# Investigating New and Existing Management and Nutritional Strategies to Improve Productive Performance and Feed Efficiency in Grower-Finisher Pigs

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# Investigating New and Existing Management and Nutritional Strategies to Improve Productive Performance and Feed Efficiency in Grower-Finisher Pigs

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que el treball de recerca i la redacció de la memòria de la tesi doctoral titulada “Investigating New and Existing Management and Nutritional Strategies to Improve Productive Performance and Feed Efficiency in Grower-Finisher Pigs” han estat realitzats sota la seva direcció per en

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**i certifiquen**

que aquest treball s’ha dut a terme en el Teagasc Pig Development Department, i en el Departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona,

**considerant**

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i perquè quedi constància, signen aquest document a Bellaterra,

a 28 de febrer del 2022.

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***“Caminante, no hay camino, se hace camino al andar”*** Antonio Machado

# Summary

Productive performance and feed efficiency are crucial for sustainability in pig production. The grower-finisher period accounts for over 65% of the total cost of production and it is the most expensive period in pig production. Thus, minor improvements in this stage result in important increases in profit for farmers. In the current thesis, several studies were conducted to improve our knowledge on different management and nutritional strategies to improve the productive performance and feed efficiency in grower-finisher pigs.

Slow growing pigs within a batch may be one of the most important factors affecting pig production and they cause increases in body weight (BW) variability within the batch of pigs. In Chapter 3, we investigated the effect of birth and weaning BW on key performance indicators of grower-finisher pigs, and determined the cut-off values for birth and weaning BW to identify slow growing pigs early in life. These methods can help pig farmers as decision-making tools to identify slow growing pigs early in life and to manage those pigs in a different way than the rest of the batch.

Pigs born and weaned small showed a poor productive performance up to slaughter, but were as feed efficient as their heavier counterparts. This served as the hypothesis for Chapter 4, which compared the response in performance of slow and fast growing pigs to an increase of dietary standardized ileal digestible (SID) lysine (Lys) / amino acid (AA) levels in isoenergetic diets at late grow-finisher stage. Slow growing pigs' feed efficiency was improved when dietary SID AA levels were increased from 0.92 up to 1.45% SID Lys/AA. The latter was not observed in fast growing pigs. Thus, nutrient requirements may vary depending on growth rate at the same age, and slow growing pigs may require higher dietary SID AA levels than fast growing pigs to present a better productive performance.

Chapter 3 and 4 served towards enhancing our understanding of how we can identify and manage slow growing pigs. Nevertheless, around 90 % of pigs in a batch are average/fast growing pigs. Therefore, Chapters 5 and 6 focused on common management strategies, such as space allowance (SA), mixing and phase feeding (PF), to understand and optimize the productive performance and feed efficiency of the whole batch.

Chapter 5 investigated the effect of SA and mixing on productive performance and body lesions, as a proxy for aggression, in pens of 10-14 pigs with a single space wet-

dry feeder during the grower-finisher stage. Mixing appeared to have considerable effect on growth performance, while SA did not affect performance. Nevertheless, high number of body lesions in the lower SA indicated that SA equal or below 0.78 $m^2$/pig are detrimental to the welfare of pigs despite following the EU legislation. Thus, animal welfare is affected before productive performance.

Chapter 6 studied the effect of SA, mixing and PF on productive performance in grower-finisher pigs, housed in pens of 10-14 pigs with a single space wet-dry feeder. It was observed that mixing and reducing SID Lys:Net Energy ratio from 0.95 to 0.82 g/MJ at 15-16 weeks of age, have a more marked impact on productive performance than a reduction in SA from 0.96 to 0.78 $m^2$/pig.

Thus far, the thesis discusses about farm adjustment strategies related to the growth rate of the grower-finisher pigs. However, the actual nutritional value of a diet for a particular pig farm is affected by many different factors, such as facilities or health status. Being able to adjust diet composition at farm level would avoid the use of suboptimal diets and the extra costs associated. Chapter 7 and 8 studied fast analysis methods to assess feed efficiency at farm level and to optimise diet formulation and productive performance of grower-finisher pigs.

Chapter 7 studied blood serum metabolite and faecal volatile fatty acid (VFA) profiles to assess feed inefficiencies in protein, AA and energy dietary content in growing and finishing pigs. Dietary changes and the pigs' age affected both blood metabolite and faecal VFA profiles. The main findings from this chapter were that serum urea nitrogen is the best indicator related to protein efficiency, increasing in growing and finishing pigs fed high protein diets with unbalances in AA profiles. Branched-chain fatty acids also increased in growing pigs fed high protein diets, but did not show the same consistency as urea.

Chapter 8 evaluated the potential use of near infrared reflectance spectroscopy (NIRS) to predict faeces chemical components and apparent total tract digestibility (ATTD) coefficients of nutrients at farm level. The results obtained were similar using freeze-dried faeces but without grounding them, which facilitates NIRS applicability.

Finally, in the general discussion, we discussed the feasibility to obtain a multivariable indicator to assess feed efficiency at farm level. The latter being based on the blood metabolite profile, the faecal VFA profile, and the faeces chemical components and ATTD coefficients of nutrients predicted by NIRS.

# Resum

El rendiment productiu i l'eficiència alimentària són crucials per la sostenibilitat de la producció porcina. El període de creixement-finalització representa més del 65% del cost total de producció, sent el període més costós en la producció porcina. Per tant, petites millores en aquesta etapa resulten en un augment dels guanys pels productors de porcs. En aquesta tesi doctoral, s'han realitzat diversos estudis sobre diferents estratègies nutricionals i de maneig per millorar el rendiment productiu i l'eficiència alimentària en porcs de creixement i finalització.

Els porcs de creixement lent poden ser un dels factors més importants que afecten la producció porcina, ja que són la causa d'una variabilitat més gran del pes viu dins d'un mateix lot de porcs. En el Capítol 3, es va investigar l'efecte del pes viu al naixement i al deslletament en indicadors claus del rendiment productiu dels porcs, i identificar valors de tall per al pes viu al naixement i al deslletament, per tal de reconèixer els porcs de creixement lent en etapes inicials del cicle productiu. Els mètodes obtinguts podrien servir com a eines de presa de decisions per identificar aquells porcs de creixement lent en una etapa inicial del cicle productiu i poder utilitzar estratègies de maneig i/o nutricionals diferents que amb la resta de porcs del lot.

Els porcs que van néixer i van ser deslletats petits van tenir un baix rendiment productiu, però la mateixa eficiència alimentària que els altres porcs més pesats. Això va servir com a hipòtesi per el Capítol 4, on es va comparar el rendiment dels porcs de creixement lent i ràpid, en base a augmentar els nivells de lisina digestible (SID Lys) / aminoàcids (AA) en dietes isoenergètiques, durant la segona etapa del període de creixement i finalització. L'eficiència alimentària dels porcs de creixement lent va millorar quan es van augmentar els nivells de SID Lys/AA de 0,92 a 1,45%. En canvi, no es va observar cap millora productiva en els porcs de creixement ràpid. D'aquesta manera, els requeriments dels nutrients poden variar segons la velocitat de creixement a la mateixa edat, i els porcs de creixement lent poden requerir de nivells més alts de SID AA en la dieta per poder aconseguir una millora en el rendiment productiu.

Els capítols 3 i 4 van servir per millorar la nostra comprensió de com podem identificar i millorar el rendiment productiu dels porcs de creixement lent. No obstant això, al voltant d'un 90% en un lot de porcs són porcs de creixement mitjà/ràpid. Per tant, els Capítols 5 i 6 tenien com a objectiu estudiar estratègies de maneig comunes, com la

densitat per corral (DC), la barreja i l’alimentació per fases (AF), per millorar el rendiment productiu i l’eficiència alimentària de tot el lot de porcs.

En el Capítol 5 es va investigar l’efecte de la DC i la barreja sobre el rendiment productiu i les lesions corporals, com a indicador d’agressió, en corrals de 10 a 14 porcs amb una menjadora amb un sistema d’alimentació humit-sec i un sol espai, durant el període de creixement i finalització. Barrejar els porcs va tenir un efecte considerable en el rendiment productiu, mentre que la DC no el va afectar. Tot i això, l’alt número de lesions corporals en les DC inferiors va indicar que una DC igual o inferior a 0,78 $m^2$/porc és perjudicial per al benestar dels porcs, malgrat seguir la legislació de la UE. Així, el benestar animal es veu afectat abans que el rendiment productiu.

En el Capítol 6 es va estudiar l'efecte de la DC, la barreja i l’AF en el rendiment productiu de porcs de creixement i finalització, allotjats en el mateix tipus de corral que en el Capítol 5. En aquest cas, es va observar que barrejar i reduir la relació SID Lys/Energia Neta de 0,95 a 0,82 g/MJ a les 15-16 setmanes d’edat, té un impacte més marcat en el rendiment productiu que una reducció de la DC de 0,96 a 0,78 $m^2$/porc.

Fins ara, la tesi ha discutit sobre diferents estratègies d’actuació en la granja segons la velocitat de creixement dels porcs. No obstant això, el valor nutricional d’una dieta per a una granja porcina en particular es pot veure afectat per diferents factors, com les instal·lacions o l’estat sanitari de la granja. Poder ajustar la formulació a nivell de granja evitaria l’ús de dietes sub-òptimes i els seus costos associats. D’aquesta manera, en els Capítols 7 i 8 tenien com a objectiu utilitzar mètodes d’anàlisi ràpids per avaluar l’eficiència alimentària a nivell de granja, i optimitzar la formulació de la dieta i el rendiment productiu dels porcs en creixement i finalització.

En el Capítol 7, es van investigar els perfils metabòlits sanguinis, i els dels àcids grassos volàtils (AGV) fecals, per avaluar ineficiències alimentàries segons el contingut de proteïna, AA i energia de la dieta en porcs de creixement i finalització. Els canvis en la dieta i l’edat dels porcs va afectar els perfils d’AGV fecals i dels metabòlits sanguinis. Els resultats principals d’aquest capítol van ser que el nitrogen ureic sèric és el millor indicador relacionat amb l’eficiència proteica, el qual augmenta quan els porcs en creixement i finalització són alimentats amb dietes altes en proteïna no balancejades per AA. A més a més, els àcids grassos de cadena ramificada van augmentar en els porcs en creixement alimentats amb dietes altes en proteïna, però no va demostrar la mateixa consistència que el nitrogen ureic sèric.

En el Capítol 8 es va avaluar l'ús de l'espectroscòpia d'infraroig proper (NIRS) per predir els components químics de les femtes i els coeficients de digestibilitat total aparent (ATTD) dels nutrients, en la granja. Els resultats obtinguts van ser similars utilitzant femtes liofilitzades, però sense moldre, fet que facilita l'ús del NIRS.

Finalment, en la discussió general, es parla sobre la possibilitat d'obtenir un indicador multivariable per avaluar l'eficiència alimentària en granja, basat en el perfil de metabòlits en sang, el perfil d'AGV en femtes, i els components químics de les femtes i els coeficients ATTD dels nutrients predits pel NIRS.

# Resumen

El rendimiento productivo y la eficiencia alimenticia son cruciales para la sostenibilidad de la producción porcina. El período de crecimiento-finalización representa más del 65% del coste total de producción, siendo el período más costoso en la producción porcina. Por lo tanto, pequeñas mejoras en esta etapa resultan en un aumento de las ganancias de los productores de cerdos. En la presente tesis doctoral, se han realizado varios estudios sobre diferentes estrategias nutricionales y de manejo para mejorar el rendimiento productivo y la eficiencia alimenticia en cerdos de crecimiento y finalización.

Los cerdos de crecimiento lento pueden ser uno de los factores más importantes que afectan la producción porcina, ya que son la causa de una mayor variabilidad del peso vivo dentro de un mismo lote de cerdos. En el Capítulo 3, se investigó el efecto del peso vivo al nacimiento y al destete en indicadores claves del rendimiento productivo de los cerdos, e identificó valores de corte para el peso vivo al nacer y al destete con tal de reconocer los cerdos de crecimiento lento en etapas tempranas del ciclo productivo. Los métodos obtenidos podrían servir como herramientas de toma de decisiones para identificar los cerdos de crecimiento lento en una etapa temprana del ciclo productivo, y así poder utilizar estrategias de manejo y/o nutricionales distintas el resto del lote de cerdos.

Los cerdos que nacieron y fueron destetados pequeños mostraron un bajo rendimiento productivo, pero tuvieron la misma eficiencia alimenticia que los otros cerdos más pesados. Esto sirvió como hipótesis para el Capítulo 4, cuyo objetivo fue comparar el rendimiento de los cerdos de crecimiento lento y rápido, en base a aumentar los niveles de lisina digestible (SID Lys) / aminoácidos (AA) en dietas isoenergéticas, en la etapa tardía del periodo de crecimiento y finalización. La eficiencia alimenticia de los cerdos de crecimiento lento mejoró al aumentar los niveles de SID Lys/AA de 0,92 a 1,45%. En cambio, no se observó ninguna mejora productiva en los cerdos de crecimiento rápido. Por lo tanto, los requerimientos de nutrientes pueden variar dependiendo de la velocidad de crecimiento a la misma edad, y los cerdos de crecimiento lento pueden requerir niveles más altos de SID AA en la dieta para presentar un mejor desempeño productivo.

Los capítulos 3 y 4 sirvieron para mejorar nuestra comprensión de cómo podemos identificar y manejar cerdos de crecimiento lento. Sin embargo, alrededor del 90% en un lote de cerdos son cerdos de crecimiento medio/rápido. Por lo tanto, los Capítulos 5 y 6 tenían como objetivo estudiar estrategias de manejo comunes, como la densidad por corral (DC), la mezcla y la alimentación por fases (AF) para mejorar el rendimiento productivo y la eficiencia alimenticia de la mayoría de todo el lote de cerdos.

En el Capítulo 5 se investigó el efecto de la DC y la mezcla sobre el rendimiento productivo y las lesiones corporales, como indicador de agresión, en corrales de 10 a 14 cerdos con un comedero húmedo-seco de un solo espacio, durante el periodo de crecimiento y finalización. La mezcla de cerdos tuvo un efecto considerable en el rendimiento productivo, mientras que la DC no lo afectó. Sin embargo, el alto número de lesiones corporales en DC inferiores indicó que una DC igual o inferior a 0,78 $m^2$/cerdo es perjudicial para el bienestar de los cerdos, a pesar de seguir la legislación de la UE. Así, el bienestar animal se ve afectado antes que el desempeño productivo.

El Capítulo 6 estudió el efecto de la DC, la mezcla y la AF en el rendimiento productivo de cerdos de crecimiento y finalización, alojados en corrales de 10 a 14 cerdos con un solo espacio de comedero húmedo-seco. Se observó que el mezclar y reducir la relación SID Lys/Energía Neta de 0,95 a 0,82 g/MJ a las 15-16 semanas de edad, tiene un impacto más marcado en el rendimiento productivo que una reducción de la DC de 0,96 a 0,78 $m^2$/cerdo.

Hasta ahora, la tesis ha discutido acerca de distintas estrategias de actuación en la granja según la velocidad de crecimiento de los cerdos. Sin embargo, el valor nutricional de una dieta para una granja porcina en particular puede verse afectado por distintos factores, como las instalaciones o el estado sanitario de la granja. Poder ajustar la formulación a nivel de granja evitaría el uso de dietas subóptimas y sus costes adicionales. De esta manera, los Capítulos 7 y 8 tenían como objetivo utilizar métodos de análisis rápidos para evaluar la eficiencia alimenticia en granja, y optimizar la formulación de la dieta y el rendimiento productivo de los cerdos en crecimiento y finalización.

En el Capítulo 7, se investigaron los perfiles de los metabolitos sanguíneos y de los ácidos grasos volátiles (AGV) fecales, para evaluar ineficiencias alimenticias en base a cambios en el contenido de energía, proteína y AA de la dieta en cerdos de crecimiento y finalización. Los cambios en la dieta y la edad de los cerdos afectaron los perfiles de AGV fecales y de metabolitos sanguíneos. Los principales hallazgos de este capítulo fueron que el nitrógeno ureico sérico es el mejor indicador relacionado con la eficiencia proteica. Este último aumenta en cerdos en crecimiento y finalización cuando son alimentados mediante dietas con niveles altos en proteína y no balanceadas en AA. Además, los ácidos grasos de cadena ramificada aumentaron en los cerdos en crecimiento que fueron alimentados con dietas altas en proteína, pero no demostraron la misma consistencia que el nitrógeno ureico sérico.

El Capítulo 8 evaluó el uso de la espectroscopia de infrarrojo cercano (NIRS) para predecir los componentes químicos de las heces y los coeficientes de digestibilidad total aparente (ATTD) de los nutrientes en granja. Los resultados obtenidos fueron similares utilizando heces liofilizadas, pero sin moler, facilitando el uso del NIRS.

Finalmente, en la discusión general, se habla sobre la posibilidad de obtener un indicador multivariable para evaluar la eficiencia alimenticia en granja. Este indicador se basaría en el perfil de metabolitos en sangre, el perfil de AGV en heces y los componentes químicos de las heces y los coeficientes ATTD de los nutrientes predichos por NIRS.

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# List of Abbreviations

| | |
|---|---|
| **AA** | Amino Acid |
| **ADFI** | Average Daily Feed Intake |
| **ADG** | Average Daily Gain |
| **AIA** | Acid Insoluble Ash |
| **AIAO** | All-in-all-out |
| **ATTD** | Apparent Total Tract Digestibility |
| **AUC** | Area Under the Curve |
| **BCFA** | Branched-chain Fatty Acids |
| **BW** | Body Weight |
| **CCW** | Cold Carcass Weight |
| **CP** | Crude Protein |
| **DM** | Dry Matter |
| **DT** | Detrending |
| **DTSW** | Days to Target Slaughter Weight |
| **ECR** | Energy Conversion Ratio |
| **EI** | Energy Intake |
| **FCR** | Feed Conversion Ratio |
| **FDG** | Freeze-dried Ground |
| **FDNG** | Freeze-dried Not Ground |
| **FNIRS** | Faecal Near Infrared Reflectance Spectroscopy |
| **FT** | Fat Thickness |
| **GE** | Gross Energy |
| **GH** | Mahalanobis Distance |
| **IUGR** | Intrauterine Growth Restriction |
| **KR** | Kleiber Ratio |
| **LM%** | Lean Meat % |
| **Lys** | Lysine |
| **MBW** | Metabolic Body Weight |
| **MC** | Muscle Content |
| **NE** | Net Energy |
| **NIR** | Near Infrared |

| | |
|---|---|
| **NIRS** | Near Infrared Reflectance Spectroscopy |
| **OM** | Organic Matter |
| **$R^2_c$** | Coefficient of Determination for Calibration |
| **$R^2_{cv}$** | Coefficient of Determination for Cross-Validation |
| **RGR** | Relative Growth Rate |
| **ROC** | Receiver Operating Characteristic |
| **RPD** | Residual Predictive Deviation |
| **SCFA** | Short-chain Fatty Acids |
| **SEC** | Standard Error of Calibration |
| **SECV** | Standard Error of Cross-Validation |
| **SID** | Standardized Ileal Digestible |
| **SNV** | Standard Normal Variate |
| **SUN** | Serum Urea Nitrogen |
| **VFA** | Volatile Fatty Acid |

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# Chapter 1

## Literature Review

> ***"The beautiful thing about learning is nobody can take it away from you"***
>
> B.B. King

# 1.1 Introduction: The Pig Production Industry

## 1.1.1 Where are we and where are we going?

The pig industry has grown dramatically worldwide during the last sixty years, thanks to improvements in nutrition, animal health and welfare, breeding, and genetics (Schultz et al., 2020). Pigs were the most produced livestock species until 2018 when poultry production took over due to consumer trends, economics and African Swine Fever in main producing countries such as China (Figure 1.1; OECD/FAO 2021b).

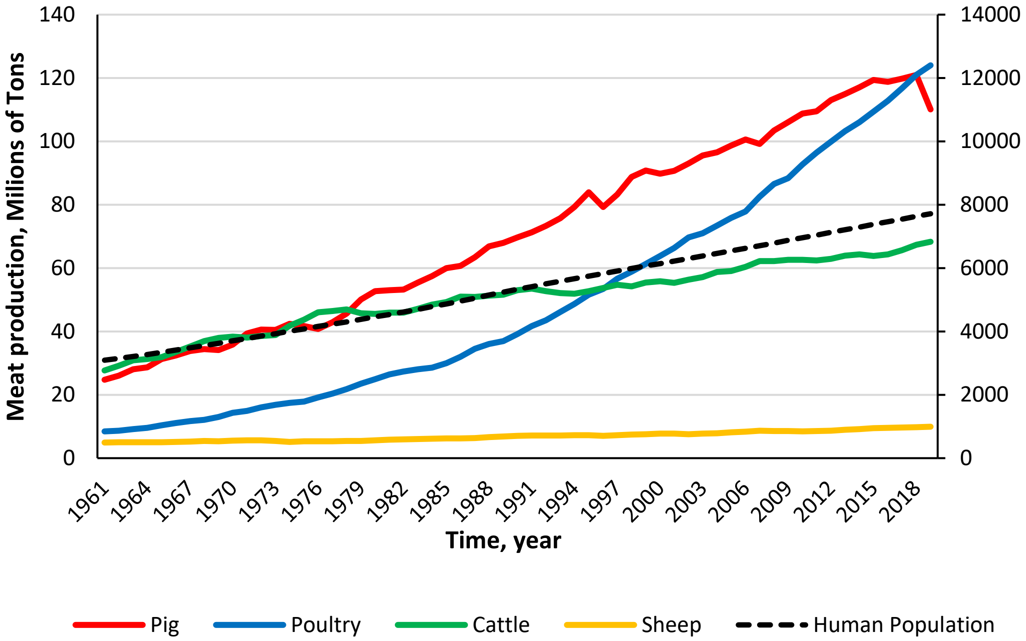

**Figure 1.1** Worldwide meat production evolution of the four main livestock species from 1961 until 2019 (OECD/FAO 2021b). The growth of human population is indicated by the right vertical axis.

However, the trend curve for pig production varies between countries or regions (Figure 1.2; OECD/FAO 2021b). Pig production will still increase in the coming years in third developing countries and big producers such as China or Vietnam to fulfil the needs of an expected increase of the world human population up to 9.7 billion by 2050 and 11.2 billion by 2100 (OECD/FAO, 2021b; United Nations, 2017). Nevertheless, in Europe, the trend curve is getting flat and it is expected to show a negative trend in coming years in some countries such as Denmark, The Netherlands, Belgium or Germany. However, pig production is still increasing in countries such as Spain due to large exports to China, and Russia is expected to expand production by a further 10% by 2030 (OECD/FAO, 2021b).

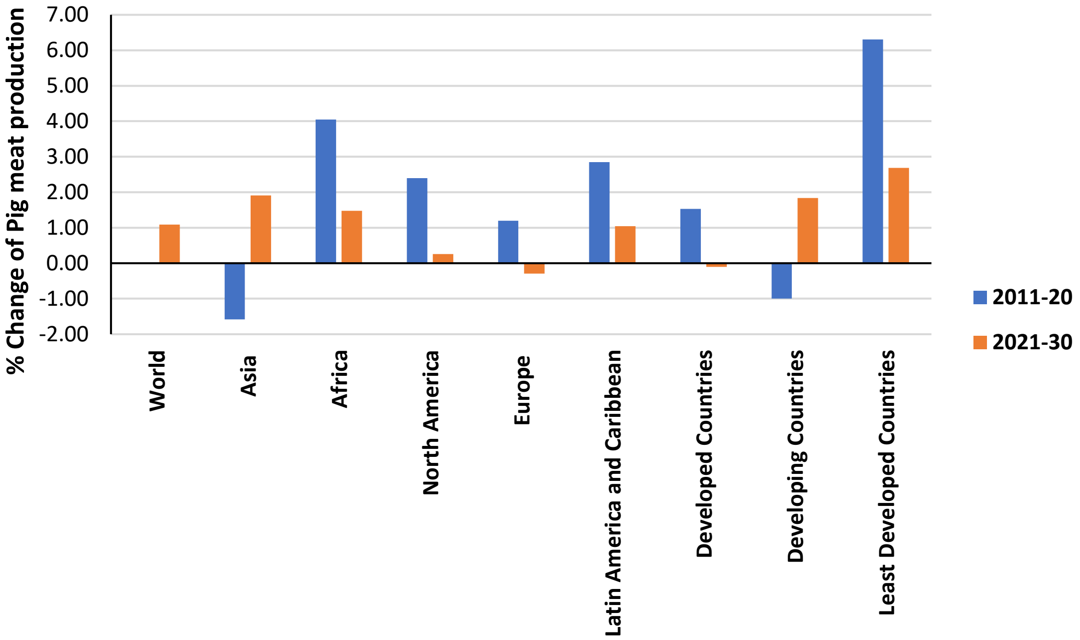

**Figure 1.2** Percentage of change in pig meat production in the world, Asia, Africa, North America, Europe, Latin America, and developed, developing and least developed countries, between 2011 and 2020, and what is expected from 2021 to 2030 (OECD/FAO, 2021a).

Moreover, society concerns towards farm sustainability, antimicrobial resistance and animal welfare are becoming more important every day (Scholten et al., 2013). Regulations in the EU are constantly updated: the ban to use zinc oxide from 2022 (Bonetti et al., 2021), restrictions in antimicrobial use (European Parliament and the Council of the European Union, 2019a, 2019b) minimum space allowance requirements (Council of the European Union, 2008) and avoiding tail-docking (European Commission, 2016) are just examples of the challenges that pig production is facing. Furthermore, carbon footprint (Bondt et al., 2020) is getting more consideration every day as part of the farm sustainability concept, and new regulations on this topic will appear in the coming years. Altogether, pig producers are considering the use of by-products (Zijlstra and Beltranena, 2013) more frequently to avoid imported raw materials, mainly from America, which currently are showing record high prices and decrease the net profit of farmers. In this context, production and feed efficiency are of a great importance to face this new challenges and to ensure economic viability of pig farms, while improving farm sustainability.

### 1.1.2 The concept of Farm Sustainability

Farm sustainability is defined as a form of production "ecologically sound, economically viable, socially just, and humane" (Appleby, 2004; Velarde et al., 2015). Thus, farm sustainability englobes animal health, environmental protection, food safety, quality and traceability, and production efficiency from a cost-benefit perspective such that consumers perceive the product as "good value for money" (Pethick et al., 2011). Moreover, animal welfare is included into the farm sustainability concept as it is of great concern for European citizens (Miele and Evans, 2010) and it is a necessary element of sustainable animal production (Broom, 2010; Velarde et al., 2015). Production and feed efficiency are discussed in this thesis as pillars of competitive pig production in Europe and part of a better farm sustainability. The concept and measurement of animal welfare will further be discussed throughout the thesis also as part of farm sustainability.

### 1.1.3 Production Efficiency, Productive Performance and Feed Efficiency in Pig Production

Production efficiency in livestock production can be defined as the efficiency at which a company/farm utilises its inputs to get animals to slaughter (Hyland et al., 2016; Lansink and Reinhard, 2004). Then, an improved production efficiency would result in an economic benefit for famers while reducing the environment pollution per unit of product (FAO, 2018). Production efficiency can be affected by factors affecting pig performance, but it will greatly depend on economic factors such as feed or pig price. Thus, the present thesis will focus on productive performance and feed efficiency in grower-finisher pigs to assess production efficiency but not considering the economics.

Productive performance describe pigs' productivity based on key performance indicators such as average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) (Rocadembosch et al., 2016).

Feed efficiency in pig production represents the cumulative efficiency with which the pig uses dietary nutrients for maintenance, lean gain and lipid accretion (Patience et al., 2015). Thus, improving feed efficiency would normally result in lower feed costs while reducing the environmental impact (Gaines et al., 2012) and would result in a better production efficiency and farm sustainability. Nevertheless, feed efficiency is not always correlated with a better production efficiency (Patience et al., 2015). Although feed efficiency has a great influence in financial returns because of feed costs, improving feed efficiency may cause financial losses as other aspects may be affected such as feed costs per pig when dietary energy concentration is increased (Gaines et al., 2012). Traditionally, feed efficiency has been measured as feed consumed per unit of BW gain (Gaines et al., 2012) which is commonly known as FCR and is widely used in the pig industry to assess productive performance. Nevertheless, there are other feed efficiency metrics such as residual feed intake (Herd and Arthur, 2009), energy conversion ratio, Kleiber ratio, and relative growth rate, among others (Calderón Díaz et al. 2017).

In pig production, the grower-finisher phase accounts for 60 to 70% of the total costs (López-Vergé et al., 2018b; Rocadembosch et al., 2016). Thus, productive performance and feed efficiency are crucial during this phase and can be affected by several factors (Patience, 2012). These factors can be classified in five main areas (Quiles, 2010):

- Physiological factors:
    - Genotype (Elbert et al., 2020; Lowell et al., 2019; Noblet et al., 1999).
    - Gender (Fix et al., 2010a; Latorre et al., 2004; Van Den Broeke et al., 2020).
    - Age (Brossard et al., 2009; Remus et al., 2020; Van Milgen et al., 2008)
    - Body weight (BW) variability (Douglas et al., 2013; López-Vergé et al., 2018a; Quiniou et al., 2002).
    - Health (Van der Meer et al., 2016; Van Der Meer et al., 2020).
- Environmental factors: Temperature, humidity, and air flow (Hyun et al., 1998b; O’Connor et al., 2010).
- Social factors: Group size and mixing (Hyun et al., 1998a; Schmolke et al., 2003; Street and Gonyou, 2008).
- Physical factors: Stocking density and feeder design (Kim et al., 2017; Thomas et al., 2017; Wastell et al., 2018).
- Nutritional Factors: Feed volume, ingredients, nutrient density, additives, contaminants, feed presentation and water ingestion (NRC, 2012; O’Meara et al., 2020; Van Milgen and Dourmad, 2015).

This thesis studies management and nutritional strategies to improve productive performance and feed efficiency (Figure 1.3). In particular, we have focused in three main approaches to improve productive performance:

- Managing slow growing pig separately to improve their performance and reduce variability.
- Optimising current management and nutrition practices in growing-finisher pigs to improve productive performance. In particular: space allowance, mixing and phase feeding.
- Exploring fast analyses methods to assess feed efficiency at farm level. In Ireland, pig production is structured on commercial individual farms without the presence of integrator companies. So, adjusting diets at farm level is of great importance.

We have reviewed the existing literature in each of these three approaches in the next three sections of this literature review.

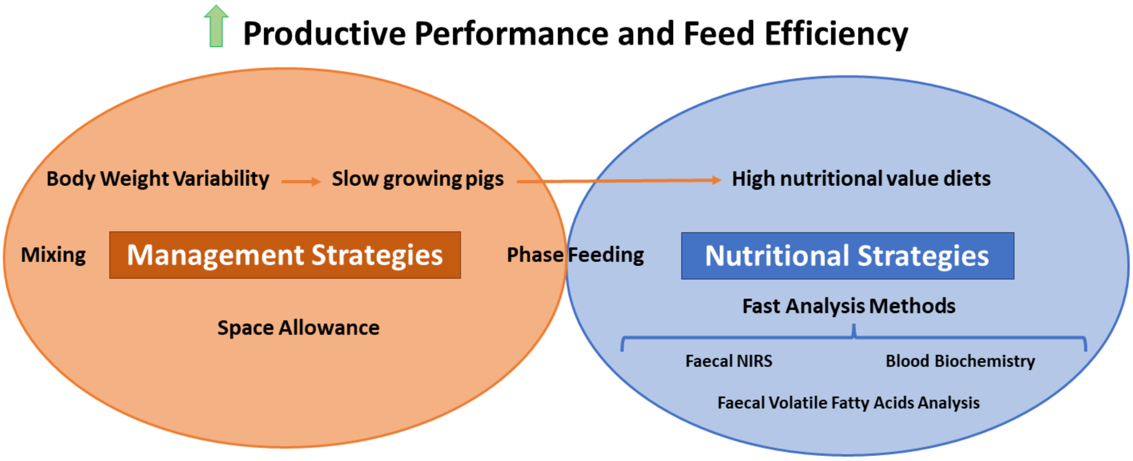

**Figure 1.3** Schematic illustration of the different nutritional and management strategies related to productive performance and feed efficiency that will be assessed during the present PhD thesis.

# 1.2 Slow Growing Pigs

### 1.2.1 Slow Growing Pigs - Definition

Slow growing pigs can be defined as those pigs that are in the lower quartile of the BW population distribution in a given batch of pigs and require extra time to reach the desirable slaughter weight in conventional pig farms (Douglas et al., 2014a; He et al., 2016). “Runt” is an arbitrary term often used in pig production and in some scientific research papers to refer to the slow growing pigs (Greenwood et al., 2010; Handel and Stickland, 1987; Powell and Aberle, 1980). Nevertheless, the present thesis will not use the word “runt” to describe this subset of pigs.

### 1.2.2 Slow Growing Pigs as a Management Problem

Variation in growth performance is common in all species in nature. Therefore, BW variability will always be present in a commercial pig farm to a certain extent. Nevertheless, the continuous genetic advancement over the last decades towards increasing sow prolificacy to increase the number of slaughtered pigs produced per sow per year (Rutherford et al., 2013) has led to a considerable decrease in average birth weight, increasing the number of lightweight piglets (Beaulieu et al., 2010a; Quiniou et al., 2002) and BW variability within litter at birth (Foxcroft et al., 2007). The latter can be observed in Figure 1.4. Those light birth BW piglets will probably remain stunted and be unable to catch up to their heavier counterparts during the entire production cycle (Beaulieu et al., 2010a; Fix et al., 2010b; Quiniou et al., 2002), therefore, becoming slow growing pigs. Coma (2017) exposes that a 6 kg of difference between the lightest 5% and the heaviest 5% at weaning results in 30 kg difference on BW at 159 days. The increased presence of slow growing pigs within a batch of pigs causes a left tail in the BW distribution at the end of the finishing phase (Figure 1.5). Thus, the increased BW variability comes from an increased presence of slow growing pigs over the last years, being an urge management problem with significant economic, welfare and environmental implications for pig farmers, especially when following an all-in-all-out (AIAO) production system (Patience et al., 2004).

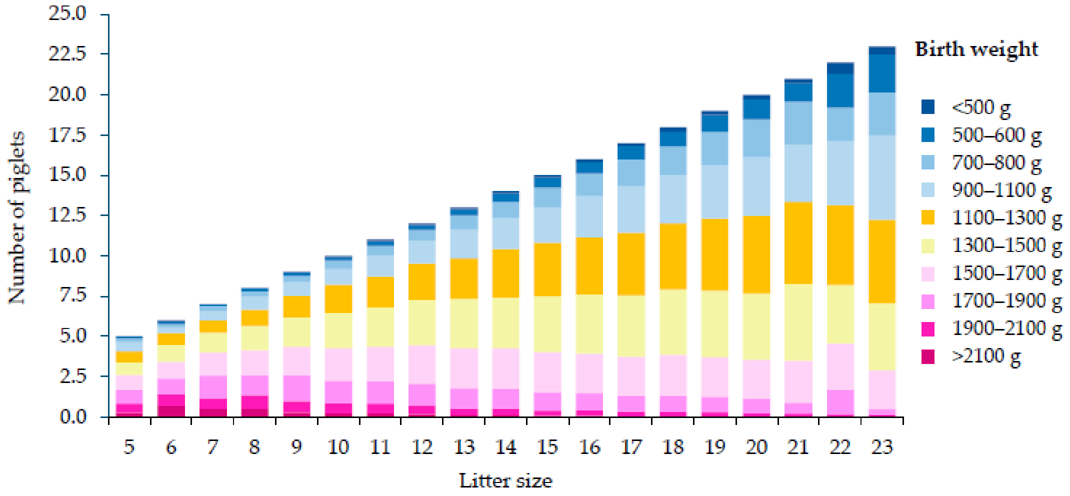

**Figure 1.4** Effect of litter size on birth weight distribution. The data were collected at Schothorst Feed Research B.V. (Lelystad, The Netherlands) from 2011 to 2019, based on 97.552 piglets born alive from 7888 litters (Huting et al., 2021).

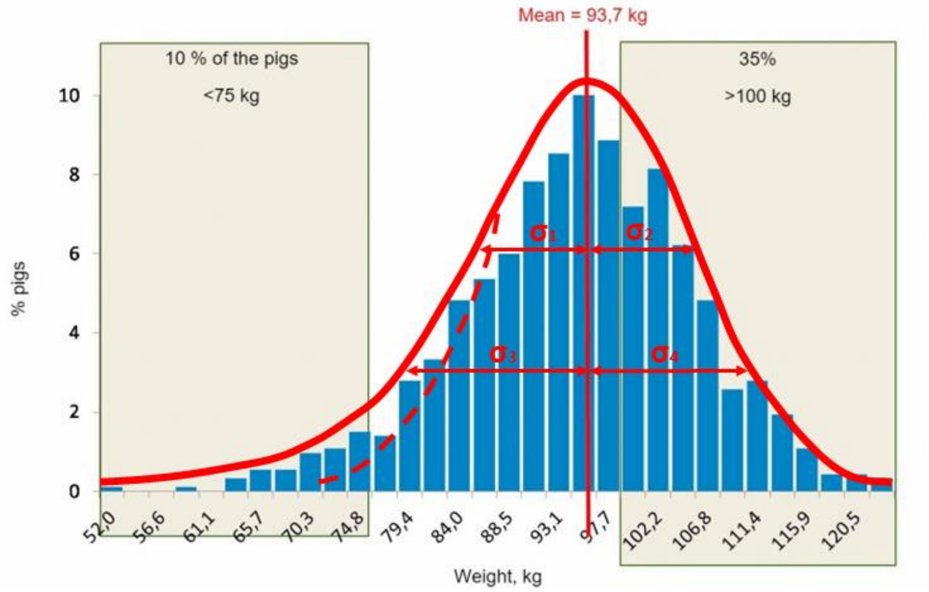

**Figure 1.5** Body weight distribution at the end of the finisher phase. The increasing presence of slow growing pigs causes a left tail in the body weight distribution. Figure adapted from Coma (2017).

#### *1.2.2.1 Risk Factors and Performance Limitations Associated with Slow Growing Pigs*

The reduced growth performance observed in slow growing pigs may occur at any stage during the production cycle from birth to slaughter. This reduction of growth leads to increased BW variability within a batch of pigs (Douglas et al., 2013). Several risks factors have been reported to cause a reduction in growth performance parallel to an increase in BW variability (Figure 1.6).

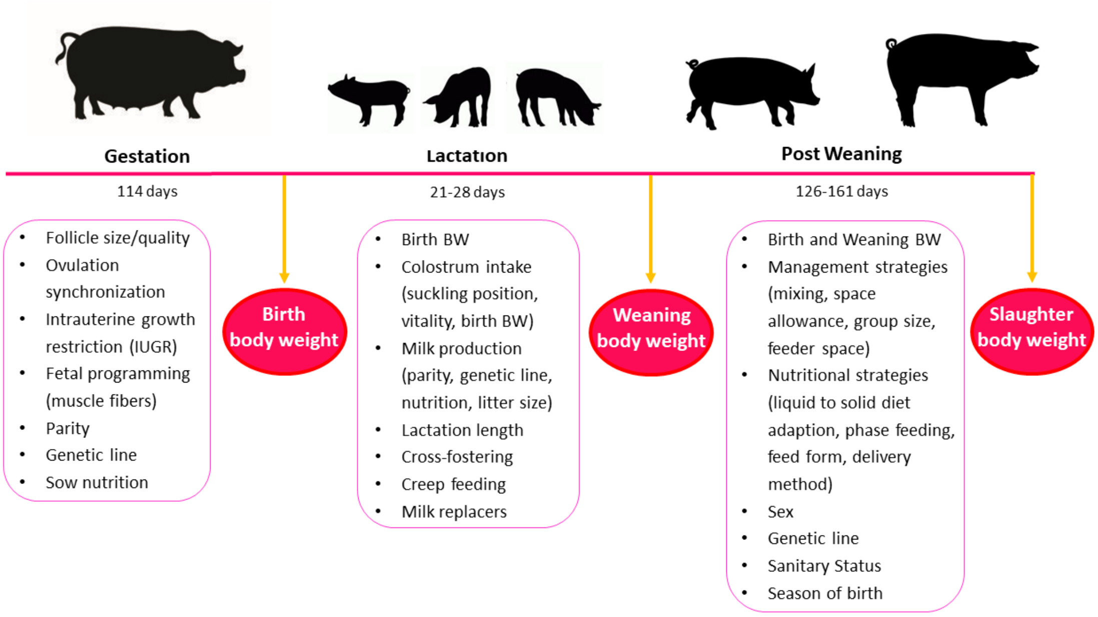

**Figure 1.6** Risk factors associated with slow growing pigs' performance at gestation, lactation and post-weaning stages.

Birth BW is one of the critical factors for postnatal growth performance (Douglas et al., 2013; He et al., 2016; Muns et al., 2016) as lightweight piglets at birth are more likely to be unable to catch up their heavier counterparts throughout the production cycle (Beaulieu et al., 2010a; Fix et al., 2010b; Quiniou et al., 2002). Indeed, a number of authors have reported that piglets weighing below 1 kg of BW at birth have a higher risk of mortality and poor growth performance over the production cycle (Calderón Díaz et al., 2017c; Larriestra et al., 2006; Zeng et al., 2019). Similarly, Paredes et al. (2012) stated that pigs with a birth BW of minus 2.5 times the standard deviation from the mean of the total population, would have no potential to exhibit an adequate growth performance during the production cycle under current practical farm conditions.

Reasons for a light BW at birth may rely on the prenatal environment, which involves factors related to the sow such as follicle size quality and ovulation synchronization (López Vergé, 2018; Vela Bello et al., 2015). The uterine capacity of the sow (Foxcroft et al., 2006) and the maternal nutrition on foetal growth through the placenta (Fowden et al., 2009; Foxcroft et al., 2007), known as placental efficiency (Wilson and Ford, 2001), are risk factors associated with intrauterine growth restriction (IUGR). The latter is impaired growth and development of the mammalian embryo/foetus or its organs during pregnancy (Wu et al., 2006). Pigs suffering of IUGR in the postnatal environment will have a reduced immune function (Cromi et al., 2009; Zhong et al., 2012), protein synthesis and cellular signalling (Wang et al., 2008), and low colostrum intake (Amdi et al., 2013). Foetal programming and development will also determine the total number and type of muscle fibres (Barker and Clark, 1997; Foxcroft and Town, 2004; Wu et al., 2006), which will be lower in low birth weight pigs (Dwyer et al., 1993; Gondret et al., 2005; Handel and Stickland, 1987; Nissen et al., 2004). Fewer number of muscle fibres at birth leads to reduced growth performance, restricting lean growth, increased fat deposits and poorer pork quality (Gondret et al., 2006; Rehfeldt et al., 2008; Rehfeldt and Kuhn, 2006). Parity (Douglas et al., 2013), genetic line (Damgaard et al., 2003), and nutrition (Solà-Oriol and Gasa, 2017) during the gestation phase of the sow may also affect the birth BW variability at farrowing.

Weaning BW has been also suggested as one of the main factors for post-weaning growth and time to reach the desirable slaughter weight (Collins et al., 2017; Smith et al., 2007; Wolter and Ellis, 2001). Larriestra et al. (2006), reported a threshold of 3.6 kg of BW at weaning (17±2 d), which maximizes sensitivity and specificity to correctly predict the likelihood of dying or of being light in weight when exiting the nursery stage. Moreover, He et al. (2016) stated that 80% of pigs weaned at 28 days of age at < 6.4 kg of BW became slow growing pigs at slaughter age. Similarly, López-Vergé et al. (2019) reported 6.88 kg of BW at weaning as a threshold to account for better productive performance in the grow-finisher period.

Weaning BW is highly influenced for what happens during the lactation period (López-Vergé et al., 2018a; Main et al., 2005) and it is highly correlated with lactation ADG (Paredes et al., 2012). During lactation, milk consumption and the sow-litter interaction will be important (López Vergé, 2018). The milk production of the sow will be influenced by the genetic line (Damgaard et al., 2003; Farmer et al., 2001), parity (Douglas et al., 2013), nutrition (Solà-Oriol and Gasa, 2017) and litter size (Auldist et al., 1998). The milk intake of the piglets will have an impact on piglet's performance towards weaning (Devillers et al., 2011; Skok et al., 2007). Piglets need to assure a good intake of colostrum (Devillers et al., 2011; Van Barneveld and Hewitt, 2016) providing energy for thermoregulation (Herpin et al., 2005; Le Dividich et al., 2005), immune protection (Devillers et al., 2011; Rooke and Bland, 2002) and a better intestinal development (Xu et al., 2000). Factors that will influence colostrum intake are the suckling position (López-Vergé et al., 2018a), the vitality (Muns et al., 2013; Panzardi et al., 2013; Theil et al., 2014) and the birth weight of the piglets (Devillers et al., 2007; Le Dividich, 1999; Quesnel et al., 2012). Piglets may also be affected due to morphometric characteristics at birth (Huting et al., 2018) showing retarded post-weaning maturation of the gastrointestinal tract (Michiels et al., 2013; Pluske et al., 2003). Strategies such as cross-fostering, creep feeding, and milk replacers may help to reduce the risk factors to become a slow growing pig (Muns and Tummaruk, 2016). Lastly, an increase of the lactation length will lead to heavier piglets at weaning, that will adapt better to the post-weaning environment, increasing their growth rates while reducing the mortality rates and probability of being slow growing pig (López-Vergé et al., 2019; Main et al., 2005, 2004).

During the post-weaning period, the risk of becoming a slow growing pig will reside on the management and nutritional strategies conducted during the nursery, grower, and finisher stage. The growth and development of the gastrointestinal tract with a good adaptation from liquid to solid diet at weaning is crucial (Blavi et al., 2021; Huting et al., 2021; Pluske, 2016). Those pigs that take a long period to start eating after weaning (Bruininx et al., 2001), may have higher mortality rates and poor post-weaning performance due to a worst adaptation of the gastrointestinal tract and immune function (Huting et al., 2021; Pluske, 2016; Wensley et al., 2021). After that period, nutritional strategies such as phase feeding (Han et al., 2000; Menegat et al., 2020), feed form and delivery method (O’Meara et al., 2020); and management strategies such as mixing (Hyun et al., 1998a, 1998b), space allowance (Carpenter et al., 2017; Kim et al., 2017; Thomas et al., 2017), group size (Schmolke et al., 2003; Street and Gonyou, 2008), and feeder space (López-Vergé et al., 2018b; Wastell et al., 2018), will be important towards improving their productive performance and feed efficiency. Finally, factors such as sex (Douglas et al., 2013; Paredes et al., 2012), pig genetic line (Damgaard et al., 2003), birth season (Douglas et al., 2013; Paredes et al., 2012) and sanitary status of the pig farm (Van der Meer et al., 2016; Van Der Meer et al., 2020) will also influence growth rate, BW variability and risk of becoming a slow growing pig.

#### *1.2.2.2 Impact of Slow Growing Pigs on the Pig Industry*

Before the use of the AIAO management, BW variability was a largely hidden cost as slow growing pigs were constantly delayed to young batches of pigs (Patience et al., 2004). The economic impact of those tail-end pigs becomes critical in AIAO production systems (Patience et al., 2004). It is estimated that, in a given batch, 10 to 15% of pigs are slow growing pigs (Calderón Díaz et al., 2017c; He et al., 2016; Schinckel et al., 2005). This subset of pigs are prone to have higher mortality rates throughout the production cycle (Calderón Díaz et al., 2017b; Larriestra et al., 2006; Muns et al., 2016), and those that survive pose management challenges in AIAO production systems

(Calderón Díaz et al., 2017c; Patience et al., 2004). For instance, farmers may hold back the slow growing pigs to a following batch of younger pigs to facilitate management, which increases the likelihood of disease spread and occurrence (Calderón Díaz et al., 2017c) and the occupation time of the facilities that results in economic losses due to inefficient pen utilisation and additional feed costs. Moreover, consumer demand for more consistent and uniform pig products prompted to produce carcasses within certain weight allowances as requirements of the slaughterhouses. Slow growing pig's carcasses failure to achieve this carcasses requirements will lead to financial penalties at the slaughterhouse due to poor carcass grading (Brumm et al., 2002; Patience et al., 2004; Tolosa et al., 2021). Altogether, slow growing pigs decrease the efficiency of the whole production cycle and farm's profitability (Douglas et al., 2014a; López-Vergé et al., 2018a; Patience et al., 2004), being a major concern in currently conventional pig farms.

### 1.2.3 Post-weaning Strategies to Optimize Slow Growing Pigs' Performance

The previous section has shown that slow growing pigs pose a management problem in the pig industry nowadays. Therefore, attempts such as regrouping pigs or culling light BW pigs at birth were done initially (Brumm et al., 2002; Rehfeldt and Kuhn, 2006). However, regrouping pigs only manage the BW variability but does not reduce it if light pigs are kept in the same management, nutrition and environment (Brumm et al., 2002; O'Quinn et al., 2001), and culling pigs has a negative animal welfare perception in the society plus economic losses for farmers. Prior studies reported that slow growing pigs may exhibit compensatory growth and be able to partially catch up their heavier counterparts (Douglas et al., 2013; López-Vergé et al., 2018b; Zeng et al., 2019). Thus, there has been an increasing amount of literature assessing management and/or nutritional strategies to improve the slow growing pigs' performance over the production cycle.

