Università degli Studi di Padova

Head Office: Università degli Studi di Padova

Department: Department of Agronomy, Food, Natural Resources, Animals and Environment | DAFNAE

Ph.D. COURSE IN: Animal and Food Science
CURRICULUM: Animal Nutrition and Feeding
SERIES: XXXV Cycle, 2019 - 2022

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, Feed Efficiency, and Dry-Cured Ham Meat Quality Traits

The thesis is written with the financial contribution of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), Università degli Studi di Padova.

Coordinator: Prof. Angela Trocino
Supervisor: Prof. Stefano Schiavon
Co-Supervisor: Assoc. Prof. Veronika Halas

Ph.D. Student: Dott. Isaac Hyeladi Malgwi
Matricola n.: 1219516

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, Feed Efficiency, and Dry-Cured Ham Meat Quality Traits

"Measure what can be measured, and make measurable what cannot be measured."

- Galileo Galilei,1642.

Supervisor: **Prof. Stefano Schiavon**
Full professor of Animal Nutrition and Feeding
Department of Agronomy, Food, Natural Resources, Animals and Environment - DAFNAE
Università degli Studi di Padova
Italy

Co-supervisor: **Assoc. Prof. Veronika Halas**
Head of Department of Farm Animal Nutrition
Institute of Physiology and Nutrition
Hungarian University of Agriculture and Life Science, MATE
Kaposvár - Campus
Hungary

"...if I have seen further, it is by standing on the shoulders of giants."

— Isaac Newton, 1675.

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, Feed Efficiency, and Dry-Cured Ham Meat Quality Traits

Isaac Hyeladi Malgwi

Department of Agronomy, Food, Natural Resources, Animals and Environment | DAFNAE | University of Padua
Campus Agripolis | Viale dell'Università 16 | 35020 Legnaro (PD) | Italy
www.unipd.it | www.dafnae.unipd.it
30 September 2022

Dott. Isaac Hyeladi Malgwi

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, the Feed Efficiency and Dry-Cured Ham Meat Quality Traits

Ph.D. Dissertation, University of Padua, Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, Campus Agripolis, Viale dell'Università 16, 35020 Legnaro (PD), Italy.
With ref. – With summary in English, Italian and Hungarian.

“…if we knew what it was we were doing, it wouldn't be called 'research,' would it?"

— Albert Einstein, 1955.

Dedication

The Almighty God is my strength, inspiration, and source of wisdom, knowledge and understanding.

This PhD Thesis is…

Dedicated to:

Mrs. Mary Malgwi, My mother
For your faith in me and your years of unwavering prayers, love, sacrifice, encouragement, and support.

In memory of:

Mr. & Mrs. Anjili Yamta Malgwi
(*My late grandparents*)

and

Mr. Nkirda Yusuf Hena
(*Who passed away, was like a father to me)*

…though death took you away from us, you forever live in our hearts, rest on.

"the Holy Scripture cannot err, and that the decrees therein contained are absolutely true and inviolable."

— Galileo Galilei, 1613.

Foreword

I would like to thank the Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE) for awarding me the "di Dipartimenti di eccellenza—Progetto CASA (Department of Excellence)" Scholarship to pursue a doctorate in Animal and Food Science at the University of Padua, Italy, XXXV Cycle, (35° ciclo) 2019–2022. Despite the absence of formal student exchange programme between the University of Padua and the Hungarian University of Agriculture and Life Sciences, MATE-Kaposvár Campus, I spent 12 months period of study abroad conducting research at MATE-Kaposvár Campus during my PhD programme. I thank both universities for this opportunity.

My sincere gratitude to Prof. Stefano Schiavon, my supervisor, for introducing me to detailed research and data analysis techniques and for the wealth of knowledge shared with me. Thank you for your advice, support, and mentoring. You and your wife, Mrs. Sara Davanzo, accepted me into your family as a son and a student. I appreciate your love, friendship, counsel, and prayers.

A heartfelt thank you to my co-supervisor, Assoc. Professor Veronika Halas. Over the years, as I have advanced in science and life, you have consistently served as an inspiration, a help, and a mentor to me. Your being there for me at all times means a lot to me.

