Study on protein and amino acid requirements in different crossbreeds of swine

Katya Eneva

Agricultural Institute – Shumen E-mail: katiq_eneva@abv.bg

Citation: Eneva, K. (2019). Study on protein and amino acid requirements in different crossbreeds of swine. *Zhivotnovadni Nauki*, *56*(5), 26-33 (Bg).

Abstract

Hybrids and different breeds of swine have distinct capacity for productivity and therefore have different nutrient requirements to satisfy their needs. Contemporary swine breeds had high genetic potential for gain and deposition of more meat which made them sensitive to the lack of protein and amino acids. Determination of protein and amino acid requirements in recently created crossbreeds was an important factor contributing to the determination of their capacity for optimal gain and utilization of feed. When establishing the requirements for new origins, the defining factors in the feeding strategy included genotype, type of used feed, protein levels in rations providing a reduction of nitrogen pollution in the environment, protein nutrition, content of intramuscular fats in carcasses and meat quality.

Key words: protein, requirements, swine, origins

Резюме

Свинете от различните породи и хибриди имат различен капацитет за продуктивност и следователно различни потребности от хранителни вещества, които да задоволяват техните нужди. Съвременните породи свине са с висок генетичен потенциал за растеж и отлагане на повече месо и това ги прави много чувствителни към недостига на протеин и аминокиселини. Определянето на потребностите от протеин и аминокиселини при новосъздаваните кръстоски е важен фактор, спомагащ за детерминиране на капацитета им за оптимален прираст и оползотворяване на фуража. При установяване потребностите на новите произходи, определящи фактори в стратегията на хранене са генотипа, вида на използваните фуражи, дажбените равнища на протеин, осигуряващи понижение на азотното замърсяване на околната среда, проте-иново хранене и съдържанието на интрамускуларни мазнини в трупа и качеството на месото.

Ключови думи: протеин, потребности, свине, произход

Hybrids and different breeds of swine have distinct capacity for productivity and therefore have different nutrient requirements to satisfy their needs for life sustainability, gain, reproduction, lactation and other functions. Factors like genetic variations, environment, presence of nutrients in rations, illnesses and others can increase the requirements for optimal productivity. Providing digestible and metabolizable energy, protein and amino acids to grower pigs, as needed according to their requirements, was an essential part of a balanced diet for good productivity and healthy nutrition. In recent decades, swine nutrition science has focused, on the one hand, on the nutrition efficiency expressed in balancing protein and energy required to obtain optimal productivity and, on the other, a balanced protein / amino acid ratio to reduce nitrogen pollution in the environment.

Crossbreeding of specialized lines in different hybridization patterns allowed the use of the maximum degree of non-additive genetic effects (Rothschild and Ruvinsky, 2010).

Nutrition requirement evaluations for fattening pigs from different hybrid combinations have had significant importance for pig breeding. Contemporary swine breeds have high genetic potential for gain and deposition of more meat which makes them sensitive to the lack of protein and amino acids. Determination of protein and amino acid requirements in recently created crossbreeds is an important factor contributing to the determination of their capacity for optimal gain and utilization of feed. Not meeting the needs for protein and nutrition has led to lower productivity values, while the application of high protein ratios can also have a negative effect on the occurrence of toxicity, antagonism and imbalance of ration, as well as to environmental pollution (Van Lunen and Cole, 2001; NRC, 2012).

Protein concentration was not only important as a source of essential amino acids, but also affected the immune system. Animal studies have confirmed that protein deficiency impairs immune status.

Determination of nutrient digestibility from ration and nitrogen balance was part of this assessment. Digestibility has been studied by a number of scientists in different hybrid combinations (Kodes, 2003; Ilchev, 2012; Ganchev and Ilchev, 2013; Shulaev et al., 2014), as well as purebred *Danube White* when testing different nutritional factors (Nedeva, 2002; Kanev, 2008; Yordanova, 2014).

Studies in the field of protein and amino acid swine nutrition were connected to the animal genotype, growth intensity, live weight and age, type of used feed and availability of amino acids in the feed.

Genotype

A main factor that determined the daily requirements for essential amino acids and energy was the protein deposition in the organism (Black and de Lange, 1995).

