Proceedings of the 15th International Symposium
Modern Trends in Livestock Production
October 29 –31, 2025, Belgrade, Serbia

STUDYING HUSBANDRY AND NUTRITION IN KRŠKOPOLJE PIG: IMPLICATIONS FOR FAT DEPOSITION AND MEAT QUALITY WITH INSIGHTS INTO METABOLISM AND GENE EXPRESSION

Martin Škrlep, Klavdija Poklukar, Bojana Savić, Marjeta Čandek-Potokar

Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia
Corresponding author: Martin Škrlep, martin.skrlep@kis.si

https://doi.org/10.5937/AnimTrend25174S

Abstract: The Slovenian autochthonous breed Krškopolje pig is receiving a growing interest from both society and consumers, as it represents an important element of agricultural biodiversity, cultural heritage and is valued for its meat quality. The present article summarizes recent studies on Krškopolje pig performance under various rearing and nutritional conditions, with comparisons to lean modern pigs. Investigations covered both intensive indoor and extensive outdoor systems, including assessments of growth, fat deposition, meat quality traits, tissue-specific enzyme activity, and gene expression related to fat metabolism. The impact of dietary protein reduction was also examined in both settings, with emphasis on physiological and molecular responses. These studies provide a comprehensive overview of how Krškopolje pig responds to different production environments and nutritional strategies, highlighting its relevance for sustainable and niche pig production.

Key words: Krškopolje pig, rearing systems, nutrition, fat deposition, gene expression, metabolism.

Introduction

The Krškopolje pig, Slovenia's only autochthonous pig breed (Figure 1), is known for its robust characteristics and good adaptability to diverse rearing conditions, positioning it as a valuable resource for sustainable pork production. Nearly extinct in the past, the breed has experienced a notable revival over the last

decades, with growing interest from breeders and researchers driving an increase in its population. Unlike modern, lean breeds, Krškopolje pig shows moderate growth rate and tends to accumulate more fat, traits that make it well-suited for extensive rearing systems using local feed resources and supporting the production of high value-added meat products (Kastelic and Čandek-Potokar, 2013; Čandek-Potokar and Nieto, 2019). Despite the revival, Krškopolje pig remains relatively understudied compared to modern cosmopolitan breeds. Recent research has begun to address this, examining its performance under different rearing and nutritional regimes and comparing it to leaner genotypes. However, key questions about its specific nutritional requirements, optimal feeding and rearing strategies, and metabolic characteristics remain unanswered, all of which are critical for more efficient and sustainable breeding practices and product quality.

The present article summarizes findings from recent studies conducted at the Agricultural Institute of Slovenia, presenting a more detailed overview of investigations into Krškopolje pig's specific traits, nutritional needs, and production capacity, either by comparing its performance with modern breeds or testing the breed under various rearing systems and/or with different feeding strategies.

Figure 1. Young specimens (app. 50 kg) of Krškopolje pig breed

Krškopolje pig compared to modern breeds

To better characterize Krškopolje pig, it is important to compare it with modern breeds under identical environmental conditions (rearing and feeding systems). However, this is challenging due to the distinct nature of these breeds, particularly their different nutritional requirements. Nevertheless, two studies have addressed this: one conducted under extensive and another under intensive rearing conditions.

