTTCTGGAAGCTTTGG	194	61	Our study
			R	CATCTGCACTGTTAACATTTGAGG			
STb	*estB*	M35586	F	CTTCTTGCATCTATGTTCGT	107	54	Our study
			R	ACTTTCCTTGGCTATTTGTC			
F4	*faeG*	M29374	F	AATGCATCTTATGCCGGTG	163	61	Stahl et al., 2011
			R	TCTTTGAATCTGTCCGAGAATATC			

[1] F = forward; R = reverse.

[2] EcoGene accession number.

for initial denaturation followed by 40 cycles of denaturation at 95°C for 15 s, primer annealing at optimal temperatures (Table 2) for 15 s (except for fimbriae F4 45 s), and extension at 72°C for 15 s. All standard curves were constructed using PCR products. To quantify total *E. coli* by qPCR, serial dilutions of DNA from 10^8 to 10^2 copies were prepared and tested in triplicate. Primer efficiency was 96.68% with an R^2 value of 0.989.

Statistical Analysis

Pig performance parameters (ADFI, ADG, and feed conversion ratio [**FCR**]) were calculated for each pen using the average pig BW, quantity of feed distributed, and the number of pigs. Multiple linear regression analyses were used for the analysis of each VFA, qPCR data, and pig performance, whereas multiple logistic regressions were used for PCR data (positive/negative) and gastric lesion (positive/negative) data. The unit of analysis was the pig, except for the performances for which it was the pen. For qPCR, data were log-transformed to scope normality. For gastric lesions, a first model was used to evaluate if the presence of ulcers vs. hyperkeratosis was associated with feed texture and particle size. In the absence of significant association, the 2 lesions were combined for modeling; otherwise, a separate model was performed for hyperkeratosis (positive/negative) and ulcers (positive/negative). The feed texture and feed particle size were included as categorical explanatory variables in all models. Their interaction was tested and was only kept in the model if statistically significant ($P < 0.05$). For VFA and qPCR data models, the pen was added as a random effect to account for potential clustering of pigs within each pen. For PCR data, estimates were also adjusted for clustering within pen using generalized estimating equations. This pen correlation effect was not considered for pig performance analyses, as the unit of analysis was the pen, or for the ulcer data, as the information on the pen of origin was not available. Residual plots were used to assess the fit of the models and to detect the presence of outliers. Least squares means or predicted probabilities were used to present the results. All statistical analyses were performed in SAS software version 9.3 (SAS Inst. Inc., Cary, NC).

RESULTS

Effect of Feed Texture and Particle Size on VFA Levels in the Intestinal Contents of Pigs

Feeding of mash to the pigs resulted in a significant increase in levels for many VFA in the cecum and colon contents compared to feeding of pellets (Table 3). Propionic and butyric acid levels were significantly greater in both the cecum and colon contents of pigs receiving the mash feed than they were in the cecum and colon contents of those receiving the pellet feed (Table 3). In addition, the valeric acid level was significantly higher in the colon contents but not in the cecum contents of pigs receiving the mash feed than it was in the colon contents of those receiving the pellet feed. With respect to the feed particle size, colon isobutyric and isovaleric acid levels were higher for the 500-μm and 750-μm diets than for the 1,250-μm diet. Moreover, a significant interaction between feed texture and particle size was observed in relation to colon butyric acid levels (data not shown). Indeed, colon butyric acid levels for the mash 1,250-μm diet were significantly higher than those for all of the other diets (24.7 ± 2.0 mmol/L versus $\leq 18.8 \pm 2.3$ mmol/L; $P < 0.05$). For all other models, the interaction between feed particle size and feed texture was not statistically significant (all P values were >0.05; data not shown). VFA levels in the ileum were similar for all feed textures and feed particle sizes tested.

