Animal 15 (2021) 100220

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Evolution of viscera and muscle fractional protein synthesis rate in lean meat selected hybrids and castrated Duroc pigs fed under moderate crude protein restriction

L. Sarri [a], J. Balcells [a], G. de la Fuente [a,*], M. Tor [a], J. Gómez-Arrue [b], A.R. Seradj [a]

[a] Departament de Ciència Animal, Universitat de Lleida- Agrotecnio-CERCA Centre, Av. Alcalde Rovira Roure 191, 25198 Lleida, Spain
[b] Instituto Aragonés de Ciencias de la Salud (IACS), Av. San Juan Bosco 13, 50009 Zaragoza, Spain

ARTICLE INFO

Article history:
Received 6 July 2020
Revised 24 February 2021
Accepted 25 February 2021
Available online 4 June 2021

Keywords:
Apparent digestibility
Pig producing type
Production phase
Protein restriction
Protein synthesis

ABSTRACT

Differences in producing performance and organoleptic meat characteristics among pig genotypes and/or producing types are widely known. These parameters are also subjected to the animal's development, feeding and management. Detailed knowledge of the effects of production phase (**PP**), pig producing type (**PT**), dietary protein availability and their interactions on nutrient digestibility, nitrogen balance and protein metabolism is essential information to improve precision feeding techniques. The experiment was a 2 (PP) × 2 (PT) × 2 (diet) factorial design conducted with 32 male pigs, 16 entire F2 pigs progeny of Pietrain sires and Duroc × Landrace dams, and 16 castrated purebred Durocs belonging to two production phases (growing: 29.5 ± 3.19 v. fattening: 88.6 ± 6.26 kg BW), and assigned to one of two dietary CP levels, either standard (**SP**: 17% in growing and 15% in fattening) or low (**LP**: 15% in growing and 13% in fattening). Viscera and muscle fractional protein synthesis rates (**FSRs**; %/day) were conducted through a single infusion of 15% L-[*ring*-$^{2}H_5$]-phenylalanine, with subsequent blood sampling from 12 to 40 min, and sample collection of liver, duodenum, *biceps femoris* and *longissimus dorsi* skeletal muscles after sacrifice. Fattening animals acquired a greater feed ingestion capacity, average daily gain ($P < 0.01$) and apparent ileal digestibility, whereas growing pigs showed higher FSRs in both viscera (duodenum and liver) and in *longissimus dorsi*. F2 pigs showed higher average daily gain, nitrogen retention rates and FSR in liver and *longissimus dorsi* ($P < 0.01$). Nevertheless, apparent ileal digestibility in all essential amino acids was lower in F2 compared with Duroc pigs ($P < 0.05$). Protein metabolism was barely influenced by dietary CP content, although animals fed LP registered the lowest apparent ileal digestibility for CP and also for most of the essential amino acids compared with SP-fed pigs. This information may reveal differences in amino acid requirements between both PTs, with Duroc pigs receiving excess of dietary amino acids.

Implications

Performance parameters and carcass quality are important features in the swine industry that are influenced by different factors such as age, genotype, gender and diet. In the present study, differences in protein metabolism, amino acid digestibility and nitrogen balance between two commercially tested productive types of pig were identified, which may evidence differences in their use of nutrients and amino acid requirements. Although further experiments are needed to validate the results obtained, our findings provide valuable information to refine rationing models in livestock precision feeding.

Introduction

Precision feeding systems are tools designed to improve feed efficiency by reducing production costs and environmental load (Pomar and Remus, 2019). They rely on accurately matching nutrient supply to animal's requirements according to its genetic merit, physiological and health status. Due to the significant diversity existing among livestock production systems, there is still a potential to improve efficiency, but it is necessary to identify what causes specific variations over a large range of systems and nutritional scenarios.

The existing literature has consistently described the influence of breed and gender on both performance and carcass quality in swine (Latorre et al., 2003); some breeds have been selected to produce low-cost lean meat, whereas others (i.e. fatty pigs from breeds like Duroc or Iberian) are transformed in high-quality

* Corresponding author.
E-mail address: gabriel.delafuente@udl.cat (G. de la Fuente).

https://doi.org/10.1016/j.animal.2021.100220
1751-7311/

products with some specific features such as higher levels of intramuscular fat (Gol et al., 2019). Diet composition (Ruiz-Ascacibar et al., 2019a) and animal's weight are also factors that might influence growth rates of tissues and carcass composition (Álvarez-Rodríguez and Teixeira, 2019), especially in terms of fat incorporation. For this reason, males chosen to produce fatty pigs and slaughtered at higher weight are submitted to castration to avoid boar taint, whereas leaner breeds usually not. Therefore, commercial pig farms can deliver two distinctive products with a direct impact on the nutritional requirements, needed to fulfill their physiological potential (i.e. fractional protein synthesis rate, **FSR**) and their resilience to nutritional changes (i.e. protein restriction). Moreover, due to the different precocity exhibited in animals subjected to both types of products, it might be reasonable to expect variations in their use of nutrients depending on their availability (i.e. level of CP) and their interaction with production phase (**PP**; growing or fattening) (Brossard et al., 2019; Poklukar et al., 2020).

In order to assess nutritional requirements in a precision feeding system, it is essential to understand, not only the mechanisms involved with nutrient absorption but also with the relationship between protein turnover and real amino acid (**AA**) requirements. The present study aims to determine the influence of two producing types (**PTs**), commercial hybrids selected for producing lean meat against castrated purebred Duroc pigs (raised as fatty animals), at two different PPs (growing v. fattening) on their digestibility, nitrogen balance and FSR, together with the effect of moderate protein restriction in both types of animals.

Material and methods

Animals and experimental diets

The study was conducted at the Swine Research Center, located in Torrelameu (CEP; Lleida, Spain) during the June and July months. 32 male pigs from two PTs and two PPs (growing and fattening) were used, where 16 were entire males [F2: Pietrain sires × (Duroc × Landrace) dams], in growing (n = 8; 30.5 ± 1.36 kg BW; mean ± SD) and in fattening (n = 8; 91.1 ± 1.23 kg BW) periods. Same number of surgically castrated purebred Duroc was used in growing and fattening periods (28.5 ± 1.03 and 86.1 ± 2.74 kg BW, respectively). For each PP, two experimental diets with different CP concentration [standard (**SP**) or low (**LP**)] were formulated to be isocaloric, and to meet the nutrient requirements recommended by the Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA, 2013). Moreover, crystalline amino acids were supplemented to ensure similar concentration of essential AAs in both diets, as well as to avoid potential effects of lowering CP supply in LP treatments. Table 1 shows the ingredients and chemical composition of the different diets given to the animals during the trial.

During the growing period, four animals from each PT were fed SP diet (17% CP), and four received LP diet (15% CP). Same pattern was conducted during the fattening period, where four animals from each PT were fed SP and the rest LP diets (15% and 13% CP for SP and LP diets, respectively). With the objective of determining their apparent digestibility coefficient, all experimental diets were supplemented with titanium dioxide (TiO_2) as external marker at the rate of 5 g TiO_2/kg DM.

Experimental design

The experiment lasted 11 days for each pig. After 5 days of diet adaptation, animals were individually weighed, catheterized (Drucafit® Splittocan, B-Braun, Melsungen, Germany) into the right external jugular vein and allotted in individual metabolic cages (2 × 1.04 m^2) for 6 days under controlled environmental and antiseptic conditions. Catheters were flushed daily with heparinized saline solution. Water and feed intake, as well as fecal and urinary excretion, was measured daily. During the last 3 days of the trial, urine was collected in H_2SO_4 (100 ml; 10% v/v) and fecal spot samples (≈50 g) were obtained by rectal stimulation. Fecal samples were stored immediately at −20 °C for further analyses.

