*Animal Science Journal*

WILEY

Animal Science Journal

RESEARCH ARTICLE OPEN ACCESS

# Reducing Protein Levels in Diets for Local Pig Breeds: A Case Study on Fat-Type Krškopolje Pig

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**Received:** 6 January 2025 | **Revised:** 28 April 2025 | **Accepted:** 27 May 2025

**Funding:** This work was supported by Ministry of Agriculture, forestry and food (V4-2201 and L7-4568), Slovenian Research and Innovation Agency (J4-3094, P4-0133, V4-2201, and L7-4568), and European Commission (H2020 and 101000236).

**Keywords:** InraPorc | Krškopolje pig | modeling | nutrition | production system

## ABSTRACT

This study investigated the effects of reduced dietary protein levels in fat-type local breed Krškopolje pig in conventional indoor and organic outdoor systems. Two tests were conducted: one in conventional system ($n=28$ pigs) and one in organic outdoor system ($n=24$ pigs). Two dietary strategies varying in crude protein (CP) level were tested: control, high protein (HP) with phases of 15%, 12.5%, and 10% CP content and low protein (LP) with phases of 15% and 10% CP content. The ingredients used were identical, but in the organic diet, they came from organic agriculture. The feed was offered ad libitum. Feed distribution was recorded, pigs were weighed, and the thickness of the backfat and longissimus lumborum muscle was measured with ultrasound. After 228 days, pigs were slaughtered, and carcass and meat quality were assessed. Modeling growth data with InraPorc indicated that protein and essential amino acid requirements were generally met, except for lysine in the LP diets, which appeared with the transition to a 10% protein diet. Lysine deficiency was more pronounced in the organic than in conventional system. Overall growth rate was similar in LP and HP groups. There were no pertinent differences in carcass or meat quality.

## 1 | Introduction

The current understanding of the performance and nutritional needs of fatty local pig breeds is limited, so their potential may not be optimally exploited. They seem to have distinct metabolic characteristics, as shown for Iberian pigs (Fernández-Fígares et al. 2007; Barea et al. 2007), and are characterized by lower growth rates and lower lean meat deposition than in commercial lines selected for lean meat growth. Local breeds are raised under diverse feeding and management conditions, often within specific production systems such as outdoor or organic settings (Candek-Potokar and Nieto Liñan 2019). These findings suggest that local breeds may have specific nutritional requirements. The response to changes in protein and energy supply has been studied in Iberian pigs (Nieto et al. 2002), but for many other local pigs, nutrient requirements to optimize growth and performance still need to be explored and determined. Modeling growth and predicting the response to nutrient supply have been developed and applied largely in modern breeds. As part of the H2020 project TREASURE, a study by Brossard et al. (2019) used a modeling approach to make a preliminary assessment of amino acid requirements for nine European local breeds. As a general rule, this study demonstrated that local breeds exhibit lower growth potential and lysine requirements, with a small proportion of energy retention dedicated to protein synthesis and a majority stored as lipids. However, there is still much work to be done to better understand the optimal dietary protein/energy ratios for different production phases and optimize the nutrition of local breeds. Owing to the high cost of protein sources and concerns about the environmental impact of pig production, implementing feeding strategies that maximize nutrient utilization is increasingly important (Pomar et al. 2021). The compensatory growth induced by lysine restriction represents a feeding strategy that improves the efficiency of nitrogen utilization for lean growth (Menegat et al. 2020). The low potential for protein deposition in local pig breeds suggests that protein levels in their diet can be lower than those in commercially available diets designed for high-performing genotypes, but how much still needs to be established.

One of the untapped local pig breeds is the Krškopolje pig, the only preserved Slovenian autochthonous pig breed. Like other fatty local breeds, it exhibits limited muscle and high fat deposition capacity and moderate growth (Batorek Lukač et al. 2019). Currently, the breed is maintained in small-scale but diverse husbandry systems, ranging from conventional indoor farming to outdoor and organic production systems (Kastelic and Čandek-Potokar 2013). Krškopolje pigs are renowned for their superior meat quality, favorable muscle pH, high intramuscular fat content, and intense meat color due to elevated myoglobin levels, which are indicative of increased oxidative muscle metabolism (Tomažin et al. 2019; Fazarinc et al. 2020; Poklukar et al. 2023). Brossard et al. (2019) reported that the Krškopolje pig breed (in an intensive system) has relatively high-protein (HP) deposition capacity compared with some other local breeds. However, these findings are preliminary and limited by the narrow weight range (40–100 kg) considered in this study. Our latest study (Škrlep et al. 2024), which compared modern lean crossbreeds with Krškopolje pigs, raised under the same conditions, suggested that protein requirements of this local breed may be significantly lower. Specifically, reducing crude protein levels to below 10% in the final fattening stage had no negative impact on their performance. To enhance our understanding of the nutritional needs of Krškopolje pigs across the broader fattening weight range that is usually practiced and with the goal of creating more sustainable diets and optimized feeding, an exploratory study was conducted to assess their response to diets with reduced amino acid and protein levels in two housing systems, conventional indoor and organic outdoor. Our hypothesis was that the control feeding strategy (despite lower protein levels as practiced in modern lean breeds) would still meet their nutritional needs, while the diet with a further reduced protein level might adversely affect their growth potential in the initial stage (25–100 kg) but potentially lead to compensatory growth once nutritional needs are fully met again in the final stage. In addition, the impacts of these nutritional strategies on carcass and meat quality properties were evaluated.