Effect of Feed Texture and Particle Size on Prevalence of E. coli Virulence Genes in the Intestinal Contents of Pigs

The detection of the various tested genes did not differ according to particle size or feed texture (all *P*

Table 3. Least squares means VFA concentration (mmol/L) ± SEM according to feed texture and particle size. Propionic and butyric acid concentrations were higher in cecal and colon contents of mash fed animals

VFA and pH	No.	Feed texture			Feed particle size			
		Mash ($n = 81$)	Pellets ($n = 83$)	P-value	500 μm ($n = 51$)	750 μm ($n = 55$)	1,250 μm ($n = 58$)	P-value
Ileum								
Acetic	163	20.53 ± 1.16	22.80 ± 1.28	0.16	21.05 ± 1.44	23.08 ± 1.43	20.87 ± 1.33	0.47
Propionic	163	2.90 ± 0.24	2.90 ± 0.24	0.99	2.93 ± 0.31	3.01 ± 0.30	2.77 ± 0.28	0.84
Butyric	163	3.11 ± 0.24	3.33 ± 0.24	0.51	3.23 ± 0.30	3.18 ± 0.30	3.25 ± 0.28	0.99
Isobutyric	163	0.36 ± 0.03	0.37 ± 0.03	0.76	0.37 ± 0.04	0.38 ± 0.04	0.33 ± 0.03	0.61
Valeric	163	0.18 ± 0.02	0.21 ± 0.02	0.44	0.18 ± 0.03	0.18 ± 0.03	0.21 ± 0.03	0.65
Isovaleric	163	0.73 ± 0.06	0.73 ± 0.06	0.94	0.80 ± 0.08	0.77 ± 0.08	0.62 ± 0.07	0.20
pH	147	6.98 ± 0.04	6.99 ± 0.04	0.84	7.03 ± 0.05	7.04 ± 0.05	6.89 ± 0.05	0.07
Cecum								
Acetic	162	86.02 ± 2.01	82.74 ± 1.99	0.25	84.90 ± 2.54	86.38 ± 2.45	81.87 ± 2.35	0.40
Propionic	162	36.74 ± 0.99	33.42 ± 0.98	0.02	34.64 ± 1.25	33.53 ± 1.20	37.07 ± 1.16	0.10
Butyric	162	16.29 ± 0.54	14.28 ± 0.53	0.01	14.25[a] ± 0.68	14.91[ab] ± 0.65	16.70[b] ± 0.63	0.03
Isobutyric	162	2.21 ± 0.09	2.25 ± 0.09	0.76	2.36 ± 0.11	2.27 ± 0.11	2.07 ± 0.11	0.17
Valeric	162	2.57 ± 0.08	2.50 ± 0.08	0.57	2.69 ± 0.10	2.51 ± 0.10	2.40 ± 0.09	0.13
Isovaleric	162	2.75 ± 0.12	2.82 ± 0.12	0.72	2.95 ± 0.16	2.86 ± 0.15	2.54 ± 0.15	0.13
pH	145	6.40 ± 0.06	6.51 ± 0.06	0.21	6.55 ± 0.07	6.46 ± 0.07	6.35 ± 0.07	0.16
Colon								
Acetic	162	83.59 ± 2.04	83.38 ± 2.04	0.94	84.89 ± 2.47	85.87 ± 2.47	79.69 ± 2.41	0.17
Propionic	162	37.05 ± 1.04	32.29 ± 1.04	<0.01	33.69 ± 1.33	33.74 ± 1.26	36.58 ± 1.22	0.18
Butyric	162	20.73 ± 0.64	17.44 ± 0.64	<0.001	17.62[a] ± 0.83	18.08[a] ± 0.78	21.57[b] ± 0.75	<0.001
Isobutyric	162	3.03 ± 0.10	2.93 ± 0.10	0.48	3.04[a] ± 0.12	3.15[a] ± 0.12	2.74[b] ± 0.12	0.04
Valeric	162	3.02 ± 0.10	2.56 ± 0.10	<0.001	2.65 ± 0.13	2.75 ± 0.12	2.98 ± 0.11	0.13
Isovaleric	162	4.18 ± 0.13	3.95 ± 0.13	0.21	4.07[ab] ± 0.16	4.33[a] ± 0.16	3.78[b] ± 0.15	0.04
pH	146	6.17 ± 0.04	6.24 ± 0.03	0.16	6.23 ± 0.04	6.20 ± 0.04	6.18 ± 0.04	0.67

[a,b]Different superscript letters between subcategories of the feed particles size indicate significant differences ($P < 0.05$) while superscripts of the same letter indicate no significant differences ($P \geq 0.05$).