On the last day of the trial, animals were submitted to the flooding dose technique to determine the FSR, following Garlick et al. (1980). In brief, an initial blood sampling was conducted in order to determine natural phenylalanine enrichment. Then, a flooding dose of phenylalanine was infused continuously for 10 min through the jugular catheter. Infusion of phenylalanine consisted of a 3.7 ml/kg BW of sterilized saline dilution containing 15 mg/kg BW of L-[*ring*-$^2H_5$]-phenylalanine ($^2H_5$-phenylalanine) (Cambridge Isotope Laboratories CIL; Andover, MA), and 85 mg/kg BW of L-phenylalanine (Sigma-Aldrich, Steinheim, Germany). Following the start of the infusion, a series of blood extractions were performed at 12, 15, 20, 25, 30 and 40 min. Then, plasma was obtained by centrifugation (3 000*g* for 10 min) and kept frozen at −80 °C until analysis. Immediately after the last blood sampling, animals were euthanized with sodium thiopental (Esteve S.A., Oudewater, Netherlands), bodies were weighed, eviscerated and liver, duodenum, *longissimus dorsi* and *biceps femoris* skeletal muscles were sampled from the left half carcass. Ileal digesta samples were also collected. All samples were immediately stored at −80 °C until further analyses. Both liver and skeletal muscles from the right half carcass were dissected and weighed.

Analytical procedures

Both feces and urine samples were thawed at 4 °C overnight and pooled into one feces and one urine sample for each animal. Both ileal and pooled fecal samples were freeze-dried and ground. Feed samples and feces were analyzed for DM (ref. 934.01), organic matter (**OM**) (ash, ref. 942.05) and ether extract (**EE**) (ref. 920.39) following the proximate analysis procedures described by the Association of Official Analytical Chemists (AOAC, 2006). The proportion of NDF was determined according to Van Soest et al. (1991), using α-amylase but not sulfites, and subtracting ashes from the residue.

The CP (nitrogen × 6.25) content in all samples, including feed, feces, ileal content, urine and tissues, was performed by Dumas combustion (Tru Spec CN; Leco Corporation, St. Joseph, MI, USA) (International Organization for Standardization (ISO), 2008). Urine samples were spun (3 500*g* for 2 min) to discard impurities before determination of the nitrogen content.

TiO_2 as a digesta marker was analyzed in ileum, feces and feed ashes using inductively coupled plasma mass spectroscopy (Agilent Technologies 7 700X model, Tokyo, Japan) following Darambazar (2019) procedure in which sample preparation consisted in a digestion process with 6.5 ml of H_2SO_4 (7.4 M, 1.5 h at 200 °C), a cooling step and the addition of 5 ml of H_2O_2 (30% (v/v)).

Amino acid analyses

Analyses of AAs were performed by means of a Multiple Reaction Monitoring method (**MRM**) implemented in an Ultra-HPLC Acquity system (Waters, Milford, MA) coupled to a triple Quadrupole mass spectrometer (Micromass MS Technologies, Manchester, UK). Plasma free AAs were obtained as described by Piraud et al. (2005). Briefly, 200 μl of plasma was mixed with 800 μl of methanol, vortexed (2 min) and maintained at room temperature for 10 min. The extract was then centrifuged (17 500*g* for 5 min) and 50 μl aliquot of the supernatant was preserved. Free AAs from

Table 1
Ingredients and chemical composition (g/kg DM) of the two-phase experimental diets, differing in 2% CP concentration (standard protein, SP v. low protein, LP) for pigs of growing and fattening production phases.

	Growing		Fattening	
	15% CP (LP)	17% CP (SP)	13% CP (LP)	15% CP (SP)
Ingredients				
Corn	294.8	246.5	190.3	99.2
Barley	290.0	287.8	466.0	512.8
Wheat	200.0	200.0	200.0	200.0
Soybean meal	137.6	195.0	65.8	113.6
Beet pulp dehydrated	30.0	30.0	30.0	30.0
Calcium carbonate	13.4	13.2	10.0	10.0
Mono Calcium phosphate	9.4	8.8	7.0	6.4
Soybean oil	9.0	6.3	17.0	17.1
L-Valine	6.8	8.0	0.0	0.0
Sodium chloride	4.6	4.6	4.1	4.1
L-Lysine HCl	4.2	2.4	3.7	2.1
Vitamin- Mineral mix[1]	4.0	4.0	4.0	4.0
L-Threonine	1.6	0.8	1.2	0.5
DL-Methionine	1.0	0.5	0.6	0.3
L-Tryptophan	0.3	0.2	0.2	0.0
Chemical composition				
DM (g/kg FM)	900.1	897.1	909.9	908.4
CP	147.2	166.9	129.27	154.95
Ether Extract	24.9	26.9	39.7	43.2
Ash	65.8	70.3	58.1	52.8
NDF	173.3	179.9	211.8	211.6
ME (kcal/kg)	3 108	3 094	3 185	3 185

Abbreviations: FM = fresh matter; ME = metabolizable energy.
[1] Vitamin mineral mixture composition (per kg of complete diet): vitamin A, 2.4 mg A; vitamin D_3, 0.02 mg; vitamin E, 40 mg; vitamin B_1, 0.8 mg; vitamin B_2, 4 mg; vitamin B_6, 1.6 mg; vitamin B_{12}, 2.4 × 10–2 mg; vitamin K_3, 1.2 mg; pantothenic acid, 8 mg; niacin, 16 mg; biotin, 0.08 mg; folic acid, 0.4 mg; citric acid, 0.264 mg; Co ($CoSO_4 \times 7H_2O$); 0.16 mg; Cu ($CuSO_4 \times 5H_2O$), 128 mg; Fe ($FeCO_4$), 72 mg; Mn (MnO_2), 23.8 mg; Zn (ZnO), 80 mg; Se (Na_2SeO_3), 0.24 mg; I (KI), 0.32 mg; choline chloride, 280 mg; 6-phytase, 600 FYT; endo-(1.4)-ß-glucanase, 2 000 BGU; endo-(1.4)-ß-xylanase, 4 800 FXU; ethoxyquin, 0.264 mg.

viscera tissues (liver, duodenum) and muscles were obtained as described by Qin et al. (2015); briefly, 300 mg of ground freeze-dried samples were homogenized (in 2 ml of distilled water and 2 ml of methanol (1/1 v/v)), incubated (4 °C for 30 min) and centrifuged (10 000*g* for 10 min). Finally, 100 µl of the supernatant was preserved.

Amino acids from tissue protein, feed and ileal digesta samples were obtained by hydrolysis of the remaining pellet after free AA extraction, feed and freeze-dried ileal digesta samples, respectively. Hydrolysis was performed following Colgrave et al. (2008), in which samples (75 mg of tissue pellet; 50 mg of feed; 50 mg of ileal digesta) were incubated (5 ml of HCl 6 N (with 0.02% phenol)) during 24 h at 110 °C under nitrogen steam. After hydrolysis, tubes were cooled and centrifuged (3 000*g* for 30 min), and 50 µl aliquots were then reserved.

All reserved aliquots were evaporated and re-diluted in 500 µl of water/acetonitrile (15/85 v/v), vortexed and filtered through a 0.20 µm hydrophilic PTFE membrane. Ultra-HPLC/MRM analyses were performed following Guo et al. (2013), on a BEH Amide column (2.1 × 150 mm; 1.7 µm). Transitions of MRM were tested in our conditions for alanine, arginine, aspartic acid, cysteine, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine.