The evaluation of the productive qualities of fattened pigs from different hybrid combinations was important in pig breeding (Dimov et al., 1986; Angelov et al., 1993; Stoykov et al., 1993; Stoykov and Gineva, 1999; Apostolov et al., 2006). In studies made by various authors (Panayotov, 1986; Shostak, 1998; Shostak et al., 1999) was established that separate hybrid combinations were with different requirements for nutrients. Knowledge of the animal requirements was one of the conditions for a complete and efficient production of pork.

Liu et al. (2015) reported that different pig genotypes had different requirements of protein and energy. Their studies showed that in *Bama* miniature pigs, unlike the *Landrace breed*, the protein/energy ratio did not affect the growth, which was lower. However, the meat quality of miniature pigs was better than that of *Landrace*.

According to Pham et al. (2010) optimal productivity for three different genotypes (*Mong Cai breed*, F1 and F2) was achieved at different levels of protein ration (from 13.1% crude protein to 18.9%). For the *Mong Cai (MC) breed*, maximum gain was obtained in 13.1% of the protein ration, while in F1-F2 – 16.0–18.0% of the protein in the compound feed. Protein levels also affected feed consumption - in the *Mong Cai breed* consumption was with the highest values – 13.1% protein and those in F1-F2 – with 16.1% protein. The authors conclude that optimum productivity for Mong Cai pigs, F1-MC x Big White and F2 – (*Landrace x MC*) x *Big White*, was achieved with different crude protein content.

Warriss et al. (1983) showed that different genotypes may respond differently to different environmental factors.

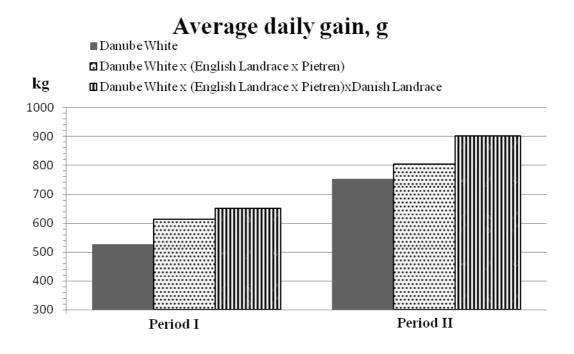
Our study (Apostolov et al., 2015) showed that the crossbreeds *Danube White x* (*EL x P*) *X Danish Landrace x Danube White x* (*EL x P*) significantly exceed over purebred swine (*DW*) in regards to average daily gain (fig.1) and feed consumption for kg gain (fig. 2).

Protein and amino acids – requirements

Precise animal husbandry has an innovative precision feeding approach that incorporates feeding techniques that take into account the nutritional composition of feed for group or individual rasing and for increasing the profitability, efficiency and sustainability of farms (Cangar et al., 2008; Hauschild et al., 2012; Pomar and Pomar, 2012; Andretta et al., 2014; Pomar et al., 2014; Andretta et al., 2016). This approach required feeding of pigs with rations that were pursuant daily with their individual requirements. According to Pomar et al. (2019), the number of feeding phases could have been increased in order to avoid excess supply of nutrients to the pigs. Daily corrections of the rations were recommended by authors, so that nutrients can be reported more precisely and nutrient efficiency can be improved.

The high level of protein in rations during the fattening period allowed animals to show their potential for muscle gain (Lebret et al., 1999).

Gloaguen et al. (2014) indicated that crude protein content in grower pigs' rations can be reduced when indispensable amino acids and total nitrogen are provided. Feeding practices in pig farming include the use of soy sources of protein to provide lysine requirements (Houmard et al., 2007), which however lead to high protein rations. Such rations lead to an excess of other amino acids and excretion of excess nitrogen in the feces and urine. On the other hand, the protein fermentation in the back of the digestive tract could lead to a decrease in intestine health. In studies made by Kerr et al. (2003), Nyachoti et al. (2006), Yue and Qiao, (2008), Fan et al. (2017) protein reduction and supplementation with indispensable amino acids (L-lysine, DL-