> 0.20), with the exception of *estA*, for which detection was higher in the mash than in the pellet feed ($P = 0.02$). The *faeG*, *F18*, and *stxA* were not tested due to the absence or very low number of positive samples (data not shown).

Quantitative PCR

For total *E. coli* enumeration by real-time PCR (Fig. 1), the number of *yccT* log gene copies per gram of cecal contents was higher for pellet-fed than for mash-fed animals ($P < 0.01$). Similarly, the number of *yccT* log gene copies per gram of colon contents was higher for pellet-fed than for mash-fed animals ($P < 0.01$). According to feed particle size, the number of *yccT* log gene copies per gram of cecal contents was higher for animals fed the 500-μm diet than for those fed the 750-μm diet ($P < 0.05$). 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. Furthermore, feed particle size had no effect on *faeG*, *estB*, and *cnf1* gene enumeration.

Pig Performance Parameters

Overall, pig performance parameters were significantly greater in pigs receiving pellet feed than in pigs receiving mash feed (Table 4), except in pigs receiving the starter feed formulation, where a similar ADFI was observed for the pellet and the mash diets ($P = 0.21$). For each feed formulation, the FCR of pigs receiving pellet feed were significantly lower ($P < 0.001$) than the FCR of pigs receiving mash feed. With respect to the feed particle size, significant differences were only observed when