The first one (Poklukar et al., 2023) compared Krškopolje pigs (KKP) with modern lean hybrid pigs (MP, crosses of Landrace, Large White and Duroc) raised in an outdoor extensive system (large fenced area with roofed shelter, 2 kg of cereal based commercial feed per pig supplemented with hay and root crops). When fattened to higher weight (165 kg), KKP exhibited superior growth (1.7 fold higher daily gain) and notably greater fat deposition with up to three times thicker backfat and nearly double the intramuscular fat compared to MP, while muscle growth was comparable (Figure 2). This greater growth performance of KKP pigs was rather unexpected, given that both groups had the same feeding regime, suggesting greater resilience and adaptability of KKP breed to outdoor rearing conditions and/or nutritionally suboptimal diets. KKP pigs also displayed higher saturated (SFA) and particularly monounsaturated fatty acid (MUFA, mainly oleic acid) content in subcutaneous fat (Figure 1) which was associated with increased stearoyl-CoA desaturase gene expression. Similar to our results, it has been attested in many local breeds, that their desaturation capacity is generally higher than in modern hybrids, and that they are also less sensitive to non-optimal dietary conditions (Poklukar et al., 2020; Benítez et al., 2017). Furthermore, genes linked to lipogenesis (ACACA, FASN), adipogenesis (PPARγ) and energy homeostasis (LEP) were overexpressed in KKP (Poklukar et al., 2023) confirming higher adipogenicity of many local breeds (Poklukar et al., 2020). Despite the increased fat deposition, lipogenic enzymes (malic enzyme – ME1, glucose-6-phosphate dehydrogenase – G6PD) in KKP were more than twofold lower than in the modern hybrids. This discrepancy may be explained by differences in physiological maturity at slaughter, as many local breeds, mature earlier and may thus reach peak lipogenic activity earlier in life than modern breeds (Lebret et al., 2014; Mourot et al., 1996).

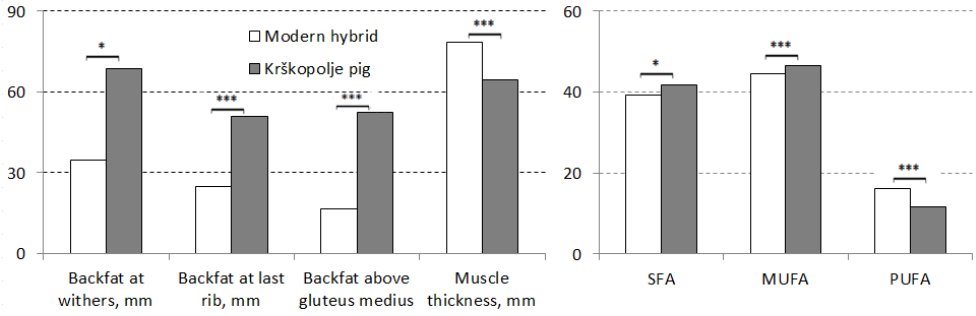

Figure 2. Carcass traits (left) and fatty acid composition of subcutaneous fat in modern hybrids and Krškopolje pigs reared in extensive outdoor conditions (adapted from Poklukar et al., 2023)

In a subsequent experiment, KKP and modern lean hybrids were reared indoor, in intensive conditions, both groups receiving daily equal amounts of the same feed mixture (i.e. 1.6 kg, 2.4 kg and 2.8 kg during three subsequent fattening phases, each lasting 60 days; Škrlep et al., 2024). Contrary to results of extensive rearing, in intensive system KKP exhibited slower growth, with 15% lower daily gain and a 13% lower slaughter weight than modern hybrids. The difference in daily gain (in average approximately 130g/day) remained consistent throughout the trial. This was associated with reduced muscle development (i.e. lower *longissimus* muscle gain resulting in a 1.7-fold smaller loin eye area and thinner muscle at *gluteus medius* level) and greater backfat accumulation throughout the entire fattening period, cumulating in over twofold thicker backfat at the end of the trial. Differences were also found for histomorphological traits, with KKP showing larger adipocyte sizes. Meat quality parameters were largely similar between the genotypes, except for darker colour of KKP meat (Figure 3), and higher myoglobin content, consistent with more oxidative muscle profile typical of local breeds (Park et al., 2007).