Period I - grower - 30 - 60 kg Period II - finisher - 60 - 110 kg

Fig. 1. Average daily gain, g

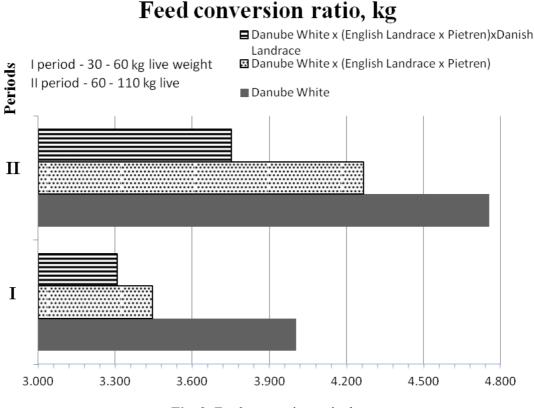


Fig. 2. Feed conversion ratio, kg

methionine, L-threonine and L-tryptophan) increased nitrogen utilization, reduced feed costs and nitrogen excretion and ensured intestine health without adversely affecting productivity.

The decrease of crude protein in pig yields has been associated with a decrease in the level of soybean sources, which according to Zuercher et al. (2006) may partially improve immune stress in weaned piglets. Ma et al. (2010) indicate soy-containing anti-nutritional factors such as trypsin inhibitor, agglutinins, antigenic proteins, isoflavones and alkaloids that reduce nutrient digestion. The undigested proteins in soybean according to Li et al. (1991) can enter the lymph and blood through gut epithelial cells, which stimulates hypersensitivity in piglets.

A number of studies (Wu et al., 1999; Kim et al., 2007; Wang et al., 2010) indicate that low levels of protein and added synthetic amino acids in rations shaped the genetic expression, decreased excess body fat, increased skeleton muscles,

improved immunological protection and minimized intestinal disorders and according to Van Der Meer et al. (2016) improved the resistance to subclinical and clinical diseases.

Yue et al. (2008) reported contradictory results when crude protein in mixtures was decreased with more than 3%. Roux et al. (2011) reported that productivity was decreased when crude protein was reduced with 4,8% and amino acids were increased.

Protein, amino acids and environment

The main environmental pollutants from pig production have been nitrogen and phosphorus pollution, heavy metals, ammonium emissions and methane (Aarnink and Vertegen, 2007). The authors state that nutrition was a key factor for reducing pollution. The main pathway for reducing nitrogen and phosphorus contamination was to reduce protein ration and the addition of limiting amino acids and phytase. The nutrition strategy, described by the authors, for reducing heavy metals included the search of alternative natural products and growth promoters to substitute Cu and Zn in combined feed and less Cd contaminated feed.

Ball et al. (2013) studied the effect of crude protein and available lysine levels on rations of grower pigs in relation to decreased N excretion. The authors reported that pigs below 40 kg live weight and 13 weeks of age require rations with higher levels of protein and lysine than those tested - 19.3% crude protein and 0.82% lysine, while those above 40 kg live weight, crude protein content can be reduced without adversely affecting productivity. The decrease of protein ration reduced the N excretion in the conducted experiments. However, finding the optimal levels of crude protein and lysine to maximize productivity while reducing nitrogen excretion, according to the Nitrates Directive (England, Northern Ireland), remains a subject of study.

Protein ration and carcass quality

Meat qualities, as well as muscle fiber type, depend on the genotype of the individual (Krasnowska G. and Salejda, 2008) on nutritional factors and movement.

In recent years nutrition sciences have been increasingly focused on the determination of the effect of protein and fat ration levels in raw materials on the content of intramuscular fat in carcasses. With this purpose, Maeda et al., 2018 carried out studies evaluating the effects of high levels of protein and fat at adequate levels of lysine on the growth capacity of fattened pigs and carcass characteristics. Maeda et al., 2018 established that cereal byproducts can be used (bread, noodles and others), which provide protein in rations with the addition of synthetic lysine for the production of marbled meat with a higher percentage of intramuscular fats.

Growth intensity and slaughter traits were influenced not only by genotype and environment but also by nutrition and in particular by the protein content. Campbell et al. (1985) reported that an increase in protein level in ration has led to the deposition of less fat in the carcasses with the same intake of exchange energy.

Wang et al. (2018) found that lysine deficiency in rations (0.43% versus 0.71% and 0.98%, respectively) increased the proportion of monounsaturated acids and decreased the proportion of polyunsaturated acids, which may be beneficial for the taste of pork.

Research of Pires et al. (2016) indicate that the use of reduced protein rations was a good strategy for increasing intramuscular fat (IMF). As a result of low protein rations, the increased intramuscular fat (IMF) has been connected to lysine restriction. The authors established that low protein rations had affected the protein quantities relating to type and structure of fibers and energy metabolism. Increased IMF has been considered to be mediated by shifting the metabolic properties of fibers from glycolytic to oxidizing.

