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REVIEW
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# Protein turnover in pigs: A review of interacting factors

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## Abstract

Protein turnover defines the balance between two continuous and complex processes of protein metabolism, synthesis and degradation, which determine their deposition in tissues. Although the liver and intestine have been studied extensively for their important roles in protein digestion, absorption and metabolism, the study of protein metabolism has focused mainly on skeletal muscle tissue to understand the basis for its growth. Due to the high adaptability of skeletal muscle, its protein turnover is greatly affected by different internal and external factors, contributing to carcass lean-yield and animal growth. Amino acid (AA) labelling and tracking using isotope tracer methodology, together with the study of myofiber type profiling, signal transduction pathways and gene expression, has allowed the analysis of these mechanisms from different perspectives. Positive stimuli such as increased nutrient availability in the diet (e.g., AA), physical activity, the presence of certain hormones (e.g., testosterone) or a more oxidative myofiber profile in certain muscles or pig genotypes promote increased upregulation of translation and transcription-related genes, activation of mTORC1 signalling mechanisms and increased abundance of satellite cells, allowing for more efficient protein synthesis. However, fasting, animal aging, inactivity and stress, inflammation or sepsis produce the opposite effect. Deepening the understanding of modifying factors and their possible interaction may contribute to the design of optimal strategies to better control tissue growth and nutrient use (i.e., protein and AA), and thus advance the precision feeding strategy.

KEYWORDS
amino acids, mechanistic target of rapamycin, muscle, pig, protein synthesis, protein turnover

## 1 | INTRODUCTION

Tissue protein metabolism has traditionally been described as the continuous balance between two energy-consuming processes, synthesis and degradation, defined as protein turnover, responsible for regulating body protein mass and potential changes in protein size. When the balance is favourable to protein synthesis, protein accretion and tissue hypertrophy occurs, whereas protein loss or tissue atrophy results from either increased rates of degradation or reduced rates of protein synthesis (Millward et al., 1976; Ten Have et al., 2019). This dynamic equilibrium is encompassed by multiple processes subjected to anabolic and catabolic stimuli, interconnected by signalling transduction networks, in which processes related to protein folding, transport and elimination are also included. Moreover, each protein has its own structure, function, lifespan, and once synthesised can undergo structural changes such as being added, reduced in size, or even destroyed. Rothman (2010) proposed that the stable concentration of manufactured and destroyed proteins may be the equilibrium by mass between the physiologically matured proteins, and those forms predisposed to degradation (i.e., modified or altered proteins).

Interest for protein metabolism and its research began in the late 1930s in order to understand the basis of tissue growth or loss, but also because of its relationship with amino acid (AA) and energy requirements. It should be considered that protein synthesis is the most costly biosynthetic process, accounting for approximately 17% of the metabolic rate of the whole animal energy cost (Garlick et al., 1976), about 0.8 kcal/g of protein for pigs. Furthermore, neonatal pigs are considered the most suitable model for paediatric nutrition and metabolism, and therefore, the study of its protein turnover has been an important issue of research in clinical medicine for decades (Odle et al., 2014).

The objective of this review is to provide an overview of tissue-protein turnover and the metabolic events involved, as well as to evaluate the effect of relevant internal and external factors from different perspectives in order to improve the understanding of this process, and make it a tool to design strategies to better control the development of pig tissues, with special emphasis on skeletal muscle.

## 2 | PROTEIN TURNOVER

### 2.1 | Protein synthesis

Protein synthesis is a complex process that requires the coordination of a large number of macromolecules, including nucleic acids, enzymes, and regulatory factors. Synthesis stimulation has been predominantly associated with the mechanistic target of rapamycin (mTOR), a highly conserved serine/threonine protein kinase that associated with other proteins constitutes two distinct complexes, mTORC1 and mTORC2, and whose activity and/or activation appears to be regulated in coordination with these partner proteins and phosphorylation of downstream effectors of the signalling pathway (Figueiredo et al., 2017). While mTORC2 regulates cell survival and actin cytoskeleton reorganisation for spatial cell growth, mTORC1 mediates cell growth by regulating translation and transcription processes, along with ribosome biogenesis, nutrient transport, and autophagy. Together with mTOR, the mTORC1 consists of two required subunits, the Raptor (mTOR—associated regulatory protein) and mLST8 (mammalian lethal with Sec. 13 protein 8), which let the translocation of mTORC1 to the lysosome surface where it is subsequently activated (Hall, 2008). Raptor enables AA sensing and the recruitment of two of the major mTOR phosphorylation substrates by which mTORC1 promotes the translational initiation process, p70 ribosomal protein S6 kinase 1 (p70S6K1) and eukaryotic initiation factor (eIF) 4E binding protein 1 (4E-BP1). Phosphorylation of 4E-BP1 prevents its own binding to eIF4E, which would lead to the formation of the inactive 4E-BP1-eIF4E complex and enables the formation of the active eIF4E-eIF4G complex responsible for recruiting mRNA to the 43 S preinitiation ribosomal complex, and the subsequent protein synthesis. Besides, phosphorylation of p70S6K1 results in phosphorylation of ribosomal protein S6, which regulates several targets involved in translation initiation and elongation, such as translation of ribosomal proteins and other components of the translational apparatus (Rudar et al., 2019; Saxton & Sabatini, 2017).

Once synthesised, proteins are released from the synthetic machinery of the ribosome into the cytosol or other cellular compartments. The process of synthesis by the ribosome is discontinuous, with no more than one chain being manufactured in the same ribosome. When the protein is released, the ribosome becomes inactive due to the dissociation of the two major subunits, until a new mRNA molecule arrives to initiate the process. The capacity for protein synthesis depends on the abundance of ribosomes and their efficiency in translating mRNA into proteins. Further molecular processes are far beyond the scope of this review article; for more details, the reader is directed to excellent reviews on this topic (Rudar et al., 2019; Saxton & Sabatini, 2017).

### 2.2 | Protein degradation

Three mechanisms of degradation of old or mistaken proteins exist in eukaryotic cells, (i) the ubiquitin-proteasome system, (ii) the autophagy-lysosome, and (iii) the calcium-mediated calpains and caspases. Degradation of most individual muscular, cytosolic and nuclear proteins is mediated by the ubiquitin-proteasome system and calpains. The 20 S proteasomes are complex proteins found in the cytosol and nucleoplasm that contain degradative enzymes and regulatory proteins. Errors in protein structure arising from environmental causes, transcription or translation mistakes are detected by ubiquitin, which are small proteins that bind to specific molecular errors and cause covalent modifications, becoming targets for protein destruction. This labelling system is regulated by certain ligases responsible for the activation of ubiquitin (E1), its conduction to the problematic proteins (E2), and its binding (E3) to make the protein recognised by the proteasome (Adams, 2003; Brooks & Myburgh, 2014). On the other hand, calpains are cysteine-proteases that are presented in different isoforms such as μ-calpain and m-calpain. The latter isoforms are ubiquitous in skeletal muscle and mostly inactive under basal conditions, however, their activity depends on certain factors such as free cytoplasmic calcium for their activation, or the presence of the polypeptide calpastatin for their inhibition (Smith et al., 2008). Although the autophagy-lysosome system is the mechanism responsible for the degradation of more complex structures (i.e., damaged organelles and protein aggregates), lysosomes are also capable of sequestering small cytoplasmic residues through invaginations of its membrane, or may even present channels to transport proteins directly into their lumen (Lőrincz & Juhász, 2020). Lysosomal enzymes and acidic pH (3–5) in the lumen of lysosomes are the mechanisms for digestion and degradation (Wesselborg & Stork, 2015). The resulting AA from protein degradation become potential substrates for recycling through biosynthesis process or energy production though catalytical activity.