To monitor protein synthesis, the transition of $^2H_5$-phenylalanine was added to the MRM method. Precursor/product ion pairs were determined obtaining the following values: 171.22 > 106.14/171.22 > 125.26. Cone voltage and collision energy were optimized for all the transitions, and the absence of crossed signal between phenylalanine and $^2H_5$-phenylalanine channels was checked.

Calibration curves were constructed from a commercial AA standard mixture (Sigma-Aldrich, St. Louis, MO, USA) spiked with $^2H_5$-phenylalanine and diluted to a series of appropriate concentrations with water/acetonitrile (20/80 v/v). Tryptophan concentration was not determined due to its partial degradation under our hydrolysis conditions. Results were processed using QuanLynx software (MassLynx, Waters Corporation, Milford, MA, USA).

Calculations

Apparent nutrient digestibility was calculated using the nutrient-to-marker ratio in diet, feces and ileum, as follows:

$$y = 1 - \left(\frac{marker_{feed}}{marker_{ds}} \times \frac{Z_{ds}}{Z_{feed}}\right)$$

where y represents the coefficient of apparent digestibility of a nutrient at a certain segment;
Z_{ds} and Z_{feed} represent the nutrient concentration in the digestive segment and in the feed, respectively;
$marker_{feed}$ and $marker_{ds}$ represent marker (TiO_2) concentrations in feed and digestive segments, respectively.

Plasma and tissue $^2H_5$-phenylalanine enrichment was expressed as molar percent excess (**MPE**), where labeled phenylalanine moles are calculated as percentage of the sum of unlabeled and labeled phenylalanine moles. The percentage of tissue protein synthesized per day (FSR, %/day) was calculated from the equation:

$$FSR = \left(\frac{MPE_{bound}}{ave\ MPE_{free}} \times \frac{100}{t}\right)$$

where MPE_{bound} is the enrichment in $^2H_5$-phenylalanine of protein-bound phenylalanine in tissues; *ave* MPE_{free} is the average of the enrichment in $^2H_5$-phenylalanine of the free phenylalanine pool in the same tissue, between time 0 until the end of the sampling per-

iod, assuming that free phenylalanine in tissues follows equivalent kinetics than in plasma; t is the labeling time in days.

Supplementary Fig. S1 shows decreases in the MPE (%) of free phenylalanine in plasma over time, as well as the MPE (%) of free phenylalanine recorded in the target tissues.

Absolute protein synthesis rate (**ASR**) as the amount of protein synthesized per day (g/day) in liver and each muscle was calculated as follows:

$$\mathrm{ASR} = \frac{\mathrm{FSR}}{100} \times \text{tissue total protein content (g)}$$

Total CP content in liver and muscle was determined after its manual dissection, sampling and nitrogen determinations.

Statistical analysis

All statistical analyses were performed using the MIXED model using SAS statistical software (v9.4; SAS Institute Inc., Cary, NC, USA), in a completely randomized design with a pig as the experimental unit. Nutrient intake, growth performance, apparent nutrient digestibility, fractional and absolute synthesis rate, and nitrogen balance data were analyzed as follows:

$$Y_{ijklmn} = \mu + PP_i + PT_j + DI_k + (PP \times PT)_l + (PP \times DI)_m + (PT \times DI)_n + \varepsilon_{ijklmn}$$

where Y is the dependent variable, μ is the mean value, PP_i is the production phase (growing and fattening), PT_j is the producing type (Duroc and F2), DI_k is the diet (SP and LP) along with their possible interactions, and ε_{ijklmn} is the error.

The three-way interaction did not affect any parameter and was removed from the final model.

Differences between least square means were assessed using Tukey multiple comparison test. Results were reported as least square means and their standard error. Significant differences and tendencies were declared at $P \leq 0.05$ and $0.05 < P < 0.10$, respectively.

Results

Intake and growth performance

Daily intake of DM, OM, CP, EE and NDF, together with the performance indices such as average daily gain and feed conversion ratio during both PPs, is shown in Table 2. Daily intake of DM, OM, CP, EE and NDF increased ($P < 0.01$) with age. Interaction effect between PP and PT tended to be significant. In growing period, DM and OM intake was numerically higher in lean pigs, while in fattening period, the opposite trend was true ($P = 0.08$ and 0.09, for DM and OM intake, respectively). As it was expected, CP intake was significantly higher in SP pigs ($P = 0.01$). Average daily gain increased with age ($P < 0.01$) and F2 males grew faster when LP diet was given (Interaction effect PT × dietary CP level, $P = 0.03$, see Fig. 1a). Pigs fed LP diets were more efficient in converting feed than SP ones expressed as feed conversion ratio ($P = 0.02$). F2 pigs showed better feed conversion than Durocs in fattening phase (Interaction effect between PP and PT, $P = 0.02$, see Fig. 1b).

Coefficients of apparent total tract and apparent ileal digestibility

Effects of PP, PT and dietary CP content on DM, OM, CP, EE and NDF apparent total tract digestibility coefficients (**ATTDs**) are presented in Table 3. Except for EE, which was more digested as animals aged ($P < 0.01$), small differences in ATTD were observed among PPs. Duroc pigs showed higher EE ($P = 0.03$) and NDF (trend, $P = 0.07$) ATTD, compared with F2. Dietary CP content also tended to affect ($P = 0.08$) CP digestibility, where SP diets showed a higher rate.

Table 4 shows the apparent ileal digestibility (**AID**) of DM, CP and essential AAs, while Supplementary Table S1 includes AID of conditionally essential and non-essential AAs. Fattening pigs showed higher AID for DM ($P < 0.01$), and growing F2 pigs the lowest DM digestibility (Interaction effect PP × PT, Supplementary Fig. S2). Rates of DM and CP digestibility were greater for Duroc animals with respect to F2 ones ($P < 0.01$). Moreover, dietary CP content also affected CP ileal digestibility, where pigs fed SP diets digested more CP in ileum ($P < 0.01$, Table 4). Except for isoleucine, methionine and valine, AID of essential AAs was affected by the PP, being greater ($P < 0.05$) in fattening pigs, but did not have influence on most of the conditionally essential and non-essential AAs. Serine ($P = 0.01$) was more digestible in the ileum of fattening pigs; however, glutamic acid showed the opposite effect ($P < 0.01$).

Duroc pigs showed higher AID for all the AAs studied (see Table 4). Histidine, leucine, phenylalanine, threonine, cysteine, proline and serine AID were lower for F2 pigs in the growing phase (Interaction effect PP × PT, $P < 0.05$, Supplementary Figs. S3 and S4). The higher CP content of SP diets also elevated the AID on most of the studied AAs, except for lysine, methionine, threonine, proline and glycine.

Viscera and muscle protein synthesis

All pigs fully recovered 1 day after catheterization surgery, and their DM intake and average daily gain remained within the range proposed for pigs of their age. Nevertheless, the jugular catheter of one of the Duroc pigs in fattening period was (accidentally) removed from its place the day before L-phenylalanine infusion;

Table 2
Average daily intake of DM, organic matter (OM), CP, ether extract (EE) and NDF, and growth performance parameters in F2 [Pietrain sires × (Duroc × Landrace) dams] and Duroc pigs at growing (29.50 kg) and fattening (88.62 kg) phases fed two experimental diets, with a standard (SP) and low (LP) CP concentration.