Figure 3. Loin cross-section in Krškopolje pig (left) and modern hybrid (right); note the difference in muscle colour and subcutaneous fat thickness

The most notable difference in chemical composition was the intramuscular fat content, which was over 2.5 times higher in KKP than in modern hybrids. As in previous experiment, KKP exhibited higher levels of SFA and MUFA but lower levels of PUFA in both backfat and muscle, though the differences were less pronounced in backfat. Notably, KKP displayed lower n-6/n-3 PUFA ratio compared to MP. While lower ratio is generally considered beneficial for health, the values in this study were still relatively high (above 20, compared to the recommended ratio being below 4; Dougan et al., 2015). Despite the higher proportion of saturated fats, elevated oxidation levels (thiobarbituric acid reactive substances - TBARS) were observed in KKP muscle, potentially due to higher overall IMF content (Fuentes et al., 2014) or increased myoglobin

concentration (Faustman et al., 2010). Lipogenic enzyme activities varied significantly by tissue; compared to MP, KKP showed lower G6PD activity in liver but higher activities of G6PD and citrate cleavage enzyme (CCL) in the *semispinalis capitis* muscle, as well as higher CCL and fatty acid synthase (FAS) activities in subcutaneous fat. Notably, FAS activity in KKP was threefold higher, consistent with their greater lipid storage capacity (Poklukar et al., 2020).

Further analyses on the same samples exploring adipose tissue gene expression differences between KKP and MP (Poklukar et al., 2025a), elucidated the key processes driving the higher fat accumulation in KKP. Among 363 genes differentially expressed between the genotypes, KKP exhibited upregulation of genes associated with lipid and energy metabolism (e.g. NR4A3, ACBD7, PTSG, CES1), adipocyte proliferation and differentiation (e.g. IL-1A, PAPPA2, NR4A3), extracellular matrix composition (e.g. COL6A5) and inflammation (e.g. CxCL8, CCL4, CXCL2). Gene ontology over-representation analysis (Figure 4) highlighted enriched biological processes, including regulation of fat cell differentiation, cell morphogenesis, transmembrane transport, and phosphatidylinositol-mediated signalling.

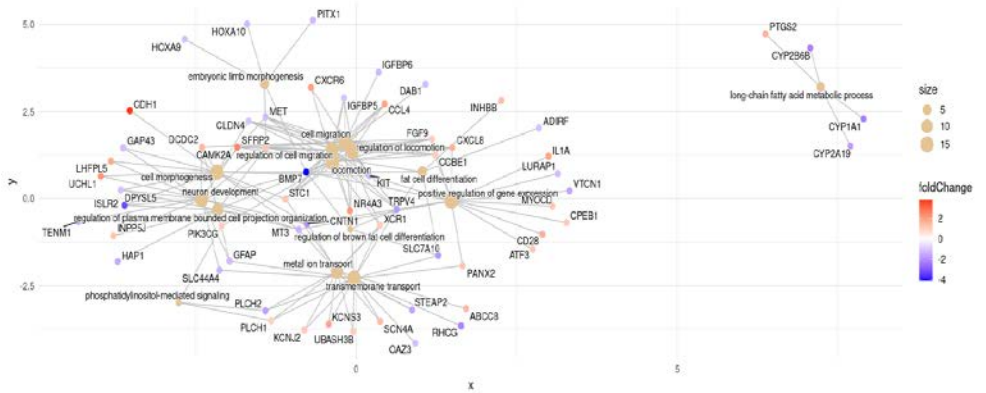

Figure 4. Gene Ontology (GO) term–gene network displaying log2 fold changes of differentially expressed genes between the local Krškopolje breed and modern hybrids. Reddish colours show upregulated and bluish colours show genes downregulated in Krškopolje pigs compared to modern hybrids. GO terms that are significantly ($p<0.05$) enriched are presented as brown circles (adapted from Poklukar et al., 2025a)

Rearing Krškopolje pig in different production systems

In the past, the traditional rearing method for Krškopolje pigs was primarily based on combined indoor housing with outdoor area and feeding predominantly with locally produced root crops. To test the effectiveness of this

rearing practice, a comparison between intensive (based on commercial feed) and extensive (cereal based feed supplemented with root crops) was conducted (Tomažin et al., 2017). The study showed that despite *ad libitum* feed access, pigs in the extensive system exhibited significantly lower growth rates after the initial phase (Figure 5), as well as reduced slaughter weight and carcass fat content. This was primarily attributed to a protein deficiency, particularly lysine, as it was estimated that extensive feeding met only 50–60% of the needs for a fatty-type pig (Nieto et al., 2015) and depends on feed availability and nutrient composition. Interestingly, in addition to the negative effects on growth, the meat from extensively reared pigs exhibited a darker colour, indicative of a more oxidative metabolism, as also observed in other local breeds (Lebret et al., 2015).

