AMINO ACIDS

Amino acids ensure piglet gut health and performance in low crude protein diets

Bart Matton

CJ EUROPE GmbH

Abstract

Managing the crude protein level in swine diets is a major tool to cope with the current challenges our industry faces. On the one hand, it will help us to reduce the environmental impact, by reducing nitrogen excretion. On the other hand, it enhances gut health as lower amounts of undigested protein will reduce the amount of toxic metabolites formed in the hindgut and reduce the dependency on antimicrobials. However, reducing crude protein needs to be done with an adequate amino acid supply as this is a prerequisite for health and performance. This article shows that amino acids will lay the foundation of a proper nutritional protein strategy to raise healthy, performing piglets without antimicrobials.

Background

As the world recovers from the pandemic, the call for better public health outcomes and organisations worldwide sounded very hard but was not new. The World Health Organisation's 'One Health' program aims already for many years to achieve this goal. One of the pillars of this approach is to limit antimicrobial resistance. Antimicrobial resistance is one of the biggest threats to global health and food security, estimated to be the major cause of death by 2050 if no action is undertaken [1]. The global action plan on antimicrobial resistance launched in 2015 aims to reduce the use of antibiotics in farm animals and is seen as the fundament for preserving effective antibiotics for humans. New and innovative ways of looking at animal health are needed to safeguard food supply across the globe. Protein and amino acid nutrition are one of the key elements in the approach to raise animals without the use of antimicrobials.

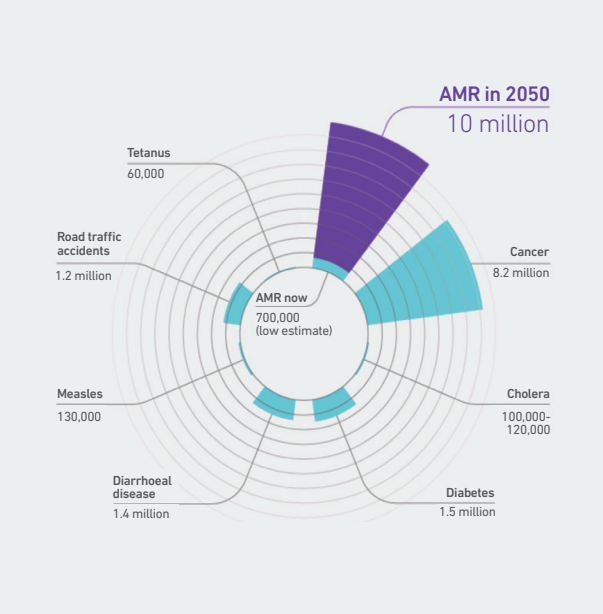

Figure 1. AMR-attributable deaths globally in 2050 compared to other major causes of mortality [1].

The pivotal role of the gut in health and performance

To fully understand how protein and amino acids will play a key role in health and performance, we need to go back more than 2000 years ago when Hippocrates (c. 460 – c. 370 BC) stated: "All disease begins in the gut". This is a statement that seems to be more and more confirmed by modern research technologies. Suggesting that the microbiome residing in the intestines is responsible for overall health, performance, well-being and many more, through different axes (so called gut-lung axis, gut-brain axis, gut-liver axis, etc.). Indeed, the intestinal tract is the largest immune organ [2], a complex yet fragile ecosystem essentially determining how our animals will thrive.

Looking more closely, the intestinal barrier can be divided into 4 protection layers (Figure 2).

The microbial barrier is a complex environment of which the metabolites such as short chain fatty acids are essential for gut health and limiting pathogen overgrowth. The chemical barrier, consisting of the mucus layer, harbours huge amounts of antibodies protecting the host from pathogens. The physical barrier, consisting of the epithelial cells, exerts the complex tasks of keeping pathogens out and absorbs nutrients and last, the immunological barrier, harbours the immune cells of the connective tissue (lamina propria). In case one or more of these protection mechanisms are compromised, we observe leaky gut and the occurrence of digestive issues such as post-weaning diarrhoea (PWD) is likely.

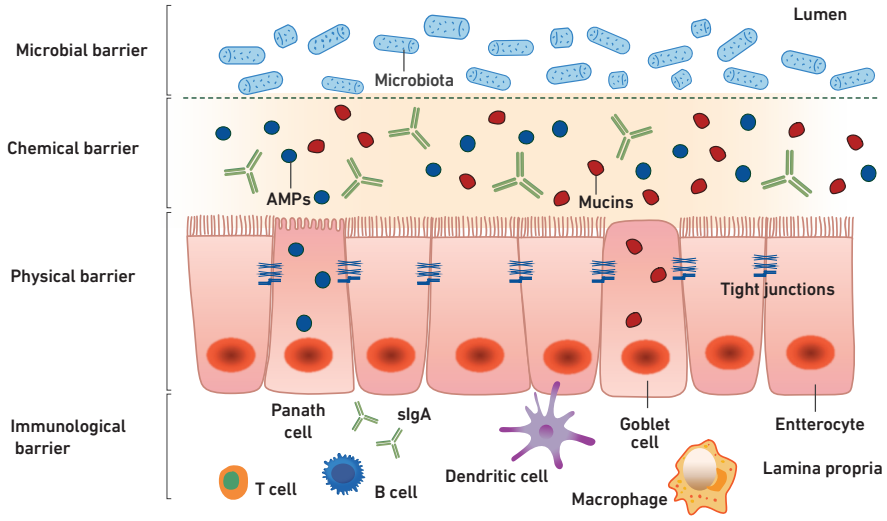

Figure 2. The different layers of the intestinal barrier, modified after Gao et al., 2020 [3]