Parameters	Phase (PP)		Producing type (PT)		Diet (DI)			*P*-value				
	Growing	Fattening	Duroc	F2	LP	SP	SEM	PP	PT	DI	PP × PT	PT × DI
Nutrient Intake (g/day)												
DM	824	2 029	1 450	1 403	1 448	1 405	50.4	<0.01	0.55	0.58	0.08	0.15
OM	768	1 916	1 364	1 320	1 361	1 323	47.6	<0.01	0.54	0.60	0.09	0.15
CP	158	321	242	236	222	257	8.6	<0.01	0.65	0.01	0.1	0.15
EE	21.4	83.9	53.9	51.4	51.7	53.6	1.99	<0.01	0.42	0.54	0.13	0.12
NDF	145.7	429.4	293.3	281.8	291.6	283.5	10.33	<0.01	0.47	0.61	0.10	0.14
Performance												
ADG (g/day)	426	933	615	743	744	615	47.6	<0.01	0.09	0.08	0.13	0.03
FCR (g/g)	2.1	2.3	2.3	2.1	2.0	2.4	0.13	0.20	0.28	0.02	0.02	0.20

Abbreviations: ADG = average daily gain; FCR = feed conversion ratio.

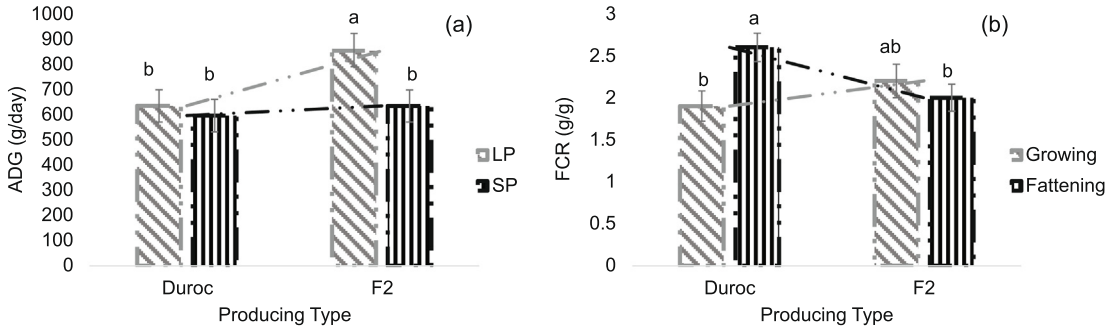

Fig. 1. Average daily gain (ADG) affected by the interaction of the productive type (PT; Duroc v. F2) and the dietary CP (standard, SP v. low, LP) (a). Feed conversion ratio (FCR) affected by two interactions: production phase (PP; Growing v. Fattening) and pig PT (b). Above each bar, different letters (^{a,b}) indicate significant differences ($P < 0.05$). Error bars = SEM.

Table 3
Apparent total tract digestibility (ATTD, %) of DM, organic matter (OM), CP, ether extract (EE) and NDF in animals of two different production phases (PP, growing v. fattening) and two producing types of pig (F2 crossbred and purebred Duroc) fed with two dietary levels of protein (low, LP v. standard, SP).

Parameters	Phase (PP)		Producing type (PT)			*P*-value		
	Growing	Fattening	Duroc	F2	SEM	PP	PT	PP × PT
DM	90.6	89.4	90.2	89.8	0.47	0.08	0.63	0.38
OM	91.5	90.4	91.1	90.8	0.42	0.09	0.60	0.51
CP	89.2	87.7	88.4	88.5	0.63	0.11	0.95	0.70
EE	70.0	81.3	78.7	72.6	1.83	<0.01	0.03	0.90
NDF	76.8	74.2	77.1	74.0	1.18	0.13	0.07	0.45

Table 4
Apparent ileal digestibility (AID, %) of DM, CP and essential amino acids (AAs) of 15 purebred Durocs and 16 F2 pigs, in growing and fattening phases and fed two dietary levels of CP (low, LP v. standard, SP).

Parameters	Phase (PP)		Producing type (PT)		Diet (DI)			*P*-value				
	Growing	Fattening	Duroc	F2	LP	SP	SEM	PP	PT	DI	PP × PT	PT × DI
DM	79.2	88.4	87.4	80.3	82.7	85.0	1.17	<0.01	<0.01	0.2	<0.01	0.42
CP	82.3	83.7	85.9	80.1	80.1	85.8	1.42	0.5	0.01	0.01	0.09	0.25
Essential AAs												
Histidine	70.9	80.9	81.8	70.0	69.9	81.9	2.45	0.01	<0.01	<0.01	0.01	0.13
Isoleucine	79.5	82.9	85.8	76.7	78.7	83.8	1.47	0.11	<0.01	0.02	0.06	0.17
Leucine	74.5	87.2	86.2	75.5	76.8	85.0	2.46	<0.01	0.01	0.03	0.03	0.41
Lysine	80.1	87.7	86.6	81.2	84.0	83.9	1.72	<0.01	0.04	0.97	0.07	0.18
Methionine	90.1	88.2	91.7	86.6	87.5	90.9	1.43	0.36	0.02	0.11	0.15	0.15
Phenylalanine	80.1	85.2	86.0	79.3	79.7	85.6	1.50	0.03	<0.01	0.01	0.04	0.28
Threonine	66.4	84.3	81.0	69.7	74.4	76.2	2.28	<0.01	<0.01	0.58	0.02	0.59
Valine	78.8	78.7	81.8	75.7	75.1	82.3	1.76	0.98	0.02	0.01	0.16	0.22

therefore, no FSR measurements were obtained from that animal. Fractional and absolute synthesis rates of protein are presented in Table 5.

Overall, FSR was numerically higher in viscera tissues than in muscles and decreased with age in most of the target tissues, except for *biceps femoris*, where the opposite trend was observed

Table 5
Fractional (FSR) and absolute protein synthesis rate (ASR) in duodenum and liver viscera tissues, and in *biceps femoris* and *longissimus dorsi* skeletal muscles in F2 crossbred pigs ($n = 8$) and purebred Duroc pigs ($n = 7$), fed with two dietary CP levels (low, LP v. standard, SP).

Tissue	Phase (PP)		Producing type (PT)		Diet (DI)			*P*-value				
	Growing	Fattening	Duroc	F2	LP	SP	SEM	PP	PT	DI	PP × PT	PT × DI
FSR (%/day)												
Duodenum	53.3	44.9	52.0	46.2	48.8	49.4	2.23	0.02	0.09	0.84	0.89	0.01
Liver	46.7	39.0	36.1	49.6	42.3	43.4	2.02	0.01	<0.01	0.71	0.01	0.50
Biceps femoris	6.6	8.1	7.2	7.6	7.0	7.7	0.25	<0.01	0.23	0.07	0.82	0.26
Longissimus dorsi	10.7	9.0	7.6	12.1	9.8	9.9	0.49	0.02	<0.01	0.86	<0.01	0.40
ASR (g/day)												
Liver	58.1	123.8	82.3	99.6	96.3	85.6	4.99	<0.01	0.02	0.15	0.15	0.28
Biceps femoris	4.1	18.3	9.6	12.8	10.9	11.6	0.43	<0.01	<0.01	0.27	0.01	0.99
Longissimus dorsi	13.9	38.3	20.3	32.0	26.4	25.8	1.84	<0.01	<0.01	0.81	0.39	0.72

($P < 0.01$; Table 5). Hybrid F2 pigs showed higher FSR in liver and in *longissimus dorsi* ($P < 0.01$) compared with Duroc. On the contrary, Duroc pigs tended ($P = 0.09$) to synthesize more protein in duodenum compared with F2 animals, becoming significant in LP diets ($P = 0.01$). Moreover, in liver and *longissimus dorsi*, FSR differences registered between Duroc and F2 at growing phase ($P < 0.01$) did disappear in fattening pigs (Interaction effect PP × PT, $P < 0.05$, Fig. 2, b) and c). Dietary CP content did not impact FSR except for *biceps femoris*, where SP diet tended to lead to higher synthesis rates ($P = 0.07$). Regarding ASR, PP influence was significant ($P < 0.01$) on all the studied tissues, showing as expected a superior body mass in the finishing phase. As happened with the FSR, F2 pigs showed higher ASRs ($P < 0.05$) compared with Duroc pigs. Absolute synthesis rate was not estimated for duodenum due to lack of exact tissue weight.

