Archivos Latinoamericanos de Producción Animal. 2022. 30 (Supl. 1)
XXXII Reunión Internacional de Producción de Carne y Leche en Climas Cálidos
6 y 7 de octubre del 2022
https://doi.org/10.53588/alpa.300507

How functional amino acids can support pigs during challenge environments

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Abstract. Pigs are often exposed to chronic sub-clinical level of diseases and climatic stress in commercial farms, causing a lower feed intake and performance. Thus, adequate supply of nutrients including amino acids (AA) must be secured in order to maintain optimal health and production levels of pigs. Functional AA function not only as building blocks for protein synthesis but also in several cellular metabolic pathways related with health, growth, development, lactation, and reproduction of organisms. The functions of sulphur amino acids methionine and cysteine, tryptophan, and threonine, beyond their role in the synthesis of protein are summarized in this review.

Keywords: pigs, functional amino acids, health, growth

Cómo los aminoácidos funcionales pueden ayudar a los cerdos en entornos de desafío

Resumen. Los cerdos a menudo están expuestos a niveles subclínicos crónicos de enfermedades y estrés climático en las granjas comerciales, lo que provoca un consumo de alimento y un rendimiento más bajos. Por lo tanto, se debe asegurar un suministro adecuado de nutrientes, incluidos los aminoácidos (AA), para mantener niveles óptimos de salud y producción de los cerdos. Los AA funcionales funcionan no solo como bloques de construcción para la síntesis de proteínas, sino también en varias vías metabólicas celulares relacionadas con la salud, el crecimiento, el desarrollo, la lactancia y la reproducción de los organismos. En esta revisión se resumen las funciones de los aminoácidos azufrados metionina y cisteína, triptófano y treonina, más allá de su papel en la síntesis de proteínas.

Palabras clave: cerdos, aminoácidos funcionales, salud, crecimiento

Introduction

Despite increased bio-security measures, pigs are often exposed to chronic sub-clinical level of diseases and climatic (heat) stress in commercial farms, causing a lower feed intake and performance. Differences in health or immune status of the pigs is one of the reasons for the large variation in the performance of pigs between commercial farms (Pastorelli et al., 2012). The optimal levels of nutrients including amino acids (AA) must be supplied with the feeds to the animals if the growth, health, and productivity of the animal are to be maintained at optimal level.

The immune system is a defense system to protect the host from invading pathogens. The gastro-intestinal (GI) tract is a key component of the body's systemic immune system and contributes to health in many ways (Bischoff, 2011). In addition to digestion, absorption of nutrients, the gut serves as an immunological barrier by secreting digestive enzymes and hormones in the enterocytes. A healthy immunity of animals is of particular importance for an efficient nutrient utilization and growth performance.

Some functional AA are involved in immune system functioning (Li et al., 2007). During immune system stimulation (ISS), nutrients are redirected away from growth and towards tissues involved in immune response (Reeds and Jahoor, 2001). This implies that the requirement some key AA for the production of compounds that are involved in the immune

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response will be increased. The application of the "functional AAs" concept is one of the possible solutions to maintain gut health and promote growth because it can enhance immune status, gut integrity for weaned pigs especially when sanitary and climatic conditions are challenging, and the antibiotic growth promoters (AGPs) are not or less used in the diets.

Protein and amino acids

Normally the amount of crude protein in ingredients and diets is calculated by analyzing the concentration of N and multiplying by 6.25 (Lewis, 2001). Proteins are formed by block chains of amino acids (AA). Amino acids are organic compounds constituent of an amino and a carboxylic acid group, as well as a side chain that is specific for each AA.

Initial thoughts over the importance of protein inclusion in diets were proved incorrect when a series of experiments in the early 1950s demonstrated that pigs fed with crystalline AA and not bound protein diets were able to grow (Shelton et al., 1950; Beeson et al., 1951, Mertz et al., 1952). Thus, suggesting the importance of amino acid supplementation more than the bound protein constituent in diets. Around 20 AA are commonly present in dietary proteins and are classified into essential and non-essential AA (Table 1). Essential is referred to AA that cannot be synthesized by the body and needs to be provided in the diet. Whereas, non-essential can be synthesize by the body, so the supplementation of this AA in diet is not required. Some AA are called conditionally essential, this means that under certain situations they can be essential, for example glutamine in younger pigs (Lewis, 2001).

Importance of formulating diets with an AA profile more than just protein level was addressed several decades ago, however, the understanding of the role of AA beyond protein synthesis for maintenance and growth is in process. Functional AA play an important role under health challenge conditions and during environmental stress. Thus, a fix ratio of AA may not be the right approach for FAA but considering a dynamic ratio may be more suitable depending on the situation.

