

Practical application of the ideal protein concept in pigs

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ABSTRACT

Objective: To determine the importance of the formulation of “ideal protein” diets for starting-growing pigs.

Design/methodology/approach: A bibliographic review of the concept of ideal protein and low-protein in pig diets was carried out to determine their practical application in commercial production.

Results: Low-protein diets in pig production are an environmentally friendly strategy. The 3 percentage units of reduction in CP is the maximum level, when only crystalline lysine, methionine, threonine, and tryptophan are available. However, when there is a greater number of synthetic amino acids, the reduction in CP can range from 4 to 5 percentage units.

Study limitations/implications: Market conditions allow the incorporation of lysine, methionine, threonine, and tryptophan into the diet. Potentially, owing to its availability and price, valine could be considered as part of commercial diets in a short time. However, the inclusion of other AA is not currently viable, as a result of their low availability and high market price.

Findings/conclusions: Low-protein diets should be used in pig production, since they maintain or improve the productive variables and reduce the environmental impact, as a result of the reduction of nitrogen excretion to the environment.

Keywords: synthetic amino acids, low-protein diets, environment.

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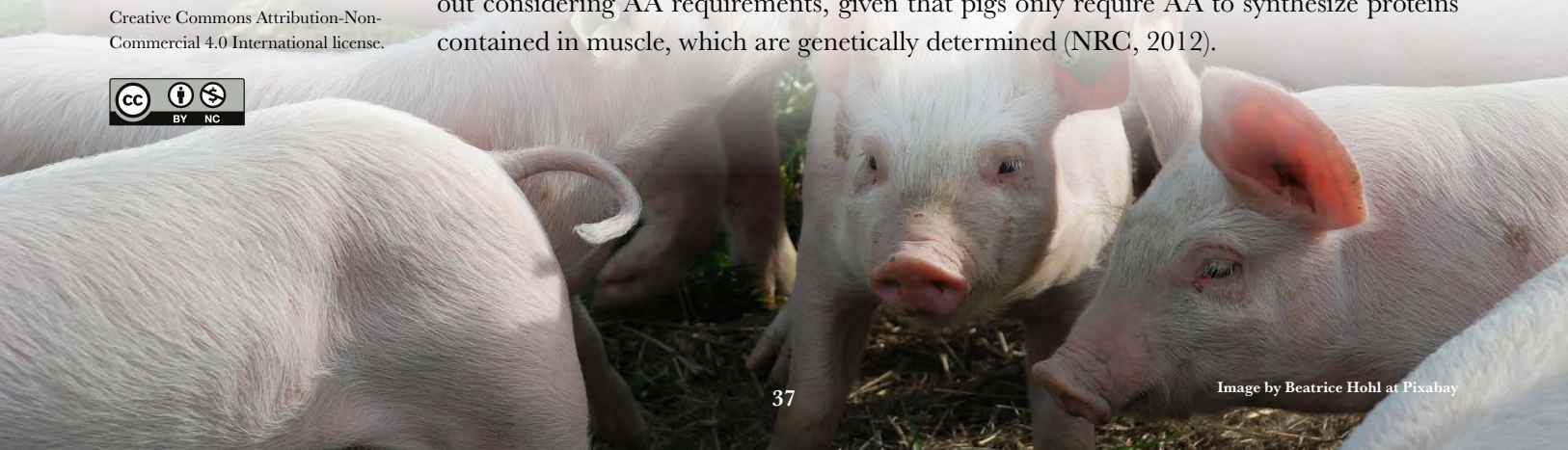
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INTRODUCTION

“Ideal protein” means that no amino acid (AA) is to be supplied in excess of requirements as compared to any other AA in pig diet: *i.e.*, an exact balance of AA, without deficiency or excess. As a consequence, protein retention (protein gain in relation to protein intake) is maximized and nitrogen excretion is minimized. This is possible through an adequate combination of protein concentrates and synthetic AA supplements (Leclercq, 1998). The use of the ideal protein concept in formulating pig diets or its practical application (low-protein diets) is an effective resource that allows a precise formulation of diets to be carried out considering AA requirements, given that pigs only require AA to synthesize proteins contained in muscle, which are genetically determined (NRC, 2012).