My PhD programme was financially supported by the European Union Rural Development Programme 2014-2020, Reg. (CE) 1305/2013—PSR Veneto DGR n. 2175—December 23, 2016, interventions 16.1.1 and 16.2.2, code 3682902. I am grateful for the institutional funding (DOR2059255/20, DOR1990028/19, DOR1845849/18) and three-year PhD grant from Dipartimento Agronomia Animali Alimenti Risorse Naturali e Ambiente, DAFNAE – “di Dipartimento di eccellenza” – progetto CASA, University of Padua, Italy.

Prof. Giovanni Bitante, Prof. Luigi Gallo, and Prof. Paolo Carnier, who are part of the PROVEN-DOP research team at DAFNAE - UNIPD, thank you for your help, support and the knowledge. A big thanks to: Prof. Angela Trocino, the coordinator of the PhD programme in Animal and Food Science at DAFNAE-UNIPD, who has been supportive throughout the programme. Prof. Piergiorgio Stevanato assisted with the Metagenomics and Next Generation Sequencing (NGS) tools while Prof. Andrea Squartini helped with the bioinformatics and comprehensive analysis of the microbial profile of the dry-cured ham. Dr. Katia Milan and Dr. Rosalba Moro (DAFNAE - UNIPD administration team) for their assistance with the necessary paperwork and formalities. My colleagues at the DAFNAE LaChi and Agricultural Biochemistry Lab colleagues. Dr. Luca Carroro and the technical staff at the farm for the guide at the stable. Attilio Fontana Hams (Attilio Fontana Prosciutti) for processing the dry-cured ham used in this study.

Prof. Dr. Melinda Kovács, H.E. Amb. Dr. Eniola Olaitan Ajayi, Prof. Attila Zsolnai, Dr. Jócsák Ildikó and Prof. Ibrahim D. Mohammed provided invaluable counsel, assistance, and support to reach this height. Thank you for the constant encouragement.

A special feeling of gratitude to my loving family, my lovely mother, Mrs. Mary Malgwi, and my siblings, Dr. Gideon Hyeladi Malgwi and Mrs. Hauwa Isa Musa, Mrs. Hyelhara Nkirda Yusuf Hena, my aunties; Mrs. Gracie Saloka and her husband, Dr. Onenee Gift Saloka, Mrs. Hirhyel Filibus and her husband, Mr. Filibus Mshelia, Dr. & Mrs. Joel Yohanna, and the entire late Anjili Yamta Malgwi's family. I am grateful for your love, prayers, and patience. Thank you for having faith in me.

To my Fiancée, Dr. Onisoyonivosekume Dede (Vosé), thank you for your love and for being part of this.

Finally, to my Hungarian and Italian friends and colleagues, Mr. & Mrs. László Hahn, Dr. Samuel Birma Mshelia, Dr. Ekpenyong Edet, Dr. Abidemi Adekoya, Dr. Chiara Broccanello, Engr. David Nkirda Hena, Mr. & Mrs. Solomon Ishaku, Celio Paolo Sasso, Lúcio Flávio Macedo Mota, Stefan Christian Prazaru, Giovanni Bertoldo, Luiza de Paula Dias Moreira, Thlama Aframai, Jimmy Reuben, Bata Nkirda Hena, Yarsmeen Y. Zeebone, Ksenia Orekhova, Tommaso Gerussi,.....the list is too long to mention everyone individually for always being there for me when I needed them. Thank you for creating a home away from home in Hungary and Italy...“*grazie mille, nagyon szépen köszönöm, thank you, nagode, usar”.*