Table 1. Nutritional classification of Amino acids into essential and nonessential for swine

Essential	Nonessential
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic acid
Leucine	Cysteine
Lysine	Glutamic acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline*
Tryptophan	Serine
Valine	Tyrosine

*Proline is essential in young pigs due to limited amount of enzymes needed to synthesis proline

Functional Amino acids

In addition to primary role of serving as a building block for protein synthesis, AA are also involved in various important metabolic pathways in the body. Amino acids that regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms are defined as functional AA (Wu, 2009). In general, AA regulate the immune system by: 1) enhancing the immune status to prevent infections, and 2) reducing or eliminating established infections such as inflammation and autoimmunity (Yoneda et al., 2009). The most important functional AA include sulfur amino acids, i.e. methionine and cysteine (SAA; Met + Cys), tryptophan (Trp), threonine (Thr), glutamine (Gln), arginine (Arg) and glycine (Gly; Li et al., 2007). In the following, the important functions beyond growth and beneficial effects of dietary supplementation with the functional AA, Met + Cys, Thr and Trp in pigs are summarized.

Sulfur-Amino acids

Methionine (Met) is an essential amino acid and is usually the third limiting amino acid after lysine and threonine in typical swine diets. When supplying Met in swine diets it is important to consider its vital metabolic functions beyond growth performance. These include serving as a methyl donor for DNA methylation, synthesis of creatine and polyamine, and synthesis of glutathione, a major intracellular antioxidant involved in immune functions. This function becomes increasingly important when pigs are exposed to immune challenge or heat stress conditions because glutathione is involved in the activation of T-lymphocytes and cytokines production, and in scavenging free radicals and reactive oxygen species (Wu et al., 2004; Lu, 2009).

Cystine (Cys) can be converted from Met, particularly when dietary Cys level is deficient. Cysteine is the precursor of glutathione (GSH), that plays an important role as the most powerful intracellular antioxidant. Glutathione is formed by glutamic acid, cysteine, and glycine (Wu et al., 2004). Normally reactive oxygen species (ROS) are produced during metabolism process inside the mitochondria, as well as during activation of the immune system by immune cells and during inflammation or induce by foreign contaminant substances (Le Floc'h et al., 2018). Glutathione (GSH) serves as scavenger of free radicals and ROS within the cell, thus GSH is the reduced form, whereas GSSG is the disulfide-oxidized form. The ratio of GSH and GSSG indicates the redox potential. A low ratio indicates that the animal is under oxidative stress (Fang et al., 2002). In addition, GSH helps with the production of cytokines activating T-lymphocytes and leukocytes (Lu, 2009; Wu et al., 2004). Cysteine is maintained at low levels inside the cell due to its unstable nature and toxic properties, enough to serve as precursor for GSH or protein synthesis. Whereas, in the extracellular fluids Cys is rapidly oxidized to the dimerized form of cystine (Grimble, 2002; Stipanuk and Ueki, 2011).

An optimal ratio of both sulfur amino acids (SAA; Met+Cys) to lysine (Lys) is usually considered for practical feed formulation. The deficiency of Met in pig diets typically results in reduced pig growth performance, and as Met cannot be converted from Cys, Met:Lys ratio should be also considered to make sure enough Met is supplied.

During the immune challenge, utilization of Met+Cys to produce compounds involved in the immune response, such as glutathione and taurine, is increased (Grimble, 2002). A recent study with piglets, Rasch et al. (2019) found that supplementation with DL-Met or L-Met to a Met-deficient diet increases the transsulfuration rate of Met to form Cys and consequently glutathione. Indeed, the need for Met+Cys to be supplied in the diet increases during the immune challenge situation. Rakhshandeh et al. (2014) reported that the immune challenge by injection of lipopolysaccharide (LPS) reduced body protein deposition but increased maintenance requirement for Met+Cys. They estimated that normal pigs need 1.63 g SID Met+Cys intake, while immune challenged pigs need 1.87 g SID Met+Cys intake to achieve the same body protein deposition of 50 g/d, which is an increase by 15% of SID Met+Cys (Figure 1). Other studies support a higher SID Met and Cys requirement for pigs raised under challenged conditions, such us in E. coli-infected pigs, the optimal SID Met+Cys:Lys requirement was observed at 70% (Capozzalo et al., 2017). This is 25% higher than 56% SID Met+Cys:Lys recommendation by NRC (2012).