## 2 | Materials and Methods

### 2.1 | Animal Ethics

This study was carried out following the Slovenian law on animal protection (Zakon o zaščiti živali, 2013). The work was undertaken within the normal running of the farms and with full owner compliance, and no procedures on animals were conducted, which would demand ethical protocols according to Directive 2010/63/EU (2010). All the tissue (meat) samples were taken after slaughter.

### 2.2 | Animals and Husbandry

Two field tests, one in a conventional indoor and the other in an organic outdoor husbandry system, were conducted to test the effects of reduced dietary protein content. A multiphase feed sequencing plan was designed considering the preliminarily available data for Krškopolje pigs (Brossard et al. 2019). A randomized block design was used to test two dietary strategies: HP treatment involving three-phase feeding with feed mixtures containing 15%, 12.5%, and 10% crude protein (CP) and low-protein (LP) treatment involving two-phase feeding with feed mixtures containing 15% and 10% CP (Figure 1).

At 102.0 ± 7.5 days of age and a body weight (BW) of 24.9 ± 4.5 kg (mean ± SD), Krškopolje pigs were purchased from local breeders and brought for fattening to an organic farm ($n$ = 24; 16 castrates and 8 females) or a conventional farm ($n$ = 28; 14 castrates and 14 females). On the organic farm, a fenced outdoor area with wooden shelters was used for fattening pigs in two groups (HP and LP), each with 12 pigs (eight castrates and four females). The available outdoor area was divided into two halves and provided approximately 35 $m^2$ per pig. On the conventional farm, the pigs (seven castrates and seven females per treatment group) were kept in four pens on a partially slatted floor (two pens per dietary treatment providing 1.6 $m^2$ per pig). The pigs were offered feed ad libitum, and the feed distribution was recorded on a group basis. The trials were conducted under field conditions; thus, it was not possible to determine feed wastage. During the trial, the feed was distributed to the pigs twice a day at 9 a.m. and 3 p.m.

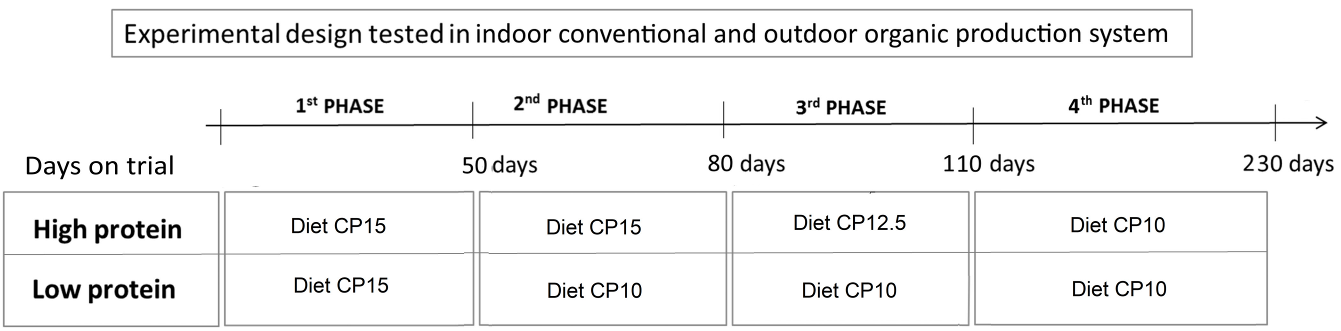


**FIGURE 1** | Design of the multiphase feeding trial with high-protein and low-protein diets in Krškopolje pigs raised in outdoor organic and indoor conventional systems. CP, crude protein.

## 2.3 | Dietary Treatments

Organic and conventional feed mixtures containing 15%, 12.5%, and 10% CP were produced by a commercial feed mill. The feed mixtures were formulated using the same ingredients but sourced from either organic or conventional agriculture (Table 1).

The diets used were based on the feeding recommendations for growing pigs (NRC 2012) but also on the results of Brossard et al. (2019), who reported a lower protein deposition capacity in Krškopolje pigs. In accordance with Regulation (EU) 2018/848 (2018), which prohibits the use of synthetic amino acids in organic farming, no synthetic amino acids have been included in feed in either organic or conventional rearing systems. We decided to follow this principle uniformly for both farming systems. The feeding plan was divided into four phases; the HP diet contained approximately 15%, 12%, and 10% CP in Phases 1–2, 3, and 4, respectively, whereas the LP diet contained 15% CP in Phase 1 and 10% CP in Phases 2–4 (Figure 1). The feed mixtures were chemically analyzed by an accredited laboratory (SIST EN 17025) according to the standard analytical procedures (Association of Official Analytical Chemists 2000) for CP, crude fat, dry matter, crude fiber, crude ash, and amino acid contents. The ingredients and chemical composition of the feedstuffs are listed in Table 1, while the detailed composition of the (essential) amino acids can be found in Figures S1–S6).

## 2.4 | Growth Monitoring

The pigs were weighed individually every 3–4 weeks. At the same time, ultrasound measurements (Draminski 4Vet mini; Draminski S.A., Olsztyn, Poland) of backfat thickness and longissimus lumborum (LL) muscle thickness at the level of the last