A diet formulation based on ideal protein and low-protein diets (LPDs) requires a vast amount and variety of high-quality ingredients or the availability of plenty essential synthetic amino acids at an affordable price for producers. LPDs supplemented with synthetic amino acids allow for a reduction in protein ingredients, at a lower feed cost and an economic impact that benefits pig farmers (Wang *et al.*, 2020). Over the last years, as a consequence of the boom in synthetic AA production, a window of possibilities has opened up for the ideal protein concept to be put into practice (Gloaguen *et al.*, 2014; Peng *et al.*, 2016). The aim of this review was to determine the importance of diet formulation for starting-growing pigs under the concept of “Ideal Protein”.

Low-protein diets (LPDs)

The reduction in crude protein (CP) in sorghum, corn, soymeal or canola-based diets — associated with an adequate addition of synthetic amino acids (AA)— allows for a reduction in AA deficiency or excess in balanced feeds (ideal protein) (Wang *et al.*, 2020), which may go hand in hand with a reduction in production costs, as well as in the excretion of fecal and urinary nitrogen to the environment (Seradj *et al.*, 2018; Wu *et al.*, 2018; Niyazov *et al.*, 2020). A reduction of 10 g kg⁻¹ of CP in diet may lower ammonia emissions in feces and urine by 8% to 10% (Wang *et al.*, 2018). Low-protein diets may also reduce the characteristic odor of pig holdings. Odor emissions were reduced by 4.2% for every percentage point of CP lowered in the diet (Trabue *et al.*, 2021), reaching up to 30% by lowering 3-4 percentage points of CP (Hayes *et al.*, 2004; Leek *et al.*, 2007).

Productive performance and diet formulation

CP reduction in diets for starting and growing pigs results in a productive performance similar to standard diets and even improves on certain productive variables (greater weight gain and feed efficiency) (Gloaguen *et al.*, 2014; He *et al.*, 2016; Peng *et al.*, 2016; Figueroa *et al.*, 2019; Wang *et al.*, 2019). This is the result of a better balance between AA for protein synthesis and the fact that AA are not used as a source of energy. However, CP reduction in diet should be no higher than 3 percentage points when only synthetic lysine, methionine, threonine, and tryptophan are used, given its impact on productive performance (associated to a lower lean meat yield and a greater fatty tissue accumulation) (Zamora *et al.*, 2011; Gloaguen *et al.*, 2014; He *et al.*, 2016). The negative response to low-CP diets may be caused by AA deficiency, given that CP reduction limits the concentration of some essential AA, as well as the amount of nitrogen needed for non-essential AA synthesis (Gloaguen *et al.*, 2014).

Although the recommendations of the NRC (2012) do not set a CP value, the sum of the values of each AA results in the approximate CP value. However, if only the four most common commercially-available synthetic AA (lysine, methionine, threonine, and tryptophan) can be included in the standard diet (NRC, 2012), the CP value provided by the formulation only allows for a reduction of approximately 1.5 percentage points of CP with regard to the NRC's (1998) recommendations, if the remaining AA requirements are to be met.

In order to find alternatives to make up for AA deficiency with a CP reduction in diet, there is now a wider variety of synthetic AA available for research or practical application (lysine, methionine, threonine, tryptophan, valine, histidine, leucine, isoleucine, proline, phenylalanine, arginine, and glutamine); however, some of these AA are not readily available in the market or they are exceedingly expensive with regards to the expected financial gain (histidine, leucine, isoleucine, proline, phenylalanine, and glutamine). Through the use of the aforementioned synthetic AA, it is theoretically feasible to balance and drastically reduce protein content in diet (NRC, 2012). However, using nearly 10 synthetic AA in diet allows for a maximum reduction of 4-5 percentage points of CP (Gloaguen *et al.*, 2014; Peng *et al.*, 2016). A higher percentage leads to the growth and development of defective organs, associated with alterations in intestinal morphology and immune function (Peng *et al.*, 2016), as well as a low availability of nitrogen for the synthesis of other AA (Gloaguen *et al.*, 2014).

The low productive performance observed with properly-balanced LPDs when a large amount of synthetic AA is added may be caused by an intact protein deficiency or an excess of free AA (Wang *et al.*, 2018). The inclusion of protein-bound AA is more efficient in sustaining nitrogen retention and protein homeostasis in the whole body than free AA (Guay *et al.*, 2006). Additionally, hydrolyzed dipeptide and tripeptide content from intact proteins correlates positively with digestive enzyme activity (Shimizu, 2004). The fastest rate of free AA absorption may induce an excessive AA oxidization, contributing to a decline in the excretion of body proteins and defective growth (Yen *et al.*, 2004).