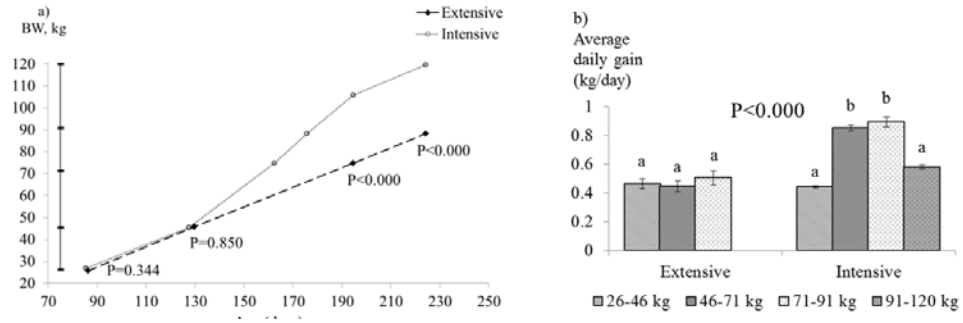

Figure 5. Body weight (left) and average daily gain (right) of Krškopolje pigs reared in intensive or extensive system (source: Tomažin et al., 2017)

A comparative study of conventional (indoor, commercial feed) and organic (outdoor access, supplemented with voluminous feed – alfalfa hay) rearing practices for the KKP (Batorek-Lukač et al., 2016) revealed slightly higher growth rates (13%) in organic system. Higher growth rate in organic system was attributed to significant alfalfa hay intake (estimated at an additional 6.0 ME ME/day) and lower feed wastage in the organic group. Except for dressing (lower in organic group) no major differences between the two rearing systems were observed in either muscle or fat-related carcass characteristics (Tomažin et al., 2019).

The differences between the systems were however more pronounced in meat quality and fatty acid composition (Tomažin et al., 2019). Meat from organic pigs had higher pH 45 minutes post-mortem, likely reflecting reduced stress due to enriched environments or physical activity (Terlouw et al., 2005; Millet et al., 2004), but lower pH 24 hours post-mortem (as to higher glycogene reserves, Bonneau and Lebret, 2010), with higher drip loss and darker meat colour. Intramuscular fat (IMF) content was similar across groups, but organic pigs

exhibited lower saturated fatty acids (SFA) and higher monounsaturated fatty acids (MUFA) in IMF, a fat profile likely driven by alfalfa supplementation and other factors such as increased physical activity (Daza et al., 2009), lower ambient temperatures (Lebret, 2008) or lower dietary lysine (Wang et al., 2018). Additionally, organic pigs showed elevated TBARS, indicating greater lipid oxidation, which, combined with lower vitamin E in backfat, suggests increased oxidation susceptibility that could compromise meat quality during storage. Backfat in organic pigs also had higher polyunsaturated fatty acids (PUFA), reflecting dietary influences from alfalfa. Additional research on products from this meat (Škrlep et al., 2018; 2019) has shown that dry-fermented sausages produced from organic pigs exhibited lower processing losses, softer texture and more intensive oxidation due to lower fat saturation. A further research on the metabolic features associated with both husbandry systems was also conducted on muscle tissue (Fazarinc et al., 2020; Figure 6). It was shown that organic production system influenced the composition of muscle fibre type with a shift towards more oxidative fibres (i.e. more SDH-positive myofibre types, with higher expression of I, IIa and IIx and lower expression of IIb myosine heavy chain isoforms), but with no apparent effect on metabolic phenotype of the muscle including expression of the energy and fat metabolism-related genes (PGC-1α, PPARγ, LPL, CPT1B, FASN, GYS1, HK2).