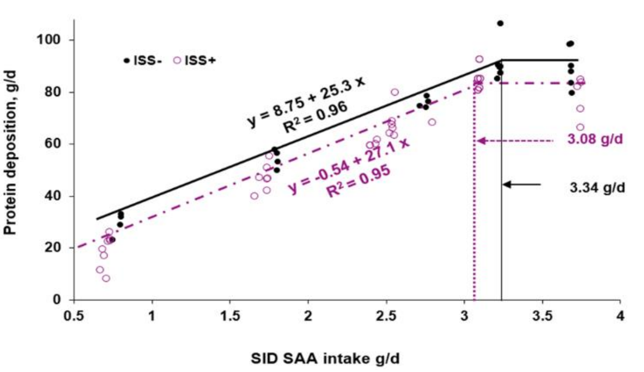

Figure 1. Impact of immune system stimulation (ISS) and standardized ileal digestible (SID).
SAA intake on body protein deposition in growing pigs

Antibiotic growth promoters have been added in pig and poultry diets to maintain gut health and to minimize infections. A higher stimulation of the immune system and a higher endogenous protein loss were observed after the ban of AGPs in animal feeds in the European Union (Roth et al., 1999). This means that pigs fed with AGP-free diets and raised under commercial conditions may be more susceptible to immune challenges. As increasing number of countries are banning AGPs in animal diets, it is important to reconsider the Met+Cys requirements for pigs fed AGP-free diets and raised under commercial conditions.

A study conducted under commercial conditions in China also showed that ADG and feed conversion ratio (FCR) of growing pigs were optimized at a SID Met+Cys:Lys ratio of 63% (Zhang et al., 2015; Table 2), which is 12.5% higher than the current NRC (2012) recommendation. Gaines et al. (2004) observed an increased in ADG and G:F by increasing SID SAA:Lys ratio from 50 to 70% in 29-45 kg pigs. Similarly, Yi et al. (2005), based on broken-line and quadratic regression analysis of ADG and G:F responses, estimated the average optimal SID SAA:Lys ratio of 61% for both 31-49 kg PIC gilts and barrows. Similarly, the dietary SID SAA:Lys to maximize body protein deposition increases from 55 to 75% when growing pigs are immune challenged with LPS (Kim et al., 2012).

Table 2. Effect of dietary SID Met+Cys:Lys ratio on the performance of growing pigs

	SID Met+Cys:Lys ratio, %				
Items	50	55	60	65	70
ADG (g/d)	697[a]	738[b]	751[bc]	764[c]	754[bc]
Feed intake (g/d)	1662	1667	1645	1650	1645
FCR (g/g)	2.38[c]	2.26[b]	2.19[a]	2.16[a]	2.18[a]

[a,b,c] Means in a row with different letters are different ($P < 0.05$).

Besides health challenge conditions, growing pigs under heat stress challenge were able to restore growth performance closer to pigs kept under thermoneutral conditions if SID Met+Cys:Lys was increased by 20% above NRC recommendations (Morales et al., 2021). Additional supplementation of SID Met+Cys:Lys by 40 and 60% above NRC recommendations did not provide any additional support under heat stress. Nonetheless, it is important to keep in mind that under non-challenge condition, excess of SAA may not be needed and may reduce performance (Baker, 2006).

In a nitrogen balance study with growing pigs, immune challenge (LPS injection) reduced protein deposition rate but the optimal dietary Met to Met+Cys ratio to maximize body protein deposition increased from 57 to 59% (Litvak et al., 2013; Figure 2). These results indicate that the needs of Met+Cys, including the Met requirement for converting to Cys, are increased during immune challenge conditions, and the impact of immune status on Met+Cys requirement should be considered when formulating diets for pigs. In addition, because Met cannot be converted from Cys, Met:Lys ratio or Met:Met+Cys should not be ignored.

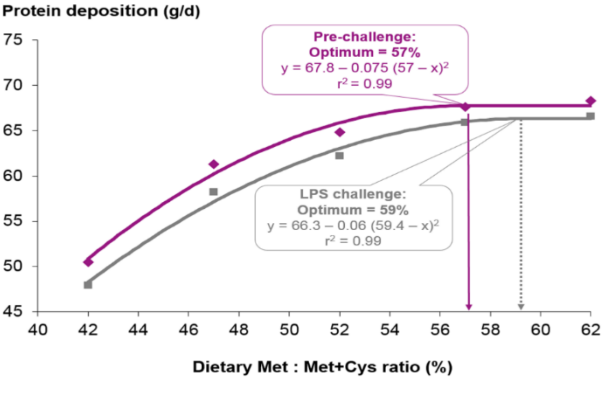

Figure 2. Effect of immune challenge and Met:Met+Cys ratios on body protein deposition

Threonine

Threonine (Thr) is usually the second- or third-limiting AA in typical cereal-based swine diets. In addition to its primary role in protein synthesis, Thr is involved in physiological functions of the gut. Among the essential AA, Thr is particularly important for synthesis of intestinal mucosal proteins and mucins. The synthesis of mucosal proteins in the GI tract includes the secretion of proteins that are secreted into the lumen, including mucins which protect the gut from injury and pathogens. Mucins are particularly rich in Thr, which represents about 30% of the total AA of mucins and 11 % of the total endogenous protein in ileal digesta of pigs (Lien *et al.*, 1997). Mucin proteins are continuously synthesized and resistant to digestion. This mean that an increase in mucus secretion will directly increase endogenous losses of AA particularly Thr. Thus, Thr plays an important role in the maintenance of intestinal health and gut barrier integrity (Bertolo et al. 1998).