The present thesis will focus on management and/or nutritional strategies applied in the post-weaning period. However, previous literature has shown also good results of improved prenatal management (Blavi et al., 2021), sow nutritional strategies (Ramanau et al., 2008; Rooney et al., 2020; Van Den Brand et al., 2009), and interventions during the lactation period such as cross-fostering (Deen and Bilkei, 2004; Heim et al., 2012; Muns et al., 2014), creep feeding (Muns and Magowan, 2018; Solà-Oriol and Gasa, 2017; Sulabo et al., 2010), using milk replacer (Blavi et al., 2015; Muns et al., 2017; Pluske et al., 2005; Wolter et al., 2002) or split suckling (Solà-Oriol and Gasa, 2017).

During the post-weaning period, feeding and management strategies were based on development of high nutritional value starter diets (Craig et al., 2020; Douglas et al., 2014c; Huting et al., 2018; Vieira et al., 2015) or increasing the quantity of the first post-weaning diets (Huting et al., 2019; Lawlor et al., 2002; Magowan et al., 2011) at nursery, and increasing nutrient specifications in diets at the beginning of the grower-finisher period (Aymerich et al., 2020; Douglas et al., 2014b). Moreover, a few studies have shown effects on the growth of slow growing pigs during the grower-finisher phase by carrying out phase feeding strategies based on a weight basis or equivalent feed consumption instead of age (Hawe et al., 2020; López-Vergé et al., 2018b). Also, Nissen and Oksbjerg (2011) concluded that slow growing pigs do not have lower nutrient requirements than average growing pigs. A review in the literature of the different post-weaning nutritional and management strategies to improve slow growing pigs' performance and reduce BW variability is presented in Table 1.1.

**Table 1.1** Review of the main studies conducted within 2000 – 2021 that studied the effect of different post-weaning management and/or nutritional strategies on productive performance of slow growing pigs versus average grower finisher pigs. The order of the studies is based on the production phase and the year of publication.[1]

| Author | Period | Intervention period, Age (d) or BW (kg) | Treatment Factor | Treatment Levels | Trial Outcomes |
|---|---|---|---|---|---|
| Beaulieu et al., 2010b | Nursery | 28 - 42 d | BW category | Light (6.44 kg) or Heavy (10.40 kg) at weaning | Light BW pigs at weaning lost less BW and showed better response to a high nutritional value diet than heavier BW weaning pigs |
| | | | Dietary treatment | High nutritional value (0, 1 or 4 first days) and/or standard starter diet | |
| Douglas et al., 2014c | Nursery | 28 - 49 d | BW category | Light (0.7-1.25 kg) or Normal (1.6-2.0 kg) at birth | Better ADG and FCR in light birth BW pigs fed high nutritional value starter diet and extra feed by the end of the nursery period with better margin cost over feed |
| | | | Dietary treatment | High nutritional value or Standard starter diet | |
| | | | Feed Allowance | Extra feed (2.5 kg/pig) or not to light BW pigs | |
| Vieira et al., 2015 | Nursery | 21 - 49 d | BW category | Light (4.5 kg) or Heavy (6.5 kg) at weaning | Better BW, ADG and ADFI in heavy piglets by the end of the trial |
| | | | Dietary treatment | 3 dietary energy density treatments: 3.4, 3.6, or 3.8 Mcal/kg ME diet | No effect on performance |
| Douglas et al., 2014b | Grower-finisher | 63 - 91 d | BW category | Normal birth BW, Normal birth BW (fed restrictly - d49 to d63), Light birth BW (< 1.2 kg) | Light BW pigs at birth do not improve performance and are less efficient than heavy BW pigs at birth fed a high nutritional value diet in the grower-finisher phase |
| | | | Dietary treatment | High (1.58% Lys) or Standard nutritional value diet (1.08% Lys) | |
| López-Vergé et al., 2018b | Grower-finisher | 63 - 163 d | BW category | Light (18.4 kg) or Heavy (22.8 kg) BW pigs | Light BW pigs "feed to weight" improve BW and ADG compared to light BW pigs fed a standard dietary regime |
| | | | Dietary treatment | Standard or "Feed to weight" | |
| Aymerich et al., 2020 | Grower-finisher | 27.6 - 62.8 kg (47 d) | BW category | Heavy (32.0 kg), Medium (27.5 kg), and Light (23.4 kg) BW pigs | Light BW pigs improved growth and feed efficiency when increasing SID Lys:NE compared to Heavy BW pigs |
| | | | Dietary treatment | 5 dietary SID Lys:NE treatments: 3.25, 3.66, 4.07, 4.47 and 4.88 g SID Lys/Mcal NE | |

*Table 1.1 continue*

| | | | | | |
|---|---|---|---|---|---|
| Brumm et al., 2002 | Grower-finisher | 70.0 - 113.6 kg | Management treatment | Sort and remixing the lightest pigs at 70 kg BW | Removing and remixing light BW pigs is ineffective in improving their performance |
| | Wean to finish | 9.0 - 116.0 kg | Management treatment | Sort and remixing the lightest pigs 3 wks post-weaning | |
| Magowan et al., 2011 | Wean to finish | 28 - 140 d | BW category | Light (7.1 kg), Medium (8.9 kg) or Heavy (10.4 kg) at weaning | Light BW pigs at weaning convert feed as efficiently as heavy BW pigs |
| | | | Dietary treatment | Standard or High nutritional value finisher diet from 11 weeks of age | Light BW pigs have higher ADFI and ADG per kg of BW than medium or heavy BW pigs |
| | | | Feed Allowance | High (12 kg/pig) or Low (6 kg/pig) allowance of starter diets post-weaning | A high allowance of starter diets improved post weaning pig performance |
| Huting et al., 2019 | Wean to finish | 34 - 140 d | BW category | Light (7.61 kg) or Heavy (10.8 kg) at weaning | Light BW pigs improve growth up to 7 wks post-weaning when fed a greater allowance of the nursery diets |
| | | | Feed Allowance | Extra (65% more) or Standard nursery and grower diet | |
| Hawe et al., 2020 | Wean to finish | 28 - 161 d | BW category | Light (< 1 kg) or Normal (1.3-1.7 kg) at birth | Better performance for normal BW pigs at birth through the period |
| | | | Dietary treatment | Standard or "Feed to weight" | Light BW pigs fed the "feed to weight" regime improve performance than those fed the standard regime at each transition |

[1] BW = body weigh; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

Table 1.1 gave us insights towards strategies which successfully or not optimise slow growing pigs' post-weaning performance. Part of literature reviewed in Table 1.1 recognizes that slow growing pigs are as feed efficient as their heavier counterparts (Magowan et al., 2011) and may improve their growth performance when higher nutrient specifications are given (Aymerich et al., 2020; Douglas et al., 2014c) and when phase feeding strategies based on a weight basis instead of age are used (Hawe et al., 2020; López-Vergé et al., 2018b). Nevertheless, knowing the key performance indicators such as ADG, ADFI, FCR and time needed to reach the target slaughter weight of slow and average growing pigs, based on their birth and weaning BW, could help the farmers to improve the management of the whole herd. Moreover, no previous research has estimated cut-off values for birth and/or weaning BW to differentiate between pigs that are born small but are able to catch up with their big counterparts and those that remain small for the whole production cycle.

Also, standard nutritional tables (De Blas et al., 2013; NRC, 2012) have previously established the nutrient requirements for the average grower-finisher pig; however, there is no information regarding specific nutrient requirements for the slow growing pigs, e.g., standardized ileal digestible (SID) lysine (Lys) / amino acid (AA) ratio. To understand if this subset of pigs has the same nutrient requirements at the same age, or instead, at the same BW compared to average grower-finisher pigs, would be helpful for farmers, veterinarians, nutritionists, and advisors working in the pig sector.

Taken together, by identifying slow growing pigs earlier in life and understanding slow growing pigs' performance indicators and nutrient requirements, farmers could design new management and nutritional strategies to improve slow growing pigs' productive performance and feed efficiency.

# 1.3 Management and Feeding Strategies to Optimize Performance, Health and Welfare

The present chapter will focus on common management and feeding strategies (such as space allowance, mixing and phase feeding) used in the pig industry, to improve productive performance and feed efficiency of a whole production batch of pigs.

## 1.3.1 Space Allowance

Space allowance is the size of a farm animal's allocated area. Space allowance plays a critical role in pig production from a productive performance, animal welfare and economic standpoint (Powell et al., 1993; Thomas et al., 2017; Vermeer et al., 2014). Increasing the number of pigs per pen reduces housing cost per pig produced (Powell et al., 1993), which is the second highest cost during the grower-finisher period after feed cost (Flohr et al., 2017). Nevertheless, for a given pen size, increasing the number of pigs per pen decreases the space allowance, and either reduces feeder space per animal in long trough systems or increases the ratio of animals per feeder in facilities with an ad libitum feeding arrangement (DeDecker et al., 2005). Thus, pig performance could be affected with a decrease on ADG driven by a decrease on ADFI (Carpenter et al., 2017; Kim et al., 2017; Thomas et al., 2017). Taken together, one of the greatest challenges for pig producers is to maximise pig production efficiency and minimise housing cost, without compromising animal welfare and farm sustainability.

### *1.3.1.1 Confounding Factors: Space Allowance, Group Size, and Feeder Space*

At commercial scale, changing the number of pigs per pen is the most common method used to change space allowances. However, this method induces confounding on whether pig productive performance is affected by space allowance, group size or feeder space (Anil et al., 2007). Usually, these three factors are confused in any commercial conditions, and also in experimental research. Flohr et al., (2017) stated that floor space allowance is the main environmental factor that influences grower-finisher pigs' performance and other factors such as group size and feeder space are not significant predictors of grower-finisher pigs' growth. However, the same authors state that potential interactions may exist between space allowance and factors such as group size and feeder space. Table 1.2 reviews the main studies conducted within 2000 – 2021 that studied the effect of space allowance, group size and/or feeder space per pig on productive performance of grower-finisher pigs.

**Table 1.2** Review of the main studies conducted within 2000 – 2021 that studied the effect of space allowance, group size and feeder space per pig on productive performance of grower-finisher pigs. The order of the studies is based on the factor studied and the year of publication.[1]

| Author | Period | Intervention period | Treatment Factor | Treatment Levels | *k*-value [2] | Trial Outcomes |
|---|---|---|---|---|---|---|
| Brumm et al., 2001 | Grower-finisher | 20 - 110 kg (110 d) | Space allowance | 0.56 (14 pigs/pen) and 0.78 (10 pigs/pen) $m^2$/pig - Mixed | 0.024 and 0.034, respectively | Reduced space allowance decreased ADFI and ADG |
| | | 22 - 110 kg (107 d) | Space allowance | 0.60 and 0.76 $m^2$/pig - Not Mixed | 0.026 and 0.033, respectively | Reduced space allowance did not affect performance |
| DeDecker et al., 2005 | Wean to finish | 5 - 117 kg (168 d) | Space allowance | 0.78 (22 pigs/pen), 0.64 (27 pigs/pen) and 0.54 (32 pigs/pen) $m^2$/pig | 0.032, 0.027 and 0.023, respectively | Increased space allowance increased growth rate after 8 wk post-weaning |
| Anil et al., 2007 | Grower-finisher | 30 - 116 kg (105 d) | Space allowance | 0.64, 0.74, 0.81 and 0.88 $m^2$/pig (19 pigs/pen) | 0.027, 0.031, 0.034 and 0.037, respectively | Pigs in 0.81 and 0.88 $m^2$/pig space allowance have higher growth rates than pigs in 0.64 $m^2$/pig, but worst pen efficiency |
| Jensen et al., 2012 | Grower-finisher | 32 - 91 kg (65 d) | Space allowance | 0.67, 0.73 and 0.79 $m^2$/pig (14 pigs/pen) | 0.033, 0.036 and 0.039, respectively | Increasing space allowance from 0.67$m^2$/pig to 0.79 $m^2$/pig did not improve performance |
| Flohr et al., 2016 | Grower-finisher | 36 - 140 kg (117 d) | Space allowance | 0.91 (15 pigs/pen) and 0.65 (21 pigs/pen) → 0.91 $m^2$/pig (3 Treatments - removing pigs) | 0.033 and 0.034, respectively | Increasing space allowance improved the growth of pigs |
| Kim et al., 2017 | Wean to finish | 11 - 25 kg | Space allowance | 0.27, 0.24, 0.21 and 0.19 $m^2$/pig (22, 25, 28, 31 pigs/pen) | 0.032, 0.028, 0.025 and 0.022, respectively | Optimum space allowance = 0.24 $m^2$/pig |
| | | 25 - 45 kg | Space allowance | 0.50, 0.44, 0.39 and 0.35 $m^2$/pig (22, 25, 28, 31 pigs/pen) | 0.039, 0.035, 0.031 and 0.028, respectively | Optimum space allowance = 0.44 $m^2$/pig |
| | | 45 - 65 kg | Space allowance | 0.73, 0.64, 0.57 and 0.52 $m^2$/pig (22, 25, 28, 31 pigs/pen) | 0.044, 0.040, 0.035 and 0.032, respectively | Optimum space allowance = 0.64 $m^2$/pig |
| | | 65 - 85 kg | Space allowance | 0.89, 0.78, 0.70 and 0.63 $m^2$/pig (22, 25, 28, 31 pigs/pen) | 0.046, 0.040, 0.036 and 0.033, respectively | Optimum space allowance = 0.78 $m^2$/pig |
| | | 85 - 110 kg | Space allowance | 0.91, 0.80, 0.71 and 0.65 $m^2$/pig (22, 25, 28, 31 pigs/pen) | 0.038, 0.034, 0.030 and 0.027, respectively | Optimum space allowance = 0.80 $m^2$/pig |
| Thomas et al., 2017 | Grower-finisher | 64 - 133 kg (71 d) | Space allowance | 0.84, 0.74 and 0.65 $m^2$/pig (9 pigs/pen) | 0.032, 0.028 and 0.025, respectively | Reduced space allowance decreased ADFI and ADG |
| Jang et al., 2017 | Grower-finisher | 30 - 98 kg (98 d) | Space allowance | 0.96 (4 pigs/pen), 0.80 (5 pigs/pen) and 0.69 (6 pigs/pen) $m^2$/pig | 0.043, 0.037 and 0.034, respectively | Optimum space allowance in a grower-finisher pigs is more than 0.80 $m^2$/pig for maximizing productive performance |

*Table 1.2 continue*

| | | | | | | |
|---|---|---|---|---|---|---|
| Johnston et al., 2017 | Grower-finisher | 27 - 138 kg (114 d) | Space allowance | 0.71, 0.80, 0.89, 0.98 and 1.07 $m^2$/pig (6 to 19 pigs/pen) | 0.027, 0.030, 0.033, 0.037 and 0.040, respectively | Optimum space allowance = 0.89 $m^2$/pig for pigs marketed at about 138 kg |
| | | 133 - 147 kg (15 d) | Space allowance | 0.71, 0.80, 0.89, 0.98 and 1.07 $m^2$/pig (4 to 11 pigs/pen) | 0.025, 0.029, 0.032, 0.035 and 0.038, respectively | Optimum space allowance = 0.98 $m^2$/pig for pigs marketed at about 147 kg |
| Carpenter et al., 2017 | Grower-finisher | 56 - 124 kg (71 d) | Space allowance | 0.91, 0.63, 0.63 → 0.91 (8 pigs/pen) and 0.63 → 0.84 (8 → 6 pigs/pen) $m^2$/pig | 0.037, 0.025, 0.037 and 0.034, respectively | Increasing space allowance improved the growth of pigs |
| Schmolke et al., 2003 | Grower-finisher | 23 - 95 kg (84 d) | Group size | 10, 20, 40 and 80 pigs/pen (0.76 $m^2$/pig) | 0.036 for all treatments | Housing grower-finisher pigs in groups of up to 80 pigs is not detrimental to productivity if space allowance is adequate |
| Street and Gonyou, 2008 | Grower-finisher | 37 - 94 kg (56 d) | Space allowance | 0.52 and 0.78 $m^2$/pig | 0.025 and 0.038, respectively | Reduced space allowance and high group sizes decrease growth performance |
| | | | Group size | 18 and 108 pigs/pen | | |
| Wastell et al., 2018 | Grower-finisher | 28 - 129 kg (100 d) | Space allowance | 0.65 and 0.78 $m^2$/pig | 0.026 and 0.030, respectively | Reduced space allowance decreased ADFI and ADG |
| | | | Feeder space | 10, 13 and 16 pigs/feeder space (2 feeders / pen) | | 10 pigs per feeder had higher ADG and ADFI compared with 13 or 16 pigs per feeder space, but FCR improved as the number of pigs per feeder space increased |
| López-Vergé et al., 2018b | Grower-finisher | 17 - 81 kg (90 d) | Feeder space | 5.5 pigs/feeder space and 2.2 pigs/feeder space (11 pigs/pen) | | Low space allowance per feeder space improves growth performance |

[1] BW = body weigh; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

[2] $k$-value = $\frac{Space\ Allowance}{Body\ weight^{0.667}}$ ; $k$-value was calculated based on the final body weight of the study

### *1.3.1.2 The k-value*

Space allowance is often expressed as $m^2$/pig although it changes as pigs grow (Gonyou et al., 2006). Kyriazakis and Whittemore (2006) reported the use of an allometric approach with a formula where space allowance in $m^2$ is expressed as: Space Allowance = $k \times BW^{0.667}$ where $k$ represents a space allowance coefficient and $BW^{0.667}$ represents the geometric conversion of BW in kg to area. Thus, the *k*-value could serve as a standard measure to compare the studies related to different space allowances and BWs in the literature. Kyriazakis and Whittemore (2006) stated that the relationship between *k*-value and feed intake is highly similar for all growing pigs, which for a 60 kg BW pig eating 2 kg of feed, for each 0.1 $m^2$ loss of floor space would suppose a change in *k*-value of 0.005 parallel to a reduction of 80 g of ADG and 100 g of potential ADFI. Table 1.2 shows the final *k*-value of the different studies in order to compare the results obtained from those previous trials. Gonyou et al. (2006) reported a critical *k*-value of 0.0336, below which productive performance is affected. This critical *k*-value of 0.0336 is quite consistent in Table 1.2, with performance of grower-finisher pigs improved or reduced when the *k*-value is above or below the critical *k*-value, respectively. However, recent studies reported that grower-finisher pigs' performance may be affected above the 0.0336 *k*-value threshold (Carpenter et al., 2017; Kim et al., 2017; Thomas et al., 2017), which may depend on the type of building, floor type, feeders system, environmental enrichment, sex, marketed BW and pig's genetics (Deen, 2005; Johnston et al., 2017; Wastell et al., 2018).

### *1.3.1.3 Where are we in Europe? – Legislation, Animal Welfare and Current Commercial Conditions*

The council of the European Union published the directive 2008/120/EC which establishes the laying down minimum standards for the protection of pigs (Council of the European Union, 2008). This directive establishes a minimum space per pig ($m^2$/pig) that will vary in each physiological phase of the production cycle (Table 1.3). A minimum space per pig of 0.65 $m^2$/pig must be applied when pigs weigh between 85 to 110 kg of BW, to improve and guarantee a minimum animal welfare. Thus, a 0.65 $m^2$/pig at least must be applied at the end of the grower-finisher phase, if pigs usually go to the abattoir when reaching a target slaughter weight around 110 kg of BW per normal practice in many European farms. Nonetheless, observe that *k*-value = 0.028 when pigs weigh 110 kg BW with 0.65 $m^2$/pig of space allowance, which could affect productive performance.

**Table 1.3** Laying down minimum standards for the protection of nursery and grower-finisher pigs in the European Union (Council of the European Union, 2008).

| **Live Weight, kg** | **Space per pig, $m^2$** | **$k$-value [1]** |
|---|---|---|
| Not more than 10 | 0.15 | 0.032 |
| More than 10 but not more than 20 | 0.20 | 0.027 |
| More than 20 but not more than 30 | 0.30 | 0.031 |
| More than 30 but not more than 50 | 0.40 | 0.029 |
| More than 50 but not more than 85 | 0.55 | 0.028 |
| More than 85 but not more than 110 | 0.65 | 0.028 |
| More than 110 | 1.00 | 0.039* |

[1] $k$-value = $\frac{Space\ Allowance}{Body\ weight^{0.667}}$ ; $k$-value was calculated based on the final body weight of the study.
*$k$-value calculated based on pigs going to slaughter with a target body weight of 130 kg.

Besides legislation, pig producers face social concerns regarding animal welfare which is in direct conflict with maximising production efficiency and minimising housing cost. The term "welfare" refers to the state of an individual in relation to its environment and should be measured in a scientific way that is independent of moral consideration (Broom, 1991). Thus, space allowance is a factor to consider when measure animal welfare (Vermeer et al., 2014).

Space allowance is part of the Welfare Quality assessment of pig production (Welfare Quality, 2009) and it is well known that insufficient space allowance can lead to adverse social behaviours directed to pen mates, resulting in skin lesions, lameness, and tail biting (Vermeer et al., 2017). These lesions can be easily measured on farm and are more sensitive indicators of pig welfare (Mkwanazi et al., 2019) than growth performance, reflecting that animal welfare may be compromised before even performance is affected (Averós et al., 2010). Thus, several studies recognise space allowance as a key factor on the number of body lesions per pig, which is an indicator of poor animal welfare (Anil et al., 2007; Fu et al., 2016; Vermeer et al., 2017). The physical damage induced by aggression may end affecting pig health and performance causing carcass condemnations and economic losses for pig producers (Chantziaras et al., 2018; Dalmau et al., 2016; Pandolfi et al., 2018). In addition, damaging behaviour may also contribute to chronic stress which affects both mental and physiologic natural state of the animals (Sutherland et al., 2006), thereby having detrimental implications to the efficiency and sustainability of pig production systems (Broom, 2010).

Currently in Europe, a penning arrangement of 10 to 14 pigs with one wet-dry feeder per pen is one of the most common types of accommodation in growing-finishing facilities. Small group pens with 10–14 grower-finisher pigs are convenient from a management point of view because they allow for a rapid monitoring of health and welfare issues in pigs, without the need to access the pen. Moreover, this pen system is usually linked to wet-dry feeders as these optimize feed efficiency (O'Meara et al., 2020). Thus, studies optimizing this system for productive performance and feed efficiency, and animal health and welfare are needed.

**Figure 1.7** Example of grower-finisher pigs given inadequate space allowance (A) (Kyriazakis and Whittemore, 2006) and adequate space allowance (B; picture taken in the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland).

### 1.3.2 Mixing

Current commercial conditions in the pig industry make mixing a common management strategy. Mixing consists in reorganising unfamiliar pigs in a same pen and it can happen several times over the production cycle. Usually, pigs are mixed at weaning and when they are transported to slaughter at least, although pigs may also be mixed at the beginning of the grower-finisher period or at late-finisher stage when regrouping light finisher pigs as heavier finisher pigs are going to slaughter (Garcia and McGlone, 2021). This management strategy is usually conducted to segregate pigs by BW or sex. Reasons to mix pigs based on BW may rely on reducing the BW variability within a pen of pigs (O'Connell et al., 2005), which may facilitate the farm management by making easier the selection of finisher pigs that reach the market slaughter weight and are ready to go to the abattoir. Nevertheless, mixing fails to reduce final BW variability in individual pig weights within a pen and it may not be necessary for achieving maximum growth performance of pigs (Anil et al., 2007; O'Quinn et al., 2001).

Mixing unfamiliar pigs leads also to aggressive behaviour, which has negative effect on physiology, productive performance and animal welfare (De Groot et al., 2001; Foister et al., 2018; Hyun et al., 1998a). In the literature, there seems to be contradictory findings related on how mixing affects productive performance in pigs (Zaragoza, 2013). Some studies reported no effect of mixing on performance when pigs are mixed at weaning and regrouped at 10 weeks of age (O'Connell et al., 2005). Moreover, Li and Johnston (2009) observed that mixing unfamiliar pigs coming from large farrowing groups at the beginning of the grower-finisher stage had no effect on performance after 14 weeks of study. However, other studies found that mixing affects productive performance by reducing ADG and ADFI in grower-finisher pigs followed in a 4-week experiment at the beginning of the grower-finisher period (Hyun et al., 1998a, 1998b). Moreover, Stookey and Gonyou (1994) also observed a depressed ADG in mixed pigs after being mixed for a 2-week period when they had 83 kg of BW. Nevertheless, the study period of these studies was relatively short and no long-term effect over 2 or 4 weeks was measured. No recent studies on how mixing affects productive performance in pigs comparing intact litters and mixed groups appear in the literature.

Mixing may also interact with other management/nutritional and environmental stressors. Hyun et al. (1998a) reported that when pigs are subjected to multiple concurrent environmental stressors such as high ambient temperature, regrouping and low space allowance, the final effect over productive performance is additive and performance could be significantly improved by removing a single stressor. Brumm et al. (2001) suggested that the response to space allowance may differ depending on how pigs are managed during the move from the nursery to the grower-finisher. Thus, low space allowances have a reduced negative effect on performance when pigs are not mixed at the beginning of the grower-finisher period (Brumm et al., 2001). Also, large group sizes of 20, 40, 80, or up to 100 pigs display a marked reduction in aggressive behavior towards foreign pigs (Schmolke et al., 2003; Street and Gonyou, 2008; Turner et al., 2001), which cause no negative effect of mixing on productive performance in grower finisher pigs.

Animal welfare may be also affected because of mixing in grower-finisher pigs. Pigs show severe aggression after re-grouping in order to establish a new social hierarchy (Driessen et al., 2020; Tong et al., 2020; Wurtz et al., 2017). This aggressive behaviour may lead to physical body lesions that can be measured to assess animal welfare in an objective way (Welfare Quality, 2009). Also, the stress related to mixing and poor establishment of new social hierarchies may lead to chronic stress (Desire et al., 2015; Foister et al., 2018; Sutherland et al., 2006), which can lead to immunosuppression and could, ultimately, have detrimental implications for pig health, performance and welfare (De Groot et al., 2001; Gimsa et al., 2018; Martínez-Miró et al., 2016). The latter may be exacerbated in low sanitary status (Van Der Meer et al., 2020). Ultimately, compromised animal welfare has detrimental implications to the sustainability of the pig production system (Broom, 2010).

Mixing is unavoidable in facilities with large groups at the finisher stage (Schmolke et al., 2003; Street and Gonyou, 2008), or it may also depend on the previous management undertake during the farrowing and nursery period (Calderón Díaz et al., 2017c). However, farrow-to-finish commercial farms with pens of 10 to 14 pigs per pen at the finishing stage, could facilitate the maintenance of intact litters from farrowing to slaughter with no mixing.

Overall, it seems necessary to investigate and fully understand how mixing affects productive performance and feed efficiency and animal welfare during the grower-finisher period, and how mixing interacts with other management/nutritional stressors such as space allowance and phase feeding, which will be further discussed in the next section of the present thesis.

### 1.3.3 Phase feeding

Phase feeding is another common management strategy used in pig production. Phase feeding is the feeding of several diets for a period of time in order to closely meet the pigs’ nutrient requirements and minimise the over- and under-feeding of nutrients (Han et al., 2000). Phase feeding started to be used as an attempt to reduce N and P excretion by feeding pigs in better agreement with age and physiological state, while providing a more economical feeding program (Han et al., 2000). Recent research also pointed out the benefits of using phase feeding in pig farms with an improved productive performance and feed efficiency, while reducing feed costs (Hong et al., 2016; Pomar et al., 2014). Consequently, phase feeding may lead to a better farm sustainability (Andretta et al., 2016; Hong et al., 2016; Pomar et al., 2014). Thus, the pig industry evolved from using one single diet to 3-5 diets over the grower-finisher period and currently, NRC (2012) recommends two growing and two finishing feeding phases.

Although there is a clear benefit from using phase feeding, the BW variability, within a batch of pigs, prevents the pigs to be fed their optimal nutrient requirements on an individual basis even with precisely designed phase-feeding strategies (Pomar and Remus, 2019). On one hand, Menegat et al. (2020) suggested that it is feasible to simplify phase-feeding up to two dietary phases considering the compensatory growth capability in grower-finisher pigs as a way to maximize efficiency of feed utilization and facilitate feed management. The latter may benefit the feed manufacturing process as a consequence of an improved feed mill efficiency and simplified mill logistics (Moore et al., 2013). Nonetheless, large changes in Lys levels in a single phase-feeding strategy may not be favorable to maximize grower-finisher pigs' productive performance and feed efficiency (Smith et al., 1999). On the other hand, phase feeding could also be used as a strategy adapted to pigs' nutrient requirements depending on BW. Aymerich et al. (2020) suggested that light grower-finisher pigs might be limited in SID Lys available for growth compared to their heavier counterparts. Then, phase feeding strategies based on a weight basis or equivalent feed consumption instead of age (Hawe et al., 2020; López-Vergé et al., 2018b) could be used in growing-finishing facilities. The latter may imply a high cost due to feeding management and facilities, but ends up being amortized in favour of improved productive performance and feed efficiency, while reducing BW variability (Aymerich et al., 2021).

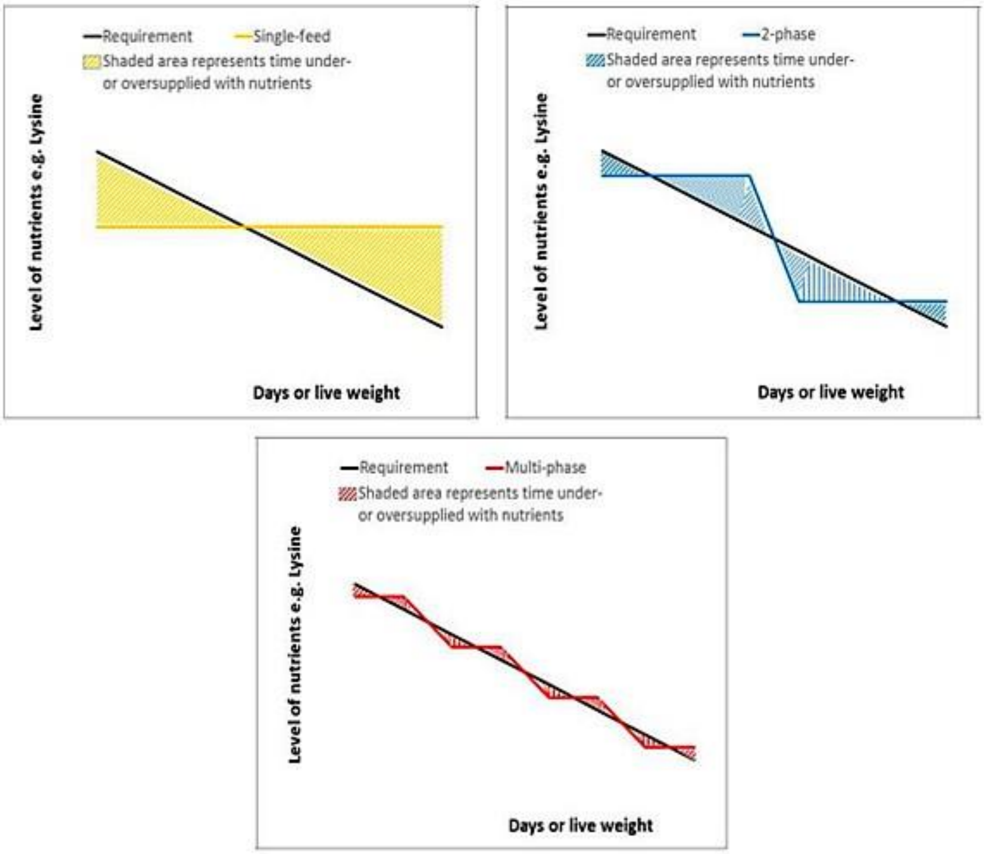

**Figure 1.8** Schematic illustration adapted from Åkerfeldt (2020), which represents the pigs' lysine requirements over the pigs' age or weight with the time that pigs are under- or over-fed of nutrients based on a single diet, a two phase-feeding or a multi-phase-feeding strategy.

Up to date, there is a large volume of published studies describing the role of phase-feeding on grower-finisher pigs. However, there is no information on how phase-feeding compares or interact with other management strategies like space allowance and mixing during the grower-finisher period. Thus, knowing how these common management strategies affect productive performance and feed efficiency, would guide farmers, veterinarians, and advisors to make better management decisions in pig production.

## 1.4 Fast Methods to Optimise Diet Formulation for Grower-Finisher Pigs at Farm Level

Diets that have been formulated following accurate nutritional values of ingredients and nutrient requirements for pigs can become suboptimal diets with inefficiencies in commercial farms when affected by different factors such as health status or environmental conditions. Suboptimal diets may induce extra costs for the pig producers and should be adjusted adequately. However, diet optimization is currently a time-consuming process including ingredient analysis, digestibility determination and on farm feed efficiency measurements. The present chapter will be focused on assessing fast analysis methods to optimize diet formulation, productive performance and feed efficiency of grower-finisher pigs at individual farm level.

### 1.4.1 Suboptimal diets in commercial pig farms, why are they produced?

The actual nutritional value of a diet for a particular pig farm is affected by different factors such as feed manufacturing (Patience et al., 2015), physicochemical characteristics of feed ingredients (Shurson et al., 2021), feed form and delivery method (O'Meara et al., 2020), management and housing conditions (Han et al., 2000; Hyun et al., 1998a, 1998b), and the animal itself (genetic, gender, weight, health status, etc.) (López-Vergé et al., 2018b; Patience et al., 2015; Van der Meer et al., 2016), among others. With so many factors affecting the nutritional value of feed, suboptimal diets may be more frequent than we think and can result in extra cost for the farmers and potential health problems for the pigs. Thus, the need of fast analysis methods to assess feed efficiency at farm level are of interest to optimise diet formulation and improve productive performance and feed efficiency, animal health, and environmental footprint, while reducing production costs (Jongbloed and Lenis, 1992; Patience et al., 2015, 2004).

To assess feed efficiency and detect suboptimal diets at farm level, one should consider:

- Sample collection: Samples must be easy to collect (blood and faecal samples).
- Cost and timeframe of the analysis.

The following sections will discuss the potential use of blood biochemistry, volatile fatty acid (VFA) analysis, and near infrared reflectance spectroscopy (NIRS) to assess feed efficiency at farm level.

### 1.4.2 The Blood Metabolite Profile

Understanding nutrient metabolism is important to maximise productive performance and feed efficiency, while wasting fewer dietary nutrients. Blood samples are an easy material to collect in pig farms and blood biochemistry is a fast analysis method that could give us valuable information directly related to energy, protein and lipid metabolism (Regmi et al., 2018; Roy et al., 2010; Zeng et al., 2013). Dietary changes affect nutrient metabolism and this should be reflected in blood serum metabolites such as total protein, albumin, serum urea nitrogen (SUN) and creatinine for the protein metabolism, and glucose, triglycerides and cholesterol for the energy and lipid metabolism (Chen et al., 1999, 1995; Coma et al., 1995; Hong et al., 2016; Kamalakar et al., 2009; Kerr et al., 2015; Mule et al., 2006; Pond et al., 1986; Regmi et al., 2018; Zeng et al., 2013).

Literature on blood serum metabolites in pigs is scarce and reference values may be useful to detect diseases accounting for different factors such as age, breed, sex, diet, geographical habitat, and methods of sample collection and laboratory measurement (Constable et al., 2017). Nevertheless, baseline blood metabolite values and how these are affected when pigs are fed different levels of protein, AAs and energy at a farm level and at a specific age to evaluate the nutritional value of a diet are not available.

**Table 1.4** Pig reference laboratory values (Constable et al., 2017) for total protein, albumin, urea nitrogen, creatinine, glucose, and cholesterol.[1]

| | **Reference Laboratory Values** | |
|---|---|---|
| **Parameters, units** | Minimum | Maximum |
| Total protein, g/L | 45.0 | 75.0 |
| Albumin, g/L | 19.0 | 40.0 |
| Urea nitrogen, mg/dL | 10.0 | 30.0 |
| Creatinine, µmol/L | 90.0 | 240.0 |
| Glucose, µmol/L | 4.70 | 8.30 |
| Cholesterol, µmol/L | 1.40 | 3.10 |

[1] Values of these parameters from healthy pigs may vary depending on many factors, including age, breed, sex, diet, geographical habitat, and methods of sample collection and laboratory measurement. The values are compiled from a variety of sources.

#### *1.4.2.1 Metabolites related to Protein Metabolism*

- **Total Protein:** Serum total protein is the sum concentration of all individual serum proteins (Constable et al., 2017). Prior studies suggested that serum total protein could be used as an indicator of the adequacy of dietary protein content in pigs (Lowrey et al., 1962). Several reports have shown that grower pigs fed insufficient crude protein (CP) diets up to 60% of the Lys requirements cause a decrease of serum total protein concentration in blood, which may persist during the subsequent finisher phases (Kamalakar et al., 2009; Yang et al., 2008). Moreover, Zeng et al. (2013) observed similar results when grower pigs were fed 65% of the Lys requirements without modifying the levels of CP between diets. However, Regmi et al. (2018), did not observe differences in serum total protein concentration in finishing pigs fed insufficient (0.32%), adequate (0.60%) or excess (0.87%) SID Lys diets, suggesting the pig age difference as the cause to explain this inconsistency in the literature.

- **Albumin:** Serum albumin is the most abundant circulating protein found in serum, which accounts for the 60% of the total plasma proteins (Constable et al., 2017). Albumin plays a major role as a modulator of the plasma colloid osmotic pressure, participating in the transport of hormones, enzymes, fatty acids, metal ions and medicinal products (Matejtschuk et al., 2000). Albumin is considered being a sensitive indicator of protein synthesis capacity of the liver (Mahdavi et al., 2012) and dietary protein nutrition in pigs (Lowrey et al., 1962). Previous research observed that growing pigs fed insufficient CP diets up to 60-80% of the requirements have a reduced albumin concentration in blood (Kamalakar et al., 2009; Mule et al., 2006; Yang et al., 2008), leading to a hypoalbuminemia in cases of severe protein restrictions with levels far below 60% of the pigs' requirements (Atinmo et al., 1976; Pond et al., 1980; Wykes et al., 1996); while hyperalbuminemia was reported with high CP diets (Mutlu et al., 2006). In finishing pigs, Regmi et al. (2018) observed reduced plasma albumin concentration when pigs were fed a 0.32% SID Lys diet during 4 weeks.

- **Serum Urea Nitrogen:** Serum urea nitrogen is the principal end product of protein catabolism. Amino acid catabolism results in ammonia that is transformed into urea, which it will be transported via blood circulation to the kidney for filtration and posterior excretion via urine (Wu, 2013). Previously, SUN has been used as a predictor of efficiency of dietary CP utilization (Chen et al., 1999, 1995; Hong et al., 2016) and dietary AA requirements (Coma et al., 1995; Regmi et al., 2018; Zeng et al., 2013), because of its short time (3 days) to achieve a constant concentration in blood after changing the diet (Coma et al., 1995). Previous literature observed increased SUN concentration due to excessive consumption of protein, which was then inefficiently used (Chen et al., 1999; Hong et al., 2016; Mule et al., 2006). The same happens when pigs are fed Lys insufficient diets, up to 60% of the pigs' requirements, due to an increase of extra free AAs that will be catabolized through deamination, after the first limiting AA is used up (Coma et al., 1995; Regmi et al., 2018; Zeng et al., 2013). Serum urea nitrogen concentration may be altered due to an increase of EI (Fang et al., 2019) or by the use of low CP diets supplemented with synthetic AA, because they could decrease the cation:anion ratio obtaining lower SUN concentrations (Coma et al., 1995). Nevertheless, the majority of previous studies were conducted using individually penned animals (Chen et al., 1999; Regmi et al., 2018; Zeng et al., 2013) or up to 4 pigs per pen (Coma et al., 1995; Hong et al., 2016; Mule et al., 2006), which may be not real commercial conditions to assess protein efficiency at farm level. Thus, SUN has not been used as a potential tool to assess protein efficiency in a group of pigs in commercial conditions and at farm level to detect suboptimal diets.

- **Creatinine:** Serum creatinine is the product result from the muscle metabolism (Constable et al., 2017) and has a positive correlation with total and striated muscle (Baxmann et al., 2008; Schutte et al., 1981). Literature on how serum creatinine concentration levels are affected because of dietary modifications is scarce. To date, Hong et al. (2016) observed that finishing pigs fed a low energy diet (13.65 MJ/ME) had higher levels of serum creatinine than pigs fed a high energy diet (14.07 MJ/ME) because of more lean tissue and less fat depositions after 13 weeks of changing the diet.

#### *1.4.2.2 Metabolites related to Energy and Lipid Metabolism*

- **Glucose:** Glucose is the principal source of energy for animal cells and the principal product of carbohydrate metabolism (Constable et al., 2017). The literature on glucose has highlighted that growing and finishing pigs have a good homoeostatic control of serum glucose concentration (Kamalakar et al., 2009; Regmi et al., 2018; Zeng et al., 2013). Only Mule et al. (2006), found decreased serum glucose concentration at the end of the finisher phase in pigs fed a high CP diet, which was attributed to a decreased amount of carbohydrates in that diet.

- **Triglycerides:** Triglycerides are involved in the energy/lipid metabolism, and they are the principal source of lipids that comes from the diet. Previous literature observed no differences in serum triglycerides concentrations when pigs were fed different dietary Lys contents (Kamalakar et al., 2009; Regmi et al., 2018; Zeng et al., 2013). Nevertheless, Mule et al. (2006), reported higher serum triglycerides concentration in finishing pigs when fed a low CP diet, which seems to be consistent with other research that found a significant correlation between dietary CP restriction and body fat deposition (Skiba, 2005).

- **Cholesterol:** Cholesterol also participates in lipid metabolism and it is derived from dietary sources and synthesized in vivo from acetyl-CoA in the liver as the main site (Constable et al., 2017). Previous literature observed reduced serum cholesterol concentration in pigs fed carbohydrate restriction hypercholesterolemic diets (DeOgburn et al., 2012; Torres-Gonzalez et al., 2008). Also, previous literature reported a hypercholesterolemic effect when pigs are subjected to dietary protein restriction (Pond et al., 1986) and AA deficient diets (Mule et al., 2006; Regmi et al., 2018), although this effect was not observed in other previous studies (Yang et al., 2008). Earlier studies suggested that the mechanism for an increased serum cholesterol in protein restricted diets could be related to the influence of dietary protein in the insulin/glucagon ratio which further regulates the serum cholesterol concentration (Sanchez and Hubbard, 1991), or that serum albumin plays a role enhancing cholesterol efflux from cells (Sankaranarayanan et al., 2013).

Taken together, blood biochemistry is a potential tool to analyse the blood serum metabolite profile and detect suboptimal diets in pig farms and correct them through dietary modifications, without the need of digestibility trials that are expensive and a time-consuming process in standard conditions and not adapted to an specific farm (Le Goff and Noblet, 2001). Thus, the present thesis will focus on this tool to assess feed efficiency at farm level.

### 1.4.3 Volatile Fatty Acids Profile

Faeces contain information about the digestive process itself and are also a very easy material to collect and work with at farm level. Moreover, VFA and other fermentation components present in faeces can be easily measured in faecal samples (Cho et al., 2015). Thus, VFA may reveal the pigs' digestion process since the nutrient composition of a diet is a key factor in the microbiome profile of the gastrointestinal tract, which will affect the faecal VFA profile (Cho et al., 2015; Dahl et al., 2020; Le et al., 2005). Literature studying the faecal VFA profile based upon dietary modifications is scarce and what we know about VFA is largely based upon experimental studies that investigated dietary strategies to reduce ammonia and odour emissions in the pig manure (Cho et al., 2015; Hobbs et al., 1996; Le et al., 2007). However, the VFA profile changes from the colon to the manure after a few days of storage (Lynch et al., 2008), so faecal VFA profile might not be comparable to the manure VFA profile. Thus, there is room to enhance our understanding towards the implications of dietary modifications in the faecal VFA profile, which gives a better assessment of pigs' digestion than the pig manure (Ziemer et al., 2009).

Carbohydrates are catabolized to short-chain fatty acids (SCFA) such as acetic, propionic or butyric acid, while protein results in a higher concentration of branched-chain fatty acids (BCFA) which are produced from the deamination of branched-chain AAs such as Leu, Ile and Val (Le et al., 2005). Therefore, high proportions of BCFA measured in faeces may indicate an excess of protein reaching distal parts of the intestine, being inefficiently used by growing pigs. In fact, previous studies reported an increased production of BCFA in manure in grower-finisher pigs fed high CP diets (Cho et al., 2015; Hobbs et al., 1996; Le et al., 2007). On the other hand, some reports indicated a decreased SCFA production in manure and colon samples in pigs fed protein restriction diets (Cho et al., 2015; Yu et al., 2019), and an increase in propionic acid (Opapeju et al., 2008) in cecum samples in weaned pigs fed low protein AA supplemented diets.

Production of VFA could also be influenced by the portion of fibre in the diet (Zhao et al., 2020b, 2020a). Indeed, a recent study reported that VFA production is positively correlated with the apparent total tract digestibility (ATTD) of insoluble dietary fibre and cellulose, being these the best factors for predicting the faecal VFA profile (Zhao et al., 2020a). Nevertheless, digestibility of dietary fibre fraction improves with increased BW and age (Zhao et al., 2020b), so insoluble dietary fibre and cellulose might have a major role in finishing pigs with a higher fermentation capacity.

Overall, there seems to be some evidence to further study the effect of suboptimal diets in energy, CP, and AAs on the faecal VFA of growing and finishing pigs to understand and assess feed efficiency at farm level.

### 1.4.4 Near Infrared Reflectance Spectroscopy

Near infrared (NIR) spectroscopy was discovered in 1800, when Herschel found that there was light beyond the visible region (Herschel, 1800). The importance of NIRS came out in the 1960s, when NIRS was introduced as an analytical method for measuring moisture in grains, allowing its application in the livestock industry (Norris and Hart, 1963). Since then, the NIRS technology has largely expanded in the number of analytical applications and possibilities. The present thesis will make a general overview of NIRS application in the livestock industry and further perspectives in this field based on the thesis' objectives.

#### *1.4.4.1 The NIRS Theory and Methodology*

The NIRS is an analytical technique based on the absorption of infrared radiations by chemical bonds in organic matter, which are polar covalent bonds between C–H, O–H, and N–H atoms (Bastianelli, 2013; Prieto et al., 2017). The near infrared region of the electromagnetic spectrum lies between the visible and mid-IR regions (Marten et al., 1989). Thus, it is generally accepted that the NIR spectrum includes wavelengths from 800 to 2500 nm (Marten et al., 1989). Recording the infrared radiation absorbed from the C–H, O–H, and N–H molecular bonds in the NIR wavelengths produces spectra which are unique to a sample, acting as a "fingerprint" (Prieto et al., 2017). Then, the collected spectrum includes data related to the chemical and physical properties of those organic molecules in the sample, which gives information on the sample composition (Prieto et al., 2017).

Once the NIR spectra is collected from a group of samples, it requires of a calibration phase, which starts with the mathematical pre-processing of the spectral data to obtain valuable information on the chemical properties of the samples. This process is named chemometrics (Fernández-Cabanás et al., 2006; Rinnan et al., 2009). During this process, methods such as smoothing, detrending (DT), derivatives, multiple scatter correction, and standard normal variate (SNV), are used to reduce possible effects that may cause noise when conducting the calibration process (Fernández-Cabanás et al., 2006; Rinnan et al., 2009). Thereafter, it is conducted a calibration, which is a regression model that allows the prediction of specific chemical properties on the spectral data, based on the chemical results obtained in the laboratory by using the reference methods (Marten et al., 1989; Williams, 2001). There are different types of regression methods to perform the NIRS calibrations being the partial least squares regression one of the most common methods (Shenk and Westerhaus, 1991a). The best calibration equations are selected according to the standard error of calibration (SEC), the coefficient of determination for calibration ($R^2_c$), the standard error of cross-validation (SECV), and the coefficient of determination for cross-validation ($R^2_{cv}$). Once the prediction model is robust and validated by using an external data set (Marten et al., 1989; Shenk et al., 2001), it can be used routinely and the precision of the measurement is not far from that of reference laboratory measurements (Bastianelli, 2013).

### *1.4.4.2 The use of NIRS in the livestock sector*

During the last four decades, the NIRS technique has greatly expanded in the livestock industry sector thankfully to the scientific knowledge, but most important the highly improvement of NIRS instrumentation and adaptation to the livestock industry requirements (Garrido-Varo et al., 2003, 1996). Nowadays, the NIRS technique is widely used to predict the nutritional value of raw materials and complete feeds in feed mills to assess control and product quality (Bastianelli, 2013; Zijlstra et al., 2011). Moreover, NIRS is also being widely used to assess the quality of livestock products such as meat and dairy products, among others, to attend the consumer demand for quality and healthfulness (Prieto et al., 2017; Pu et al., 2021). The great success of this technique relies on a set of advantages (Bastianelli, 2013; Garrido-Varo et al., 2017; Marten et al., 1989):

- Non-destructive: The sample is recovered intact after the analysis so it can be analysed several times.
- Fast: The NIRS measurement can be made typically between 30 seconds to 3 minutes, allowing an immediate prediction of the chemical composition. Moreover, a high number of samples can be analysed every day.
- Simplicity of sample preparation: It does not require a large sample quantity and use of chemicals.
- Multiplicity of analyses in one operation.
- Possibility of analysing samples at different places: Samples may be analysed at the laboratory or at field level, thanks to the development of portable NIRS instruments.
- Cheap: Once the initial investment (cost of the NIRS instrument) and the development (or purchase) of the calibration equations for each chemical parameter is done, the marginal cost of analysis for a sample is extremely low.