Another important aspect to be considered is that, under the concept of diet formulation for pigs at a minimum cost, it is economically more feasible to exceed the NRC's recommendations (2012) of certain AA in diet than to attempt to reach the lowest (optimal) values (Dubeau *et al.*, 2011), since basic ingredients (sorghum, corn, soy, and canola) have a high concentration of these AA. Therefore, when attempting to include a large amount of synthetic AA in diets, the fact that this leads to a reduction in base protein ingredients (Gloaguen *et al.*, 2014; Peng *et al.*, 2016; Figueroa *et al.*, 2019) must be considered. Consequently, an economic assessment must be carried out to compare the substitution of one raw material for another.

Low Protein Diets and meat quality

A CP reduction of less than two percentage points in pig diet does not affect the percentage of lean meat nor fat in the carcass (Zamora *et al.*, 2011; Qin *et al.*, 2015; Figueroa *et al.*, 2019). There is also evidence that neither sex nor genetics impact the reduction of 2 percentage points of CP over meat percentage, meat yield in the carcass or backfat thickness (Molist *et al.*, 2016). However, González *et al.* (2016) observed that a reduction of more than three percentage points of CP in pig diet lowered lean meat percentage while increasing fat in the carcass, possibly as a result of a greater availability of net energy for the accumulation of fatty tissue (Li *et al.*, 2016). In a work carried out by Li *et al.* (2018), a reduction of more than three percentage points of CP increased the red color value (a^*), intramuscular fat, monounsaturated fatty acid content, and AA content

related to meat flavor, while lowering the content of polyunsaturated fatty acids and the cutting force of meat.

Gut health

Diet manipulation regarding CP content has been suggested as part of a general strategy for the nutritional management of weaned pigs in order to improve gut health. The use of LPD minimizes the amount of undigested dietary protein that enters the large intestine and is subjected to bacterial fermentation; this is important because protein fermentation leads to the production of toxic metabolites and encourages the proliferation of pathogenic bacteria, causing enteric problems such as post-weaning diarrhea. LPD feeding interferes with enterotoxigenic *E. coli* adhesion to intestinal mucosa, thus minimizing its ability to cause disease (Nyachoti and Lee, 2020).

A moderate restriction of dietary CP can modify the composition of intestinal microbiota (Zhou *et al.*, 2016) and improve the function of the ileal barrier in adult pigs (Fan *et al.*, 2017). A low-protein diet is recommended over a diet with proteins exceeding nutritional needs. The highest levels and undigested protein lead to an increase in pathogenic microorganisms, increasing the associated risk of developing a metabolic disease (Zhao *et al.*, 2019). With moderate CP restriction, pigs are able to adjust their absorption and intake of nutrients to sustain growth, whereas extremely low-protein diets suppress appetite, alter intestinal morphology, decrease lactobacilli and streptococci, and reduce energy intake in pigs (Yu *et al.*, 2019).

On the whole, adequate LPD management can reduce the incidence of post-weaning diarrhea, sustain gut health, and modify intestinal morphology and microbiota (Ren *et al.*, 2015; Wang *et al.*, 2018). The use of LPD reduces 2-3 percentage points of CP in diet through synthetic AA supplements for post-weaning pigs and, therefore, can be effective in reducing diarrhea, while improving fecal consistence (Lynegaard *et al.*, 2021). In addition, moderate protein restriction (−3 percentage points) can optimize the structure of ileal microbiota, strengthening beneficial microbial populations and suppressing the growth of harmful bacteria, as well as modifying the proliferation of epithelial cells (Chen *et al.*, 2018).

There is evidence that reducing CP levels in diet within a range no greater than three percentage points does not modify the integrity of intestinal morphology (Ren *et al.*, 2015; Chen *et al.*, 2018). However, a reduction greater than four percentage points results in reduced villi height in the duodenum and the jejunum, even when diets are complemented with isoleucine, valine, histidine, and phenylalanine (Fan *et al.*, 2017).

The reduction in villi height in LPD-fed pigs is probably related with a lower number of the proteins or AA that sustain the architecture of the intestinal epithelium (Wang *et al.*, 2018). However, including nine synthetic AA in nursery piglet diets can reduce CP by five percentage points (from 20% to 15%) with no detrimental effect on immune response; nevertheless, a greater reduction (up to 14%) led to the growth and development of defective organs, associated with modifications of intestinal morphology and immune function (Peng *et al.*, 2016).