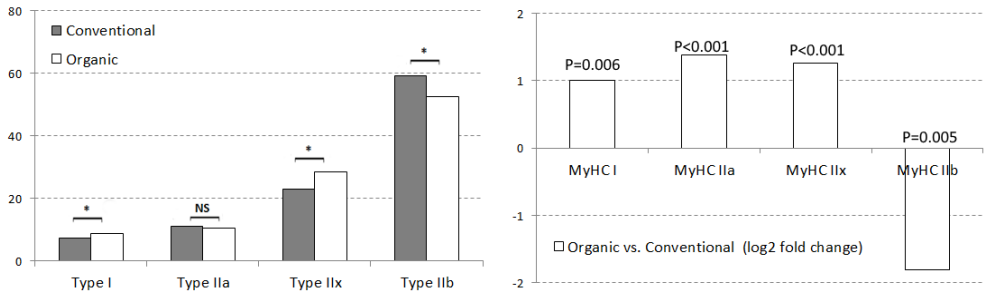

Figure 6. Effect of rearing practice on percentages of myofiber types (left) and myosine heavy chain isoforms gene expression (MyHC, right) in the longissimus dorsi muscle of Krškopolje pigs (adapted from Fazarinc et al., 2020)

Dietary protein reduction

Due to their slower growth, greater fat deposition potential, and higher resilience to feed scarcity, local pig breeds typically exhibit lower protein requirements. However, these requirements are relatively unknown for most local breeds. In addition, there is considerable variation in protein requirements among

local breeds. For instance, the Italian local breed Cinta Senese requires on average as little as 10% crude protein throughout the fattening period (Aquilani et al., 2019). A preliminary study by Brossard et al. (2019) found that KKP, compared to nine other local breeds, have relatively high protein needs, approximately 65% higher than those of Cinta Senese pigs. However, subsequent studies (e.g., Škrlep et al., 2024) suggest that KKP protein requirements are likely much lower. A reduction of approximately 2% points in crude protein (reaching as low as 9.3% in the final fattening stage) did not result in any differences in growth rate, tissue deposition, carcass characteristics, meat quality, biochemical composition, or metabolic response. Furthermore, modelling of amino acid nutritional needs revealed no deficiencies.

In a subsequent study (Škrlep et al., 2025) the effects of dietary protein reduction in two distinct rearing systems (indoor conventional vs. outdoor organic, receiving feeds formulated using the same ingredients but of conventional or organic origin, Figure 7) were investigated to test for the interaction between breed and environment.

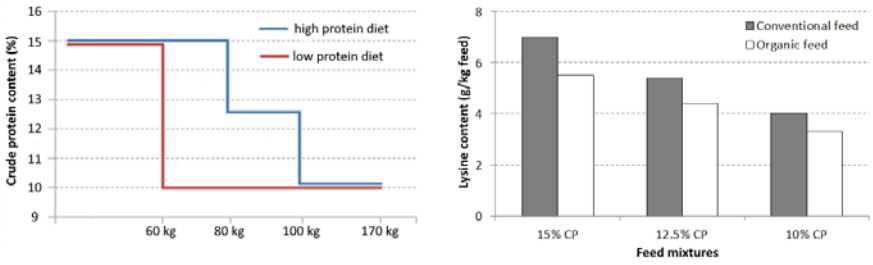

Figure 7. Scheme of the multiphase feeding trial with high and low protein diet in Krškopolje pigs and the lysine content of the feed mixtures with different crude protein (CP) content (adapted from Škrlep et al., 2025)

By modelling growth data (e.g. using InraPorc®), requirements for proteins and essential amino acids can be assessed (Figure 8). The results of modelling showed that, except for lysine in low protein diet, the essential amino acid requirements were covered with control “high protein” diets used throughout the fattening and that lysine deficiency in the case of low protein diet was more pronounced in the outdoor organic system, resulting in different effect of protein reduced diet in conventional and organic rearing system.