comparing the FCR and the ADFI of pigs receiving the 500-μm feed size with those of pigs receiving the 750- or 1,250-μm feed size. Cumulative results for particle size from all feed formulations demonstrated that the 500-μm feed particle size was associated with a lower FCR than that observed for the 750- and 1,250-μm feed particle sizes ($P < 0.001$). Also, the ADFI for the 500-μm feed particle size was significantly lower than that observed for the 1,250-μm feed particle size ($P < 0.01$) Moreover, cumulative results for the 4 different feed formulations showed significant interaction between feed texture and feed particle size with respect to the FCR (data not shown). Thus, the impact of feed texture on the FCR observed
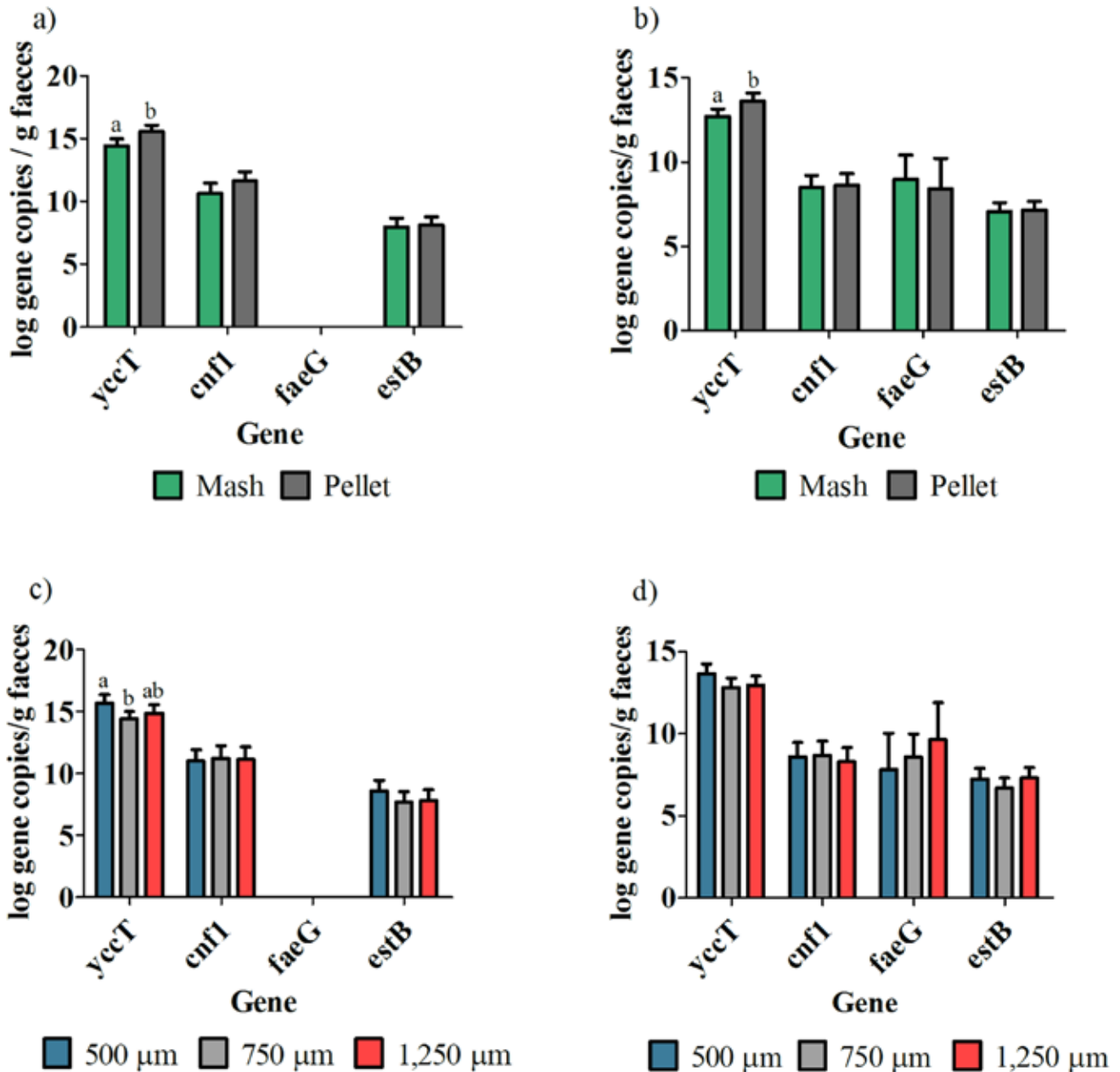
Figure 1. Quantification of specific *E. coli* genes in cecal and colon contents of animals according to feed texture and particle size diets. (a) Effect of feed texture on cecal *E. coli* populations. (b) Effect of feed texture on colon *E. coli* populations. (c) Effect of feed particles size on cecal *E. coli* populations. (d) Effect of feed particles size on colon *E. coli* populations. Multiple linear regression SAS 9.3 (SAS Inst. Inc., Cary, NC)—Different superscript letters between subcategories of the feed particles size or feed texture indicate significant differences ($P < 0.05$) while superscripts of the same letter indicate no significant differences ($p \geq 0.05$).

