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Nutritional strategies to optimize gut health and performance of weaned piglets
Part 3. A holistic approach to improve gut health and performance

Introduction

Swine industry has been trying to find alternative solutions to overcome the bann or restrictive use of antimicrobial growth promoters (AGP) and zinc oxide (ZnO) in weaned pig diets to control the incidence of diarrhea. In the previous two chapters, the nutritional concepts such as low crude protein diets (LCP), fortified diets with functional amino acids (FAA), balancing with soluble and insoluble fiber, as well as the use of feed additives, such as probiotic and organic acids, are suggested as some of the alternatives. However, these are not exclusive among each other, thus combinations of two or more of these strategies may bring further benefits and a more consistent response to improve gut health and performance of piglets. Therefore, different combinations will be discussed in this chapter.

Combination of low protein diets with functional amino acids

Under immune challenge conditions, the utilization amino acids (AA) supplied in the feed is redirected primarily to support the immune response at the expense of using for growth. Thus, under these circumstances, extra supplementation of FAA may improve growth performance of pigs. As previously mentioned in part 1, reducing dietary CP may lower susceptibility of weaned pigs to enteric pathogens. In a recent study, the combination of LCP diets with extra supplementation of selected FAA in pigs challenged with *Salmonella typhimurium* was tested (Rodriguez *et al.*, 2020). Treatments consisted of high protein (HP;19 % CP) and LCP (16 % CP) diets with (Thr, Met, and Trp at 120 % of NRC, 2012 requirement) or without (Thr, Met, and Trp at 100 % of NRC, 2012 requirement) supplementation of FAA. No interactions were observed between level of CP and FAA level. However, regardless of CP level, feeding diets supplemented with FAA to challenged pigs increased ADG, gain:feed (G:F), and reduced glutathione:oxidized glutathione (GSH:GSSG) ratio, and decreased serum haptoglobin concentrations, plasma superoxide dismutase, and fecal shedding and *S. typhimurium* counts in colon, compared to pigs fed unsupplemented diets (Figures 1 and 2). Thus, this indicates that additional supplementation with FAA has positive effects on both immune response and performance in weaned pigs challenged with an enteric pathogen. Interestingly, lowering dietary CP content tended to reduce ($P = 0.06$) the concentration of myeloperoxidase, i.e. a pro-inflammatory biomarker, in digesta of ileum, cecum and colon at day 7 post-inoculation, which indicates a potential to reduce intestinal inflammation, however, other additional effects were not observed when FAA supplementation was combined with LP diets. The idea of potential synergistic effects between LCP diets and supplementation of FAA should not be excluded, as in this particular case, the experiment was done with older pigs with an average body weight (BW) of 15 kg compared with a typical BW of about 8 kg at weaning. Older pigs may not be as sensitive to dietary protein levels as younger weaned pigs. Thus, further investigation of this synergistic effect should be tested with younger weaned pigs.

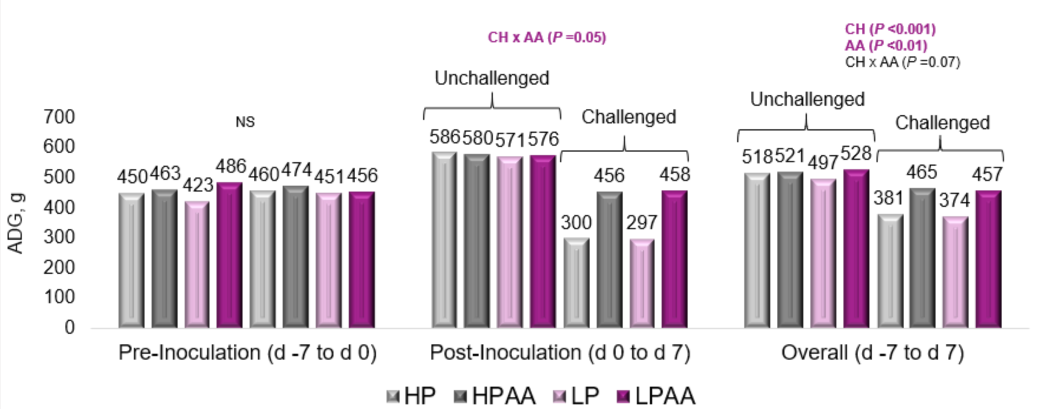

Figure 1: Effects of high (HP) and low (LP) crude protein level and extra supplementation of FAA on average daily gain (ADG) under challenged (*Salmonella typhimurium*) or unchallenged conditions