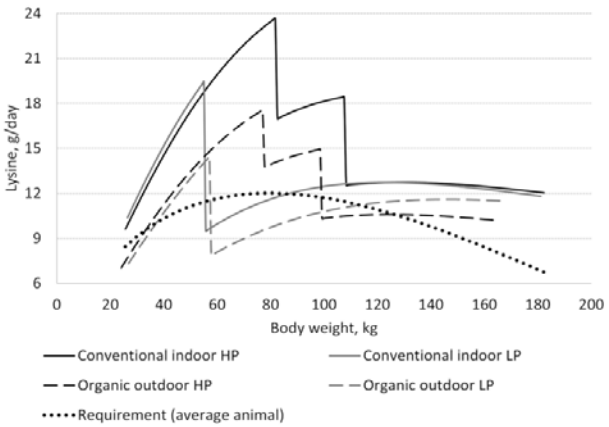

Figure 8. Lysine supply and requirements for Krškopolje pigs reared in outdoor organic or indoor conventional system, fed high (HP) or low (LP) protein diet; lysine requirement was estimated on the profile of conventional indoor HP animals (adapted from Škrlep et al., 2025)

In the outdoor organic system, pigs on low protein diet exhibited slower growth during intermediate phases but achieved compensatory growth in the final fattening stage. Differences in carcass composition and meat quality were minimal, with slightly increased fat deposition and thicker backfat in low protein group. These differences were evident also on biochemical and histological level as higher activity of lipogenic enzymes (Figure 9), increased MUFA content in the backfat and larger adipocytes, while transcriptomic analysis revealed upregulation of genes involved in lipid metabolism, including fatty acid synthesis (ACYL, ACACA, FASN, ACSM5), triglyceride synthesis (MOGAT2), and fatty acid desaturation (SCD, ELOVL6, consistent with higher MUFA), alongside genes related to oxidative stress and mitochondrial function (Poklukar et al., 2025b).

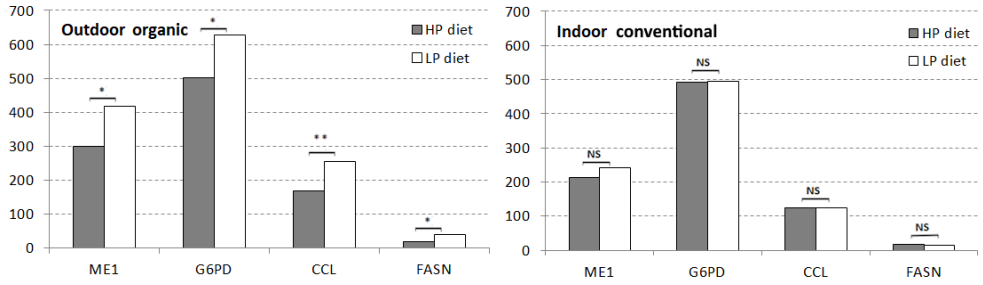

Figure 9. Lipogenetic enzyme activities (ME1 – malic enzyme, G6PD – glucose-6-phosphate dehydrogenase, CCL – citrate cleavage enzyme, FASN – fatty acid synthase) in subcutaneous adipose

tissue of Krškopolje pigs from different rearing systems (outdoor organic, indoor conventional) fed high (HP) or low (LP) protein diet (adapted from Poklukar et al., 2025b)

In accordance with smaller amino acid deficit, animals in the indoor intensive system, showed no diet-related differences in growth, tissue deposition, carcass composition, or biochemical properties (Škrlep et al., 2025). Transcriptomic responses nevertheless differed, with upregulation of heat shock proteins (HSPA6, HSP70) and downregulation of genes associated with the innate immune system (MSR1, CSF3R, TREM) in low protein compared to high protein animals (Poklukar et al., 2025b).

Conclusion

Based on the research results, it can be concluded that KKP demonstrated distinct adaptability and fat deposition characteristics either compared to modern breeds or when subjected to different rearing and feeding practices. The effects observed for growth, fat deposition and composition may be utilized to manage breed's aptitude and potential for specialized and sustainable production.