Inadequate dietary Thr supply to piglets caused increased incidence of diarrhea, decreased mucosal weight and mucin secretion along the GIT (Law *et al.*, 2007), and reduced villus height and villus height to crypt depth ratio in the ileum (Hamard *et al.*, 2007). Feeding wheat bran and barley-based diets which are high in fiber (hemicellulose) increase endogenous losses of Thr in growing pigs compared with those fed a casein-based diet (Myrie *et al.*, 2008). Mathai et al. (2016) reported that the average optimum SID Thr:Lys ratio for ADG and G:F of 25-50 kg pigs was 67% when fed high fiber diet (contained 16.7% NDF) while 65% was optimal for pigs fed a low fiber diet (7.8% NDF) indicating that feeding diets high in fiber increases the demand for Thr for mucin production relative to body growth in pigs. Zhang et al. (2013) observed a clear response of ADG and G:F by increasing SID Thr:Lys ratio from 55 to 75% in low CP-AGP free diets fed to 25-50 kg pigs raised under commercial conditions in China. The average SID Thr:Lys ratio to optimize ADG and G:F was 70% based on broken-line and quadratic regression. These estimates are 11 to 17% higher than the NRC (2012) recommendations of 60 and 63% for the corresponding BW ranges.

Use of dietary AGP helps maintaining animal's health status. Feeding animals AGP-free diets may allow higher microbial growth in the gut and potentially affect the gut health and AA utilization. Bikker et al., (2007) reported that the SID Thr:Lys ratio to optimize ADG was 9% higher (71%) for 25-110 kg pigs fed AGP-free diet compared with those fed AGP-added diet (65%; Figure 3). It is possible that more dietary Thr is utilized to enhance the immune system through its incorporation into immunoglobulin (Li et al., 1999).

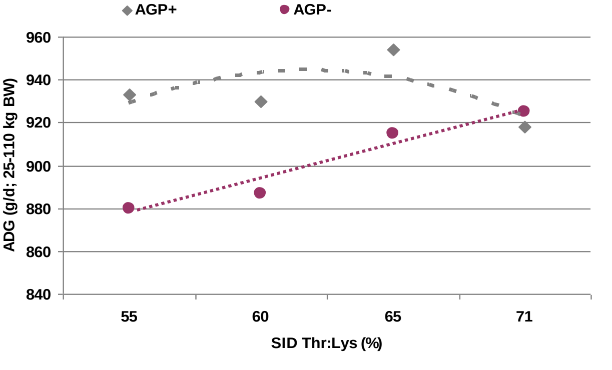

Figure 3. Effect of dietary SID Thr:Lys ratio on average daily (ADG) gain of pigs fed diets with or without AGP (Bikker et al., 2007)

Tryptophan

Tryptophan (Trp) is usually considered the 4th limiting AA in typical cereal-based swine diets. Tryptophan is required not only for protein synthesis but also involved in various metabolic pathways including immune response via its metabolism through the kynurenine pathway and the formation of serotonin which involves in behaviour and feed intake regulation (Baranyiova, 1991; Henry et al. 1992; Koopmans et al., 2006). Around 5% dietary Trp is utilized for protein synthesis, whereas the other 95% of enters the Kynurenine pathway (Botting, 1995). Furthermore, Trp can be used to synthesize a neurohormone melatonin, which may act as free radical scavengers and have antioxidant properties (Le Floc'h and Seve, 2007).

Pigs kept under poor sanitary conditions can induce a moderate inflammatory response in pigs. Le Floc'h et al. (2007) reported that the optimal feed intake and ADG of weaned pigs kept under poor sanitary conditions were achieved at 21% Trp:Lys ratio whereas 18% was sufficient for pigs kept under good sanitary conditions (Figure 4).

The variations in optimum dietary Trp:Lys ratio among published data may be attributed to differences in health status, statistical models, and the use of AGPs and digestibility of Trp in feed ingredients used.