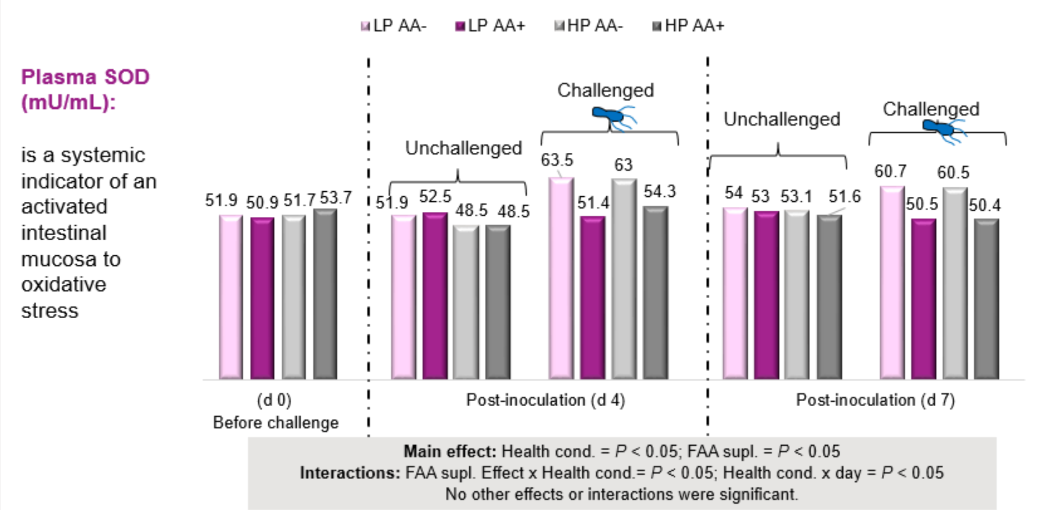

Figure 2: Effects of high (HP) and low (LP) crude protein level and extra supplementation of FAA on plasma superoxide dismutase (SOD) under challenged (*Salmonella typhimurium*) or unchallenged conditions

In a follow-up study, the effect of the duration of an adaptation period to FAA supplementation before pigs were challenged with *S. typhimurium* was evaluated (Rodrigues *et al.*, 2021). All diets contained low CP (16 %) level, and pigs were fed with either 100 % (no FAA+) or 120 % of Met, Thr, and Trp (FAA+) NRC requirements (2012) provided at different time lengths for 21 days in total. One group of pigs received 100 % FAA for 21 days and the other 3 groups of pigs were fed instead a 120 % FAA diet since day 0, 7, or 14 before the challenged (FAA+ at day 0, at d -7, and at day -14 as illustrated in Figure 3). Results indicated that pigs with the longest adaptation period (14 day) had the greatest ADG and G:F among all treatments (Figure 4), as well as the lowest serum haptoglobin concentration and the lowest quantity of *S. typhimurium* in the colon (Table 1). In addition, all pigs fed diets supplemented with 120 % FAA, regardless of the adaptation period, had decreased plasma superoxide dismutase concentrations and increased GSH:GSSG ratio. Overall, these results with extra supplementation of FAA were associated with beneficial effects on gut health, attenuation of the acute-phase response, and reduction of *Salmonella* colonization and shedding.