**TABLE 1** | Ingredient and nutritional composition of experimental diets.

| | Conventional diet | | | Organic diet | | |
|---|---|---|---|---|---|---|
| **Diet** | **CP15** | **CP12.5** | **CP10** | **CP15** | **CP12.5** | **CP10** |
| Ingredient (inclusion level; %) | | | | | | |
| Alfalfa, dehydrated | 8.10 | 8.54 | 8.98 | 8.10 | 8.54 | 8.98 |
| Maize | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Barley | 45.0 | 51.0 | 57.0 | 45.0 | 51.0 | 57.0 |
| Wheat | 6.00 | 6.50 | 7.00 | 6.00 | 6.50 | 7.00 |
| Wheat feed flour | 5.88 | 7.94 | 10.0 | 5.88 | 7.94 | 10.0 |
| Soybean meal | 12.0 | 6.00 | / | 12.0 | 6.00 | / |
| Sunflower meal | 6.00 | 3.00 | / | 6.00 | 3.00 | / |
| Molasses | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Sodium chloride | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 | 0.33 |
| Monocalcium phosphate | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Calcium carbonate | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 |
| Vitamins and trace mineral mixtures | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Analytical values, g/kg feed | | | | | | |
| Dry matter | 879.0 | 892.1 | 891.0 | 886.8 | 879.6 | 881.9 |
| Crude ash | 73.0 | 73.2 | 70.5 | 68.0 | 72.0 | 64.0 |
| Crude protein | 148.0 | 130.9 | 106.8 | 144.7 | 125.0 | 106.6 |
| Ether extract | 30.0 | 20.3 | 21.8 | 32.0 | 24.0 | 17.0 |
| Crude fiber | 67.6 | 70.4 | 64.0 | 69.4 | 63.4 | 62.0 |
| Nutritional traits | | | | | | |
| ME,[a] MJ/kg of feed | 11.3 | 11.4 | 11.5 | 11.6 | 11.3 | 11.4 |
| NE,[a] MJ/kg of feed | 8.4 | 8.5 | 8.6 | 8.5 | 8.4 | 8.5 |
| Lysine,[b] g/kg of feed | 7.0 [5.7] | 5.4 [4.2] | 4.0 [2.9] | 5.5 [4.5] | 4.4 [3.5] | 3.3 [2.5] |

Abbreviation: CP, crude protein.
[a]Calculated from ingredient and gross chemical composition according to Sauvant et al. (2004).
[b]Values in brackets represent standard ileal digestibility values estimated with InraPorc. For all essential amino acids, the values can be found in Figures S1–S6.

rib (measured on the left and right sides of the back lateral to the spine and then averaged per pig) were performed. The difference in backfat thickness and LL muscle thickness divided by the number of days elapsed between measurements was calculated to evaluate daily backfat gain and daily muscle gain in the total and per phase.

### 2.5 | Modeling Nutritional Requirements

Data on the feed distribution (group level) and growth performance of Krškopolje pigs were recorded in InraPorc, a tool for assessing performance and nutrient utilization (van Milgen et al. 2008), to determine the extent to which the tested feeds meet the assumed nutrient requirements. The calculation of nutrient requirements requires the creation of animal profiles. The animal profile describes the phenotypic growth and feed intake potential (or phenotypic performance potential) of the pigs studied, assuming ideal conditions. Specific model parameters must be determined for each production setting. When the animal profiles were calibrated, the model parameters were estimated using the observed feed usage and growth data. The ad libitum net energy daily feed intake was modeled with InraPorc using the gamma function of BW (Figure 2), which expresses the daily feed intake relative to the maintenance energy requirement. After the profiles for each treatment group were created (Figure S7), the lysine requirements of Krškopolje pigs in each production system were evaluated based on the profile of the HP group (Figure 3).

### 2.6 | Carcass and Meat Quality Traits

At an average age of 330 ± 13 days, pigs were transported to a commercial slaughterhouse (transport duration of 30 min). The animals were slaughtered according to the routine abattoir procedures (electrical stunning, exsanguination, vapor scalding, and dehairing). After evisceration, the carcasses were split in half, the leaf fat was removed and weighed, the carcass weight was recorded, and the backfat thickness was measured at the withers (between the first thoracic vertebra and the last cervical vertebra), last rib and thinnest point over the gluteus medius muscle (GM). Muscle thickness was measured as the shortest distance between the cranial edge of the GM and the dorsal edge of the vertebral canal.

Muscle pH was measured in LL at the level of the last rib 45 min (pH 45) and 24 h (pH 24) after slaughter with MP120 pH meter and a combined glass puncture electrode (Sensor InLab Solids) equipped with a temperature probe (Mettler-Toledo GmbH, Schwarzenbach, Switzerland). Marbling, subjective, and instrumental color parameters (CIE L*, a*, b*) were measured with a Minolta chromameter (with D65 illuminant). Images of the loin eye and the overlying subcutaneous fat area (cross-section at the last rib) were taken for subsequent measurement of the surface. Two 2.5-cm-thick portions of LL were removed and carefully cleaned from superficial connective and fat tissues for determination of water holding capacity (drip, thawing, and cooking loss), shear force, and chemical traits. All the measurements were performed in duplicate, and the quality criteria for the methods are reported in Tomažin et al. (2019).

Near-infrared spectroscopy was employed to assess the intramuscular fat (IMF) content of LL using the NIR Systems 6500 Monochromator (Foss NIR System, Silver Spring, Maryland, United States). Internal calibrations (with a prediction performance of Sy.x = 0.03 and $R^2 = 0.97$) developed by the Agricultural Institute of Slovenia were used.

As the meat water holding capacity is affected by oxidation (Bao and Ertbjerg 2018), indicators of protein and lipid oxidation were assessed. To measure protein oxidation, myofibril isolates were prepared from LL samples as described in Tomažin et al. (2019). The concentration of carbonyl groups in DNPH-treated samples was measured at 370 nm using a fluorescence bioSpectrometer (Eppendorf GmbH, Wesseling-Berzdorf, Germany) and expressed in nmol/mg protein. The protein concentration was determined from the absorbance measured at 280 nm, based on standard BSA concentrations in 6-M guanidine HCl. Oxidation of muscle lipids in LL was assessed by spectrophotometric determination of TBARS (thiobarbituric acid reactive substances), with absorbance measured at 535 nm using BioSpectrometer Fluorescence. TBARS concentrations were calculated using a standard curve prepared with 1,1,3,3-tetraethoxypropane (for details, see Tomažin et al. 2019).