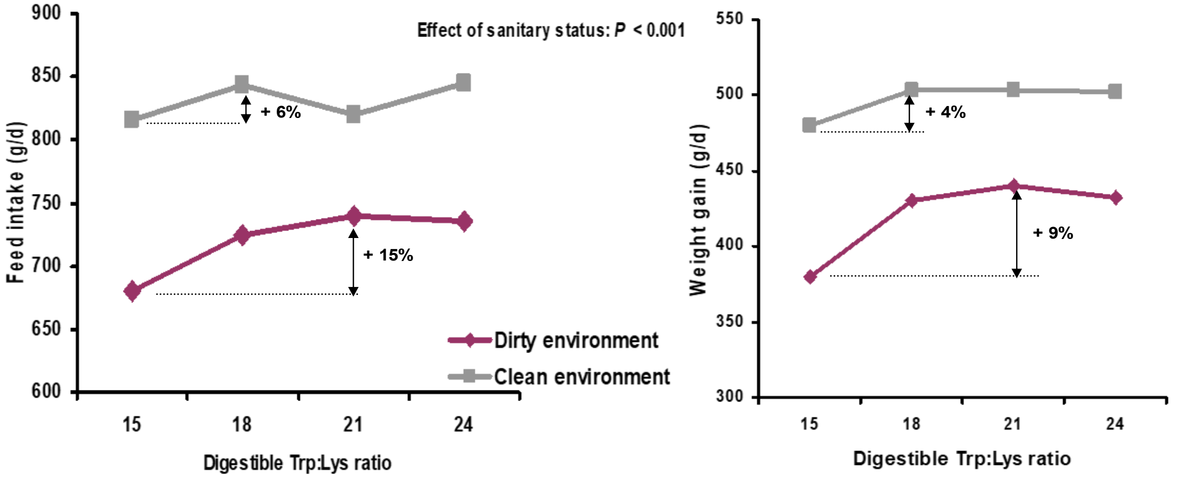

Figure 4. Effect of sanitation and dietary SID Trp:Lys ratio on feed intake and body weight gain of pigs

Millet et al. (2015) reported the average SID Trp:Lys ratio to optimize the ADG and FCR of 20-55 kg high lean gain pigs based on broken-line regression was 21%. Quant et al. (2012) evaluated the effect of ingredient composition on Trp:Lys ratio of 25-40 kg pigs fed diets containing AGP. They found an average optimal SID Trp:Lys ratio of 18% when pigs were fed corn, pea, SBM diets whereas the optimal ratio was 17% when corn, pea, barley, wheat diets were fed. Zhang et al. (2012) reported the average SID Trp:Lys of 21% to optimize the ADG, FCR or N retention of 25-50 kg crossbred pigs fed AGP-free diets (Table 3). Similarly, van der Aar et al. (2012), based on broken-line regression, observed that the ADG, FCR and feed intake of 25-55 kg crossbred gilts maximized at 20% SID Trp:Lys ratio. More recently, Xie et al. (2014), based on broken-line and quadratic regressions, estimated 22% SID Trp:Lys as optimal for ADG and FCR of 67-96 kg pigs fed low CP-AGP free diets. Additionally, effects of Trp under high stress levels due to weaning and mixing have been evaluated.

Pigs fed extra supplementation of Trp (5 g/kg of feed, as-fed basis) had less intestinal atrophy than control, most likely by the influence in blood flow by the sympathetic nervous system (Koopmans et al., 2006). In addition, less cortisol levels and less physical activity on day 10 were observed.

Table 3. Effect of dietary SID SID Trp:Lys ratio on the performance of growing pigs

	SID Trp:Lys ratio, %				
Items	13	16	19	22	25
ADG (g/d)	559[c]	629[b]	684[a]	706[a]	697[a]
Feed intake (g/d)	1353	1463	1534	155 3	1545
FCR (g/g)	2.42[c]	2.33[b]	2.24[a]	2.20 a	2.22[a]

[a,b] Means in a row with different letters are different ($P < 0.05$).

Combine supplementation of FAA

As addressed before, the role of each FAA (Met+Cys, Thr, and Trp) is essential during challenged conditions, however, a combine approach of these AA may result more beneficial as it will support immune system, antioxidant status, and gut protection. This combine approach was demonstrated in recent experiments with pigs challenged with *Salmonella* Typhimurium fed diets containing either standard AA profile or 20% extra supplementation of Met+Cys, Thr, and Trp diets (Rodrigues et al., 2021a, 2021b, 2022). Pigs fed extra FAA supplementation were able to reduce the infection with *Salmonella* Typhimurium more than pigs fed standard AA profile (Rodrigues et al., 2021a). This resulted in less oxidant stress and inflammation and therefore a better growth performance was observed. Because pigs were fed experimental diets 14 days prior to the challenge, it was suggested that pigs fed extra FAA supplementation were better prepared in terms of immune support, antioxidant status, and gut protection at the time of the challenge. Thus, a follow up study was conducted to evaluate the influence of pre-exposure to higher FAA ratios before a challenged (Rodrigues et al., 2021b). Pigs were fed diets containing 20% extra FAA supplementation either 14, 7, or 0 days before challenge with *Salmonella* Typhimurium as well as during the challenge. Pigs fed the longest period, 14 d pre-challenge, with extra FAA supplementation resulted in the least fecal Salmonella shedding and serum haptoglobin, resulting in the best growth performance. Interestingly, the antioxidant status was not different between 0- and 14-days pre-challenge of FAA supplementation. However, FAA supplementation during challenge resulted beneficial increasing GSH:GSSG compared to non-supplemented pigs. Pigs challenged with PRRS were fed with either standard AA, 25% or 50% extra supplementation of Met, Thr, Trp, Arg, and His of NRC (2012) recommendations (Weaver et al., 2022). Diet with 25% extra FAA supplementation increased performance and livability of PRRS challenged pigs compared to the other 2 treatments. Response to extra FAA supplementation is more consistent in normal weight pigs compared to low weight pigs (Rodrigues et al., 2022). Thus, different levels of FAA or complementary strategies should be considered to support low weight pigs under challenged conditions.