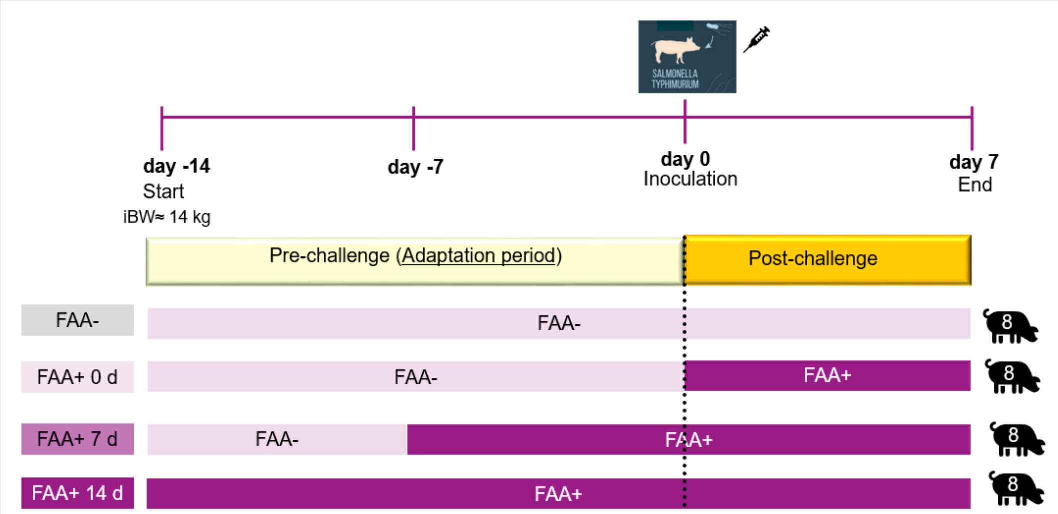

Figure 3: Experimental design of extra supplementation of FAA in pre-and post-challenged pigs with *Salmonella typhimurium*

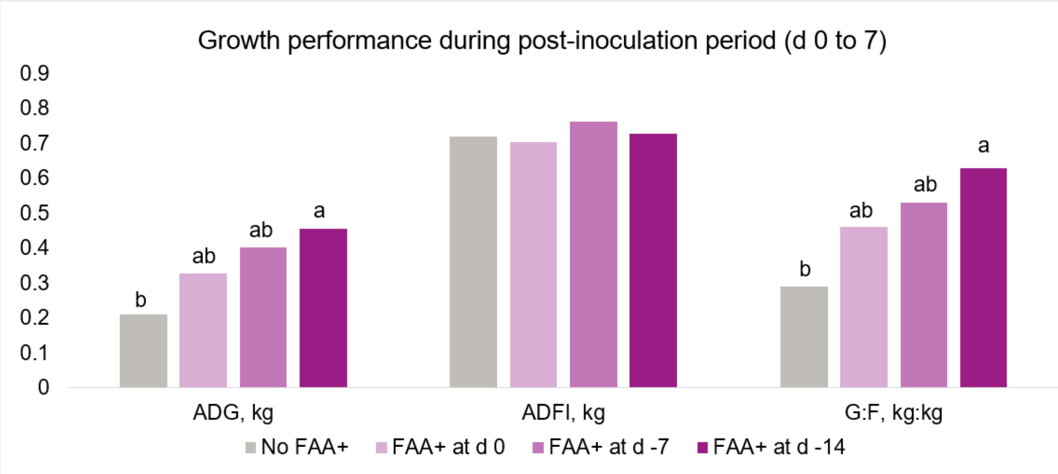

Figure 4: Effects of adaptation period to extra supplementation of FAA on growth performance under challenged (*Salmonella typhimurium*) pigs

Table 1: *Salmonella typhimurium* quantification in intestinal contents (Log10 CFU/g; d 7 post-inoculation) of *Salmonella*-inoculated pigs fed diets with a basal amino acid profile (No FAA+) or a supplemented functional amino acid profile (FAA+) for 0, 7, or 14 days pre-inoculation

Item	No FAA+	FAA+ d 0	FAA+ d-7	FAA+ d-14	SEM	*P*-value
Ileum	5.47	4.61	4.95	3.92	1.273	0.78
Cecum	4.55	4.09	3.98	3.79	0.434	0.63
Colon	6.28[a]	6.03[a]	4.42[ab]	3.32[b]	0.517	< 0.01

Combination of low protein diet with probiotics

As discussed in part 1, one of the strategies to lower the risk of post-weaning diarrhea is by reducing dietary CP level while balancing to meet all AA requirements for proper growth and gut health. A reduced amount of undigested protein is observed in the large intestine with LCP diets, which limits the proliferation of pathogenic bacteria that cause diarrhea. In addition, as discussed in chapter 2, the inclusion of probiotics may benefit the intestinal health of the pig by modulating the microbiota and enhancing the development and integrity of the gut. Therefore, in a study (Facts & Figures 14132; Tang *et al.*, 2019), the LCP concept was combined with the use of probiotics. High (20.5 % CP prestarter and 19.5 % CP starter) and low CP diets (18 % CP prestarter and 17 % CP starter) were fed with or without 500 g/ton of feed with a probiotic (*Bacillus Subtilis* DSM32315). Improvements on the microbiota profile and gut integrity in piglets supplemented probiotic were observed, especially in low CP diets. Low CP diets supplemented with probiotic had the greatest counts of *Bacillus* and *Bifidobacterium* in colon among all diets (Figure 5). *Lactobacillus* counts tended to increase with the supplementation of probiotic regardless of the CP level. However, no differences were observed in *E. coli* counts, and only a numerical reduction was observed in the low CP diets with probiotic.