in pigs receiving the mash 500-μm diets was significantly lower than those observed in pigs receiving the mash 750- and mash 1,250-μm diets ($P = 0.04$).

Gastric Lesions

Gastric lesions were evaluated at slaughterhouse in 733 pigs. The presence of stomach hyperkeratosis was noted in 110 pigs (graded as moderate [$n = 28$] or severe [$n = 82$]), whereas 189 pigs had gastric ulcers graded as mild ($n = 126$), moderate ($n = 42$), or severe ($n = 21$). The particle size and texture were not as-

sociated with the presence of ulcers when compared to hyperkeratosis as the baseline; thus, the 2 lesions were combined for the final model. The presence of gastric lesions (hyperkeratosis or ulcers) was significantly associated with feed particle size and texture; a significant interaction was also observed between the 2 variables (all $P < 0.001$). Overall, the probability of observing gastric lesions was higher with a low particle size and in pellet feed; moreover, the effect for the mash feed was larger in pigs receiving 750-μm diets compared to other particle sizes. The predicted probabilities from the model are presented in Fig. 2.

Table 4. Least squares means ± SEM of ADG, ADFI, and feed conversion ratio (FCR) according to feed texture and particle size (n = 84 pens)

Feed formulation	Feed texture			Feed particle size			
	Mash (n = 42)	Pellets (n = 42)	P-value	500 μm (n = 28)	750 μm (n = 28)	1,250 μm (n = 28)	P-value
Prestarter							
ADG, kg	0.684 ± 0.007	0.711 ± 0.007	0.01	0.707 ± 0.009	0.693 ± 0.009	0.694 ± 0.009	0.49
ADFI, kg	1.344 ± 0.011	1.311 ± 0.011	0.04	1.340 ± 0.0134	1.323 ± 0.013	1.319 ± 0.013	0.51
FCR	1.968 ± 0.014	1.846 ± 0.014	<0.001	1.901 ± 0.018	1.911 ± 0.018	1.908 ± 0.018	0.91
Starter							
ADG, kg	0.880 ± 0.007	0.925 ± 0.007	<0.001	0.912 ± 0.008	0.896 ± 0.008	0.899 ± 0.008	0.38
ADFI, kg	2.061 ± 0.012	2.040 ± 0.012	0.21	2.036 ± 0.015	2.041 ± 0.015	2.045 ± 0.015	0.13
FCR	2.347 ± 0.014	2.207 ± 0.014	<0.001	2.238[a] ± 0.017	2.282[ab] ± 0.017	2.311[b] ± 0.017	0.01
Grower							
ADG, kg	1.021 ± 0.006	1.048 ± 0.006	<0.01	1.044 ± 0.008	1.030 ± 0.008	1.031 ± 0.008	0.35
ADFI, kg	2.875 ± 0.023	2.776 ± 0.023	<0.01	2.769[a] ± 0.028	2.839[ab] ± 0.028	2.867[b] ± 0.028	0.05
FCR	2.817 ± 0.020	2.650 ± 0.020	<0.001	2.654[a] ± 0.025	2.761[b] ± 0.025	2.786[b] ± 0.025	<0.001
Finisher							
ADG, kg	0.932 ± 0.011	0.968 ± 0.011	0.03	0.933 ± 0.014	0.951 ± 0.014	0.966 ± 0.014	0.27
ADFI, kg	3.104 ± 0.037	2.892 ± 0.037	<0.001	2.890[a] ± 0.046	3.007[ab] ± 0.046	3.098[b] ± 0.046	<0.01
FCR	3.337 ± 0.034	2.993 ± 0.034	<0.001	3.101[a] ± 0.042	3.176[ab] ± 0.042	3.218[b] ± 0.042	<0.01
Cumulative							
ADG, kg	0.903 ± 0.004	0.936 ± 0.004	<0.001	0.923 ± 0.005	0.915 ± 0.005	0.919 ± 0.005	0.57
ADFI, kg	2.431 ± 0.016	2.328 ± 0.016	<0.001	2.334[a] ± 0.019	2.385[ab] ± 0.019	2.420[b] ± 0.019	<0.01
FCR	2.648 ± 0.014	2.448 ± 0.014	<0.001	2.493[a] ± 0.017	2.564[b] ± 0.017	2.587[b] ± 0.017	<0.001

[a,b]Different superscript letters between subcategories of the feed particles size or feed texture indicate significant differences ($P < 0.05$) while superscripts of the same letter indicate no significant differences ($P \geq 0.05$).

