RESEARCH REPORT
PORK SAFETY

Title: Corn Particle Size and Pelleting Influence on Fecal Shedding and Enteric Colonization of *Salmonella enterica* serovar Typhimurium - **NPB #02-046**

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Abstract: Ninety-six pigs (initially 13.8 lb.) were used in a 28-d trial to determine the interactive effects between pelleting and particle size on *Salmonella* serovar Typhimurium shedding and colonization in a young growing pig model. The experiment was a 2 x 2 factorial arrangement consisting of meal or pelleted diets with fine or coarse ground corn. Pigs were fed the diets 1 wk pre-salmonella inoculation and allotted based on weight to one of four dietary treatments. For the main effect of particle size, pigs fed finer ground corn had significantly improved feed efficiency ($P < 0.01$) than pigs fed coarser ground corn for the 28 d trial. Pigs fed meal diets had greater ADG, ADFI, and greater F/G ($P < 0.05$) than the pigs fed pelleted diets. Fecal shedding of salmonella was low and variable with no significant differences between main effects ($P > 0.26$) or in treatments ($P > 0.82$). There was no difference in salmonella infection scores of mesenteric lymph nodes obtained on d 28 between treatments or main effects. Finer grinding and meal diets generally improved growth, feed intake, and feed efficiency compared to pigs fed coarser ground or pelleted feeds. However, particle size or diet form did not alter the fecal shedding or mesenteric lymph node infection rates of salmonella in this study.

Introduction: Each year, an estimated 1.4 million human salmonella infections occur in the United States, causing an estimated 80,000 to 160,000 persons to seek medical attention, resulting in 16,000 hospitalizations and nearly 600 deaths.[1] Foods of animal origin are the dominant source of human salmonellosis and little person-to-person transmission occurs.[2] Since most contamination of meat products occurs because of fecal contamination, reduction of salmonella shedding and colonization in gastro-intestinal tracts of animals is critically important for reducing human infections.

Feed has long been implicated as a possible source of salmonella contamination.[3] Therefore, heat treatment such as pelleting has been advocated for reducing feed related Salmonella infection.[4] Reduction in salmonella infection has been associated with feeding pelleted feed in poultry, even though salmonella was not detected in raw ingredients. However, survey information in swine seems to indicate that pelleted feeds are a significant risk factor associated with higher salmonella seroprevalence.[5,6] It is not know if these results are due to delivery of contaminated feed or an effect of the pelleted diet on factors that promote salmonella growth in the gastrointestinal tract.

Feed processing techniques such as pelleting and grinding have been shown to influence the fluidity of stomach contents and the pH of colonic contents.[7,8,9] The ph of the intestinal microenvironment has been shown to influence growth of salmonella in the intestine.[10,11] **Currently, feeding coarse ground grains, higher fiber containing diets, and mash diets are being advocated in European salmonella reduction programs.** Recently, researchers in Denmark have evaluated some of these dietary factors in wheat and barley based diets.[12,13,14,15] However, their results are unequivocal and difficult to interpret since these experiments relied on field studies in naturally infected herds and the degree of challenge doses or other confounding factors is unknown. Nevertheless, as expected these dietary interventions were shown to significantly impact productivity in a negative manner. Furthermore, it is unknown how these results translate to corn based diets.

In contrast, feeding diets containing highly digestible ingredients (rice and corn) to pigs can reduce colonization by the highly pathogenic spirochete *B. hyodysenteriae*, and partially protects against clinical signs of swine dysentery.[16,17] Feeding higher fiber containing diets appears to be associated with increased clinical expression of swine dysentery. It is known that incompletely digested resistant starch, non-starch polysaccharides and oligosaccharides (which are higher in feedstuffs that contain dietary fiber) in the foregut reach the hindgut where they undergo breakdown by microbial fermentation releasing volatile fatty acids. [16,17] This factor is thought to be the key reason for the highly digestible diets causing a protective effect with *B. hyodysenteriae*. Feed processing techniques such as pelleting and particle size reduction lead to more complete digestion of starches and fewer starches available for microbial fermentation in the hindgut. Therefore, biologic evidence is not clear as to how feed-processing techniques such as pelleting and particle size may impact salmonella shedding and colonization.