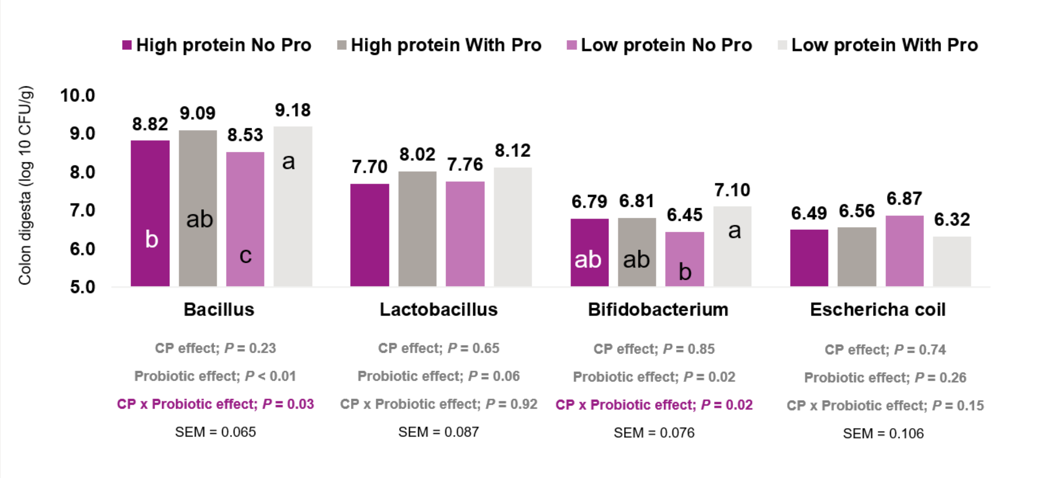

Figure 5: Microbial count (log 10 CFU/g) in colon digesta of piglets fed high or low CP diets with or without Probiotic (*Bacillus Subtilis* DSM32315) supplementation

An increase in the propionic and butyric acid production in the large intestine was observed when probiotic was added. Whereas, in the ileum, an increase in acetic acid production was only observed with probiotic in the LCP diets. The acetic acid produced may be utilized by bacteria, which in turn produce butyric acid through cross-feeding.

The production of short chain fatty acids (SCFA) influences epithelial cell proliferation, which may explain the increase in villus height in the jejunum and ileum in LCP diets with probiotic *Bacillus Subtilis* DSM32315. Probiotic supplementation, regardless of CP level, increased villus height: crypt depth ratio (VH:CD) in jejunum, whereas in ileum this effect was only observed in the LCP diet with probiotic.

In the ileum, the relative mRNA expression of tight junction-related genes, such as Occludin-1, and intestinal tract development-related genes, such as EGF and IGF-1R, increased to a greater extent when probiotic was added to LCP diets compared to when probiotic was added to HCP diets. The relative mRNA expression of ZO-1 and GLCP-2 increased when pigs were fed with *Bacillus Subtilis* DSM32315 regardless of the CP level.

Benefits on the integrity of the intestinal barrier function and gut development may be a result of the increased mRNA expression of tight junction- and intestinal tract development-related genes, and of the increase in SCFA production. As a result of a better gut integrity, an increase in the apparent total tract digestibility (ATTD) of dry matter and gross energy with probiotic, regardless of CP level, and an increase in the ATTD of ether extract in pigs fed probiotic in LCP diets were observed.