Protein reduction and amino acid nutrition

As more and more antimicrobials are banned from (preventive) usage in animal nutrition, more attention needs to be given to how to maintain a good balance in the gut. Chain partners agree that there is no silver bullet to keep (gut)homeostasis, but an interplay of management and feed is needed. Looking into feed more specifically, protein nutrition is a fundamantal and effective strategy to start from [4]. In fact, the amount of undigested protein flux into the intestines needs to be considered in combination with amino acid nutrition beyond the ideal protein approach. The undigested flux of protein is determined by a combination of protein quality and quantity. Even though keeping an eye on protein quality might sound relatively simple, the pressure on environmentally sustainable feed formulation is leading to usage of often less known, less digestible protein sources containing more or other anti-nutritional factors compared to widely used soybean meal.

Limiting protein quantity and working on an ideal amino acid profile with crystalline amino acids, sometimes leads to unexpected loss of performance. However, the recent enlargement of commercially available BESTAMINO™ crystallin amino acids e.g. isoleucine (Ile) and histidine (His), but also arginine (Arg) makes it possible to reduce CP levels further in a safe way without loss in performance.

A recent trial conducted at Wageningen Livestock Research in the Netherlands by CJ Bio and extensively discussed in the Q1 CJ Bio bulletin showed that it is possible to reduce dietary CP concentration to 15 % in nursery piglets without the use of a high dose of ZnO or antimicrobials if the diets are fortified with crystalline AA to recommendation level. Although this study did not look specifically at differences in protein levels, adequate amino acid supply according to the assumed recommendation showed the highest average daily gain and feed intake compared to imbalanced amino acid profiles.

A recently published study from Denmark by Lynegaard et al. (2021) [5] shows again how crucial it is to watch amino acid supply critically in ZnO free diets. In this post-weaning trial (phase 1: 6-9 kg; phase 2: 9-15 kg; phase 3: 15-30 kg) the authors compared in phase 1 and 2 a positive control (PC) containing a high dosage of ZnO (2500 ppm in phase 1)

with a normal CP level (±18.8%) to negative control (NC) containing normal CP (±18.8%) but not containing high dosage of ZnO and to low CP level (LCP) diets (15.1-15.4% CP) not containing ZnO. Phase 3 had the same CP level (±19%).

The performance of the LCP group decreased significantly compared to the other groups (Table 1) despite the theoretical addition of the next limiting amino acids even up to phenylalanine and tyrosine. The authors indicate that they unintendedly undersupplied the added amino acids particularly (Leu, Ile, His, Phe, and Tyr) by 5 to 12%. This means the piglets were not fed their theoretical requirements for performance, this provoked an extra challenge for the piglets in the low CP diets. Earlier research [6-7] shows that pigs kept under low sanitary conditions or being challenged have higher amino acid requirements. Despite this undersupply event in the Lynegaard study, the piglets receiving the LCP diet had no pen diarrhoea treatments in the first phase, likewise to the PC group, compared to the NC group which had 6.7% pen diarrhoea treatments.
In the second phase, only 4% of pen diarrhoea treatments were observed in the LCP group compared with almost 27% in the PC group and almost 45% in the NC group.

Looking at the overall treatment days per pig over the experiment (6-30 kg), the PC group had 2.6 days and the LCP only 1.7 days differing significantly ($P < 0.05$) from the NC group having 4.6 days (Table 1). These results indicate that lowering CP levels has positive effects on intestinal health, however, to keep the performance up, correct amino acids supplementation is needed.

Table 1. Body weight at the start and end of the trial. Percentage of pen diarrhoea treatments and treatment days per pig.

Adapted from Lynegaard et al., 2021

	PC	NC	LCP
BW at entry	6.9	6.9	6.9
BW at exit	30.7^{a}	30.7^{a}	29.2^{b}
Pen diarrhoea treatments %			
Phase 1, 6-9 kg	0	6.7	0
Phase 2, 9-15 kg	26.7^{b}	44.6^{a}	4.0^{b}
Treatment days per pig			
Total period 6-30 kg	2.6^{b}	4.6^{a}	1.7^{b}

a,b Values within a row with different superscripts differ significantly at $p<0.05$ in relation to group NC.

Amino acid nutrition with gut health challenge in mind

To determine the amino acid requirements of an animal, requirement studies are most of the time conducted in near to ideal conditions in terms of sanitary condition and health of the animals. As stated above, challenging conditions increase specific amino acid requirements.