Oxidative phosphorylation is also a relevant pathway to modulate cellular metabolism, energy homoeostasis and cell number. This pathway controls the rates of programmed cell death or apoptosis, and thus protein degradation. Cellular aerobic metabolism regulated by mitochondria generates the production of free radicals such as reactive oxygen (ROS) and reactive nitrogen species. These species spontaneously oxidise nucleic acids, lipids, and proteins, modifying its structure, and consequently losing their function or enzymatic activity. Under physiological conditions, cells counteracts this macromolecular damage with antioxidant production or degradation mechanisms, mainly the 20 S proteasome system and calpain/caspases (Smuder et al., 2010). Mitochondrial proteins are particularly exposed to this oxidation, and their withdrawal is crucial to maintain cellular integrity. Excessive ROS production due to mitochondrial dysfunction, together with the deficient antioxidant defense of cells, leads to oxidative stress occurring mostly during the aging process or in pathological conditions (Hao et al., 2021). In pig, certain factors that induce oxidative stress have been identified as birth, weaning stress, dietary mycotoxin exposition and other environment or social factors (Hao et al., 2021).

## 3 | METHODS TO DETERMINE *IN VIVO* PROTEIN TURNOVER

Classic methodologies such as nitrogen balance or RNA-to-protein ratio are still in use today for determining whole body protein balance, growth and dietary protein requirements (Anthony, 2016), however, more sophisticated techniques have been developed to allow a more direct monitoring of protein metabolism *in vivo*. The application of radioactive (e.g., $^3H$, $^{14}C$, or $^{35}S$) or stable (e.g., $^{13}C$, $^{15}N$, or $^2H$) isotope tracers has contributed to the tracking of transport, downstream metabolism and turnover, as these molecules are chemically and functionally identical to the tracee (i.e., unlabelled molecules). The different number of neutrons in the atomic nucleus between tracee and tracer allow them to be distinguished by their differential molecular weight through high sensitive analytical platforms, such as gas or liquid chromatography coupled to mass spectrometry, or nuclear magnetic resonance spectroscopy (Violante et al., 2019). These methodologies allow measuring rates of protein and DNA synthesis and degradation, also at the level of cellular organelles (e.g., mitochondria), and detect differences in muscle fibre type.

The precursor-product approach or the direct incorporation method, is considered the 'gold standard' method for measuring protein metabolism (Holmes et al., 2015), and consists of administering an isotopically labelled AA tracer, in one bolus or by continuous infusion, flooding the AA pool and reaching the isotopic steady state (i.e., constant labelling pattern). This molecule will act as a metabolic substrate for newly synthesised proteins, but will also be part of other essential AA functions, such as cell signalling, gene expression, and metabolic regulation (Wu et al., 2014). Subsequent tissue sampling is performed over a relatively short and defined period of time to quantify the fraction of newly synthesised labelled AA, considering the pre-existing natural isotope enrichment. In addition, the rates at which the tracer reaches the tissue of interest or 'product', as well as the reservoir where the polymer or 'precursor' is manufactured (i.e., tissue fluid pool or blood) are measured. With this approach, the fractional synthesis rate (FSR) is commonly defined quantitatively as the fraction of a specific protein or protein group synthesised per unit of time (%/t), and these estimations would allow to define basal protein and AA requirements (Wilkinson et al., 2021). The essential AA phenylalanine (Phe) has been one of the preferred tracers, especially in muscle, since its metabolism basically consists of its protein incorporation and release, with almost absence of intermediary metabolism (Wilkinson et al., 2021). For further information authors redirect the readers to additional reviews on methods to determine protein synthesis and degradation (Holmes et al., 2015; Rathmacher, 2000).

High turnover rates of AA tracers limit the assessment of the dynamics of longer half-live proteins with more complex post-translational maturation (e.g., collagenous proteins). This may be overcome by using orally administered deuterated water ($^2H_2O$), as it has a slower disappearance rate than free AA (Holm & Kjaer, 2010). Deuterium atoms ($^2H$) from water are quickly transferred to covalent C–H bonds of free AA and AA precursors (i.e., ketoacids), via hydrogen exchange reactions, which will subsequently be part of newly synthesised proteins (Holm et al., 2019). The advantages of using this method over the former are the reduced invasiveness, as it avoids catheterisation of the animals and allows free living conditions, as well as reduced intracellular recycling of the AA tracer (Holm et al., 2019). However, the metabolic tracer approaches are expensive and time consuming. Therefore, the more affordable cost and greater accessibility to genome-wide sequencing technique has made it a powerful tool for establishing the expression and activity of specific factors and proteins that regulate protein synthesis and degradation (Anthony, 2016).

## 4 | FACTORS INFLUENCING PROTEIN TURNOVER RATES

### 4.1 | Organs and tissue type

The mechanisms responsible for protein turnover vary for each type of protein, resulting in differences among organs and tissues. There is wide evidence that skeletal musculature has a relatively slow turnover rate in comparison with several visceral organs

(Bregendahl et al., 2008; Cross et al., 2020; Garlick et al., 1976; Ten Have et al., 2019). According to Garlick et al. (1976), protein FSR of skeletal muscle are 77–86% lower than those of spleen, kidney, liver and lungs, and 47% lower than those of the brain. Similarly, skeletal muscle FSR is 76–93% lower than liver and 79–97% than upper gut sections (duodenum and jejunum), taking in consideration the references collected in Table 1 of the present review. In general, jejunum has the highest FSR, followed by liver, ileum, heart and skeletal muscle (Cross et al., 2020). Nevertheless, skeletal muscle is the major contributor to whole-body protein synthesis in the pig (50%; Huber, Rudar, et al., 2018; Huber, Squires, et al., 2018) as it is the main reservoir of total body mass protein, while the liver contributes about 10% (Garlick et al., 1976).

Early growth and maturation of these more active tissues (e.g., liver or intestinal tract) have important implications for nutrient availability to peripheral tissues (Reeds et al., 2000). Moreover, increased protein synthesis allows these organs to better adapt to stimuli or environmental changes (Ten Have et al., 2019). However, the higher rates of protein synthesis in specific viscera can also be attributed to its secretory activity, including the synthesis of digestive enzymes by the exocrine pancreas, or the synthesis of mucus or mucins by the mucosal epithelial cells of the gastrointestinal tract (Sans et al., 2004; Wang et al., 2007). The latter proteins are released into the intestinal lumen and do not belong to constitutive tissue cells (Reeds et al., 1993). In addition, the mucus and mucins lining the gastrointestinal epithelium are constantly subjected to erosion and proteolytic degradation, which are counteracted by a high protein synthesis activity (130%/day) (Montagne et al., 2004). As for the liver, it should be considered the main site of AA metabolism (Hou et al., 2020) and synthesises not only hepatic proteins, but also plasma proteins such as albumin and acute-phase proteins (McNurlan et al., 1980; Ten Have et al., 2019), although they are released shortly after being synthesised. Furthermore, although skeletal muscle has no secretory activity, a certain proportion of the synthetic activity is applied for the physiological function of the tissue (Reeds et al., 1993). In a recent study, it was found that the elongation process of mRNA translation showed differences of more than 50% between different organs or tissues (Gerashchenko et al., 2021), with the liver being the organ with the highest elongation rate, followed by kidney and skeletal muscle.