The myoglobin concentration of the LL muscle was determined spectrophotometrically by measuring the absorbance at 370 and 409 nm in a solution prepared according to the method described in Tomažin et al. (2019).

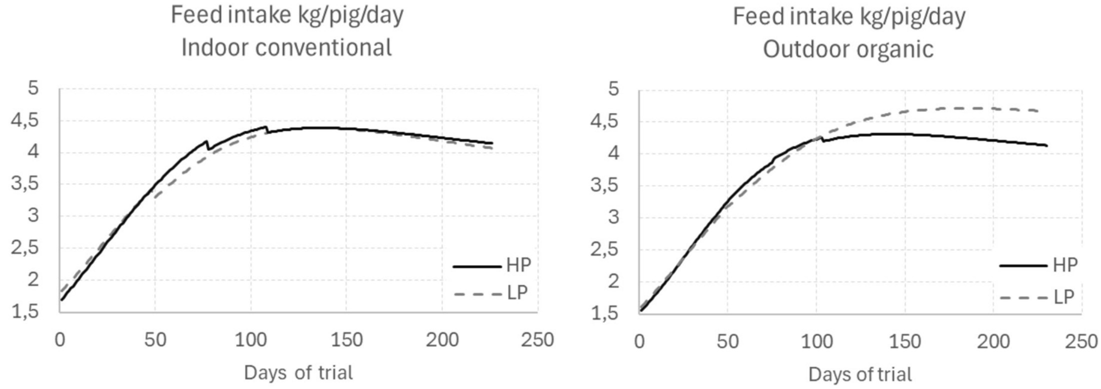


**FIGURE 2** | Daily feed intake estimated with InraPorc based on feed distribution and growth data. HP, high-protein diet; LP, low-protein diet.

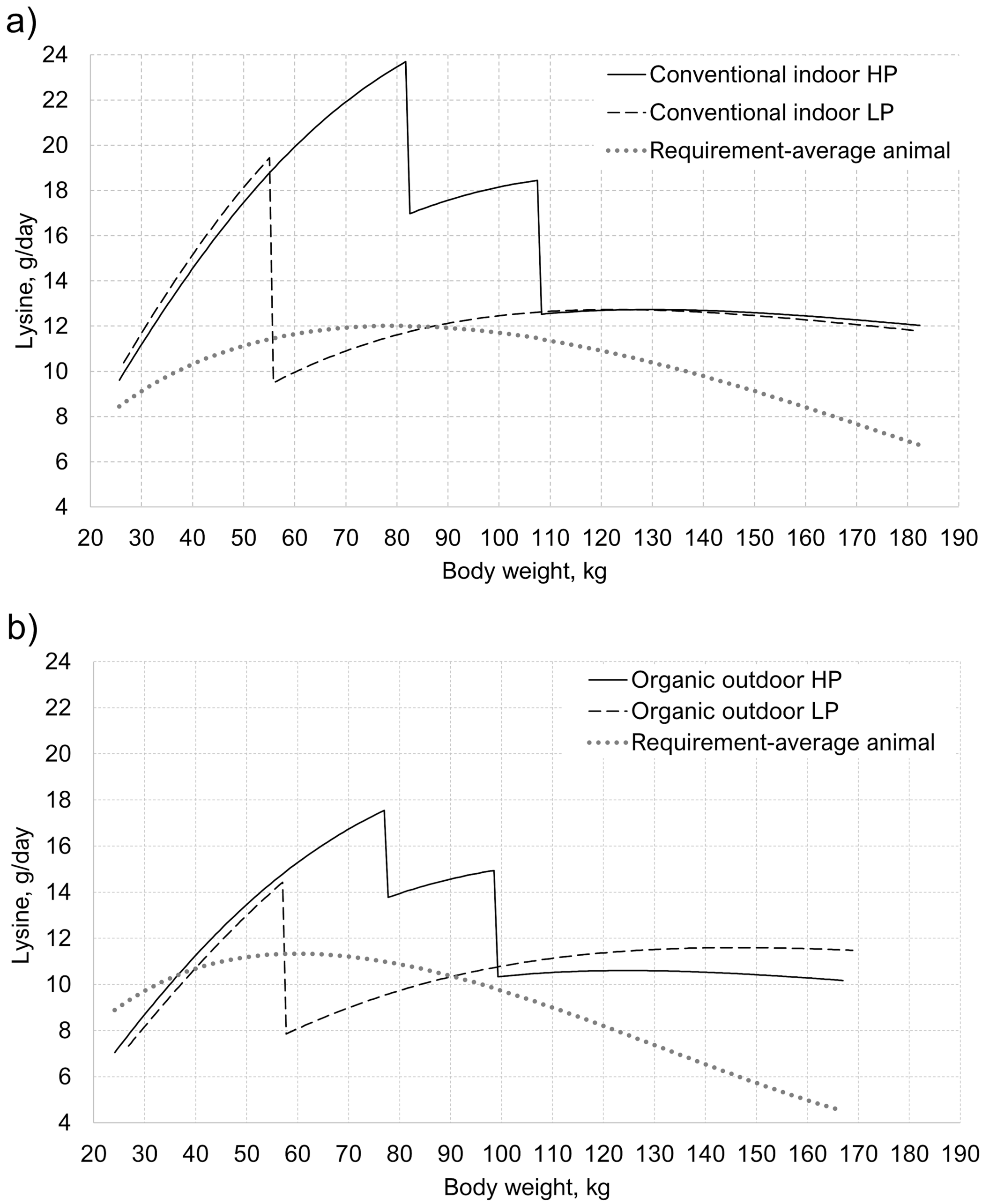


**FIGURE 3** | Assumed lysine (expressed as standardized digestible lysine) requirements and supply for Krškopolje pigs. Lysine requirements are estimated based on the profile of HP animals in (a) conventional indoor and (b) organic outdoor rearing systems. HP, high-protein diet; LP, low-protein diet.

