The influence of grinding intensity and compaction of diets on the microbial community in the gastrointestinal tract of young pigs

S. J. Sander,* 2 J. Bullermann,* M. Arlinghaus,* J. Verspohl,† and J. Kamphues*

*Institute of Animal Nutrition, †Institute of Microbiology, University of Veterinary Medicine Hannover, Foundation, 30173 Hannover, Germany

ABSTRACT: Sixty weaned piglets (33 d, 7.96 ± 1.09 kg BW) were divided into 4 groups with 15 pigs each and fed identical diets in which meal was coarsely ground (CM), coarsely ground and pelleted (CP), finely ground and pelleted (FP), or coarsely ground and extruded (CE) for 4 wk. At the end of the trial the pigs were killed and samples of the digesta were taken from the stomach, the end of the small intestine, and the cecum for microbiological, DM, pH, and lactic acid analyses. Differences (P < 0.05) regarding the counts of bacteria were mainly found between the CM and the FP group, but the CP and the CE diet mostly resulted in intermediate values. Pigs fed the CM diet had the highest numbers of lactobacilli in the stomach content (P < 0.01) and the cecal digesta (P < 0.05). Perhaps due to a more efficient stomach barrier, characterized by high lactobacilli counts and a marked pH gradient in the stomach content (cardia, 5.15 ± 0.475; pylorus, 2.83 ± 1.06; P < 0.01), the lowest counts of coliform bacteria were found in the distal part of the small intestine in pigs fed the CM diet (P < 0.05).

Key words: grinding intensity, intestinal microflora, particle size, pig

INTRODUCTION

Feeding and housing strategies that support the health of animals have become important in ongoing discussions on the amount of antibiotics used in livestock production. It was shown that feeding a coarsely ground meal diet decreased salmonella prevalence in finishing pigs (Visscher et al., 2009) and effectively prevented gastric ulcers (Maxwell et al., 1970). The aim of this study was to evaluate potential influences of different grinding intensities (coarse or fine) and further compaction on shifts in the intestinal flora to obtain basic data of healthy animals before conducting challenge tests with experimental infections.

MATERIAL AND METHODS

The project was approved by the Ethics Committee on Animal Welfare of the Hannover District Government in accordance with German legislation on animal welfare.

Animals and Diets

Sixty weaned female piglets (Piétrain × Large White × Landrace) with an initial age of 33 d and a BW of 7.96 ± 1.09 kg were used in this study. The animals were divided into 4 groups with 15 pigs each and fed identical diets as meal that was coarsely ground (CM), coarsely ground and pelleted (CP), finely ground and pelleted (FP), or coarsely ground and extruded (CE) for 4 wk ad libitum during the whole trial. At the end of the trial (age, 61 d; BW, 21.5 ± 3.16 kg) the animals were anesthetized and subsequently euthanized, and samples for microbiological analyses were taken. Diets were based on wheat (48.5%), barley (25.0%), soybean meal (21.0%), and soya oil (2.0%) and were supplemented with a mineral and vitamin premix containing amino acids to fulfill requirements. Nutrient content was 213 g crude protein, 43.2 g crude fiber, and 13.6 MJ ME/kg diet as fed in all 4 groups. Particle size distribution was calculated after wet sieve analysis as follows: 50 g of the diet diluted in 1 L of water for 1 h, put on the top
of 8 sieves (3.15, 2.0, 1.5, 1.0, 0.8, 0.56, 0.4, 0.2 mm), and rinsed with 10 L of water through the sieves. After
the sieves were dried, the remaining fraction of the diet
on each sieve was weighed and calculated as the propor-
tion of the sample put on the top sieve. The percentage
missing to 100% represents the proportion of particles
that were <0.2 mm. The discrete mean of the particles
(dMEAN), expressed in millimeters, was determined ac-
cording to Fritz et al. (2011).