Likewise, there are also differences between different types of muscle tissue, with cardiac musculature showing higher rates of protein synthesis than skeletal muscles, which is supported by the continuous and indispensable function of the heart

**TABLE 1** Fractional synthesis rates (FSR) of skeletal muscle, liver, and upper intestine obtained from pigs of different body weights.

| Reference | Body weight (kg) | Genetics | Sex | Method | FSR muscle (%/day) | FSR liver (%/day) | FSR upper gut (%/day) |
|---|---|---|---|---|---|---|---|
| (Bregendahl et al. [2008]) | 7.2 | YK | Gilts | Flooding dose $^{2}H_{5}$-Phe | 20.3 (b.f.)<br>21.8 (l.d.) | 91.2 | 109.5 (prox. jejunum) |
| (Wang et al. [2007]) | 11.8 | LW × LD | Barrows | Flooding dose $^{2}H_{5}$-Phe (i.p) | 9.8 (l.d.) | 136.8 | 115.8 (jejunum) |
| (Deng et al. [2009]) | 12.0 | DU × LD × YK | – | Flooding dose $^{2}H_{5}$-Phe | 11.8 (l.d.) | 83.5 | 59.7 (prox. small gut) |
| (Rivera-Ferre et al. [2005]) | 23.5 | IB | Gilts | Flooding dose $^{2}H_{5}$-Phe | 7.8 (l.d.)<br>8.2 (b.f.) | 46.6 | 60.5 (duodenum) |
| (Rivera-Ferre et al. [2005]) | 27.1 | LD | Gilts | Flooding dose $^{2}H_{5}$-Phe | 6.4 (l.d.)<br>6.3 (b.f.) | 46.0 | 65.8 (duodenum) |
| (Sarri et al. [2021]) | 28.5 | DU | Barrows | Flooding dose $^{2}H_{5}$-Phe | 6.5 (l.d.)<br>6.4 (b.f.) | 36.0 | 52.0 (duodenum) |
| (Sarri et al. [2021]) | 30.5 | PI × LD × DU | Boars | Flooding dose $^{2}H_{5}$-Phe | 14.0 (l.d.)<br>6.9 (b.f.) | 57.3 | 50.2 (duodenum) |
| (Sève et al. [1993]) | 64.6 | PI × LW | Barrows | Flooding dose $^{13}C$-Val | 4.1 (l.d.) | 43.4 | 118.9 (duodenum) |
| (Sarri et al. [2021]) | 86.1 | DU | Barrows | Flooding dose $^{2}H_{5}$-Phe | 8.7 (l.d.)<br>7.9 (b.f.) | 36.2 | 47.8 (duodenum) |
| (Sarri et al. [2021]) | 91.1 | PI × LD × DU | Boars | Flooding dose $^{2}H_{5}$-Phe | 9.3 (l.d.)<br>8.3 (b.f.) | 41.9 | 42.2 (duodenum) |
| (Huber, Rudar, et al. [2018]; Huber, Squires, et al. [2018]) | 183.0 | YK/YK × LD | Sows | Continuous infusion $^{2}H_{5}$-Phe | 2.2 (l.d.)<br>2.4 (g.n) | 29.9 | – |

Abbreviations: b.f., *biceps femoris*; DU, Duroc; g.n., *gastrocnemius*; l.d., *longissimus dorsi*; IB, Iberian; i.p., intraperitoneal; LD, Landrace; LW, Large White; PI, Pietrain; YK, Yorkshire.

(Garlick et al., 1976; Yuan et al., 2008). It should also be considered that there are differences in protein synthesis rates among different anatomical parts of the same organ, due to distinct structure and functions. In this sense, Garlick et al. (1976) concluded that the renal medulla synthesised less protein than the cortex, as did the pons of the brain compared to the cerebellum and cerebrum. In addition, differences have been found between segments of the small intestine in their rates of protein synthesis and degradation, decreasing from proximal to distal regions (Stoll et al., 2000).

### 4.1.1 | Muscle fibre type

There are two main processes by which skeletal muscle mass grows, either by increasing the number of myofibers or by increasing their size, called hyperplasia and hypertrophy, respectively. Myofibers are the functional contractile unit of skeletal muscle, accounting for 75–90% of this tissue (Joo et al., 2013). The process of hyperplasia occurs mainly during the fetal period, and the total number of myofibers is considered to be stablished at around 90 days of gestation (Gondret et al., 2020), remaining practically constant during the postnatal period. However, myofiber size appears to remain almost unaltered during the prenatal period, with hypertrophy occurring mainly after birth (Lefaucheur, 2010). Myofiber hypertrophy is accompanied by the appearance of new nuclei that are provided by the satellite cells, which are myogenic stem cells located under the basal lamina of the myofibers, constantly required for growth and maintenance of muscle tissue. Activation of satellite cells leads to proliferation, becoming myoblasts that differentiate into mononucleated myocytes and further fuse with myofibers or with each other, resulting in multinucleated myotubes and subsequently matured into contractile myofibers (Murach et al., 2018; Zammit et al., 2004). A second population of satellite cells, however, is often in a quiescent state, becoming activated under regenerative or anabolic stimuli such as exercise or damage (Neal et al., 2012), not being influenced by age or sex.

Differences in protein metabolism have been reported among skeletal muscles, which differ in their location and functionality, and even within the same muscle (Beermann et al., 1990; Garlick et al., 1989). It has been attributed to the heterogeneous composition in different types of myofibers, which are characterised by the expression of the myosin heavy chain (MyHC) isoform. Four main types of myofibers (I, IIa, IIb, and IIx) are recognised in pigs, which are differentiated by their metabolic enzyme profile, morphological features and contractile properties (Choi & Kim, 2009), although myofibers may also contain more than one MyHC isoform.

Based on the speed of contraction, myofibers are classified into slow-twitch type I fibres and fast-twitch type II fibres (IIa, IIb, and IIx), with the following order of shortening speed: I < IIa < IIx < IIb (Fazarinc et al., 2020). Type I fibres are predominantly oxidative and contain a higher number of mitochondria, myonuclei and myoglobin (Choi & Kim, 2009). These fibres are characterised by a tendency to be smaller and the use of lipids as aerobic metabolic fuel. Type II fibres contain a greater amount of glycogen and glucose, giving them higher glycolytic activity and higher myosin ATPase activity. While IIb fibres are the most glycolytic type, IIa and IIx are metabolically intermediate between type I and IIb fibres, although type IIa fibres or fast oxido-glycolytic fibres have higher oxidative capacity than the IIx fibre type. In the pig, deeper postural muscles are generally more oxidative than the more superficial ones, responsible for fast movements, and have higher proportion of type I fibres (Joo et al., 2013).

Although myofiber composition is genetically defined for each individual, myofibers adapt their phenotype and MyHC isoform in response to internal or external factors such as sexual condition, age, nutrition, environment, and physical activity (Fazarinc et al., 2020). In addition, myofibers are dynamic structures and their MyHC expression can be reversibly converted into others by the action of long-non-coding RNAs, as follows: from MyHC I to IIa type, from MyHC IIa to IIx type, and from MyHC IIx to IIb type (Pette & Staron, 2001). The relative composition in myofiber types determines the metabolic properties, and meat characteristics at slaughter of each muscle (Joo et al., 2013; Scheffler & Gerrard, 2007), with protein synthesis rates following this order according to myofiber type: IIb < IIx < IIa ≈ I (Goodman et al., 2011).

On the other hand, Mittendorfer et al. (2005) suggested that other internal factors, such as the amount of RNA and myofiber nuclei, muscle function and location, are more suggestive of the basal rate of protein synthesis. Substantial advances in high-throughput sequencing techniques have identified IGF-1-Akt-mTOR as one of the major pathways regulating, but mostly increasing protein synthesis by modulating translation initiation (Manning & Toker, 2017).

## 4.2 | Developmental stage

Animal growth and development occur continuously beyond reaching sexual maturity, being associated with high rates of protein synthesis and deposition; however, this does not take place uniformly. Whole-body protein synthesis reaches its highest rates during the early postnatal period, related to higher rates in skeletal muscle than in other body tissues (Burrin et al., 1995; Rudar et al., 2019), coinciding when its proportion in the body is of great importance. This phenomenon has been widely documented in different animal species such as pigs (Davis et al., 1996), chickens (Kang et al., 1985), rats (Millward et al., 1975), sheep and humans (Reeds et al., 2000). In addition, neonatal skeletal muscle is characterised by a high glycogen content to ensure sufficient energy in the first moments of the newborns (Herpin et al., 2002), which decreases rapidly within a few days while lipid and protein content increases.