Conclusions

In conclusion, AAs are involved in important metabolic pathways beyond growth, such as the regulation body's immune system, antioxidant status, and gut protection. This means that some key AAs such as Met+Cys, Trp, and Thr, under challenged conditions, are prioritized to form compounds involved in the immune response, antioxidant capacity and gut mucus resulting in compromised growth. This redirection of key AAs implies that dietary supply of these AAs should be increased to maintain optimal immune status and antioxidant capacity, minimizing reduction on growth. Recent trials with 20% extra supplementation of Met+Cys, Trp, and Thr have reduced the negative impact on animal performance under challenge conditions. Future research is warranted to quantitatively estimate the increased need of these functional AA to excerpt improvement in immunity, antioxidant capacity, gut health and performance of pigs raised under sub-optimal conditions. Furthermore, there is a need to access the interaction or synergetic effect of various AA on animal's health and performance of pigs raised under sub-optimal conditions and further research is needed to develop strategies how to support low weight pigs.

Literature Cited

Baker, D. H. 2006. Comparative Species Utilization and Toxicity of Sulfur Amino Acids. J. Nutr.136:1670S–1675S, https://doi.org/10.1093/jn/136.6.1670S

Baranyiova, E. 1991. Effect of serotonin on food intake by piglets during the early postnatal period. Acta Vet. (Brno) 60:127–136.

Beeson, W. M., E. T. Mertz, and D. C. Shelton. 1951. Effect of arginine, leucine, phenylalanine, and valine on growth of weanling pigs. J. Anim. Sci. 10:1037.

Bertolo, R. F., C. Z. Chen, G. Law, P. B. Pencharz, and R. O. Ball. 1998. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. Journal of Nutrition 128:1752-1759.

Bikker, P., J. Fledderus, L. Le Bellego, and M. Rovers. 2007. Growth response of pigs to dietary threonine:lysine ratio is affected by the withdrawal of antimicrobial growth promoters. European Association for Animal Production 124:557-560.

Bischoff, S. C. 2011. Gut health: a new objective in medicine? BMC Medicine. 9:24.

Botting, N. P. 1995. Chemistry and neurochemistry of the kynurenine pathway of tryptophan metabolism. Chem. Soc. Reviews. 24:401-412.

Capozzalo, M.M., Resink, J.W., Htoo, J.K., Kim, J.C., de Lange, F.M., Mullan, B.P., Hansen, C.F. and J. R. Pluske 2017. Determination of the optimum standardized ileal digestible sulphur amino acids to lysine ratio in weaned pigs challenged with enterotoxigenic Escherichia coli. Animal Feed Science and Technology, 227: 118-130.

Fang, Y. Z., S. Yang, and G. Wu. 2002. Free radicals antioxidants, and nutrition. Nutrition 8, 872-879.

Gaines, A.M., G.F. Yi, B.W. Ratliff, P. Srchana, G.L. Allee, C.D. Knight, and K.R. Perryman, 2004. Estimation of the true ileal digestible sulfur amino acid:lysine ratio for growing pigs weighing 29 to 45 kilograms. J. Anim. Sci. 82(Supp. 1):294-295.

Grimble, R. F. 2002. Sulphur amino acids, glutathione and immune function. Pages 133-150 in: Nutrition and Immune Function; Calder PC, Field CJ, Gill HS. (eds.); CABI Publishing: New York, NY, USA.

Hamard, A., B. Seve and N. Le Floch. 2007. Intestinal development and growth performance of early-weaned piglets fed a low-threonine diet. Animal 1: 1134-1142.

Henry, Y., B. Sève, Y. Colléaux, P. Ganier, C. Saligaut, and P. Jego. 1992. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 70: 1873-1887.