### 2.7 | Data Analysis

For the results presented in Tables 2–4, the data were analyzed with SAS statistical software (SAS Institute Inc., Cary, North Carolina, United States) using the PROC MIXED procedure. We chose to designate the individual animal as the experimental unit, assuming independence (or negligible dependence) between animals on the measured outcomes (Gosselin 2019), as the pigs were provided with ample feeder space and overall space. The effect of the diet was evaluated by the production system, that is, separately for conventional indoor and organic outdoor rearing systems. In both cases, the model included fixed effects of diet and sex (blocking factor). For the analysis of growth performance traits in the conventional indoor system, the pen within the dietary treatment was also included as a random effect in the model. Least square means were calculated and compared using the LSMEANS procedure with the PDIFF option. Significant values were declared if $p < 0.05$, and $0.05 < p < 0.10$ was considered a tendency.

## 3 | Results

### 3.1 | Simulation With InraPorc

A simulation was conducted to estimate the protein and amino acid requirements and their provision within the studied

**TABLE 2** | Growth performance traits of Krškopolje pigs according to dietary treatment in indoor conventional and outdoor organic rearing systems.

| | Indoor conventional | | | | Outdoor organic | | | |
|---|---|---|---|---|---|---|---|---|
| | **HP** | **LP** | **RMSE** | ***p*** | **HP** | **LP** | **RMSE** | ***p*** |
| *N* | 14 | 14 | | | 12 | 12 | | |
| Body weight, kg | | | | | | | | |
| Initial | 25.0 | 24.9 | 4.65 | 0.991 | 23.9 | 24.8 | 2.64 | 0.417 |
| Phase 1 | 56.8 | 55.5 | 7.98 | 0.695 | 58.8 | 59.8 | 4.56 | 0.587 |
| Phase 2 | 82.3 | 77.7 | 10.31 | 0.363 | 77.9 | 76.4 | 5.43 | 0.530 |
| Phase 3 | 108.5 | 103.1 | 11.43 | 0.338 | 98.6 | 94.7 | 6.38 | 0.155 |
| Phase 4 | 184.0 | 182.5 | 13.84 | 0.796 | 165.2 | 165.3 | 12.42 | 0.977 |
| Daily gain, g/day | | | | | | | | |
| Phase 1 | 637 | 610 | 104.7 | 0.567 | 711 | 714 | 56.7 | 0.911 |
| Phase 2 | 879 | 768 | 113.6 | 0.124 | 763 | 665 | 71.6 | 0.003 |
| Phase 3 | 846 | 819 | 100.6 | 0.551 | 739 | 653 | 99.8 | 0.046 |
| Phase 4 | 636 | 667 | 86.42 | 0.476 | 522 | 554 | 71.7 | 0.285 |
| Total, g/day | 696 | 688 | 66.52 | 0.788 | 616 | 612 | 55.4 | 0.888 |
| LL muscle thickness gain, mm/day | | | | | | | | |
| Phase 2 | 0.24 | 0.14 | 0.080 | 0.140 | 0.24 | 0.20 | 0.117 | 0.522 |
| Phase 3 | 0.21 | 0.18 | 0.072 | 0.343 | 0.12 | 0.15 | 0.095 | 0.520 |
| Phase 4 | 0.11 | 0.14 | 0.040 | 0.218 | 0.09 | 0.09 | 0.044 | 0.946 |
| Total, mm/day | 0.15 | 0.14 | 0.023 | 0.525 | 0.12 | 0.10 | 0.026 | 0.136 |
| Backfat thickness gain, mm/day | | | | | | | | |
| Phase 2 | 0.13 | 0.17 | 0.056 | 0.228 | 0.18 | 0.14 | 0.043 | 0.040 |
| Phase 3 | 0.10 | 0.12 | 0.044 | 0.715 | 0.17 | 0.13 | 0.067 | 0.117 |
| Phase 4 | 0.11 | 0.10 | 0.026 | 0.319 | 0.10 | 0.12 | 0.030 | 0.052 |
| Total, mm/day | 0.11 | 0.11 | 0.016 | 0.947 | 0.12 | 0.13 | 0.025 | 0.560 |

*Notes:* Phase 1 denotes transition to a 10% crude protein diet in the LP group; Phase 2 means transition to a 12.5% crude protein diet in the HP group; and Phase 3 indicates transition to a 10% crude protein diet in the HP group.
Abbreviations: HP, high-protein diet; LP, low-protein diet; LL, longissimus lumborum.