Nitrogen balance

Daily nitrogen intake and excretion (feces and urine) together with the nitrogen balance, as the difference between input and output, are presented in Supplementary Table S2. As expected, animals during fattening period ingested, excreted, and retained more nitrogen ($P < 0.01$) than growing animals. At fattening, more nitrogen was excreted via urine in Duroc pigs compared with F2 ($P < 0.01$, Supplementary Fig. S5), reflecting also in less retention in the former ($P < 0.01$). Feeding SP diets to animals significantly increased nitrogen intake ($P < 0.01$) and tended to increase its retention ($P = 0.08$).

Discussion

Methodological approach

The main objective of this trial was to refine protein requirements in pigs by analyzing the specific impact of genotype (i.e. PT), dietary CP restriction (2% reduction) and their potential interactions. The experiment was conducted in two different stages of development (phases), (a) when animal retains mostly protein (20–30 kg BW) and (b) when fat becomes the principal component of the daily body gain (80–90 kg BW). To detect potential differences in protein metabolism and to refine requirements, FSR [in muscle (*longissimus dorsi*, *biceps femoris*) and viscera (liver and duodenum)] together with ileum AA-digestibility and nitrogen balance were determined.

Our experimental approach involved both surgery and confinement in metabolic cages to the pigs under study to get digestibility, nitrogen balance and FSR results; such a complex experimental procedure limited the number of available animals for the experiment. Authors are aware that having 32 animals for a 2 × 2 × 2 factorial design might have been a limiting factor to reach consistency in both results and conclusions; however, the absence (or scarce impact) of interaction effects among main factors would indicate that, in this scenario, experimental underpowering was not a relevant issue, and hence the number of animals used was considered enough to achieve the proposed objectives.

In relation to the genotypes employed it is necessary to point out that some Duroc-crossbred lines have been selected to improve lean deposition, although pure Duroc breed remains the most common line exploited as a fatty pig to produce high-quality products with high intramuscular fat infiltration (Latorre et al., 2003). To commercially promote such features, Duroc males are castrated early and slaughtered at higher ages, while lean breed males, destined for leaner pork cuts, are not. Since one of the main objectives of the present assay was to study protein metabolism under commercial conditions, Duroc males were surgically castrated. Moreover, castration may involve maximizing potential differences between PTs. The anabolic effects of testicular hormones seem to impact feed efficiency, nitrogen retention and protein deposition in entire males (Ruiz-Ascacibar et al., 2019b). Hence, definition of breed throughout the text has been replaced by PT, which includes the effect of genotype plus castration in the case of Duroc males.

Effect of production phase

As expected, fattening pigs (88.6 ± 6.26 kg BW) showed higher level of feed intake and average daily gain (Table 2), along with higher AID of essential AAs in ileum; however, growing pigs (29.

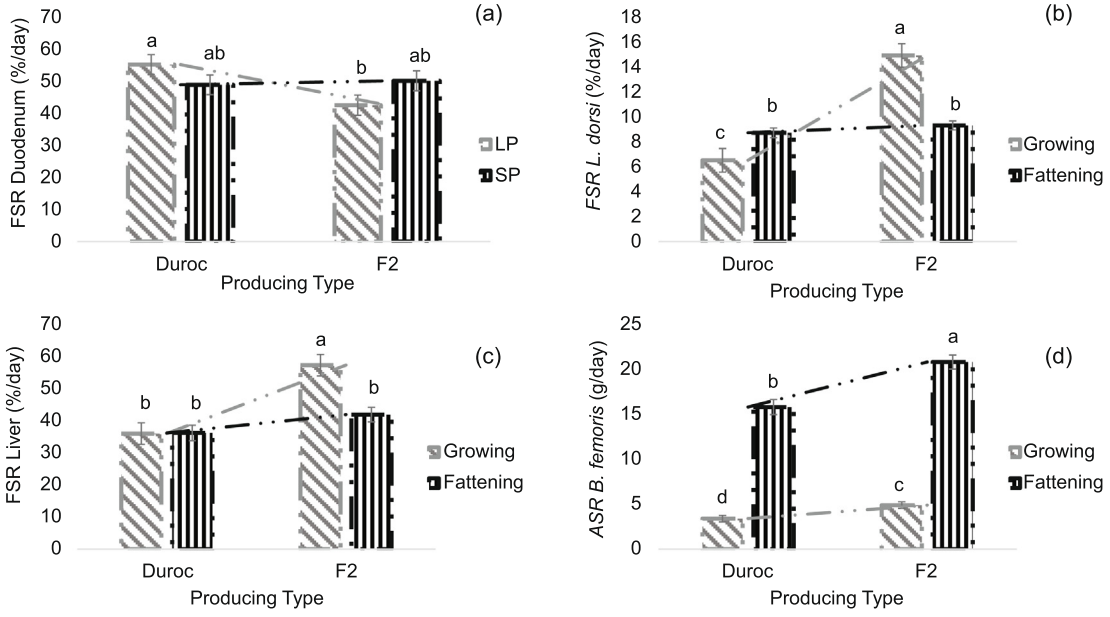

Fig. 2. Fractional synthesis rate (FSR) of duodenum affected by the interaction of producing type (PT; Duroc v. F2) and dietary CP (standard, SP v. low, LP) (a). FSR of *longissimus dorsi* (b) and liver (c) affected by the interaction of production phase (PP; Growing v. Fattening) and pig PT. Absolute synthesis rate (ASR) of *biceps femoris* affected by the interaction of PP and PT (d). Above each bar, different letters (^{a,b}) indicate significant differences ($P < 0.05$). Error bars = SEM.

5 ± 3.19 kg BW) showed an improved FSR in almost all tissues analyzed. Only ATTD for EE was significantly higher in fattening pigs, which is consistent with the existing literature (Noblet et al., 2013). Several studies have reported that nutrient digestibility increases linearly with the animal's development, mainly due to a lessening in the digesta transit (Noblet et al., 2013) and/or the development of hindgut microbiota (Knecht et al., 2020). In the present study, fattening pigs showed better AID for DM as well as for most of the essential AAs (histidine, leucine, lysine, phenylalanine and threonine). Digestibility coefficients for AAs were variable but did agree with previous studies (Liu et al., 2016). Such variability could be explained by differential affinity either for molecular transport through the brush border membrane (Bröer, 2008), or the variation in AA richness in the endogenous fraction (Mosenthin et al., 2000).