DISCUSSION

In the present study, we demonstrate that administration of mash rather than pellet feed is strongly associated with higher intestinal propionic and butyric acid levels, specifically in the colon and cecum contents. The effect of mash feed was specific to propionic and butyric acids, and no significant effects were observed for acetic, isobutyric, valeric, and isovaleric acids. It is possible that mash feeding may promote an increase in bacteria producing specific VFA and, thus, contribute to gastrointestinal health by preventing the proliferation and/or virulence of harmful bacteria such as *Salmonella* spp. and pathogenic *E. coli* (Lo Fo Wong et al., 2004; Mikkelsen et al., 2007). LeBel et al. (2013) observed a higher prevalence of *Salmonella* in the colon of pigs fed with pellet feed in contrast to those fed with mash feed ($P = 0.026$). An increase in the concentration of VFA in association with mash structure and higher particle size diets has also already been observed (Canibe et al., 2005). In fact, Canibe et al. (2005) showed that feeding a coarse, nonpelleted diet to finishing pigs stimulated the growth of total anaerobes and lactic acid bacteria at the same time as enhancing lactic, acetic, propionic, and butyric acid concentrations in the stomach. However, these authors observed no significant effect of the coarse, nonpelleted diet on VFA concentrations in the cecum and the colon contents. The feeding of a higher particle size mash diet, which results in a slower gastric passage rate leading to an increased proliferation of certain microorganisms of the microbiota associated with a decrease in pH, may explain the changes in *E. coli* populations and the observed effects on *Salmonella* excretion (LeBel et al., 2013). LeBel et al. (2013) demonstrated that pigs fed with mash 1,250-μm feed had significantly more *Bifidobacterium* spp. in their feces during the fattening period than pigs receiving pellet feed. Interestingly, the bacteria of the *Bifidobacterium* spp. genus have often been associated with an increase in antibacterial compounds such as VFA (Mountzouris et al., 2006). Furthermore, results from our study showed that a 1,250-μm diet, mash or pellet, is also associated with higher intestinal butyric acid production. The effect of a greater feed particle size on increasing butyric acid concentrations in the cecum and the colon contents found in our study is in accordance with another study on the impact of feed on microbial ecology in pigs (Mikkelsen et al., 2004). Similarly, grinding (coarse vs. fine diets) also resulted in higher acetic ($P < 0.001$), propionic ($P < 0.05$), and butyric ($P < 0.10$) acid concentration in the stomach. These results suggest that change in feed presentation could be associated with microbiota modification (different composition and/or metabolic activities) in the gastrointestinal tract of pigs. No significant effect of a 1,250-μm diet was observed for the other VFA examined in our study. These marked effects of mash feed and greater feed particle size on butyric acid levels suggest promising strategies to reduce *Salmonella*
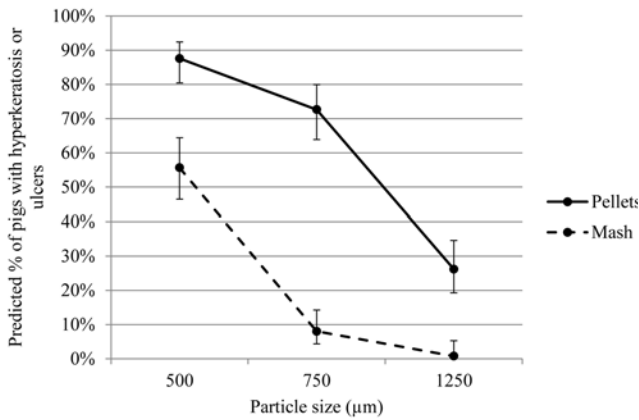
Figure 2. Predicted probabilities of detecting hyperkeratosis or ulcer lesions in pigs at the slaughterhouse according to feed texture and feed particle size. Multiple logistic regression SAS 9.3 (SAS Inst. Inc., Cary, NC)—All predicted probabilities were statistically different ($P < 0.01$) from each other.

spp. colonization, as these have also been reported in a study where coated butyric acid used as a feed supplement decreased the levels of *Salmonella* Typhimurium excretion and intestinal colonization of piglets (Boyen et al., 2008). *Salmonella* Typhimurium virulence gene expression, as demonstrated by colonization and invasion, can also be decreased and inhibited in vitro in response to higher butyrate concentrations. A study showed that pigs fed a nonpelleted diet are less susceptible to *Salmonella enterica* serovar Typhimurium DT12 colonization than pigs fed a pelleted diet (Hedemann et al., 2005). Gantois et al. (2006) showed that the effects of butyric acid on *Salmonella* spp. are caused by the specific downregulation of *Salmonella* pathogenicity island 1 (**SPI1**) gene expression. Reduction of the pH obviously inhibited the dissociation of the VFA, resulting in an increase in the quantity of nondissociated VFA molecules, which seemed to be responsible for bactericidal activity. In the pig colon contents, the pH may vary between 5.6 and 7.2, depending on the metabolic status, and may also be influenced by feeding. Antibacterial activity is observed only between pH 5.6 and 6.6; above pH 6.6, the proportion of nondissociated VFA molecules is so low that the antibacterial effect ceases. This may be an explanation for our results in the ileum samples showing no effect of the feed diets on the concentrations of VFA and indicating that the pH in this section of the gastrointestinal tract was around 7, suggesting an impact on VFA molecules. For the colon and cecum contents, no statistically significant effects of the feed texture or the feed particle size on the pH were observed in our study. However, the pH was always between 5.6 and 6.6. Prohászka (1980, 1986) demonstrated that, when compared at identical pH values, VFA from the rabbit, pig, and human intestinal tract exerted an antibacterial effect of similar intensity on all members of the *Enterobacteriaceae* family. The antibacterial defense mechanism functioning in the colon is sensitive to all