Madeira et al. (2017) conducted an experiment with 40 male pigs (60–93 kg live weight) to evaluate the pig genotype and the level of protein ration on the content of IMF. With this research they established that protein ration decrease has increased IMF in red muscles without impacting the transformation of glycolytic fibers into oxidizing.

Conclusion

Establishing nutrient and energy requirements for different crossbreeds and breeds is necessary to ensure productive traits, health and carcass composition and meat quality, while protecting the environment from nitrogen and phosphorus pollution. Effective pork production mainly is connected to nutrition efficiency and type of used feed and supplements. Protein and amino acid levels in rations are a fundamental factor in nutrition efficiency. Other factors also have to be taken into account, such as genotype, live weight at the beginning and at the end, level of feed intake, growth intensity, immunological status, etc. It is clear that, given this large number of factors, all of them have to be taken into account when determining the nutrition strategy.

REFERENCES

Aarnink, A. J. A., & Verstegen, M. W. A. (2007). Nutrition, key factor to reduce environmental load from pig production. *Livestock Science*, *109*(1-3), 194-203.

Andretta, I., Pomar, C., Rivest, J., Pomar, J., & Radünz, J. (2016). Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. *Animal*, *10*(7), 1137-1147.

Andretta, I., Pomar, C., Rivest, J., Pomar, J., Lovatto, P. A., & Radünz Neto, J. (2014). The impact of feeding growing–finishing pigs with daily tailored diets using precision feeding techniques on animal performance, nutrient utilization, and body and carcass composition. *Journal of Animal Science*, *92*(9), 3925-3936.

Angelov, I., Stoykov, A., Shostak, B., G. Petkova, G., & Kozhuharova, P. (1993). Results of the three-breed hybridization of pigs of the Danube White, the English Large White pigs and Landrace (Line S). *Bulgarian Journal of Animal Husbandry, 30:* 1-2, 12-17.

Apostolov, A., Nedeva, R., Yordanova, G., & Eneva, K. (2015). Effect of blood introduction on the fattening ability of purebred pigs and crossbreeds with different origin. *Bulgarian Journal of animal husbandry, 5.* 8-11(Bg).

Apostolov, A., Slanev, S., & Nedeva, R. (2006). Comparative investigation of the fattening abilities and the level of direct heterosis effect in pigs from the breeds Danube White, Landrace and their crosses. *Journal of Animal Science. 5*, 36-39(Bg).

Ball, M. E. E., Magowan, E., McCracken, K. J., Beattie, V. E., Bradford, R., Gordon, F. J., Robinson, M. J., Smyth, S., & Henry, W. (2013). The effect of level of crude protein and available lysine on finishing pig performance, nitrogen balance and nutrient digestibility. *Asian-Australasian journal of animal sciences*, *26*(4), 564.

Black, J. L., & De Lange, C. F. M. (1995). Introduction to the principles of nutrient partitioning for growth. In (Moughan, P. J., Verstegen, M. W. A., & Visser-Reyneveld, M. I. Eds.): Modelling Growth in the Pig. Wageningen Pers, Wageningen, The Netherlands. pp. 33.

Campbell, R. G., Taverner, M. R., & Curic, D. M. (1985). The influence of feeding level on the protein requirement of pigs between 20 and 45 kg live weight. *Animal Science*, *40*(3), 489-496.

Cangar, Ö., Aerts, J. M., Vranken, E., & Berckmans, D. (2008). Effects of different target trajectories on the broiler performance in growth control. *Poultry science*, 87(11), 2196-2207.

Dimov, Y., Vangelov, K., Slanev, S. (1986). Fattening and slaughter qualities and meat quality of parent combinations of different combinations. *Bulgarian Journal of Animal Husbandry, 33*(5), 49-55.

Hauschild, L., Lovatto, P. A., Pomar, J., & Pomar, C. (2012). Development of sustainable precision farming systems for swine: estimating real-time individual amino acid requirements in growing-finishing pigs. *Journal of animal science*, 90(7), 2255-2263.

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*, 43412.