### *1.4.4.3 Faecal NIRS to predict feed digestibility*

As discussed above, diet optimization is currently a time-consuming process including ingredient analysis, digestibility determination and on farm feed efficiency measurements. The nutritional value of raw materials and complete feed for livestock is currently analysed mainly via NIRS (Bastianelli, 2013; Zijlstra et al., 2011) and by using chemical analysis of the feed and prediction equations for each type of animals (Le Goff and Noblet, 2001). However, feed efficiency will vary from farm to farm as the actual nutritional value of a diet will be affected by many different factors. Thus, several lines of evidence suggest the potential use of NIRS to predict feed digestibility in animals by analysing faeces (Bastianelli et al., 2007; Decruyenaere et al., 2015; Gil-Jiménez et al., 2015). The latter would be of great interest to avoid the high costs of digestibility trials for in vivo measurements of nutrients and energy (Bastianelli, 2013), and improve productive performance and feed efficiency, animal health and farm sustainability.

Faeces are an easy material to collect at farm level and contain information about the digestive process since faeces contain non-digested feed components. Faecal NIRS (FNIRS) has been assessed in different species such as cattle (Boval et al., 2004; Coates and Dixon, 2011; Decruyenaere et al., 2015), small ruminants (Landau et al., 2006), poultry (Bastianelli, 2013; Bastianelli et al., 2007), and rabbits (Gil-Jiménez et al., 2015; Núñez-Sánchez et al., 2012). Literature on the use of FNIRS to predict digestibility in pigs is scarce and only four studies have been conducted to our knowledge. Bastianelli et al., (2015) showed that the use of FNIRS can provide useful information as it accounts for digestibility due to animals' factors with a moderate accuracy. Moreover, FNIRS technique is feasible for use in pig nutrition research for predicting the chemical composition of diet and faeces, as well as to determine the ATTD coefficients with a moderate accuracy (Nirea et al., 2018; Paternostre et al., 2021; Schiborra et al., 2015). Thus, FNIRS is a cost-effective promising tool for measuring feed efficiency and digestibility (Nirea et al., 2018).

The FNIRS studies conducted in pigs are presented in Table 1.5 and they are compared by using the residual predictive deviation (RPD) value. The latter allows SECV to be standardized (standard deviation of the reference data / SECV), describing the accuracy of the calibration equations and being able to compare the results obtained by different studies with different experimental data (means, standard deviations, ranges, etc.) (Williams, 2001). Williams (2001) established a RPD > 3 to be acceptable, although Chang et al. (2001) suggested a good accuracy when RPD > 2.0. Minasny and McBratney

(2013) suggested that a good calibration equation and RPD value are subjected to author's interpretation. Moreover, difference between SEC and SECV values should not be more than 20% to be a good calibration equation. The latter was not accounted for some calibration equations of previous studies (Bastianelli et al., 2015; Nirea et al., 2018; Schiborra et al., 2015). Table 1.5 shows that some constituents like CP are better predicted than others in faeces. Moreover, faeces chemical components seem to be better predicted than ATTD coefficients. Nevertheless, depending on the objective of the calibrations, a moderate accuracy could usefully distinguish between high, medium and low levels in faecal samples, which in practical conditions could serve as a tool for early detection of digestive problems and/or improve performance.

**Table 1.5** Comparison of the standard error of calibration (SEC), standard error of cross-validation (SECV), coefficient of determination for cross-validation ($R^2$cv), and residual predictive deviation (RPD) values from the faecal NIRS studies conducted in pigs.

| | Bastianelli et al., 2015 | | | | Schiborra et al., 2015 | | | | Nirea et al., 2018 | | | | Paternostre et al., 2021 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Constituent [1] | SEC | SECV | $R^2$cv | RPD | SEC | SECV | $R^2$cv | RPD | SEC | SECV | $R^2$cv | RPD | SEC | SECV | $R^2$cv | RPD |
| Faeces chemical values | | | | | | | | | | | | | | | | |
| DM, g/kg | | | | | | | | | | | | | 4.60 | 4.90 | 0.92 | 3.40 |
| OM, g/kg | | | | | 10.02 | 13.72 | 0.73 | 1.90 | 48.50 | 56.50 | 0.92 | 3.44 | 7.80 | 8.80 | 0.86 | 2.30 |
| N, g/kg | 0.80 | 0.90 | 0.85 | 2.44 | | | | | | | | | | | | |
| CP, g/kg | | | | | 11.84 | 15.03 | 0.74 | 2.00 | 18.10 | 18.80 | 0.89 | 2.96 | 7.90 | 8.80 | 0.90 | 2.90 |
| GE, MJ/kg | | | | | | | | | 1.20 | 1.40 | 0.91 | 3.14 | 0.23 | 0.27 | 0.81 | 2.10 |
| FAT, g/kg | | | | | | | | | 11.70 | 12.30 | 0.66 | 1.74 | 3.80 | 4.20 | 0.91 | 2.90 |
| CF, g/kg | | | | | 12.94 | 14.38 | 0.90 | 3.10 | | | | | 11.10 | 12.60 | 0.84 | 2.20 |
| NDF, g/kg | | | | | 23.66 | 30.29 | 0.90 | 3.10 | 55.00 | 60.20 | 0.93 | | | | | |
| ADF, g/kg | | | | | 15.34 | 20.80 | 0.94 | 3.90 | | | | | | | | |
| NSP, g/kg | | | | | | | | | | | | | 12.20 | 13.00 | 0.81 | 2.80 |
| ATTD coefficients | | | | | | | | | | | | | | | | |
| DM | 0.097 | 0.104 | 0.58 | 1.54 | | | | | | | | | | | | |
| OM | 0.079 | 0.097 | 0.69 | 1.86 | 0.018 | 0.024 | 0.77 | 2.10 | 0.055 | 0.067 | 0.91 | 3.45 | 0.015 | 0.018 | 0.82 | 2.00 |
| N | 0.104 | 0.120 | 0.76 | 2.08 | | | | | | | | | | | | |
| CP | | | | | 0.019 | 0.024 | 0.82 | 2.40 | 0.023 | 0.027 | 0.51 | 1.48 | 0.023 | 0.025 | 0.79 | 2.00 |
| GE | 0.087 | 0.107 | 0.66 | 1.68 | | | | | 0.023 | 0.026 | 0.85 | 2.62 | | | | |
| NE, MJ/kg | | | | | | | | | | | | | 0.380 | 0.390 | 0.59 | 1.50 |
| FAT | | | | | | | | | 0.060 | 0.068 | 0.74 | 1.94 | 0.043 | 0.046 | 0.48 | 1.30 |
| CF | | | | | 0.076 | 0.086 | 0.71 | 1.90 | | | | | 0.059 | 0.066 | 0.75 | 1.80 |
| NDF | | | | | 0.085 | 0.106 | 0.57 | 1.50 | 0.077 | 0.088 | 0.53 | | | | | |
| ADF | | | | | 0.071 | 0.099 | 0.61 | 1.60 | | | | | | | | |
| NSP | | | | | | | | | | | | | 0.030 | 0.036 | 0.80 | 1.90 |

[1] DM = Dry Matter; OM = Organic Matter; N = Nitrogen; CP = Crude Protein; GE = Gross Energy; NE = Net Energy; CF = Crude Fibre; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; NSP = Non-Starch Polysaccharides.

All previous research conducted using the FNIRS technique has dried or freeze-dried the faecal samples, with a grounding process to obtain a homogeneous and low particle size in faecal samples. However, no research has assessed the feasibility of using dried or freeze-dried faecal samples not ground for FNIRS analysis to evaluate the chemical composition and ATTD coefficients. The possibility to obtain similar results could avoid a workload of grounding faecal samples previous to analyse them via NIRS. Previous literature reported the feasibility to obtain similar nutritional value results from intact or ground raw feed material (Garrido-Varo et al., 2003). The analysis of fresh faeces samples using FNIRS could also be a significant step.

Moreover, previous studies in pigs conducted an external validation to assess the quality and robustness of the calibration equations (Bastianelli et al., 2015; Nirea et al., 2018). However, the external validation used in these previous studies was a subset of the total data set that was not used for the calibration process. Thus, no studies assessed the validation of a calibration predicting faeces chemical components and ATTD coefficients of nutrients by using a complete external validation data set to understand how accurate the calibration is and to quantify how much faecal samples are needed to obtain a robust calibration feasible to be implemented in practical conditions.

FNIRS could be used to differentiate suboptimal diets in protein and energy levels and establish the accuracy level needed for the calibration equations to differentiate, for instance, when the animals are fed excess or low levels of protein at farm.

Finally, a multivariable indicator could be developed based on fast analysis methods conducted on blood and faecal samples such as blood biochemistry, VFA, and FNIRS analysis, to assess the probability of suboptimal diets being fed in pigs at farm level, and therefore, optimise diet formulation, productive performance and feed efficiency, animal health, farm sustainability, and pig producer’s income.

# Chapter 2

## Hypothesis and Objectives

> ***"The man who asks a question***
> ***is a fool for a minute,***
> ***the man who does not ask is a***
> ***fool for life"***
>
> Confucius

After reviewing the main factors influencing the productive performance and feed efficiency in grower-finisher pigs, the objective of this thesis is to study new and existing management and nutritional strategies to improve the pig productive performance and feed efficiency in the grower-finisher phase. Based on this main objective, the general hypothesis of the present thesis is that management and nutritional strategies adapted to growth rate will improve the productive performance and feed efficiency in pigs during the grower-finisher phase. Therefore, the hypothesis assessed along the present thesis were:

1. Slow growing pigs can be identified by having cut-off values for birth and weaning weight lower than the population mean (Chapter 3).
2. Productive performance of slow growing pigs will improve in response to an increase of the SID Lys/AA levels of growing-finishing diets, while fast growing pigs will show a saturated performance response (Chapter 4).
3. Space allowance, mixing and phase feeding interact each other. Space allowance and mixing have an effect on grower-finisher pigs' productive performance and animal welfare, while a two phase feeding strategy will result in a better feed efficiency and same growth performance than a non-phase feeding strategy on grower-finisher pigs (Chapter 5 and 6).
4. Dietary changes in energy, CP, and AA content can be differentiate by the blood metabolite and faecal VFA profiles in growing and finishing pigs (Chapter 7).
5. The NIRS technique can successfully predict faeces chemical composition and ATTD coefficients of nutrients in grower-finisher pigs using freeze-dried not ground faecal samples (Chapter 8).
6. Integrating all the results obtained using the FNIRS, VFA, and blood biochemistry analysis gives us a multivariable indicator to assess feed efficiency at farm level (Chapter 9: General Discussion).

Thus, the specific objectives of the present thesis were:

1. To investigate the effect of birth and weaning BW on key performance indicators of grower-finisher pigs and identify cut-off values for birth and weaning BW to identify slow growing pigs early in life. (Chapter 3).

2. To compare the response in productive performance of slow and fast growing pigs to an increase of dietary SID Lys/AA levels in isoenergetic diets at late grower-finisher stage (Chapter 4).
3. To investigate and quantify the effect and interactions of space allowance, mixing and phase feeding on productive performance and body lesions, as a proxy for aggression, in single wet-dry feeder pens during the grower-finisher stage (Chapter 5 and 6).
4. To use the blood serum metabolite and faecal VFA profiles to identify differences in energy, CP and AA dietary content in growing and finishing pigs (Chapter 7).
5. To use the NIRS technique to assess faeces chemical composition and ATTD coefficients of nutrients in grower-finisher pigs and comparing the use of freeze-dried not ground and freeze-dried ground faecal samples (Chapter 8).

The following chapters will describe the methodology used to assess the hypothesis and objectives commented previously, and the principal findings observed in the present thesis. The present thesis and all of its experimental studies were conducted at the Teagasc Pig Research Facility in Ireland. This is a conventional commercial farm, which facilities are similar to other European farms. Thus, the results and conclusions that will be reported in this thesis might be of relevance for other pig commercial farms in Europe. The findings and conclusions from each chapter will further be discussed in Chapter 9: General Discussion.

# Chapter 3

# Predicting productive performance in grow-finisher pigs using birth and weaning body weight

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## 3.1 Background

Improving production efficiency during the grow-finisher stage is crucial. This stage is the most expensive period in pig production, accounting for over 65% of the total cost of production (Rocadembosch et al., 2016), and minor improvements result in important increases in profit for farmers. One of the main factors impacting the production efficiency is increased BW variability. Additionally, birth and weaning BW are among the most critical factors for lifetime growth performance in pigs (Collins et al., 2017; Douglas et al., 2014a; He et al., 2016). The continuous genetic advancement over the last decade has increased litter size at birth, leading to a considerable decrease in average birth weight, increased percentage of piglets born with light weight (Beaulieu et al., 2010a; Quiniou et al., 2002) and increased BW variability at birth (Foxcroft et al., 2007). Piglets that are born small often remain stunted and are unable to catch up to their big counterparts during the entire production cycle (Beaulieu et al., 2010a; Fix et al., 2010b; Quiniou et al., 2002). Some studies have established ≤ 1.25 kg as low birth BW (Beaulieu et al., 2010b; Douglas et al., 2014c; He et al., 2016), while recent work suggests that 15 to 25% of new-born piglets are born under 1.1 kg of BW (Wang et al., 2017). Moreover, Quiniou et al., (2002) stated that the proportion of piglets weighing less than 1 kg at birth increases from 7 to 23% in large litters (≥ 16 piglets per sow). Similarly, several studies also indicated weaning BW as a critical factor for post-weaning growth and time to reach target slaughter weight (Collins et al., 2017; Smith et al., 2007; Wolter and Ellis, 2001).

Light birth and/or weaning BW pigs that are in the lower quartile of the BW population distribution and require extra time to reach target slaughter weight are often referred to as slow growing pigs (Douglas et al., 2014a; He et al., 2016). It is estimated that, in a given batch, 10 to 15% of pigs are slow growing pigs (Calderón Díaz et al., 2017c; He et al., 2016; Schinckel et al., 2005). These pigs are susceptible to higher mortality rates throughout the production cycle (Calderón Díaz et al., 2017b; Larriestra et al., 2006; Muns et al., 2016), and those that survive pose management challenges in AIAO production systems (Patience et al., 2004). For instance, contrary to AIAO principles, farmers may hold back slow growing pigs to a similarly sized following batch of younger animals to facilitate management (Calderón Díaz et al., 2017c). Calderón Díaz et al. (2017c) reported that carcasses from pigs repeatedly delayed during the production cycle were 10 kg lighter than pigs that were not delayed. In addition, such practice increases the likelihood of disease spread and occurrence (Calderón Díaz et al., 2017c) and increases the occupation time of the facilities. This leads to increased feed costs and the production of fewer finisher pigs per pig space per year. Additionally, carcasses from slow growing pigs are more likely to have poor grading due to higher fat content as a result of the extended time they require to reach target slaughter weight (He et al., 2016), thus decreasing the efficiency of the whole production cycle (Douglas et al., 2014a; López-Vergé et al., 2018a).

Knowing slow growing pigs key performance indicators, such as ADG, ADFI, FCR and time needed to reach target slaughter weight, would allow farmers to improve the management of the whole herd. There is conflicting information in the scientific literature regarding slow growing pigs key performance indicators (Douglas et al., 2014a; Gondret et al., 2006). Some authors reported that light weight pigs at birth and/or at weaning have poor growth performance compared to their heavier counterparts (Douglas et al., 2013; He et al., 2016; Quiniou et al., 2002), while other authors reported that light birth BW pigs are able to catch up their bigger counterparts and to have a similar BW by the end of the production cycle (Huting et al., 2018; Surek et al., 2019; Zeng et al., 2019). However, previous research has not estimated cut-off values for birth and/or weaning BW to differentiate between pigs that are born small but are able to catch up with their big counterparts and those that remain small for the whole production cycle. By identifying slow growing pigs earlier in life, farmers could design new management and nutritional strategies to improve slow growing pigs growth performance, improving farm production efficiency.

We hypothesize that pigs with low BW at birth and at weaning would have decreased growth performance in the whole grow-finisher period compared with their heavier counterparts at birth or at weaning. We hypothesize that slow growing pigs will be identified by having cut-off values for birth and weaning weight lower than the population mean. Thus, the objectives of this study were to (1) investigate the effect of birth and weaning BW on key performance indicators of grow-finisher pigs and (2) identify cut-off values for birth and weaning BW in order to recognize slow growing pigs early in life.

## 3.2 Material & Methods

### 3.2.1 Care and Use of Animals

The study received ethical approval from the Teagasc Animal Ethics Committee (TAEC 184/2018), and it was conducted at the Teagasc Pig Research Unit in Fermoy, Co. Cork, Ireland. A total of 370 pigs [194 females and 176 males; Danish Duroc × (Large White × Landrace)] born within one week were individually ear-tagged, and their BW was recorded within 24 h after birth. Information on sow parity and litter size was also recorded. Pigs were classified according to their birth BW as small (SMALL; 0.9 ± 0.23 kg) if BW $\leq$ 1.15 kg, or big (BIG; 1.4 ± 0.20 kg) if BW > 1.15 kg. The 1.15 kg BW cut-off value was selected considering previous literature research (Beaulieu et al., 2010b; He et al., 2016; Wang et al., 2017) and the lower quartile of the birth BW population distribution from the batch of pigs used for this study. Pigs were weaned at approximately 28 days, individually weighed and re-classified as SMALL (4.3 ± 1.11 kg) if BW $\leq$ 5.5 kg or BIG (7.4 ± 1.86 kg) if BW > 5.5 kg, yielding a 2 × 2 factorial arrangement. The 5.5 kg BW cut-off value at weaning was selected considering the lower quartile of the weaning BW population distribution from the batch of pigs used for this study. Pigs were matched according to sow parity and litter size, and 144 pigs (64 males and 80 females) were selected. Pigs were classified into one of four groups (n = 36 pigs/group): SMALL–SMALL (15 males and 21 females), SMALL–BIG (15 males and 21 females), BIG–SMALL (17 males and 19 females) and BIG–BIG (16 males and 20 females). Descriptive statistics by group are provided in Table 3.1. At weaning, pigs were fitted with a transponder and transferred to the nursery stage accommodation. Pigs were housed in mixed sex pens (n = 12 pigs per pen; 0.55 $m^2$ per pig) with fully slatted plastic floor equipped with individual feeding stations (MLP-ECO, ASR 500, Schauer, Prambachkirchen, Austria) to record individual daily feed intake. Pigs underwent a training period of 15 days to get habituated to use the feeding stations as per normal practice at the Teagasc Pig Research Facility. During this period, feeding stations were switched off and feed bins were manually filled, and thus, feed intake was not recorded. Pigs received a common starter diet [20.0% CP, 12.34 MJ of Net Energy (NE) and 1.40% SID Lys per kg of feed] for seven days, link diet (19.0% CP, 10.96 MJ/NE and 1.28% SID Lys per kg of feed) for 18 days and soybean meal–barley–wheat based nursery diet (17.75% CP, 10.63 MJ/NE and 1.04% SID Lys per kg of feed) for 28 days. At 53 days post-weaning, pigs were transferred to the finisher accommodation, where they remained until reaching the target slaughter weight, which was at least 110 kg of BW as per normal practice in Irish pig farms. During the finisher stage, pigs were housed in the same mixed sex groups with a minimum space of 0.95 m$^2$ per pig in pens with fully slatted concrete floor equipped with individual feeding stations (MLP-ECO, ASR 500, Schauer, Prambachkirchen, Austria). Pigs were fed ad libitum a common soybean meal–maize–wheat based finisher diet (16.18% CP, 9.67 MJ/NE and 0.92% SID Lys per kg of feed). In the nursery and finisher stages, the temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller.

**Table 3.1** Descriptive statistics for birth body weight (BW), weaning BW, average daily gain (ADG) during lactation, sow parity and litter size [Mean ± Standard deviation (SD)] for pigs (n = 36 per group) classified according to their birth BW as SMALL (BW ≤ 1.15 kg) or BIG (BW > 1.15 kg), and re-classified according to their weaning BW as SMALL (BW ≤ 5.5 kg) or BIG (BW > 5.5 kg) yielding a 2 × 2 factorial arrangement.

| | SMALL-SMALL | | SMALL-BIG | | BIG-SMALL | | BIG-BIG | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P*-value |
| Birth BW, kg | $0.9^{b}$ | 0.13 | $1.0^{b}$ | 0.13 | $1.4^{a}$ | 0.22 | $1.4^{a}$ | 0.19 | < 0.001 |
| Weaning BW, kg | $4.0^{d}$ | 0.95 | $6.8^{b}$ | 0.99 | $4.6^{c}$ | 0.74 | $8.0^{a}$ | 0.83 | < 0.001 |
| ADG lactation, g | $121.1^{c}$ | 39.00 | $228.3^{b}$ | 33.39 | $128.5^{c}$ | 29.43 | $257.3^{a}$ | 31.15 | < 0.001 |
| Sow parity | 3.7 | 2.01 | 3.0 | 2.17 | 4.0 | 2.04 | 3.9 | 2.09 | 0.189 |
| Litter size | 16.9 | 2.55 | 16.9 | 2.67 | 16.7 | 2.81 | 17.5 | 2.43 | 0.614 |

$^{a\text{-}b}$ Within rows, significant differences between groups ($P < 0.05$).

### 3.2.2 Measurements

Pigs were individually weighed using a digital scale (R323, Rinstrum, Langenfeld, Germany) every two weeks starting at 16 days post-weaning until they reached target slaughter weight (i.e., at least 110 kg). Average daily gain was calculated for every two-weeks interval. Feed intake was recorded on a daily basis, and ADFI was calculated for every two-week period. For each two-week period, FCR was calculated. Additionally, the days to target slaughter weight (DTSW) were recorded.

### 3.2.3 Statistical Analyses

#### *3.2.3.1 Body Weight, Feed Intake and Feed Efficiency Traits*

Each pig was considered as the experimental unit for all data analyses. Residuals were tested for normality using the Shapiro–Wilk test and by examining the normal probability plot using the UNIVARIATE procedure of SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Two different analyses were performed: Model 1 included data from

16 days post-weaning up to 20 weeks of age, when the first group of pigs reached 110 kg of BW and were sent to slaughter. Predicted variables included BW, ADG, ADFI and FCR. Mixed model equation methods accounting for repeated measurements were used in PROC MIXED of SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Model 2 included data from 15 days post-weaning until all pigs reached target slaughter weight, and the same predicted variables were investigated as per Model 1 plus DTSW. Data were also analysed using mixed model equation methods in PROC MIXED of SAS v9.4 (SAS Institute Inc., Cary, NC, USA). For both analyses, models included birth and weaning BW classification, observation day and their interaction, and sex as fixed effects. Pig was included as a random effect. Multiple means comparisons were done using Tukey-Kramer's correction. Results for the fixed effects are reported as least square means ± standard error. Alpha level for determination of significance and trends were 0.05 and 0.10, respectively.

#### *3.2.3.2 Birth and Weaning Body Weight Cut-Off Values*

Two different analyses were used to estimate cut-off values for birth and weaning BW to identify slow growing pigs, namely regression tree analysis and Receiver Operating Characteristic (ROC) Curves analysis. For the regression tree analysis, data were analysed using the rpart package (Therneau et al., 2019) of R v3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). The model included DTSW as the outcome variable and birth and weaning BW as predictor variables. Then, analysis of variance (ANOVA) was performed using the stats package in R v3.5.2 to confirm that the groups created were statistically different from each other. ROC curve analysis was used to estimate cut-off values for birth and weaning BW to identify pigs that would reach target slaughter weight at 22 weeks of age. The age to slaughter was selected based on Irish commercial criteria and the farm performance. First, pigs were dichotomized based on whether or not reaching target slaughter weight at 22 weeks, and data were analysed using the pROC package (Turck et al., 2011) of R v3.5.2. Univariable and bi-variable models were used in this analysis. To evaluate the overall performance of the models, sensitivity and specificity were calculated at various cut-off values. Sensitivity was defined as the proportion of pigs correctly classified as reaching target slaughter BW at 22 weeks of age. Specificity was defined as the proportion of pigs correctly classified as not reaching target slaughter BW at 22 weeks of age. The accuracy of the models was assessed by calculating the area under the curve (AUC). Values of AUC were interpreted as non-accurate (AUC

= 0.5), less accurate (0.5 < AUC ≤ 0.7), moderately accurate (0.7 < AUC ≤ 0.9), highly accurate (0.9 < AUC < 1) and perfect (AUC = 1) (Greiner et al., 2000). The Youden Index was used to identify the optimal cut-off value that would separate the sample into two populations (Fluss et al., 2005).

## 3.3 Results

### 3.3.1 Body Weight, Feed Intake and Feed Efficiency Traits

#### *3.3.1.1 Model 1*

There was an interaction between birth and weaning BW when predicting BW, ADG, ADFI and FCR. There were no differences for ADG and ADFI between SMALL–BIG, BIG–SMALL and BIG–BIG pigs from 6 to 20 weeks of age (*P* > 0.05; Table 3.2). Pigs classified as SMALL–SMALL were 15.8 kg lighter than the other groups at 20 weeks of age (*P* < 0.05); they tended to gain 97.4 g less per day (*P* < 0.10), and consumed 337 g less feed per day compared to the other groups from 6 to 20 weeks of age (*P* < 0.001; Table 3.2). Pigs classified as BIG–SMALL had higher FCR compared to the other groups (*P* < 0.001), and no difference was observed for FCR between SMALL–SMALL, SMALL–BIG and BIG–BIG pigs (*P* > 0.05; Table 3.2). Male pigs gained 47.8 g more per day (*P* = 0.025) and had lower FCR compared to female pigs (*P* < 0.001). There was no difference in BW and ADFI between male and female pigs at 20 weeks of age (*P* > 0.05).

**Table 3.2** Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) from 6 to 20 weeks of age (Least square means [LS mean] ± Standard error mean [SEM]) for four groups of pigs classified according to their birth BW as SMALL (BW ≤ 1.15 kg) or BIG (BW > 1.15 kg) and re-classified according to their weaning BW as SMALL (BW ≤ 5.5 kg) or BIG (BW > 5.5 kg).

| | Birth BW × Weaning BW | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Trait | SMALL-SMALL | SMALL-BIG | BIG-SMALL | BIG-BIG | SEM | Birth BW | Weaning BW | Interaction |
| BW, kg | | | | | | | | |
| 6 wk | 8.3 $^{d}$ | 9.9 $^{c}$ | 11.7 $^{b}$ | 12.9 $^{a}$ | 0.29 | < 0.001 | < 0.001 | 0.479 |
| 20 wk [1] | 86.1 $^{b}$ | 100.3 $^{a}$ | 100.6 $^{a}$ | 104.7 $^{a}$ | 2.22 | < 0.001 | < 0.001 | 0.025 |
| ADG, g | 868.4 $^{b}$ | 975.6 $^{a}$ | 944.1 $^{a}$ | 977.6 $^{a}$ | 21.02 | 0.001 | 0.066 | 0.081 |
| ADFI, g | 1690.1 $^{b}$ | 1948.7 $^{a}$ | 2133.2 $^{a}$ | 1999.5 $^{a}$ | 52.41 | 0.235 | < 0.001 | < 0.001 |
| FCR | 1.91 $^{b}$ | 1.96 $^{b}$ | 2.19 $^{a}$ | 2.00 $^{b}$ | 0.02 | 0.005 | < 0.001 | < 0.001 |

[1] 20 weeks of age corresponds to the time when the first group of pigs reached 110 kg of BW and were sent to slaughter.

$^{a\text{-}b}$ Within rows, significant differences between groups (*P* < 0.05).

### *3.3.1.2 Model 2*

There was an interaction between birth and weaning BW when predicting ADG, ADFI, FCR and DTSW. Pigs classified as SMALL–SMALL gained 79.9 g less per day during the grow-finisher period compared to the other groups (*P* < 0.05; Table 3.3). Additionally, SMALL–SMALL pigs needed 14.2 days more to reach target slaughter weight than the other groups (*P* < 0.001), and no difference was observed in DTSW among SMALL–BIG, BIG–SMALL and BIG–BIG pigs (*P* > 0.05; Table 3.3). Pigs classified as BIG–SMALL consumed 191.5 g more per day (*P* < 0.001) and had higher FCR during the grow-finisher period compared to the other groups (*P* < 0.05; Table 3.3). Male pigs consumed 83.3 g less per day (*P* = 0.015), had lower FCR (*P* < 0.001), and they reached target slaughter weight 4.1 days earlier (*P* = 0.047) than female pigs.

**Table 3.3** Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and days to target slaughter weight (DTSW) (Least square means [LS mean] ± Standard error mean [SEM]) from six weeks of age until all pigs reached 110 kg of target slaughter weight for four groups of pigs classified according to their birth BW as SMALL (BW ≤ 1.15 kg) or BIG (BW > 1.15 kg) and re-classified according to their weaning BW as SMALL (BW ≤ 5.5 kg) or BIG (BW > 5.5 kg).

| | Birth BW × Weaning BW | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Trait | SMALL-SMALL | SMALL-BIG | BIG-SMALL | BIG-BIG | SEM | Birth BW | Weaning BW | Interaction |
| ADG, g | 849.6 $^{b}$ | 939.7 $^{a}$ | 911.2 $^{a}$ | 937.7 $^{a}$ | 14.01 | < 0.001 | 0.035 | 0.025 |
| ADFI, g | 1787.7 $^{b}$ | 1906.1 $^{b}$ | 2051.1 $^{a}$ | 1884.9 $^{b}$ | 33.72 | 0.48 | < 0.001 | < 0.001 |
| FCR | 2.12 $^{b}$ | 2.04 $^{b}$ | 2.26 $^{a}$ | 2.01 $^{b}$ | 0.03 | < 0.001 | 0.073 | 0.015 |
| DTSW, d | 167.1 $^{b}$ | 153.0 $^{a}$ | 155.3 $^{a}$ | 150.4 $^{a}$ | 2.04 | < 0.001 | < 0.001 | 0.025 |

$^{a\text{-}b}$ Within rows, significant differences between groups (*P* < 0.05).

## 3.3.2 Cut-Off Values for Birth and Weaning Body Weight

### *3.3.2.1 Regression Tree Analysis*

Weaning BW was the main predictor variable to classify pigs based on their age at target slaughter weight. The regression tree classified pigs into three distinctive groups (*P* < 0.001; Figure 3.1). A first cut-off value was obtained at 3.7 kg of BW at weaning, with pigs with a weaning BW lower than this cut-off value taking 177 days to reach 110 kg of BW and representing 12.4% of pigs (Figure 3.1). This group of pigs would correspond to slow growing pigs. A second cut-off value was observed for pigs with a weaning BW ≥ 3.7 kg, which were further classified into two separate groups based on their birth BW (*P* < 0.05; Figure 3.1). Pigs with a weaning BW ≥ 3.7 kg and birth BW ≥ 1.0 kg (61.2% of pigs) took 152 days to reach 110 kg of BW, while pigs with a weaning

BW ≥ 3.7 kg and birth BW < 1.0 kg (26.4% of pigs) needed eight more days to reach 110 kg of BW (Figure 3.1).

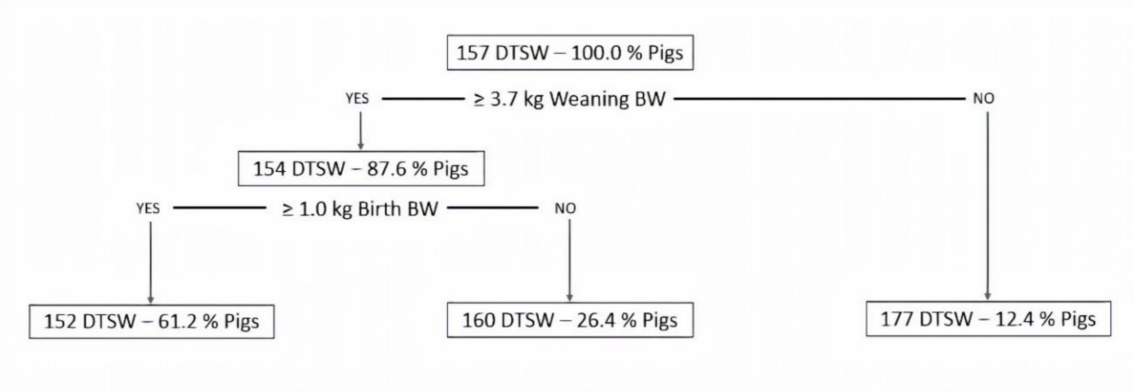

**Figure 3.1** Regression tree analysis used to estimate cut-off values for birth and weaning BW to identify slow growing pigs within a batch. Model included the days to target slaughter weight (DTSW; i.e., 110 kg of BW) as the outcome variable, and birth body weight and weaning body weight as predictor variables. Pigs with body weight lower than 3.7 kg of BW at weaning (i.e., 28 days of age) would be considered slow growing pigs. Regression tree analysis was performed using the rpart package (Therneau et al., 2019) of R v3.5.2.

### *3.3.2.2 Receiver Operating Characteristic (ROC) Curve Analysis*

Cut-off values for birth and weaning BW were estimated to identify pigs that would reach target slaughter weight at 22 weeks of age. The AUC for the three models ranged from 68.4% (weaning BW as predictor) to 76.3% (birth BW plus weaning BW as predictors), and they were all significantly different from 0.5 (Table 3.4). The optimal cut-off value for the predictor variables and their associated sensitivity and specificity are also shown in Table 3.4. When comparing ROCs, no difference was observed between the AUC of the three models (*P* > 0.05). ROCs are shown in Figure 3.2.

**Table 3.4** Performance [Area under the curve (AUC) and 95% confidence interval (CI)], *P*-value, sensitivity and specificity for the optimal cut-off value to identify pigs that would reach target slaughter weight [i.e., 110 kg of body weight (BW)] at 22 weeks of age considering birth BW, weaning BW and birth BW + weaning BW as predictor variables.

| Predictor variable | AUC, % (95 % CI) | *P*-value | Sensitivity, % | Specificity, % | Optimal cut-off value, kg |
|---|---|---|---|---|---|
| Birth BW | 72.7 (64.0-81.5) | < 0.001 | 71.6 | 70.3 | 1.1 |
| Weaning BW | 68.4 (59.4-77.5) | 0.001 | 77.6 | 53.1 | 6.7 |
| Birth + Weaning BW | 76.3 (67.8-84.8) | < 0.001 | - | - | - |

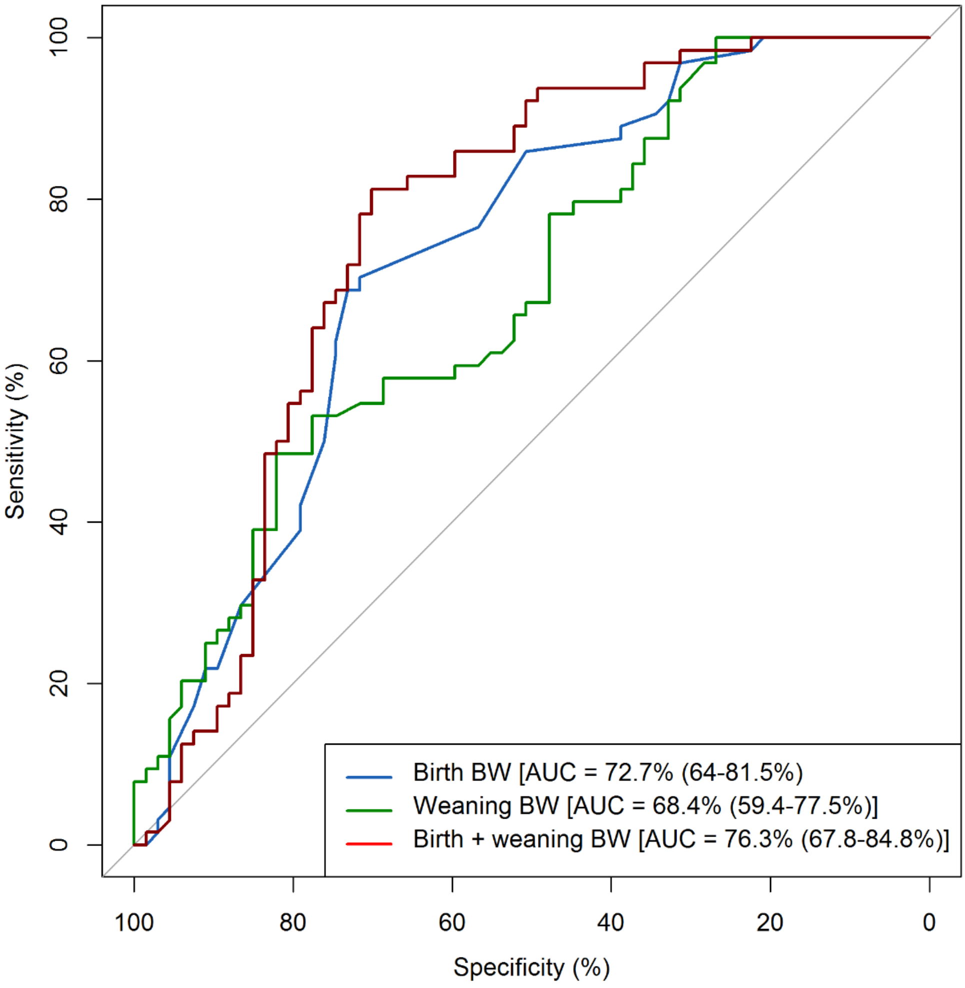

**Figure 3.2** Receiver Operating Characteristic (ROC) curve representing the predictive performance of three different models for identifying pigs that would reach 110 kg of body weight (BW) at 22 weeks of age. Models included birth BW, weaning BW and birth BW + weaning BW as predictor variables. The ROC curve was estimated using the pROC package (Turck et al., 2011) of R v3.5.2. AUC = area under the curve (95% CI); the diagonal line represents an AUC of 0.5.

## 3.4 Discussion

Under the conditions of this study, pigs born and weaned small continued to have decreased growth performance during the grow-finisher period compared with pigs that were classified as big either at birth or at weaning. This result is in line with those of previous studies (Douglas et al., 2013; He et al., 2016; Huting et al., 2018). Some light birth weight pigs have fewer muscle fibres (Gondret et al., 2006, 2005; Nissen et al., 2004) that result in reduced future growth performance, restricting lean growth, increasing fat deposits and resulting in poorer pork quality (Gondret et al., 2006; Rehfeldt et al., 2008; Rehfeldt and Kuhn, 2006). Moreover, light birth weight pigs can have an inadequate colostrum intake due to delaying the first suckle and have a lower ability to access the best teats and to stimulate them to have higher milk consumption (Le Dividich, 1999; Muns et al., 2016). Furthermore, they may show retarded post-weaning maturation of the gastrointestinal tract (Michiels et al., 2013; Pluske et al., 2003), which could contribute to the poorer growth performance observed after weaning in the SMALL–SMALL pigs. On the contrary, SMALL–BIG pigs were able to compensate their light birth weight by having higher ADG during lactation than the average of the batch. Comparisons of the findings with those of other studies (Paredes et al., 2012; Surek et al., 2019) confirm that this subset of pigs have the potential to grow as fast as heavier pigs at birth during nursery and the grow-finisher period. Previous research pointed out that low birth weight pigs can show various degrees of compensatory growth to finally meet or exceed target slaughter BW of their heavier counterparts (Douglas et al., 2013; Handel and Stickland, 1988; Pardo et al., 2013). However, they will only be able to show such compensatory growth if their ADG during lactation is above the average level (Zeng et al., 2019), as we observed with the SMALL–BIG pigs in this study. Explanations to why some piglets can exhibit varying degrees of compensatory growth are reliant on the number of muscle fibres present at birth (Dwyer et al., 1993; Handel and Stickland, 1988) and/or morphometric characteristics at birth (Huting et al., 2018). Additionally, one should discriminate between piglets that have been born light for their gestational age and are proportionally small (Foxcroft et al., 2006) from those that have suffered uterine growth restriction and may remain stunted throughout the production cycle (Wu et al., 2006).

Pigs with heavier birth weight and lighter weaning weight had similar ADG during lactation to SMALL–SMALL pigs. The causes of this lower growth of the BIG–SMALL pigs were not evaluated since several factors can influence ADG during suckling, such as differences in colostrum and milk production among sows and teats, creep feed intake by the piglets, human handling, as well as health conditions (Muns et al., 2016; Muns and Tummaruk, 2016; Nuntapaitoon et al., 2018). However, regardless of the underlying causes, the lower growth of BIG–SMALL pigs shows an interesting reflection on the growth dynamics of piglets during the suckling phase. Nonetheless, in agreement with previous studies (Huting et al., 2018; Surek et al., 2019; Zeng et al., 2019), BIG–SMALL pigs showed compensatory growth during the grow-finisher period, achieving a similar BW to heavier weaning weight pigs by the end of the production cycle.

In Model 1, SMALL–SMALL pigs had lower ADFI compared with the other groups. This result agrees with those observed in earlier studies (Douglas et al., 2014c; Paredes et al., 2014). However, in Model 2, SMALL–SMALL pigs had similar ADFI as SMALL–BIG and BIG–BIG pigs. Daily feed intake increases as pigs get heavier. Thus, for the same age, SMALL–SMALL pigs have different ADFI because they are smaller than the other groups. However, for the same BW, SMALL–SMALL pigs have the same ADFI as their heavier counterparts as a result of spending more time in the facilities to reach target slaughter weight. Interestingly, BIG–SMALL pigs had higher ADFI when they were followed until they reached target slaughter weight, despite their ADFI being similar to SMALL–BIG and BIG–BIG pigs until 20 weeks of age. Pigs classified as BIG–SMALL had higher FCR for the whole grow-finisher period, indicating that they were less efficient in energy utilization although they showed compensatory growth during the grow-finisher stage. This high feed intake but poorer feed efficiency of the BIG–SMALL pigs could be the consequence of a long-term effect caused during the suckling phase. On the contrary, SMALL–SMALL pigs were as feed-efficient as BIG–BIG and SMALL–BIG pigs in both Model 1 and Model 2. This finding is in accordance with previous results reported by Douglas et al. (2014c), Collins et al. (2017) and Paredes et al. (2014) but contrary to those reported by Gondret et al. (2006), who suggested that the poor growth performance of slow growing pigs was due to poorer feed efficiency. Feed efficiency is a key factor in pork production, with economic and environmental implications. Feed efficiency is affected by many factors, such as diet composition, body composition, feed intake, growth rate, thermal environment, immunological status, feed processing and delivery (Patience et al., 2015). Therefore, strategies to improve the growth performance of slow growing pigs could include improving the diet and nutrient composition in the grow-finisher stage (Camp Montoro et al., 2020b).

On average, faster growing pigs reached target slaughter weight at 22 weeks of age, while SMALL–SMALL pigs required 14.2 days extra to reach target slaughter weight. This is similar to previous results where slow growing pigs required 10 to 20 days extra to achieve target slaughter weight (He et al., 2016; Mahan and Lepine, 1991; Quiniou et al., 2002). We estimated cut-off values for birth and weaning weight that could be used to identify slow growing pigs early in life. Regression tree analysis was used to calculate a cut-off value for birth and weaning weight considering age at slaughter. Weaning weight appeared to be a better predictor variable than birth weight to classify pigs based on age at slaughter. A cut-off of 3.7 kg of BW at weaning would allow farmers to identify slow growing pigs that would need 23 days extra to achieve target slaughter BW. This finding is consistent with that of Larriestra et al. (2006), who established a cut-off value of 3.6 kg of BW at weaning, which maximizes sensitivity and specificity to correctly predict the likelihood of dying or of being light in weight when exiting the nursery stage. Additionally, pigs below 3.6 kg of BW at weaning will require a higher level of management and more complex diets (Damgaard et al., 2003). Using the regression tree analysis, 12.4% of pigs were identified as slow growing pigs. This is in accordance with previous studies that reported 10–15% of slow growing pigs within a batch (Calderón Díaz et al., 2017c; He et al., 2016; Larriestra et al., 2006). Most farmers weigh pigs at weaning but not at birth, albeit weighing pigs individually is not a common practice in commercial pig farms. Nonetheless, farmers usually sort pigs by size or BW at weaning. Therefore, the cut-off value of 3.7 kg of BW at weaning could be a valuable indicator for farmers to identify slow growing pigs.

ROC curve method was used to estimate cut-off values for birth and weaning BW to identify pigs that would reach target slaughter weight at 22 weeks of age. A cut-off value of 6.7 kg of BW was obtained using weaning weight as the only predictor variable. This is consistent with previous reports which stated that 80% of pigs weaned at 28 days of age at <6.4 kg of BW became slow growing pigs at slaughter age (He et al., 2016). Recently, López-Vergé et al. (2019) reported 6.88 kg of BW at weaning as a threshold to account for better productive performance in the grow-finisher period. A cut-off value of 1.1 kg of BW was obtained when birth BW was considered as the only predictor variable in the ROC curve analysis. This is similar to previous results showing 1 kg of BW at birth as a critical value for higher risk of mortality and poor growth performance (Calderón Díaz et al., 2017c; Larriestra et al., 2006; Zeng et al., 2019). Nevertheless, no difference was observed between the AUCs for the univariable models including either birth or weaning BW as predictors, indicating that either variable could be used to identify the pigs that would require more time to reach target slaughter weight. However, farmers could find the weaning BW cut-off value more valuable because most farms sort pigs by size or BW at weaning as a routine management practice. It is worth mentioning that the AUCs obtained in our study are considered less-moderate accurate (Greiner et al., 2000). This was likely due to the number of “controls” (i.e., animals not reaching target slaughter weight at 22 weeks of age) being only three more pigs than the number of “cases” (i.e., pigs reaching target slaughter weight at 22 weeks of age), when, ideally, the number of controls should be twice the number of cases. Moreover, it is likely that the less-moderate accurate AUCs observed in this study reflect the fact that birth and/or weaning BW predict subsequent growth performance only to a certain extent, as growth performance is also influenced by other factors such as husbandry practices, nutritional strategies and animal health. Future studies are therefore required where other factors affecting reaching target slaughter weight in a timely manner are also included in the analyses.

We acknowledge that the cut-off values obtained in the present study using the regression tree and ROC curve analyses may not be extrapolated to other farms with a different production system, genetics, management strategies or sanitary status, as growth performance can be affected by other factors not presented in this study. Nevertheless, pig farmers could use regression tree and/or ROC curve analyses as decision-making tools to identify slow growing pigs earlier in life. The cut-off values identified could be used as a first indicator for pig producers to determine, from an economic standpoint, whether to cull low birth weight pigs or implement new management and nutritional strategies for slow growing pigs. As a result, two production flows could be created, always treating the slow growing pigs “off-site” from the normal production flow in an AIAO production systems (Owsley et al., 2013). Re-grouping slow growing pigs does not improve their growth performance (Brumm et al., 2002; O’Quinn et al., 2001; Patience et al., 2004), unless different management and nutritional strategies are implemented, such as milk supplementation (Douglas et al., 2014a), cross-fostering (Huting et al., 2017), the development of high specifications diets post-weaning (Beaulieu et al., 2009; Nissen and Oksbjerg, 2011; Vieira et al., 2015), increasing feeder space or establishing different phase feeding strategies during the grow-finisher period (López-Vergé et al., 2018b). These strategies could improve the growth performance of slow growing pigs, leading them to partially catch up with their faster growing counterparts, maximizing financial returns.

## 3.5 Conclusion

This study provides a better understanding of the key performance indicators for grow-finisher pigs classified as small or big at birth and weaning based on their BW. Pigs that are born small and wean small have poorer lifetime growth performance and are not able to catch up with their heavier counterparts classified as big either at birth or at weaning. In addition, birth BW might not be the best predictor of subsequent growth performance, as some light birth BW pigs can show compensatory growth. Nevertheless, slow growing pigs had similar feed efficiency to pigs that were heavier at weaning. However, they spent more time in the facilities to reach target slaughter weight. This may lead to production inefficiencies. Pigs that were born heavier but were light at weaning showed compensatory growth during the grow-finisher period but had higher ADFI and FCR compared to the rest of the batch. Future research should focus on this group of pigs in economic terms and in relation to their carcass traits. The methods and cut-off values obtained for birth and weaning weight in this study may aid pig farmers as a decision-making tools to identify slow growing pigs early in life. As a result, pig farmers could design new management and/or nutritional strategies targeting slow growing pigs to improve their performance, thereby increasing production efficiency and farm profitability.