Acknowledgement
The authors acknowledge funding from European Union's Horizon 2020 research and innovation program (GEroNIMO project, grant agreement No 101000236), the Slovenian Research and Innovation Agency (grants J4-3094, P4-0133, V4-2201, Z4-60178) and the Ministry of Agriculture, Forestry and Food (V4-2201).

Conflict of interest
The authors declare no conflict of interest.

References

Aquilani C., Sirtori F., Franci O., Acciaioli A., Bozzi R. 2019. Effects of different protein levels on the nitrogen balance, performance and slaughtering traits of Cinta Senese growing pigs. *Animals*, 9, 1021.

Batorek-Lukač N., Tomažin U., Prevolnik-Povše M., Škrlep M., Čandek-Potokar M. 2016. Effect of production system (organic vs. conventional) and roughage supplementation in diet on performance of growing-finishing Krškopolje pigs. In Book of abstracts of the *9th International Symposium on Mediterranean Pig*, Portalegre, 3-5 November, p 36.

Benítez R., Fernández A., Isabel B., Núñez Y., De Mercado E., Gómez-Izquierdo E., García-Casco J., López-Bote C., Óvilo C. 2017. Modulatory effects of breed,

feeding status, and diet on adipogenic, lipogenic, and lipolytic gene expression in growing Iberian and Duroc pigs. *International Journal of Molecular Science*, 19, 22.

Bonneau M., Lebret B. 2010. Production systems and influence on eating quality of pork. *Meat Science*, 84, 293–300.

Brossard L., Nieto R., Charneca R., Araujo J.P., Pugliese C., Radović Č., Čandek-Potokar, M. 2019. Modelling nutritional growth requirements of growing pig from local breeds using InraPorc. *Animals*, 9, 169.

Čandek-Potokar M., Nieto R. 2019. European local pig breeds – diversity and performance: A study of project Treasure. IntechOpen, London, 141-154.

Daza A., Rey A.I., Olivares A., Cordero G., Toldrá F., López-Bote C.J. 2009. Physical activity-induced alterations on tissue lipid composition and lipid metabolism in fattening pigs. *Meat Science*, 81, 641–646.

Dougan M.E.R., Vahmani P., Turner T.D., Mapiye C., Juárez M., Prieto N., Beaulieu A.D., Zijlstra R.T., Patience J.F., Aalhus J.L. 2015. Pork as a source of omega-3 (n-3) fatty acids. *Journal of Clinical Medicine*, 4, 1999–2011.

Faustman C., Sun Q., Mancini R., Suman S.P. 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, 86, 86–94.

Fazarinc G., Vrecl M., Poklukar K., Škrlep M., Batorek-Lukač N., Brankovič J., Tomažin U., Čandek-Potokar M. 2020. Expression of myosin heavy chain and some energy metabolism-related genes in the longissimus dorsi muscle of Krškopolje pigs: Effect of the production system. *Frontiers in Veterinary Science*, 7, 533936.

Fuentes V., Utrera M., Estévez M., Ventanas J., Ventanas S. 2014. Impact of high pressure treatment and intramuscular fat content on colour changes and protein and lipid oxidation in sliced and vacuum-packaged Iberian dry-cured ham. *Meat Science*, 97,468–474.

Kastelic A., Čandek-Potokar M. 2013. Application of quality labels in support of conservation of local breeds – a challenge for Slovenian Krškopolje pig. *Acta Agriculturae Slovenica*, Suppl. 4, 205-209.

Lebret B., Ecolan P., Bonhomme N., Méteau K., Prunier A. 2015. Influence of production system in local and conventional pig breeds on stress indicators at slaughter, muscle and meat traits and pork eating quality. *Animal*, 9, 1404–1413.

Lebret B., Dourmad J.Y., Mourot J., Pollet P.Y., Gondret F. 2014. Production performance, carcass composition, and adipose tissue traits of heavy pigs: Influence of breed and production system. *Journal of Animal Science*, 92, 3543-3556.

Lebret B. 2008. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal* 2, 1548–1558.