nutritional factors, which alter the pH of the colon. This underlines the importance of VFA-producing anaerobic bacterial flora, mainly consisting of species of the genus *Bacteroides*, which is considered to play an important role in the maintenance of gastrointestinal health through the production of butyric acid and the consumption of lactic acid (Guilloteau et al., 2010). It is tempting to speculate that modification of gut flora by modifying feed texture to produce more butyric acid could reduce invasive infections by enteric bacteria such as *E. coli* in growing pigs. Additionally, this study reports an effect of nonpelleted feed on propionic acid levels in different phases of growing pigs. Interestingly, it has recently been shown that propionic acid directly affects *Salmonella* transcriptional regulators, reducing the bacterial penetration of cultured epithelial cells (Hung et al., 2013). Consequently, enteric bacteria like *Salmonella* spp. and certain *Escherichia coli* virotypes may be killed or reduced in number in the gastrointestinal tract by this diet change. However, it is important to evaluate the impact of this alternative strategy on potentially pathogenic subpopulations of *E. coli* to avoid selection pressure on bacteria potentially linked to animal health problems or human disease.

This study is the first to report the effect of feed texture combined with the effect of feed particle size on *E. coli* prevalence in pigs. Total *E. coli* bacteria were quantified using qPCR because of the ease and rapidity of qPCR compared with traditional culture and because qPCR does not rely on the ability of bacteria to grow. Moreover, qPCR offers the option of storing samples until their analysis, which is an important advantage in field conditions. The results showed that total *E. coli* load is markedly lowered in both the caecal and colon contents of mash-fed animals. However, quantification of virulence genes revealed that there is no evidence that mash feed affected the proliferation of pathogenic *E. coli* populations, as consistent effects of feed texture and feed particles size were not observed for *faeG, estB*, and *cnf1* gene copies in caecal and colon contents. This raises the question of the biological significance of a greater representation of the subset of *E. coli*-carrying virulence genes in the intestinal contents of mash-fed pigs. Interestingly, another study reported an influence of nonstarch polysaccharides in the diet on swine-pathogenic *E. coli* by quantifying similar virulence factors (Metzler-Zebeli et al., 2010). This shows that diet may have an impact on *E. coli* virulence gene levels. More importantly, virulence genes such as *faeG* and *estB* are found on plasmids, which can be horizontally transferred (Fernández-Alarcón et al., 2011). Thus, the impact of feed modifications may be different for the quantification of virulence genes carried on plasmids than for the quantification of bacterial population count. This suggests that by reducing the total load of *E. coli*, the risk of spreading virulence genes in the *E. coli* popula-

tion and the potential risk of dissemination of pathogenic and/or zoonotic bacteria is decreased. Nevertheless, the present study showed a feed texture effect on the F4 fimbriae gene cecal prevalence as demonstrated by enrichment PCR. The fimbriae F4 was only detectable in the cecum contents of pigs fed a pelleted diet and not in those of pigs fed a mash diet. These fimbriae are an important virulence factor, as they allow the bacteria to bind to specific receptors on intestinal epithelial cells, resulting in colonization and subsequently in the secretion of enterotoxins such as STa, STb, and LT leading to diarrhea in piglets (Melkebeek et al., 2013). As previously mentioned, enterotoxigenic *E. coli* (**ETEC**) strains causing diarrhea are more often detected in neonatal and newly weaned pigs (Amezcua et al., 2002; Martins et al., 2010). However, reducing the prevalence and the persistence of ETEC in growing pig herds may contribute to protecting pigs from contamination between production cycles and to reducing the risk of cross-contamination of piglets in the production network. Nevertheless, it would be interesting to compare the effect of the feed texture on animals experimentally infected with ETEC to better understand the mechanism involved to increase animal health and to target strategic production periods in pigs for use of this diet without performance loss.