production systems based on "average animal" (Figure 3). The results indicated that neither protein nor any essential amino acids, except for lysine, limited growth. Thus, we present the results only for this amino acid. For conventional indoor fattening (Figure 3), the simulation indicated that the HP diet met the lysine requirements of Krškopolje pigs whereas a deficiency of lysine was observed in the case of the LP diet in Phase 2 (between approximately 55 and 90 kg BW). This lysine deficit appeared with the transition to a diet containing 10% protein. However, this had no effect on the global deposition of protein or lipids (Figure 4), which was similar in HP and LP diets (83 vs. 81 g/day for protein and 394 vs. 403 g/day for lipids, respectively). In the case of the organic outdoor system and HP diet, the simulation showed some deficit in lysine, from the start up to approximately 35 kg BW (Figure 3). However, this deficit was overall balanced, as no deficit was observed on average in the first phase. In the case of the organic outdoor system and LP diet, the lysine deficit was more pronounced than in the conventional indoor system; in addition to lysine deficit at the beginning (as in HP diet), a substantial deficit was noted when switching to a diet with 10% CP, which persisted from approximately 60 to 90 kg BW (Phases 2 and 3); this deficit was estimated to be (on average) 1.9 g/day. As a result, the simulation revealed overall 8% lower protein deposition in the LP group than in the HP group, that is, 62 versus 67 g/day, respectively (Figure 4).

## 3.2 | Growth Performance

Overall, daily weight gain differed (numerically) between the indoor conventional and outdoor organic systems and was 13% greater in the conventional system (Table 2). Interestingly, the

**TABLE 3** | Carcass traits of Krškopolje pigs according to dietary treatment in organic and conventional rearing systems.

| | Indoor conventional | | | | Outdoor organic | | | |
|---|---|---|---|---|---|---|---|---|
| | HP | LP | RMSE | *p* | HP | LP | RMSE | *p* |
| Number of pigs | 14 | 14 | | | 12 | 12 | | |
| Carcass weight, kg | 149.8 | 148.9 | 11.19 | 0.825 | 136.2 | 136.5 | 10.19 | 0.926 |
| Leaf fat, kg | 4.28 | 4.75 | 0.95 | 0.199 | 5.37 | 5.60 | 0.817 | 0.420 |
| Backfat thickness, mm[a] | | | | | | | | |
| Average | 45 | 43 | 5.2 | 0.236 | 49 | 54 | 5.2 | 0.058 |
| At withers | 57 | 56 | 8.8 | 0.733 | 64 | 70 | 9.6 | 0.106 |
| Last rib | 40 | 39 | 5.8 | 0.540 | 44 | 47 | 4.7 | 0.111 |
| Over GM[b] | 38 | 34 | 5.6 | 0.035 | 41 | 44 | 5.8 | 0.215 |
| Muscle depth, mm[c] | 78 | 74 | 6.2 | 0.079 | 71 | 70 | 6.9 | 0.519 |
| Loin eye area, $cm^2$[d] | 48.9 | 49.2 | 7.17 | 0.922 | 40.8 | 40.4 | 4.06 | 0.808 |
| Fat area, $cm^2$[d] | 51.1 | 50.8 | 8.55 | 0.914 | 43.9 | 45.1 | 6.55 | 0.656 |

Abbreviations: GM, gluteus medius muscle; HP, high-protein diet; LP, low-protein diet.
[a]Measured at the carcass split line.
[b]Minimum backfat thickness over the gluteus medius muscle measured at the carcass split line.
[c]The shortest distance between the dorsal edge of the vertebral canal and the cranial edge of the gluteus medius muscle measured at the carcass split line.
[d]Measured at the last rib cross-section.

**TABLE 4** | Meat quality (LL muscle) traits according to diet in indoor conventional and outdoor organic rearing systems of Krškopolje pigs.

| | Indoor conventional | | | | Outdoor organic | | | |
|---|---|---|---|---|---|---|---|---|
| | HP | LP | RMSE | *p* | HP | LP | RMSE | *p* |
| Number of pigs | 14 | 14 | | | 12 | 12 | | |
| Intramuscular fat, % | 4.7 | 4.9 | 1.09 | 0.715 | 5.1 | 5.7 | 1.01 | 0.171 |
| TBARS, μ MDA/kg | 0.85 | 0.82 | 0.152 | 0.685 | 0.78 | 0.78 | 0.171 | 0.925 |
| Carbonyl groups, nmol/mg protein | 1.48 | 1.42 | 0.196 | 0.409 | 1.30 | 1.16 | 0.241 | 0.189 |
| Myoglobin, mg/g | 1.8 | 1.8 | 0.25 | 0.988 | 2.3 | 2.3 | 0.32 | 0.860 |
| pH 45 | 6.36 | 6.15 | 0.207 | 0.010 | 6.23 | 6.34 | 0.182 | 0.137 |
| pH 24 | 5.47 | 5.47 | 0.092 | 0.808 | 5.47 | 5.43 | 0.045 | 0.049 |
| Marbling | 4.1 | 3.7 | 1.08 | 0.365 | 3.4 | 3.9 | 1.11 | 0.283 |
| Color score (1–6) | 3.5 | 3.3 | 0.75 | 0.535 | 3.7 | 3.6 | 0.43 | 0.814 |
| L* | 51.2 | 52.7 | 3.73 | 0.303 | 50.8 | 51.6 | 3.46 | 0.566 |
| a* | 8.8 | 9.2 | 1.24 | 0.422 | 9.9 | 10.2 | 1.31 | 0.566 |
| b* | 4.9 | 5.4 | 1.28 | 0.337 | 5.4 | 5.8 | 1.33 | 0.536 |
| Drip loss in 24 h, % | 3.2 | 5.6 | 2.22 | 0.008 | 4.1 | 4.6 | 1.57 | 0.505 |
| Thawing loss, % | 11.8 | 12.6 | 2.41 | 0.351 | 13.1 | 14.0 | 1.74 | 0.267 |
| Cooking loss, % | 29.4 | 29.1 | 1.79 | 0.704 | 25.9 | 25.3 | 2.74 | 0.587 |
| WB shear force, N | 46.2 | 48.3 | 7.9 | 0.508 | 44.4 | 37.5 | 8.11 | 0.049 |

*Notes:* pH 45 means pH value measured 45 min after slaughter; pH 24 indicates pH value measured 24 h after slaughter; L*a*b* denotes color parameters measured with a Minolta chromameter.
Abbreviations: HP, high-protein diet; LL, longissimus lumborum; LP, low-protein diet; MDA, malondialdehyde; TBARS, thiobarbituric reactive substances; WB, Warner–Bratzler.