It is after birth when skeletal musculature is particularly sensitive to anabolic stimuli, such as hormones and nutrient availability (Thivierge et al., 2005), coinciding with the ingestion of colostrum that is highly nutrient-dense and contains a high concentration of growth-regulating compounds (Reeds et al., 2000).

Similarly, some vital organs such as the brain and heart have also shown increased rates of protein synthesis when piglets are fed colostrum rather than other substitutes, including mature milk or synthetic formula (Burrin et al., 1997). Increased protein synthesis at this stage has also been associated with a higher RNA-to-DNA ratio and a higher content of cellular ribosome and mitochondria; the later associated with the higher predominance of oxidative fibres in young animals (Skjaerlund et al., 1994). Conversely, many negative regulators are less abundant in the skeletal muscle of these younger pigs (Davis et al., 2008), with mTORC1 suppressing protein catabolism, particularly autophagy (Saxton & Sabatini, 2017). All these aspects result in increased muscle protein deposition and explain the rapid relative growth of these animals, achieving the highest nutritional efficiency for muscle protein accretion. In this sense, Davis et al. (1996) found a decreased FSR in skeletal muscles (*longissimus dorsi* and *gastrocnemius*), heart, and liver of piglets between 7 and 26 days of age, whereas no differences were found in the jejunum and pancreas. Due to the scarcity of studies comparing FSR between ages, FSR data from several previous studies have been compiled in Table 1. As can be observed, protein synthesis of both skeletal muscle and relevant visceral tissues, such as liver and upper intestine, tends to decrease throughout the growth and maturation of the pig. In most of these studies, the intravenous flooding dose technique was used, applying mainly Phe as a tracer. However, Wang et al. (2007) employed the intraperitoneal route, through which they obtained a lower muscle FSR and a higher visceral FSR than expected. When Bregendahl et al. (2008) compared the intravenous and intraperitoneal routes, they obtained a higher FSR in skeletal muscles in individuals submitted to the intravenous route than by the intraperitoneal route, while in liver and jejunum, the differences were not significant. The authors associated the differences between both routes to a greater induction of cortisol when the tracer administration was intraperitoneal. However, it could be hypothesised that through the latter route, a non-negligible part of the tracer can be transported directly from the intraperitoneal cavity to the intracellular space of internal organs, increasing the free-pool tracer isotopic enrichment. On the other hand, Huber, Rudar, et al. (2018); Huber, Squires, et al. (2018) opted to use a primed continuous infusion. Although this technique requires a much longer tracer infusion protocol, it has been described that the high amount of AA administered with the flooding dose technique stimulate by itself muscle FSR, especially when essential AA are used as tracer (Jahoor et al., 1992).

Transcriptome studies have indicated that gene expression changes considerably throughout pig life, but especially during the perinatal period (Mohammadabadi et al., 2021). The intense evolution of FSR during this life period is explained by different reasons. First, the decrease in ribosome abundance between birth and weaning (i), together with their lower translational efficiency under feeding stimulation (Reeds et al., 2000; Srivastava, 2017). The significant decrease in the number of satellite cells with age (ii) (Neal et al., 2012). Satellite cells proliferate and differentiate intensively during the postnatal period, contributing to myofiber hypertrophy by fusing with them (Gondret et al., 2020). Finally, modification of the MyHC myofiber profile (iii) (Brocks et al., 2000; Saxton & Sabatini, 2017). During the embryonic and fetal period, the predominant MyHC isoform is slow-twitch type I, although by late gestation the adult fast-twitch types IIa and IIx isoforms appear. It is at birth that a large part of the type I MyHC isoforms mature to fast-twitch isoforms, and type IIb MyHC isoform also emerge (Gondret et al., 2020). The intensity with which the proportion of slow-twitch myofibers changes to fast-twitch myofibers decreases with pig maturation (Fazarinc et al., 2017). On the other hand, the shift in myofiber profile has shown to reverse with aging, with increased atrophy of type II myofibers, and increased grouping and cross-sectional area of type I myofibers (Kelly et al., 2018).

Regarding muscle protein degradation rates, although they exist in the neonatal period, are much lower than those of synthesis and decay more slowly than synthesis rates as the animal grows (Fiorotto et al., 2000; Skjaerlund et al., 1994).

Skeletal muscle development throughout growth and adulthood is affected by its genetic background and by external factors to which individuals are constantly subjected (e.g., nutrition, hormones, activity, and injury) (Wallace et al., 2016). It is generally suggested that protein synthesis and its regulatory machinery decreases throughout animal maturity (Attaix et al., 1988; Connors et al., 2008). In this sense, the decrease in FSR between growing lambs and mature ewes obtained by Connors et al. (2008) was attributed to down-regulation of translation process, with decreased RNA concentration and RNA:protein ratio, which is a measure of synthetic capacity. Recent studies performed mostly in mice also suggest that the decrease in protein synthesis is mainly attributed to changes in different steps of the translation process (Gonskikh & Polacek, 2017), both in the translation initiation and elongation (Anisimova et al., 2020; Gerashchenko et al., 2021). These changes are also evident at gene expression and transcriptome level (Anisimova et al., 2020), by downregulation of ribosome biogenesis and components of the protein synthesis machinery, resulting in a gradual decrease in translation efficiency.

Upregulation of genes associated with inflammation, extracellular matrix (ECM) development and lipid metabolism has been identified as the animal matures, to the detriment of genes involved in myofibers proliferation and differentiation. (Ayuso et al., 2016). Subsequently, Benítez et al. (2021) described that as the maturity progresses, the expression of genes involved in the chronological processes of myogenesis changed, such that upregulated genes in transition pigs were more associated with proliferation and early stages of differentiation, whereas upregulated genes in grower pigs were more involved in advanced differentiation, hypertrophy, and ECM organisation. Development of ECM is generally associated with hypertrophy of muscle growth since it is involved in signalling pathways, activating/inhibiting enzymatic activities, being the site of binding hormones and enzymes, regulating the interaction of several ligands with their receptors, and also regulating the mechanical support between muscle cells (Csapo et al., 2020). To all these changes must be included the accumulation of molecular damage in cells and proteins with aging, preventing their normal functioning, and causing the appearance of aggregates or toxic products (Anisimova et al., 2018; Hipp et al., 2019).

## 4.3 | Genetics

Comparison of protein metabolism between pig breeds or genotypes is not a simple issue since they can exhibit differences in growth potential and tissue development, which implies substantial changes in protein turnover and nutritional requirements throughout the different growth stages. Therefore, it is important to work with comparable ages or physiological states. Most of the comparative studies between pig breeds aim to compare protein metabolism between leaner or modern breeds versus fatty or autochthonous pig breeds, due to potential differences in the quality of their meat products.

In an initial study, Rivera-Ferre et al. (2005) found that fatty Iberian pigs had a higher skeletal muscle FSR than leaner purebred Landrace pigs. Considering that Landrace pigs had a larger protein pool than Iberian, they concluded that the higher FSR registered in Iberian pigs was unrelated to higher protein accretion but was associated with higher rates of protein degradation. Consistent with this study, lower protein FSR were reported in fast-growing genotypes in other species such as steers (Lobley et al., 2000). However, in chickens, higher growth rates in fast-growing strains were associated with lower FDR compared to slower-growing strains (Klasing et al., 1987). Subsequently, when Rivera-Ferre et al. (2006) studied whole-body protein synthesis and degradation based on metabolic body size, they found that it was lower in Iberian than in Landrace pigs at 28 kg body weight (BW), without significant breed differences in FSR and FDR. Authors attributed the inconsistency with their previous study to the different body protein mass, with Iberian pigs having 20–32% smaller muscles than Landrace (Rivera-Ferre et al., 2005). Our group (Sarri et al., 2021) recently compared two distinct producing types: castrated purebred Duroc pigs as fatty type against entire males from a leaner crossbreed (Pietrain sires × [Duroc × Landrace]), and obtained that the leaner type had higher FSR in liver and *longissimus dorsi* muscle than the fatty one, especially in the growing phase with approximately 30 kg BW.