Despite the variability in FSR data in the existing literature due to differences in animal age and analytical procedures, our FSR (%/day) findings were within the range proposed by previous authors (Rivera-Ferre et al., 2005; Wang et al., 2007). Differences in FSR between viscera and skeletal muscle tissues have also been reported, being superior to the former tissues mainly due to their higher metabolic activity and protein turnover. Increased protein metabolism, fast growth and highly efficient protein synthesis in young mammals have been widely documented, peaking at birth and decreasing rapidly afterwards (Davis et al., 2008). In our study, FSR in *biceps femoris* was an exception, being significantly higher in the fattening period. Authors are not aware of previous data to confirm the reported exception, although muscles from the hind limb show a later maturation than those from the rest of the body (Darinskii, 1975).

Effect of producing type

Previous experimental evidence (Morales et al., 2002) has been suggested that fatty pigs (i.e. Iberian or Duroc) have higher voluntary feed intake than modern lean lines (i.e. Landrace or Pietrain). This is consistent with our findings during the fattening phase, although the differences recorded did not reach statistical significance; however, the opposite trend was detected during the growing phase. It has traditionally been accepted that Duroc is a fatter breed, due to its high intramuscular fat content. However, genetic selection exercised on Duroc pigs to ameliorate leanness and reduce fat content has improved lean gain in Duroc lines. Several studies have evidenced that Duroc or Duroc-crossbred pigs grow at a similar rate (Čandek-Potokar et al., 1998) or even faster than lean breeds (Latorre et al., 2003).

Authors are not aware of data showing differences in nutrient digestibility among PTs, but in our case, Duroc pigs showed higher ATTD (for EE and NDF) and AID (for DM, CP and all the AAs), although it was not translated to better CP retention rates or lower nitrogen excretion. Intestine is a very active metabolic organ responsible of releasing AAs into the peripheral blood for the rest of the organism, but it has been detected that the quantity and proportion of AAs in the portal system differ from that absorbed by the intestine (Baracos, 2004). Considering the high tendency for Duroc pigs to have higher duodenum FSR, an important part of the AAs absorbed by the intestine may have been used for its own protein metabolism.

Fractional synthesis rate values obtained from both liver and *longissimus dorsi* muscle in F2 pigs were higher than those of Duroc pigs, suggesting that F2 pigs synthesized a higher proportion of protein on a daily basis (%/day: 37% and 59%, for liver and *longissimus dorsi*, respectively). Moreover, differences were more pronounced in young pigs, and diminished with age and/or development (PP × PT interaction). In *biceps femoris* muscle, FSR differences did not reach statistical significance, although ASR also differs between PTs (on average 33%, see Fig. 2d) because of the greater protein pool in F2.

The higher FSR and ASR (just ASR in the case of *biceps femoris*) shown by lean pigs should explain their tendency to grow faster. In this sense, Rivera-Ferre et al. (2006) also reported greater protein deposition in Landrace than Iberian gilts, but in contradiction with the present results, no breed differences were reported in FSR, when nitrogen flows were calculated by cumulative urinary isotope excretion after an oral dose of [^{15}N]-glycine. Likewise, Rivera-Ferre et al. (2005), using an identical $^{2}H_5$-phenylalanine protocol than the current study, concluded that Iberian pigs showed higher synthesis rates in muscles and viscera than Landrace pigs. According to the existing literature, the type of muscular fiber could affect FSR; thus those animals with predominantly slow-twitch oxidative fibers (type I) showed greater FSR than those with fast-twitch glycolytic (type II) ones (Goodman et al., 2012). Nonetheless, Duroc pigs seem to have a higher proportion of slow-twitch oxidative fibers than Pietrain boars (Werner et al., 2010); however, the possibility that crossbred F2 [Pietrain × (Duroc × Landrace)] have higher proportion of slow-twitch oxidative fibers than purebred Duroc pigs seems to be negligible. Therefore, the mechanism underlying the differences in FSR between Duroc and F2 should be related with additional factors, such as the effect of gender or castration on protein metabolism that needs to be further elucidated.

Nevertheless, the predominant type of muscle fiber in the animal may not be the only factor conditioning the rate of protein synthesis. In this sense, Liu et al. (2015) found that some Landrace lines showed higher abundance of protein precursors related to protein deposition and muscle growth in their tissues, such as p-AKT, mTOR, p70S6K, than a Chinese indigenous fatty pig (Bama mini-pig). The abundance of such positive regulators also decreases with age and thus reduces the protein synthesis in skeletal muscle. In fact, mTOR (serine/threonine protein kinase) is an important mechanism for the regulation of protein synthesis in cells (Deng et al., 2014), and when it phosphorylates other proteins such as ribosomal protein p70S6K coordinates gene transcription and protein translation, involved in the regulation of growth, proliferation and differentiation of cells.

Effect of dietary CP

Experimental diets were formulated to meet the nutrition requirements for growing/fattening pigs (FEDNA, 2013). Although CP concentration in LP diets was reduced by 2%, requirements of essential AAs were covered in both diets by using crystalline AAs. The CP reduction improved both average daily gain and feed conversion ratio (see Table 2). A reduction to certain levels (2–3%) with optimal adjustment of AAs is widely accepted, and allows to maintain animals growth and performance, promote nitrogen accretion (Wang et al., 2019), and in consequence reduce both cost of feeding and nitrogen waste. An interaction in feed conversion ratio between PP and dietary CP content was seen, being differences between diets more pronounced in the growing period. Better feed efficiencies were provided by LP diets, which is consistent with Wang et al. (2019) who showed that pigs in growing phase were more sensitive to CP diet changes than in fattening phase.

The effect of dietary CP on AID of DM, CP, and AAs was investigated. When dietary CP was reduced by two percentage units, the AID of DM was maintained, as was the ATTD of DM and the rest of nutrients, except for CP. Pigs fed LP diet presented lower AID for most AAs, including histidine, isoleucine, leucine, phenylalanine, and valine.

Although the precise relationship between dietary CP supply and AID is open to discussion, several studies agree with our findings by describing how protein AID increases linearly with dietary

CP until reaching a plateau (Furuya and Kaji, 1989; Li et al., 1993; Fan et al., 1994). Moreover, Furuya and Kaji (1989) stated that influence of endogenous AAs on AID becomes negligible at CP levels like those proposed in our study. In this sense, Fan et al. (1994) described that both CP and AAs AID tend to reach a plateau, but not simultaneously: CP, methionine, threonine and tyrosine ileal digestibility leveled off when dietary CP reached values of 15.36, 13.70, 15.53 and 16.29%, respectively; anyway, this plateau is over the CP level reached in LP diets (14.7 and 12.9% for growing and fattening animals, respectively). On the other hand, the opposite effect was also described by Li et al. (1993), although the study is based on early-weaned pigs and they applied higher levels of CP inclusion (19.5–25.5% of CP), obtaining a reduction in AID when dietary CP increased. In their study, authors claim that the total supply of AAs from the high protein diets may have exceeded the maximum capacity of efficiency of AA transport throughout the intestinal mucosa. Our study never reached those CP levels, and our animals exhibited a more developed digestive tract.

Incorporation of free AAs from crystalline form varied among experimental diets (1.4 and 1.2 in growing and 0.57 and 0.29% in fattening pigs, for LP and SP, respectively). This fact might have influenced AID since crystalline AAs are rapidly and completely absorbed (Otto et al., 2003). However, incorporation of free synthetic AAs may imply less secretion of proteolytic enzymes, due to a lower intact dietary protein present in the small intestine, and that fact might have influenced AID (Yen et al., 2004). Therefore, a long-term effect of crystalline AAs inclusion on AID is unpredictable (Wang et al., 2019).