Objectives: Our objective was to study the interactive effects between pelleting and corn particle size on *Salmonella enterica* serovar Typhimurium shedding and colonization in a young growing pig model. In our experience cereal grain particle size and pelleting are confounded. In order to improve pellet mill efficiency and promote pellet quality, cereal grain particle size is generally reduced to an average of < 600 microns in pelleted diets. However, when feeding meal diets, particle size is generally limited to a lower minimum of 600 microns with a target of 600 to 800 microns. Feed handling becomes difficult with finely ground feed. Examples include feeders plugging and bins bridging, leading to interruptions in feed intake, facilitating ulcer development. **Therefore, our specific aim was to determine if meal or pellet and fine or coarse ground feed have an effect on salmonella shedding or colonization.**

Materials & Methods: Ninety-six pigs (initially 13.8 lbs.) were blocked by initial weight and allotted to one of four dietary treatments. Two pigs were assigned to a pen with a total of 12 replicates per treatment. The four dietary treatments were arranged in a 2 x 2 factorial, with main effects being fine or coarse ground corn and either a meal or pelleted form. All diets were identical in formulation (Table 1) with the only difference being the grain particle size and feed form. To insure feed was not contaminated within the feed mill, swab samples were taken and cultured for the presence of salmonella within the mixer, pellet, cooler, as well as the feed ingredients and complete feed samples from each of the diets. All pigs were housed in two environmentally controlled rooms. One feeder and one water-nipple were in each pen so as to allow ad libitum access to feed and water. Prior to inoculation, fecal samples were obtained to insure that all pigs were not shedding salmonella. Pigs were acclimated to the test diets one week pre-inoculation. Pigs were inoculated on d 7 with 1.9×10^7 CFU of *Salmonella enterica* serovar Typhimurium.

Rectal temperatures and feed intakes were determined daily for the first week post inoculation. Pigs were weighed and average daily feed intake (ADFI), average daily gain (ADG), and feed/gain (F/G) were determined on d 0, 7, 14, 21, and 28. Fecal samples were collected on days 14, 21, and 28 days to be cultured for the presence of salmonella. A semi-quantitative method for evaluating salmonella shedding was developed for the experiment. Salmonella growth was classified into one of four categories that included confluent growth over the whole plate (Score 3), any number of wells with growth (Score 2), only growth that was obtained on enrichment (Score 1), or no growth detected (Score 0). On d 28 of the study, in addition to the fecal sample, an ileo-colic lymph node was collected and cultured for the presence of salmonella. All data was analyzed as a 2 x 2 factorial in a randomized complete block design replicating over time using the Mixed model procedure of SAS. All means presented are least-squares means.

Table 1. Diet Composition (As-Fed)[ab]

Ingredient	% of Diet
Corn	51.74
Soybean meal, 46.5% CP	27.94
Spray dried whey	10.00
Select menhaden fish meal	4.50
Soy oil	3.00
Monocalcium phosphate, 21% P	1.20
Limestone	0.68
Salt	0.35
Vitamin premix	0.25
Trace mineral premix	0.15
Lysine HCl	0.15
DL-Methionine	0.05
Total	100.00%
Calculated Analysis	
Lysine, %	1.39%
ME, kcal/lb	1553
Calcium, %	0.90
Phosporous, %	0.80

[a] Diet fed for d 0 to 28 post-weaning

[b] Diets did not contain antimicrobials or growth promoting levels of zinc oxide or copper sulfate.

Results: A particle size x diet form x week interaction was observed for ADG (P< 0. 01), F/G (P< 0. 01), and a tendency in ADFI (P< 0. 08; Table 2). In the first week of the trial, the pigs fed coarse pelleted diets had lower ADG (P< 0. 01) and worse F/G (P< 0. 01) than all other treatments. While the growth and feed efficiency of this treatment only tended to be the lowest in the other weeks after challenge. The magnitude of the difference in of this treatment in the week prior to challenge was responsible for the three way interaction. Also, there were no significant interactions between week of the study and the main effects of corn particle size or diet form. Therefore, the main effects for pig growth performance corn particle size and diet form were further evaluated (Figure 1 and 2). For the overall d 0 to 28 period pigs fed meal diets grew faster (P < 0.05) compared to those fed pelleted diets (Figure 1). Main effect of corn particle size indicated pigs fed fine ground corn having improved feed efficiency (P< 0.01) compared to pigs fed the coarse ground corn (1.57 vs. 1.85, respectively; Figures 2). Meal fed pigs had better (P < 0.05) F/G than those fed pelleted diets.