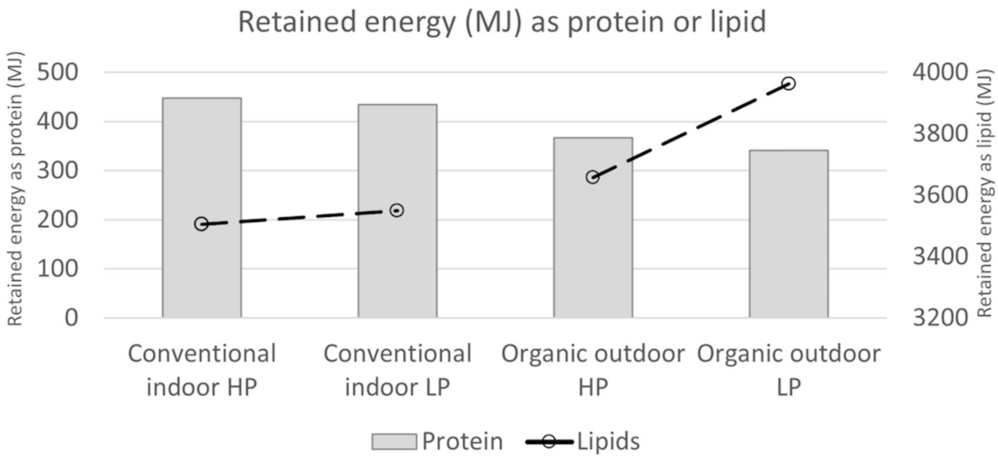


**FIGURE 4** | Retained energy (MJ) as protein or lipid or lipid in Krškopolje pig raised in outdoor organic and indoor conventional systems. HP, high-protein diet; LP, low-protein diet.

opposite was observed for Period 1. With respect to the effect of dietary protein level, the results for growth performance over the entire trial duration (Table 2) revealed no significant difference between the HP and LP diets in either of the rearing systems. However, when we analyzed the growth rate according to the feed sequence plan, we noted some differences. In the indoor conventional rearing system, passage to a diet with 10% CP in Phase 2 resulted in a daily gain that was 111 g (14%) lower ($p = 0.12$) and a muscle thickness gain that was 0.10 mm/day (42%) lower ($p = 0.14$) in the LP group than in the HP group. However, overall, the differences in the rates of muscle and backfat thickness gain were small and not statistically significant. In outdoor organic rearing, the daily gain was significantly lower in pigs on the LP diet during Phases 2 and 3 (98 and 86 g, respectively; $p < 0.05$). A lower daily gain was accompanied by a smaller rate of backfat thickness gain during this period. In the last period of fattening (Phase 4), pigs on the LP diet presented approximately 5% greater daily gains ($p > 0.10$) in both the conventional and organic systems (31 and 32 g, respectively).

### 3.3 | Carcass and Meat Quality

No important and consistent differences were observed in carcass traits, regarding either muscle or fat tissue indicators (Table 3). The observed tendency toward greater muscle thickness in the HP group ($p = 0.08$) in the conventional system was not supported by the loin eye area ($p = 0.92$). Similarly, greater backfat thickness at the GM level ($p = 0.04$) in the HP group of pigs in the conventional system was not confirmed by adiposity indicators at other sites along the carcass split line, resulting in similar average backfat thicknesses. In pigs raised in the organic system, there was a trend of thicker backfat at the withers, last rib, and on average ($p < 0.10$) of pigs on the LP diet. There were no major differences in meat quality traits (Table 4) in relation to dietary protein levels. However, in the conventional system, pigs on the LP diet exhibited a lower pH 45 ($p = 0.01$) and reduced water-holding capacity ($p = 0.01$) compared to those on the HP diet. In the organic system, pigs on the LP diet showed a lower pH 24 ($p = 0.05$) and reduced shear force ($p = 0.05$) compared to those on the HP diet.

## 4 | Discussion

Knowledge about the nutritional requirements of fat-type local breeds is limited. Owing to their lower protein deposition capacity, slower growth rate, and relatively high maintenance needs, they are expected to have a lower protein-to-energy requirement, as reported for Iberian pigs (Nieto et al. 2002; Nieto et al. 2012). Compared to genetically improved, modern lean pig breeds, lower protein requirements (and consequently a reduced need for soybean meal) have also been determined for other local pig breeds using the modeling approach (Brossard et al. 2019). However, more data are needed for different production settings, as local breeds are often raised under diverse conditions: indoor, outdoor, conventional, and organic systems. When the diets used in this study were formulated, data available for the Krškopolje pig (Brossard et al. 2019) were considered. The diets were isoenergetic and isoproteic in both production systems to ensure comparability not only in nutrient composition but also in the ingredients used. However, the use of ingredients from two production systems led to differences in amino acid content between the diets formulated for the conventional and organic systems (Figures S1–S6). The levels of lysine, the first limiting amino acid, were generally low (≤ 7 g/kg diet), especially for the first phase of rearing, as no synthetic amino acids were added. Nevertheless, lysine requirements were met during the early fattening stage in both the HP and LP diets in the conventional indoor system. In contrast, a slight deficiency could be noted in the organic system, yet it was accompanied by a higher daily gain compared to the conventional indoor system. This is a noteworthy observation, although the underlying causes may be different, potentially involving differences in growth potential and/or early adaptation of the pigs. Overall, we can conclude that the HP diet was sufficient to meet the requirements of the "average" Krškopolje pigs in the present experimental setup. However, to address the requirements at the population level, a somewhat greater lysine supply should be considered (e.g., InraPorc suggests 10%) to meet the needs of pigs with above-average requirements, particularly during the critical stages of production. In contrast, the deficit caused by the LP diet was evident from the transition to the 10% protein diet in both systems. This was intended, as we aimed to assess how much growth performance would be affected and, potentially, whether the pigs could compensate for the deficit in the later stages.