Millet S. 2004. Influence of nutrition on performance, product quality and immunocompetence in organic pig production, PhD thesis, Ghent University, Ghent, Belgium.

Mourot J., Kouba M., Bonneau M. 1996. Comparative study of in vitro lipogenesis in various adipose tissues in the growing Meishan pigs: comparison with the Large White pig (Sus domesticus). *Comparative Biochemicsty and Physiology Part B: Biochemicstry and Molecular Biology*, 115, 383-388.

Nieto R., Martínez-Pérez M., Haro A., Lara L., Aguilera J. F. 2012. Effects of protein intake on rate of growth, protein deposition and carcass traits of heavy Iberian pigs. *Journal of Animal Science*, 93, 3471–3482

Park B.Y., Kim N.K., Lee C.S., Hwang I.H. 2007. Effect of fiber type on post-mortem proteolysis in longissimus muscle of Landrace and Korean native black pigs. *Meat Science*, 77, 482–491.

Poklukar K., Škrlep M., Vrecl M., Čandek-Potokar, M. 2025a. Transcriptomic differences in the adipose tissue of Krškopolje and modern hybrid pigs. In Book of abstracts of the *3rd Regional Meeting of the European Federation of Animal Science*, April 9-11, Krakow, p 41.

Poklukar K., Čandek-Potokar M., Vrecl M., Brankovič J., Uršič M., Škrlep M. 2025b. The effect of reduced dietary protein on adipose tissue in local Krškopolje pigs. *International Journal of Molecular Sciences*, 26, 4440.

Poklukar K., Čandek-Potokar M., Batorek Lukač N., Škrlep, M. 2023. Biochemical and gene expression differences associated with higher fat deposition in Krškopolje pigs in comparison with lean hybrid pigs. *Livestock Science*, 272, 105247.

Poklukar K., Čandek-Potokar M., Batorek Lukač N., Tomažin U., Škrlep M. 2020. Lipid deposition and metabolism in local and modern pig breeds: A review. *Animals*, 10, 424.

Škrlep M., Poklukar K., Millet S., Čandek-Potokar M. 2025. Reducing protein levels in diets for local pig breeds: A case study on fat-type Krškopolje pig. *Animal Science Journal*, 96, e70077.

Škrlep M., Poklukar K., Vrecl M., Brankovič J., Čandek-Potokar M. 2024. Growth performance, carcass quality, and lipid metabolism in Krškopolje pigs and modern hybrid pigs: Comparison of genotypes and evaluation of dietary protein reduction. *Animals*, 14, 3331.

Škrlep M., Čandek-Potokar M., Batorek-Lukač, N., Tomažin U., Flores M. 2019. Aromatic profile, physicochemical and sensory traits of dry-fermented sausages produced without nitrites using pork from Krškopolje pigs reared in organic and concentional husbandry. *Animals*, 9, 55.

Škrlep M., Čandek-Potokar M., Tomažin U., Batorek Lukač N., Flores M. 2018. Properties and aromatic profile of dry-fermented sausages produced from

Krškopolje pigs reared under organic and conventional rearing regime. *Animal*, 12, 1316–1323.

Terlouw C. 2005. Stress reactions at slaughter and meat quality traits in pigs: genetic background and prior experience. A brief review of recent findings. *Livestock Production Science*, 94, 125–135.

Tomažin U., Škrlep M., Batorek Lukač N., Prevolnik Povše, M., Čandek-Potokar M. 2017. Performance of Krškopolje pigs in extensive and intensive production systems. *Agriculturae Conspectus Scientificus*, 82, 211-215.

Tomažin U., Batorek-Lukač N., Škrlep M., Previlnik-Povše M., Čandek-Potokar M. 2019. Meat and fat quality of Krškopolje pigs reared in conventional and organic production systems. *Animal*, 13, 1103-1110.

Wang T., Crenshaw M.A., Regmi N., Rude B.J., Hasan M.S., Sukumaran A.T., Dinh T., Liao S.F. 2018. 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, 241–249.