Because variation in fibre type composition among genotypes may affect both, meat organoleptic characteristics and protein turnover rates, it has been extensively studied (Chang et al., 2003; Joo et al., 2013). In this sense, intramuscular fat content and backfat thickness correlate positively with the expression of MyHC I and IIa isoforms, as opposed to the expression of MyHC IIb (Qi et al., 2019). Accordingly, the proportion of glycolytic fibres, especially type IIb fibres, increases with those genotypes highly selected for improved growth rate, lean carcass meat and feed efficiency (Fazarinc et al., 2017; Ruusunen & Puolanne, 2004). Examples for this assumption are: Serra et al. (1998) reported a higher proportion of type I fibres in Iberian pigs than in Landrace, while Landrace contained a higher proportion of type IIb fibres. Ryu et al. (2008) noted that Berkshire pigs had greater percentage of type I fibres and lower type IIb fibres than Landrace, Yorkshire and Landrace × Yorkshire × Duroc crossbred pigs. Guo et al. (2011) observed higher mRNA abundance of oxidative myofibers and lower expression of glycolytic myofibers in Jinhua pigs compared to Landrace pigs. Wojtysiak and Połtowicz (2014) also found that autochthonous Puławska pigs presented a higher proportion of type I fibres than muscles from Polish Large White pigs. And more recently, Fazarinc et al. (2017) demonstrated the transition of European wild pig myofibers toward increased expression of oxidative myofibers with maturation, whereas the domestic Large White pig expressed more type IIb myosin heavy chain after the same time period.

Although oxidative type I fibres have a higher transcriptional potential and a greater capacity to synthesise myofibrillar proteins, including a higher density of mitochondria and myonuclei per fibre, higher RNA and mRNA content, higher α-actin mRNA expression and a larger satellite cell population during postnatal development, they exhibit a smaller fibre size and lower cross-sectional area than glycolytic type IIb fibres (van Wessel et al., 2010). This contradiction seems to be related to higher rates of protein degradation and thus, higher protein turnover, as certain degradative machinery is more abundant in oxidative muscle fibres, especially calpains and calpastatins (Smuder et al., 2010; van Wessel et al., 2010), resulting in lower net protein deposition.

Gene expression is mainly behind the muscle fibre type profile and other phenotypic characteristics, upregulating or downregulating key genes involved in signalling pathways throughout the animal's growth that can alter protein metabolism among pig genotypes. In this regard, the heritability of certain traits such as the percentage of type I ($h^2 = 0.46$) and IIb myofibers ($h^2 = 0.58$) are relatively high (Larzul et al., 1997). According to SanCristobal et al. (2015), the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, mostly involved in the regulation of muscle mass growth, is the most relevant signalling pathway showing variable expression between modern pig breeds. In addition, genes associated with muscle growth, muscle anabolism, and cell proliferation and differentiation are overexpressed in leaner breeds (Zhao et al., 2011). The higher expression of collagen in this latter type of breeds is also related to their leaner growth and lower intramuscular fat deposition. On the other hand, genes related to fatty acid synthesis, adipogenesis, energy or glucose metabolism, cytoskeleton organisation and microtubule dynamics are overexpressed in fatty breeds (Ayuso et al., 2016; Srivastava, 2017). In addition, expression of genes related to growth and development in earlier stages have also been identified in these later breeds, which may be associated with their earlier developmental stage, as fatty and autochthonous breeds are more precocious than leaner breeds. As for genes associated with protein degradation, showed upregulation in fatty breeds (Srivastava, 2017), as well as genes regulating myogenesis inhibition (Zhao et al., 2011).

## 4.4 | Sex

Differences in muscle growth and carcass characteristics among sexes have been widely reported (Boler et al., 2014; Kress et al., 2020;

Pauly et al., 2008; Suster et al., 2006), being frequently attributed to the effects of androgen hormones, like testosterone (Bonneau, 1998; Claus & Weiler, 1994; Xue et al., 1997). The effect of androgens on tissue protein balance has been of great interest especially in the human species, to give a solution to different types of hypogonadism in men, which is a clinical condition, congenital or acquired, characterised by reduced production or availability of androgens. Affected patients suffer a decrease in their muscular mass, leading to muscle atrophy or sarcopaenia, and immunocastration in male pigs is currently serving as a model to study this condition in men (Batorek-Lukač et al., 2022). Supplementation with testosterone and other pharmacological derivates are proposed to reverse the clinical signs, although the underlying molecular mechanisms are complex, with numerous experimental inconsistencies, and remain to be stablished. Existing studies suggest that the anabolic effect of testosterone acts through different pathways, as can be: increasing AA availability, stimulation of myoblast growth and differentiation (Herbst & Bhasin, 2004), activation and proliferation of satellite cells (Rosa-Caldwell & Greene, 2019), stimulation of synthesis through leucine-induced pathway (Jiao et al., 2009), and restoring or increasing protein FSR (Wendowski et al., 2017) activating mTORC1 signalling pathway through upstream effectors (Basualto-Alarcón et al., 2013; Steiner et al., 2017; White et al., 2013). In this sense, Basualto-Alarcón et al. (2013) demonstrated that stimulation of myotube cultures with testosterone resulted in increased hypertrophy through activation of the mTOR/p70S6K1 pathway via PI3K/Akt signalling.

In this regard, one of the first studies in pigs was conducted by Mulvaney (1984) to look for differences in protein turnover rates between boars and barrows. They reported, through *in vitro* procedures, an average reduction of 56% and 9% in FSR and FDR, respectively, in barrows compared to boars in the prepubertal stage (40 kg BW); and 33% and 26%, respectively, in the pubertal stage (75 kg BW) using the *semitendinosus* muscle. However, when Skjaerlund et al. (1994) further studied differential rates of *in vivo* protein turnover in younger barrows and boars (1 to 4 weeks old), they found no differences in FSR, FDR or accretion rates despite the tendency of barrows to have lower protein content than boars. Although the authors agreed with other studies (Colenbrander et al., 1978; Ford, 1983) that circulating testosterone picked between the second and third week of age, the low levels in this early stage may be insufficient to detect significant effects on protein turnover.

When comparisons are made with females, male individuals have shown greater mRNA expression associated with differentiation and hypertrophy (myogenin and MyoD) and higher proliferation capacity (Lee et al., 2011; Manzano et al., 2011), which is reflected by increased myonuclei number. In addition, it has also been suggested that androgens mediate the increased expression of their receptors and the activation and proliferation of satellite cells (Mulvaney et al., 1988; Rosa-Caldwell & Greene, 2019). In this regard, uncastrated males exhibit higher satellite cell proliferation and differentiation than females and castrated males (Lee et al., 2011); and male individuals show a higher satellite cell content per fibre than females, particularly those specialised in muscle growth and muscle maintenance (Neal et al., 2012). However, the lack of differences between young females and males in protein turnover, obtained in multiple studies, indicates that other mechanisms may exist. As for the skeletal muscle fibre type profile, females tend to have a more oxidative profile, especially a predominance of type I MyHC isoform than males (Haizlip et al., 2015). Therefore, females have more mitochondrial content and activity than males, which may contribute to differences in protein metabolism, however, further research is required to confirm that aspect (Rosa-Caldwell & Greene, 2019). In addition, it has been hypothesised that female hormones (e.g., estrogens and progesterone) may compensate their lower levels of testosterone (Hansen, 2018). Both, estrogens and progesterone have been proposed to exert hypertrophic effects and enhance muscle function, with progesterone increasing protein synthesis and estrogens reducing protein degradation and increasing cell sensitivity to anabolic stimuli (Hansen, 2018; Rosa-Caldwell & Greene, 2019).