Ganchev, G., & Ilchev, A. (2013). Comparative investigations on feeding efficiency in growing and fattening DanBred and Topigs hybrid pigs. *Agricultural Science & Technology (1313-8820)*, 5(4), 400-404

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.

Houmard, N. M., Mainville, J. L., Bonin, C. P., Huang, S., Luethy, M. H., & Malvar, T. M. (2007). Highlysine corn generated by endosperm-specific suppression of lysine catabolism using RNAi. *Plant biotechnology journal*, 5(5), 605-614.

Ilchev, A., (2012). Study on the influence of nutrition, age and genotype on the nitrogen retention and excretion and minerals in growing and fattening pigs. *Abstract for the award of the scientific degree Ph. D.*, Trakia University – Stara Zagora.

Kanev, D., (2008). Study on the influence of some protein components on the performance of growing pigs. *Dissertation*, Sofia, 155p.

Kerr, B. J., Southern, L. L., Bidner, T. D., Friesen, K. G., & Easter, R. A. (2003). Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. *Journal of animal science*, *81*(12), 3075-3087.

Kim, S. W., Mateo, R. D., Yin, Y. L., & Wu, G. (2006). Functional amino acids and fatty acids for enhancing production performance of sows and piglets. *Asian*-*Australasian Journal of Animal Sciences*, *20*(2), 295-306.

Kodeš, A., Hučko, B., Mudřik, Z., Eberová, J., & Neužil, T. (2003). Digestibility of nutrients in hybrid pigs. *Scientia Agriculturae Bohemica*, *34*(3), 90-93.

Krasnowska, G., & Salejda, A. (2008). The quality of pork from various pig genetic lines. In *III Baltic Con*- *ference on Food Science and Technology "Foodbalt* (pp. 144-147).

Lebret, B., Lefaucheur, L., & Mourot, J. (1999). La qualité de la viande de porc. Influence des facteurs d'élevage non génétiques sur les caractéristiques du tissu musculaire. *Productions Animales 1 (12), 11-28.(1999)*.

Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Klemm, R., & Goodband, R. D. (1991). Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. *Journal of Animal Science*, *69*(10), 4062-4069.

Liu, Y., Kong, X., Jiang, G., Deng, J., Yang, X., Li, F., ... & Yin, Y. (2015). Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. *Journal of Animal Science and Biotechnology*, 6(1), 36.

Ma, X., Sun, P., He, P., Han, P., Wang, J., Qiao, S., & Li, D. (2010). Development of monoclonal antibodies and a competitive ELISA detection method for glycinin, an allergen in soybean. *Food Chemistry*, *121*(2), 546-551.

Madeira, M. S., Lopes, P. A., Costa, P., Coelho, D., Alfaia, C. M., & Prates, J. A. M. (2017). Reduced protein diets increase intramuscular fat of psoas major, a red muscle, in lean and fatty pig genotypes. *animal*, *11*(11), 2094-2102.

Maeda, K., Yamanaka, K., Masanaritoyoshi, & Irie, M. (2018). Effects of dietary protein and fat levels on growth performance and meat quality in finishing pigs while maintaining sufficient lysine. *International J. Anim. Sci.* 2(2), 1020.

Nedeva, R. (2002). Influence of different levels of calcium and phosphorus on the performance of growing and fattening pigs. Sofia, p.150, *Dissertation PhD* (Bg).

Nyachoti, C. M., Omogbenigun, F. O., Rademacher, M., & Blank, G. (2006). Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *Journal of Animal Science*, *84*(1), 125-134.

Panayotov, P. (1986). Study on productivity qualities, nutrient digestion and the nitrogen balance of fattening pigs from two combinations of crossbreeding. *Bulgarian Journal of Animal Husbandry*, *33*(5) 56-61.

Pham, K. T., Hoang, N. D., Le Duc, N., Hendriks, W. H., Van Der Peet-Schwering, C. M. C., & Verstegen, M. W. A. (2010). Effect of genotype and dietary protein level on growth performance and carcass characteristics of fattening pigs in Central Vietnam. *Asian-Australasian Journal of Animal Sciences*, 23(8), 1034-1042.

Pires, V. M. R., Madeira, M. S., Dowle, A. A., Thomas, J., Almeida, A. M., & Prates, J. A. M. (2016). Increased intramuscular fat induced by reduced dietary protein in finishing pigs: effects on the longissimus lumborum muscle proteome. *Molecular BioSystems*, 12(8), 2447-2457.

