Effect of mash feed on swine intestinal microflora and non-specific immune response.

Letellier A.¹*, Collazos J.A², Ménard J³, Quessy S¹.

¹Faculté de Médecine Vétérinaire de l’Université de Montréal, 3200 Sicotte, J2S 7C6, St-Hyacinthe, Canada.
²University of Léon, 24071 León, Spain
³F. Ménard Inc., 251 route 235, Ange-Gardien, Québec, J0E 1E0, Canada.

*corresponding author: ann.letellier@umontreal.ca

Abstract

Pelleting of feed was recommended in the past to reduce the risk of introduction of Salmonella in swine herds. However, it was shown more recently that consumption of pelleted feed was associated with an increased probability of seropositivity. Furthermore, several studies showed that the prevalence of Salmonella is decreased when mash feed is used. The objective of this study was to evaluate the effect of mash feed as a pre-harvest intervention strategy to prevent Salmonella colonization, to modify of intestinal microflora and to stimulate of the immune system in swine. Two experimental groups of 45 and 43 piglets were given respectively conventional corn-based pelleted feed or mash feed from 10 weeks of age to slaughter. Rectal swabs and blood samples were taken periodically from each pig. Fecal swabs were cultured for the presence of Salmonella while a semi-quantitative evaluation of various fecal bacterial populations was also done. Phagocytosis rates of FITC marked Salmonella using whole blood of both groups of animals were evaluated by flow cytometry as an indirect measurement of non-specific immune response. At slaughter, mesenteric lymph nodes (MLN) were collected and cultured for Salmonella and an evaluation of presence of stomach ulcers or hyperkeratosis was done for each group. Although prevalence of Salmonella in both groups was too low to observe differences in prevalence, our results indicated that mash feed promoted some gram positive bacterial populations in comparison to pelleted feed group. The percentages of phagocytosis by PMN in the mash feed group was higher than in the pelleted feed group. In the mash feed fed group, all stomachs were normal while in the pelleted fed group, only 40% of pig stomachs were normal. These results suggest that mash feed influence bacterial content of intestine by promoting protective microbial flora; it positively affect the stomach mucosal integrity as well as it may stimulate non specific immune system of pigs.

Introduction

Salmonella infections can cause clinical and sub-clinical diseases in pig that may result in contamination of pork products. Feed had already been considered as a significant source of Salmonella infections in swine and can therefore potentially spread Salmonella to a large number of farms. For this reason, the pelleting of feed was recommended to reduce the introduction of Salmonella in farms during decades (Edel et al., 1974). However, it was shown more recently that pelleting of feed was associated with an increased risk of seropositivity for Salmonella at slaughter (Leonides et al., 2003; Lo Fo Wong et al., 2004). Jørgensen et al., 2002 showed that prevalence of Salmonella is decreased when coarse feeds rather than fine feeds are fed, suggesting that the stomach acts as a barrier that decreases the occurrence of pathogenic bacteria (Mikkelsen et al., 2004). Moreover, nonpelleted diets change the level of mucin secretion in the small intestine, creating conditions that decrease binding of Salmonella (Hedemann et al., 2005). The objective of this study was to evaluate the effect of mash feed as a pre-harvest intervention strategy to prevent Salmonella colonization, to modify of intestinal microflora and to stimulate of the immune system in swine.
Material and methods

**Animals:** Two groups (45 and 43 piglets) from six pens in the nursery unit were randomly selected (three pens each group) and the animals were identified individually. Piglets were then moved to the fattening unit and each group was located in two different fattening units. Piglets (20-25 kg, approximately 10 weeks of age) of each group were placed randomly in three pens. The fist sampling was performed in the nursery and all piglets were tested in bacteriology individually. The next samplings were done in the fattening units. Randomly, 40 blood samples (20 each group) and 40 blood samples (20 each group) were taken. The fecal samples were taken by rectal swabs. Two different blood samples were taken by animal: with heparin to test phagocytosis rates and without anticoagulant to obtain serum. In the nursery, animals were feed with the same feed, while in the fattening units one group received commercial pelleted feed (PF) and the other group received commercial mash feed (MF) (most of particles had a size larger than 1000 µm). Management and housing conditions were the same for each group of pigs.

**Bacteriological culture:** Rectal swabs and MLN were incubated in 9 ml (BWP) at 37°C for 24h. 0.1ml of culture of pre-enrichment was transferred to 9, 9 ml of RV broth and incubated at 41,5°C for 24h. Then, 10 µl of the selective enrichment media was inoculated on BGS containing novobiocin at 20µg/ml and incubated for 24 h at 37°C. Pooled fecal samples from pens (25g) was placed into 225ml de BWP and for feed samples, 100g were put on 900ml BWP, and also incubated for 24 h at 37°C. The selective enrichment was done with two selective media (TBG and RV broth); 1ml was transferred to TGB and 0.1 ml to RV and incubated at 41.5°C for 24h. Finally, 10 µl were plated into BGS with 20µg/ml at 37°C for 24h. Three suspected colonies by plate were tested for urease production and for typical reaction on Triple sugar iron media, and the typical colonies were tested by slide agglutination with polyvalent O-antisera (Poly A1-Vi, Difco).