The influence of CP levels in diet on intestinal microbiota has been more broadly studied in weaned piglets, because the bacterial composition of growing and finishing pigs remains relatively stable (Wang *et al.*, 2018). A reduction of five percentage points of CP in recently weaned piglet diet lowered counts of *Clostridium leptum* (Pieper *et al.*, 2012), while pigs fed with 14% CP displayed a lower count of *Firmicutes* and *Clostridium cluster*, than those feed with 20% CP, with a minimal impact on other bacterial populations (Luo *et al.*, 2015). For their part, Opapeju *et al.* (2015) report that a reduction of five percentage points of CP in diets for weaned pigs infected with *Escherichia coli* reduced its proliferation and fixation in intestinal mucosa.

Diet formulation for pigs in starting and growing stages should take into consideration a reduction of three percentage points of CP through the use of the four essential amino acids (lysine, methionine, threonine, and tryptophan) which are readily available for purchase. When other essential or functional amino acids (arginine, valine, leucine, isoleucine, etc.) become readily available or accessible, they should be assessed for application in the formulation, in order to determine if it is economically or functionally feasible to include them in the diet. Although the use of some synthetic amino acids in diet has shown favorable results during experiments, their price and availability are neither feasible nor permissible within current market conditions. Therefore, a greater inclusion of synthetic amino acids will depend on their cost-benefit ratio, in the context of productive performance, sustainability, animal health, and well-being.

CONCLUSIONS

The application of the ideal protein concept in starting and growing pigs —through the reduction of protein in diet and the inclusion of synthetic amino acids— improves parameters and productive efficiency, benefits pig health, and implements an environmentally friendly production. A reduction of three percentage points of CP is the maximum level attainable when only synthetic lysine, methionine, threonine, and tryptophan are available. However, when a wider variety of commercial amino acids is available, CP reduction may reach 4-5 percentage points. Pigs fed on conventional diets are unable to synthesize enough amino acids for an optimal gut health and growth. Therefore, an increase in these amino acid levels —through supplements at certain productive stages or physiological stress states— may improve performance response and animal health.

REFERENCES

- Chen, X., Song, P., Fan, P., He, T., Jacobs, D., Levesque, C. L., Johnston, L.J., Ji, L., Ma, N., Chen, Y., Zhang, J., Zhao, J., & Ma, X. (2018). Moderate dietary protein restriction optimized gut microbiota and mucosal barrier in growing pig model. *Front. Cell. Infect. Microbiol.*, 8, 246. Doi: 10.3389/fcimb.2018.00246
- Dubeau, F., Julien, P.O., & Pomar, C. (2011). Formulating diets for growing pigs: economic and environmental considerations. *Annals of Operations Research*, 190(1), 239-269. Doi: 10.1007/s10479-009-0633-1
- Fan, P., Liu, P., Song, P., Chen, X., & Ma, X. (2017). Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. *Scientific Reports*, 7(1), 1-12. Doi: 10.1038/srep43412
- Figuroa, J.L., Martinez, J.A., Sanchez-Torres, M.T., Cordero, J.L., Martinez, M., Valdez, V.M., & Ruiz, A. (2019). Evaluation of reduced amino acids diets added with protected protease on productive performance in 25-100 kg barrows. *Austral Journal of Veterinary Sciences*, 51(2), 53-60. Doi: 10.4067/S0719-81322019000200053