Pomar, C., & Remus, A. (2019). Precision pig feeding: a breakthrough toward sustainability. *Animal Frontiers*, 9(2), 52-59.

Pomar Gomà, J., & Pomar, C. (2012). Sustainable precision livestock farming: a vision for the future of the Canadian swine industry. *Advances in Pork Production,* 23, 207-213.

Pomar, C., Pomar, J., Dubeau, F., Joannopoulos, E., & Dussault, J. P. (2014). The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing–finishing pigs. *Animal*, 8(5), 704-713.

Rothschild, M. F., & Ruvinski, A. (2010). The genetics of the pig. *2-nd Edition*. p 342-348.

Roux, M. L., Donsbough, A. L., Waguespack, A. M., Powell, S., Bidner, T. D., Payne, R. L., & Southern, L. L. (2011). Maximizing the use of supplemental amino acids in corn-soybean meal diets for 20-to 45-kilogram pigs. *Journal of animal science*, *89*(8), 2415-2424.

Shostak, B., 1998. Study on fattening and meat production qualities and meat quality of three-breed hybrid pigs. *Bulgarian Journal of Animal Husbandry, 2* 14-17.

Shostak, B., Nedeva, R., Doichev, V., & V. Katzarov, V. (1999). Fattening and slaughter performance of hybrid pigs fattened with different protein and equal lysine level feeds, Curent trends in the development of fundamental and applied sciences, *VI. Stook-breeding, Plant –growing and veterinary medicine*, Stara zagora. 114-120 (Bg).

Shulaev, G. M., Engavatov, V. F., Betin, A. N., & Milushev, R. K. (2014). Vegetable protein concentrate – an alternative to fishmeal. *Pig breeding*, *4*, 73-74.

Stoykov, A., & Gineva, E. (1999). Study on fattening abilities and slaughter qualities of two-, three-, four-breed cross hybrids. *Bulgarian Journal of Animal Husbandry*, *36*(3-4), 21-25.

Stoykov, A., Slanev, S., Apostolov, A., & Gineva, E. (1993). Comparative study on the fattening and slaughter qualities of two and three line hybrid pigs. *Bulgarian Journal of Animal Husbandry, 30*(8) 35-39.

Van der Meer, Y., Lammers, A., Jansman, A. J. M., Rijnen, M. M. J. A., Hendriks, W. H., & Gerrits, W. J. J. (2016). Performance of pigs kept under different sanitary conditions affected by protein intake and amino acid supplementation. *Journal of animal science*, *94*(11), 4704-4719.

Van Lunen, T. A., & Cole, D. J. A. (2001). Energyamino acid interactions in modern pig genotypes. In: Wiseman, J., Garnsworthy, P.C., editors. *Recent Developments in Pig Nutrition 3. Nottingham University Press.* pp. 439–466. Wang, W., Zeng, X., Mao, X., Wu, G., & Qiao, S. (2010). Optimal dietary true ileal digestible threonine for supporting the mucosal barrier in small intestine of wean-ling pigs. *The Journal of nutrition*, *140*(5), 981-986.

Wang, T., Crenshaw, M. A., Regmi, N., Rude, B. J., Shamimul Hasan, M., Sukumaran, A. T., Dinh, T. & Liao, S. F. (2017). Effects of dietary lysine level on the content and fatty acid composition of intramuscular fat in late-stage finishing pigs. *Canadian journal of animal science*, *98*(2), 241-249.

Warriss, P. D., Kestin, S. C., & Robinson, J. M. (1983). A note on the influence of rearing environment on meat quality in pigs. *Meat Science*, *9*(4), 271-279.

Wu, G., Ott, T. L., Knabe, D. A., & Bazer, F. W. (1999). Amino acid composition of the fetal pig. *The Journal of nutrition*, *129*(5), 1031-1038.

Yordanova, G. (2014). Effect of testing biological active components on performance in growing and finishing pigs, Shumen, p. 136, *Dissertation PhD* (BG).

Yue, L. Y., & Qiao, S. Y. (2008). Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. *Livestock Science*, *115*(2-3), 144-152.

Zuercher, A. W., Fritsche, R., Corthésy, B., & Mercenier, A. (2006). Food products and allergy development, prevention and treatment. *Current opinion in biotechnology*, *17*(2), 198-203.