Experimental diets were formulated to cover all essential AA requirements, however a slight but significant diet effect on FSR was observed. Protein restriction tended to decrease FSR in *biceps femoris* (Table 5); moreover, in lean animals, duodenal-FSR was also restricted when giving LP diet (PT × dietary CP content, Fig. 2a). In that sense, the observed restriction cannot be explained by essential AAs metabolism (such fraction did not differ among diets) although it can be hypothesized that the observed reduction in FSR could be a consequence of a possible restriction of non-essential AAs supply in lean (F2) pigs to attain the maximum potential of protein accretion. In relation to the existing literature, no effect of CP or lysine restriction on FSR was reported by Rivera-Ferre et al. (2005), although, Sève et al. (1986) observed that by decreasing CP supply in young pigs (17 days old), duodenal protein metabolism experimented a FSR depression, but with increases in protein accretion due to a concomitant decrease in fractional degradation rate. In agreement with the former author, Li et al. (2016) demonstrated that diet may be relevant at longer experimental periods since skeletal muscle can be stimulated by the availability of AAs (i.e. arginine, lysine or leucine). Such stimulation would disappear with animal's maturity (De Bandt, 2016).

Conclusions

Most of the parameters studied were influenced by the PP, where fattening pigs had higher growth, apparent digestibility coefficients and tissue protein accretion, whereas growing pigs showed more active protein metabolism. Although Duroc pigs showed higher AID for all the AAs, growing F2 pigs got higher FSRs in liver and *longissimus dorsi* and higher nitrogen retention rates with lower nitrogen waste. This information may reveal differences in AA requirements between both PTs, with Duroc pigs receiving excess of dietary AAs. A 2% reduction of dietary CP concentration in the LP diets resulted in improved average daily gain and feed conversion ratio. Although this dietary CP reduction was detrimental for AA, apparent digestibility and nitrogen retention hardly affected the protein synthesis rates.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100220.

Ethics approval

Protocols and experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Lleida (Ref: CEEA 09–05/16). The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD 53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental purposes.

Data and model availability statement

None of the data were deposited in an official repository. Data can be available upon request.

Author ORCIDs

L. Sarri: https://orcid.org/0000-0002-4894-6925.
J. Balcells: https://orcid.org/0000-0002-2126-7375.
G. de la Fuente: https://orcid.org/0000-0003-0083-776X.
M. Tor: https://orcid.org/0000-0002-2572-9274.
J. Gómez-Arrue: https://orcid.org/0000-0001-9014-6457.
A.R. Seradj: https://orcid.org/0000-0001-8104-4571.

Author contributions

Conceptualization and Methodology: **L.S., J.B., G.F., M.T., A.R.S.**; Investigation: **L.S., J.B., G.F., M.T., A.R.S., J.G.-A.**; Formal analysis: **L. S., A.R.S., M.T.**; Writing – original draft: L.S., Writing – review & editing: **J.B., G.F., M.T., A.R.S., J.G.-A.**; Funding acquisition; **J.B., G.F.**

Declaration of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to express their sincere gratitude to the staff of the Centre d'Estudis Porcins (CEP, Spain), Mr. Joan Carles Melo, Ms. Anna Ñaco and Ms. Teresa Giró for their technical assistance and Ms. Francesca Vilaró for LC/MS analysis.

Financial support statement

This study was a part of the Feed-a-Gene project and received funding from the European Union's H2020 program under National Institutes of Health (grant number 633531), as well as Spanish National funding by MINECO (AGL2017-89289-R). L. Sarri is recipient of a research training grant from the Generalitat de Catalunya-European Social Funds (2019 FI_B 00416).

References

Álvarez-Rodríguez, J., Teixeira, A., 2019. Slaughter weight rather than sex affects carcass cuts and tissue composition of Bisaro pigs. Meat Science 154, 54–60. https://doi.org/10.1016/j.meatsci.2019.04.012.
Association of Official Analytical Chemists (AOAC), 2006. Official Methods of Analysis. AOAC, Gaithersburg, MD, USA.
Baracos, V.E., 2004. Animal models of amino acid metabolism: a focus on the intestine. American Society for Nutritional Science 134, 1656S–1659S.

Bröer, S., 2008. Amino acid transport across mammalian intestinal and renal epithelia. Physiological Reviews 88, 249–286. https://doi.org/10.1152/physrev.00018.2006.

Brossard, L., Nieto, R., Charneca, R., Araujo, J.P., Pugliese, C., Radović, Č., Čandek-Potokar, M., 2019. Modelling nutritional requirements of growing pigs from local breeds using InraPorc. Animals 9, 1–17. https://doi.org/10.3390/ani9040169.

Čandek-potokar, M., Žlender, B., Bonneau, M., 1998. Effects of breed and slaughter weight on longissimus muscle biochemical traits and sensory quality in pigs. Annales de Zootechnie 47, 3–16.

Colgrave, M.L., Allingham, P.G., Jones, A., 2008. Hydroxyproline quantification for the estimation of collagen in tissue using multiple reaction monitoring mass spectrometry. Journal of Chromatography A 1212, 150–153. https://doi.org/10.1016/j.chroma.2008.10.011.

Darambazar, E., 2019. Determination of Titanium Dioxide as a Livestock Digestibility Marker. Assay Protocol. University of Saskatchewan, Saskatchewan, Canada. https://doi.org/10.13140/RG.2.2.14265.31842.

Darinskii, N.V., 1975. Order of maturation of skeletal-muscle groups in rats during postnatal development. Bulletin of Experimental Biology and Medicine 80, 731–733.

Davis, T.A., Suryawan, A., Orellana, R.A., Nguyen, H.V., Fiorotto, M.L., 2008. Postnatal ontogeny of skeletal muscle protein synthesis in pigs. Journal of Animal Science 86, E13–E18. https://doi.org/10.2527/jas.2007-0419.

De Bandt, J., 2016. Leucine and mTOR-dependent activation of muscle protein synthesis in aging. The Journal of Nutrition 146, S1–S9. https://doi.org/10.3945/jn.116.234518.1S.

Deng, H., Zheng, A., Liu, G., Chang, W., Zhang, S., Cai, H., 2014. Activation of mammalian target of rapamycin signaling in skeletal muscle of neonatal chicks: effects of dietary leucine and age. Poultry Science 93, 114–121. https://doi.org/10.3382/ps.2013-03287.

Fan, M.Z., Sauer, W.C., Hardin, R.T., Lien, K.A., 1994. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. Journal of Animal Science 72, 2851–2859. https://doi.org/10.2527/1994.72112851x.

Fundación española para el desarrollo de la nutrición animal (FEDNA), 2013. Necesidades nutricionales para ganado porcino. Normas FEDNA, Madrid, Spain.

Furuya, S., Kaji, Y., 1989. Estimation of the true ileal digestibility of amino acids and nitrogen from their apparent values for growing pigs. Animal Feed Science and Technology 26, 271–285. https://doi.org/10.1016/0377-8401(89)90040-0.

Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [3H] phenylalanine. Biochemical Journal 1, 719–723.

Gol, S., González-Prendes, R., Bosch, L., Tor, M., Reixach, J., Pena, R.N., Estany, J., 2019. Linoleic acid metabolic pathway allows for an efficient increase of intramuscular fat content in pigs. Journal of Animal Science and Biotechnology 10, 1–9.

Goodman, C.A., Kotecki, J.A., Jacobs, B.L., Hornberger, T.A., 2012. Muscle fiber type-dependent differences in the regulation of protein synthesis. PLoS ONE 7, 1–11. https://doi.org/10.1371/journal.pone.0037890.