Degradative processes also appear to be influenced by sex, with females showing higher autophagy-lysosome related protein degradation, but lower ubiquitin-proteasome activity than males (Rosa-Caldwell & Greene, 2019), although testosterone treatment at the human elderly was seen to decrease protein degradation through ubiquitin-proteasome system (Kruse et al., 2020). In addition, higher calpain expression and lower calpastatin expression were shown in boars in comparison with barrows, evidencing their faster protein synthesis and degradation (dos Santos et al., 2021). Such characteristics are also noticeable in meat between sexes, with boar meat being less fatty but with a higher tenderness and lower shear force than barrows (dos Santos et al., 2021), considering the persistence of degradative mechanisms after the animal slaughter, contrary to synthesis. Finally, testosterone also reduce protein degradation through the hepatic urea cycle (Birzniece et al., 2011; Rossetti et al., 2017). In this sense, testosterone restrict the action of certain enzymes present in the urea cycle, decreasing the synthesis of hepatic urea and thus, reduces protein degradation and the loss of AA and nitrogen through urine. Therefore, it may increase the amount of AA susceptible for muscle anabolism (Lam et al., 2017).

### 4.5 | Feeding level

It was early noticed that whole body protein synthesis is influenced by feeding and fasting periods (Millward et al., 1975). Colostrum or milk intake in the neonate is the main stimuli responsible for enhancing whole-body protein synthesis by promoting mTORC1 activation, as they provide a high source of nutrients, including AA, which consist of the biochemical building blocks and precursors for protein accretion and energy storage (Burrin et al., 1997). In addition, colostrum contains potential bioactive compounds, including those related to passive immunity (e.g., immunoglobulins (IgG, IgA and IgM) and leukocytes), bacteriostatic agents, hormones and growth factors, such as epidermal growth factor, insulin-like growth factors (IGF-1/2) and transforming growth factors (Burrin et al., 1995, 1997). The latter growth factors have been linked to increased intestinal maturation and benefit ribosomal RNA synthesis (Xu et al., 2002).

Connors et al. (2008) suggested that the increased FSR with feed intake in growing and mature ewes was due to an increased translational initiation rate, increasing the proportion of ribosomes actively translating mRNA. This is explained by increased formation of the eIF4E-eIF4G complex, and increased phosphorylation of mTORC1, 4E-BP1, S6, and eIF4G (Anthony, 2016; Wilson et al., 2009). However, the effect of feeding level on FSR is not homogeneous over time, decreasing as the postnatal period progresses (Davis et al., 1996). In neonates, protein synthesis in skeletal muscle peaks at 30 min postprandial and remains constant during 90 min (Wilson et al., 2009). Some studies suggested that skeletal muscles, especially those with predominantly fast-twitch glycolytic fibres, along with the brain, are the tissues most stimulated by feeding (Burrin et al., 1997; Fiorotto et al., 2000). As for visceral tissues, such as liver and intestine, FSR increment to feeding level have also been reported (Burrin et al., 1997; Widdowson et al., 1976), which was suggested to be a result of forcing these organs to a greater metabolic response (Nyachoti et al., 2000). It is noteworthy that, (i) 30–50% of dietary AA are used by intestinal tissues, and ii) the intestinal tract is directly exposed to dietary nutrients, hormones (i.e., insulin and IGF-1) and growth factors. Therefore, the absence or reduced availability of lumen nutrients that occur in parenterally fed piglets alters protein metabolism in the small intestine (Stoll et al., 2000). In addition, the dependence on lumen nutrients was greater in the proximal (jejunum) than in the distal (ileum) segments, which is explained by the normal decrease in nutrient availability as nutrients are absorbed along the small intestine. This is consistent with registered changes in intestinal villus height (Stoll et al., 2000).

It has also been proposed that increased postprandial insulin and IGF-1 stimulate skeletal muscle protein synthesis (Davis et al., 2002; Rennie et al., 2004). These two hormones stimulate the phosphorylation of S6K1 and 4E-BP1 (Han et al., 2008), which activates mTORC1 and the subsequent translation initiation process, with an efficient binding of the mRNA to the 43 S ribosomal complex. Although the stimulation of protein synthesis by insulin is exclusive to immature skeletal muscle (Reeds et al., 2000), decreased whole-body protein degradation by insulin has been detected at more mature ages as well (Reeds et al., 2000).

Regarding degradation rates, feeding was also reported to reduce whole-body protein degradation in neonates (Thivierge et al., 2005), mainly in visceral tissues, although protein degradation increased or was unaffected in skeletal musculature (Rudar et al., 2019; Wilson et al., 2009). Protein degradation actually plays a critical role in maintaining protein accretion rates, as this process maintains the free AA pool, although the preferred AA for muscle and whole-body protein synthesis are dietary rather those coming from intracellular degradation (Groen et al., 2015). Nevertheless, increased rates of protein degradation are below protein synthesis rates, leading to protein deposition (Zhang et al., 2014).

On the other hand, fasting states inhibit mTORC1 signalling, as well as low ATP levels in order to conserve limited resources (Allen et al., 2010; Saxton & Sabatini, 2017), therefore, skeletal muscle synthesis rates decrease in all myofiber types, but especially in types IIx and IIb (Goodman et al., 2012). Decreases in FSR have also been reported in liver during fasting states, however, no differences were found in myocardial tissue, even though animals were subjected to chronic dietary restriction (Yuan et al., 2008). Under these circumstances, heart showed few reductions in 4E-BP1 and eIF2α phosphorylation.

In addition, upregulation of atrophy-related genes have been identified under prolonged feed deprivation in mice (Allen et al., 2010). As for protein FDR during fasting states, it was proposed to increase in order to permit the released AA to be converted into glucose through gluconeogenesis, since maintaining the circulating glucose is essential for the proper functioning of certain organs (Allen et al., 2010). Undernutrition during the gestation period results in lower postnatal growth performance, decreasing the number of secondary fibres and muscle growth (Brown, 2014; Du et al., 2010), whereas offspring from overfed gestating sows have also shown lower total number of myofibers (Cerisuelo et al., 2009).

## 4.6 | Dietary CP content

Reducing the dietary CP level by balancing non-bound AA (i.e., synthetic and crystalline AA) is a commonly used feeding strategy in pig production to conform to EU regulations (Council Directive 91/676/EEC of the 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources,/676/EEC of the 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources, 1991), directed to decrease nitrogen waste through manure. However, the reduction of CP in the diet may compromise the adequate provision of essential AA. Essential AA play a critical role in protein synthesis, and their imbalance could delay or cease the RNA translation process due to polyribosome disaggregation (Deng et al., 2009; Escobar et al., 2007), thus, adequate supplementation of non-bound AA is crucial (Deng et al., 2009). Understanding the physiological functions of AA and their requirements becomes of great interest to optimise the AA profile in dietary formulations.

AA are signalling regulators that stimulate protein FSR of skeletal muscles and most visceral tissues throughout life (Davis et al., 2002, 2008) by mTORC1 activation (Rennie et al., 2004; Saxton & Sabatini, 2017), however, the response to this stimuli loses intensity with age, which has been linked to a change in the mTOR-associated signalling pathway (Rudar et al., 2019). Previous assays have shown a decrease in protein synthesis with decreasing dietary CP level (Rennie et al., 2004; Rivera-Ferre et al., 2006; Sève et al., 1986). Accordingly, Li et al. (2016) described downregulation of certain AA transceptors when CP level was reduced more than 4%, which modulate the mTORC1 activation pathway, even though crystalline AA were supplemented to cover the limiting essential AA.

The same authors also reported that pigs fed this same diet had lower expression of key myogenesis regulatory genes (MyoD and MyoG), and higher expression of genes related to proteolysis.