In addition, the synergistic effects between low CP diets and supplementation of probiotics *Bacillus Subtilis* DSM32315 during the first 14 days were observed and resulted in the greatest ADG and G:F among all treatments. For the next 28 days and for the overall 42-day period, increased ADG and G:F in pigs fed diets supplemented with probiotic was observed, regardless of the level of CP (Table 2). Overall, the data indicate that the benefits of probiotic supplementation on growth performance and gut health parameters were more pronounced when supplemented to a LCP diet compared with a high CP diet. Although it is not very clear, this may be because feeding the piglets with LCP diets can reduce the amount of undigested protein and the pH in the hindgut and minimizing the risk of post-weaning diarrhea.

These findings offer a potential nutritional strategy to improve performance and gut health for weaned piglets, especially for those producers looking to maintain or improve the intestinal health of their livestock and reduce nitrogen emissions.

Table 2: Supplementation of probiotics (*Bacillus Subtilis* DSM32315) improved performance in weaned piglets in high protein and low protein diets

Item	Diets				SEM	*P*-value		
	High CP		Low CP					
	Without Probiotic	With Probiotic	Without Probiotic	With Probiotic		CP	PROB	CP×PROB
Initial BW (kg)	7.61	7.60	7.61	7.61	0.10	0.991	0.973	0.991
Phase 1 (d 1 to 14)								
ADFI (g/d)	378.96	354.26	375.92	371.80	3.36	0.226	0.022	0.092
ADG (g/d)	255.68[a]	248.64[a]	250.48[a]	270.93[b]	2.74	0.065	0.141	<0.01
FCR (g/g)	1.48[c]	1.43[b]	1.50[c]	1.37[a]	0.01	0.246	<0.01	0.024
Phase 2 (d 15 to 42)								
ADFI (g/d)	727.28	736.95	721.97	736.15	8.45	0.866	0.513	0.901
ADG (g/d)	453.21	473.11	441.25	485.35	7.54	0.992	0.036	0.406
FCR (g/g)	1.61	1.56	1.64	1.52	0.02	0.861	0.010	0.189
Overall (d 1to 42)								
ADFI (g/d)	611.17	609.39	606.63	614.70	5.49	0.968	0.791	0.678
ADG (g/d)	387.37	398.29	377.66	413.30	5.22	0.758	0.021	0.195
FCR (g/g)	1.56	1.53	1.61	1.49	0.01	0.696	<0.01	0.104

Combination of dietary fiber with amino acids or probiotics

When diets are rich in fiber, nutrient requirements should be adjusted to optimize gut integrity and performance of pigs. For example, production of mucin increases with high fiber diets, and therefore the threonine requirement increases (Mathai *et al.*, 2016; Wellington *et al.*, 2018, 2020). Mucin secretion helps to protect the intestines from physical damage and pathogens; however, limited knowledge is available to understand interactions between fiber and levels of Thr under a pathogen challenge on mucin production (Wellington *et al.*, 2018, 2020). To elucidate this interaction, in a study, pigs were fed low fiber (13 % total dietary fiber (TDF) or high fiber diets (20 % TDF, partly replacing corn with 5 % wheat bran and 10 % sugar beet pulp) with either basal Thr level (0.65 % standardized ileal digestible; SID) or a higher supplementation of Thr (0.78 % SID) under an enteric challenge using *Salmonella* Typhimurium (Wellington *et al.*, 2020). Interestingly, pigs fed with high fiber diets increased fecal mucin output with high Thr level compared to low Thr level (Wellington *et al.*, 2020). Whereas in low fiber diets, fecal mucin output was not different between both Thr levels (Figure 6). This interaction may be associated to a higher production of SCFA with high fiber diets, that may stimulate mucus production by a higher MUC2 gene expression (Wellington *et al.*, 2020).