Guo, S., Duan, J.-A., Qian, D., Tang, Y., Qian, Y., Wu, D., Su, S., Shang, E., 2013. Rapid determination of amino acids in fruits of ziziphus jujuba by hydrophilic interaction ultra-high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry. Journal of Agricultural and Food Chemistry 61, 2709–2719. https://doi.org/10.1021/jf305497r.

International Organization for Standardization (ISO), 2008. Food Products-Determination of the Total Nitrogen Content by Combustion According to the Dumas Principle and Calculation of the Crude Protein Content, ISO 16634-1. ISO, Geneva, Switzerland.

Knecht, D., Cholewińska, P., Jankowska-Mąkosa, A., Czyż, K., 2020. Development of swine's digestive tract microbiota and its relation to production indices—A review. Animals 10, 1–13. https://doi.org/10.3390/ani10030527.

Latorre, M.A., Medel, P., Fuentetaja, A., Lázaro, R., Mateos, G.G., 2003. Effect of gender, terminal sire line and age at slaughter on performance, carcass characteristics and meat quality of heavy pigs. Animal Science 77, 33–45. https://doi.org/10.2527/2003.81102448x.

Li, S., Sauer, W.C., Fan, M.Z., 1993. The effect of dietary crude protein level on amino acid digestibility in early-weaned pigs. Journal of Animal Physiology and Animal Nutrition 70, 26–37. https://doi.org/10.1109/MSPEC.2009.4768861.

Li, Y.H., Wei, H.K., Li, F.N., Kim, S.W., Wen, C.Y., Duan, Y.H., Guo, Q.P., Wang, W.L., Liu, H.N., Yin, Y.L., 2016. Regulation in free amino acid profile and protein synthesis pathway of growing pig skeletal muscles by low-protein diets for different time periods. Journal of Animal Science 94, 5192–5205. https://doi.org/10.2527/jas2016-0917.

Liu, Y., Li, F., Kong, X., Tan, B., Li, Y., Duan, Y., Blachier, F., Hu, C.A., Yin, Y., 2015. Signaling pathways related to protein synthesis and amino acid concentration in pig skeletal muscles depend on the dietary protein level, genotype and developmental stages. PLoS ONE 10, 1–21. https://doi.org/10.1371/journal.pone.0138277.

Liu, Z., Lv, S., Zhang, S., Liu, J., Zhang, H., 2016. Effects of dietary cellulose levels on the estimation of endogenous amino acid losses and amino acid digestibility for growing pigs. Animal Nutrition 2, 74–78. https://doi.org/10.1016/j.aninu.2016.04.001.

Morales, J., Pérez, J.F., Baucells, M.D., Mourot, J., Gasa, J., 2002. Comparative digestibility and lipogenic activity in Landrace and Iberian finishing pigs fed ad libitum corn– and corn–sorghum–acorn-based diets. Livestock Production Science 77, 195–205.

Mosenthin, R., Sauer, W.C., Blank, R., Huisman, J., Fan, M.Z., 2000. The concept of digestible amino acids in diet formulation for pigs. Livestock Production Science 64, 265–280. https://doi.org/10.1016/S0301-6226(99)00139-6.

Noblet, J., Gilbert, H., Jaguelin-Peyraud, Y., Lebrun, T., 2013. Evidence of genetic variability for digestive efficiency in the growing pig fed a fibrous diet. Animal 7, 1259–1264. https://doi.org/10.1017/S1751731113000463.

Otto, E.R., Yokoyama, M., Ku, P.K., Amest, N.K., Trottier, N.L., 2003. Nitrogen balance and ileal amino acid digestibility in growing pigs fed diets reduced in protein concentration. American Society of Animal Science 81, 1743–1753.

Piraud, M., Vianey-Saban, C., Petritis, K., Elfakir, C., Steghens, J.P., Bouchu, D., 2005. Ion-pairing reversed-phase liquid chromatography/electrospray ionization mass spectrometric analysis of 76 underivatized amino acids of biological interest: A new tool for the diagnosis of inherited disorders of amino acid metabolism. Rapid Communications in Mass Spectrometry 19, 1587–1602. https://doi.org/10.1002/rcm.1957.

Poklukar, K., Candek-Potokar, M., Batorek, N., Tomažin, U., Škrlep, M., 2020. Lipid deposition and metabolism in local and modern pig breeds: A review. Animals 10, 1–20.

Pomar, C., Remus, A., 2019. Precision pig feeding: a breakthrough toward sustainability. Animal Frontiers 9, 52–59. https://doi.org/10.1093/af/vfz006.

Qin, C., Huang, P., Qiu, K., Sun, W., Xu, L., Zhang, X., Yin, J., 2015. Influences of dietary protein sources and crude protein levels on intracellular free amino acid profile in the longissimus dorsi muscle of finishing gilts. Journal of Animal Science and Biotechnology 6, 1–10. https://doi.org/10.1186/s40104-015-0052-x.

Rivera-Ferre, M.G., Aguilera, J.F., Nieto, R., 2006. Differences in whole-body protein turnover between Iberian and Landrace pigs fed adequate or lysine-deficient diets. Journal of Animal Science 84, 3346–3355. https://doi.org/10.2527/jas.2005-405.

Rivera-Ferre, M.G., Aguilera, J.F., Nieto, R., 2005. Muscle fractional protein synthesis is higher in Iberian than in Landrace growing pigs fed adequate or lysine-deficient diets. Journal of Nutrition 135, 469–478. https://doi.org/10.1093/jn/135.3.469.

Ruiz-Ascacibar, I., Stoll, P., Bee, G., 2019a. Effects of dietary CP and amino acid restriction on the growth dynamics of organs and body components in entire male, castrated and female pigs. Animal 13, 2223–2231. https://doi.org/10.1017/S1751731119000132.

Ruiz-Ascacibar, I., Stoll, P., Kreuzer, M., Bee, G., 2019b. Dietary CP and amino acid restriction has a different impact on the dynamics of protein, amino acid and fat deposition in entire male, castrated and female pigs. Animal 13, 74–82. https://doi.org/10.1017/S1751731118000770.

Sève, B., Reeds, P.J., Fuller, M.F., Cadenhead, A., Hay, S.M., 1986. Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early-weaning. Possible connection to the energy metabolism. Reproduction Nutrition Development 26, 849–861.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Wang, X., Qiao, S., Yin, Y., Yue, L., Wang, Z., Wu, G., 2007. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. The Journal of Nutrition 137, 1442–1446.

Wang, Y.M., Yu, H.T., Zhou, J.Y., Zeng, X.F., Wang, G., Cai, S., Huang, S., Zhu, Z.P., Tan, J.J., Johnston, L.J., Levesque, C.L., Qiao, S., 2019. Effects of feeding growing-finishing pigs with low crude protein diets on growth performance, carcass characteristics, meat quality and nutrient digestibility in different areas of China. Animal Feed Science and Technology 256, 1–12. https://doi.org/10.1016/j.anifeedsci.2019.114256.

Werner, C., Natter, R., Wicke, M., 2010. Changes of the activities of glycolytic and oxidative enzymes before and after slaughter in the longissimus muscle of Pietrain and Duroc pigs and a Duroc-Pietrain crossbreed. Journal of Animal Science 88, 4016–4025. https://doi.org/10.2527/jas.2010-3136.

Yen, J.T., Kerr, B.J., Easter, R.A., Parkhurst, A.M., 2004. Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. Journal of Animal Science 82, 1079–1090. https://doi.org/10.2527/2004.8241079x.