**Serological status:** Salmonella seroprevalence was evaluated with the Diakit Salmonella-ELISA test (Maxivet Inc, Québec, Canada) on 40 sera, 20 from each group, on first sampling and last sampling.

**Ulcer and Hyperkeratosis evaluation:** For each slaughtered pig, the stomach was evaluated and signs of hyperkeratosis and ulcer were noted (normal stomach, hyperkeratosis mild or severe and light, mild or severe ulcer).

**Fecal flora evaluation:** The evaluation of fecal bacterial populations was done by smearing rectal swabs on glass slide with subsequent Gram staining. A total of four groups of bacterial population were evaluated depending of shape and gram stain: coccoid gram positive, coccoid gram negative, rods gram positive and rods gram negative. The evaluation was done on 5 fields at 1000 x magnification.

**Phagocytosis evaluation:** One ml of whole blood was incubated with a suspension (100µl) of S. Typhimurium (1-5 10⁸ ufc/ml) labeled with FITC, for 1h at 37°C o at 4°C for the control. Phagocytosis was stopped by addition of ice-cold PBS. The samples were read in the flow cytometer, and the ratio of phagocytosis were obtained by subtracting the percentage of phagocytosis at 4°C from the percentage of phagocytosis obtained at 37°C. The different populations of cells were identified by their forward- scatter and side-scatter characteristics, always considering that the normal percentage of PMN was 25-40% and monocytes were 5-8%.

Results and discussion

**Bacteriological and serological prevalence of salmonella:** In both groups the prevalence of Salmonella (bacteriology and serology) was very low (<2,5%). In MF group, the same animal was found positive to Salmonella (group E) in the sampling 1 and 3. In PF group, Salmonella (group B) was found. All feed and pooled fecal samples from pens were negative to Salmonella. At slaughter, the prevalence of Salmonella in MLN was 0% in MF group and 20% in PF group (Salmonella group B).

**Ulcer and Hyperkeratosis evaluation:** In the MF group, all stomachs were normal while in the PF group, only 40% of pig stomach were normal. 20% were noted as having hyperkeratosis signs and 40% of pig stomach had ulcer, indicating that the MF positively affect the stomach mucosal integrity.

**Fecal flora evaluation:** There were differences observed between groups. In the MF, the populations of Gram positive bacteria were higher than in the PF group. The populations of Gram...
negative bacteria increased through samplings 1 to 4 while the populations of Gram negative bacteria decreased. In the MF group, changes were observed in different subgroups of bacteria. The populations of Gram positive coccioids were very important in the first sampling, but decreased in the next sampling. Overall, the populations of Gram positive rods generally increased over time with the different samplings in MF group. This effect was not apparent in the PF group. The populations of Gram-negative rods in both groups were stable through different sampling. While the populations of Gram negative coccioids decreased slightly.

Figure 1. Evaluation of bacterial microflora in feces of swine fed with different diets (MF: mash feed or PF: pelleted feed).

Similar results were obtained by Mikkelsen et al, 2004. They studied the effect of different diets on the populations of acid lactic bacteria and coliform bacteria. The population of acid lactic bacteria of the animals fed with a coarse feed (MF) was higher than the population of the pigs fed with a PF. On other hand, the number of coliforms in pigs fed with coarse feed (MF) was lower than in pigs fed with PF, the population of Gram positive bacteria in the group of MF was higher and the population of Gram negative bacteria was lower than the PF group. Letellier et al, 2000 also found that prebiotics and probiotics induced changes in the microflora and a predominantly Gram positive bacterial flora was noted in pigs supplemented with these products.

Table 1. Phagocytosis of FITC-Salmonella Typhimurium by swine whole-blood phagocytes

<table>
<thead>
<tr>
<th>% Phagocytosis by polymorphonuclears (PMN)</th>
<th>Pellet feed group</th>
<th>Mash feed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>sampling</td>
<td>4°C</td>
<td>37°C</td>
</tr>
<tr>
<td>1</td>
<td>6,0</td>
<td>31,6</td>
</tr>
<tr>
<td>2</td>
<td>7,8</td>
<td>19,6</td>
</tr>
<tr>
<td>3</td>
<td>8,4</td>
<td>15,8</td>
</tr>
<tr>
<td>4</td>
<td>12,3</td>
<td>24,8</td>
</tr>
</tbody>
</table>

The percentage of phagocytosis by PMN in the MF group was higher than in the PF group. Neutrophils or PMN play an important role in the first step of immune and inflammatory response. Stabel et al, 2002 showed that swine infected with S. choleraesuis, the phagocytosis by neutrophils was increased 2 days post inoculation. Therefore, this may contribute to the establishment of a carrier status or to clinical infection in swine.

Conclusion

These results suggest that mash feed influence bacterial content of intestine by promoting protective microbial flora; it positively affect the stomach mucosal integrity as well as it may stimulate non specific immune system of pigs.
Reference


STABEL T.J., et al. 2002 Neutrophil Phagocytosis Following Inoculation of Salmonella choleraesuis into Swine, *Veterinary Research Communications*, 26 (2), 103 - 109