Kim, J. C., B. P. Mullan, B. Frey, H. G. Payne and J. R. Pluske. 2012. Whole body protein deposition and plasma amino acid profiles in growing and/or finishing pigs fed increasing levels of sulfur amino acids with and without *Escherichia coli* lipopolysaccharide challenge. Journal of Animal Science 90:362-365.

Koopmans, S. J., A. C. Guzik, J. van der Meulen, R. Dekker, J. Kogut, B. J. Kerr, and L. L. Southern. 2006. Effects of supplemental L-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. J. Anim. Sci. 84:963–971

Law, G. K., R. F. Bertolo, A. Adjiri-Awere, P. B. Pencharz and R. O. Ball. 2007. Adequate oral threonine is critical for mucin production and gut function in neonatal piglets. American Journal of Physiology-Gastrointestinal and Liver Physiology 292: G1293-G1301.

Le Floc'h, N., and B. Seve. 2007. Biological roles of tryptophan and its metabolism. Potential implications for pig feeding. Livest. Sci. 112:23-32.

Le Floc'h, N., D. Melchior, L. Le Bellego, J. J. Matte and B. Sève. 2007. Does sanitary status have an effect on tryptophan requirement for growth of postweaning piglets? (in French, with English abstract). Journees Rech. Porcine en France 39:125-132.

Le Floc'h, N., D. Melchior, B. Seve. 2008. Dietary tryptophan helps to preserve tryptophan homeostasis in pigs suffering from lung inflammation. J. Anim. Sci. 86, 3473–3479. https://doi.org/10.2527/jas.2008-0999

Lewis, A. J. 2001. Amino acids in swine nutrition. Pages 131-150 in Swine Nutrition. Edited by A. J. Lewis, and L. L. Southern, 2nd ed. Boca Raton, Florida.

Li, D. F., C. T. Xiao, S. Y. Qiao, J. H. Zhang, E. W. Johnson and P. A. Thacker. 1999. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. Animal Feed Science and Technology 78:179-188.

Li, P., Y-L. Yin, D. Li, S. W. Kim and G. Wu. 2007. Amino acids and immune function. Br J Nutr 98, 237-252.

Lien, K. A., W. C. Sauer and M. Fenton. 1997. Mucin output in ileal digesta of pigs fed a protein-free diet. Zeitschrift für Ernährungswissenschaft 36: 182-190.

Litvak, N., A. Rakhshandeh, J. K. Htoo and C. F. M. de Lange. 2013. Immune system stimulation increases the optimal dietary methionine to methionine plus cysteine ratio in growing pigs. Journal of Animal Science 91:4188-4196.

Lu, S. C. 2009. Regulation of glutathione synthesis. Molecular Aspects of Medicine. 30:42-59.

Mathai, J. K., J. K. Htoo, J. E. Thomson, K. J. Touchette and H. H. Stein. 2016. Effects of dietary fiber on the ideal standardized ileal digestible threonine:lysine ratio for twenty-five to fifty kilogram growing gilts. J. Anim. Sci. 2016.94 (doi:10.2527/jas2016-0680).

Mertz, E. T., W. M. Beeson, and H. D. Jackson. 1952. Classification of essential amino acids for the weanling pig. Arch. Biochem. Biophys. 38:121.

Millet, S., A. Marijke, E. L. Gall, E. Corrent, J. de Sutter and B. Ampe. 2015. The tryptophan requirement of growing pigs (20 - 55 kg). Journées Recherche Porcine, 47, 137-138.

Morales, A., V. Sánchez, B. Pérez, L. R. Camacho, J. C. González-Vega, J. K. Htoo, and M. Cervantes. 2021. Effect of Supplemental Dl-met Above Requirement on Performance and Serum Concentration of Amino Acids in Heat Stressed Pigs. J. Anim. Sci. Issue Suppl. 1:59-60 https://doi.org/10.1093/jas/skab054.099

Myrie, S. B., R. F. Bertolo, W. C. Sauer and R. O. Ball. 2008. Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine. Journal of Animal Science 86: 609-619.

NRC. 2012. Nutrient requirements of swine. 12th ed. Natl. Acad. Press, Washington, DC.

Pastorelli, H., J. van Milgen, P. Lovatto and L. Montagne. 2012. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. Animal 6, 952-961.

Quant, A. D. M. D. Lindemann, B. J. Kerr, R. L. Payne and G. L. Cromwell. 2012. Standardized ileal digestible tryptophan-to-lysine ratios in growing pigs fed corn-based and non-corn-based diets. J. Anim. Sci. 90:1270-1279.

Rakhshandeh, A., J. K. Htoo, N. Karrow, S. P. Miller and C. F. M. de Lange. 2014. Impact of immune system stimulation on ileal nutrient digestibility and utilization of methionine plus cysteine intake for whole body protein deposition in growing pigs. Br J Nutr 111, 101-110.