- Gloaguen, M., Le Floc'h, N., Corrent, E., Primot, Y., & van Milgen, J. (2014). The use of free amino acids allows formulating very low crude protein diets for piglets. *Journal of Animal Science*, 92(2), 637-644. Doi: 10.2527/jas.2013-6514
- González, M., Figueroa, J.L., Vaquera, H., Sánchez-Torres, M.T., Ortega, M.E., Copado, J.M.F., & Martínez, J.A. (2016). Meta-análisis del efecto de dietas bajas en proteína y adicionada con aminoácidos sintéticos para cerdos machos castrados en finalización. *Archivos de Medicina Veterinaria*, 48(1), 50-58.
- Guay, F., Donovan, S.M., & Trotter, N.L. (2006). Biochemical and morphological developments are partially impaired in intestinal mucosa from growing pigs fed reduced-protein diets supplemented with crystalline amino acids. *Journal of Animal Science*, 84(7), 1749-1760. Doi: 10.2527/jas.2005-558
- Hayes, E.T., Leek, A.B.G., Curran, T.P., Dodd, V.A., Carton, O.T., Beattie, V.E., & O'Doherty, J.V. (2004). The influence of diet crude protein level on odour and ammonia emissions from pig houses. *Bioresource Technology*, 91(3), 309-315. Doi: 10.1016/s0960-8524(03)00184-6
- He, L., Wu, L., Xu, Z., Li, T., Yao, K., Cui, Z., Yin, Y., & Wu, G. (2016). Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino Acids*, 48(1), 21-30. Doi: 10.1007/s00726-015-2059-1
- Leek, A.B., Hayes, E.T., Curran, T.P., Callan, J.J., Beattie, V.E., Dodd, V.A., & O'Doherty, J.V. (2007). The influence of manure composition on emissions of odour and ammonia from finishing pigs fed different concentrations of dietary crude protein. *Bioresource Technology*, 98(18), 3431-3439. Doi: 10.1016/j.biortech.2006.11.003
- Li, Y. H., Li, F. N., Duan, Y. H., Guo, Q. P., Wen, C. Y., Wang, W. L., Huang, X. G., & Yin, Y. L. (2018). Low-protein diet improves meat quality of growing and finishing pigs through changing lipid metabolism, fiber characteristics, and free amino acid profile of the muscle. *Journal of Animal Science*, 96(8), 3221-3232. Doi: 10.1093/jas/sky116
- Li, Y., Li, F., Chen, S., Duan, Y., Guo, Q., Wang, W., Wen, C., & Yin, Y. (2016). Protein-restricted diet regulates lipid and energy metabolism in skeletal muscle of growing pigs. *Journal of Agricultural and Food Chemistry*, 64(49), 9412-9420. Doi: 10.1021/acs.jafc.6b03959
- Luo, Z., Li, C., Cheng, Y., Hang, S., & Zhu, W. (2015). Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Archives of Animal Nutrition*, 69(3), 212-226. Doi: 10.1080/1745039X.2015.1034521
- Lynegaard, J.C., Kjeldsen, N.J., Bache, J.K., Weber, N.R., Hansen, C.F., Nielsen, J.P., & Amdi, C. (2021). Low protein diets without medicinal zinc oxide for weaned pigs reduced diarrhoea treatments and average daily gain. *Animal*, 15(1), 100075. Doi: 10.1016/j.animal.2020.100075
- Molist, F., Pijlman, J., van der Aar, P.J., Rovers, M., Ensink, J., & Corrent, E. (2016). Effect of low crude protein diets on growth performance and carcass characteristics of grower-finisher pigs. *Journal of Animal Science*, 94 (3). 226-229. Doi: 10.2527/jas.2015-9733
- Niyazov, N.S.A., Cherepanov, G.G., & Ostrenko, K.S. (2020). Use of low-protein diets for growing pigs to reduce fecal nitrogen excretion. *Ukrainian Journal of Ecology*, 10, 313-316.
- Nyachoti, M., & Lee, J. (2020). 13 Opportunities for using low-protein diets for weanling pigs to improve intestinal health. *Journal of Animal Science*, 98 (3). 18-19. Doi: 10.1093/jas/skaa054.031
- NRC, National Research Council. (1998). Nutrient requirements of swine. 10th Ed. National Academy Press. Washington DC, USA. 212 p.
- NRC, National Research Council. (2012). Nutrient Requirements of Swine. 11th Ed. National Academy Press. Washington, DC, USA. 400p.
- Opapeju, F.O., Rodriguez-Lecompte, J.C., Rademacher, M., Krause, D.O., & Nyachoti, C.M. (2015). Low crude protein diets modulate intestinal responses in weaned pigs challenged with *Escherichia coli* K88. *Canadian Journal of Animal Science*, 95(1), 71-78. Doi: 10.4141/cjas-2014-071
- Peng, X., Hu, L., Liu, Y., Yan, C., Fang, Z. F., Lin, Y., Wu, C.M., Chen, D.W., Sunn, H., Wu, D., & Che, L.Q. (2016). Effects of low-protein diets supplemented with indispensable amino acids on growth performance, intestinal morphology and immunological parameters in 13 to 35 kg pigs. *Animal*, 10(11), 1812-1820. Doi: 10.1017/S1751731116000999
- Pieper, R., Kröger, S., Richter, J.F., Wang, J., Martin, L., Bindelle, J., Htoo, J.K., von Smolinski, D., Vahjen, W., Zentek, J., & van Kessel, A. G. (2012). Fermentable fiber ameliorates fermentable protein-induced changes in microbial ecology, but not the mucosal response, in the colon of piglets. *The Journal of Nutrition*, 142 (4). 661-667. Doi: 10.3945/jn.111.156190
- Qin, C., Huang, P., Qiu, K., Sun, W., Xu, L., Zhang, X., & Yin, J. (2015). Influences of dietary protein sources and crude protein levels on intracellular free amino acid profile in the longissimus dorsi muscle of finishing gilts. *Journal of Animal Science and Biotechnology*, 6(52), 1-10.