# Chapter 4

# High levels of standardized ileal digestible amino acids improve feed efficiency in slow growing pigs at late grow-finisher stage

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## 4.1 Background

Pigs that, at the same age, are smaller than the rest of the batch and require additional time to be sent to slaughter in conventional pig farms are often referred to as slow growing pigs (Collins et al., 2017; Douglas et al., 2014a; He et al., 2016). It is estimated that between 10% and 12.5% of pigs on a given batch are slow growing pigs (Calderón Díaz et al., 2017c; Camp Montoro et al., 2020a; He et al., 2016). This subset of pigs increases weight heterogeneity within batch (Fix et al., 2010b; López-Vergé et al., 2018a; Paredes et al., 2012) and poses a management challenge to pig producers because it increases the occupation time of the facilities and makes AIAO difficult to implement in swine production systems (Calderón Díaz et al., 2017c; Patience et al., 2004). Moreover, slow growing pigs are prone to have high mortality rates (Calderón Díaz et al., 2017b; Larriestra et al., 2006; Muns et al., 2016) and may spread disease in a farm if they are deferred to the subsequent batch of pigs (Calderón Díaz et al., 2017c). Furthermore, slow growing pigs are associated with increased risk of carcass penalties in the abattoir as a result of higher fat content due to prolonged time in the facilities to reach the target slaughter weight (Gondret et al., 2006; He et al., 2016; Rehfeldt et al., 2008). The extended occupation time in the farm facilities leads to increased feed costs and to produce fewer pigs per place per year, impacting the efficiency of the production cycle and farm's profitability (Douglas et al., 2014a; López-Vergé et al., 2018a; Patience et al., 2004).

The continuous genetic advancement in pig production has led to increased litter size at birth resulting in increased numbers of light birth weight piglets (Beaulieu et al., 2010a; Quiniou et al., 2002). Those pigs are at high risk of being slow growing pigs considering that birth BW is one of the most critical factors for postnatal performance (Douglas et al., 2013; He et al., 2016). Moreover, weaning BW has been indicated as a critical factor for lifetime post-weaning growth performance (Collins et al., 2017; Smith et al., 2007; Wolter and Ellis, 2001) and low weaning BW has also been related to slow growing pigs (Camp Montoro et al., 2020a). Nevertheless, previous studies reported that slow growing pigs can exhibit compensatory growth and partially catch up with their big counterparts (Douglas et al., 2013; López-Vergé et al., 2018b). Thus, there has been an increasing amount of literature assessing management and/or nutritional strategies to improve the slow growing pigs' performance over the production cycle, mostly focused at nursery (Douglas et al., 2014c; Huting et al., 2019; Vieira et al., 2015) and the beginning of the grower–finisher phase (Aymerich et al., 2020; Douglas et al., 2014b). In addition, few studies have shown effects on the growth of slow growing pigs during the grower–finisher phase by carrying out phase feeding strategies based on a weight basis or equivalent feed consumption instead of age (Hawe et al., 2020; López-Vergé et al., 2018b).

There is a growing body of literature that recognizes that slow growing pigs are as feed efficient as their big counterparts (Camp Montoro et al., 2020a; Collins et al., 2017; Paredes et al., 2014). An important question is whether slow growing pigs' feed efficiency could be improved by increasing the SID AA dietary levels based on the ideal protein profile during the grower-finisher stage. Standard nutritional tables (De Blas et al., 2013; NRC, 2012; PIC, 2016) have previously established the SID AA requirements for the average grower–finisher pig; however, there is no information regarding specific SID AA requirements for the slow growing pigs. Aymerich et al. (2020) observed that slow growing pigs may use more efficiently high dietary SID Lys levels based on the ideal protein profile compared to fast growing pigs. However, it is not clear yet at which SID Lys/AA levels the slow growing pigs maximize their performance. Understanding how productive performance is affected by increasing the dietary SID Lys/AA levels on slow growing and fast growing pigs may help pork producers to make better management and feeding strategies.

In the present study, we hypothesized that slow growing pigs would have an improved productive performance in response to an increase of the dietary SID Lys/AA levels, while fast growing pigs would show a saturated response, in a high sanitary status farm. Therefore, the aim of the study was to compare the productive performance of slow growing and fast growing pigs to an increase of dietary SID Lys/AA levels in isoenergetic diets at late grower-finisher stage.

## 4.2 Material & Methods

### 4.2.1 Care and Use of Animals

The experiment received ethical approval from the Teagasc Animal Ethics Committee (TAEC 204/2018) and it was conducted at the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland. A total of 421 pigs born within one week were followed per pen as intact litters from birth until 11 weeks of age. Pigs were weaned at approximately 28 days and received a starter diet (20.0% CP, 12.3 MJ/NE and 1.40% SID

Lys per kg of feed) for seven days, link diet (19.0% CP, 11.0 MJ/NE and 1.28% SID Lys per kg of feed) for 18 days and soybean meal–barley–wheat based nursery diet (17.8% CP, 10.6 MJ/NE and 1.04% SID Lys per kg of feed) for 28 days. At 11 weeks of age, a total of 84 pigs [48 females and 36 males; Danish Duroc × (Large White × Landrace)] out of 324 pigs were weighed, ear tagged individually and classified by growth rate as slow growing (n = 48; 24.1 ± 1.38 kg) or fast growing pigs (n = 36; 42.7 ± 1.63 kg). Criteria selection was based on the upper and lower quartile of the BW population distribution from the batch of pigs used for this study. Pigs were matched according to gender, litter size and pen of origin. At 11 weeks of age, pigs were moved to the finisher accommodation and they were housed in 6 mixed sex pens (n = 14 pigs per pen; 0.81 $m^2$ / pig) with fully slatted plastic floor equipped with one nipple drinker and individual feeding stations (MLP-ECO, ASR 500, Schauer, Prambachkirchen, Austria) to record daily individual feed intake. Water and pelleted feed were provided ad libitum. Temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller. Pens were enriched with a larch wood post. Pigs were fed ad libitum a soybean meal-maize-wheat based finisher diet (16.2% CP, 9.7 MJ/NE and 0.92% SID Lys per kg of feed) from 11 to 15 weeks of age. Pigs were assigned to three different dietary treatments in a 3×2 factorial arrangement at 15 weeks of age. Diet and growth rate (slow growing or fast growing) were considered as fixed factors, and pig as the experimental unit. Each diet was assigned to two pens, each pen containing 8 slow growing (40.1 ± 1.72 kg) and 6 fast growing (63.2 ± 2.04 kg) pigs. Diets were formulated by increasing the ideal protein profile (Van Milgen and Dourmad, 2015) based on the following SID Lys levels: 0.92, 1.18 and 1.45% (Table 4.1). All three diets were isoenergetic (9.68 MJ/NE) based on wheat, maize and soybean meal, and were formulated to meet or exceed the minimum nutrient requirements (NRC, 2012). Shown in Table 4.1 are the analysed compositions of selected nutrients contained in the three experimental diets. The experiment finished at 21 weeks of age when fast growing pigs were sent to slaughter after reaching target slaughter weight which was set at 110 kg of BW as per normal practice in Irish pig farms.

**Table 4.1** Ingredient, calculated and analysed nutrient composition on an as-fed basis of the three dietary treatments fed in grow-finisher pigs from 15 to 21 weeks of age.

| | Diets | | |
|---|---|---|---|
| | 0.92 % SID Lys / AA | 1.18 % SID Lys / AA | 1.45 % SID Lys / AA |
| Ingredients, g/kg | | | |
| Wheat | 435.0 | 318.5 | 310.0 |
| Maize | 300.0 | 370.0 | 360.0 |
| Soybean meal 48 | 171.0 | 210.0 | 220.0 |
| Soybean hulls | 71.0 | 70.0 | 68.0 |
| Vegetable Oil | 0.00 | 0.90 | 0.80 |
| Calcium carbonate | 11.0 | 11.0 | 11.0 |
| Dicalcium phosphate anhydrous | 1.00 | 1.00 | 1.00 |
| Sodium chloride | 3.00 | 3.00 | 3.00 |
| L-Lysine HCl | 3.75 | 5.90 | 9.10 |
| L-Threonine | 1.70 | 3.10 | 4.95 |
| DL-Methionine | 0.93 | 1.63 | 2.53 |
| L-Tryptophan | 0.15 | 0.60 | 1.05 |
| L-Valine | 0.00 | 1.30 | 3.10 |
| L-Arginine | 0.00 | 1.60 | 4.00 |
| Vitamin and trace mineral mixture‡ | 1.47 | 1.47 | 1.47 |
| | | | |
| Composition†, % as fed or as specified | | | |
| Dry Matter, analysed | 88.10 | 87.70 | 87.70 |
| NE, MJ/kg | 9.67 | 9.68 | 9.70 |
| Crude Protein, analysed | 15.90 | 17.60 | 18.30 |
| Total Lys, analysed | 1.02 | 1.22 | 1.44 |
| Total Thr / Lys ratio, analysed | 0.70 | 0.70 | 0.73 |
| Total Met / Lys ratio, analysed | 0.32 | 0.32 | 0.33 |
| Total Trp / Lys ratio, analysed | 0.13 | 0.13 | 0.14 |
| Total Val / Lys ratio, analysed | 0.67 | 0.67 | 0.68 |
| Total Arg / Lys ratio, analysed | 0.89 | 0.82 | 0.73 |
| Total Lys | 1.03 | 1.29 | 1.57 |
| SID Lys | 0.92 | 1.18 | 1.45 |
| SID Thr / Lys ratio | 0.70 | 0.70 | 0.70 |
| SID Met / Lys ratio | 0.34 | 0.34 | 0.34 |
| SID Trp / Lys ratio | 0.19 | 0.19 | 0.19 |
| SID Val / Lys ratio | 0.69 | 0.69 | 0.69 |
| SID Arg / Lys ratio | 0.96 | 0.96 | 0.96 |
| Fat, analysed | 2.70 | 2.90 | 2.50 |
| Crude Fibre, analysed | 4.10 | 4.40 | 4.00 |
| NDF | 14.60 | 14.30 | 14.10 |
| Calcium | 0.68 | 0.69 | 0.69 |
| Digestible Phosphorus | 0.23 | 0.23 | 0.23 |
| SID Lys:NE, g/MJ | 0.95 | 1.21 | 1.50 |

† NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fibre.

‡ Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin $D_3$, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin $B_{12}$, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin $B_1$, 3 mg Vitamin $B_6$.

### 4.2.2 Feed Analysis

Feed samples of each diet were collected from the feeders and analysed for dry matter (DM), crude ash, CP, crude fibre, and fat at the Dairy Gold Feed Laboratory (Lombardstown, Co. Cork, Ireland). Dry matter was determined by oven drying for 4 h at 103°C (Thiex, 2009). Crude ash was determined via combustion in a muffle furnace at 550°C (Thiex et al., 2012). Crude protein was determined as N × 6.25 using the Automated Kjeldahl method (Thiex et., 2002). Crude fibre was measured by a Fibertec semi-automatic system using the gravimetric method (Thiex, 2009). Fat was determined using light petroleum ether and Soxtec instrumentation (Thiex, 2009). Total AA profile was analysed based on high performance liquid chromatography technique (Otter, 2012) at the Sciantec Analytical Services (Stockbridge Technology Centre, Cawood, Yorkshire, UK).

### 4.2.3 Body Weight, Feed Intake and Feed Efficiency Traits

Pigs were individually weighed using a digital scale (R323, Rinstrum, Langenfeld, Germany) every 2 weeks from 15 to 21 weeks of age. Feed intake was recorded individually on a daily basis. Average daily gain, ADFI and FCR were calculated for every 2 weeks interval. Average daily feed intake was multiplied by the NE density of the diet to calculate the energy intake (EI; MJ NE/day). Moreover, metabolic BW (MBW) was calculated for each animal as $BW^{0.6}$ (Noblet et al., 1999; NRC, 2012). Finally, three feed efficiency ratio traits were calculated based on Calderón Díaz et al., (2017a):

- Relative growth rate (RGR):

$$100 \times [(\log10\text{BW at end of trial} - \log10\text{BW at start of trial}) / (\text{age at end of trial} - \text{age at start of trial})]$$

- Kleiber ratio (KR), which relates ADG to the cost of maintenance energy:

$$\text{KR} = \text{ADG} / \text{MBW}$$

- Energy conversion ratio (ECR):

$$\text{ECR} = \text{EI} / \text{ADG}$$

### 4.2.4 Statistical Analysis

Each pig was considered as the experimental unit for all data analyses. All analysis were carried out using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Data was tested for normality using the Shapiro-Wilk test and by examining the normal probability plot. The analysed model included diet, growth rate group and their interaction, and sex as fixed effects, and pig as random effect. Models for BW, ADG, ADFI, FCR, MBW, EI, RGR, KR, and ECR variables were analysed using general linear mixed model accounting for repeated measurements in PROC MIXED of SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Multiple means comparisons were done using Tukey-Kramer's correction in all cases. Alpha level for determination of significance was 0.05 and trends were identified as alpha of 0.10. Results for fixed effects are reported as least square means ± standard error mean.

## 4.3 Results

Final BW, ADG and ADFI did not show an interaction or diet effect during the whole period of the trial (all $P > 0.05$; Table 4.2). Nevertheless, fast growing pigs were 33.7 kg heavier than slow growing pigs at 21 weeks of age ($P < 0.001$; Table 4.2). Moreover, fast growing pigs gained 255 g more per day ($P < 0.001$) and consumed 625.5 g more per day ($P < 0.001$) than slow growing pigs from 15 to 21 weeks of age (Table 4.2). Feed conversion ratio showed an interaction tendency between SID Lys/AA dietary treatments and growth rate group. Feed conversion ratio was 0.3 lower for slow growing pigs fed the 1.45% SID Lys/AA dietary treatment compared to the slow growing pigs fed the 0.92% SID Lys/AA dietary treatment ($P = 0.002$; Figure 4.1), however, FCR did not differ within the fast growing pigs fed with the different SID Lys/AA dietary treatments ($P > 0.05$; Figure 4.1).

**Table 4.2** Effect of increasing the standardized ileal digestible lysine based on the ideal protein profile (SID Lys/AA) on final body weight (BW), average daily gain (ADG) and average daily feed intake (ADFI) (Least square means ± Standard error mean [SEM]) from 84 grower-finisher pigs grouped by diet and growth rate from 15 to 21 weeks of age.

| | Dietary treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.92 % SID Lys/AA | | 1.18 % SID Lys/AA | | 1.45 % SID Lys/AA | | | *P*-value | | |
| Trait | SG$^{\dagger}$ | FG$^{\ddagger}$ | SG | FG | SG | FG | SEM | Growth Rate | Diet | Interaction |
| BW, kg | | | | | | | | | | |
| 15 wks | 41.0 | 64.2 | 39.5 | 61.7 | 39.7 | 63.6 | 1.88 | | | |
| 21 wks | 79.0 $^{b}$ | 111.8 $^{a}$ | 77.2 $^{b}$ | 111.3 $^{a}$ | 78.5 $^{b}$ | 112.9 $^{a}$ | 1.89 | < 0.001 | 0.712 | 0.953 |
| ADG, g | 910.0 $^{b}$ | 1149.7 $^{a}$ | 910.9 $^{b}$ | 1183.6 $^{a}$ | 951.4 $^{b}$ | 1204.1 $^{a}$ | 32.05 | < 0.001 | 0.335 | 0.873 |
| ADFI, g | 2073.3 $^{b}$ | 2618.6 $^{a}$ | 1952.3 $^{b}$ | 2527.0 $^{a}$ | 1936.8 $^{b}$ | 2696.1 $^{a}$ | 89.07 | < 0.001 | 0.463 | 0.440 |

$^{\dagger}$ SG = Slow growth rate.

$^{\ddagger}$ FG = Fast growth rate.

$^{a\text{-}b}$ Within rows, significant differences between groups ($P < 0.05$).

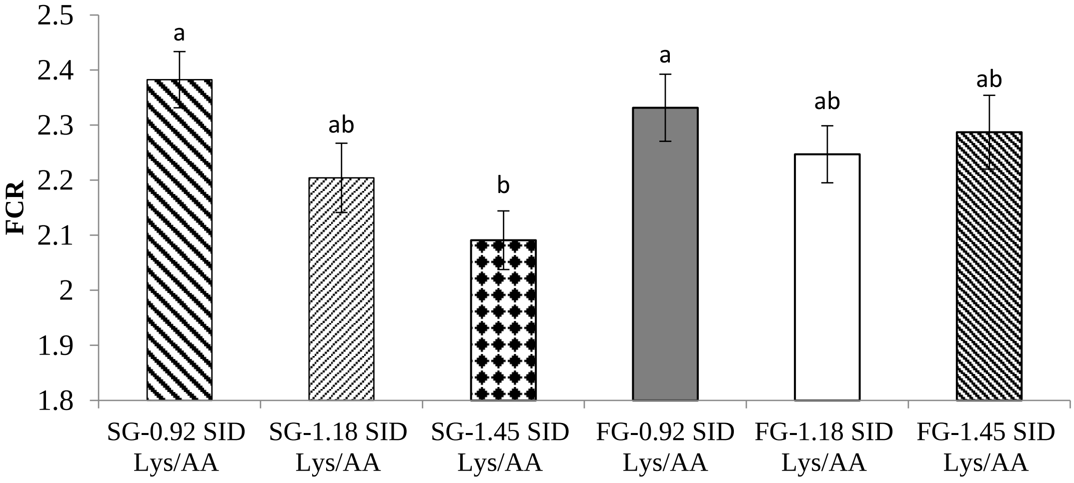

**Figure 4.1** Effect of increasing the standardized ileal digestible lysine based on the ideal protein profile (SID Lys/AA) on feed conversion ratio (FCR) from 84 grower-finisher pigs grouped by growth rate (SG = Slow-growing; FG = Fast-growing) and diet (0.92, 1.18, 1.45% SID Lys/AA) from 15 to 21 weeks of age. $^{a,\ b}$ Significant differences between treatments ($P < 0.05$).

Final MBW and EI did not show an interaction or diet effect during the whole period of the trial (all $P > 0.05$; Table 4.3). However, fast growing pigs had 3.3 kg of MBW more than slow growing pigs at 21 weeks of age ($P < 0.001$; Table 4.3); and fast growing pigs had an EI of 6 MJ NE/day more than slow growing pigs from 15 to 21 weeks of age ($P < 0.001$; Table 4.3). The KR did not differ between treatments from 15 to 21 weeks of age ($P > 0.05$; Table 4.3). Nevertheless, RGR was 0.10 higher for slow growing pigs compared to fast growing pigs ($P < 0.001$; Table 4.3). Moreover, ECR showed an interaction tendency between SID Lys/AA dietary treatments and growth rate group. Energy conversion ratio was 2.7 lower for slow growing pigs fed the 1.45% SID Lys/AA dietary treatment compared to the slow growing pigs fed the 0.92% SID Lys/AA dietary treatment (*P* = 0.003; Table 4.3), however, ECR did not differ within the fast growing pigs fed with the different SID Lys/AA dietary treatments (*P* > 0.05; Table 4.3).

**Table 4.3** Effect of increasing the standardized ileal digestible lysine based on the ideal protein profile (SID Lys/AA) on final metabolic body weight (MBW), energy intake (EI), relative growth rate (RGR), Kleiber ratio (KR) and energy conversion rate (ECR) (Least square means ± Standard error mean [SEM]) from 84 grower-finisher pigs grouped by diet and growth rate from 15 to 21 weeks of age.

| | Dietary Treatment | | | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.92 % SID Lys/AA | | 1.18 % SID Lys/AA | | 1.45 % SID Lys/AA | | | | | |
| Trait | SG[†] | FG[‡] | SG | FG | SG | FG | SEM | Growth Rate | Diet | Interaction |
| MBW, kg | | | | | | | | | | |
| 15 wks | 9.3 | 12.1 | 9.1 | 11.9 | 9.1 | 12.1 | 0.21 | | | |
| 21 wks | 13.7 $^{b}$ | 17.0 $^{a}$ | 13.6 $^{b}$ | 16.9 $^{a}$ | 13.7 $^{b}$ | 17.1 $^{a}$ | 0.21 | < 0.001 | 0.757 | 0.964 |
| EI, MJ NE/d | 20.1 $^{b}$ | 25.3 $^{a}$ | 19.0 $^{b}$ | 24.5 $^{a}$ | 18.8 $^{b}$ | 26.2 $^{a}$ | 0.86 | < 0.001 | 0.466 | 0.433 |
| Ratio feed efficiency traits | | | | | | | | | | |
| RGR | 0.69 $^{a}$ | 0.58 $^{b}$ | 0.70 $^{a}$ | 0.61 $^{b}$ | 0.72 $^{a}$ | 0.61 $^{b}$ | 0.02 | < 0.001 | 0.221 | 0.828 |
| KR | 74.2 | 74.8 | 75.2 | 78.0 | 78.1 | 78.3 | 1.82 | 0.422 | 0.140 | 0.768 |
| ECR | 23.0 $^{a}$ | 22.5 $^{a}$ | 21.3 $^{ab}$ | 21.8 $^{ab}$ | 20.3 $^{b}$ | 22.2 $^{ab}$ | 0.56 | 0.187 | 0.017 | 0.107 |

[†] SG = Slow growth rate.
[‡] FG = Fast growth rate.
$^{a\text{-}b}$ Within rows, significant differences between groups (*P* < 0.05).

## 4.4 Discussion

Birth and weaning BW are two of the most critical factors affecting productive performance (Collins et al., 2017; Douglas et al., 2013; He et al., 2016). Slow growing pigs have been related with a low birth and weaning BW (Camp Montoro et al., 2020a; Douglas et al., 2013), although they can exhibit several degrees of compensatory growth and partially catch up with their big counterparts (Douglas et al., 2013; López-Vergé et al., 2018b). Knowing that slow growing pigs have a lower feed intake, but are as feed efficient as their big counterparts, Camp Montoro et al., (2020a), set the basis for our hypothesis that slow growing pigs' performance may be improved by increasing the dietary SID Lys/AA levels in the late grow-finisher stage. It is worth to mention that the present study has been carried out in a high sanitary status farm and selected fast and slow growing pigs were in good health conditions, although we cannot rule out subclinical issues in some individuals. The latter could be a reason for slow growth due to low health conditions which may affect specific AA requirements of the pigs such as methionine, threonine and tryptophan (Van der Meer et al., 2016), and the efficiency of nitrogen utilization for body protein deposition (Van Der Meer et al., 2020). Nevertheless, the increased dietary SID Lys/AA levels should be helpful in case of pigs with subclinical issues.

Fast growing pigs weighed, gained, and consumed considerably more than slow growing pigs, which is in line of previous studies (Camp Montoro et al., 2020a; Douglas et al., 2013; He et al., 2016) and states the importance of the slow growing pigs' management to reduce the BW variability within a batch (López-Vergé et al., 2018a). Although fast growing pigs gained much more than slow growing pigs, slow growing pigs had a high RGR during the trial period. This finding is in accordance with previous literature and further supports the idea that RGR tends to be greater in lighter BW animals, although it does not necessarily mean that those pigs are more efficient (Calderón Díaz et al., 2017a). Nevertheless, slow growing pigs improved their feed and ECR when dietary SID AA levels were increased from 0.95 to 1.45% SID Lys/AA, while no improvement was observed in the fast growing pigs. The KR did not differ between growth rates which means that both fast and slow growing pigs have the same growth for the same cost of energy maintenance (Tedeschi et al., 2006). Therefore, a possible explanation for the slow growing pigs' improvement in terms of feed efficiency may rely on that nutrient requirements were better matched in the 1.45% than the 0.92% SID Lys/AA diet due to their BW or physiological stage, while fast growing pigs nutrient requirements are already matched in the 0.92% SID Lys/AA diet (López-Vergé et al., 2018b). So, increasing the SID AA levels in the diet of slow growing pigs may match better their nutrient requirements optimizing their protein deposition and feed efficiency.

Although SID Lys/AA levels used in the present study differ from other studies, Aymerich et al. (2020), pointed out that slow growing pigs may improve their growth performance by increasing from 0.80 to 1.20% the dietary SID Lys levels in isoenergetic diets, while fast growing pigs showed a saturated response to increased dietary SID Lys levels from 28 to 63 kg of BW. Nonetheless, this finding is contrary to that of Douglas et al. (2014b), who found that slow growing pigs failed to improve their performance increasing the total Lys dietary levels from 0.98 to 1.48% during the first 30 days post-weaning period. It is difficult to explain this discrepancy, but it might be related to the experimental design itself and the management of the pigs during the lactation period where all the pigs in the study were cross-fostered into a litter according to their birth weight within the first 48 hours, reducing limiting factors such as competition for access to teats during suckling, and receiving also supplementary milk. This management could have had a post-weaning effect, not observed when pigs are kept in intact litters. Other reasons may be an unbalanced AA diet when increasing SID Lys to such levels, or the sanitary conditions of the farm where the study was carried out. Low sanitary conditions and subclinical infections may affect the AA requirements and efficiency of protein deposition in pigs (Van der Meer et al., 2016; Van Der Meer et al., 2020). Despite that study of Douglas et al. (2014b), other studies carried out during the nursery period also observed that high specifications diets targeted to slow growing pigs improved their productive performance (Beaulieu et al., 2010b; Douglas et al., 2014c).

Taken together the results from the present study and the ones from previous studies (Aymerich et al., 2020; Beaulieu et al., 2010b; Douglas et al., 2014c), it can therefore be assumed that the slow growing pigs may require higher SID Lys/AA requirements than previous established for average grower-finisher pigs (De Blas et al., 2013; NRC, 2012; PIC, 2016). Therefore, the present study suggests that requirements for pigs are different depending on the growth rate at the same age, and further research should be undertaken to assess and establish the nutrient requirements for those pigs with reduced growth rate. The scope of this study was limited in terms of number of treatments and several questions remain unanswered as for example which is the plateau where slow growing pigs achieve their maximum performance.

Despite the fact that slow growing pigs were more efficient when increasing the dietary SID AA levels, final BW and ADG were only numerically different from other slow growing pigs’ dietary treatments. This may be explained by the fact that total growth is mainly related with feed intake, while lean deposition, which highly affects feed efficiency, is more related with feed composition and total AA supply (Patience et al., 2015). Lopéz-Vergé et al., (2018b) investigated a feed management strategy for the slow growing pigs based on changing the feeds on the basis of an equivalent feed consumption to the average pigs, instead of age, during the whole grower-finisher period. With this strategy based on feeding the diets in terms of feed consumption and BW, slow growing pigs increased their growth rate and partially catch up with their big counterparts, decreasing the variability within the batch. Future research might explore the application of high specific diets and/or the accuracy in feeding management to the slow growing pigs starting from the nursery or the beginning of the grow-finisher period, giving time to this subset of pigs to partially catch up their big counterparts by fulfilling their nutrient requirements.

Noteworthy, increasing the protein diet to such levels may increase the levels of $NH_3$ and affect the environment and farm sustainability (Lee et al., 2020; Liu et al., 2015). Then, to reach the described levels of SID Lys/AA may not be feasible, although the environmental impact could be minimised by formulating diets using synthetic AAs which allows to reduce the CP levels while maintaining the nutrient requirements, performance and carcass quality (Prandini et al., 2013). Moreover, it would be crucial but challenging to identify the slow growing pigs early in life (Camp Montoro et al., 2020a) and re-grouping them in separated pens with a tailored management/nutritional strategy. Although mixing may have a negative effect on productive performance (Camp Montoro et al., 2021a), high specification diets from weaning onwards may improve the slow growing pigs' productive performance (Aymerich et al., 2020; Beaulieu et al., 2010b; Douglas et al., 2014c). Ultimately, further studies could be focused on undertaking an economic assessment of the cost/gain of the slow growing pigs fed with high specification diets to have a better knowledge of its worthy and feasibility in commercial diets.

## 4.5 Conclusion

This study set out to compare the productive performance between slow and fast growing pigs to different levels of dietary SID AA in isoenergetic diets at late grow-finisher stage. Slow growing pigs' feed efficiency is improved when dietary SID AA levels are increased from 0.92 up to 1.45% SID Lys/AA. Such a response is not present in fast growing pigs. Thus, nutrient requirements may vary depending on growth rate at the same age, and slow growing pigs may require higher dietary SID AA levels than fast growing pigs to present a better productive performance. Further work needs to be done to establish the nutrient requirements for slow growing pigs and investigate new nutritional strategies towards slow growing pigs.

# Chapter 5

# Effect of space allowance and mixing on growth performance and body lesions of grower-finisher pigs in pens with a single wet-dry feeder

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## 5.1 Background

One of the greatest challenges for pig producers is to maximise pig production efficiency and minimise housing cost, without compromising animal welfare and farm sustainability. Housing costs are the second highest cost during the grower-finisher period after feed cost (Flohr et al., 2017). A higher number of pigs per pen, reduces the housing cost per pig as pens are used more efficiently (Powell et al., 1993). However, for a given pen size, increasing the number of pigs per pen causes a reduction in the space allowance, and either a reduction in feeder space per animal in long trough systems or an increase in the ratio of animals per feeder in facilities with an ad libitum feeding arrangement (DeDecker et al., 2005). This may result in a reduction in productive performance (Carpenter et al., 2017; Kim et al., 2017; Thomas et al., 2017). Changing the number of pigs per pen may induce confounding on whether productive performance is affected by space allowance, feeder space or group size (Anil et al., 2007), although this method is the most common applied in commercial conditions. Nevertheless, recent research pointed out no effect of group size and feeder space on productive performance (Flohr et al., 2017; Schmolke et al., 2003; Wastell et al., 2018).

Kyriazakis and Whittemore (2006) reported a formula where space allowance in $m^2$ is expressed as: space allowance = $k \times BW^{0.667}$ where $k$ represents a space allowance coefficient and $BW^{0.667}$ represents the geometric conversion of BW in kg to area. Gonyou et al. (2006) reported that below 0.0336 $m^2/BW^{0.667}$ productive performance is affected. However, these thresholds are likely to change for each type of building, floor type, feeders, environmental enrichment, sex and pig's genetics (Deen, 2005; Wastell et al., 2018), and animal welfare may be compromised before performance is affected. Space allowance is part of the Welfare Quality assessment of pig production (Welfare Quality®, 2009) and it is well known that insufficient space allowance can lead to adverse social behaviours directed to pen mates, resulting in skin lesions, lameness, and tail biting (Vermeer et al., 2017). These lesions are more sensitive indicators of pig welfare (Mkwanazi et al., 2019) than growth performance. Existing research recognises the critical role played by space allowance (Anil et al., 2007; Fu et al., 2016; Vermeer et al., 2017) and mixing (Tong et al., 2020) on the number of body lesions per pig as an indicator of poor animal welfare. The physical damage induced by aggression may end affecting pig performance causing carcass condemnations and economic losses for pig producers

(Dalmau et al., 2016). Moreover, damaging behaviour may contribute to chronic stress which affects both mental and physiologic natural state of the animals (Sutherland et al., 2006), thereby having detrimental implications to the efficiency and sustainability of swine production systems (Broom, 2010).

Mixing is a common strategy used in pig production to sort pigs by weight to reduce variability and facilitate management in the grower-finisher stage, even though it has minimal impact on the final variability in individual pig weights within a pen (Anil et al., 2007; O'Quinn et al., 2001). In fact, there are indications that mixing affects productive performance by reducing ADG and ADFI (Hyun et al., 1998b, 1998a) and it also affects animal welfare as pigs show severe aggression after re-grouping in order to establish a new social hierarchy (Tong et al., 2020). Mixing is unavoidable in facilities with large groups at the finisher stage (Schmolke et al., 2003; Street and Gonyou, 2008), or it may also depend on the previous management undertake during the farrowing and nursery period (Calderón Díaz et al., 2017c). However, farrow-to-finish commercial farms with pens of 10 to 14 pigs per pen at the finishing stage, could facilitate the maintenance of intact litters from farrowing to slaughter with no mixing. Despite no references are available, such a penning arrangement with one single space wet-dry feeder is a common type of accommodation for grower-finisher pigs in Europe. Thus, studies optimizing this system for efficiency and animal health and welfare are needed.

Previous literature showed the effects of space allowance on growth performance in grower-finisher pigs (Flohr et al., 2017; Gonyou et al., 2006; Thomas et al., 2017) but the information on how mixing affects growth performance in grower-finisher pigs is scarce and little attention has been paid to whether space allowance and mixing interact with each other (Hyun et al., 1998b, 1998a). Understanding how productive performance is affected by different space allowances and mixing in each system is important for pig producers, veterinarians and advisors to make better management decisions. In the present study we hypothesised that an interaction between mixing and space allowance exists and it affects pig productive performance and animal welfare. Therefore, the aim of the present study was to investigate the effect of space allowance and mixing on growth performance and body lesions, as a proxy for aggression, in pens with a single space wet-dry feeder during the grower-finisher stage.

## 5.2 Material & Methods

### 5.2.1 Care and Use of Animals

Two experiments were conducted at the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland. Both experiments received ethical approval from the Teagasc Animal Ethics Committee (TAEC 204/2018). Danish Duroc × (Large White × Landrace) grower-finisher pigs were housed in mixed sex pens with fully slatted concrete floor (2.4 x 4.2 m) containing a single wet-dry feeder [330 mm (Width) × 370 mm (Depth) × 1000 mm (Height); MA37, Verba, Netherlands] and one supplementary nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller. Pens were enriched with a larch wood post. Pigs were fed a single soybean meal-maize-wheat based finisher diet (162.0 g of CP, 9.7 MJ/NE and 9.2 g of SID Lys per kg of feed) and remained in the facility until the first group of pigs reached 110 kg of BW and were sent to slaughter in both experiments.

In experiment 1, a total of 216 pigs were used and moved as intact litter pens to the finisher accommodation at 10 weeks of age (26.3 ± 2.26 kg BW). Pigs were assigned per pen to three different space allowances; 0.96 $m^2$/pig (10 pigs/pen; n = 6), 0.84 $m^2$/pig (12 pigs/pen; n = 6) and 0.72 $m^2$/pig (14 pigs/pen; n = 6), in a randomized design. Litter pens were adjusted to the space allowance treatments by removing pigs in case it was necessary. All space allowances were above the minimum space per pig set by European legislation based on live weight (Council of the European Union, 2008).

In experiment 2, a total of 230 pigs were used in a 2×2 factorial randomized design considering space allowance and mixing as treatments. Pigs were moved to the finisher accommodation at 11 weeks of age (34.3 ± 3.25 kg BW). Pigs were assigned to two different space allowances; 0.96 $m^2$/pig (n = 10 pens; 10 pigs/pen) and 0.78 $m^2$/pig (n = 10 pens; 13 pigs/pen), all above the minimum space per pig set by European legislation based on live weight (Council of the European Union, 2008). Mixing was applied randomly to 5 pens of each space allowance while the rest of the pens remained as litter pens. Litter pens were adjusted to the space allowance treatments by removing pigs in case it was necessary.

In both experiments, space allowances were adjusted by changing the number of pigs per pen as it would happen in field conditions. Space allowance coefficient (*k*) for each treatment were calculated using the formula space allowance = $k \times \text{BW}^{0.667}$ (Kyriazakis and Whittemore, 2006) and are reported in Table 5.1.

**Table 5.1** Initial and final space allowance coefficient (k) for each treatment in experiment 1 and 2.

| | Experiment 1 | | | Experiment 2 | |
|---|---|---|---|---|---|
| Number of pigs/pen | 10 | 12 | 14 | 10 | 13 |
| Space allowance, $m^2$/pig | 0.96 | 0.84 | 0.72 | 0.96 | 0.78 |
| Space allowance coefficient, $k$ [1] | | | | | |
| Initial [2] | 0.110 | 0.095 | 0.079 | 0.090 | 0.074 |
| Final | 0.042 | 0.037 | 0.031 | 0.042 | 0.034 |

[1] The allometric expression of the space coefficient is as follows: $k$ = Space allowance ($m^2$) / $\text{BW}^{0.667}$ (kg).

[2] Initial body weight in experiment 1 was: 25.6 ± 1.38 kg (0.96 $m^2$/pig), 26.1 ± 1.38 kg (0.84 $m^2$/pig) and 27.4 ± 1.38 kg (0.72 $m^2$/pig). Initial body weight in experiment 2 was: 34.6 ± 0.95 kg (0.96 $m^2$/pig) and 34.1 ± 0.95 kg (0.78 $m^2$/pig).

### 5.2.2 Measurements

#### *5.2.2.1 Body Weight, Feed Intake and Feed Efficiency Traits*

In both experiments, pigs were weighed per pen and BW was recorded every two weeks until the first group of pigs reached 110 kg of BW and were sent to slaughter. Average daily gain was calculated for every 2 weeks interval. Feed intake was recorded daily at a pen level, added for every 2 week period and ADFI was calculated. Feed conversion ratio was calculated as for each 2-week period.

#### *5.2.2.2 Pen Efficiency*

Overall pen efficiency was calculated for each treatment in both experiment 1 and 2. Pen efficiency was calculated as $\frac{\text{kg daily gain}}{\text{sq m space}}$.

#### *5.2.2.3 Body Lesion Counts*

Following the Welfare Quality® criteria (Welfare Quality®, 2009), the body of the pigs was divided into anterior, mid and posterior part. Body lesion was defined as either surface penetration of the epidermis or penetration of the muscle tissue (Welfare Quality®, 2009). Then, all skin lesions in each location were counted individually as body lesions and recorded on a check sheet (Welfare Quality®, 2009). In experiment 1, body lesions were counted at 20 weeks of age before pigs started to go to slaughter. In experiment 2, body lesions were counted at 12, 16 and 21 weeks of age.

### 5.2.3 Statistical Analyses

All data were analysed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Each pen was considered as the experimental unit for all data analyses. In experiment 1, the models included space allowance as fixed effects. In experiment 2, models included space allowance, mixing and their interaction as fixed effects. Models for BW, ADG, ADFI, FCR and pen efficiency were analysed using general linear mixed model accounting for repeated measurements in both experiments. Initial BW was used as a covariable for BW, ADG, ADFI and FCR. Body lesions were analysed using a generalized linear mixed model in both experiments. Difference between treatment groups on body lesions were calculated as $\frac{\text{Group A } - \text{ Group B}}{\text{Group B}}$. Multiple means comparisons were done using Tukey-Kramer's correction in all cases. Alpha level for determination of significance was 0.05 and trends were identified as alpha of 0.10. Results for fixed effects are reported as least square means ± standard error mean.

## 5.3 Results

### 5.3.1 Body Weight, Feed Intake and Feed Efficiency Traits

Final BW, ADG, ADFI and FCR from 10 to 20 weeks of age, were not affected by space allowance in experiment 1 (*P* > 0.05; Table 5.2); although pigs with 0.96 $m^2$/pig were numerically heavier and had lower FCR than those pigs with 0.84 and 0.72 $m^2$/pig by the end of the trial.

**Table 5.2** Effect of space allowance on productive performance and body lesion counts in experiment 1.[1]

| | Space Allowance, $m^2$/pig [2] | | | | |
|---|---|---|---|---|---|
| Trait | 0.96 | 0.84 | 0.72 | SEM | *P*-value |
| BW, kg, 20 wk | 103.5 | 100.6 | 99.8 | 1.38 | 0.162 |
| ADG, g | 1211.8 | 1155.0 | 1141.0 | 35.88 | 0.396 |
| ADFI, g | 2566.2 | 2558.9 | 2580.2 | 70.34 | 0.979 |
| FCR | 2.15 | 2.20 | 2.26 | 0.07 | 0.578 |
| Body lesions [3] 20 wk | | | | | |
| Anterior | 2.0 $^b$ | 2.3 $^b$ | 4.1 $^a$ | 0.40 | < 0.001 |
| Mid | 1.0 | 1.0 | 1.6 | 0.26 | 0.263 |
| Posterior | 0.8 $^b$ | 1.1 $^{ab}$ | 1.7 $^a$ | 0.23 | 0.021 |
| Total | 3.8 $^b$ | 4.3 $^b$ | 7.5 $^a$ | 0.78 | 0.003 |

[1] Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and body lesion counts from 216 grow-finisher pigs (6 pens/treatment; Least square means ± Standard error mean [SEM]) from 10 to 20 weeks of age, when the first group of pigs reached 110 kg of BW and were sent to slaughter.

[2] 0.96 $m^2$/pig = 10 pigs/pen; 0.84 $m^2$/pig = 12 pigs/pen; 0.72 $m^2$/pig = 14 pigs/pen.

[3] Mean of the total number of body lesions counted at anterior, mid, posterior and total body regions on both sides of the body.

[a,b] Within rows, significant differences between groups (*P* < 0.05).

Final BW showed an interaction between space allowance and mixing in experiment 2. Non-mixed pigs with 0.78 $m^2$/pig were 6.1 and 6.5 kg heavier than mixed pigs with 0.96 and 0.78 $m^2$/pig at 21 weeks of age ($P < 0.001$; Table 5.3). This interaction was not present for ADG, ADFI or FCR. Non-mixed pigs gained 74 g more per day ($P = 0.004$) and consumed 101.8 g more of feed per day ($P = 0.007$) than mixed pigs from 11 up to 21 weeks of age (Table 5.3). Non-mixed pigs tended to have lower FCR compared to mixed pigs from 11 to 21 weeks of age ($P = 0.079$; Table 5.3).

**Table 5.3** Effect of space allowance × mixing on productive performance and body lesion counts in experiment 2.[1]

| | Space Allowance [2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.96 $m^2$/pig | | 0.78 $m^2$/pig | | | *P*-value | | |
| Trait | Mixed | Non-Mixed | Mixed | Non-Mixed | SEM | Mixing | Space Allowance | Interaction |
| BW, kg, 21 wk | 102.1 $^{b}$ | 106.4 $^{ab}$ | 101.7 $^{b}$ | 108.2 $^{a}$ | 0.95 | < 0.001 | 0.472 | < 0.001 |
| ADG, g | 983.0 | 1034.1 | 955.4 | 1052.3 | 21.82 | 0.004 | 0.836 | 0.309 |
| ADFI, g | 2150.3 | 2222.3 | 2125.6 | 2257.1 | 32.57 | 0.007 | 0.880 | 0.374 |
| FCR | 2.18 | 2.12 | 2.19 | 2.11 | 0.04 | 0.079 | 0.974 | 0.692 |
| Body lesions [3] 12 wk | | | | | | | | |
| Anterior | 4.2 | 4.0 | 6.8 | 7.3 | 0.70 | 0.960 | < 0.001 | 0.622 |
| Mid | 2.2 | 2.3 | 2.7 | 2.5 | 0.47 | 0.903 | 0.457 | 0.777 |
| Posterior | 1.0 | 0.7 | 1.1 | 1.1 | 0.28 | 0.590 | 0.371 | 0.505 |
| Total | 7.6 | 7.0 | 10.8 | 10.9 | 1.23 | 0.828 | 0.004 | 0.748 |
| Body lesions 16 wk | | | | | | | | |
| Anterior | 3.8 | 3.7 | 5.6 | 5.3 | 0.71 | 0.801 | 0.019 | 0.894 |
| Mid | 1.8 | 3.0 | 3.6 | 3.4 | 0.56 | 0.210 | 0.038 | 0.119 |
| Posterior | 2.2 | 1.9 | 2.9 | 2.8 | 0.36 | 0.600 | 0.021 | 0.641 |
| Total | 7.7 | 8.6 | 12.0 | 11.4 | 1.51 | 0.886 | 0.018 | 0.598 |
| Body lesions 21 wk | | | | | | | | |
| Anterior | 2.6 | 2.0 | 4.2 | 4.0 | 0.59 | 0.380 | 0.002 | 0.497 |
| Mid | 0.8 | 1.0 | 2.3 | 1.8 | 0.39 | 0.983 | 0.003 | 0.410 |
| Posterior | 0.6 | 1.0 | 2.0 | 1.8 | 0.36 | 0.569 | 0.001 | 0.316 |
| Total | 4.1 | 3.9 | 8.6 | 7.5 | 1.21 | 0.622 | < 0.001 | 0.834 |

[1] Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and body lesion counts (Least square means ± Standard error mean [SEM]) from 230 grow-finisher pigs in 20 pens grouped by space allowance × mixing from 11 up to 21 weeks of age, when the first group of pigs reached 110 kg of BW and were sent to slaughter.

[2] 0.96 $m^2$/pig = 10 pigs/pen; 0.78 $m^2$/pig = 13 pigs/pen.

[3] Mean of the total number of body lesions counted at anterior, mid, posterior and total body regions on both sides of the body.

[a,b] Within rows, significant differences between groups ($P < 0.05$).

### 5.3.2 Pen Efficiency

Pen efficiency increased by reducing space allowance during the grower-finisher period in both experiments ($P < 0.001$). Pigs wit 0.72 m$^2$/pig had higher overall pen efficiency compared to those pigs with 0.96 and 0.84 m$^2$/pig in experiment 1 ($P < 0.001$; Figure 5.1). Moreover, in experiment 2, non-mixed pigs with 0.78 m$^2$/pig had higher overall pen efficiency than mixed pigs with 0.78 m$^2$/pig ($P = 0.049$) and non-mixed and mixed pigs with 0.96 m$^2$/pig ($P < 0.001$; Figure 5.2). Also, mixed pigs with 0.78 m$^2$/pig had higher overall pen efficiency than non-mixed and mixed pigs with 0.96 m$^2$/pig ($P < 0.05$; Figure 5.2).

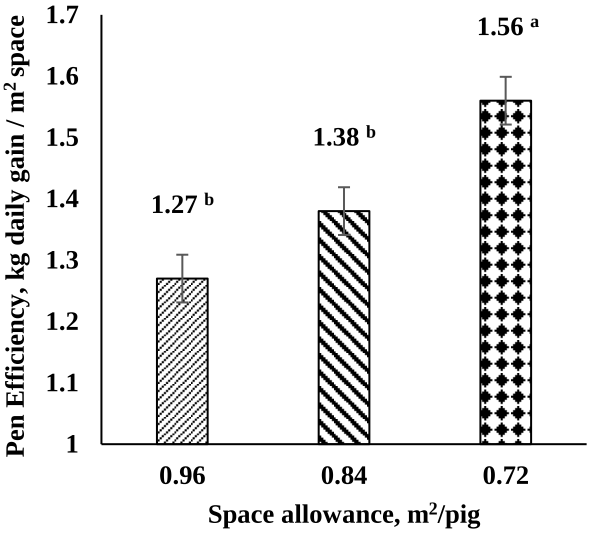

**Figure 5.1** Pen efficiency of the space allowance treatments from 10 to 20 weeks of age in experiment 1. $^{a, b}$ Significant differences between treatments ($P < 0.05$).

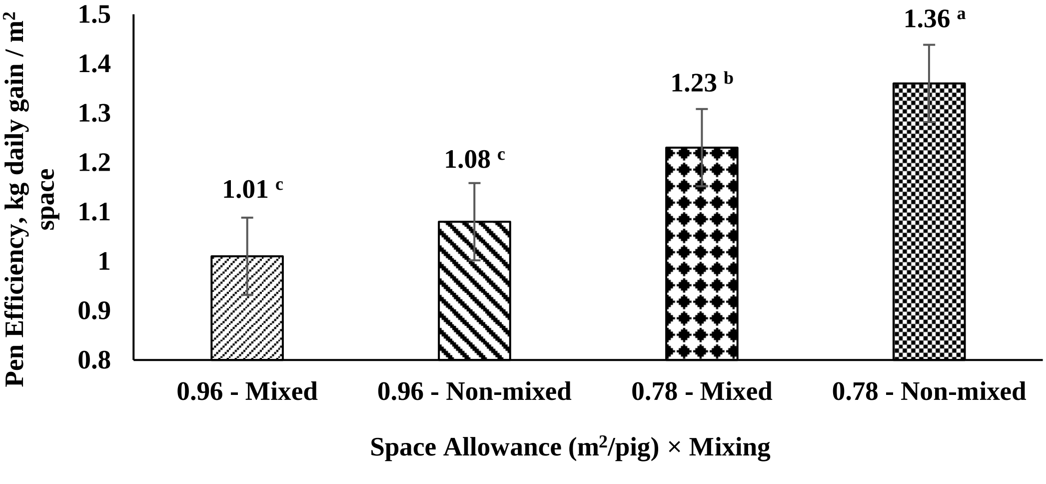

**Figure 5.2** Pen efficiency of the space allowance × mixing treatments from 11 to 21 weeks of age in experiment 2. $^{a, b}$ Significant differences between treatments ($P < 0.05$).

### 5.3.3 Body Lesion Counts

Body lesion counts were higher at the lower space allowances at 20 weeks of age in experiment 1 (Table 5.2). Pigs with 0.72 $m^2$/pig had 78.3% and 105.0% more lesions than those pigs with 0.84 and 0.96 $m^2$/pig on the anterior body region respectively ($P < 0.001$). There was no difference in counts on the mid body region between space allowances ($P > 0.05$). On the posterior body region, pigs with 0.72 $m^2$/pig had 112.5% more lesions than pigs with 0.96 $m^2$/pig ($P = 0.021$), although no differences were observed between pigs with 0.84 $m^2$/pig and the other groups ($P > 0.05$). In total, pigs with 0.72 $m^2$/pig had 74.4% and 97.4% more lesions than those pigs with 0.84 and 0.96 $m^2$/pig, respectively ($P < 0.01$). In experiment 2 (Table 5.3), body lesion counts were higher at lower space allowances, however no mixing and interaction effect were observed ($P > 0.05$). Pigs with 0.78 $m^2$/pig had 72% more lesions on the anterior body region ($P < 0.001$) and 48.6% more lesions in total ($P = 0.004$) than pigs with 0.96 $m^2$/pig at 12 weeks of age. There was no difference in the mid and posterior body region between space allowance treatments at 12 weeks of age ($P > 0.05$). At 16 weeks of age, pigs with 0.78 $m^2$/pig had 45.3, 45.8, 39.0 and 43.6% more lesions than pigs with 0.96 $m^2$/pig on the anterior, mid, posterior and total body region respectively ($P < 0.05$). Pigs with 0.78 $m^2$/pig had 78.3, 127.8 and 137.5% more lesions than pigs with 0.96 $m^2$/pig on the anterior, mid and posterior body region respectively at 21 weeks of age ($P < 0.01$). In total, pigs with 0.78 $m^2$/pig had 101.3% more lesions than pigs with 0.96 $m^2$/pig at the end of the trial ($P < 0.001$). Body lesion counts decreased from 16 to 21 weeks of age in all treatments ($P < 0.05$).