Digesta Analyses
Digesta were sampled from the stomach, the end of
the small intestine, and the cecum for microbiological
analyses (lactobacilli, enterococci, coliform bacteria) and
for determination of DM, pH, and particle size distribu-
tion. Bacteria (cfu/g digesta) were determined by common
culture techniques. Serial 10-fold dilution steps of each
digesta sample were set up, and 0.1 mL of every step was
plated on the specific agar (enterococci: selective blood
agar; coliform bacteria: Gassner agar; lactobacilli: Rogosa
agar, anaerobe conditions). After 24 h (coliforms, enter-
ococci) or 48 h (lactobacilli) of incubation, cfu per gram
of digesta were assessed and results expressed as log
10 to
obtain a normally distributed data set. Identi-
fication of the distinct bacteria was confirmed by morphology of the colo-
nies and the bacteria itself, lactose and oxidase reaction
(coliforms), or catalase reaction (enterococci, lactobacilli).
Particle size distribution in the digesta was determined by
wet sieve analysis including 4 sieves (2.0, 1.0, 0.4, 0.2
mm). Proportions were calculated according to the sieve
analysis of the diets.

Statistical Analyses
All data were analyzed using SAS (SAS Inst. Inc.,
Cary, NC). Differences were considered significant when
P ≤ 0.05. The effects of feed structure were analyzed in
relation to DM content and pH of the digesta as well as
microbial counts in different parts of the gastrointestinal
tract by GLM procedure. Comparison of the pH in the
gastric content at the cardia and the pylorus within a
group was performed by Student’s t test.

RESULTS AND DISCUSSION
As shown in Table 1, particle size distribution dif-
fered markedly between the finely ground and the 3
coarsely ground diets, whereas only minor effects of sec-
ondary grinding due to further compaction occurred (di-
ets CP and CE). These differences regarding the particle
size distribution of the diets were reflected throughout
the whole intestine with the highest dMEAN in the diges-
ta of animals fed the CM diet. Although the extrusion of
the coarsely ground meal resulted in a smaller dMEAN
of the CE diet compared to the CP diet (pelleting), the
dMEAN of the digesta does not reflect this. In the stom-
ach as well as in the colon, the particle size distribution
of the digesta resulted in a higher dMEAN after feed-
ing the CE diet than the CP diet (Table 2). These results
suggest differences in the retention (stomach) and accu-
mulation (colon) of large particles depend on the post-
grinding treatment of the diet.

Although the dMEAN of the CM and the CP diet
revealed only minor differences, the DM content of the
digesta in the stomach differed significantly (P < 0.05)

| Table 1. Particle size distribution and dMEAN\(^1\) of the 4 different diets |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Mill type                   | Fine, pelleted              | Coarse, meal                | Coarse, pelleted            | Coarse, extruded            |
|                            | Hammer mill                 | Roller mill                 | Roller mill                 | Roller mill                 |
| >1 mm, %                   | 8.97                        | 45.8                        | 41.6                        | 29.3                        |
| <0.2 mm, %                 | 42.4                        | 27.2                        | 32.7                        | 43.7                        |
| dMEAN, mm                  | 0.463                       | 0.880                       | 0.836                       | 0.659                       |

\(^1\)dMEAN = discrete mean particle size according to Fritz et al. (2011).