- Ren, M., Zhang, S. H., Zeng, X. F., Liu, H., & Qiao, S. Y. (2015). Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. *Asian-Australasian Journal of Animal Sciences*, 28(12), 1742-1750. Doi: 10.5713/ajas.14.0131
- Seradj, A.R., Balcells, J., Morazan, H., Alvarez-Rodriguez, J., Babot, D., & De la Fuente, G. (2018). The impact of reducing dietary crude protein and increasing total dietary fiber on hindgut fermentation, the methanogen community and gas emission in growing pigs. *Animal Feed Science and Technology*, 245, 54-66. Doi: 10.1016/j.anifeedsci.2018.09.005
- Shimizu, M. (2004). Food-derived peptides and intestinal functions. *Biofactors*, 21, 43-47.
- Trabue, S.L., Kerr, B.J., Scoggin, K.D., Andersen, D., & Van Weelden, M. (2021). Swine diets impact manure characteristics and gas emissions: Part I protein level. *Science of the Total Environment*, 755 (2), 142528. Doi: 10.1016/j.scitotenv.2020.142528
- Wang, Y., Zhou, J., Wang, G., Cai, S., Zeng, X., & Qiao, S. (2018). Advances in low-protein diets for swine. *Journal of Animal Science and Biotechnology*, 9, 1-14.
- Wang, Y.M., Yu, H.T., Zhou, J.Y., Zeng, X.F., Wang, G., Cai, S., Huang, S., Zhu, Z.P., Tan, J.J., Johnston L.J., Levesque C.L., & Qiao, S.Y. (2019). Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. *Animal Feed Science and Technology*, 256, 114256. Doi: 10.1016/j.anifeedsci.2019.114256
- Wang, H., Long, W., Chadwick, D., Velthof, G.L., Oenema, O., Ma, W., Wang, J., Qin, W., Hou, Y., & Zhang, F. (2020). Can dietary manipulations improve the productivity of pigs with lower environmental and economic cost? A global meta-analysis. *Agriculture, Ecosystems & Environment*, 289, 106748.
- Wu, L., Zhang, X., Tang, Z., Li, Y., Li, T., Xu, Q., Zhen, J., Huang, F., Yang, J., Chen, C., Wu, Z., Li, M., Sun, J., Chen, C., An, R., Zhao, S., Jiang, Q., Zhu, W., Yin, Y., & Sun, Z. (2018). Low-protein diets decrease porcine nitrogen excretion but with restrictive effects on amino acid utilization. *Journal of Agricultural and Food Chemistry*, 66(31), 8262-8271.
- Yen, J.T., Kerr, B.J., Easter, R.A., & Parkhurst, A.M. (2004). Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. *Journal of Animal Science*, 82, 1079-90.
- Yu, D., Zhu, W., & Hang, S. (2019). Effects of long-term dietary protein restriction on intestinal morphology, digestive enzymes, gut hormones, and colonic microbiota in pigs. *Animals*, 9(4), 180.
- Zamora, V., Figueroa, J.L., Reyna, L., Cordero, J.L., Sánchez-Torres, M.T., & Martínez, M. (2011). Growth performance, carcass characteristics and plasma urea nitrogen concentration of nursery pigs fed low-protein diets supplemented with glucomannans or protease. *Journal of Applied Animal Research*, 39, 53-56.
- Zhao, J., Zhang, X., Liu, H., Brown, M.A., & Qiao, S. (2019). Dietary protein and gut microbiota composition and function. *Current Protein and Peptide Science*, 20(2), 145-154.
- Zhou, L., Fang, L., Sun, Y., Su, Y., & Zhu, W. (2016). Effects of the dietary protein level on the microbial composition and metabolomic profile in the hindgut of the pig. *Anaerobe*, 38, 61-69