## 5.4 Discussion

Small group pens with 10-14 grower-finisher pigs are convenient from a management point of view because they allow for a rapid monitoring of health and welfare issues in pigs, without the need to access the pen. This pen system is normally linked to wet-dry feeders as these optimize feed efficiency (O’Meara et al., 2020). Despite no references are available, the authors’ experience in groups like EUPIG (https://www.eupig.eu/) or the ECPHM suggests that this is one of the most common systems in growing-finishing units in the EU. To understand how space allowance and mixing influence growth performance and welfare of pigs in this system, pigs were subjected to three space allowances (0.96, 0.84 and 0.72 $m^2$/pig) in a first trial, and two space allowances (0.96 and 0.78 $m^2$/pig) combined with mixing in the second trial. These space allowances were chosen above the 0.65 $m^2$/pig minimum set by European legislation based on live weight (Council of the European Union, 2008) which is already criticised from a welfare point of view because of very low amount of shared space (Averós et al., 2013). The space allowances were adjusted by changing the number of pigs per pen as it would be observed in current field situations. This fact could induce confounding on whether growth performance is affected by space allowance, feeder space or group size (Anil et al., 2007). However, these factors are usually confused in any commercial conditions. Nevertheless, Schmolke et al. (2003) observed no detrimental effect on growth performance of pigs housed at 10, 20, 40 and 80 pigs per pen with a space allowance of 0.76 $m^2$/pig and one single wet-dry feeder provided for every 10 pigs. Moreover, Flohr et al. (2017) also reported no effect of group size on ADG, ADFI and FCR in similar conditions to the present study. This results suggests that group size would not affect productive performance in the present study. Restricted feeder space could also impact growth performance (DeDecker et al., 2005). Wastell et al. (2018) compared group sizes of 20 and 26 pigs per pen and did not find any detrimental effect on growth performance with 10 pigs per wet-dry feeder space compared to 13 pigs per wet-dry feeder space considering a space allowance of 0.65 or 0.78 $m^2$/pig. This results suggest that feeder space would not affect growth performance in the present study. Hence, the present study discusses space allowance as the main factor to affect growth performance regarding the pen system studied.

In terms of space allowance, previous studies observed that decreasing space allowance resulted in a poorer growth performance in pigs with space allowances of 0.65 $m^2$/pig and similar slaughter weights (Kim et al., 2017; Thomas et al., 2017; Wastell et al., 2018) or 0.80 $m^2$/pig when marketed to slaughter weights up to 138 kg of BW (Johnston et al., 2017). Overall, all these studies can be compared using the allometric approach expressing space allowance as a coefficient ($k$) (Kyriazakis and Whittemore, 2006). Gonyou et al. (2006) stated that the critical $k$-value below which growth performance is affected as space allowance is further decreased, ranges from 0.0317 to 0.0348 over all data sets analysed using a broken-line analysis. In our study, pigs with 0.72 and 0.78 $m^2$/pig reached the critical $k$-value by the end of the trial when the first group of pigs reached the marketed weight (i.e. 110 kg of BW) and were sent to slaughter. Thus, the growth performance of the pigs would not be compromised during the grower-finisher period if space allowance is established based on the critical *k*-value at the marketed weight.

Mixing affected growth performance during the whole grower-finisher period in experiment 2. The drop in ADG and ADFI is consistent with previous literature which observed that mixed pigs had decreased ADG and ADFI when they were followed in a 4 week experiment at the beginning of the grower-finisher period (Hyun et al., 1998b, 1998a). Stookey and Gonyou (1994) also observed a depressed ADG in mixed pigs after being mixed for a 2 week period when they had 83 kg of BW. The present study showed that mixing causes a severe effect on growth performance in currently modern facilities and genetics, and strategies to avoid mixing or mitigate it are an important issue for future research (Peden et al., 2019, 2018).

The underlying hypothesis in this study was that space allowance and mixing interact with each other in current field situations. The study found that mixing effect on final BW is exacerbated at lower space allowances (i.e. 0.78 $m^2$/pig). However, this interaction did not show up in any of the other variables and should be checked for repeatability in further experiments. Hyun et al. (1998b) reported that when pigs are subjected to multiple concurrent environmental stressors such as high ambient temperature, regrouping and low space allowance, the final effect over productive performance is additive.

In terms of animal welfare, the current study found that the number of body lesions increased at lower space allowances. This finding is in accordance with previous literature which reported a strong relationship between space allowance and body lesions (Anil et al., 2007; Fu et al., 2016; Vermeer et al., 2017). Anil et al. (2007) stated that animal welfare is enhanced at higher space allowances in terms of postural behaviour, lower injuries and aggression. Space allowance affected the number of body lesions during the whole grower-finisher period. Nevertheless, the number of body lesions decreases as the pigs get heavier which is in accordance with previous studies (Meyer-Hamme et al., 2016). A possible explanation for this might be related to the pigs' experience and ability to adapt to their social environment and being in a stable group for a long time which benefits the long term welfare of the pigs (Godyń et al., 2019; Tennessen, 1989). The present study raises the possibility that there is a threshold between 0.78 and 0.84 $m^2$/pig which an increase in the number of body lesions due to space allowance is observed. Still, the number of body lesions as a proxy for aggression may vary because of other factors not controlled in the present study, and moreover, it could also be related to the pen design and the wet-dry feeder space per pig.

One interesting finding was that highest body lesion counts were seen in the anterior body region which is consistent with fighting for access to the feeder (Botermans and Svendsen, 2000). Single space wet-dry feeders may allow to accommodate a high number of pigs per feeder space without having a detrimental effect on growth performance (Gonyou and Lou, 2000; Wastell et al., 2018). However, López-Vergé et al. (2018b) observed that pigs allotted to more feeder spaces had low body lesion counts and tended to have low BW variability within pen by the end of the grower-finisher period.

The results provided in the present study indicate that animal welfare may be compromised before growth performance is affected. Averós et al. (2010) suggested a critical *k*-value of 0.039 for lying behaviour with a broken-line analysis. This *k*-value is higher than the 0.0336 reported by Gonyou et al. (2006) below which productive performance is affected. High number of body lesions caused by competition or aggression, are likely associated with detrimental implications for pig health and performance due to immunosuppression caused by the social stress (De Groot et al., 2001; Gimsa et al., 2018; Martínez-Miró et al., 2016). This fact may be exacerbated in farms that have more infectious diseases in comparison to the farm where the trial was performed, which is free of the main infectious diseases. Ultimately, compromised animal welfare has detrimental implications to the sustainability of the swine production system (Broom, 2010).

Mixing pigs leads to agonistic social behaviour mainly within the first 24 h (Turner et al., 2017). However, the current study observed that the number of body lesions due to aggression in mixed groups is the same as the non-mixed groups after one week of being mixed. This finding is consistent with previous studies which may be explained by the establishment of the social hierarchy (Driessen et al., 2020; Foister et al., 2018; Wurtz et al., 2017). Nevertheless, mixed pigs showed a poor growth performance compared to their counterparts in the study. This could be related to the social network properties and chronic stress that are not shown in body lesions (Desire et al., 2015; Foister et al., 2018; Sutherland et al., 2006).

Regarding pen efficiency, this study supports evidence from previous observations (Anil et al., 2007) which showed that pigs in lower space allowances had higher overall pen efficiency. In addition, pen efficiency showed an interaction between space allowance and mixing which indicates that pen efficiency in lower space allowance may be affected when pigs are mixed. These results may encourage pig producers to seek optimal space allowances to optimize overall efficiency and reduce housing costs. Nevertheless, the present study also observed that pig welfare is aggravated in low space allowances. This and if other environmental stressors such as high ambient temperature, mixing or the farm sanitary status are considered, pig performance and pig producer's income may be mitigated even with improved pen efficiency. Therefore, further studies considering economic analyses on how environmental stressors affect pig performance and welfare in different space allowances are needed.

## 5.5 Conclusion

This study provides a deeper insight into how space allowance and mixing affect growth performance and animal welfare in pens of 10-14 grower-finisher pigs with one single wet-dry feeder. Mixing appears to have a considerable effect on growth performance although the number of body lesions is not affected once social hierarchy is established. Strategies to avoid or mitigate it are recommended in current field situations with a similar pen design. Space allowance will not compromise growth performance if it is established based on the critical *k*-value at the marketed weight. Nevertheless, the increase on the number of body lesions in lower space allowances indicates that 0.72 and 0.78 $m^2$/pig are detrimental to the welfare of pigs in single wet-dry feeder pens despite being compliant with the EU legislation. Animal welfare is affected before productive performance. Then, farmers should take it into account to maximise growth performance and overall efficiency of the facility without sacrifice animal welfare as a market concept.

# Chapter 6

# Effect of phase feeding, space allowance and mixing on productive performance of grower-finisher pigs

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## 6.1 Background

Adjusting space allowance, mixing and phase feeding are common strategies that have been used over the years in pig production to facilitate management and reduce production costs. Space allowance plays a critical role in pig production from a productive performance, animal welfare and economic standpoint (Powell et al., 1993; Thomas et al., 2017; Vermeer et al., 2014). Increasing the number of pigs per pen is correlated with a decrease in the facility cost per pig produced (Powell et al., 1993), which is the second most expensive cost after feed cost in the grower-finisher period (Flohr et al., 2017). However, increasing the number of pigs per pen decreases the space allowance and feeder space per animal and pig performance could be affected with a decrease in ADG, driven by a decrease on ADFI (Carpenter et al., 2017; Kim et al., 2017; Thomas et al., 2017). Space allowance is normally expressed as $m^2$/pig although it changes as pigs grow (Gonyou et al., 2006). Then, space allowance in $m^2$ can be expressed using the following formula: space allowance = $k \times BW^{0.667}$, where $k$ is a coefficient and $BW^{0.667}$ represents the geometric conversion of BW in kg to area (Kyriazakis and Whittemore, 2006). Previous research reported a critical *k*-value of 0.0336, below which productive performance is affected (Gonyou et al., 2006). Nevertheless, recent studies reported that performance may be affected above the 0.0336 *k*-value (Carpenter et al., 2017; Thomas et al., 2017; Wastell et al., 2018). A minimum space per pig ($m^2$/pig) is required by the EU legislation (Council of the European Union, 2008), which varies for each physiological phase and it must be of 0.65 $m^2$/pig at least when pigs weigh between 85 to 110 kg of BW, in order to improve and guarantee a minimum animal welfare. With the current societal concerns regarding animal welfare, pig producers have to maximize production efficiency and minimize facility cost, while at the same time, meeting animal welfare and farm sustainability requirements.

Mixing is a common management strategy used to sort pigs by weight in order to decrease BW variability and facilitate farm management practices. However, previous research stated that mixing fails to reduce final BW variability in individual pig weights within a pen (Anil et al., 2007; O’Quinn et al., 2001), and that it may affect growth performance (Camp Montoro et al., 2021a; Hyun et al., 1998b, 1998a) and pig welfare due to the establishment of a new social hierarchy after re-grouping (Foister et al., 2018).

Phase feeding is another common management strategy used in pig production. Grower-finisher feeding programs normally include between one to four diets. NRC (2012) recommends two growing and two finishing feeding phases. However, Garry et al. (2007) found no differences in growth performance between using one diet or two to four diets in Irish pig farms. On the other hand, recent research pointed out that phase feeding reduces the feed cost and improves growth performance as it matches the pig's nutrient requirements that change as a consequence of age or size (Hong et al., 2016; Pomar et al., 2014). Consequently, feed efficiency is improved, which leads to better farm sustainability because of reduced ammonia emissions, as the requirements of CP are reduced by age (Andretta et al., 2016; Hong et al., 2016; Pomar et al., 2014). Menegat et al. (2020) stated that it is feasible to simplify phase feeding up to two dietary phases considering the compensatory growth capability in grower-finisher pigs. The latter would maximize feed efficiency utilization and facilitate feed management.

Previous studies reported findings on the effects of space allowance and phase feeding on growth performance in grower-finisher pigs (Flohr et al., 2017; Menegat et al., 2020; Thomas et al., 2017). However, very little attention has been paid to the role of mixing on productive performance in grower-finisher pigs over the last two decades (Camp Montoro et al., 2021a; Hyun et al., 1998a, 1998b). Moreover, there has been little discussion about how space allowance and mixing or whether space allowance and phase feeding interact with each other in field conditions (Camp Montoro et al., 2021a; Hyun et al., 1998b). Knowing how these common management strategies affect productive performance, would guide farmers, veterinarians, and advisors to make better management decisions in pig production.

We hypothesized that, for grower-finisher pigs, space allowance and mixing would have an effect on pig productive performance, and that phase feeding would result in a better feed efficiency and similar growth performance to non-phase feeding. Therefore, the present study aimed to investigate and quantify the effect of space allowance, mixing and phase feeding on productive performance in single wet-dry feeder pens during the grower-finisher stage.

## 6.2 Material & Methods

### 6.2.1 Animals, Experimental Design and Diets

Three trials were conducted at the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland. All trials received ethical approval from the Teagasc Animal Ethics Committee (TAEC 204/2018). A schematic illustration of the experimental designs for trials 1, 2 and 3 is provided in Figure 6.1.

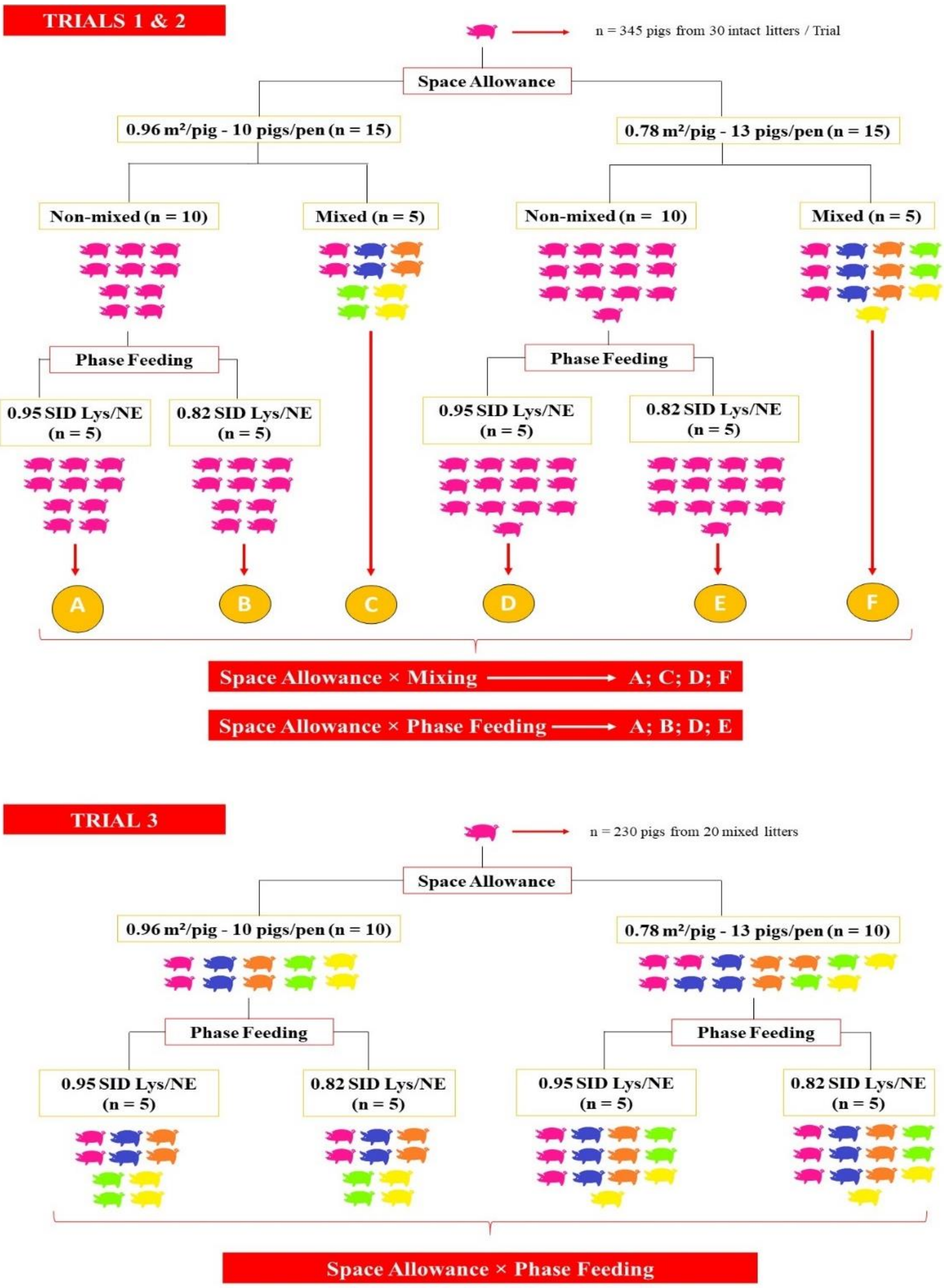

**Figure 6.1** Schematic illustration of the experimental design in trial 1, 2 and 3. In trials 1 and 2, experimental design was a two 2×2 factorial arrangement with space allowance and mixing as treatments and space allowance and phase feeding as treatments. In trial 3, experimental design was a 2×2 factorial arrangement with space allowance and phase feeding as treatments, and mixing was applied to all the pens. Space allowances were above the minimum space per pig set by European legislation based on live weight (Council of the European Union, 2008).

Trials 1 and 2 had the same experimental design. Danish Duroc × (Large White × Landrace) pigs born within one week (Birth weight = 1.46 ± 0.28 kg; average litter size = 15.2 ± 4.0; 9.7% piglets with <1 kg birth weight) were followed per pen as intact litters from birth until 11 weeks of age. Pigs were weaned at approximately 28 days and received a starter diet (200.0 g CP, 12.3 MJ/NE and 14.0 g SID Lys per kg of feed) for seven days, link diet (190.0 g CP, 11.0 MJ/NE and 12.8 g SID Lys per kg of feed) for 18 days and soybean meal–barley–wheat based nursery diet (178.0 g CP, 10.6 MJ/NE and 10.4 g SID Lys per kg of feed) for 28 days. At 11 weeks of age, pigs were moved to the grower-finisher stage. Pigs were housed in pens with fully slatted concrete floor (2.4 × 4.2m) containing one wet-dry feeder [330mm (Width) × 370mm (Depth) × 1000mm (Height); MA37, Verba, Netherlands] and one nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was automatically controlled by a Big Dutchman 135pro ventilation controller (Vechta, Germany), with water heating, air intake via a perforated ceiling, and mechanical exhaustion of stale air via a fan. Temperature in the finisher accommodation ranged from 17 °C and 21 °C. Artificial lighting (LED) was provided at a minimum light intensity level of no less than 40 Lux and typically averaging 140–160 Lux for a minimum continuous period of eight hours per day. Lighting was provided between 07:00 a.m.–18:00 p.m. every day to coincide with natural day light. Pens were enriched with a 1.20 m fixed larch wood post on one of the walls without impairing on the available floor space. Pigs were fed a soybean meal-maize-wheat based grower-finisher diet (161.8 g CP, 9.67 MJ/NE and 9.2 g SID Lys per kg of feed; Table 6.1) in both trials.

When moved to the grower-finisher stage at 11 weeks of age, pigs (N = 345 pigs, 34.4 ± 3.96 kg of BW in Trial 1; N = 345 pigs, 31.2 ± 4.20 kg of BW in Trial 2) were weighed per pen and were randomly assigned to two different space allowances balanced by BW: 0.96 $m^2$/pig (n = 15 pens; 10 pigs/pen) and 0.78 $m^2$/pig (n = 15 pens; 13 pigs/pen). Litter pens were adjusted to the space allowance treatments by removing pigs in case it was necessary, keeping the same average BW per pen and similar standard deviation, and balanced per sex. Both space allowances were above the minimum space per pig (0.65

$m^2$/pig) set by the European legislation based on live weight (Council of the European Union, 2008). The 15 pens in each space allowance treatment were arranged as follows (Figure 6.1):

- Five pens were left as intact litters and fed a single finisher diet (0.95 g/MJ SID Lys:NE) up to slaughter.
- Five pens were mixed balancing by weight and sex and fed a single finisher diet (0.95 g/MJ SID Lys:NE) up to slaughter. This resulted in a 2 × 2 factorial arrangement with space allowance and mixing as main factors.
- Five pens were left as intact litters and phase feeding was applied with a second diet (0.82 g/MJ SID Lys:NE) introduced at 15 weeks of age in trial 2 (50.6 ± 5.93 kg of BW) and at 16 weeks of age in trial 1 (66.2 ± 5.65 kg of BW). This resulted in a 2 × 2 factorial arrangement with space allowance and phase feeding as main factors.

Table 6.1 shows the composition of the diets in both trials. Diets were formulated to meet or exceed the minimum requirements established by the standard nutritional tables (NRC, 2012). Pigs remained in the facility until they reached at least 110 kg of BW when they were sent to slaughter. Recording of the performance data was terminated when the first group of pigs went to slaughter.

In trial 3, Danish Duroc × (Large White × Landrace) grower-finisher pigs (N = 230) born within one week were moved to the grower-finisher stage at 11 weeks of age (32.9 ± 5.92 kg of BW), mixed, balanced by BW and sex, and randomly assigned to the same two space allowances (0.78 and 0.96 $m^2$/pig; n = 10 pens) as in trials 1 and 2. Pigs received the same management from birth until 11 weeks of age as in the previous trials. Mixing criteria and housing conditions were also the same as in the previous trials. Within each space allowance, pens were allocated to a single diet or phase feeding, leading to a 2 × 2 factorial arrangement considering space allowance and phase feeding. The diets used in trial 3 were the same as in trials 1 and 2 (Table 6.1). The second diet in the case of phase feeding was introduced at 15 weeks of age (53.7 ± 9.02 kg of BW). Pigs remained in the facility until they reached at least 110 kg of BW when they were sent to slaughter. Recording of the performance data was terminated when the first group of pigs went to slaughter.

In all trials, space allowance coefficient (*k*) for each treatment were calculated using the formula space allowance = $k \times BW^{0.667}$ (Kyriazakis and Whittemore, 2006). The *k*-values are reported in Table 6.2.

**Table 6.1** Composition on an as-fed basis of the experimental diets.

| | Diets | |
|---|---|---|
| | High SID Lys:NE ratio [1] | Low SID Lys:NE ratio [2] |
| Ingredients, g/kg | | |
| Wheat | 435.0 | 435.0 |
| Corn | 300.0 | 351.0 |
| Soybean meal 48 | 171.0 | 120.0 |
| Soybean hulls | 71.0 | 71.0 |
| Calcium carbonate | 11.0 | 11.0 |
| Dicalcium phosphate anhydrous | 1.00 | 1.00 |
| Sodium chloride | 3.00 | 3.00 |
| L-Lysine HCl | 3.75 | 3.75 |
| L-Threonine | 1.70 | 1.70 |
| DL-Methionine | 0.93 | 0.93 |
| L-Tryptophan | 0.15 | 0.15 |
| Vitamin and trace mineral mixture [3] | 1.47 | 1.47 |
| Calculated Composition [4], % as fed or as specified | | |
| Dry Matter | 87.34 | 87.26 |
| NE, MJ/kg | 9.67 | 9.83 |
| Crude Protein | 16.18 | 14.28 |
| Total Lys | 1.03 | 0.90 |
| SID Lys | 0.92 | 0.80 |
| SID Thr | 0.64 | 0.58 |
| SID Met | 0.31 | 0.29 |
| SID Trp | 0.17 | 0.15 |
| Fat | 2.23 | 2.33 |
| Crude Fibre | 5.06 | 4.87 |
| NDF | 14.60 | 14.51 |
| Calcium | 0.58 | 0.57 |
| Digestible Phosphorus | 0.23 | 0.22 |
| SID Lys:NE, g/MJ | 0.95 | 0.82 |

[1] Higher ratio SID Lys diet was given to the pigs all the way or during 11 to 15-16 weeks of age to the phase feeding treatment group.

[2] Lower ratio SID Lys diet was given to the phase feeding treatment group from 15-16 weeks of age until slaughter.

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin D3, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin B12, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin B1, 3 mg Vitamin B6.

[4] The calculated composition is based on reference data provided by INRAE. NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fibre.

**Table 6.2** Initial and final space allowance coefficient (*k*) for each treatment in trial 1, 2 and 3.

| | Trial 1 | | Trial 2 | | Trial 3 | |
|---|---|---|---|---|---|---|
| Number of pigs/pen | 10 | 13 | 10 | 13 | 10 | 13 |
| Space allowance, $m^2$/pig | 0.96 | 0.78 | 0.96 | 0.78 | 0.96 | 0.78 |
| Space allowance coefficient, *k* [1] | | | | | | |
| Initial [2] | 0.090 | 0.074 | 0.098 | 0.078 | 0.093 | 0.077 |
| Final [3] | 0.043 | 0.035 | 0.044 | 0.036 | 0.044 | 0.036 |

[1] The allometric expression of the space coefficient is as follows: $k$ = Space allowance ($m^2$) / $BW^{0.667}$ (kg).
[2] Initial body weight in trial 1 was: 34.6 ± 3.76 kg (0.96 $m^2$/pig), 34.1 ± 4.12 kg (0.78 $m^2$/pig). In trial 2 was: 30.8 ± 4.00 kg (0.96 $m^2$/pig) and 31.5 ± 4.36 kg (0.78 $m^2$/pig). In trial 3 was: 33.3 ± 6.00 kg (0.96 $m^2$/pig) and 32.5 ± 5.84 kg (0.78 $m^2$/pig).
[3] Final space allowance coefficient was predicted based on the average pen weight when the first group of pigs reached 110 kg of BW and were sent to slaughter.

### 6.2.2 Measurements

#### *6.2.2.1 Body Weight, Feed Intake and Feed Efficiency Traits*

Pigs were weighed per pen every two weeks until pigs started to go to slaughter at 21 weeks of age when the first pigs reached the target slaughter weight, 110 kg. Feed intake was recorded daily at pen level. Average daily gain, ADFI and FCR were calculated for every 2 weeks interval.

#### *6.2.2.2 Carcass Traits*

Cold carcass weight (CCW), fat thickness (FT), muscle content (MC) and percentage of lean meat % (LM%) were recorded by the slaughterhouse personnel. Cold carcass weight, FT and MC were measured immediately after the processing line. Fat thickness and MC were measured using a Fat-O-Meat'er (Carometec Food Technology, Carometec A/S, Hasselunden 9, Smørum, Denmark). Percentage of lean meat was calculated according to the formula established by the European Communities Pig Carcass Grading Amendment Regulations (Statutory Instruments, 2001):

$$\%\,\text{lean meat} = 60.30 - (0.847 \times \text{fat thickness}) + (0.147 \times \text{muscle})$$

### 6.2.3 Statistical Analyses

All data were analyzed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). In trial 1 and trial 2, the model included space allowance, phase feeding or mixing, and their interaction as fixed effects. In trial 3, the model included space allowance, phase feeding, and their interaction as fixed effects. Initial BW was used as a co-variable for BW, ADG, ADFI and FCR, and each pen was considered as the experimental unit. Data were analyzed using general linear mixed models, accounting for repeated measurements in all trials. In terms of carcass traits, predicted variables included LM%, FT and MC, and each pen was considered as the experimental unit. In trial 1 and trial 2, model included age at slaughter, CCW, space allowance, phase feeding or mixing, and the space allowance × phase feeding or mixing interaction as fixed effects. In trial 3, model included age at slaughter, CCW, space allowance, phase feeding, and the space allowance × phase feeding interaction as fixed effects. Data were analyzed using general linear models. Multiple means comparisons were carried out using Tukey-Kramer's correction. Alpha level for determination of significance was 0.05, and from 0.05 to 0.10 for trends. Results for fixed effects are reported as least square means ± standard error.

## 6.3 Results

### 6.3.1 Body Weight, Feed Intake and Feed Efficiency Traits

Only general results of the whole trial period for each productive performance variable are presented.

#### *6.3.1.1 Space Allowance × Mixing (Trials 1 and 2)*

Body weight showed an interaction between space allowance and mixing in trial 1. Non-mixed pigs with 0.78 $m^2$/pig space allowance were 6.1 and 6.5 kg heavier than mixed pigs with 0.96 and 0.78 $m^2$/pig space allowance, respectively, at 21 weeks of age ($P < 0.001$; Table 6.3). On average, non-mixed pigs gained 74 g more per day ($P = 0.004$) and consumed 101.8 g more of feed per day ($P = 0.007$) than mixed pigs for the whole period of the trial (Table 6.3). Non-mixed pigs tended to have lower FCR compared to mixed pigs from 11 to 21 weeks of age ($P = 0.079$; Table 6.3).

In trial 2, an interaction between space allowance and mixing on BW was observed ($P = 0.005$) but was not significant after adjustment for multiple mean comparison ($P > 0.05$; Table 6.3). Non-mixed pigs were 5.25 kg heavier than mixed pigs at 21 weeks of age ($P < 0.001$; Table 6.3). On average, non-mixed pigs gained 76.6 g more per day ($P = 0.029$) and consumed 144.4 g more of feed per day ($P = 0.046$) than mixed pigs for the whole period of the trial (Table 6.3). Pigs with 0.78 $m^2$/pig space allowance had lower FCR compared to pigs with 0.96 $m^2$/pig space allowance for the whole period of the trial ($P < 0.001$; Table 6.3).

In both trials, the mixing effect was observed as a long-lasting effect, with the difference between mixed and non-mixed increasing towards the end of the grower-finisher period, and not at the beginning when the pigs were mixed (Figure 6.2).

**Table 6.3** Effect of space allowance and mixing on bodyweight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Least square means (LS means) ± Standard error mean (SEM)) in trial 1 and trial 2 from 11 to 21 weeks of age.

| | Space Allowance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.96 $m^2$/pig | | 0.78 $m^2$/pig | | | *P*-value | | |
| Trait | Mixed (n = 5) | Non-Mixed (n = 5) | Mixed (n = 5) | Non-Mixed (n = 5) | SEM | Mixing | Space Allowance | Interaction |
| Trial 1 | | | | | | | | |
| BW [1], kg, 21 wk | 102.1 $^{b}$ | 106.4 $^{ab}$ | 101.7 $^{b}$ | 108.2 $^{a}$ | 0.95 | < 0.001 | 0.472 | < 0.001 |
| ADG, g | 983.0 | 1034.1 | 955.4 | 1052.3 | 21.82 | 0.004 | 0.836 | 0.309 |
| ADFI, g | 2150.3 | 2222.3 | 2125.6 | 2257.1 | 32.57 | 0.007 | 0.880 | 0.374 |
| FCR | 2.18 | 2.12 | 2.19 | 2.11 | 0.04 | 0.079 | 0.974 | 0.692 |
| Trial 2 | | | | | | | | |
| BW, kg, 21 wk | 97.5 | 104.3 | 97.8 | 101.5 | 1.50 | < 0.001 | 0.400 | 0.005 |
| ADG, g | 914.5 | 1014.3 | 929.7 | 983.1 | 31.90 | 0.029 | 0.809 | 0.476 |
| ADFI, g | 2120.7 | 2314.7 | 2055.1 | 2149.9 | 66.80 | 0.046 | 0.110 | 0.468 |
| FCR | 2.36 | 2.32 | 2.23 | 2.18 | 0.03 | 0.172 | < 0.001 | 0.825 |

[1] Initial body weight in trial 1 was: 34.2 ± 3.25 kg ($P = 0.545$). In trial 2 was: 30.8 ± 4.31 kg ($P = 0.645$).

$^{a\text{–}b}$ Within rows, significant differences between groups ($P < 0.05$).

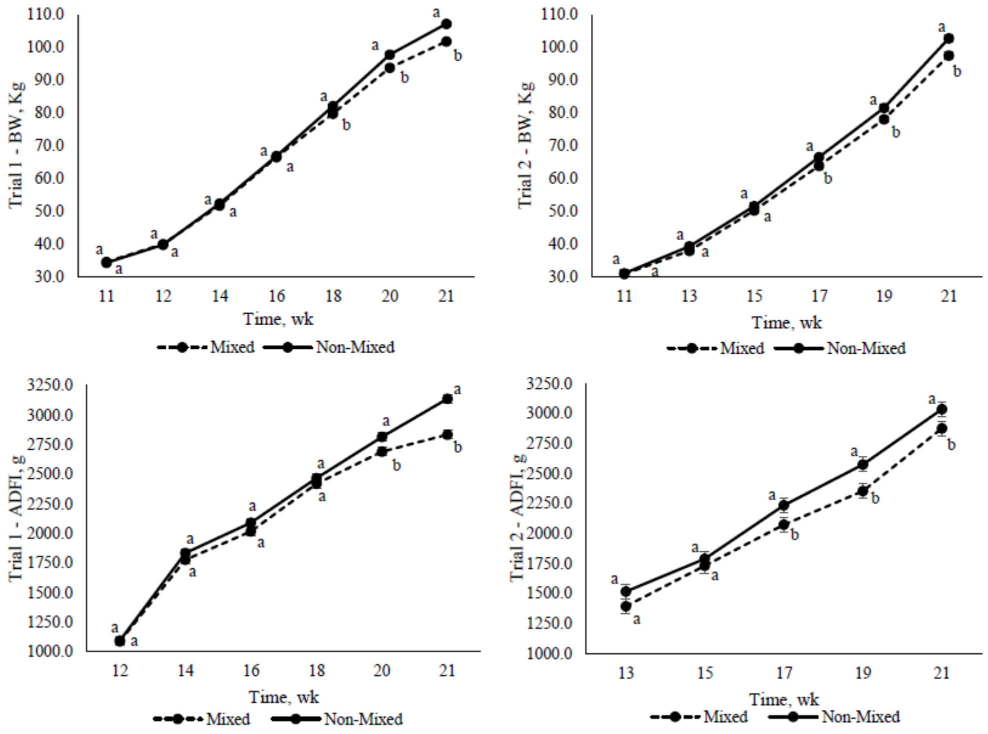

**Figure 6.2** Effect of mixing on body weight (BW) and average daily feed intake (ADFI) in trial 1 and trial 2 (n = 5) from 11 to 21 weeks of age, when the first group of pigs reached 110 kg of BW and were sent to slaughter; $^{a\text{–}b}$ Significant differences between groups ($P < 0.05$).

### *6.3.1.2 Space Allowance × Phase Feeding (Trials 1, 2 and 3)*

Body weight showed an interaction between space allowance and phase feeding in trial 1. Pigs with 0.78 m$^2$/pig space allowance and 0.95 g/MJ SID Lys:NE diet were 4.4 and 4.5 kg heavier than pigs with 0.82 g/MJ SID Lys:NE diet and 0.78 or 0.96 m$^2$/pig space allowance, respectively, at 21 weeks of age ($P < 0.001$; Table 6.4). On average, pigs that received the 0.95 g/MJ SID Lys:NE diet gained 90.1 g more per day ($P = 0.011$) and had lower FCR ($P = 0.005$) compared to pigs that received the 0.82 g/MJ SID Lys:NE diet from 16 to 21 weeks of age (Table 6.4). No differences were observed on ADFI between treatments ($P > 0.05$; Table 6.4).

In trial 2, an interaction between space allowance and phase feeding on BW was observed. Pigs with 0.96 m$^2$/pig space allowance and 0.95 g/MJ SID Lys:NE diet were 5.6 and 5.2 kg heavier than pigs with 0.82 g/MJ SID Lys:NE diet and 0.78 or 0.96 m$^2$/pig space allowance, respectively, at 21 weeks of age ($P < 0.001$; Table 6.4). On average, pigs that received the 0.95 g/MJ SID Lys:NE diet gained 83.9 g more per day ($P = 0.013$) and had lower FCR ($P = 0.031$) compared to pigs that received the 0.82 g/MJ SID Lys:NE diet from 15 to 21 weeks of age (Table 6.4). No differences were observed on ADFI between treatments ($P > 0.05$; Table 6.4).

In trial 3, BW showed an interaction tendency between space allowance and phase feeding at 21 weeks of age ($P = 0.093$; Table 6.4). Average daily gain, ADFI and FCR results for the whole trial period were not affected by space allowance and phase feeding ($P > 0.05$; Table 6.4).

**Table 6.4** Effect of space allowance and phase feeding on bodyweight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Least square means (LS means) ± Standard error mean (SEM)) in trial 1 from 16 to 21 weeks of age, and in trial 2 and trial 3 from 15 to 21 weeks of age.[1]

| Trait | Space Allowance | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 0.96 m$^2$/pig | | 0.78 m$^2$/pig | | | | | |
| | 0.82 SID Lys:NE (n = 5) | 0.95 SID Lys:NE (n = 5) | 0.82 SID Lys:NE (n = 5) | 0.95 SID Lys:NE (n = 5) | | Phase feeding | Space Allowance | Interaction |
| Trial 1 | | | | | | | | |
| BW[2], kg, 21 wk | 103.2 $^{b}$ | 105.7 $^{ab}$ | 103.3 $^{b}$ | 107.7 $^{a}$ | 0.84 | < 0.001 | 0.208 | < 0.001 |
| ADG, g | 1084.7 | 1157.1 | 1094.3 | 1202.0 | 30.89 | 0.011 | 0.391 | 0.575 |
| ADFI, g | 2754.9 | 2748.0 | 2758.1 | 2839.1 | 42.19 | 0.398 | 0.280 | 0.312 |
| FCR | 2.56 | 2.39 | 2.54 | 2.37 | 0.05 | 0.005 | 0.662 | 0.943 |

*Table 6.4 continue*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Trial 2 | | | | | | | | |
| BW, kg, 21 wk | 97.6 $^{b}$ | 102.8 $^{a}$ | 97.2 $^{b}$ | 100.1 $^{ab}$ | 0.92 | < 0.001 | 0.094 | < 0.001 |
| ADG, g | 1064.6 | 1174.7 | 1063.0 | 1120.4 | 29.59 | 0.013 | 0.361 | 0.386 |
| ADFI, g | 2524.1 | 2665.7 | 2510.0 | 2505.6 | 59.40 | 0.269 | 0.164 | 0.236 |
| FCR | 2.37 | 2.27 | 2.37 | 2.24 | 0.05 | 0.031 | 0.770 | 0.760 |
| Trial 3 | | | | | | | | |
| BW, kg, 21 wk | 100.7 | 101.0 | 98.0 | 100.6 | 0.93 | 0.120 | 0.095 | 0.093 |
| ADG, g | 1098.2 | 1102.5 | 1036.7 | 1094.0 | 21.20 | 0.164 | 0.118 | 0.230 |
| ADFI, g | 2521.6 | 2530.0 | 2409.9 | 2535.1 | 54.83 | 0.242 | 0.347 | 0.304 |
| FCR | 2.29 | 2.28 | 2.32 | 2.31 | 0.03 | 0.773 | 0.479 | 0.988 |

[1] In trial 1 and trial 2 pigs were kept in litters, while in trial 3 pigs were mixed.

[2] Initial body weight in trial 1 was: 33.6 ± 4.61 kg ($P = 0.718$). In trial 2 was: 30.7 ± 4.30 kg ($P = 0.610$). In trial 3 was: 32.4 ± 5.93 kg ($P = 0.966$).

[a-b] Within rows, significant differences between groups ($P < 0.05$).

## 6.3.2 Carcass Traits

### *6.3.2.1 Space Allowance × Mixing (Trials 1 and 2)*

Lean meat %, FT and MC were not affected by space allowance and mixing on both trial 1 and trial 2 ($P > 0.05$; Table 6.5).

**Table 6.5** Effect of space allowance and mixing on lean meat %, fat thickness and muscle content (Least square means (LS means) ± Standard error mean (SEM)) from trial 1 and 2.[1]

| | Space Allowance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.96 $m^2$/pig | | 0.78 $m^2$/pig | | | *P*-value | | |
| Trait | Mixed (n = 5) | Non-Mixed (n = 5) | Mixed (n = 5) | Non-Mixed (n = 5) | SEM | Mixing | Space Allowance | Interaction |
| Trial 1 | | | | | | | | |
| Lean meat, % | 57.71 | 57.98 | 58.07 | 57.60 | 0.273 | 0.741 | 0.984 | 0.173 |
| Fat thickness, % | 13.57 | 13.56 | 13.31 | 14.03 | 0.358 | 0.390 | 0.784 | 0.297 |
| Muscle content, % | 45.93 | 48.61 | 47.60 | 48.35 | 0.746 | 0.059 | 0.364 | 0.187 |
| Trial 2 | | | | | | | | |
| Lean meat, % | 58.62 | 58.49 | 58.52 | 58.77 | 0.342 | 0.853 | 0.824 | 0.565 |
| Fat thickness, % | 12.55 | 12.70 | 12.65 | 12.46 | 0.450 | 0.965 | 0.895 | 0.705 |
| Muscle content, % | 47.29 | 47.21 | 47.07 | 48.29 | 0.773 | 0.454 | 0.629 | 0.394 |

[1] Model included age at slaughter, cold carcass weight, space allowance, mixing and their interaction as fixed effects.

#### *6.3.2.2 Space Allowance × Phase Feeding (Trials 1, 2 and 3)*

Lean meat % and FT were not affected by space allowance and phase feeding in trial 1, trial 2 and trial 3 (*P* > 0.05; Table 6.6). Muscle content was not affected on both trial 1 and trial 2 (*P* > 0.05; Table 6.6). However, MC showed an interaction between space allowance and phase feeding in trial 3 (*P* = 0.010), although no significant difference was observed in the least square means (*P* > 0.05; Table 6.6).

**Table 6.6** Effect of space allowance and phase feeding on lean meat %, fat thickness and muscle content (Least square means (LS means) ± Standard error mean (SEM)) from trial 1, 2 and 3.[1]

| Trait | Space Allowance | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 0.96 m$^2$/pig | | 0.78 m$^2$/pig | | | | | |
| | 0.82 SID Lys:NE (n = 5) | 0.95 SID Lys:NE (n = 5) | 0.82 SID Lys:NE (n = 5) | 0.95 SID Lys:NE (n = 5) | | Phase feeding | Space Allowance | Interaction |
| Trial 1 | | | | | | | | |
| Lean Meat, % | 57.16 | 58.03 | 57.90 | 57.77 | 0.293 | 0.230 | 0.426 | 0.106 |
| Fat thickness, % | 14.37 | 13.46 | 13.24 | 13.92 | 0.397 | 0.789 | 0.410 | 0.065 |
| Muscle content, % | 47.37 | 48.19 | 45.97 | 47.64 | 0.681 | 0.100 | 0.167 | 0.545 |
| Trial 2 | | | | | | | | |
| Lean Meat, % | 58.08 | 58.62 | 57.83 | 58.87 | 0.451 | 0.121 | 0.996 | 0.588 |
| Fat thickness, % | 13.17 | 12.53 | 13.65 | 12.18 | 0.614 | 0.131 | 0.920 | 0.512 |
| Muscle content, % | 46.48 | 47.18 | 47.65 | 47.06 | 0.826 | 0.952 | 0.535 | 0.444 |
| Trial 3 | | | | | | | | |
| Lean Meat, % | 57.80 | 58.49 | 58.02 | 58.07 | 0.220 | 0.112 | 0.662 | 0.161 |
| Fat thickness, % | 13.49 | 12.86 | 13.50 | 13.19 | 0.302 | 0.140 | 0.605 | 0.600 |
| Muscle content, % | 46.06 | 48.45 | 48.53 | 46.55 | 0.744 | 0.785 | 0.718 | 0.010 |

[1] In trial 1 and trial 2 pigs were kept in litters, while in trial 3 pigs were mixed. Model included age at slaughter, cold carcass weight, space allowance, phase feeding and their interaction as fixed effects.

## 6.4 Discussion

### 6.4.1 Body Weight, Feed Intake and Feed Efficiency Traits

This study was carried out to assess the importance of space allowance, mixing and phase feeding on productive performance of grower-finisher pigs. A pen system of 10-13 grower-finisher pigs linked to one wet-dry feeder, which optimizes feed efficiency (O’Meara et al., 2020) and allows a good assessment of health and welfare issues in pigs, was chosen because it is one of the most common systems in growing-finishing units in the EU. The latter it is suggested by the authors’ experience in groups such as ECPHM or EUPIG (https://www.eupig.co.uk). Moreover, space allowances in the present study were not chosen to ensure a negative effect on productive performance and were chosen above the 0.65 $m^2$/pig minimum set by the current EU legislation based on live weight (Council of the European Union, 2008). This decision was taken as a consequence that a space allowance of 0.65 $m^2$/pig is already considered negative from a welfare point of view because of a very low amount of shared space (Averós et al., 2013), and its final *k*-value is below the critical *k*-value when pigs reach the market slaughter weight, which was 110 kg of BW per commercial practice. Furthermore, under the conditions of the study, space allowances were adjusted by changing the number of grower-finisher pigs per pen. This method ensures a feasibility at producer level because it is how would happen in field conditions, although it can induce confounding of space allowance with group size (Anil et al., 2007). Nevertheless, previous studies have suggested that group size does not have a detrimental effect on growth performance of grower-finisher pigs (Schmolke et al., 2003; Street and Gonyou, 2008), and it is not a predictor for ADG, ADFI and FCR (Flohr et al., 2017). Moreover, a higher number of pigs per pen decreases the feeder space, which could have also affected productive performance (DeDecker et al., 2005). However, Wastell et al. (2018) observed that feeder space did not have a detrimental effect on growth performance between 10 and 13 pigs per wet-dry feeder space in a space allowance of 0.78 $m^2$/pig. Together, these studies suggest that group size and feeder space would not affect the productive performance of grower-finisher pigs under the conditions of this study. Therefore, and regarding the pen system studied, the present study discusses space allowance as the main factor to affect productive performance, instead of group size or feeder space.

Space allowance did not affect final BW and overall ADG and ADFI, which is in line with results obtained from a previous study using similar space allowances (Camp Montoro et al., 2021a). However, previous literature suggested that further decreasing space allowances results in poorer productive performance (Jang et al., 2017; Kim et al., 2017; Thomas et al., 2017). These studies subjected pigs to restricted space allowances below 0.70 $m^2$/pig, which may explain the difference in results. Moreover, other studies found that 0.80 $m^2$/pig space allowance affected productive performance when pigs were marketed to at least 138 kg of target slaughter BW (Johnston et al., 2017). Overall, all these studies could be compared using the allometric approach which can relate space allowance to BW (Kyriazakis and Whittemore, 2006). Gonyou et al. (2006), using a broken-line analysis, reported a critical *k*-value of 0.0336, below which productive performance was affected. This *k*-value was almost reached in pigs with 0.78 $m^2$/pig by the end of the trial when they reached the target slaughter weight (i.e., 110 kg). Then, productive performance may not be affected during the grower-finisher period when space allowance is established based on the critical *k*-value at the target slaughter weight. Nevertheless, recent studies reported that productive performance restriction could occur above the critical *k*-value of 0.0336 (Flohr et al., 2016; Thomas et al., 2017), which may depend on the genetics and feeder system (Wastell et al., 2018). Moreover, it has been suggested that the critical *k*-value could also underestimate the space allowance requirement for marketed heavy pigs with BWs over 120 kg (Johnston et al., 2017). Nonetheless, BWs over 120 kg were not reached in the present study.

Pigs in the lower space allowance had a better feed efficiency in trial 2, although this finding is not consistent in the study as it was not observed in trial 1. Similarly, there are several conflicting reports in previous literature of FCR not being affected by space allowance (Carpenter et al., 2017; Gonyou et al., 2006; Thomas et al., 2017), being increased by decreasing space allowance (Flohr et al., 2016; Hyun et al., 1998a; Kim et al., 2017), or being improved with lower space allowances (Wastell et al., 2018). These discrepancies within the literature could rely on the experimental design itself of each prior study and which space allowances were considered low or high. Moreover, environmental and management factors such mixing or the farm health status may have their role in feed efficiency (Camp Montoro et al., 2021a; Van Der Meer et al., 2020).

Mixing appears to have a considerable effect on productive performance in grower-finisher pigs. This finding is in accordance with previous studies which found reduced performance when pigs were mixed at the beginning of the grower-finisher period and followed in a 4-wk experiment with space allowances of 0.25 and 0.52 $m^2$/pig (Hyun et al., 1998a, 1998b) or when pigs had 83 kg of BW and were mixed and followed in a 2-wk experiment (Stookey and Gonyou, 1994). Thus, the present study suggests that the strategy of mixing should be reconsidered in current modern facilities and genetics. Moreover, the issue of mixing segregating by sex, which it is a common practice, is an intriguing one which could be usefully explored in further research. Apart of the detrimental effect on productive performance (Camp Montoro et al., 2021a), mixing affects animal welfare (Driessen et al., 2020) and sorting by weight fails to improve final variability in individual pig weights within a pen (O'Quinn et al., 2001), although it may facilitate farm management. Therefore, strategies to avoid or mitigate mixing, such as maintaining intact litters, should be considered in future research. The management strategy of keeping pigs in intact litters is possible in farrow-to-finish farms, but it may be more difficult in farms with the 3 production stages separated, where animals need to be transported using a truck.