Rasch, I., S. Görs, A. Tuchscherer, J. K. Htoo, B. Kuhla, C. Metges. 2019. Substitution of dietary sulfur amino acids by DL-2-hydroxy-4methylthiobutyric acid increases remetylation and decreases transsulfuration in weaned piglets. J. Nutr. 149:432-440.

Reeds, P. J. and F. Jahoor. 2001. The amino acid requirements of disease. Clin Nutr 20 (S1): 15-22.

Rodrigues, L. A., M. O. Wellington, J. C. González-Vega, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2021a. Functional amino acid supplementation, regardless of dietary protein content, improves growth performance and immune status of weaned pigs challenged with *Salmonella* Typhimurium. J. Anim. Sci. 99:1-13 doi:10.1093/jas/skaa365

Rodrigues, L. A., M. O. Wellington, J. C. González-Vega, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2021b. A longer adaptation period to a functional amino acid-supplemented diet improves growth performance and immune status of Salmonella Typhimurium-challenged pigs. J. Anim. Sci. 99, 1–11.

Rodrigues, L. A., J. C. Panisson, L. A. Kpogo, J. C. González-Vega, J. K. Htoo, A. G. Van Kessel, and D. A. Columbus. 2022. Functional amino acid supplementation postweaning mitigates the response of normal birth weight more than for low birth weight pigs to a subsequent Salmonella challenge. Animal 16: 100566 https://doi.org/10.1016/j.animal.2022.100566

Roth, F. X., G. G. Gotherbarm, W. Windisch, and M. Kirchgissner. 1999. Whole-body protein turnover and nitrogen balance in growing pigs supplied with an antibiotic feed additive (Avilamycine). Journal of Animal Physiology and Animal Nutrition 82:88-93

Shelton, D. C., W. M. Beeson, and E. T. Mertz. 1950. Growth of weanling pigs on a diet containing ten purified amino acids. Arch. Biochem. Biophys., 26:446

Stipanuk, M. H., and I. Ueki. 2011. Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. J. Inherited Met. Diseases 34(1):17-32.

Van der Aar, P., P. Bikker, M. Rovers and E. Corrent. 2012. Tryptophan requirements of growing and finishing pigs. Journées Recherche Porcine, 44, 205-206.

Weaver, E., R. Samuel, and B. Thaler. 2022. Research has found that amino acid supplementation may benefit feed intake, gut health and immunity when disease has altered the health status of the animal. National Hog Farmer July 14, 2022.

Wu, G., Y. Fang, S. Yang, J. R. Lupton, and N. D. Turner. 2004. Glutathione metabolism and its implications for health. Journal of Nutrition 134:489-492.

Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids. 37:1-17.

Xie, C. Y., G.J. Zhang, F.R. Zhang, S.H. Zhang, X.F. Zeng, P.A. Thacker, S.Y. Qiao. 2014. Estimation of the optimal ratio of standardized ileal digestible tryptophan to lysine for finishing barrows fed low protein diets supplemented with crystalline amino acids. Czech J. Anim. Sci., 59 (1): 26–34.

Yi, G. F., A. M. Gaines, B. W. Ratliff, P. Srichana, G. L. Allee, C. D. Knight, and K. R. Perryman. 2005. Estimation of the true ileal digestible sulfur amino acid:lysine ratio for growing pigs weighing 28-49 kilograms. J. Anim. Sci. 83(Suppl. 1):213.

Yoneda, J., A. Andou and K. Takehana. 2009. Regulatory roles of amino acids in immune response. Curr Rheumatol Rev 5:252-258.

Zhang, G. J., Q. L. Song, C. Y. Xie, L. C. Chu, P. A. Thacker, J. K. Htoo and S. Y. Qiao. 2012. Estimation of the ideal standardized ileal digestible tryptophan to lysine ratio for growing pigs fed low crude protein diets supplemented with crystalline amino acids. Livestock Science 149: 260–266.

Zhang, G. J., C.Y. Xie, P.A. Thacker, J.K. Htoo, S.Y. Qiao. 2013. Estimation of the ideal ratio of standardized ileal digestible threonine to lysine for growing pigs (22–50 kg) fed low crude protein diets supplemented with crystalline amino acids. Animal Feed Science and Technology 180: 83– 91.

Zhang, G. J., C. J. Cai, C. Y. Xie, L. C. Chu, P. A. Thacker, J. K. Htoo, and S. Y. Qiao. 2015. Estimation of the optimal standardized ileal digestible sulfur amino acids ratio in low crude protein, amino acid supplemented diets for 25- to 50-kg pigs. Czech J. Anim. Sci. 60 (7): 302-310.