Moreover, Deng et al. (2009) described a differential sensitivity among tissues to dietary CP concentrations. While gastrointestinal tract (including small intestine and colon) was relatively insensitive in weaning pigs, skeletal muscle (*longissimus dorsi*), liver, pancreas and kidneys reduced their protein FSR when animals were fed low-CP diets, even though pigs were supplemented with deficient crystalline essential AA. The authors suggested that the underlying mechanisms were related to the reduced phosphorylation of mTOR found in liver and of 4E-BP1 in both muscle and liver. However, the formation of the active eIF4E-eIF4G complex only decreased in liver, but not in muscle, which was associated with a tissue-specific response (Deng et al., 2009). In this sense, scarce or no differences were found in skeletal muscles (*longissimus dorsi* and *biceps femoris*) and viscera (duodenum and liver) protein FSR, when moderate differences in CP content were tested in growing (15 vs. 17% CP) and fattening pigs (13 vs. 15% CP) in our recent study (Sarri et al., 2021). These results agreed with those of Huber, Rudar, et al. (2018); Huber, Squires, et al. (2018), who identified no FSR differences in *longissimus dorsi*, *gastrocnemius*, liver or mammary gland protein when lactating sows were fed a low-CP diet, although the apparent efficiency of nitrogen utilisation for milk protein production was enhanced. In both assays, crystalline AA were supplemented in low CP diets to meet essential AA requirements. The short feeding period or moderate CP variance between treatments in Sarri et al. (2021) could have been the reasons for the lack of effects, since Li et al. (2016) found consistent changes in muscle AA transceptor expression and mTORC1 pathway when the CP level drops below 3% and when the feeding period reached 25 days.

Important signalling roles of certain AA in protein turnover have been identified, mainly mediated through the mTORC1 pathway, especially for leucine, arginine, glutamine, and proline (Rezaei et al., 2013). Leucine stimulates protein synthesis by enhancing mTORC1 activation through phosphorylation of mTOR and its downstream effectors S6K1 and 4E-BP1 (Han et al., 2008; Rudar et al., 2019; Suryawan et al., 2008), especially in neonatal pigs. In addition, leucine downregulates the expression of proteolytic-related genes linked to ubiquitin-proteasome and autophagy-lysosome in skeletal muscle (Nakashima et al., 2005), and some metabolites from its catabolism (α-ketoisocaproate and β-hydroxy-β-methylbutyrate) are also involved in the activation of translation initiation factors (Zheng et al., 2017). The deficiency in lysine and methionine have also been linked with decreases in both FSR and FDR (Mazor et al., 2018; Rivera-Ferre et al., 2006; Roy et al., 2000), although Palma-Granados et al. (2019) observed an increase in the proportion of oxidative myofibers in pigs fed a lysine-deficient diet. In addition, different sensitivities to a lysine-deficient diet have been found among pig genotypes, being more sensitive the highly selected pigs in comparison with the autochthonous ones (Rivera-Ferre et al., 2006). Besides Lys and Met, the supplementation of low-CP diets with specific nonessential AA, such as glutamine and arginine, have shown maintenance of mTORC1 signalling activation for optimal protein synthesis (Deng et al., 2009; Rudar et al., 2019).

## 4.7 | Rearing system and physical activity

The growing concern of consumers for animal welfare on farms has increased their preference and demand for meat products coming from organic or free-range rearing systems (Akaichi et al., 2019; García-Gudiño et al., 2021). Similar to free-range systems, organic production enables animals access to outdoor areas and/or pastures, which implies regular or spontaneous exercise (*Regulation 2018/848/ EU of the European Parliament and of the Council*, 2018). Physical activity promotes the use of dietary AA for de novo protein synthesis in muscle (Holwerda et al., 2019), and influences muscle characteristics regulating the myofiber composition. Fazarinc et al. (2020) noticed that Slovenian Krškopolje pigs reared in organic production systems presented lower glycolytic-to-oxidative myofiber ratio. This muscle phenotype corresponded to mRNA expression levels, with upregulation of MyHC I, IIa and IIx isoforms and downregulation of MyHC IIb isoform in organic-reared pigs. According to these results, Qi et al. (2019) also demonstrated that pig production in a semi free-grazing system increased the percentage of type I and IIx fibres than those reared on an indoor farm.

Previous studies also reported increased oxidative capacity when pigs were subjected to treadmill training while reared in conventional indoor facilities (Mcallister et al., 1997; Petersen et al., 1998). Under this same training, Mcallister et al. (1997) found a lower proportion of type IIb fibres and an increased composition in type IIx fibres in the *triceps brachii*, while Petersen et al. (1998) detected a higher ratio of type IIa-to-IIb/IIx in the *longissimus dorsi* of pigs. However, correlation between muscular fibre profile and physical activity is controversial and other authors could not evidence any difference. Gentry et al. (2002) showed no differences in the percentage of muscle fibre types when pigs were reared in long pens with 10 times more space allowance, even though they noticed higher physical activity than those reared in conventional dimensions. Similarly, neither Cross et al. (2020) found differences between inherently low-active and high-active inbred strain mice; while low-active mice had a higher kidney FSR. In addition to increased physical activity, access to the outdoors may lead to greater exposure to temperature fluctuations. In this regard, prolonged exposure to cold temperatures increased the proportion of oxidative myofibers (Herpin & Lefaucheur, 1992; Mizunoya et al., 2014; Yu et al., 2021), as well as increased protein turnover rates in chicks, which may result in increased nutrient burning for caloric energy (Aoyagi et al., 1988).

## 4.8 | Health status

Protein and AA metabolism is significantly affected by injury, inflammatory states, and the presence of disease (Rudar et al., 2019).

Such effects have been studied mainly through sepsis and bacterial lipopolysaccharide models by the administration of endotoxins, live bacteria, and bacterial fragments (e.g., lipopolysaccharides); or through induction of local inflammation by administration of substances such as dextran sulfate, turpentine, or tumour necrosis factor (TNF-α). When the immune system is stimulated, a greater proportion of nutrients that were being destined to maintenance, growth, or reproduction are redirected to support the immune system to enable the production of specific proteins and metabolites (e.g., proinflammatory cytokines and leucocytes). Although ileal digestibility appears not to be affected during immune system stimulation (Rakhshandeh et al., 2014), immune-challenged pigs significantly reduce their voluntary feed consumption (de Ridder et al., 2012). Decrease in feed intake and AA utilisation efficiency reduce animal growth and protein retention rates under pathological circumstances (de Ridder et al., 2012; McGilvray et al., 2019). This results in decreased rates of skeletal muscle protein synthesis (McGilvray et al., 2019; Rudar et al., 2019), which is primarily related to a suppression of mTORC1 signalling and changes in local and circulating growth factor levels (Rudar et al., 2019). Although skeletal muscle reduces its contribution (Breuillé et al., 1994), whole-body protein synthesis is generally maintained in comparison with nonchallenged animals, as synthesis in some viscera compensates.

Protein metabolism of viscera in response to immune challenge is highly variable and depends on each organ or tissue. Increases in the protein synthesis rate have been described especially in the liver (Breuillé et al., 1998; Mackenzie et al., 2003), due to increased AA demands (e.g., gluconeogenic AA) and energy expenditure directed to the synthesis of acute-phase proteins such as fibrinogen and albumin (Ten Have et al., 2019). Indeed, the action of cytokines and glucocorticoids during infection and inflammatory processes increases blood flow and AA transport in the liver, increasing its AA uptake at the expense of peripheral tissues (Spurlock, 1997). The degree of increase in its synthetic rates can vary widely, being more pronounced in septic than in inflammatory models (Breuillé et al., 1998). Along with the liver, the spleen is also involved in the immune response, filtering the blood to remove antigens. This organ undergoes cell hypertrophy and even hyperplasia, as a result of increased protein synthesis (Breuillé et al., 1998; Obled, 2003). Another splanchnic tissue involved in immune response is the gastrointestinal tract, which also undergoes relevant morphological and physiological changes under immune system stimulation, affecting mucins production, epithelial cells and proteins related to intestinal repair and protection against bacterial translocation (Ten Have et al., 2019). Under systemic inflammation caused by acute bacteremia-induced sepsis (e.g., *Escherichia coli*), increases in FSR of the whole intestine have been found in rats (Breuillé et al., 1998). However, Ten Have et al. (2019) could not confirm previous results and noticed no change in FSR in the ileum during *Pseudomonas aeruginosa*-induced sepsis, along with substantial decreases in FSR (29%) and FDR in the jejunal mucosa. Furthermore, the lungs experienced increased protein synthesis in the early phases of bacteremia-induced sepsis (Breuillé et al., 1998; Ten Have et al., 2019).