Consequently, in addition to the pig's gastrointestinal tract health status, this study considered pig performance and economic consequences linked with feed texture and particle size. Our study showed that mash feed generated reduced pig performance to a greater extent than pellet feed and, therefore, had an impact on the profitability of the farms. However, there have been few reports on the study of pigs in intensive conditions and on the impact of particle size on pig performance as in our study. FCR associated with mash feed diets were higher than those associated with pellet feed diets. This supports data from previous studies (Wondra et al., 1995a, 1995b; Mikkelsen et al., 2004), although feed texture did not appear to affect the daily feed intake in pigs receiving the starter feed formulation. Nevertheless, mash feeding could be used at strategic time points at the beginning of the growing phase to reduce the excretion of potential pathogens in pigs and, consequently, to reduce environmental contamination and exposure of pigs and humans, without generating significant effects on the FCR. Further, mash 500-μm diets showed lower FCR than the 2 other mash diets. Consequently, the mash 500-μm diet would represent the more economically reliable mash diet with considerable gastrointestinal tract health benefits. Interestingly, for the larger particle size mash diet, no reduction of voluntary intake by the pigs compromised the energy intake during the growing stage, as was observed by Anguita et al. (2007). Gregory et al. (1990) demonstrated that the retention time of food in the digestive tract of the pig simulta-

neously increases with the size of the feed particle. Pigs would feel satiated and satisfied for a longer period, resulting in a drop in voluntary intake of the food. However, these analyses are not consistent with the results of our study. Indeed, for the grower diet formulation, pigs fed the 1,250-μm particle size diet have average daily consumption indices significantly higher than those of pigs fed the 500-μm diet. Pigs fed the 1,250-μm diet therefore ingested greater quantities, which contributes to reduced growth performance (ADG and FCR).

In conclusion, this study is the first to demonstrate that mash feed diets are associated with higher propionic and butyric acid levels and with a decrease of total *E. coli* load in both the cecum and the colon contents of growing pigs raised in field-intensive conditions. This approach could participate in reducing *E. coli* intestinal load and consequently have a beneficial impact on animal health and/or human disease. Contribution to an increase of the microbiological quality and shelf life of the meat product is still to be investigated. Optimistically, economic disadvantages of mash feeding can be countered by optimizing strategies, such as the use of mash feed for curative purposes or in maternity to reduce piglet exposure to potential pathogens and during stressful periods associated with greater vulnerability of animals. Hence, such strategies provide interesting alternatives to increase global health of pigs and consequently help to improve use of antimicrobial agents at the farm level.

APPENDIX

Diet formulation

Item	Prestarter	Starter	Grower	Finisher
Ingredient, %				
Corn	47.18	47.96	53.53 or 54.06	52.70
Soybean meal 47%	21.00	16.60	11.40	13.00
Corn DDGS[1]	15.00	20.00	20.00	20.00
Wheat	10.00	10.00	10.00	10.00
Animal fat	2.40	2.20	1.60	1.60
Limestone	1.50	1.60	1.60	1.30
Lysine sulfate 65	0.90	0.60	0.60	0.60
Phosphate dical-21%	0.50	0.25	–	–
Salt	0.45	0.45	0.45	0.45
Vit/min premix	0.15	0.15	0.15	0.13
Threonine	0.13	–	–	0.06
Methionine hydroxy analog 88%	0.10	–	–	0.00
Phytase 1000 FTU	0.05	0.05	0.05	0.05
Copper chloride 58%	0.02	0.02	0.02	–
Drug or additive, mg/kg				
Chlortetracycline	1,210	–	1,210 or 0	–
Salinomycin	–	60	0 or 25	–
Ractopamine	–	–	–	10

[1]DDGS = distillers dried grains with solubles.

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