The observed lysine deficit in the LP diets affected growth performance during the period of protein reduction, and this impact was more pronounced in the organic system than in the conventional system. Specifically, in Phase 3, in the conventional system, a 3.3% reduction in daily gain was noted for the LP diet, whereas in the organic system, a 13.2% reduction in daily gain was noted for the LP diet. In Phase 4, we noted a slight (5%) increase in daily gain in LP-fed pigs in both systems, suggesting some compensatory growth during the final experimental period. This ultimately led to similar final BWs and similar overall daily gains for the HP and LP dietary regimens. A thorough review by Menegat et al. (2020) indicated that for effective compensatory growth, the degree of lysine restriction should be substantial (between 10% and 30%) and should be applied before pigs reach their maximum protein deposition. The duration of the restriction should be brief (less than 40%–45% of the overall rearing duration), whereas the recovery period should be relatively long (at least 55%–60% of the overall rearing duration). Additionally, lysine levels during the recovery period should meet or exceed the estimated requirements. These conditions were met in our study. In conventional indoor rearing, the degree of restriction was 10% in Period 2, whereas in organic outdoor rearing, it was 20% and 25% in Periods 2 and 3, respectively. Additionally, the duration of the lysine restriction was short compared with the overall fattening period. Despite differences in the degree and duration of lysine restriction, the impact on performance was similar in both systems (5% and 6% greater daily gains in Period 4 in the LP groups subjected to conventional and organic rearing, respectively). While a direct comparison of rearing systems is difficult with the chosen design, the 13% greater growth rate in the conventional indoor system than in the organic outdoor system may be attributed partly to mild lysine deficiency and partly to outdoor rearing. Pigs reared outdoors generally exhibit a lower growth rate because of the increased energy demands for thermoregulation and physical activity (Pugliese et al. 2003; Bee et al. 2004). The observed differences in protein and lipid deposition (Figure 4) between HP and LP diets (the former being greater in conventional indoor rearing than in organic outdoor rearing) are consistent with the observed difference in growth performance.

Diet plays a pivotal role in shaping body composition, and a protein- or lysine-deficient diet offered ad libitum increases the fatness (Lebret and Čandek-Potokar 2022). The absence of any substantial difference in protein and lipid deposition between the HP and LP diets, regardless of the rearing system (Figure 4), is consistent with the small impact of dietary strategy on carcass traits. This may be of practical importance; a faster switch to a cheaper and more sustainable feed may only have limited effects on performance while potentially enhancing efficiency and reducing environmental impact. With some minor exceptions, no notable differences were observed in meat quality traits. Technological meat quality, such as pH and water-holding capacity, is predominantly determined by the post-mortem biochemical conversion of muscle to meat, with limited evidence in the literature supporting a direct influence of dietary protein levels (see review by Lebret and Čandek-Potokar 2022). Nonetheless, the observed lower LL shear force in the LP group in the organic system aligns with findings that LP diets or lysine deficient diets, when not energy restricted, enhance pork tenderness, because of increased intramuscular fat content (Alonso et al. 2010; Madeira et al. 2013).

Modeling the data acquired with InraPorc showed that protein and essential amino acid requirements were met in the control (HP) diet, despite a lower protein level than commonly practiced. However, lysine was deficient in the LP group after transitioning to a 10% protein diet, although this deficiency was temporary and did not persist in the final fattening stage. This could be related to the reduced protein requirements when muscle growth stagnates and weight gain is primarily driven by fat accumulation. Lysine deficiency was more pronounced in the organic system (feed ingredients in the diet from organic agriculture), affecting growth rates during the deficiency period in the organic system, but without any effect on global growth rate. Reduced-protein diets had no major impact on growth, carcass, or meat quality, but this approach could help reduce the final amount of nitrogen excretion, promoting more sustainable farming practices of this fat-type breed. This study provided valuable insights into the nutritional requirements of Krškopolje pigs and similar untapped local pig breeds, which is important for better nutrient utilization and more sustainable and optimized feeding system of fat-type breeds. In practical terms, a conventional diet CP12.5 could be fed starting from 40 kg of BW and CP10 from 90 kg onwards. For diets with organic ingredients, due to the lower lysine content, CP15 diet was not sufficient until around 35 kg BW, while CP12.5 could be fed from 55 kg onwards, and CP10 from 90 kg onwards. Variations in ingredients and husbandry circumstances should be taken into account when formulating the diet. It is also important to consider additional margin if we want to cover the needs of above-average animals.

**Acknowledgments**

The authors are thankful to Jani Debeljak for the technical help and to farms B.V. and Korenika for implementing the indoor and outdoor trials, respectively. This study has received 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, and L7-4568), and the Ministry of Agriculture, Forestry, and Food (V4-2201 and L7-4568).

**Conflicts of Interest**

The authors declare no conflicts of interest.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.