Contrary to expectations, mixing was found to have a long-term effect instead of an initial effect on growth performance associated with aggression. Mixed pigs had a poorer growth performance during the second period of the grower-finisher stage. This might be explained due to chronic stress related to aggression and social hierarchy (Foister et al., 2018; Gimsa et al., 2018; Martínez-Miró et al., 2016). Chronic stress can lead to immunosuppression and could, ultimately, have detrimental implications for pig health and performance (De Groot et al., 2001; Gimsa et al., 2018; Martínez-Miró et al., 2016). Recently, Foister et al. (2018) suggested that high betweenness centralization after mixing represents poorly established dominance relationships between pen mates. This leads to chronic stress due to prolonged aggression behavior. Nonetheless, several studies have reported that mixing does not have an effect on health and performance traits when it is applied to larger group sizes of 20, 40, or 80 pigs (Schmolke et al., 2003), or up to 108 pigs (Street and Gonyou, 2008). Turner et al. (2001) observed that mixed pigs in large group size (i.e., 80 pigs) displayed a marked reduction in aggressive behavior towards foreign pigs, perhaps due to different social dynamics in larger groups. The latter may explain the differences between the present study when there are only 10 or 13 pigs per group and the previous studies with higher group sizes.

Although this study aimed to find whether space allowance and mixing interacted, the interaction observed between space allowance and mixing in final BW in trials 1 and 2 were contradictory in terms of space allowance. Moreover, the interaction did not show up in the ADG, ADFI or FCR, and it could be checked for repeatability in further experiments. Previous research suggested that the effects of reduced space allowance and mixing were additive (Hyun et al., 1998b).

Reducing the SID Lys:NE ratio from 0.95 to 0.82 g/MJ at 15-16 weeks of age had a detrimental effect on growth performance in trials 1 and 2. This outcome is contrary to previous studies, which have reported that a single phase feeding strategy reduces overall performance (Menegat et al., 2020) or has no impact on growth performance compared to multiple dietary phases (Garry et al., 2007; Moore et al., 2013; O’Connell et al., 2005). Nevertheless, the divergence between previous research and the present study may rely on differences in the SID Lys:NE ratio, weight ranges and the experimental conditions.

Previous research considered compensatory growth during the grower-finisher stage and provided low SID Lys:NE ratio initially and adequate or excess SID Lys:NE ratio later in the grower-finisher period (Garry et al., 2007; Menegat et al., 2020; O'Connell et al., 2005). However, this was not the case in the present study, as pigs received an adequate SID Lys:NE ratio (i.e., 0.95 g/MJ) at the beginning of the trial and the 0.82 g/MJ SID Lys:NE ratio applied as phase feeding strategy at 15-16 weeks of age was adjusted to meet or exceed the requirements established by the standard nutritional tables (NRC, 2012). Therefore, it is somewhat surprising that productive performance was affected using this phase feeding strategy. A possible explanation for these results might be related to the genetic advancements, where high lean gain potential pig genetics have higher SID Lys requirements (Gonçalves et al., 2017; Ho et al., 2019; Htoo, 2018). Nowadays, the Lys requirements for those high lean gain potential pig genetics are already higher than the NRC recommendations (Gonçalves et al., 2017; Ho et al., 2019; Landero et al., 2016), and it should be considered to what extent current recommendations are aligned with the current genetic pig potential. Nonetheless, different genotypes may derive to different results. Thus, future studies could consider predicting dietary specifications using the NRC model by estimating the lean gain potential of the pigs being studied. Another possible explanation for the results observed in the present study may be due to large changes in the SID Lys:NE ratio, which may not be favorable to maximize grower-finisher pig productive performance (Smith et al., 1999). Moreover, environmental factors such as management, feedstuffs, facilities or the farm health status may have an effect on pig's nutrient requirements (Ferket et al., 2002; Li et al., 2012; Liu et al., 2015). Management reasons could explain that no differences in productive performance were observed between pigs that were fed one-single diet and pigs that had the phase feeding in trial 3 where all pigs were mixed. Mixing effect on productive performance was even more severe than reducing SID Lys:NE from 0.95 to 0.82 g/MJ in trial 1 and 2. Mixing could have had an impact on productive performance in both groups that may have concealed the phase feeding effect in trial 3. Likewise, the interaction observed between space allowance and phase feeding in final BW in both trials 1 and 2 was not evident when all pigs were mixed in trial 3. Therefore, the relationship between space allowance, phase feeding and productive performance may vary between batches, due to interactions of different environmental/management factors such as mixing and should be checked for repeatability in further studies. Finally, it is worth mentioning that the high levels of SID Lys:NE ratio used at the late finishing stage may increase the levels of $NH_3$ (Lee et al., 2020; Liu et al., 2015; Prandini et al., 2013) which is contrary to farm sustainability because it contributes to the environment pollution (Andretta et al., 2016).

### 6.4.2 Carcass Traits

Space allowance × mixing and space allowance × phase feeding were not a source of variation for carcass traits. These results match those observed in earlier studies which did not find differences in carcass traits using different phase feeding strategies (Garry et al., 2007; Menegat et al., 2020; Moore et al., 2013). Nevertheless, previous literature also found that LM% increases as space allowance decreases (Thomas et al., 2017), and fat thickness increases as SID Lys:NE ratio decreases (Cho et al., 2012; Lee et al., 2020; Li et al., 2012). Although these findings were not observed in the present study, an interaction tendency was observed between space allowance and phase feeding on LM% and fat thickness in trial 1, and these two traits were numerically different in trial 2 when reducing the SID lys:NE ratio from 0.95 to 0.82. Therefore, further work is needed to fully understand the implications of space allowance, phase feeding and mixing on carcass traits.

## 6.5 Conclusion

The present study compared the effects of space allowance, mixing and phase feeding during the grower-finisher period in pens of 10-13 grower-finisher pigs with one single wet-dry feeder. Space allowance of 0.78 $m^2$/pig did not affect productive performance compared to 0.96 $m^2$/pig. Space allowance may not compromise growth performance if the critical *k*-value is considered at the marketed weight. Mixing causes a negative and long-term impact on productive performance. Reducing SID Lys:NE ratio from 0.95 to 0.82 g/MJ at 15-16 weeks of age had an effect on productive performance when pigs were not mixed. Nevertheless, carcass traits were not affected as a consequence of mixing or reducing SID Lys:NE ratio from 0.95 to 0.82 g/MJ at 15-16 weeks of age. Finally, high lean gain potential pig genetics may require a higher SID Lys:NE ratio than current nutrient requirement recommendations during the grower-finisher period.

# Chapter 7

# Blood and faecal biomarkers to assess dietary energy, protein and amino acid efficiency of utilization by growing and finishing pigs levels

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## 7.1 Background

Pig diets are formulated to optimise growth, health, and welfare of the animals by meeting their nutritional requirements while avoiding the excess of nutrients, especially nitrogen and phosphorus, that result in environmental pollution. The actual nutritional value of a diet for a particular pig farm is affected by different factors such as quality of ingredients (Shurson et al., 2021), feed form and delivery method (O'Meara et al., 2020), BW variability (Camp Montoro et al., 2021b, 2020a), farm management (Camp Montoro et al., 2022, 2021a), or health status of the farm (Van der Meer et al., 2016; Van Der Meer et al., 2020) among others. With so many factors affecting nutritional value of feed, suboptimal diets are not rare and can result in extra cost for the farmers, potential health and welfare problems for the pigs and environmental contamination.

The use of low protein diets, including more synthetic AAs, is one of the most effective measures to reduce the incidence of diarrhoea in pigs (Rist et al., 2013) and to reduce ammonia emissions in pig farms (Prandini et al., 2013). However, the optimum levels of AAs and the balance between AAs and energy can vary depending on the particularities of each farm and the same low protein diet can be adequate or not depending on the farm. Thus, methods to optimise diets at farm level are needed. Classical methods for feed assessment, such as digestibility trials, are expensive, time consuming and are not suited for use in commercial farms (Le Goff and Noblet, 2001). Fast methods to optimise diets at individual farm level would be of interest to improve production efficiency, animal health and welfare, and to reduce the environmental footprint and production costs (Jongbloed and Lenis, 1992; Patience et al., 2015, 2004).

Blood biochemistry is a fast analysis method used regularly in clinical practice for many animal species and it can provide parameters directly related to energy and protein metabolism. Dietary changes affect blood metabolites such as total protein (Kamalakar et al., 2009; Regmi et al., 2018; Zeng et al., 2013), albumin (Kamalakar et al., 2009; Mule et al., 2006; Regmi et al., 2018), SUN (Chen et al., 1999; Coma et al., 1995; Hong et al., 2016) and creatinine (Hong et al., 2016) for the protein metabolism, and glucose (Kamalakar et al., 2009; Regmi et al., 2018; Zeng et al., 2013), triglycerides (Kerr et al., 2015; Mule et al., 2006) and cholesterol (Mule et al., 2006; Regmi et al., 2018) for the energy metabolism.

Faecal samples are easy to collect in pig farms and fermentation components present in faeces, like VFAs can be easily measured and are directly affected by the composition of the diet and the metabolism of nutrients by the animal (Cho et al., 2015; Dahl et al., 2020; Le et al., 2005). Carbohydrates are catabolized to SCFA such as acetic, propionic or butyric acid, while protein results in a higher concentration of BCFA produced from the deamination of branched-chain amino acids such as leucine, isoleucine and valine (Le et al., 2005). Therefore, high proportions of BCFA measured in faeces could indicate an excess of protein reaching distal parts of the intestine and being inefficiently used by pigs.

Blood serum metabolites and faecal VFA profiles could be potential biomarkers to identify diets that are suboptimal at individual farm level. Therefore, we hypothesise that dietary energy, protein, and AA content could be optimised using blood metabolite and faecal VFA profiles in growing and finishing pigs. The objective of the present study was to evaluate the use the blood serum metabolite and faecal VFA profiles to identify differences in the levels of dietary NE, CP, and AA within normal ranges for growing and finishing pigs.

## 7.2 Material & Methods

### 7.2.1 Animals, diets and experimental design

Two studies were conducted in the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland. Both studies received ethical approval from the Teagasc Animal Ethics Committee (TAEC 244/2019). In both studies, pigs were housed in pens with fully slatted concrete floor (2.4 × 4.2 m) containing a single wet-dry feeder [330mm (Width) × 370mm (Depth) × 1000mm (Height); MA37, Verba, Netherlands] and one supplementary nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller. Pens were enriched with a larch wood post.

The first study was conducted in finishing pigs from 18 to 20 weeks of age. A total of 220 Danish Duroc × (Large White × Landrace) growing pigs born within one week were moved to the grower-finisher stage at 11 weeks of age and housed in balanced mixed sex pens. Pigs were fed a single soybean meal-maize-wheat based grower-finisher diet (9.67 MJ/NE, 161.8 g CP, and 9.2 g of SID Lys per kg of feed) from 11 to 18 weeks of age before the study started. The study started at 18 weeks of age when pigs were weighed per pen as a group (n = 20; 11 pigs/pen; 87.0 ± 4.10 kg BW), assigned per pen based on BW to five different dietary treatments, and followed for 10 days. Diets were formulated to obtain a control diet (**C1**; 10.03 MJ of NE, 160 g of CP, and 9.5 g of SID Lys per kg of feed) which met or exceed the minimum nutrient requirements (NRC, 2012), and 4 modifications of this diet: low protein (**LP1**) by reducing CP to 132 g per kg of feed and AAs to 7.5 g of SID Lys per kg of feed; high protein (**HP1**) by increasing CP to 188 g per kg of feed and AAs to 11.5 g of SID Lys per kg of feed; low energy (**LE1**) by reducing NE to 9.61 MJ/NE per kg of feed; and high energy (**HE1**) by increasing NE to 10.45 MJ/NE per kg of feed. Ingredient, calculated and analysed nutrient diet composition is shown in Table 7.1. Feed was given to each pen by bags ensuring that pigs were fed ad libitum during the whole trial. Pigs went back to the common management of the Teagasc Pig Research Facility after the 10-day trial period.

The second study was conducted in growing pigs from 12 to 15 weeks of age. A total of 308 Danish Duroc × (Large White × Landrace) growing pigs born within one week were mixed and moved to the grower-finisher stage at 11 weeks of age and housed in balanced mixed sex pens. After one week of adaptation, pigs were assigned per pen (n = 28 pens; 11 pigs/pen; 41.3 ± 2.60 kg BW) based on BW to seven different dietary treatments at 12 weeks of age, and pigs were followed for 20 days. Diets were formulated to obtain a control diet (**C2**; 10.03 MJ/NE, 165 g of CP, and 9.5 g of SID Lys per kg of feed) which met or exceed the minimum nutrient requirements (NRC, 2012), and 6 modifications of this diet: low protein (**LP2**) by reducing CP to 140 g of CP per kg of feed; high protein (**HP2**) by increasing CP to 190 g of CP per kg of feed; low AA (**LA2**) by reducing AAs to 7.5 g of SID Lys per kg of feed; high AA (**HA2**) by increasing AAs to 11.5 g of SID Lys per kg of feed; low energy (**LE2**) by reducing NE to 9.61 MJ/NE per kg of feed; and high energy (**HE2**) by increasing NE to 10.45 MJ/NE per kg of feed. When the level of AA was modified all AAs were adjusted following the ideal protein concept. Ingredient, calculated and analysed nutrient diet composition is shown in Table 7.2. Feed was given to each pen by bags ensuring that pigs were fed ad libitum during the whole trial. Pigs went back to the common management of the Teagasc Pig Research Facility after the 20-day trial period.

**Table 7.1** Ingredient, calculated and analysed nutrient composition on an as-fed basis of the five dietary treatments in study 1.[1]

| | Diets [2] | | | | |
|---|---|---|---|---|---|
| | C1 | LP1 | HP1 | LE1 | HE1 |
| Ingredients, g/kg | | | | | |
| Wheat | 350.0 | 350.0 | 350.0 | 330.0 | 306.2 |
| Barley | 282.5 | 345.0 | 0.0 | 310.5 | 200.0 |
| Maize | 150.0 | 150.0 | 286.6 | 100.0 | 275.5 |
| Soybean meal 47.5 | 172.4 | 95.7 | 254.1 | 175.1 | 172.4 |
| Soybean hulls | 14.2 | 29.7 | 63.9 | 58.3 | 0.0 |
| Vegetable Oil | 5.0 | 5.0 | 17.6 | 0.0 | 21.5 |
| Calcium carbonate | 12.3 | 12.7 | 12.2 | 10.7 | 11.7 |
| Dicalcium phosphate anhydrous | 0.50 | 0.50 | 1.00 | 3.00 | 0.50 |
| Sodium chloride | 4.50 | 4.40 | 3.20 | 4.40 | 3.70 |
| L-Lysine HCl | 4.30 | 3.80 | 5.30 | 4.15 | 4.40 |
| L-Threonine | 1.60 | 1.20 | 2.20 | 1.15 | 1.60 |
| DL-Methionine | 1.30 | 0.70 | 2.20 | 1.30 | 1.20 |
| L-Tryptophan | 0.20 | 0.10 | 0.20 | 0.20 | 0.10 |
| L-Valine | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 |
| Vitamin and trace mineral mixture [3] | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| | | | | | |
| Calculated / Analysed Composition [4], % as fed or as specified | | | | | |
| Dry Matter, analysed | 88.00 | 87.70 | 88.30 | 87.90 | 87.90 |
| Ash, analysed | 3.90 | 3.60 | 4.00 | 4.10 | 3.90 |
| NE, MJ/kg | 10.03 | 10.03 | 10.03 | 9.61 | 10.45 |
| SID Lys:NE, g/MJ | 0.95 | 0.75 | 1.15 | 0.99 | 0.91 |
| Crude Protein, analysed | 13.40 | 11.60 | 16.20 | 14.50 | 14.30 |
| Total Lys, analysed | 1.05 | 0.88 | 1.31 | 1.08 | 1.02 |
| Total Thr / Lys ratio, analysed | 0.58 | 0.58 | 0.56 | 0.57 | 0.62 |
| Total Met-Cys / Lys ratio, analysed | 0.64 | 0.64 | 0.63 | 0.65 | 0.68 |
| Total Trp / Lys ratio, analysed | 0.14 | 0.15 | 0.15 | 0.14 | 0.14 |
| Total Val / Lys ratio, analysed | 0.60 | 0.65 | 0.65 | 0.67 | 0.69 |
| Total Leu / Lys ratio, analysed | 1.09 | 1.14 | 1.08 | 1.14 | 1.14 |
| Total Ile / Lys ratio, analysed | 0.54 | 0.55 | 0.57 | 0.58 | 0.60 |
| Total His / Lys ratio, analysed | 0.34 | 0.38 | 0.37 | 0.36 | 0.38 |
| SID Lys | 0.95 | 0.75 | 1.15 | 0.95 | 0.95 |
| SID Thr / Lys ratio | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| SID Met-Cys / Lys ratio | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| SID Trp / Lys ratio | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| SID Val / Lys ratio | 0.66 | 0.67 | 0.65 | 0.66 | 0.66 |
| SID Leu / Lys ratio | 1.16 | 1.20 | 1.11 | 1.16 | 1.15 |
| SID Ile / Lys ratio | 0.56 | 0.55 | 0.57 | 0.57 | 0.57 |
| SID His / Lys ratio | 0.36 | 0.36 | 0.34 | 0.35 | 0.36 |
| Fat, analysed | 2.79 | 2.74 | 3.78 | 2.21 | 4.19 |
| Crude Fibre, analysed | 2.90 | 3.40 | 4.20 | 4.20 | 2.40 |

*Table 7.1 Continue*

| | | | | | |
|---|---|---|---|---|---|
| NDF | 12.96 | 14.15 | 13.54 | 15.02 | 12.00 |
| Calcium | 0.75 | 0.75 | 0.80 | 0.77 | 0.72 |
| Digestible Phosphorus | 0.22 | 0.22 | 0.22 | 0.25 | 0.22 |

[1] Diets were fed in finishing pigs during 10 days at 18 weeks of age.

[2] C1 = Control; LP1 = Low Crude Protein; HP1 = High Crude Protein; LE1 = Low Net Energy; HE1 = High Net Energy

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin $D_3$, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin $B_{12}$, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin $B_1$, 3 mg Vitamin $B_6$.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fibre.

**Table 7.2** Ingredient, calculated and analysed nutrient composition on an as-fed basis of the seven dietary treatments in study 2.[1]

| | Diets[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | C2 | LP2 | HP2 | LA2 | HA2 | LE2 | HE2 |
| Ingredients, g/kg | | | | | | | |
| Maize | 272.9 | 401.1 | 254.9 | 350.0 | 423.1 | 300.0 | 332.1 |
| Barley | 251.8 | 230.0 | 252.2 | 295.9 | 150.0 | 246.1 | 225.7 |
| Wheat | 218.1 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 |
| Soybean meal 47.5 | 209.3 | 137.2 | 286.7 | 135.9 | 197.6 | 209.8 | 218.7 |
| Soybean hulls | 20.0 | 44.0 | 20.0 | 38.9 | 39.8 | 70.4 | 20.0 |
| Vegetable Oil | 5.00 | 5.00 | 17.6 | 5.00 | 5.00 | 0.00 | 31.0 |
| Calcium carbonate | 10.6 | 14.0 | 10.5 | 13.6 | 12.3 | 11.3 | 10.6 |
| Dicalcium phosphate anhydrous | 0.50 | 0.50 | 0.50 | 0.50 | 0.70 | 0.50 | 0.50 |
| Sodium chloride | 4.80 | 5.00 | 4.30 | 4.50 | 5.20 | 4.90 | 4.50 |
| L-Lysine HCl | 3.00 | 5.20 | 0.80 | 2.60 | 6.00 | 2.90 | 2.90 |
| L-Threonine | 1.30 | 2.30 | 0.40 | 1.00 | 2.80 | 1.30 | 1.30 |
| DL-Methionine | 1.20 | 1.80 | 0.70 | 0.60 | 2.50 | 1.30 | 1.20 |
| L-Tryptophan | 0.10 | 0.50 | 0.00 | 0.10 | 0.60 | 0.10 | 0.10 |
| L-Valine | 0.00 | 0.90 | 0.00 | 0.00 | 1.30 | 0.00 | 0.00 |
| L-Isoleucine | 0.00 | 0.90 | 0.00 | 0.00 | 0.70 | 0.00 | 0.00 |
| L-Leucine | 0.00 | 0.00 | 0.00 | 0.00 | 0.60 | 0.00 | 0.00 |
| L-Histidine | 0.00 | 0.20 | 0.00 | 0.00 | 0.40 | 0.00 | 0.00 |
| Vitamin and trace mineral mixture[3] | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 |
| Calculated / Analysed Composition[4], % as fed or as specified | | | | | | | |
| Dry Matter, analysed | 87.30 | 87.70 | 88.10 | 87.70 | 88.00 | 87.50 | 88.30 |
| Ash, analysed | 3.60 | 4.10 | 4.20 | 4.10 | 4.20 | 4.10 | 3.80 |
| NE, MJ/kg | 10.03 | 10.07 | 10.03 | 10.03 | 10.07 | 9.61 | 10.45 |
| SID Lys:NE, g/MJ | 0.95 | 0.94 | 0.95 | 0.75 | 1.14 | 0.99 | 0.91 |
| Crude Protein, analysed | 15.4 | 15.1 | 17.7 | 13.7 | 16.6 | 16.2 | 16.3 |
| Total Lys, analysed | 1.02 | 1.12 | 1.08 | 0.86 | 1.32 | 1.07 | 1.02 |
| Total Thr / Lys ratio, analysed | 0.71 | 0.69 | 0.73 | 0.74 | 0.67 | 0.68 | 0.72 |
| Total Met-Cys / Lys ratio, analysed | 0.62 | 0.62 | 0.63 | 0.64 | 0.58 | 0.60 | 0.63 |

*Table 7.2 Continue*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total Trp / Lys ratio, analysed | 0.19 | 0.15 | 0.18 | 0.09 | 0.15 | 0.16 | 0.17 |
| Total Val / Lys ratio, analysed | 0.75 | 0.68 | 0.87 | 0.76 | 0.67 | 0.74 | 0.75 |
| Total Leu / Lys ratio, analysed | 1.19 | 0.98 | 1.38 | 1.27 | 1.04 | 1.18 | 1.23 |
| Total Ile / Lys ratio, analysed | 0.63 | 0.56 | 0.76 | 0.64 | 0.57 | 0.63 | 0.64 |
| Total His / Lys ratio, analysed | 0.41 | 0.37 | 0.48 | 0.40 | 0.37 | 0.41 | 0.42 |
| SID Lys | 0.95 | 0.95 | 0.95 | 0.75 | 1.15 | 0.95 | 0.95 |
| SID Thr / Lys ratio | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| SID Met-Cys / Lys ratio | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| SID Trp / Lys ratio | 0.19 | 0.19 | 0.22 | 0.19 | 0.19 | 0.19 | 0.19 |
| SID Val / Lys ratio | 0.69 | 0.65 | 0.81 | 0.72 | 0.65 | 0.69 | 0.69 |
| SID Leu / Lys ratio | 1.14 | 0.98 | 1.33 | 1.23 | 0.99 | 1.15 | 1.16 |
| SID Ile / Lys ratio | 0.59 | 0.55 | 0.71 | 0.60 | 0.52 | 0.59 | 0.60 |
| SID His / Lys ratio | 0.37 | 0.32 | 0.43 | 0.38 | 0.32 | 0.36 | 0.37 |
| Fat, analysed | 2.28 | 2.75 | 3.91 | 2.64 | 2.89 | 2.40 | 5.00 |
| Crude Fibre, analysed | 2.90 | 3.50 | 3.20 | 3.70 | 3.20 | 4.80 | 2.90 |
| NDF | 12.52 | 13.72 | 12.00 | 14.00 | 12.72 | 14.81 | 12.00 |
| Calcium | 0.70 | 0.80 | 0.70 | 0.80 | 0.77 | 0.75 | 0.70 |
| Digestible Phosphorus | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 |

[1] Diets were fed in growing pigs during 20 days at 12 weeks of age.

[2] C2 = Control; LP2 = Low Crude Protein; HP2 = High Crude Protein; LA2 = Low Amino Acid; HA2 = High Amino Acid; LE2 = Low Net Energy; HE2 = High Net Energy

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin $D_3$, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin $B_{12}$, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin $B_1$, 3 mg Vitamin $B_6$.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fibre.

### 7.2.2 Body Weight, Feed Intake and Feed Efficiency Traits

Pigs were weighed per pen at the beginning and at the end of the trial period. Feed intake was recorded per pen at the beginning and at the end of the trial period. Feed bags were weighed before feeding the pigs, and before weighing the pigs per pen, to know exactly the feed intake per pen on a week basis. On both studies, study 1 and 2, ADG, ADFI and FCR were calculated for the overall trial period.

### 7.2.3 Feed Analysis

Feed samples of each diet were collected per duplicate and analysed for DM, ash, CP, crude fibre, fat, and total AA profile at the Sciantec Analytical Services (Stockbridge Technology Centre, Cawood, Yorkshire, UK). Dry matter was measured by oven drying for 4 h at 103°C (Thiex, 2009); ash was measured via combustion in a muffle furnace at 550°C (Thiex et al., 2012); CP was determined as N × 6.25 based on the DUMAS method (Ebeling, 1968) using LECO FP-628 analyser (Leco Instruments Ltd., Stockport, UK);

crude fibre was determined by a Fibertec semi-automatic system (Tecator, Hoganas, Sweden) using the gravimetry method (Thiex, 2009); fat was measured using Randall/Soxtec/Submersion method (Thiex et al., 2003); and total AA profile was determined based on ion exchange HPLC (Otter, 2012) using the Biochrom AA Analyser Sodium System (Biochrom Ltd., Cambridge, UK).

### 7.2.4 Blood sample collection and blood serum analysis

Blood samples were collected via venepuncture of the external jugular vein (approximately 10 ml/pig) from 2 pigs/pen selected randomly at 20 (study 1) and 14 (study 2) weeks of age. A total of 40 and 56 (n = 8 per treatment) blood samples were collected in study 1 and 2 respectively. Blood samples were collected early in the morning in a non-fasting state as per commercial practice. Blood samples were kept immediately on ice at 4°C after collection until serum was separated by centrifugation for 15 min at 2000 rcf. Blood serum samples were analysed the same day using the ABX Pentra 400 Clinical Chemistry benchtop analyser (HORIBA Medical, Irvine, California, USA) and ABX Pentra 400 re-agents (HORIBA ABX SAS, Montpellier, France) at the Teagasc Chemistry Lab in Fermoy, Co. Cork, Ireland. Selected blood serum metabolites (and techniques): Albumin [bromocresol green dye-binding procedure (Doumas et al., 1971)], glucose [hexokinase method (Todd et al., 1979)], triglycerides [enzymatic method (Fossati and Prencipe, 1982)], cholesterol [enzymatic photometric test (Deeg and Ziegenhorn, 1983)], SUN [enzymatic UV test (Talke and Schubert, 1965)], total protein concentration [Biuret reaction (Gornall et al., 1949)], and creatinine [enzymatic method (Guder et al., 1986)].

### 7.2.5 Faecal sample collection and volatile fatty acid analysis

Faecal samples were collected using BioFreeze$^{TM}$ vials (Alimetrics Diagnostics Ltd, Espoo, Finland) from 2 pigs/pen selected randomly at 20 (study 1) and 14 (study 2) weeks of age. A total of 40 and 56 (n = 8 per treatment) faecal samples were collected in study 1 and study 2 respectively. BioFreeze$^{TM}$ vials enable to collect the fresh samples and stop all biological activity at ambient temperature until the analysis. Faecal VFA analysis was conducted via gas chromatography using pivalic acid as an internal standard (Czerkawski, 1976) at Alimetrics Diagnostics. The VFA profile included acetic, propionic, butyric, valeric, BCFA and total VFA. In study 2, SCFA were also analysed.

### 7.2.6 Data management and statistical analysis

Performance, blood serum and faecal VFA data analyses were carried out using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Plots were created using R v4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Pen was considered as the experimental unit for all performance, serum and faecal data analyses. Alpha level for determination of significance was 0.05 and trends were identified as alpha of 0.10. Data were tested for normality using the Shapiro-Wilk test and by examining the normal probability plot. Initial BW data were analysed using general linear models including treatment as fixed effect. Models for BW, ADG, ADFI and FCR variables were analysed using general linear models including treatment diet as fixed effect and initial BW as a co-variable. For blood serum, models for albumin, glucose, triglycerides, cholesterol, SUN, creatinine, and total protein were analysed using general linear models including treatment diet as fixed effect. For faecal VFA, models for acetic, propionic, butyric, valeric, SCFA (only study 2), BCFA and VFA were analysed using general linear models including treatment diet as fixed effect. Multiple means comparisons were done using Tukey-Kramer's correction in all cases. Results for fixed effects are reported as least square means ± standard error mean.

Receiver Operating Characteristic Curves analysis was used to identify biomarkers that discriminated between diets. Data were analysed using the *pROC* package (Turck et al., 2011) for R v4.0.2. Univariable ROC curves were calculated for SUN and BCFA comparing LP1 vs HP1, LE1 vs HE1, LP2 vs HP2, LE2 vs HE2, and LA2 vs HA2. The accuracy of the models was assessed by calculating the AUC. Values of AUC were interpreted as non-accurate (AUC = 0.5), less accurate ($0.5 < \text{AUC} \leq 0.7$), moderately accurate ($0.7 < \text{AUC} \leq 0.9$), highly accurate ($0.9 < \text{AUC} < 1$) and perfect (AUC = 1). The AUC is significant when the confidence interval does not include 50%. Cut-off concentrations were calculated for each ROC curve and the corresponding sensitivities and specificities, and 95% confidence intervals were obtained.

# 7.3 Results

## 7.3.1 Body Weight, Feed Intake and Feed Efficiency Traits

In study 1, there were no differences on BW, ADG, ADFI and FCR between dietary treatments (Table 7.3). In study 2, pigs fed the LA2 diet were 3.5 kg lighter and gained 171.0 g/d less in average than those pigs fed with the C2, LP2, HP2, HA2, and HE2 diets at the end of the trial ($P < 0.01$; Table 7.4). Moreover, pigs fed the LP2 diet consumed 229.3 g/d more than HP2 pigs ($P = 0.035$) at the end of the trial. Finally, pigs fed the LA2 diet had higher FCR than pigs fed with the C2, HP2, HA2, LE2, and HE2 diets ($P < 0.001$); while HP2 pigs had lower FCR than those pigs fed with the C2, LP2, LA2, and LE2 dietary treatments ($P < 0.001$) at the end of the trial.

**Table 7.3** Productive performance of finishing pigs grouped by dietary treatment in study 1.[1]

| | Dietary Treatment [2] | | | | | | |
|---|---|---|---|---|---|---|---|
| Traits | C1 | LP1 | HP1 | LE1 | HE1 | SEM | *P*-value |
| BW 128 d, kg | 87.1 | 88.1 | 87.8 | 85.4 | 86.9 | 2.32 | 0.926 |
| BW 138 d, kg | 100.2 | 99.9 | 101.0 | 100.2 | 100.2 | 0.70 | 0.828 |
| ADG, g | 1315.0 | 1283.7 | 1399.1 | 1312.1 | 1317.7 | 71.49 | 0.825 |
| ADFI, g | 3024.2 | 3078.4 | 3110.0 | 3284.8 | 2977.7 | 128.86 | 0.531 |
| FCR | 2.33 | 2.39 | 2.23 | 2.48 | 2.27 | 0.08 | 0.228 |

[1] Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) (SEM = Standard error mean [SEM]). Pigs were followed from 128 to 138 days of age (n = 4).

[2] Dietary treatments: C1 (Control; 10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LP1 (Low Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HP1 (High Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LE1 (Low Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE1 (High Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed).

**Table 7.4** Productive performance of growing pigs grouped by dietary treatment in study 2.[1]

| | Dietary Treatment [2] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Traits | C2 | LP2 | HP2 | LA2 | HA2 | LE2 | HE2 | SEM | *P*-value |
| BW 84 d, kg | 41.1 | 41.7 | 41.3 | 41.5 | 41.1 | 41.2 | 41.3 | | |
| BW 104 d, kg | $62.6^{a}$ | $62.1^{a}$ | $62.7^{a}$ | $59.3^{b}$ | $63.2^{a}$ | $61.5^{ab}$ | $63.2^{a}$ | 0.57 | 0.001 |
| ADG, g | $1061.0^{a}$ | $1038.9^{a}$ | $1075.5^{a}$ | $900.9^{b}$ | $1090.1^{a}$ | $1011.5^{ab}$ | $1093.8^{a}$ | 28.90 | 0.002 |
| ADFI, g | $1932.3^{ab}$ | $1950.2^{a}$ | $1720.9^{b}$ | $1819.7^{ab}$ | $1933.2^{ab}$ | $1850.3^{ab}$ | $1878.4^{ab}$ | 46.76 | 0.030 |
| FCR | $1.84^{b}$ | $1.89^{ab}$ | $1.61^{c}$ | $2.03^{a}$ | $1.78^{bc}$ | $1.85^{b}$ | $1.73^{bc}$ | 0.04 | < 0.001 |

[1] Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (SEM = Standard error mean [SEM]). Pigs were followed from 84 to 104 days of age (n = 4).

[2] Dietary treatments: C2 (Control; 10.03 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), LP2 (Low Protein; 10.07 MJ/kg NE; 140.0 g of CP; 9.5 g of SID Lys per kg of feed), HP2 (High Protein; 10.03 MJ/kg NE; 190.0 g of CP; 9.5 g of SID Lys per kg of feed), LA2 (Low Amino Acid; 10.03 MJ/kg NE; 135.0 g of CP; 7.5 g of SID Lys per kg of feed), HA2 (High Amino Acid; 10.07 MJ/kg NE; 165.0 g of CP; 11.5 g of SID Lys per kg of feed), LE2 (Low Energy; 9.61 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE2 (High Energy; 10.45 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed).

[a-b] Within rows, significant differences between groups ($P < 0.05$).

### 7.3.2 Blood serum metabolites

Blood metabolite profile for study 1 and 2 are presented in table 7.5 and 7.6, respectively. Albumin and glucose did not differ between dietary treatments, although HE1 pigs tended to have higher glucose concentration levels than LE1 pigs ($P = 0.063$). For creatinine and triglycerides, HE1 pigs had higher levels than LE1 pigs ($P < 0.05$) however, HE1 pigs had lower total protein levels than C1 pigs ($P = 0.033$). Furthermore, LP1 pigs had higher cholesterol concentration levels than LE1 pigs ($P = 0.015$) while HP1 pigs had higher SUN (13.63 ± 0.951 mg/dL) than the other dietary treatments (7.47 ± 0.951 mg/dL; $P < 0.001$; Figure 7.1). In study 2, glucose and creatinine did not differ between dietary treatments. For albumin, LA2 pigs had lower concentrations than pigs fed with HP2, HA2, LE2, and HE2 diets ($P < 0.001$). Also, LA2 pigs had lower total protein levels than HP2 pigs ($P = 0.05$). Pigs fed HP2 diet had higher SUN (11.60 ± 0.613 mg/dL) than the rest of the dietary treatments ($P < 0.001$; Figure 7.1) while LP2 pigs had lower SUN (5.2 ± 0.61 mg/dL) than those pigs fed with C2 (8.2 ± 0.61), HA2 (8.7 ± 0.61), LE2 (8.8 ± 0.61), HE2 (8.5 ± 0.61), and HP2 ($P < 0.001$). Finally, C2 pigs showed lower triglycerides levels than pigs fed with LE2 and HE2 dietary treatments ($P < 0.01$) however, HE2 pigs had higher cholesterol concentration levels than C2 and LE2 pigs ($P < 0.05$).

### 7.3.3 Volatile fatty acids profile

Total VFA and VFA profiles for study 1 and study 2 are presented in table 7.5 and 7.6, respectively. Total VFA (mmol/kg) did not differ between dietary treatments. Pigs fed C1 had lower percentage of acetic than LP1 and HP1 pigs ($P < 0.05$) but higher percentage of valeric than LE1 pigs ($P = 0.010$). Pigs fed LE1 showed lower percentage of BCFA (4.4 ± 0.38) than C1 and HE1 pigs (6.4 ± 0.38; 6.1 ± 0.38, respectively; P < 0.01; Figure 7.1). In study 2, total VFA (mmol/kg) did not differ between dietary treatments ($P > 0.05$). Moreover, acetic, butyric, valeric, and SCFA (as % of total VFA) did not differ between dietary treatments ($P > 0.05$). Nevertheless, LA2 pigs had higher % of propionic of total VFA than those pigs fed with C2, HP2, HA2, LE2, and HE2 dietary treatments ($P < 0.001$). Finally, HP2 pigs had higher % of BCFA (6.61 ± 0.408) of total VFA than HA2 and LE2 pigs (4.84 ± 0.408; 4.84 ± 0.408, respectively; $P < 0.05$; Figure 7.1); and HP2 pigs tended to have higher % of BCFA of total VFA than LP2 and LA2 pigs (5.06 ± 0.408; 5.01 ± 0.408, respectively; $P < 0.10$; Figure 7.1).

**Table 7.5** Blood serum and faecal volatile fatty acid (VFA) profile from 40 finishing pigs grouped by dietary treatment (n = 4) in study 1.[1]

| | Dietary Treatment [2] | | | | | | |
|---|---|---|---|---|---|---|---|
| Traits | C1 | LP1 | HP1 | LE1 | HE1 | SEM | *P*-value |
| Blood Serum | | | | | | | |
| Albumin, g/L | 36.54 | 37.08 | 38.04 | 36.04 | 36.53 | 0.880 | 0.565 |
| Glucose, mmol/L | 5.31 | 5.24 | 5.39 | 4.61 | 5.68 | 0.273 | 0.105 |
| Triglycerides, mmol/L | 0.38 $^{ab}$ | 0.35 $^{ab}$ | 0.37 $^{ab}$ | 0.28 $^{b}$ | 0.42 $^{a}$ | 0.030 | 0.023 |
| Cholesterol, mmol/L | 2.12 $^{ab}$ | 2.44 $^{a}$ | 2.27 $^{ab}$ | 2.03 $^{b}$ | 2.24 $^{ab}$ | 0.084 | 0.021 |
| Creatinine, µmol/L | 123.03 $^{ab}$ | 127.50 $^{ab}$ | 123.85 $^{ab}$ | 117.20 $^{b}$ | 133.81 $^{a}$ | 3.966 | 0.070 |
| Total Protein, g/L | 67.36 $^{a}$ | 64.20 $^{ab}$ | 63.61 $^{ab}$ | 61.46 $^{ab}$ | 60.86 $^{b}$ | 1.509 | 0.035 |
| Faecal VFA, % of total VFA | | | | | | | |
| Acetic | 57.4 $^{b}$ | 61.6 $^{a}$ | 62.0 $^{a}$ | 60.6 $^{ab}$ | 58.5 $^{ab}$ | 1.01 | 0.012 |
| Propionic | 21.9 | 21.4 | 21.2 | 22.0 | 21.7 | 0.59 | 0.827 |
| Butyric | 11.4 | 9.8 | 9.3 | 10.9 | 11.0 | 0.55 | 0.061 |
| Valeric | 2.9 $^{a}$ | 2.3 $^{ab}$ | 2.4 $^{ab}$ | 2.1 $^{b}$ | 2.6 $^{ab}$ | 0.15 | 0.010 |
| VFA, mmol/kg | 193.0 | 158.2 | 166.9 | 170.5 | 154.7 | 14.98 | 0.451 |

[1] Blood and faecal samples were collected from 2 pigs/pen selected randomly at 20 weeks of age (Means ± Standard error mean [SEM]).

[2] Dietary treatments: C1 (Control; 10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LP1 (Low Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HP1 (High Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LE1 (Low Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE1 (High Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed).

[a-b] Within rows, significant differences between groups ($P < 0.05$).

**Table 7.6** Blood serum and faecal volatile fatty acid (VFA) profile from 56 growing pigs grouped by dietary treatment (n = 4) in study 2.[1]

| | Dietary Treatment [2] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Traits | C2 | LP2 | HP2 | LA2 | HA2 | LE2 | HE2 | SEM | *P*-Value |
| Blood Serum | | | | | | | | | |
| Albumin, g/L | 36.04 $^{ab}$ | 34.76 $^{ab}$ | 38.05 $^{a}$ | 32.10 $^{b}$ | 39.08 $^{a}$ | 37.25 $^{a}$ | 37.19 $^{a}$ | 1.083 | < 0.001 |
| Glucose, mmol/L | 5.37 | 5.83 | 5.72 | 5.94 | 6.05 | 5.69 | 5.57 | 0.253 | 0.559 |
| Triglycerides, mmol/L | 0.33 $^{b}$ | 0.55 $^{ab}$ | 0.44 $^{ab}$ | 0.57 $^{ab}$ | 0.47 $^{ab}$ | 0.67 $^{a}$ | 0.61 $^{a}$ | 0.059 | 0.004 |
| Cholesterol, mmol/L | 2.34 $^{b}$ | 2.61 $^{ab}$ | 2.45 $^{ab}$ | 2.52 $^{ab}$ | 2.39 $^{ab}$ | 2.35 $^{b}$ | 2.80 $^{a}$ | 0.107 | 0.046 |
| Creatinine, µmol/L | 111.01 | 100.29 | 117.56 | 113.89 | 112.97 | 113.00 | 107.34 | 4.422 | 0.168 |
| Total Protein, g/L | 61.98 $^{ab}$ | 64.60 $^{ab}$ | 65.95 $^{a}$ | 59.98 $^{b}$ | 63.49 $^{ab}$ | 61.64 $^{ab}$ | 63.44 $^{ab}$ | 1.391 | 0.077 |
| Faecal VFA, % of total VFA | | | | | | | | | |
| Acetic | 55.99 | 55.22 | 54.64 | 54.66 | 56.84 | 56.37 | 56.01 | 1.192 | 0.798 |
| Propionic | 21.15 $^{b}$ | 23.18 $^{ab}$ | 21.40 $^{b}$ | 24.53 $^{a}$ | 21.19 $^{b}$ | 21.57 $^{b}$ | 21.51 $^{b}$ | 0.567 | < 0.001 |
| Butyric | 13.53 | 12.57 | 13.25 | 11.81 | 13.42 | 13.04 | 13.42 | 0.710 | 0.608 |
| Valeric | 4.08 | 3.96 | 4.09 | 3.99 | 3.72 | 4.18 | 3.49 | 0.367 | 0.844 |
| SCFA [3] | 101.56 | 101.32 | 101.43 | 100.80 | 101.43 | 101.27 | 101.21 | 0.221 | 0.310 |
| VFA, mmol/kg | 93.78 | 88.44 | 91.55 | 86.58 | 95.75 | 99.86 | 88.38 | 6.281 | 0.754 |

[1] Blood and faecal samples were collected from 2 pigs/pen selected randomly at 14 weeks of age (Means ± Standard error mean [SEM]).

[2] Dietary treatments: C2 (Control; 10.03 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), LP2 (Low protein; 10.07 MJ/kg NE; 140.0 g of CP; 9.5 g of SID Lys per kg of feed), HP2 (High protein; 10.03 MJ/kg NE; 190.0 g of CP; 9.5 g of SID Lys per kg of feed), LA2 (Low amino acid; 10.03 MJ/kg NE; 135.0 g of CP; 7.5 g of SID Lys per kg of feed), HA2 (High amino acid; 10.07 MJ/kg NE; 165.0 g of CP; 11.5 g of SID Lys per kg of feed), LE2 (Low energy; 9.61 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE2 (High energy; 10.45 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed).

[3] SCFA = Short-chain fatty acids.

[a-b] Within rows, significant differences between groups ($P < 0.05$).

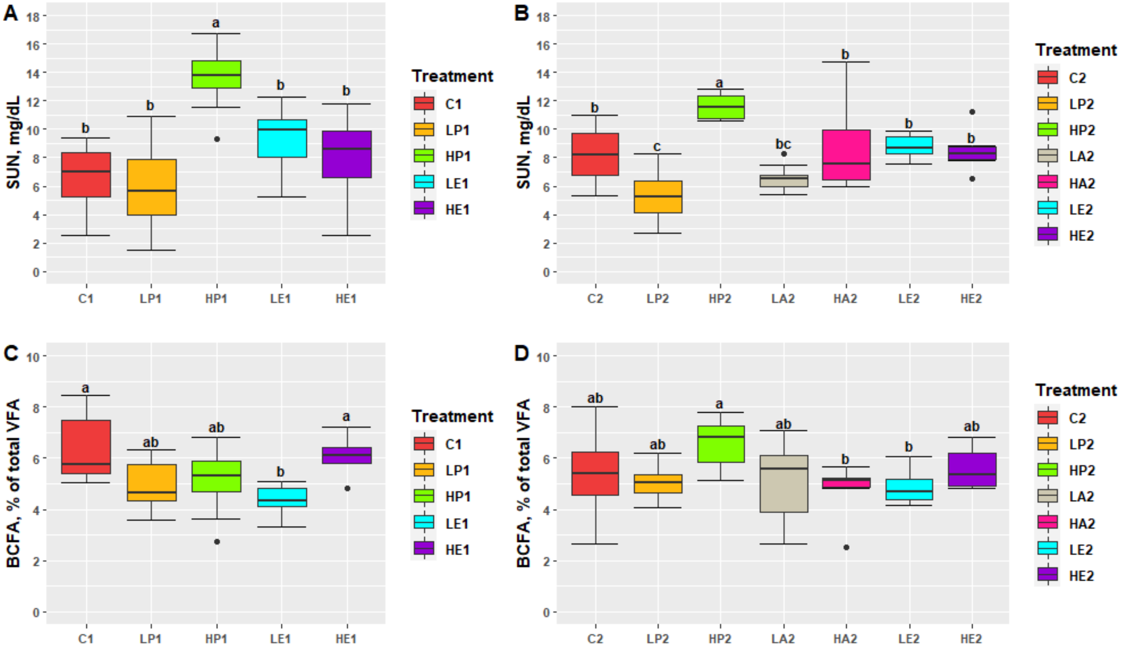

**Figure 7.1** Serum urea nitrogen (SUN; mg/dL) and branched-chain fatty acids (BCFA; % of total VFA) levels grouped by dietary treatment (n = 4) from study 1 (**A**, **C**) and study 2 (**B**, **D**). Results are presented as Means ± Standard error mean. [a, b] Significant differences between treatments ($P < 0.05$). Blood and faecal samples were collected from 2 pigs/pen selected randomly at 20 weeks of age (study 1) and 14 weeks of age (study 2). Dietary treatments from study 1: C1 (Control; 10.03 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), LP1 (Low Protein; 10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), HP1 (High Protein; 10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), LE1 (Low Energy; 9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE1 (High Energy; 10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed). Dietary treatments from study 2: C2 (Control; 10.03 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), LP2 (Low protein; 10.07 MJ/kg NE; 140.0 g of CP; 9.5 g of SID Lys per kg of feed), HP2 (High protein; 10.03 MJ/kg NE; 190.0 g of CP; 9.5 g of SID Lys per kg of feed), LA2 (Low amino acid; 10.03 MJ/kg NE; 135.0 g of CP; 7.5 g of SID Lys per kg of feed), HA2 (High amino acid; 10.07 MJ/kg NE; 165.0 g of CP; 11.5 g of SID Lys per kg of feed), LE2 (Low energy; 9.61 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), and HE2 (High energy; 10.45 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed).

### 7.3.4 ROC curve analysis

The ROC curve analysis for study 1 is presented in Figure 7.2. The AUC for SUN was of 98.4% with two optimal cut-offs of 9.4 mg/dL (100% sensitivity, 87.5% specificity) and 11.2 mg/dL (87.5% sensitivity, 100% specificity) that could serve to differentiate the LP1 vs HP1 dietary treatments. Branched-chain fatty acids showed a high accuracy (AUC = 96.9%) to differentiate the LE1 vs HE1 dietary treatments. The ROC curve analysis for study 2 is presented in Figure 7.3. The AUC for SUN was 100% with an optimal cut-off of 9.4 mg/dL to differentiate LP2 vs HP2 dietary treatments. Branched-chain fatty acids showed a moderate-high accuracy to differentiate LP2 vs HP2, and LE2 vs HE2 dietary treatments (AUC = 87.5% and 81.2%, respectively).