Protein degradation, particularly of muscle or skin, has been described as a potential AA-contributor of acute-phase proteins synthesis, which are plasma proteins produced mainly by hepatocytes that act destroying or inhibiting the pathogens growth, or contributing to the negative feedback of the inflammatory response. In this sense, several authors have reported increased rates of protein degradation in skeletal muscle (Breuillé et al., 1998; Llovera et al., 1993; Rudar et al., 2019) promoted by the action of cytokines that stimulate the formation of ROS and the activation of the nuclear factor kB (Bernacchioni et al., 2021; Li et al., 1999). However, other authors described no changes in whole body protein degradation (Rudar et al., 2017), or even a reduction in whole-body protein degradation rates in immune-challenged pigs (McGilvray et al., 2019; Ten Have et al., 2019). In this regard, increases in calpain activity were reported during sepsis, particularly in skeletal muscle, which has been linked to decreased calpastatin activity inhibition (Smith et al., 2008), whereas increased activity of the ubiquitin-proteasome system has also been observed (Voisin et al., 1996).

Finally, alteration in muscle fibre type profile during disease has also been described (McGilvray et al., 2019), with type I fibres increasing their proportion towards type II, especially type IIx fibres, in immune-challenged pigs. On the other hand, specific muscle pathologies such as muscular dystrophies or cancer cachexia show their inherent muscle fibre type changes (Rosa-Caldwell & Greene, 2019).

## 4.9 | Thermal Stress

The limited number of functional sweat glands, thick subcutaneous fat layer, and intense metabolism prevent pigs from adequate body heat dissipation (Renaudeau et al., 2006), making these animals susceptible to heat stress (HS). Temperatures above the pig's thermoneutral zone significantly reduce their appetite and, consequently, their voluntary feed intake as a biological strategy aimed at avoiding the generation of additional heat from ingestion, absorption, and metabolism (Huynh et al., 2005; Pearce, Gabler, et al., 2013). The gastrointestinal tract is particularly sensitive to HS, suffering a loss of epithelial integrity (Liu et al., 2009; Pearce, Mani, et al., 2013) and impairing its normal functioning, leading to lower apparent and standardised ileal digestibility of certain AA (Morales, Cota, et al., 2016; Morales, Pérez, et al., 2016).

Within this framework, reduced growth rates and protein deposition in pigs under these environmental conditions have been associated with lower feeding levels and subsequent potential nutrient deficiency (Cervantes et al., 2018). However, this hypothesis is currently questioned because other works suggested that HS itself may induce other metabolic alterations caused by intestinal barrier disruption, inflammatory state, postabsorptive metabolism and endocrine responses (Liu et al., 2022; Qaid & Al-Garadi, 2021). In addition, HS seems to cause oxidative stress and mitochondrial dysfunction by increasing ROS production in muscle cells (Kikusato & Toyomizu, 2013; Kim et al., 2021), whose intracellular signal transduction contributes to protein catabolism. It is noteworthy that pigs with higher genetic potential for lean growth, as well as heavier and older pigs appear to have less tolerance to HS (Renaudeau et al., 2011). Similarly, the effect of HS on carcass composition is also age-dependent, being irrelevant in young pigs but pronounced in older pigs (Christon, 1988). These results have been confirmed in other species such as chicks (Temim et al., 1999; Yunianto et al., 1997) or rodents (Katsumata et al., 1990).

Regarding protein metabolism, the authors of the present review are not aware of studies in pigs describing the direct impact of HS on FSR and FDR, however, there is extensive related literature in chickens, which can be used as a homoeothermic animal model. Broilers are also greatly affected by high ambient temperatures due to their underdeveloped sweat glands and the high body heat expenditure of modern commercial lines. The low growth and protein retention of broilers subjected to chronic HS conditions results from a pronounced depression of FSR (Temim et al., 2000), which has been associated with lower protein synthesis capacity (i.e., RNA-to-protein ratio; Temim et al., 2000), and reduced S6K1 mRNA expression in tissue (Ma et al., 2021). Maharjan et al. (2020) and Temim et al. (2000) noticed that HS affected the FSR to a greater extent than the FDR.

In terms of FDR, several authors have described the increase of this rate through distinct indicators among which we highlight an increased concentration of circulating markers of muscle catabolism, such as $N_{\tau}$-methylhistidine, creatinine and blood urea nitrogen (Nakashima et al., 2004; Pearce, Gabler, et al., 2013), higher expression of muscle atrophy genes (e.g., MAFbx; Ma et al., 2021), increment in the activity of calpain, cathepsin D and proteasome (Nakashima et al., 2004), increased postabsorptive concentration of serum AA metabolites (e.g., 3-methyl-Histidine, OH-Pro, OH-Lys; Morales et al., 2016a), higher hepatic deamination through aminotransferase enzymes (e.g., GOT and GPT), and higher corticosterone level in serum (Ma et al., 2021). As seen in the other circumstances where feed intake is negatively affected, skeletal muscle proteolysis is aimed at redirecting AA to hepatic gluconeogenesis for energy provision (Ma et al., 2021), where corticosterone plays a critical role (Lin et al., 2004). High circulating concentration of this glucocorticoid has been shown to accelerate protein degradation, and disrupt protein synthesis (Dong et al., 2007; Liu et al., 2004; Qaid & Al-Garadi, 2021), consequences that could be attributed to any type of stress as the hypothalamic-pituitary-adrenal cortex axis is activated.

## 5 | CONCLUSIONS

Maximising protein retention continues to be one of the main challenges for science and the meat industry. Protein deposition in muscle is essentially explained by the balance between protein synthesis and degradation, which are greatly affected by several internal and external factors, as reviewed. Reduced activation of the mTORC1 pathway during fasting, animal aging, inflammation or sepsis, inactivity and stress has been shown to reduce protein synthesis and thus protein mass in the pig. However, the opposite effect has been demonstrated during the perinatal period, feeding a higher proportion of dietary CP, the effect of certain hormones such as testosterone and physical activity. Muscle mass is the result of the sum of all the effects and factors affecting the animal, and it is important to take them into account holistically. In addition, understanding the mechanisms and dynamics of protein metabolism in response to the several effects helps to advance the knowledge of physiology and metabolism. Increased accessibility and development of highly accurate and sensitive techniques allow better detailing of the molecular mechanisms, open the door to new advances in the prediction of general changes in protein mass, and could be used for its modulation through genetic, hormonal or environmental tools to maximise pig production, or even for *in vitro* meat production.

Despite the great advances, it is complex to translate this information to the animal's protein requirements for application in precision livestock farming. Most of the techniques used to determine protein metabolism still have shortcomings, are costly and have great experimental difficulties. This is possibly the reason why there is still a lack of information on the differences in protein metabolism mainly due to internal animal effects (e.g., between sexes, breeds, ages, myofiber types) and their implication on meat quality. Moreover, although much is known about the mechanisms of protein synthesis and its rates, protein degradation remains difficult to analyse.

## AUTHOR CONTRIBUTIONS

**Laura Sarri**: Conceptualisation; writing—original draft preparation. **Joaquim Balcells**: Conceptualisation; writing—review and editing. **Ahmad Reza Seradj**: Writing—review and editing. **Gabriel de la Fuente**: Writing—review and editing; final approval.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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