Table 2. Interactive Effects of Particle Size and Dietary Form on Growth Performance of *Salmonella* Challenged Weanling Pigs[ab]

	Meal		Pelleted		SE
Item, Micron	500	1000	500	1000	
D 0 to 7					
ADG, lb	0.56[c]	0.55[c]	0.51[c]	0.28[d]	0.038
ADFI, lb	0.78[c]	0.89[d]	0.73[c]	0.76[c]	0.049
F/G	1.44[c]	1.63[c]	1.44[c]	2.89[d]	0.107
D 7 to 14					
ADG, lb	0.98[c]	0.99[c]	0.77[d]	0.70[d]	0.065
ADFI, lb	1.37[cd]	1.56[c]	1.29[c]	1.22[c]	0.079
F/G	1.41[c]	1.61[cd]	1.72[d]	1.85[de]	0.077
D 14 to 21					
ADG, lb	1.08[c]	1.06[de]	0.93[d]	0.98[e]	0.054
ADFI, lb	1.63	1.73	1.54	1.56	0.091
F/G	1.51	1.64	1.66	1.60	0.057
D 21 to 28					
ADG, lb	1.17[c]	1.10[ce]	0.92[d]	0.99[e]	0.054
ADFI, lb	1.86[c]	1.97[c]	1.52[d]	1.77[cd]	0.102
F/G	1.59[c]	1.81[d]	1.78[d]	1.79[d]	0.068
D 0 to 28					
ADG, lb	0.95[c]	0.93[c]	0.78[d]	0.74[d]	0.040
ADFI, lb	1.41[cd]	1.54[c]	1.27[d]	1.33[d]	0.067
F/G	1.49[c]	1.67[d]	1.65[d]	2.03[e]	0.046

[a]Ninety-six weanling pigs (initially 13.8 lbs) were used with two pigs per pen and 12 replications (pens) per treatment and inoculated on d 7 with 1.9 x 10^7 CFU of *Salmonella enterica* serotype Typhimurium.

[b] A Particle Size*Diet Form*Week interaction was observed for ADG (P< 0.01), F/G (P< 0.01), and a tendency for ADFI (P< 0.08).

[cde] Treatment means in the same row with different superscripts diff. (P<0.05).

Fecal samples evaluated for shedding of salmonella were collected on d 14, 21, and 28. Since there were no differences in shedding scores by treatment across week or interactions between corn particle size and diet form (P> 0.79) main effects are presented in Figure 3. There were no differences (P> 0.23) in fecal shedding scores due to grain particle size or diet form. In general, shedding was low and variable. An interaction between grain particle size and diet form was not observed for lymph node infection rate (P>0.82). Lymph nodes were collected on d 28 for presence of salmonella organisms and no significant differences were found for main effects of particle size (P> 0.50) and diet form (P> 0.26; Figure 4).

Effects of Particle Size and Dietary Form on Growth Performance of *Salmonella* Challenged Weanling Pigs[ab]

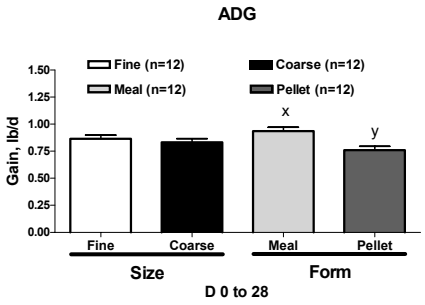

Figure 1.

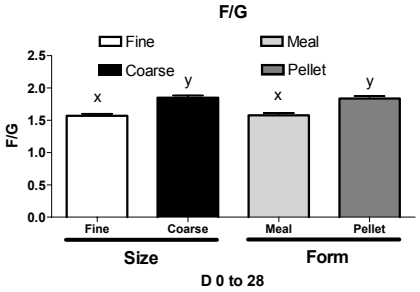

Figure 2.