More recently Rodrigues and co-workers [8] investigated the effect of supplying piglets with or without Met, Thr, Trp 120% above NRC recommendation in combination with a salmonella challenge in a low (16%) and high protein (20%) diet. Adding functional amino acids had not only positive effects on perfor-

mance but also on immune status, independently of the CP level. Methionine, part of the sulphur containing amino acids and precursor of cysteine, plays an important role in the intestinal epithelial antioxidative status by the formation of glutathione (GSH), the most present cellular antioxidant in the intestines of piglets and chicks. Less optimal sanitary condition in combination with the stressful weaning period increases oxidative stress in the gut, jeopardizing gut functionality, provoking leaky gut and leading to PWD. CJ's BESTAMINO™, L-Methionine, has shown to act positively on gut morphology [9] and will, due to its 100% L-isomer, be available directly to the intestinal cells for the production of glutathione [10].

Threonine, on the other hand, is an important constituent of the mucus layer and therefore adequate supply of Thr supports the chemical barrier function. Recently Liu and co-workers [11] showed that tryptophane has a positive effect on gut health in piglets challenged with *E. coli* lipopolysaccharide amongst others, improving the anti-oxidant status and alleviating inflammation. Therefore, paying attention even to well-known and commonly used amino acids in your formulation will help to support the defence mechanism in the gut.

In the last two decades, more and more studies are focusing on how specific amino acids modulate overall gut health. Recent reviews published in 2021 by Liao from Mississippi State University, and Montout and co-workers from INRAe, summarize in a comprehensive way how amino acids play a crucial role in gut health and immune response in pigs and other livestock. For example, the established amino acids mentioned above, but also Arg and Ile, are important contributors to gut homeostasis and thus cannot be neglected when thinking about healthy weaning without anti-microbials. Arginine on the one hand, has shown good effects on gut morphology, barrier function and inflammation whilst isoleucine has shown positive effects on the immune function of infected animals. Thus, AA requirements of animals not being supported preventively with antimicrobials, such as therapeutic dosages of ZnO face more immunological stress, and will increase compared to the current AA recommendations, largely determined by good sanitary conditions or limited challenge. On top, new ways of formulating feed, such as the higher inclusion of fiber to support gut health or the use of alternative protein sources, demand an extra eye on amino acid incorporation to keep performance up.

Conclusions

Reducing the usage of anti-microbials to prevent antibiotic resistance will continue to grow worldwide. Feeding low CP diets is a very effective strategy to reach these goals when amino acid supply is closely watched. This means, taking into account that the largest immune organ will face more stress and that amino acids play a crucial role in the immune system. This extra stress is caused by: no more preventive usage of antimicrobials, new formulation habits (e.g. fibre usage) focussing on health and taking into account welfare and sustainability regulations. The cocktail above requires us to look further in formulation than we currently do. Beyond any doubt, make use of newly available amino acids such as Ile and His to correct your formulation for performance, but also go beyond that and make use of amino acid nutrition as a key strategy to improve gut health and reduce post-weaning diarrhoea of young piglets.

Reference

1. Pullon, Humphrey W., et al. "Antimicrobial resistance in New Zealand: the evidence and a call for action." The New Zealand Medical Journal 129.1444 (2016): 103-110.
2. Chassaing, Benoit, et al. "Mammalian gut immunity." Biomedical journal 37.5 (2014): 246.
3. Gao, Yanan, et al. "The compromised intestinal barrier induced by mycotoxins." Toxins 12.10 (2020): 619
4. Heo, Jung M., et al. "Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds." Journal of animal physiology and animal nutrition 97.2 (2013): 207-237.
5. Lynegaard, Julie C., et al. "A Very Low CP Level Reduced Diarrhoea and Productivity in Weaner Pigs, but No Differences between Post-Weaning Diets Including Soybean Meal or Soy Protein Concentrate Were Found." Animals 11.3 (2021): 678.
6. Capozzalo, M. M., et al. "Determination of the optimum standardised ileal digestible sulphur amino acids to lysine ratio in weaned pigs challenged with enterotoxigenic Escherichia coli." Animal Feed Science and Technology 227 (2017): 118-130.
7. Van der Meer, Y., et al. "Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation." Journal of Animal Science 94.11 (2016): 4704-4719.
8. Rodrigues, Lucas A., et al. "Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with Salmonella Typhimurium." Journal of Animal Science 99.2 (2021): skaa365.
9. Shen, Y. B., A. C. Weaver, and S. W. Kim. "Effect of feed grade L-methionine on growth performance and gut health in nursery pigs compared with conventional DL-methionine." Journal of Animal Science 92.12 (2014): 5530-5539.
10. Shen, Y. B., et al. "Effects of feed grade L-methionine on intestinal redox status, intestinal development, and growth performance of young chickens compared with conventional DL-methionine." Journal of Animal Science 93.6 (2015): 2977-2986.
11. Liu, Guangmang, et al. "Dietary Tryptophan Supplementation Improves Antioxidant Status and Alleviates Inflammation, Endoplasmic Reticulum Stress, Apoptosis, and Pyroptosis in the Intestine of Piglets after Lipopolysaccharide Challenge." Antioxidants 11.5 (2022): 872.