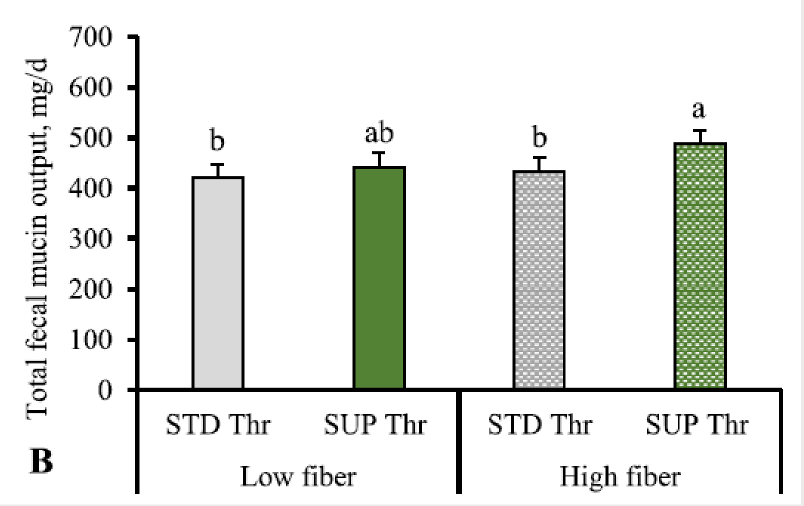

Figure 6: Interaction between supplementation of L-threonine in low and high fiber diets on fecal mucin output of pigs

However, no interactions between fiber and Thr levels were observed on performance of pigs. High fiber diets reduced pig performance before and during the enteric disease challenge with *Salmonella* (Wellington *et al.*, 2019). The fibrous ingredients, sugar beet pulp and wheat bran, that were used in the high fiber diet reduced feed intake, reducing growth rate and feed efficiency. Supplying extra Thr (20 % above NRC requirement) improved pig performance regardless of the fiber content in the diets, but only during the challenge period and overall period (Figure 7). Therefore, under challenge conditions, when voluntary feed intake is reduced, an effective immune response and growth may be compromised. Thus, increased dietary Thr could play an important role in alleviating the negative effects of the disease challenge by improving growth performance, gut health, and immune status.

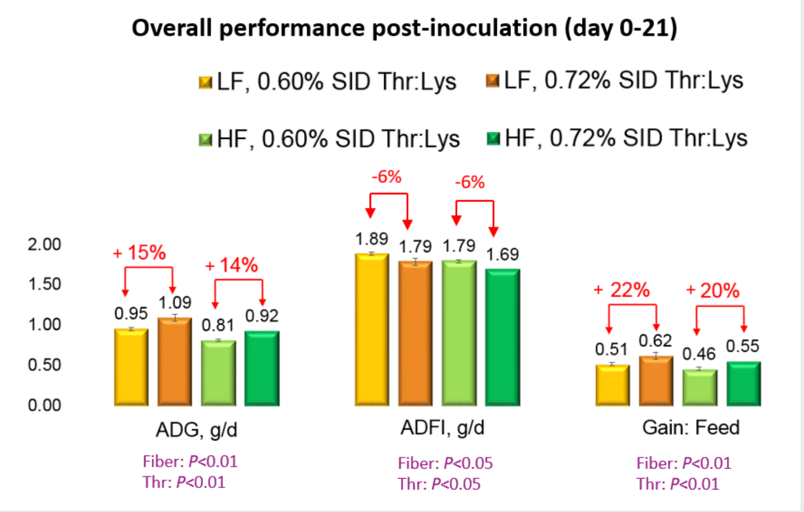

Figure 7: Effects of supplementation level of L-threonine in low and high fiber diets on growth performance of pigs challenged with *Salmonella* typhimurium at day 7

A similar study was conducted but this time using an *E. coli* lipopolysaccharide (LPS) as a systemic immune challenge (Wellington *et al.*, 2020). Pigs fed high fiber diets had lower urinary Lactulose:Mannitol ratio than low fiber diets during LPS challenge, whereas before challenge no differences were observed among fiber levels (Figure 8). Lactulose and mannitol ratio are used as indicators of intestinal barrier permeability, a high ratio in the urine indicates a leaky gut. In addition, high fiber diets had more mucin secretion than low fiber diets, regardless of systemic challenge. Fiber, therefore, may help to improve intestinal barrier of pigs.

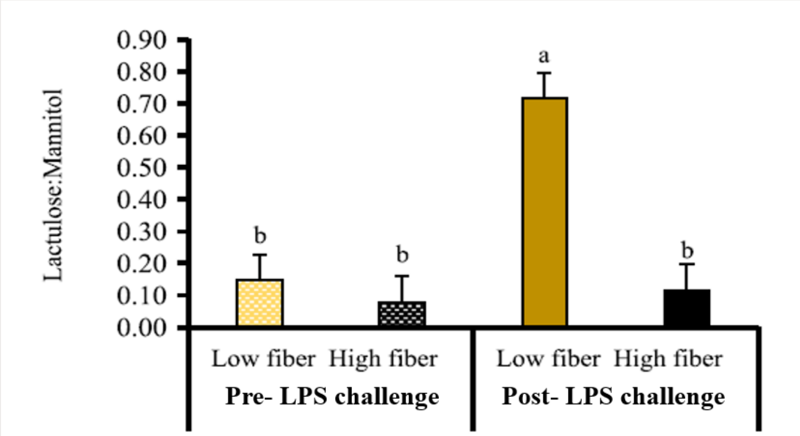

Figure 8: Effects of low and high fiber diets during pre- and post- LPS challenge on urinary Lactulose:Mannitol ratio of pigs