Contents

Abstract

In Italy, pigs must be slaughtered at 160 ± 16 kg body weight (BW) at 9 months of age for dry-cured ham production (control, C). In Chapter 1, we investigated three alternatives based on different feeding conditions to address the implications of changing the age and weight at slaughter of heavy pigs on carcass and green ham quality traits: 1) allowing pigs to express their growth potential by allowing them to achieve 160 ± 16 kg slaughter weight (SW) at younger slaughter age (SA) (younger Age, YA); 2) allowing pigs to express their growth potential by maximizing their SW at 9 months SA (greater weight, GW); 3) increasing the SA required to achieve 160 ± 16 kg SW (older age, OA). Pigs (336 C21 Goland, 95 kg initial body weight) were slaughtered at 257, 230, 257, and 273 d SA and 172.7, 172.3, 192.9, and 169.3 SW kg for the four treatments, respectively. C pigs had an average daily gain (ADG) of 715 g/d and feed efficiency (FE) of 0.265 (gain to feed). Compared to C, YA pigs had higher ADG (+32%), FE (+7.5%), and better ham adiposity; GW pigs had higher carcass weight (+12%), ADG (+25%), trimmed ham weight (+10.9%), and better ham adiposity. OA treatment affected ADG (−16.4%), FE (−16.6%), and trimmed ham weight (−3.6%). YA and GW could be promising alternatives to C as they improved FE and ham quality traits.

In Chapter 2, a total of 159 C21 Goland pigs (gilts and barrows) at 95 ± 9.0 kg BW from three batches were used to investigate the impact of ad libitum feeding on SW, growth performance, feed efficiency, and carcass and green ham characteristics. Diets contained 10 MJ/kg of net energy and 7.4 and 6.0 g/kg of SID-lysine in early and finishing periods. Slaughter weight classes (SWC) included <165, 165–180, 180–210 and >210 kg BW. In each batch, pigs were sacrificed at 230 or 258 d of age. Left hams were scored for round shape, fat cover thickness, marbling, lean colour, bicolour and veining. Data were analyzed with a model considering SWC, sex and SWC × Sex interactions as fixed factors and the batch as a random factor. The linear, quadratic and cubic effects of SWC were tested, but only linear effects were found. Results showed that pigs with greater SWC had greater average daily gain and feed consumption, with similar feed efficiency and better ham quality traits: greater ham weight, muscularity, and fat covering in correspondence of *semimembranosus* muscle. Barrows were heavier and produced hams with slightly better characteristics than gilts.

The current National Research Council (NRC) nutrient recommendations are based on pigs fed ad libitum up to 140 kg BW. It is unclear whether this applies to pigs weighing more than 140 kg in BW raised under different conditions. This was addressed in Chapter 3 using a mathematical modelling approach based on repeated BW and backfat (BF) measurements to estimate: i) Protein (Pd) and lipid (Ld) depositions over the course of the growth; ii) Metabolizable energy requirement for maintenance (MEm) and growth (MEg); iii) Standardized ileal digestible lysine (SID lysine) requirement, and partitioning the body protein and lipid accretions of 90 and 200 kg BW using 224 Goland C21 heavy pigs when exposed to different rearing conditions. The control pigs (C) received diets limiting ME up to 170 kg in slaughter weight (SW) at 9 months of age (SA); older (OA) pigs had restricted diets limiting ME and SID lysine up to 170 kg in SW at >9 months SA; younger (YA) pigs were fed nonlimited amounts of ME and SID lysine up to 170 kg in SW at <9 months SA; and greater weight (GW) pigs were fed as the YA group, with 9 months SA at >170 kg in SW. We confirmed that the estimated MEm averaged 1.03 MJ/kg$^{0.60}$. An 11% increase in MEm was observed in OA pigs compared to the controls. Energy restriction had negligible effects on the estimated MEm. The marginal efficiency of SID lysine utilization for Pd averaged 0.725, corresponding to a 9.8 g/100 g Pd SID lysine requirement.

Nutrients can be matched more accurately with inherited genes to optimise metabolic functions and improve health and economically important traits in animals. Furthermore, biological, and nutritional pathways related primarily to fat metabolism have confirmed that matching nutriome (nutrient intake combination) in pigs to enhance cellular metabolic functions and desired genetic responses can be successful. It is difficult to unravel the complex nature of nutrient-gene interaction and the underlying molecular mechanisms involved in fatty acid synthesis and marbling in pigs. While existing knowledge on QTLs and SNPs of genes associated with fat metabolism and IMF development is still being harmonised, the scientific explanations for the nature of the existing correlation between nutrients, genes, and environment remain ambiguous, inconclusive, or lacking precision. Nonetheless, nutritional effects can be measured in pigs to optimise growth performance, backfat thickness, IMF deposition, disease resistance, and meat quality traits by fine-tuning gene expression and regulating genome responses. In Chapter 4, nutrigenetics, nutrigenomics and epigenetic mechanisms controlling fat metabolism and IMF accretion in pigs was discussed. We emphasised the potential application of these concepts in pig nutritional research for nutritional intervention for swine production and the improvement of economically important traits in animals. The question remains, however, as to how prepared we are to use this science as a tool in animal nutrition and feeding.