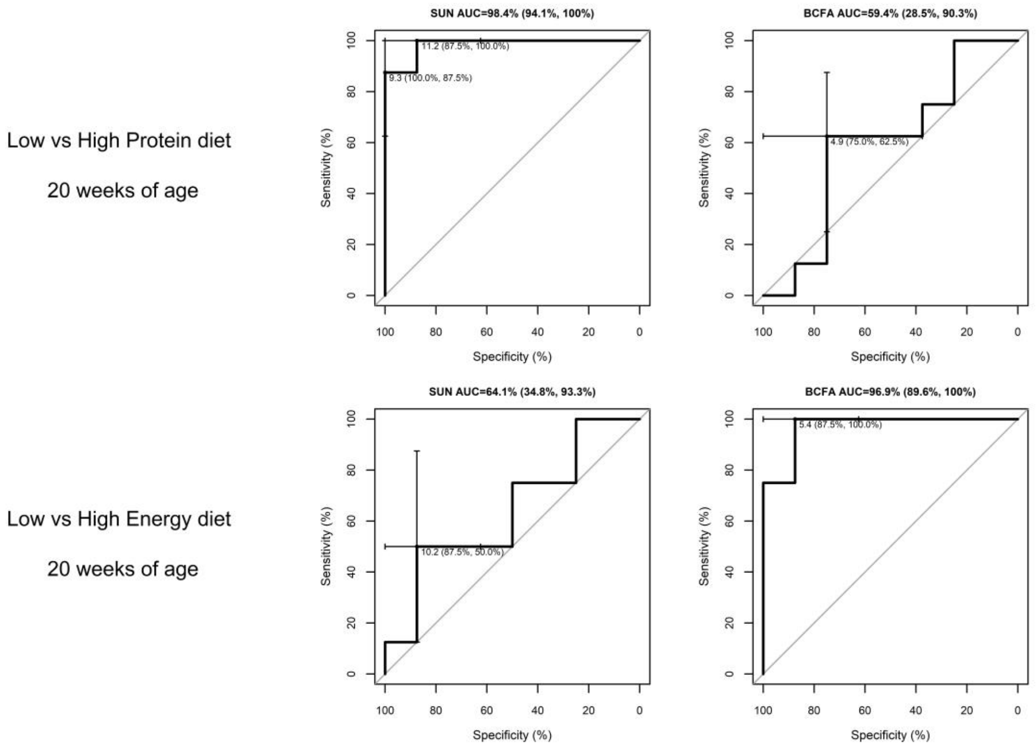

**Figure 7.2** Receiver operating characteristic (ROC) curves for serum urea nitrogen (SUN) and branched-chain fatty acids (BCFA) biomarkers used to differentiate low vs high protein and energy diets in finishing pigs at 20 weeks of age (Study 1). Dietary treatments from study 1: Low Protein (10.03 MJ/kg of NE; 132.0 g of CP; 7.5 g of SID Lys per kg of feed), High Protein (10.03 MJ/kg of NE; 188.0 g of CP; 11.5 g of SID Lys per kg of feed), Low Energy (9.61 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed), and High Energy (10.45 MJ/kg of NE; 160.0 g of CP; 9.5 g of SID Lys per kg of feed). Headings include the area under the curve (AUC) and the 95% confidence interval (CI). The AUC is significant when the CI does not include 50%. Within each graph, the optimal cut-off concentration and the corresponding specificity and sensitivity (parenthesis) are shown.

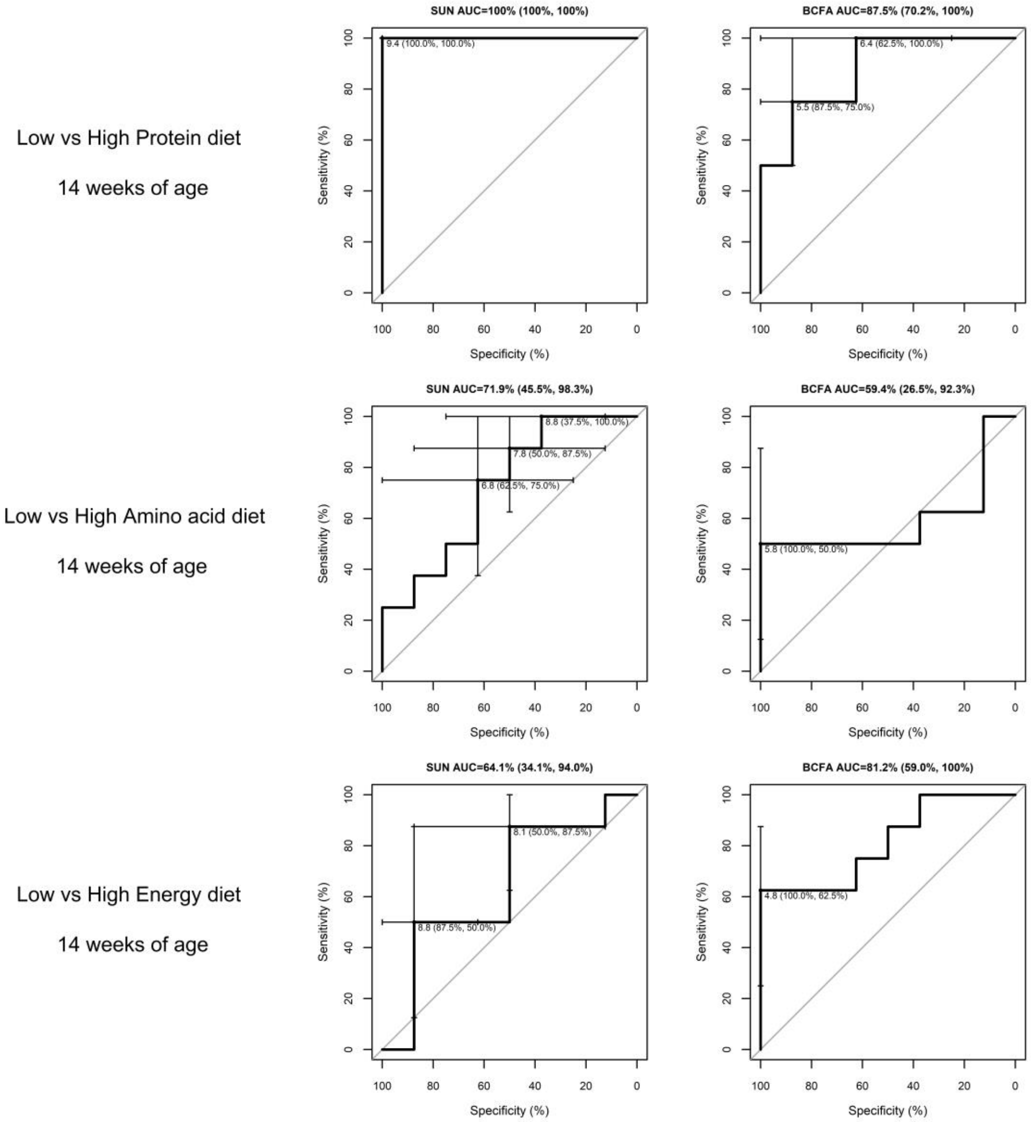

**Figure 7.3** Receiver operating characteristic (ROC) curves for serum urea nitrogen (SUN) and branched-chain fatty acids (BCFA) biomarkers used to differentiate low vs high protein, amino acids, and energy diets in growing pigs at 14 weeks of age (Study 2). Dietary treatments from study 2: Low protein (10.07 MJ/kg NE; 140.0 g of CP; 9.5 g of SID Lys per kg of feed), High protein (10.03 MJ/kg NE; 190.0 g of CP; 9.5 g of SID Lys per kg of feed), Low amino acid (10.03 MJ/kg NE; 135.0 g of CP; 7.5 g of SID Lys per kg of feed), High amino acid (10.07 MJ/kg NE; 165.0 g of CP; 11.5 g of SID Lys per kg of feed), Low energy (9.61 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed), and High energy (10.45 MJ/kg NE; 165.0 g of CP; 9.5 g of SID Lys per kg of feed). Headings include the area under the curve (AUC) and the 95% confidence interval (CI). The AUC is significant when the CI does not include 50%. Within each graph, the optimal cut-off concentration and the corresponding specificity and sensitivity (parenthesis) are shown.

## 7.4 Discussion

Although these trials were not designed to study differences in productive performance due to the short period of study and low sample size, productive performance was monitored and there were some interesting findings. In study 1, there were no differences in productive performance among diets. Thus, for study 2, the authors decided to use younger pigs which are more likely to be affected by reductions in dietary AA levels similar to those used in study 1 (from 1.15 to 0.75 g SID Lys per MJ). Pigs fed LA2 diet were the lightest and had the worst efficiency of all the diets in study 2 probably because the levels of AAs were not enough to meet the requirements of the pigs (NRC, 2012). Pigs in study 2 were also followed for a slightly longer period of time which may have allowed them to show differences in productive performance. It is interesting to note that productive performance of pigs fed LP2 diet was not affected compared to HP2. This diet was supplemented with AAs and both LP2 and HP2 had the same level of SID Lys (9.5 g per MJ). Thus, LP2 would achieve a lower risk of abnormal protein fermentations and lower emissions than HP2 with no negative effects in performance.

Serum metabolites have been used in pigs for research purposes but the literature on their use for clinical purposes is scarce and reference values are needed considering different factors such as age, breed, sex, diet, and methods of sample collection and analysis (Constable et al., 2017). In this study, the authors aimed to use serum metabolites as biomarkers to discriminate between diets differing on CP, AA, and NE levels with the final intention to use these biomarkers in daily practice as indicator of suboptimal diets. In study 1, SUN was the clearest indicator of differences between diets with high and low CP discriminate in the ROC curve analysis with AUC close to 1. The principal end product of protein catabolism is SUN (Wu, 2013), thus it makes sense that SUN increases when the diet has an excess of CP that cannot be used by the animal due to CP excess or AA imbalances. Diets LP1 and HP1 differed in both, the level of CP and the level of AAs. To separate these 2 effects, in study 2, LP2 and HP2 were formulated to have different levels of CP but the same levels of AAs and LA2 and HA2 were formulated to differ on AA levels. As expected, diet HP2, with an excess of CP, resulted in an increased SUN level compared to LP2. However, diet HA2 compared to LA2 did not induce the same increase in SUN that HP2 compared to LP2, despite having a similar increase in CP. The increase in CP in diet HA2 could be used by the pig for growth because it was achieved by increasing AAs levels according to ideal protein profile. Overall, SUN may be a useful indicator of protein efficiency at farm level when pigs are fed suboptimal dietary CP diets not balanced in AAs. The use of SUN is also an advantage because of its short time to achieve a constant concentration in blood after changing the diet (Coma et al., 1995).

Total protein and albumin are also involved in protein metabolism and were studied as interesting biomarkers. There were no differences in study 1 in total protein and albumin between diets. The latter is in agreement with Regmi et al. (Regmi et al., 2018), who did not observe differences in serum total protein concentration in finishing pigs, of similar age to those in study 1, fed insufficient (0.32%), adequate (0.60%) or excessive (0.87%) SID Lys diets during 4 weeks, and only observed reduced plasma albumin concentration in finishing pigs when fed the 0.32% SID Lys diet which is far below the SID Lys levels of the dietary treatments of the present study. Nevertheless, in study 2, pigs fed higher amounts of CP showed an increase in serum total protein and albumin levels. These findings are in accordance with some of the previous literature (Kamalakar et al., 2009; Mule et al., 2006). Thus, the age of the pig may affect serum total protein and albumin levels and finishing pigs may be able to show a homeostatic control besides the dietary CP content. Moreover, finishing pigs have already reached the maximum protein deposition (Brossard et al., 2009; Remus et al., 2020; Van Milgen et al., 2008) and their metabolism may not be focused on protein turn-over contrary to early stages of the grower-finisher period. Albumin was a good biomarker to differentiate between LA2 and HA2 in study 2, but it was not as consistent as SUN. Further research is needed to explore its use in multivariable biomarkers in combination with SUN.

Serum creatinine is also related to protein metabolism because is the product from muscle metabolism (Constable et al., 2017) and has a positive correlation with total and striated muscle (Baxmann et al., 2008). In the current studies, creatinine did not show any clear patterns and may not be as good as SUN as a biomarker.

Concerning energy, serum glucose did not show clear patterns between dietary treatments either which agrees with previous literature (Kamalakar et al., 2009; Regmi et al., 2018; Zeng et al., 2013) and shows a good homoeostatic control of serum glucose concentration by either growing and finishing pigs in commercial conditions fed ad libitum. Triglycerides and cholesterol are both metabolites involved in lipid metabolism (Constable et al., 2017). They did not show any consistent pattern despite showing some differences between diets differening in NE levels. The incosistency in the results may be related to the age of the animals. The hypercholesterolemic effect observed in study 2 pigs but not so clear in study 1 is in agreement with previous literature (Kamalakar et al., 2009; Mule et al., 2006; Regmi et al., 2018), although the exact mecanishim of this effect is not clear yet. Further research should be carried out in order to further define these results.

No differences in total VFAs concentrations were observed between the dietary treatments in any of the two trials, and none of the individual VFAs showed difference worth discussing except for BCFA. The authors hypothesised that BCFA would show CP excess in the diets. However, the pattern differed between trials. Growing pigs in study 2 fed HP2 diet showed a higher percentage of BCFA than pigs fed the HA2 and LE2 diets. Moreover, HP2 pigs had numerically greatly amounts of BCFA compared to those pigs fed the LP2 and LA2 diets. ROC curve analysis showed that BCFA has a moderate-high accuracy to differentiate LP2 and HP2 diets in growing pigs. These findings agree with previous literature that reported an increased production of BCFA in manure in grower-finisher pigs fed high CP diets (Cho et al., 2015; Le et al., 2007). The low production of BCFA in pigs fed the HA2 diet compared to those fed the HP2 diet might be related to a fast absorption rate of free AA and a lower level of CP available for fermentation which agrees with the pattern observed for SUN. In study 1, finishing pigs fed the HP1 diet did not show higher percentage of BCFA than any other dietary treatment. This absence of differences in BCFA might be explained by the fact that older pigs have a more developed gastrointestinal tract with a high fermentation capacity that makes it difficult to observe differences between the dietary treatments at faecal level. The differences may exist in cecum or proximal colon but are not present in faeces. On the other hand, a higher percentage of BCFA between pigs was found in pigs fed diet HE1 when compared to LE1. This difference could be related to the added fibre in the LE1 diet. In this line, a recent study reported that the increased BW and age of the pigs resulted in an improved digestibility of dietary fibre fractions (Zhao et al., 2020b), which will influence the VFA profile as it is positively correlated with the ATTD of insoluble dietary fibre and cellulose (Zhao et al., 2020a). Therefore, the fermentation of soybean hulls could have produced a shift in the VFA profile reducing the BCFA production by the microbial population. Although BCFA did not show the same consistency as SUN, more research is warranted. The deamination of branched AAs may also cause a shift in the microbiome population to increase production of BCFA (Le et al., 2005) which may be a more sensitive biomarker.

## 7.5 Conclusion

This study aimed to assess the feasibility of blood serum metabolite and faecal VFA profiles as biomarkers for suboptimal diets on energy, protein, and AAs in growing and finishing pig at farm level. Out of all the blood serum metabolites studied, SUN seems to be the best indicator to assess protein efficiency at farm level. Regarding the faecal VFA profile, BCFAs could be a potential indicator for high CP diets but may be affected by the age of the pig. Further studies at commercial scale are needed to fully understand the applicability of SUN in a cohort of commercial farms. The use of other biomarkers as part of a multivariable indicator should also be considered.

# Chapter 8

# Predicting chemical composition and apparent total tract digestibility of freeze-dried not ground faeces using near-infrared spectroscopy in pigs

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## 8.1 Background

Diet optimization is currently a time-consuming process including ingredient analysis, digestibility determination and on farm feed efficiency measurements. Describing the nutritional value of ingredients for livestock should be performed *in vivo* and using chemical analysis of the feed and prediction equations for each type of animals (Le Goff and Noblet, 2001). Nonetheless, feed efficiency will vary from farm to farm as the actual nutritional value of a diet will be affected by different factors related to the diet (feed manufacturing, physiochemical characteristics of feed ingredients, feed form and delivery method, etc.) (O'Meara et al., 2020; Patience et al., 2015; Shurson et al., 2021), the management and housing conditions (Camp Montoro et al., 2022, 2021a, 2020a), and the animal (genetic, gender, weight, health status) (Camp Montoro et al., 2021b; Patience et al., 2015; Van der Meer et al., 2016), among others. Thus, fast analysis methods to assess feed digestibility at farm level are of great interest to avoid the high costs of in vivo digestibility trials for nutrients and energy (Bastianelli, 2013), and improve production efficiency and farm sustainability.

Near-infrared reflectance spectroscopy is widely used to predict the nutritional value of raw materials and complete feeds in feed mills to monitor product quality (Bastianelli, 2013; Zijlstra et al., 2011). The success of this technique relies on its non-destructive and fast analysis of samples, the low cost per sample with little or no sample preparation, the no need of using chemicals, and the possibility of analysing samples at different places (Garrido-Varo et al., 2017). Thus, the same sample can be analysed several times and a high number of samples can be analysed every day.

Recent research has focused on assessing feed digestibility using faeces analysed via NIRS in different animal species (Bastianelli et al., 2007; Decruyenaere et al., 2015; Gil-Jiménez et al., 2015). Faeces contain information about the digestive process itself and are an easy material to collect at farm level. Bastianelli et al. (2007; 2013) showed in chickens and in pigs (2015) that the use of FNIRS can provide useful information as it accounts for digestibility due to animals' factors with acceptable accuracy. Moreover, FNIRS technique is feasible for use in pig nutrition research for predicting the chemical composition of diet and faeces, as well as to determine the ATTD coefficients with a moderate accuracy (Nirea et al., 2018; Paternostre et al., 2021; Schiborra et al., 2015).

Thus, FNIRS is a cost-effective promising tool for measuring feed efficiency and digestibility (Nirea et al., 2018).

All previous research conducted using the FNIRS technique has dried the faecal samples by drying or freeze-drying methods, followed by a grounding process to obtain a homogenic and low particle size faecal samples. However, no research has assessed the feasibility of using dried or freeze-dried faecal samples without grounding for FNIRS to assess chemical composition and ATTD coefficients. If results for ground and not ground samples were similar, we could avoid an important workload before analysis. Previous literature reported the feasibility to obtain similar nutritional value results from intact or ground raw feed material (Garrido-Varo et al., 2003).

Therefore, we hypothesise that similar results in faeces chemical composition and ATTD coefficients will be obtained from analysing via NIRS freeze-dried not ground (FDNG) and freeze-dried ground (FDG) faecal samples. The objective of the present study was to compare the FNIRS technique using faecal samples in two forms, FDNG and FDG, to predict the faeces chemical composition and ATTD coefficients of nutrients.

## 8.2 Material & Methods

### 8.2.1 Care and Use of Animals, Diets, and Faecal Sampling

The study was conducted at the Teagasc Pig Research Facility in Fermoy, Co. Cork, Ireland, and received ethical approval from the Teagasc Animal Ethics Committee (TAEC 244/2019). Two batches of pigs were used in the present study. In both batches, 220 Danish Duroc × (Large White × Landrace) grower-finisher pigs born within one week were housed in mixed sex pens with fully slatted concrete floor (2.4 × 4.2 m) containing a single wet-dry feeder [330mm (Width) × 370mm (Depth) × 1000mm (Height); MA37, Verba, Netherlands] and one supplementary nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was controlled by a mechanical ventilation system with fan speed and air inlet area regulated by a climate controller. Pens were enriched with a larch wood post.

The first batch of pigs was weighed per pen (n = 20 pens; 11 pigs/pen; 50.1 ± 3.44 kg BW) at 13 weeks of age. The second batch of pigs was weighed per pen (n = 20 pens; 11 pigs/pen; 87.0 ± 4.10 kg BW) at 18 weeks of age. For both batch 1 and 2, pens were assigned based on BW to five different dietary treatments and pigs were followed for 2 weeks. Diets were formulated to obtain a control diet (10.03 MJ/NE, 160.0 g of CP, and 9.5 g of SID Lys per kg of feed) which met or exceed the minimum nutrient requirements

(NRC, 2012), and 4 suboptimal diets which were: low CP (10.03 MJ/NE, 132.0 g of CP, and 7.5 g of SID Lys per kg of feed), high CP (10.03 MJ/NE, 188.0 g of CP, and 11.5 g of SID Lys per kg of feed), low NE (9.61 MJ/NE, 160.0 g of CP, and 9.5 g of SID Lys per kg of feed) and high NE (10.45 MJ/NE; 160.0 g of CP, and 9.5 g of SID Lys per kg of feed). Calculated and analysed nutrient diet composition is shown in Table 8.1. After a 10-day adaptation period, in both batch 1 and 2, faecal samples were collected from the pen floor during the 6 following days. Each day, one faecal sample was collected from each pen (n = 20/day) adding up to a total of 120 faecal samples (n = 24/ treatment) from batch 1 at 15 weeks of age, and 120 faecal samples (n = 24/ treatment) from batch 2 at 20 weeks. Pigs went back to the common management of the Teagasc Pig Research Facility after the 15 day’s trial period.

**Table 8.1** Ingredient, calculated and analysed nutrient composition on an as-fed basis of the five dietary treatments.[1]

| | Diets [2] | | | | |
|---|---|---|---|---|---|
| | Control | LCP | HCP | LNE | HNE |
| Ingredients, g/kg | | | | | |
| Wheat | 350.0 | 350.0 | 350.0 | 330.0 | 306.2 |
| Barley | 282.5 | 345.0 | 0.0 | 310.5 | 200.0 |
| Maize | 150.0 | 150.0 | 286.6 | 100.0 | 275.5 |
| Soybean meal 47.5 | 172.4 | 95.7 | 254.1 | 175.1 | 172.4 |
| Soybean hulls | 14.2 | 29.7 | 63.9 | 58.3 | 0.0 |
| Vegetable Oil | 5.0 | 5.0 | 17.6 | 0.0 | 21.5 |
| Calcium carbonate | 12.3 | 12.7 | 12.2 | 10.7 | 11.7 |
| Dicalcium phosphate anhydrous | 0.50 | 0.50 | 1.00 | 3.00 | 0.50 |
| Sodium chloride | 4.50 | 4.40 | 3.20 | 4.40 | 3.70 |
| L-Lysine HCl | 4.30 | 3.80 | 5.30 | 4.15 | 4.40 |
| L-Threonine | 1.60 | 1.20 | 2.20 | 1.15 | 1.60 |
| DL-Methionine | 1.30 | 0.70 | 2.20 | 1.30 | 1.20 |
| L-Tryptophan | 0.20 | 0.10 | 0.20 | 0.20 | 0.10 |
| L-Valine | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 |
| Vitamin and trace mineral mixture [3] | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| Calculated / Analysed Composition [4], % as fed or as specified | | | | | |
| Dry Matter, analysed | 88.00 | 87.70 | 88.30 | 87.90 | 87.90 |
| Ash, analysed | 3.90 | 3.60 | 4.00 | 4.10 | 3.90 |
| NE, MJ/kg | 10.03 | 10.03 | 10.03 | 9.61 | 10.45 |
| SID Lys:NE, g/MJ | 0.95 | 0.75 | 1.15 | 0.99 | 0.91 |
| Crude Protein, analysed | 13.40 | 11.60 | 16.20 | 14.50 | 14.30 |
| Total Lys, analysed | 1.05 | 0.88 | 1.31 | 1.08 | 1.02 |

*Table 8.1 Continue*

| | | | | | |
|---|---|---|---|---|---|
| Total Thr / Lys ratio, analysed | 0.58 | 0.58 | 0.56 | 0.57 | 0.62 |
| Total Met-Cys / Lys ratio, analysed | 0.64 | 0.64 | 0.63 | 0.65 | 0.68 |
| Total Trp / Lys ratio, analysed | 0.14 | 0.15 | 0.15 | 0.14 | 0.14 |
| Total Val / Lys ratio, analysed | 0.60 | 0.65 | 0.65 | 0.67 | 0.69 |
| Total Leu / Lys ratio, analysed | 1.09 | 1.14 | 1.08 | 1.14 | 1.14 |
| Total Ile / Lys ratio, analysed | 0.54 | 0.55 | 0.57 | 0.58 | 0.60 |
| Total His / Lys ratio, analysed | 0.34 | 0.38 | 0.37 | 0.36 | 0.38 |
| SID Lys | 0.95 | 0.75 | 1.15 | 0.95 | 0.95 |
| SID Thr / Lys ratio | 0.65 | 0.65 | 0.65 | 0.65 | 0.65 |
| SID Met-Cys / Lys ratio | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| SID Trp / Lys ratio | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| SID Val / Lys ratio | 0.66 | 0.67 | 0.65 | 0.66 | 0.66 |
| SID Leu / Lys ratio | 1.16 | 1.20 | 1.11 | 1.16 | 1.15 |
| SID Ile / Lys ratio | 0.56 | 0.55 | 0.57 | 0.57 | 0.57 |
| SID His / Lys ratio | 0.36 | 0.36 | 0.34 | 0.35 | 0.36 |
| Fat, analysed | 2.79 | 2.74 | 3.78 | 2.21 | 4.19 |
| Crude Fibre, analysed | 2.90 | 3.40 | 4.20 | 4.20 | 2.40 |
| NDF | 12.96 | 14.15 | 13.54 | 15.02 | 12.00 |
| Calcium | 0.75 | 0.75 | 0.80 | 0.77 | 0.72 |
| Digestible Phosphorus | 0.22 | 0.22 | 0.22 | 0.25 | 0.22 |

[1] Diets were fed in growing and finishing pigs during 15 days at 13 and 18 weeks of age, batch 1 and batch 2 respectively.

[2] LCP = Low Crude Protein; HCP = High Crude Protein; LNE = Low Net Energy; HNE = High Net Energy.

[3] Provided per each kg of feed: 60 mg Copper sulphate, 80 mg Ferrous sulphate monohydrate, 50 mg Manganese oxide, 100 mg Zinc oxide, 0.5 mg Potassium iodate, 0.4 mg Sodium selenite, 2 MIU Vitamin A, 0.5 MIU Vitamin $D_3$, 40 MIU Vitamin E, 4 mg Vitamin K, 0.015 mg Vitamin $B_{12}$, 2 mg Riboflavin, 12 mg Nicotinic acid, 10 mg Pantothenic acid, 2 mg Vitamin $B_1$, 3 mg Vitamin $B_6$.

[4] NE = Net Energy; SID = Standardized Ileal Digestible; NDF = Neutral Detergent Fibre.

### 8.2.2 Feed Analysis

Feed samples of each diet were collected (duplicate) from the feeders and analysed for DM, ash, CP, crude fibre, fat, total AA profile, and acid insoluble ash (AIA) at the Sciantec Analytical Services (Stockbridge Technology Centre, Cawood, Yorkshire, UK). Dry matter was determined by oven drying for 4 h at 103°C (Thiex, 2009). Ash was determined via combustion in a muffle furnace at 550°C (Thiex et al., 2012). Organic matter (OM) was calculated as *1000 – Moisture – Ash*. Crude protein was determined as N × 6.25 based on the DUMAS method (Ebeling, 1968) using LECO FP-628 analyser (Leco Instruments Ltd., Stockport, UK). Crude fibre was measured by a Fibertec semi-automatic system (Tecator, Hoganas, Sweden) using the gravimetric method (Thiex 2009). Gross energy (GE) was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA). Fat was determined using Randall/Soxtec/Submersion method (Thiex et al., 2003). Amino acid determination was carried out based on ion exchange high performance liquid chromatography technique (Otter, 2012) using the Biochrom Amino Acid Analyser Sodium System (Biochrom Ltd., Cambridge, UK). Acid insoluble ash was determined according to McCarthy et al. (1974).

### 8.2.3 Faecal analysis

Faecal samples were frozen at -20 °C after collection, then freeze-dried and ground using a FOSS Cyclotec 1093 Sample Mill (Foss, Denmark) with 1 mm sieve. A total of 10 faecal samples had to be discarded because of a technical problem of the freeze-drier machine. Faecal chemical analyses were conducted at Sciantec Analytical Services (Stockbridge Technology Centre, Cawood, Yorkshire, UK). Dry matter, ash, OM, CP, GE, fat and AIA parameters were determined or calculated using the same methods previously described in the feed analysis section.

### 8.2.4 Determination of nutrient and energy digestibility

The chemical analyses of diets and faeces allowed for the determination of ATTD coefficients for all the analysed nutrients and energy. The ATTD of the nutrients was calculated using the following equation (Zhang and Adeola, 2017):

$$ATTD\ coefficient = 1 - \left(\frac{Nutrient\ in\ faeces}{Nutrient\ in\ feed}\right) x \left(\frac{AIA\ in\ feed}{AIA\ in\ faeces}\right)$$

### 8.2.5 Faecal NIRS analysis

Faecal NIRS spectres were obtained from the same samples that were used for chemical analysis. Faecal samples were scanned via NIRS two times, first as FDNG and then as FDG. Faecal samples were scanned on a FOSS monochromatic spectrometer NIRSystem 6500 (Foss NIRSystems, Denmark) in reflectance mode from 1100 to 2498 nm (with 2 nm steps). The analysis of FDNG and FDG faecal samples was carried out using the 1/4 rectangular cup transport cell that was 4.6 cm wide and 5.7 cm long. Two replicates were measured for each sample, using the average of spectra for calibrating. Spectral absorbance values were obtained as log (1/*R*), where *R* is sample reflectance. Spectra data were collected using the WinISI software package (version 4.10.0, Infrasoft International LLC, State College, PA, USA). Log (1/*R*) average spectra of FDG and FDNG are shown in Figure 8.1.

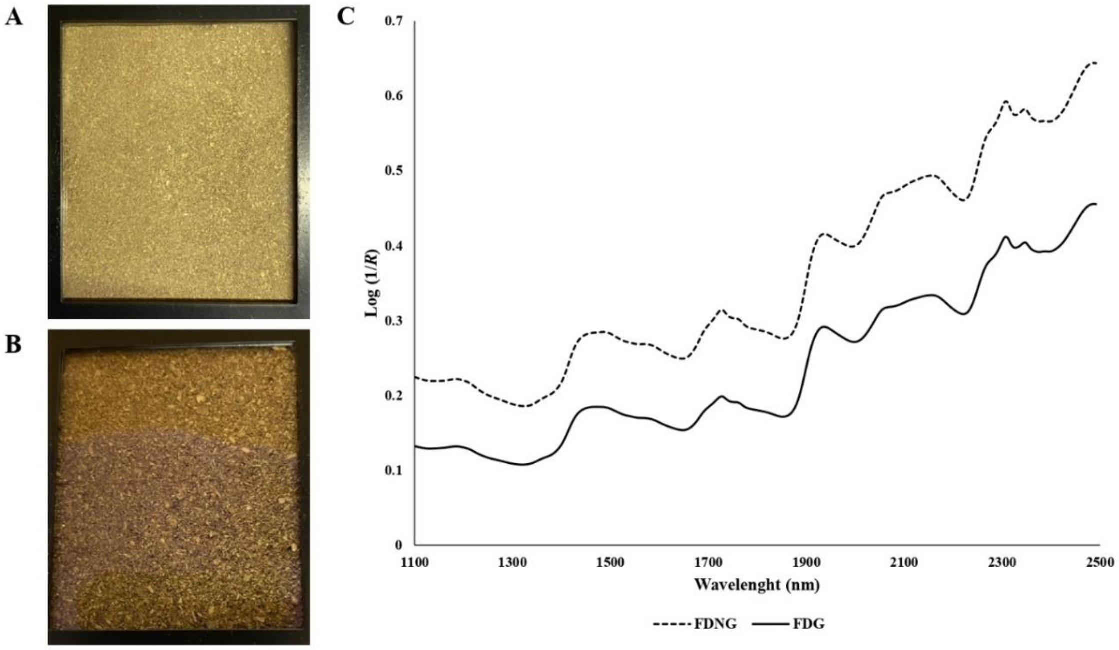

**Figure 8.1** Log (1/*R*) average spectra of freeze-dried not ground faeces (FDNG; B) and freeze-dried faeces (FDG; A).

### 8.2.6 Development of Faecal NIRS calibration equations

Prior to do the calibration procedures, spectral data was subjected to an analysis of its structure and variability of the sample population using the CENTER algorithm included in the WinISI software package. Thus, a principal component analysis and calculation of the Mahalanobis distance (GH) was performed. The latter calculates the distance of each spectrum sample from the center of the population in an n-dimensional space. Then, samples with a statistical value of more than 3 GH were considered outliers or anomalous spectra (Shenk and Westerhaus, 1991a). For this analysis, SNV and DT methods were used for scatter correction (Barnes et al., 1989). Moreover, a first-derivative treatment "1,5,5,1" was used. The first digit is the derivative number, the second digit is the gap over which the derivative is calculated, the third digit is the number of data points in a moving average or first smoothing, and the fourth digit is the second smoothing (Shenk and Westerhaus, 1991b). During this process, a total of 7 samples were deleted from both FDNG and FDG data set, so 223 faecal samples were finally available for the calibration procedures. Descriptive statistics of the final calibration data set are provided in Table 8.2.

**Table 8.2** Descriptive statistics of faeces chemical composition and apparent total-tract digestibility (ATTD) of nutrients for the final calibration data set and each experimental diet.[1]

| | Chemical Analysis | | | | | ATTD | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Constituent [2] | Mean | SD | Min | Max | CV | Mean | SD | Min | Max | CV |
| Total data set, n = 223 | | | | | | | | | | |
| DM, g/kg | 930.0 | 16.40 | 901.0 | 957.0 | 0.018 | 0.83 | 0.038 | 0.71 | 0.90 | 0.046 |
| CP/DM, g/kg | 266.0 | 23.90 | 218.0 | 320.0 | 0.090 | 0.71 | 0.065 | 0.52 | 0.83 | 0.091 |
| OM/DM, g/kg | 971.0 | 14.50 | 940.0 | 1012.0 | 0.015 | 0.85 | 0.035 | 0.74 | 0.91 | 0.041 |
| GE/DM, MJ/Kg | 20.3 | 0.38 | 19.1 | 21.3 | 0.019 | 0.79 | 0.045 | 0.63 | 0.88 | 0.056 |
| FAT/DM, g/kg | 47.0 | 7.30 | 32.0 | 79.0 | 0.157 | 0.77 | 0.061 | 0.62 | 0.90 | 0.079 |
| Control diet, n = 46 | | | | | | | | | | |
| DM, g/kg | 929.0 | 17.00 | 903.0 | 951.0 | 0.018 | 0.87 | 0.016 | 0.82 | 0.90 | 0.019 |
| CP/DM, g/kg | 258.0 | 21.00 | 221.0 | 304.0 | 0.081 | 0.77 | 0.029 | 0.72 | 0.82 | 0.037 |
| OM/DM, g/kg | 975.0 | 16.00 | 944.0 | 1005.0 | 0.016 | 0.88 | 0.016 | 0.84 | 0.91 | 0.018 |
| GE/DM, MJ/Kg | 20.3 | 0.22 | 19.9 | 20.8 | 0.011 | 0.83 | 0.020 | 0.77 | 0.87 | 0.024 |
| FAT/DM, g/kg | 45.0 | 5.30 | 36.0 | 61.0 | 0.118 | 0.81 | 0.034 | 0.74 | 0.88 | 0.042 |
| LCP diet, n = 47 | | | | | | | | | | |
| DM, g/kg | 931.0 | 16.10 | 911.0 | 955.0 | 0.017 | 0.82 | 0.029 | 0.73 | 0.88 | 0.036 |
| CP/DM, g/kg | 246.0 | 12.20 | 218.0 | 268.0 | 0.050 | 0.66 | 0.053 | 0.52 | 0.78 | 0.080 |
| OM/DM, g/kg | 975.0 | 15.10 | 946.0 | 1012.0 | 0.015 | 0.84 | 0.027 | 0.76 | 0.90 | 0.032 |
| GE/DM, MJ/Kg | 20.3 | 0.26 | 19.7 | 20.8 | 0.013 | 0.75 | 0.038 | 0.63 | 0.84 | 0.051 |
| FAT/DM, g/kg | 46.0 | 6.80 | 32.0 | 67.0 | 0.146 | 0.73 | 0.051 | 0.62 | 0.82 | 0.071 |
| HCP diet, n = 41 | | | | | | | | | | |
| DM, g/kg | 928.0 | 17.50 | 901.0 | 955.0 | 0.019 | 0.79 | 0.042 | 0.71 | 0.88 | 0.053 |
| CP/DM, g/kg | 298.0 | 13.30 | 262.0 | 320.0 | 0.045 | 0.66 | 0.062 | 0.55 | 0.80 | 0.094 |
| OM/DM, g/kg | 967.0 | 11.60 | 940.0 | 992.0 | 0.012 | 0.81 | 0.039 | 0.74 | 0.89 | 0.047 |
| GE/DM, MJ/Kg | 20.2 | 0.37 | 19.3 | 21.1 | 0.018 | 0.76 | 0.045 | 0.68 | 0.86 | 0.059 |
| FAT/DM, g/kg | 48.0 | 6.70 | 37.0 | 69.0 | 0.140 | 0.77 | 0.061 | 0.62 | 0.86 | 0.080 |
| LNE diet, n = 44 | | | | | | | | | | |
| DM, g/kg | 935.0 | 15.50 | 909.0 | 957.0 | 0.017 | 0.84 | 0.022 | 0.80 | 0.90 | 0.027 |
| CP/DM, g/kg | 271.0 | 14.20 | 243.0 | 310.0 | 0.052 | 0.73 | 0.035 | 0.67 | 0.83 | 0.048 |
| OM/DM, g/kg | 965.0 | 11.70 | 945.0 | 997.0 | 0.012 | 0.86 | 0.020 | 0.82 | 0.91 | 0.024 |
| GE/DM, MJ/Kg | 20.0 | 0.34 | 19.1 | 21.1 | 0.017 | 0.80 | 0.026 | 0.76 | 0.88 | 0.032 |
| FAT/DM, g/kg | 41.0 | 5.30 | 32.0 | 55.0 | 0.129 | 0.73 | 0.054 | 0.62 | 0.87 | 0.073 |
| HNE diet, n = 45 | | | | | | | | | | |
| DM, g/kg | 929.0 | 15.20 | 904.0 | 952.0 | 0.016 | 0.83 | 0.032 | 0.75 | 0.90 | 0.039 |
| CP/DM, g/kg | 263.0 | 20.40 | 225.0 | 300.0 | 0.078 | 0.72 | 0.051 | 0.59 | 0.83 | 0.071 |
| OM/DM, g/kg | 975.0 | 13.70 | 949.0 | 1010.0 | 0.014 | 0.85 | 0.030 | 0.77 | 0.91 | 0.035 |
| GE/DM, MJ/Kg | 20.7 | 0.39 | 19.7 | 21.3 | 0.019 | 0.80 | 0.036 | 0.71 | 0.88 | 0.045 |
| FAT/DM, g/kg | 53.0 | 6.80 | 41.0 | 79.0 | 0.129 | 0.81 | 0.045 | 0.67 | 0.90 | 0.056 |

[1] SD = Standard Deviation; Min = Minimum; Max = Maximum; CV = Coefficient of Variation.

[2] LCP = Low Crude Protein; HCP = High Crude Protein; LNE = Low Net Energy; HNE = High Net Energy; DM = Dry Matter; CP = Crude Protein; OM = Organic Matter; GE = Gross Energy.

The calibration procedure was performed using the modified partial least squares regression method. Using this method, the NIR residuals at each wavelength are standardized before calculating the next factor (Shenk and Westerhaus, 1991a). Then, FNIRS calibration were performed to predict DM, CP, OM, GE, fat, and the ATTD for DM, CP, OM, GE and fat, based on the FDG and FDNG faeces spectra. The objective of the study was to compare the FDG versus the FDNG calibrations on pig faeces, thus, all data was used to perform the calibration and it was not divided into calibration and validation data sets. Calibrations were performed based on cross-validation methods. Scatter correction was applied to all calibrations using SNV and DT (Barnes et al., 1989). The SNV approach is used to remove multiplicative interferences of scatter and particle size, while DT is used to remove variations in the baseline shift and curvilinearity that are usually found in the reflectance spectra (Barnes et al., 1989). Moreover, a total of eight derivative mathematical treatments were tested for the calibration procedure: 1,4,4,1; 1,8,4,1; 1,5,5,1; 1,10,5,1; 2,4,4,1; 2,8,4,1; 2,5,5,1; and 2,10,5,1.

For both FDG and FDGN calibration data sets, cross-validation was performed using two different methods. The first method consisted of randomly split the data into four equal cross-validation groups. The second method was the leave-one-out cross-validation. The number of terms was limited to 15 for the faeces calibrations (Paternostre et al., 2021). The best calibration equations were selected according to SEC, $R^2_c$, SECV, and $R^2_{cv}$. Moreover, RPD was calculated to describe the accuracy of the calibration equations and calculated as the ratio of the standard deviation of the reference data to the SECV (Williams, 2001).

Finally, the best and final selected calibration equations for each faeces chemical and ATTD components for FDNG and FDG were compared using the Fisher's Test (De La Haba et al., 2006; Fearn, 1996). $F$ value is calculated as:

$$F = \frac{(SECV_2)^2}{(SECV_1)^2}$$

where $SECV_1$ and $SECV_2$ are from two different models (FDNG and FDG) and $SECV_1 < SECV_2$. Then, $F$ is compared to the $F_{\text{critical}}$ $(1 - P, n_1 - 1, n_2 - 1)$ with $P = 0.05$ and $n - 1$ degrees of freedom. The $F_{\text{critical}}$ can be obtained in the $F$ table where $P$ is the significance level, $n_1$ the times that the measure is repeated in the FDNG model and $n_2$ the times that the measure is repeated in the FDG model. Differences between the SECV values are significant when $F > F_{\text{critical}}$.

## 8.3 Results

### 8.3.1 Faeces chemical and ATTD nutrient composition

Descriptive statistics of each experimental diet for the faeces chemical components and ATTD nutrient composition are provided in Table 8.2. Dietary treatment and pigs' age affected all parameters (*P* < 0.001; Table 8.3). An interaction effect was observed for all ATTD coefficients (*P* < 0.05; Table 8.3). Diets were formulated to generate an important variation in the calibration data set for both chemical components of faeces and ATTD of nutrients. A wide range in nutrient composition was observed especially for CP (218.0-320.0 g/kg) and fat (32.0-79.0 g/kg) for faeces chemical components, and all ATTD nutrients. However, standard deviation was low for most parameters and the highest coefficients of variation were observed for CP (9.0% and 9.1%) and fat (15.7% and 7.9%) for both chemical and ATTD components, respectively.

**Table 8.3** Faeces chemical composition and apparent total-tract digestibility (ATTD) coefficients of nutrients grouped by dietary treatment (n = 4) from grower-finisher pigs at 20 weeks of age.[1]

| | Dietary Treatment [2] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | High Protein | | Low Protein | | High Energy | | Low Energy | | | *P*-Value | | |
| Constituent [3] | 15 wk | 20 wk | 15 wk | 20 wk | 15 wk | 20 wk | 15 wk | 20 wk | 15 wk | 20 wk | SEM | Diet | Age | Interaction |
| Faeces Chemical Components | | | | | | | | | | | | | | |
| DM, % | 94.48 | 91.16 | 94.47 | 91.14 | 94.67 | 91.56 | 94.25 | 91.37 | 94.92 | 91.94 | 0.114 | < 0.001 | < 0.001 | 0.204 |
| CP/DM, % | 27.18 | 24.38 | 30.65 | 29.01 | 25.47 | 23.83 | 27.23 | 24.94 | 27.94 | 26.13 | 0.571 | < 0.001 | < 0.001 | 0.815 |
| OM/DM, % | 96.05 | 99.01 | 95.78 | 97.57 | 96.16 | 98.68 | 96.47 | 98.67 | 95.64 | 97.40 | 0.250 | < 0.001 | < 0.001 | 0.108 |
| GE/DM, MJ/kg | 20.47 | 20.12 | 20.48 | 20.01 | 20.38 | 20.21 | 20.87 | 20.49 | 20.20 | 19.87 | 0.093 | < 0.001 | < 0.001 | 0.614 |
| FAT/DM, % | 4.23 | 4.75 | 4.57 | 5.01 | 4.36 | 4.81 | 5.20 | 5.32 | 3.83 | 4.34 | 0.167 | < 0.001 | < 0.001 | 0.746 |
| ATTD coefficients of nutrients, % | | | | | | | | | | | | | | |
| DM | 87.53 $^{a}$ | 85.59 $^{a}$ | 82.42 $^{bc}$ | 75.60 $^{d}$ | 82.49 $^{bc}$ | 80.43 $^{c}$ | 84.79 $^{ab}$ | 80.21 $^{c}$ | 84.88 $^{ab}$ | 82.13 $^{bc}$ | 0.642 | < 0.001 | < 0.001 | 0.003 |
| CP | 77.68 $^{ab}$ | 76.84 $^{bc}$ | 70.55 $^{cd}$ | 61.35 $^{e}$ | 66.15 $^{de}$ | 64.72 $^{de}$ | 74.45 $^{bc}$ | 69.52 $^{cd}$ | 74.28 $^{bc}$ | 71.70 $^{bcd}$ | 1.431 | < 0.001 | < 0.001 | 0.041 |
| OM | 89.03 $^{a}$ | 86.93 $^{ab}$ | 84.52 $^{bcd}$ | 78.10 $^{e}$ | 84.68 $^{bcd}$ | 82.43 $^{d}$ | 86.58 $^{abc}$ | 82.14 $^{d}$ | 86.75 $^{abc}$ | 84.06 $^{cd}$ | 0.576 | < 0.001 | < 0.001 | 0.003 |
| GE | 83.81 $^{a}$ | 81.61 $^{ab}$ | 79.73 $^{bc}$ | 72.48 $^{e}$ | 76.10 $^{cde}$ | 73.52 $^{de}$ | 82.21 $^{ab}$ | 77.26 $^{cd}$ | 81.52 $^{ab}$ | 78.54 $^{bc}$ | 0.776 | < 0.001 | < 0.001 | 0.015 |
| FAT | 83.30 $^{a}$ | 78.35 $^{bc}$ | 81.26 $^{ab}$ | 71.22 $^{d}$ | 75.29 $^{c}$ | 69.94 $^{d}$ | 83.37 $^{a}$ | 77.78 $^{bc}$ | 76.79 $^{c}$ | 69.06 $^{d}$ | 0.732 | < 0.001 | < 0.001 | 0.007 |

[1] Results [Least Square Means ± Standard Error of the Mean (SEM)]. Pen was considered the experimental unit (n = 4). Data analyses were carried out using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Models for DM, CP, OM, GE, FAT, and their respective ATTD coefficients were analysed using general linear models including treatment diet as fixed effect. Alpha level for determination of significance was 0.05 and trends were identified as alpha of 0.10.

[2] Dietary treatments: Control (10.03 MJ/NE, 160.0 g of CP, and 9.5 g of SID Lys per kg of feed); Low Protein (10.03 MJ/NE, 132.0 g of CP, and 7.5 g of SID Lys per kg of feed); High Protein (10.03 MJ/NE, 188.0 g of CP, and 11.5 g of SID Lys per kg of feed); Low Energy (9.61 MJ/NE, 160.0 g of CP, and 9.5 g of SID Lys per kg of feed); High Energy (10.45 MJ/NE; 160.0 g of CP, and 9.5 g of SID Lys per kg of feed).

[3] DM = Dry Matter; CP = Crude Protein; OM = Organic Matter; GE = Gross Energy.

[a-b] Within rows, significant differences between groups ($P < 0.05$).

### 8.3.2 Faecal NIRS calibrations

Faecal NIRS calibration equations were successfully developed for all parameters and statistics of the selected calibration equations to predict the faeces chemical composition and ATTD coefficients are provided in Table 8.4. Similar results were obtained using both cross-validations with 4 random groups and leave-one-out methods. Therefore, only leave-one-out cross-validation results will be further discussed. Overall, faeces chemical components were better predicted than ATTD coefficients in both FDG and FDNG faeces.

Chemical components such as DM and CP were successfully predicted with $R^2_{cv}$ close to 0.9 and RPD values close or higher than 3 for both FDG and FDNG faeces. However, predictions of OM and GE were less accurate with $R^2_{cv}$ values between 0.7-0.8 and RPD values closer to 2. Lastly, fat had the lowest accurate prediction, with $R^2_{cv}$ values close to 0.6 and RPD values lower than 2.

The ATTD coefficients had a moderate prediction accuracy with $R^2_{cv}$ values ranging from 0.54 to 0.67 and RPD values lower than 2 in FDG faeces, while $R^2_{cv}$ values ranged between 0.60 to 0.75 and DM and OM had RPD values higher than 2 in FDNG faeces.

### 8.3.3 Freeze-dried not ground vs Freeze-dried ground models

Results from the Fisher's Test to differentiate FDG and FDNG models for the different faeces chemical and ATTD parameters are reported in Table 8.5. Chemical components calibration equations for DM, OM, GE and fat were not different between FDG and FDNG ($P > 0.05$), while CP differed between FDG and FDNG ($P < 0.05$) with SECV being lower in the FDG model. The ATTD coefficients calibration equations for CP, GE and fat were not different between FDG and FDNG ($P > 0.05$); however, ATTD DM and OM coefficients differed between FDG and FDNG ($P < 0.05$) with SECV being lower in the FDNG model in both cases.