It is important to carefully understand all these interactions, especially with Thr, as it was reported that requirement for SID Thr to maximize protein deposition of growing pigs fed low fiber diets was increased from 0.68 to 0.76 % by LPS challenge compared with un-challenged pigs, whereas when feeding high fiber diets, the estimated SID Thr requirement was 0.78 and 0.72 % pre- and post-LPS challenge, respectively (Wellington *et al.*, 2018). These responses indicate that high fiber level may modify the effects of immune challenge on Thr requirement for immune response and protein deposition.

Further interactions with type of fiber on gut health has been reported. Supplementing a moderate amount of insoluble fiber (4 % wheat bran) and soluble fiber (2 % sugar beet pulp) to a low CP diets increased growth performance and SCFA concentrations in the hindgut while fecal *E. coli* counts were reduced compared to diets without fibers (Hermes *et al.*, 2009). Interactions between type of fiber and probiotic has also been observed in a study conducted in Spain. Sugar beet pulp (44.6 % insoluble dietary fiber (IDF) and 4.0 % soluble dietary fiber (SDF)) and wheat bran (48 % IDF and 2.9 % SDF) were fed to weaned piglets at 10 % inclusion level into a common basal diet with or without probiotics (*Bacillus Subtilis* DSM32315). It was observed that inclusion of the probiotic mitigate the negative effects on G:F when 10 % wheat fiber was included in the diets (Figure 9). However, this effect was not observed when pigs were fed 10 % sugar beet pulp (unpublished data). This may be explained by different effects that have different type of fibers not only on microbiota changes, but also on passage rate. Thus, it is important to understand the interactions between sources of fiber, AA, and probiotics, as fibers play an important role on gut health and microbiota modulation of pigs.

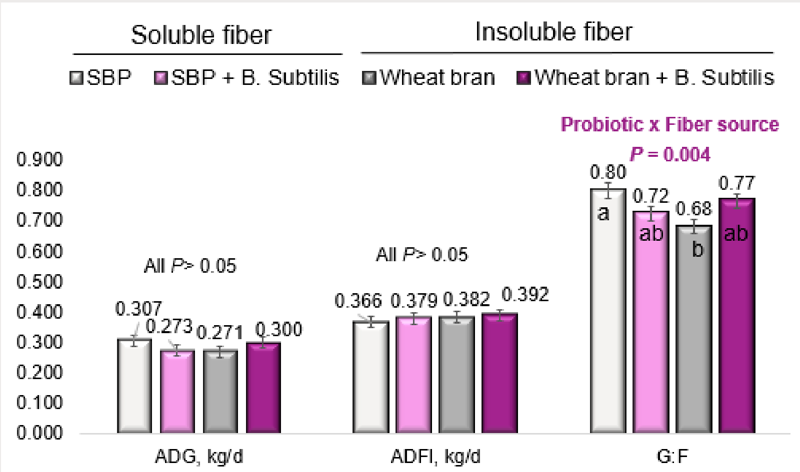

Figure 9: Effect of type of fiber and probiotic (*Bacillus Subtilis* DSM32315) on growth performance of weaned pigs

Conclusions

Nutritional strategies need to be developed and combined to cope with the restrictive use of antibiotics and zinc oxide, thus enabling good gut health status and mitigating the negative effects of weaning. The holistic gut health approach, i.e. extra supplementation of functional amino acids, applying low protein diets particularly in combination with feed additives such as probiotics and/or some beneficial fibers, are some of the strategies that have indicated potential improvements in gut integrity, microbiota modulation, and immune response. However, further studies need to be conducted to fully understand the interactions and possible synergistic effects of different concepts and feed additives under different production conditions.

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