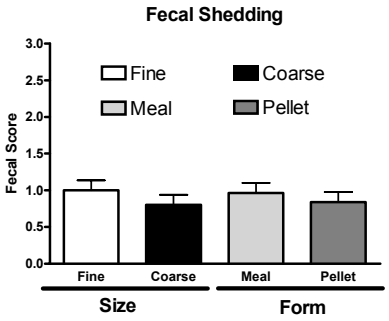

Figure 3.

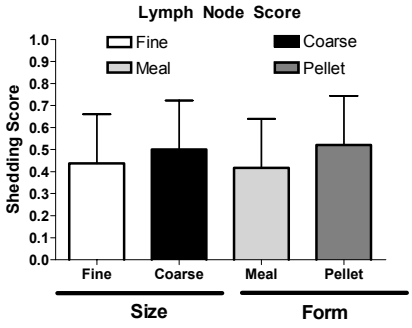

Figure 4.

[a]Ninety-six weanling pigs (initially 13.8 lbs) were used with two pigs per pen and 12 replications (pens) per treatment and inoculated ond d 7 with 1.9 x 10^7 CFU of *Salmonella enterica* serotype Typhimurium.

[xy] Main effects with different superscipts differ (P<0.05).

Discussion: Finer grinding of the corn improved feed conversion compared to the coarse grinded corn diets. This response to the decreased particle size was as expected since the smaller particles allow for a greater particle surface area to aid digestion of starches. Meal fed pigs showed improved ADG, ADFI, and feed conversion over the pigs fed pelleted diets. While this result is contradictory to many other studies, we believe this was the result of a feeder by pelleted diet interaction occurred which may explain this phenomenon rather than a physical effect of the pelleting process. This was especially evident feeding the coarse ground pelleted diet in the first week of the

study. The coarse pelleted diets had a large amount of fines that led to a significant amount of feed wastage. In subsequent weeks pigs fed the pelleted diets had a significant amount of fines in their feed pan. These fines were collected and weighed back against the amount of feed consumed. However, some of the fines were pushed out of the feeder by the pigs in order to consume more pellets. This loss of fines may explain for the discrepancy of F/G and we believe the poorer F/G was due to feed wastage of the pelleted diets.

This study seems to indicate that the increased amount of salmonella shedding associated with pelleted feeds may be due to other factors than an effect of the feed processing methods on the gastro-intestinal environment of the pig. For example, the humid environment of the pellet cooler and holding bins may increase the risk of post pelleting contamination. In our study we extensively sampled the feed processing equipment prior to the manufacture to ensure that the feed was not contaminated during the manufacturing process. Additionally, we evaluated the individual ingredients and the complete feed after manufacture to reduce the risk of introducing a source of salmonella other than the challenge. Also, some of the studies evaluating feed processing as a risk factor may have confounded the processing method with the source of ingredients. In these studies, meal based diets were more likely to be manufactured on-farm while pelleted diets were more likely to be obtained from a large centralized commercial feed mill. These large feed mills may have a higher probability of obtaining ingredients from a larger number of sources and using alternative products that are known to have a higher risk of salmonella contamination. Another factor may be the type of cereal grain. Many of the studies associating the increased risk have been with wheat or barley based diets. Differences in carbohydrate composition of the diet have been shown to influence the composition of the intestinal micro flora. Wheat and barley have different carbohydrate composition, especially in regard to non-starch poly saccharides that may influence the rate of salmonella shedding.

Lay Interpretation: Grinding corn to a finer particle size has been shown to have beneficial effects on growth, feed efficiency, and digestibility. The trial demonstrated improvements in growth and efficiency. However, in contrast to previous research, pelleting did not result in an improvement in growth and feed efficiency. Using this model, we were unable to detect influences of feed processing on fecal shedding and colonization of mesenteric lymph nodes with salmonella. Therefore, it appears that the increased risk of finer grinding and pelleting of feeds associated with salmonella shedding reported in other studies may be due to factors other than those confined to the intestinal tract environment.

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