**Table 8.4** Faecal NIRS calibration statistics of the selected calibration equations to predict the faeces chemical composition and the apparent total tract digestibility coefficients of grower-finisher pigs using freeze-dried ground and not ground faeces.[1]

| Constituent [2] | Mean$_{cv4}$ | SD$_{cv4}$ | SEC$_{cv4}$ | R$^2$c$_{cv4}$ | SECV$_{cv4}$ | R$^2$cv$_{cv4}$ | RPD$_{cv4}$ | Mean$_{lo}$ | SD$_{lo}$ | SEC$_{lo}$ | R$^2$c$_{lo}$ | SECV$_{lo}$ | R$^2$cv$_{lo}$ | RPD$_{lo}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Freeze-dried ground faeces - Faeces chemical components | | | | | | | | | | | | | | |
| DM, g/kg | 930.0 | 16.50 | 5.00 | 0.91 | 5.50 | 0.89 | 2.98 | 930.0 | 16.50 | 4.60 | 0.92 | 5.40 | 0.89 | 3.02 |
| CP/DM, g/kg | 266.0 | 23.80 | 4.80 | 0.96 | 5.60 | 0.95 | 4.31 | 267.0 | 23.90 | 5.10 | 0.96 | 5.40 | 0.95 | 4.45 |
| OM/DM, g/kg | 971.0 | 13.70 | 5.90 | 0.81 | 6.70 | 0.76 | 2.17 | 971.0 | 14.20 | 6.10 | 0.82 | 6.70 | 0.77 | 2.15 |
| GE/DM, MJ/kg | 20.3 | 0.36 | 0.16 | 0.80 | 0.18 | 0.75 | 2.18 | 20.3 | 0.36 | 0.16 | 0.79 | 0.18 | 0.74 | 2.11 |
| FAT/DM, g/kg | 46.0 | 6.10 | 3.40 | 0.69 | 3.80 | 0.62 | 1.95 | 46.0 | 6.10 | 3.60 | 0.66 | 3.80 | 0.61 | 1.91 |
| Freeze-dried ground faeces - Apparent total tract of nutrient digestibility | | | | | | | | | | | | | | |
| dDM | 0.83 | 0.034 | 0.017 | 0.75 | 0.019 | 0.70 | 2.05 | 0.83 | 0.036 | 0.020 | 0.71 | 0.022 | 0.64 | 1.75 |
| dCP | 0.71 | 0.062 | 0.037 | 0.63 | 0.040 | 0.58 | 1.62 | 0.71 | 0.061 | 0.036 | 0.65 | 0.041 | 0.54 | 1.57 |
| dOM | 0.85 | 0.032 | 0.016 | 0.75 | 0.017 | 0.71 | 2.04 | 0.85 | 0.031 | 0.016 | 0.73 | 0.018 | 0.67 | 1.98 |
| dGE | 0.79 | 0.041 | 0.024 | 0.66 | 0.026 | 0.59 | 1.70 | 0.79 | 0.039 | 0.022 | 0.69 | 0.024 | 0.62 | 1.85 |
| dFAT | 0.77 | 0.058 | 0.028 | 0.77 | 0.033 | 0.68 | 1.87 | 0.77 | 0.058 | 0.028 | 0.77 | 0.033 | 0.67 | 1.83 |
| Freeze-dried not ground faeces - Faeces chemical components | | | | | | | | | | | | | | |
| DM, g/kg | 931.0 | 16.20 | 4.90 | 0.91 | 5.70 | 0.88 | 2.85 | 930.0 | 16.30 | 5.00 | 0.91 | 5.70 | 0.88 | 2.86 |
| CP/DM, g/kg | 266.0 | 23.40 | 6.10 | 0.93 | 6.40 | 0.93 | 3.77 | 266.0 | 23.60 | 6.10 | 0.93 | 6.60 | 0.92 | 3.62 |
| OM/DM, g/kg | 971.0 | 14.00 | 6.60 | 0.78 | 7.30 | 0.73 | 2.00 | 971.0 | 14.20 | 6.40 | 0.80 | 7.40 | 0.73 | 1.96 |
| GE/DM, MJ/kg | 20.3 | 0.35 | 0.17 | 0.77 | 0.18 | 0.74 | 2.14 | 20.3 | 0.36 | 0.19 | 0.73 | 0.20 | 0.69 | 1.92 |
| FAT/DM, g/kg | 46.0 | 6.20 | 3.40 | 0.70 | 3.70 | 0.64 | 1.97 | 46.0 | 6.50 | 4.00 | 0.63 | 4.20 | 0.57 | 1.73 |
| Freeze-dried not ground faeces - Apparent total tract of nutrient digestibility | | | | | | | | | | | | | | |
| dDM | 0.83 | 0.036 | 0.017 | 0.76 | 0.019 | 0.70 | 1.97 | 0.83 | 0.035 | 0.015 | 0.81 | 0.018 | 0.73 | 2.09 |
| dCP | 0.71 | 0.058 | 0.030 | 0.73 | 0.034 | 0.66 | 1.91 | 0.71 | 0.062 | 0.035 | 0.68 | 0.039 | 0.61 | 1.67 |
| dOM | 0.85 | 0.033 | 0.015 | 0.78 | 0.017 | 0.72 | 1.99 | 0.85 | 0.032 | 0.013 | 0.83 | 0.016 | 0.75 | 2.21 |
| dGE | 0.79 | 0.039 | 0.019 | 0.76 | 0.023 | 0.65 | 1.96 | 0.79 | 0.039 | 0.019 | 0.77 | 0.023 | 0.67 | 1.96 |
| dFAT | 0.77 | 0.059 | 0.029 | 0.76 | 0.034 | 0.67 | 1.83 | 0.77 | 0.059 | 0.028 | 0.78 | 0.033 | 0.67 | 1.85 |

[1] Faecal NIRS calibrations were performed based on two cross-validation methods: Cross-validation in groups of 4 (CV4) and leave-one-out cross-validations (Lo). SD = Standard Deviation; SEC = Standard Error Calibration; R$^2$c = Coefficient of Determination for Calibration; SECV = Standard Error Cross-Validation; R$^2$cv = Coefficient of Determination for Cross-Validation; RPD = Residual Predictive Deviation.

[2] DM = Dry Matter; CP = Crude Protein; OM = Organic Matter; GE = Gross Energy.

**Table 8.5** Fisher's test statistical comparison ($P \leq 0.05$) between the leave-one-out standard error cross-validation ($SECV_{lo}$) values obtained for the best models for predicting faeces chemical and apparent total tract digestibility parameters using freeze-dried not ground (FDNG) and freeze-dried ground (FDG) faeces samples.[1]

| | $SECV_{lo}$ | | | |
|---|---|---|---|---|
| Constituent [2] | FDG | FDNG | $F$ | $F_{critical}$ |
| Faeces chemical components | | | | |
| DM, g/kg | 5.40 | 5.70 | 1.10 | 1.25 |
| CP/DM, g/kg | 5.40 | 6.60 | 1.51 | 1.25 |
| OM/DM, g/kg | 6.70 | 7.40 | 1.20 | 1.25 |
| GE/DM, MJ/kg | 1.80 | 2.00 | 1.19 | 1.25 |
| FAT/DM, g/kg | 3.80 | 4.20 | 1.21 | 1.25 |
| Apparent total tract of nutrient digestibility | | | | |
| dDM | 0.022 | 0.018 | 1.43 | 1.25 |
| dCP | 0.041 | 0.039 | 1.14 | 1.25 |
| dOM | 0.018 | 0.016 | 1.26 | 1.25 |
| dGE | 0.024 | 0.023 | 1.13 | 1.25 |
| dFAT | 0.033 | 0.033 | 1.01 | 1.25 |

[1] Differences between the SECV values are significant when $F > F$critical. Low SECV values improve the quality of the calibration equations.
[2] DM = Dry Matter; CP = Crude Protein; OM = Organic Matter; GE = Gross Energy.

## 8.4 Discussion

The results from the present study show that it is possible to successfully predict with good accuracy the chemical components of faeces and with moderate accuracy the ATTD coefficients by using FNIRS. Furthermore, similar prediction equations can be obtained by using FDG and FDNG faeces via NIRS. It is worth mentioning that the FNIRS use is not novel since several studies have been conducted in cattle (Boval et al., 2004; Coates and Dixon, 2011; Decruyenaere et al., 2015), poultry (Bastianelli, 2013; Bastianelli et al., 2007), or rabbits (Gil-Jiménez et al., 2015; Núñez-Sánchez et al., 2012) among others. Bastianelli et al. (2015), Schiborra et al. (2015), Nirea et al. (2018), and Paternostre et al. (2021) are the only studies on the use of FNIRS in pigs that appear in the literature. These studies may be compared with the present study by using the RPD value. The latter allows SECV to be standardized and compare the results obtained by previous reports that used different data (means, standard deviations, ranges, etc.) obtained in different conditions from the data used in the present study (Williams, 2001). Williams (2001) established a RPD value to be acceptable when it is above 3.0, although Chang et al. (2001) suggested a good accuracy when RPD > 2.0, moderate accuracy when

RPD ranges between 1.4 – 2.0, and poor accuracy when RPD < 1.4. Minasny and McBratney (2013) suggested that a good calibration equation and RPD value are subjected to the author's interpretation. Moreover, the RPD value will be influenced by the kind of sample, its preparation and how is presented to the NIRS instrument, the variance observed in the data set used for calibration, and the possible error of the reference method (Esbensen et al., 2014). Also, Shenk et al. (2001) suggested an excellent calibration when $R^2_{cv} \geq 0.90$, a good calibration when $R^2_{cv} = 0.70 – 0.89$, while a calibration with a $R^2_{cv} = 0.50 – 0.69$ could establish a classification with a good separation between the high, medium and low values of the parameters being analysed. In the present study, we consider a RPD value above 3 with a $R^2_{cv} \geq 0.90$ an excellent accuracy calibration, a RPD value between 2.0 – 3.0 with a $R^2_{cv} = 0.70 - 0.89$ a good accuracy calibration, a RPD value between 1.5 – 2.0 with a $R^2_{cv} = 0.50 - 0.69$ a moderate accuracy calibration, and a RPD value below 1.5 with a $R^2_{cv} = 0.50$ a poor accuracy calibration.

Faecal CP showed an excellent accuracy with RPD values above 3.0 and $R^2_{cv} > 0.90$ in the present study. This outcome is in agreement with previous studies using pigs, which found RPD and $R^2_{cv}$ values close to 3.0 and 0.90, respectively (Nirea et al., 2018; Paternostre et al., 2021). Differences with other authors who obtained lower RPD and $R^2_{cv}$ values in CP compared to the present study (Bastianelli et al., 2015) could be related to a low standard deviation of the reference values of the calibration set as RPD and $R^2_{cv}$ are dependent on the range of values (Dardenne, 2010). Bastianelli et al. (2015) assessed feed digestibility using FNIRS accounting for animal factors but fed with the same diet. The latter might explain the low standard deviation of their calibration data set, but at the same time, the potential use of FNIRS for animal genetics digestibility trials as an example. In agreement with previous studies (Paternostre et al., 2021; Schiborra et al., 2015), the accuracy for DM was excellent, while for OM was good. Gross energy calibrations were good with RPD values close to 2.0 and $R^2_{cv}$ values between 0.70 – 0.75, similar to those obtained by Paternostre et al. (2021). Nirea et al. (2018) obtained higher values of RPD and $R^2_{cv}$ for OM and GE that are explained with range of their calibration data set (Dardenne, 2010), which also resulted in a higher SECV. Fat calibrations had a moderate accuracy similar to previous literature (Nirea et al., 2018), which could be enough to distinguish between high, medium and low levels in faecal samples.

Calibrations for ATTD coefficients of nutrients had a moderate accuracy with RPD values between 1.5 – 2.0 and $R^2_{cv}$ between 0.55 – 0.75 in the present study. These findings are similar to previous studies in pigs (Bastianelli et al., 2015; Nirea et al., 2018;

Schiborra et al., 2015) and differences between them may rely on the range and variability of the reference data used for calibration (Dardenne, 2010). The digestibility results obtained in the present study could be improved by combining the faeces and feed spectra as has been previously demonstrated in ruminants (Decruyenaere et al., 2009), poultry (Coulibaly et al., 2013), and recently in pigs (Paternostre et al., 2021). Nevertheless, depending on the objective of the calibrations, a moderate accuracy could usefully distinguish between high, medium and low levels of ATTD coefficients of nutrients in faecal samples, which in practical conditions could serve as a tool for early detection of digestive problems and/or to improve performance. Also, further research could explore the correlation between high, medium, and low ATTD coefficients of nutrients with factors such as farm management, feeding management, feed ingredients, farm facilities, health status, environment, and season, among others.

Overall, the present study reaffirms FNIRS as a potential tool to evaluate faeces chemical components and ATTD coefficients of nutrients at farm level by collecting faeces from the pen floor. Further studies might explore the possibility to differentiate suboptimal diets in protein and energy levels by using FNIRS and establish the level of accuracy needed for the calibration equations to differentiate, for instance, when the animals are fed high levels of protein above their nutrient requirements at farm level.

A limitation of the present study was the absence of a complete external validation data set to corroborate the robustness of the calibrations. Some previous studies using FNIRS in pigs conducted internal validations to assess the quality and robustness of the calibration equations (Bastianelli et al., 2015; Nirea et al., 2018) using a subset of the total data set, not used for the calibration process. However, no previous study has assessed the quality and robustness of the calibration equations by using a complete external validation data set. In the present study, the robustness of the calibrations were assessed by using two cross-validation methods (4 random groups and leave-one-out). In both cases, the SECV obtained for each parameter were similar. A further study could assess the validation of a calibration predicting faeces chemical components and ATTD coefficients of nutrients by using a complete external validation data set to understand how accurate the calibration is and to quantify how many faecal samples are needed to obtain a robust calibration feasible to be implemented in practical conditions.

The present study compared the faeces chemical and ATTD coefficients from analysing via FNIRS faecal samples in FDNG and FDG form. The concern was that the difference in particle size between FDNG and FDG faecal samples could cause a scatter effect in FDNG faecal samples due to a deviation of light from a straight trajectory into different paths (Garrido-Varo et al., 2003). Nonetheless, the pre-treatment of the raw spectra (Fernández-Cabanás et al., 2006) by using SNV, DT and derivative methods allows to reduce the differences observed in the raw spectra and obtain similar prediction results for both FDNG and FDG faecal samples. However, differences in some parameters were observed when comparing the prediction equations of FDNG and FDG. In any case, the magnitude of the differences and the possible loose of precision and accuracy seems minor for the advantages obtained by using FDNG faecal samples in FNIRS, which are faster analysis while reducing the amounts of workload that suppose the faecal grounding process. Further research should explore the possibility to predict faeces chemical components and ATTD coefficient of nutrients by using FNIRS technique with fresh faecal samples which would be an important step towards facilitating the sample procedure and analysis while reducing the workload, and early detection of health problems related to the digestion process. With the appearance and advances of different types of NIRS instruments adapted to different circumstances (Pu et al., 2021), one NIRS instrument could be able to analyse these fresh faeces and even do it at the farm level and not in the laboratory.

## 8.5 Conclusion

This study has shown that faeces chemical components and ATTD of nutrients are successfully predicted using FNIRS with freeze-dried not ground faecal samples. This outcome facilitates the FNIRS analysis being faster with less workload because it avoids the grounding process. Further research might explore the use of fresh faecal samples analysed via NIRS. Moreover, the present study reaffirms the FNIRS as a potential tool to evaluate faeces chemical components and ATTD coefficients of nutrients.

# Chapter 9

## General Discussion

> ***"Jo només sé que no sé res"***
>
> Sòcrates

The hypothesis and objectives (Chapter 2) tested in the present thesis were based upon two main lines of work: farm adjustment and farm assessment. Figure 9.1 shows an illustrated schematic summary that relates the different strategies studied in the present thesis differentiating between farm adjustment or assessment, and management and/or nutritional strategies. In the following pages, we discuss in greater detail the outcomes of the strategies studied in this thesis (Chapter 3 to Chapter 8), how these are related and which ones may have more impact on productive performance and feed efficiency, limitations of the conducted studies, and future perspectives and possible lines of investigation work in each topic studied.

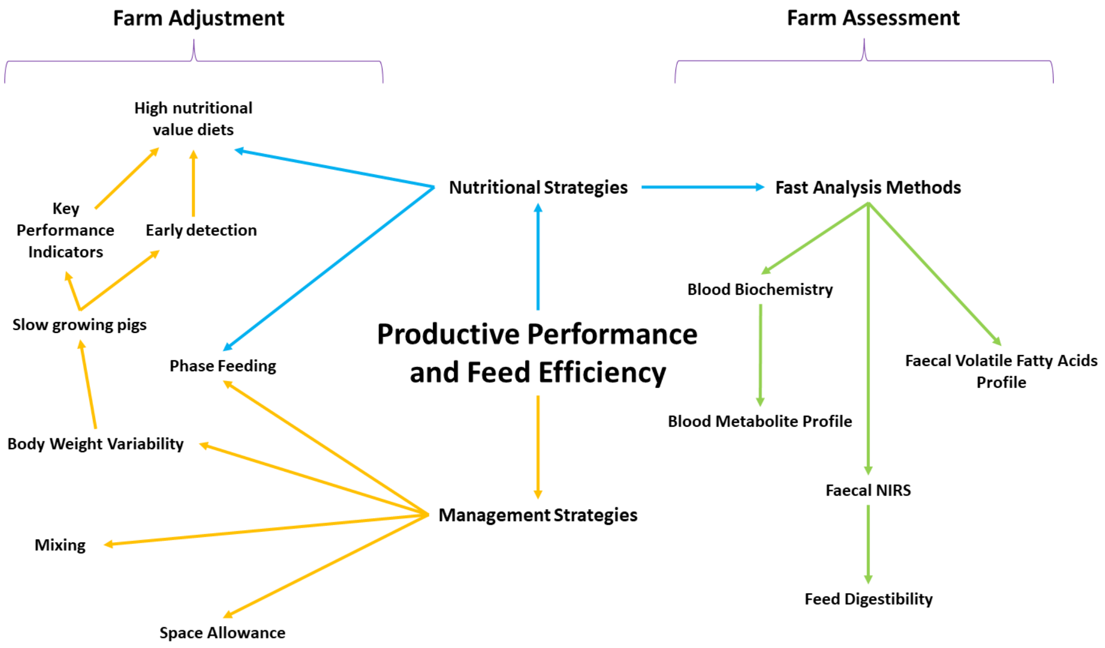

**Figure 9.1** Schematic summary of the different nutritional and/or management strategies related to productive performance and feed efficiency assessed in this PhD thesis.

## 9.1 Facing the Slow Growing Pigs

Slow growing pigs within a batch of pigs may be one of the most important factors impacting the commercial pig production cycle. This subset of pigs is the cause of an increased BW variability within a batch of pigs. The latter is not a hiding cost anymore since the AIAO implementation, and it is a management problem that will ultimately affect production efficiency, and facility costs (Patience et al., 2004). Slow growing pigs will increase the occupation time of the facilities and they will frequently be sent to slaughter before reaching the target weight and/or carcass quality, which may have economic penalties (Douglas et al., 2014a; He et al., 2016; Tolosa et al., 2021).

To manage this subset of pigs, first we need to identify them at earlier stages such as at birth or weaning, to implement a different management and/or nutritional strategy. Second, it would be important to quantify and compare the slow growing pigs' performance indicators versus the average/fast growing pigs, in order to understand which is the best strategy to improve slow growing pigs' performance. These were the two main objectives of Chapter 3.

Predicting productive performance in grower-finisher pigs by using birth and weaning weight drew us some clear conclusions. First, the regression tree and ROC curves statistical approaches were successful on predicting cut-offs based on the pigs' birth and weaning weights. These cut-offs may aid pig farmers as a decision-making tool to identify slow growing pigs early in life. However, the cut-offs obtained in the present study should not be extrapolated to farms with different commercial conditions. Moreover, the sample size in the experimental trial was the same in each subset of pigs (Small-Small, Big-Small, Small-Big, and Big-Big), which would not happen in commercial conditions considering a normal distribution. Then, the percentage of predicted slow growing pigs in the regression tree might be lower than the 12.4% stated in the trial. Nevertheless, this value will also depend on the commercial objectives and target slaughter weight of each farm. ROC curves cut-off values were predicted to distinguish between pigs that would go or not to slaughter at 22 weeks of age (110 kg of BW), with a moderate sensitivity and specificity. Birth and weaning weight cut-off values changed using the same data set as the study from Chapter 3 but setting a target slaughter age of 23 or 24 weeks of age (Table 9.1).

Further research should use the same statistical approaches at a great scale commercial trial and considering other factors affecting performance that were not measured in Chapter 3 such as sex, birth season, genetic potential, management/nutrition strategies, colostrum intake, suckling positions, among others (Douglas et al., 2013; López-Vergé et al., 2018a; Paredes et al., 2012). All these factors explain part of the slaughter weight variability and taken together, a more reliable result could be obtained with an increased sensitivity and specificity.

Weighing pigs at birth and at weaning may not be feasible for all farms. Nonetheless, weaning weight predicted better the target weight or age at slaughter than birth weight in Chapter 3. Then, farmers may be able to identify by sight those light piglets at weaning and separate them to conduct another management and/or nutritional strategy. This "semi-precision" strategy based on two group of pigs within a batch (slow and average/fast growing pigs) differentiated by weaning weight could imply a high cost due to feeding management and facilities, but may end up being amortized in favour of improved productive performance and feed efficiency (Aymerich et al., 2021). Another option for large pig companies could be to create two production flows depending on the growth rate and treating the slow growing pigs “off-site” from the normal production flow in AIAO production systems. However, this strategy may not be logistically feasible and mixing different farm origins may be detrimental to the farm sanitary status.

**Table 9.1** Performance [Area under the curve (AUC) and 95% confidence interval (CI)], *P*-value, sensitivity and specificity for the optimal cut-off value to identify pigs that would reach target slaughter weight [i.e., 110 kg of body weight (BW)] at 22, 23 or 24 weeks of age considering birth BW, weaning BW and birth BW + weaning BW as predictor variables.[1]

| Predictor variable | AUC, % (95 % CI) | *P*-value | Sensitivity, % | Specificity, % | Optimal cut-off value, kg |
|---|---|---|---|---|---|
| 22 weeks of age, | | | | | |
| Birth BW | 72.7<br>(64.0-81.5) | < 0.001 | 71.6 | 70.3 | 1.1 |
| Weaning BW | 68.4<br>(59.4-77.5) | < 0.001 | 77.6 | 53.1 | 6.7 |
| Birth + Weaning BW | 76.3<br>(67.8-84.8) | < 0.001 | - | - | - |
| 23 weeks of age, | | | | | |
| Birth BW | 64.9<br>(53.6-76.3) | 0.010 | 55.9 | 75.3 | 1.0 |
| Weaning BW | 72.9<br>(61.7-84.1) | < 0.001 | 44.1 | 97.9 | 3.8 |
| Birth + Weaning BW | 74.5<br>(63.1-85.9) | < 0.001 | - | - | - |
| 24 weeks of age, | | | | | |
| Birth BW | 65.4<br>(65.4-80.8) | 0.037 | 38.9 | 93.8 | 0.8 |
| Weaning BW | 72.9<br>(57.4-88.3) | 0.001 | 50.0 | 96.5 | 3.5 |
| Birth + Weaning BW | 74.7<br>(59.1-90.4) | < 0.001 | - | - | - |

[1] Data set used for the analysis was the same used in Chapter 3 of the present thesis.

The second conclusion from Chapter 3 was that pigs born and weaned small were the majority of the slow growing pigs and had a lower feed intake but were as feed efficient as their bigger counterparts. The latter agrees with previous literature (Aymerich et al., 2020; Magowan et al., 2011) and gives us some clues on how management and nutritional strategies towards the slow growing pigs should be undertaken. Currently, feed is changed based on age and not BW or growth rate in most weaner/grower-finisher facilities. Thus, current phase-feeding strategies comprise the nutrient requirements of the majority of average grower-finisher pigs, but not those nutrient requirements needed for slow growing pigs. Also, standard nutritional tables (De Blas et al., 2013; NRC, 2012) have previously established the SID Lys/AA requirements for the average grower-finisher pig, but not for the slow growing pigs. There may be a need for 2 tables considering average/fast or slow growth rate pigs. Prior studies observed that slow growing pigs may use more efficiently high SID Lys/AA ratios than fast growing pigs (Aymerich et al., 2020; Douglas et al., 2014c). Then, could slow growing pigs improve their growth performance by increasing the SID Lys/AA dietary levels? and at which level would they achieve their maximum growth potential? This set out the objective of Chapter 4.

Slow growing pigs showed an improved feed efficiency when increasing SID Lys/AA levels while average/fast growing pigs showed a saturated response. Then, slow growing pigs' SID Lys/AA requirements might be higher than the average/fast growing pigs, and nutrient requirements may be related on growth rate or BW at the same age. However, the feed efficiency improvement in the slow growing pigs fed high SID Lys/AA dietary levels was not translated with a better final BW and ADG. Moreover, the SID Lys/AA levels used in the present thesis may not be reproduceable in standard commercial farms for different reasons:

- Feed costs.
- Feed volumes to be manufactured could suppose a logistic problem in feed mills.
- Feasibility to give two or more feed diets in pig farms.

Altogether, the nutritional strategy presented in the thesis might not be the best solution to improve the slow growing pigs' performance. However, the same strategy but starting it at nursery or the beginning of the grower-finisher period, could give time to the slow growing pigs to respond to it. Nevertheless, the use of a phase feeding strategy based on a weight basis or equivalent feed consumption instead of age may be the most practical approach after a good identification of the slow growing pigs earlier in life as reported by López-Vergé et al., (2018b). Precision feeding would be an optimal solution, but it is not feasible in current commercial conditions (Coma, 2017)

## 9.2 The Importance of Common Management Strategies in Productive Performance and Feed Efficiency

The 88-90% of pigs in a batch are average/fast growing pigs. Productive performance and feed efficiency of these pigs can be affected by common management strategies such as space allowance, mixing and phase feeding. These strategies may not be reviewed often enough on many occasions. In Chapter 5 and 6, we studied the effect of space allowance, mixing and phase feeding on productive performance and body lesions, as a proxy for aggression, in single wet-dry feeder pens with 10 to 14 pigs during the grower-finisher period.

Regarding space allowance, the results obtained showed that decreasing space allowance down to 0.72 $m^2$/pig (0.65 $m^2$/pig is the EU minimum requirement) has no effect on productive performance and feed efficiency, but animal welfare is affected by pigs having more body lesions. This would lead to two different point of views:

1. Attend productive performance and feed efficiency and reduce space allowance per pig as much as possible. Thus, pen efficiency is improved and space allowance is still within EU animal welfare legislation.
2. Attend to animal welfare by increasing space allowance. Thus, animal welfare is improved and can be used as an added value.

Nonetheless, it is worth mentioning that the study was conducted in high sanitary status farm. In farms with a lower sanitary status and/or different management/facilities, stress could affect pigs' health affecting growth performance and animal welfare (Boyle et al., 2022; Van der Meer et al., 2016). Finally, the *k*-value may be a good measure to standardised and establish space allowance in different kind of pig farms with different target slaughter BWs. Group sizes and feeder space could have been confounding factors with space allowance, but previous research suggested no effect of them using similar pen systems (Flohr et al., 2017; Schmolke et al., 2003; Wastell et al., 2018).

Mixing had a considerable effect on performance and feed efficiency in grower-finisher pigs in both Chapter 5 and 6. Very little was published of the effects of mixing before this thesis. Previous literature reported that mixing may affect pig performance the following weeks after mixing due to the stress caused to establish a new hierarchy at the pen (Hyun et al., 1998a; Stookey and Gonyou, 1994). However, mixing showed a long term effect in the present thesis, which could be explained due to poorly established dominance relationships between pen mates (Foister et al., 2018). This leads to chronic stress which can lead to immunosuppression and could have detrimental implications for pig performance (Gimsa et al., 2018; Martínez-Miró et al., 2016). Body lesions between intact and mixed litters were similar after one week period in Chapter 5. This could indicate that body lesions only measure a physical welfare problem, but do not reflect a mental-physiologic welfare problem. Then, a further line of investigation could be the assessment of welfare physiologic measures, such as ropes to take saliva samples to measure stress levels in pigs and to detect a possible chronic stress after weeks of mixing.

The effect of mixing on productive performance and feed efficiency was similar to the effect of reducing SID Lys/AA dietary levels from 0.92 to 0.80% in Chapter 6. In both cases, final BW was 5 kg lighter than those pigs kept in intact litters or fed with the 0.92% SID Lys/AA dietary treatment all the way. Thus, mixing should be avoided and strategies to mitigate it should be considered in pig farms. The management strategy of keeping pigs in intact litters is possible in farrow-to-finish farms with the adequate pen size, but it may be more difficult in farms with the 3 production stages separated, where animals need to be transported using a truck. Then, mixing should be avoided as much as possible. Mixing pigs only at weaning could mitigate its effect, avoiding the mix of pigs at the transfer from nursery to fattening facilities. In fact, mixing is a time consuming process that farmers could dedicate to other tasks, since sorting pigs by weight has little effect on individual BW variability (O’Quinn et al., 2001). Another possible option to mitigate mixing may be previous socialization of piglets during lactation (Li and Johnston, 2009), although management should be strict and biosecurity high to avoid possible spread of diseases that would have a greater impact on the production batch. Also, mixing is unavoidable in facilities that include pens of more than 20 pigs. Nevertheless, previous literature reported no differences in growth performance between different group sizes of 20, 40, 80 or even 100 pigs (Schmolke et al., 2003; Street and Gonyou, 2008). Finally, another question that raises the present thesis is the issue of mixing segregating by sex, which it is a common practice that could be usefully explored in further research.

Phase feeding strategies are widely used in pig production and several studies have reported that phase feeding improved productive performance and feed efficiency while reducing feed costs (Hong et al., 2016; Pomar et al., 2014). However, pigs showed an improved productive performance and feed efficiency when they were fed a single diet all the way (0.95 SID Lys/NE), instead of two diets (0.95 → 0.82 SID Lys/NE) adjusting the nutrient requirements at 60 kg of BW, in Chapter 6. This outcome was discussed in

Chapter 6 and might be related to the genetic potential or environmental factors (Ho et al., 2019; Liu et al., 2015), or large changes in the SID Lys/NE levels (Smith et al., 1999). Nevertheless, it seems clear that phase feeding as nutritional strategy should be adapted to the growth rate of the animal and its potential as discussed for slow growing pigs (Chapter 4). Thus, phase feeding continues to be a good strategy, but nutrient requirements should be established based on pigs' growth potential.

Phase feeding effect was not observed when pigs were mixed. Thus, mixing could have had an impact on productive performance that may have concealed the phase feeding effect in Chapter 6. This fact suggests the importance of mixing in pig production, which it has been usually hidden by other management/nutritional strategies and pig commercial facilities when the 3 production stages are separated.

Thus far, Chapter 3 to 6 have argued different management and/or nutritional strategies that require of farm adjustments and were focused on two groups of pigs based on their growth rate: average/fast and slow growing pigs. Overall, the present thesis suggests that pig nutrient requirements may be established based on pigs' growth rate instead of age. The latter is based on the results obtained in Chapter 4 with slow growing pigs, and Chapter 6 with average/fast growing pigs when using a phase feeding strategy. From all the management strategies studied in the present thesis, identification of slow growing pigs and further strategies are important because of the impact of this subset of pigs in the pig production cycle. Moreover, mixing appears to have an important effect on productive performance and feed efficiency in grower-finisher pigs, at least, when space allowance is maintained above the EU requirements.

## 9.3 The Potential of Fast Analysis Methods to Assess Feed Efficiency at Farm Level

Feed during the grow-finisher period accounts for over 60% of the total cost of production in pig farms (Rocadembosch et al., 2016) and small adjustments in the diets have important effects on farm costs. Yet, diets are formulated based on general nutrient requirements without considering specific factors present in each farm. Then, the use of suboptimal diets may not be rare and may induce extra costs to pig producers. Diet optimization is currently expensive and time-consuming process including ingredient analysis, digestibility determination and on farm feed efficiency measurements. Thus, the use of fast analysis methods to assess feed efficiency at farm level may have an important role to optimize diets for a particular farm.

Chapter 7 and 8 explored this idea by assessing feed efficiency at farm level based on blood biochemistry, VFA, and FNIRS analysis on cheap samples easy to collect. Blood samples are regularly taken in pig farms due to detection of pathologies such as PRRS, and collecting faeces is not a time consuming or an expensive process. Also, these are fast analyses with some being already available using hand-held devices.

In Chapter 7, results obtained showed that blood metabolites and faecal VFAs are affected by dietary changes and the physiological age of the pigs. Out of all the blood serum metabolites studied, SUN seems to be the best indicator for protein efficiency in grower-finisher pigs. At both 14 and 20 weeks of age, SUN was increased in pigs fed the high CP diet in commercial conditions, with an AUC close to 1. The fact that a SUN constant concentration is reached after 3 days of changing the diets (Coma et al., 1995), makes SUN a reliable indicator of diets with too much CP. Regarding the faecal VFA profile, BCFA increased in growing pigs fed high CP diets with an AUC of 0.88, but this was not observed in finishing pigs. The latter may be explained by the fermentation capacity difference between ages (Zhao et al., 2020b). Other blood metabolites and VFAs studied could be interesting indicators of suboptimal diets, but further studies are needed beyond this thesis. Overall, further research should be conducted at commercial scale to obtain a range of serum metabolites and VFAs values considering dietary specifications and pigs' age, and understand the implications of specific dietary nutrients. Other factors related to farm management and environment may also be considered.

Chapter 8 assessed the faeces chemical composition and ATTD coefficients of nutrients in grower-finisher pigs using NIRS. The results obtained in the study reaffirmed FNIRS as a potential tool to evaluate faeces chemical components and ATTD coefficients of nutrients at farm level. Moreover, freeze-dried not ground faecal samples gave good calibrations. This is an important progress because faeces can be analysed faster, reducing the workload significantly and making FNIRS a more attractive technique in commercial settings. Further research in our group is exploring the use of FNIRS by analysing fresh faeces. This would be a significant step because it would avoid the freeze-dried process.

Faeces chemical composition and ATTD coefficients of nutrients differed between suboptimal diets (in protein and energy) and pigs' age (14 or 20 weeks of age). The key would be to find these differences using the NIRS calibration equation results. Some previous studies using FNIRS in pigs conducted internal validations to assess the quality and robustness of the calibration equations (Bastianelli et al., 2015; Nirea et al., 2018) using a subset of the total data set, not used for the calibration process. This thesis includes an internal validation, and for the first time, an external validation using a completely external data set. The latter would be useful to understand how accurate a calibration is and how much faecal samples are needed to obtain a robust calibration feasible to be implemented in commercial conditions.

Generally, for calibration groups comprising 100 or more samples and validation groups containing nine or more samples, the following control limits are assumed: Limit Control SEP(c) = 1.30 × SEC, Limit Control bias = ±0.60 × SEC, minimum value of 0.6 for $r^2$ v and slope value between 0.90–1.1 (Garrido-Varo et al., 2017). Validation results for DM, OM, CP, and GE, were within or close to the control limits from the subset of the total data set. However, results from the complete external data set were completely outside of the control limits, which means that the calibration equations would not be robust enough to analyse faecal samples obtained in different conditions than the ones used for calibration. Only CP validation results were acceptable and within or close to the control limits, in exception of the bias. The latter could be attributed to low variability in the calibration data set, and that faecal samples were collected in different experimental conditions. Crude protein reference versus predicted values are represented in Figure 9.2. Overall, calibration equations may need to be built with high variability faecal sample values in order to achieve a good prediction when using faecal samples not related to the ones used in the calibration set. The combination of faeces and feed spectra could also help to build more robust equations (Paternostre et al., 2021). Another option could be to account for, or have specific calibration equations, related to pigs' age, BW, sex, genetic, health, farm, and other environmental factors.

Previously, it was commented that the NIRS technique has a set of advantages that makes this technique interesting for digestibility research and industry companies such as feed mills and integrators. However, the initial investment is expensive because of the NIRS instrument cost and the development (if not purchase) of calibration equations for each chemical parameter. Then, further research could address the question of how many samples and how much variability should be necessary to obtain robust calibration equations that could be used and implemented in commercial situations for parameters such as DM, OM, CP, GE, fat, and fibre, among others. The latter would be useful to understand the feasible applicability of FNIRS in commercial conditions. The use of NIRS in pig production could be further explored in research for other possible applications such as blood metabolites or AA identification in blood samples.

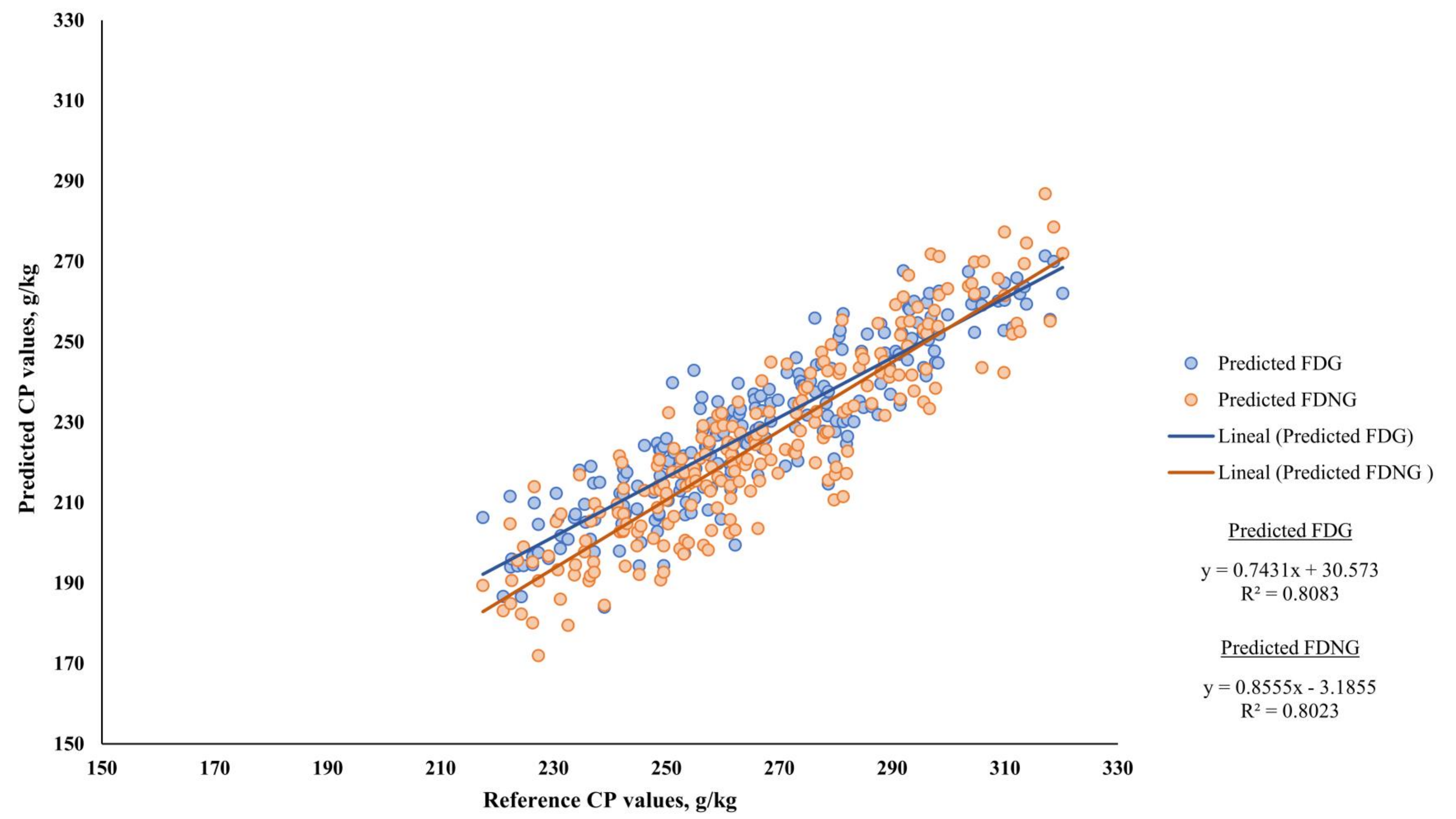

**Figure 9.2** Crude protein (CP) reference values (n = 228) versus NIR predicted data using freeze-dried ground (FDG) and freeze dried not-ground (FDNG) faeces. Validation analyses were conducted using a complete external validation data set.

Finally, the present thesis hypothesised that integrating all the results obtained using the FNIRS, VFA, and blood biochemistry analysis gives us a multivariable indicator to assess feed efficiency at farm level. Correlation coefficients were assessed using the data from Chapter 7 and 8, but no significant outcomes were obtained in this thesis. Nevertheless, further research in our group will be undertaken to investigate possible multivariable indicators to assess feed efficiency at farm level considering nutrition and pig's age, among other factors that may imply variability, such as genetic, sex and environmental factors.

Finally, regarding the possibility to use these analyses in practical conditions in the near future, this could depend on the country's pig production systems. In countries such as Spain, Italy, USA or Brasil, vertical integration companies may be the most interested in using these analyses since they control the entire production chain from the feed mills to the slaughterhouse. Results could serve to optimize diet formulation, e.g., excess of protein, and could even be used to classify farms for reduced emissions. However, at the same time, the search to simplify the number of diets to facilitate feed mills' management and logistics, could be an objection on going to a specific farm level. Other countries such as Ireland, Belgium or the Netherlands, don't have such a marked integration system in exception of some big companies that have their own feed mills. Then, feed mills may be the ones interested in this kind of analyses in order to adjust protein levels to reduce costs and offer a differentiation to clients. A possible disadvantage would appear on the relationship between feed mills and farms, although it could be a "win-win". Premix/feed additives companies could also be interested in this kind of analyses as a complementary service. On the other hand, familiar pig farms may not be interested to conduct these analyses because of the price cost, unless they are part of an association or cooperative. Finally, these analyses could be used in research as a cost-effective resource that allows to have a larger sample size in digestibility studies and favours the application of the three R's.

# Chapter 10

# Conclusions

> ***“La feina ben feta, no té fronteres”***
>
> Edgar Garcia Manzanilla

The results of the studies described in Chapters 3 to 8 led to the following conclusions:

1. The regression tree and ROC curve analyses can be used to obtain cut-off values for birth and weaning weight to classify slow growing pigs and other subgroups early in life.
2. Slow growing pigs are as feed efficient as their bigger counterparts in the same batch.
3. Increasing dietary SID Lys/AA levels from 0.92 to 1.45% improves FCR but not ADG and ADFI in slow growing pigs from 40 to 78.2 kg of BW (15 to 21 weeks of age, respectively), while it does not improve productive performance in fast growing pigs from 63.2 to 112 kg of BW.
4. Space allowances of 0.72 and 0.78 $m^2$/pig do not affect productive performance but welfare is affected in terms of increased body lesions in grower-finisher pigs from 10-11 to 20-21 weeks of age. Space allowances of 0.84 and 0.96 $m^2$/pig did not result in welfare issues.
5. Mixing at the beginning of the grower-finisher period causes a long term negative effect on productive performance reducing by 5.1% final BW, 7.4% ADG and 5.5% ADFI, while increasing by 2.6% FCR in grower-finisher pigs from 11 to 21 weeks of age. However, number of body lesions is not affected once social hierarchy is established.
6. Reducing SID Lys:NE ratio from 0.95 to 0.82 g/MJ at 15-16 weeks of age has a negative effect on productive performance, when pigs are not mixed, reducing by 3.6% final BW and 7.5% ADG, while increasing by 6.0% FCR in grower-finisher pigs from 11 to 21 weeks of age.
7. Serum urea nitrogen is a good indicator related to protein efficiency in growing and finishing pigs, while BCFA has a moderate accuracy to detect crude protein excess but only in growing pigs.
8. Faeces chemical composition and ATTD coefficients of nutrients can be predicted with NIRS either using freeze-dried ground or not ground faeces.
9. NIRS calibration equations can predict faeces chemical components and ATTD coefficients of nutrients using an internal validation data set, but further research is needed to successfully predict faeces chemical components and ATTD coefficients of nutrients when using a complete external validation data set.

# Chapter 11

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# **Chapter 12**

## List of Publications

## 12.1 Scientific Peer Reviewed Publications

**Camp Montoro, J.**, Manzanilla, E.G., Solà-Oriol, D., Muns, R., Gasa, J., Clear, O., Calderón Díaz, J.A. 2020. Predicting Productive Performance in Grow-Finisher Pigs Using Birth and Weaning Body Weight. Animals. 10(6):1017. https://doi.org/10.3390/ani10061017

**Camp Montoro, J**., Boyle, L.A., Solà-Oriol, D., Muns, R., Gasa, J., Manzanilla, E.G. 2021. Effect of space allowance and mixing on growth performance and body lesions of grower-finisher pigs in pens with a single wet-dry feeder. Porc. Heal. Manag. 7, 7. https://doi.org/10.1186/s40813-020-00187-7

**Camp Montoro, J**., Solà-Oriol, D., Muns, R., Gasa, J., Llanes, N., Manzanilla, E.G. 2021. High levels of standardized ileal digestible amino acids improve feed efficiency in slow-growing pigs at late grower-finisher stage. J. Anim. Physiol. Anim. Nutr. 00:1-8. https://doi.org/10.1111/jpn.13610

**Camp Montoro, J**., Pessoa, J., Solà-Oriol, D., Muns, R., Gasa, J., Manzanilla, E.G. 2022. Effect of phase feeding, space allowance and mixing on productive performance of grower-finisher pigs. Animals. 12(6): 390. https://doi.org/10.3390/ani12030390

## 12.2 Scientific publications currently with journal editors

**Camp Montoro, J**., Solà-Oriol, D., Muns, R., Gasa, J., Llanes, N., Manzanilla, E.G. 2022. Blood and faecal biomarkers to assess dietary energy, protein and amino acid efficiency of utilization by growing and finishing pigs levels.
*Submitted to Porcine Health Management.*

**Camp Montoro, J**., Solà-Oriol, D., Muns, R., Gasa, J., Llanes, N., Manzanilla, E.G. 2022. Predicting chemical composition and apparent total tract digestibility of freeze-dried not ground faeces using near-infrared spectroscopy in pigs.
*Submitted to Animal Feed Science and Technology.*

## 12.3 Conference Oral Presentations

**Camp Montoro, J.**, Manzanilla, E.G., Boyle, L.A., Clear, O., Solà-Oriol, D., Calderón Díaz, J.A. 2019. Birth body weight does not always determine subsequent growth performance in grow-finisher pigs. 70th Annual Meeting of the European Federation of Animal Science. 26-30th of August. ICC, Ghent, Belgium.

**Camp Montoro, J.**, Solà-Oriol, D., Boyle, L.A., Muns, R., Llanes, N., Manzanilla, E.G. 2020. Effect of the number of growing-finisher pigs per pen using wet-dry feeders on growth performance and body lesions. British Society of Animal Science Annual Conference. 30th of March - 1st of April. East Midlands Conference Centre, Nottingham, England.

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Llanes, N., Manzanilla, E.G. 2020. Slow growing pigs show a response on feed conversion ratio to high levels of standard ileal digestive lysine. British Society of Animal Science Annual Conference. 30th of March - 1st of April. East Midlands Conference Centre, Nottingham, England.

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2020. Effect of space allowance, mixing and phase feeding on productive performance and body lesions of grow-finisher pigs in pens with a single wet-dry feeder. ASAS-CSAS-WSASAS 2020 Virtual Annual Meeting and Trade Show. 19th-23rd of July. In: Journal of Animal Science, Volume 98, Issue Supplement_4, November 2020, Pages 176–177. https://doi.org/10.1093/jas/skaa278.325

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2020. Interaction effect of phase feeding, space allowance and mixing on productive performance of grow-finisher pigs. 24th Congress of the European Society of Veterinary and Comparative Nutrition. 17-19th September. UTAD, Vila Real, Portugal.

**Camp Montoro, J.**, Boyle, L.A., Levacher, A., Manzanilla, E.G. 2021. Effect of lameness on feed intake and growth performance of grow-finisher pigs. 72nd Annual Meeting of the European Federation of Animal Science. 30th August - 3rd September. Davos Congress, Davos, Switzerland.

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2021. Blood serum metabolites and faecal VFA as tools to detect unbalanced diets in grower-finisher pigs. 72nd Annual Meeting of the European Federation of Animal Science. 30th August - 3rd September. Davos Congress, Davos, Switzerland.

## 12.4 Conference Poster Presentations

**Camp Montoro, J.**, Boyle, L.A., Pessoa, J., Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2021. Group size effect on growth performance and body lesions of grower-finisher pigs in pens with a single wet-dry feeder. 8th International Conference on the Assessment of Animal Welfare at Farm and Group level. 16-19th August. UCC, Cork, Ireland.

## 12.5 Technical Reports

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2020. Estrategias de manejo y alimentación en cerdos de engorde: Mejora en el rendimiento productivo, salud y bienestar. Edición Noviembre. Revista nutriNews.

**Camp Montoro, J.**, Solà-Oriol, D., Muns, R., Manzanilla, E.G. 2021. Estrategias de manejo y alimentación en cerdos de engorde: Mejora en el rendimiento productivo, salud y bienestar. Edición Marzo. Revista porciNews.