| Table 2. dMEAN\(^1\) (mm) in the digesta of stomach and colon, DM, pH, and lactic acid in the ingesta of the stomach\(^2\) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | Fine, pelleted              | Coarse, meal                | Coarse, pelleted            | Coarse, extruded            |
| Stomach                    | 0.314 ± 0.024\(^a\)         | 0.885 ± 0.101\(^b\)         | 0.503 ± 0.074\(^c\)         | 0.675 ± 0.068\(^d\)         |
| Colon                      | 0.323 ± 0.040\(^a\)         | 0.920 ± 0.154\(^b\)         | 0.439 ± 0.080\(^c\)         | 0.553 ± 0.108\(^d\)         |
| DM, %                      | 19.5 ± 5.42\(^a\)           | 29.9 ± 3.17\(^b\)           | 22.0 ± 5.62\(^c\)           | 22.8 ± 4.78\(^d\)           |
| pH cardia                  | 3.79 ± 0.557\(^a\)          | 5.15 ± 0.475\(^b\)          | 4.00 ± 0.979\(^a\)          | 3.79 ± 0.838\(^a\)          |
| pH pylorus                 | 3.76 ± 0.716\(^a\)          | 2.83 ± 1.06\(^b\)           | 3.62 ± 0.630\(^a\)          | 3.31 ± 0.634\(^a\)          |
| Lactic acid, mmol/kg       | 5.71 ± 6.01\(^a\)           | 17.6 ± 8.28\(^b\)           | 9.69 ± 14.0\(^a\)           | 18.0 ± 11.7\(^b\)           |

\(^1\)dMEAN = discrete mean particle size according to Fritz et al. (2011).

\(^2\)Means without a common superscript within a row (lowercase letters) or within a column (uppercase letters) differ
(P < 0.05).
Sander et al. (Table 2). Previously observed by Flatlandsmo and Slagsvold (1971). Due to the higher DM content after feeding the CM diet, the digesta formed layers within the stomach, specified by the highly significant pH gradient between the cardia and the pyloric regions \( (P < 0.001) \). A comparable effect did not occur in the 3 other diets, probably due to the low DM content.

Lactobacilli, enterococci, and coliform bacteria were chosen because these bacteria represent major groups of the microbial community in the gastrointestinal tract. An influence of the feed structure was detected on lactobacilli in the stomach and the cecum and on enterococci and coliform bacteria in the distal part of the small intestine. Significantly different occurred between the FP and the CM group, whereas the 2 coarsely ground and further processed diets (CP and CE) resulted in absolute numbers between the 2 other diets (Table 3). Perhaps due to a more efficient stomach barrier, characterized by high lactobacilli counts, high lactic acid content, and a marked pH gradient in the stomach content, the significantly lowest counts of coliform bacteria \( (P < 0.05) \) were found in the distal part of the small intestine after feeding the CM diet, although this group showed the significantly highest number of these bacteria in the stomach \( (P < 0.05) \). Mikkelsen et al. (2004) also showed that feeding a coarsely ground diet led to higher counts of lactobacilli accompanied by higher lactate contents in the stomach resulting in an increased death rate of Salmonellae. The high lactobacilli counts in the stomach after feeding the CM diet are likely to be associated with the layering of the digesta and an increased retention time (Maxwell et al. 1970). Significantly higher numbers of lactobacilli in the cecum in all 3 groups fed the coarsely ground diets (CM, CP, CE) is probably due to a higher substrate availability in the hindgut from preceally undigested starch.

In conclusion, feed structure influenced the resident intestinal microbial community of young pigs represented by lactobacilli, enterococci, and coliform bacteria. The shift to higher numbers of lactobacilli and lower counts of enterococci and coliforms after feeding the coarsely ground diets might contribute to positive effects on gut health arising from a more efficient stomach barrier and prebiotic effects of more starch reaching the hindgut.

**LITERATURE CITED**


<table>
<thead>
<tr>
<th>Bacterial counts (log (_10) cfu/g digesta) at different sections of the gastrointestinal tract (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Section</td>
</tr>
<tr>
<td>Lactobacilli</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Small intestine</td>
</tr>
<tr>
<td>Cecum</td>
</tr>
<tr>
<td>Coliform bacteria</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Small intestine</td>
</tr>
<tr>
<td>Cecum</td>
</tr>
<tr>
<td>Enterococci</td>
</tr>
<tr>
<td>Stomach</td>
</tr>
<tr>
<td>Small intestine</td>
</tr>
<tr>
<td>Cecum</td>
</tr>
</tbody>
</table>

\(^1\)Means without a common superscript within a row differ \( P < 0.05 \).