General Introduction

General Introduction:

Dry-cured ham is a meat product with a long history of association with global food culture, particularly in Europe. It is made from salted pork legs (ham), as has been done since before the Roman era. The top producers and consumers of dry-cured ham in Europe are Spain, Italy, France, Germany, Poland, and Greece [1]. In Italy, this product is known as "Prosciutto Crudo." Its manufacturing process, which includes pre-salting, salting, drying, and maturing, is characterised by an extended curing and drying period of at least 12 months. This process is carried out under strictly controlled environmental conditions (air, light, relative humidity, pressure, and temperature) [2-5]. On the one hand, achieving pigs with the desired green ham traits and better growth and carcass characteristics is most preferred by the dry-cured ham industry. On the other, setting target slaughter weight (SW) and slaughter age (SA) is a critical management decision that impacts the productivity and profitability of pig rearing [6]. Several studies over the past decades have shown that the availability of lean pig lines has led to an increase in SW in several countries in order to reduce production costs per pig [6]. Nonetheless, modern high-lean pig genotypes have intensive growth due to their ability to deposit protein at a high rate for an extended period. As a result, purebred and traditional crossbred pigs achieve their maximal protein deposition (Pd) at approximately 60-70 kg of body weight, but intensive hybrids continue to acquire protein at that body weight and reach the plateau of Pd at around 90-100 kg [7,8]. The feed efficiency of the high-lean pig genotypes is favourable for a longer time, and it is economically worth slaughtering them at a higher bodyweight. Therefore, the more efficient the pig is, the higher the bodyweight is applied at slaughter. However, this raises the question of whether the meat from a younger animal is suitable for high-quality dry-cured ham production. Heavy SW, and advanced SA are required to ensure adequate ham size, ham fat covering depth, and lean tissue maturity in the dry-cured ham pig production chain [4,9]. The SW and SA are often mandatory criteria for the quality assurance of hams and other quality traits of the pork [10]. Thus, in practice, heavy BW, and advanced ages at slaughter are required to ensure adequate ham size, the fat covering depth and lean tissue maturity.

An extensive overview of some critical aspects of dry-cured ham has been summarised in [2,4]. Briefly, regarding the processing, seasoning attributes and quality, rearing and green ham processing conditions are critical factors that determine the quality of the dry-cured ham product [11,12,13]. Literature indicates that the aptitude for ham seasoning is influenced by the pigs' genotype, initial characteristics of the green ham such as weight, adipose tissue thickness and composition, physicochemical properties (such as pH and water holding capacity) and intrinsic properties of the muscle (like moisture, fat, and enzyme activities) and so on [14-17]. Therefore, if the dry-curing conditions are optimal, the natural qualities of the fresh green ham determine its qualities at the end of the ageing process during dry-curing [4]. Notwithstanding, existing literature indicates that a few critical factors to consider include adequate fat covering, fat thickness, marbling (intramuscular fat content), salt content, and the historical relationships between SW, growth performance, feed efficiency, and general carcass and green ham characteristics [8,12,19].

Green hams are evaluated at the slaughterhouse for their weight (both before and after trimming), fat colour, the fat covering thickness, degree of marbling, presence of veins, and degree of haemorrhage. Prior to beginning the salting process, a similar evaluation is made when visitors arrive at the ham factory. At the first stage of salting, the ham exudes water rapidly and diffuses the salt into the ham through the muscles. This is accompanied by a series of water losses as the ham ages through evaporation [15]. Similarly, muscle proteolysis and lipolysis are also associated with the salt intake of the ham [4,16,20]. A detailed overview of green ham processing, the type of ham evaluation, and the dry-curing processes at the slaughterhouse and the ham factory, are presented in Figures 1 and 2 respectively.

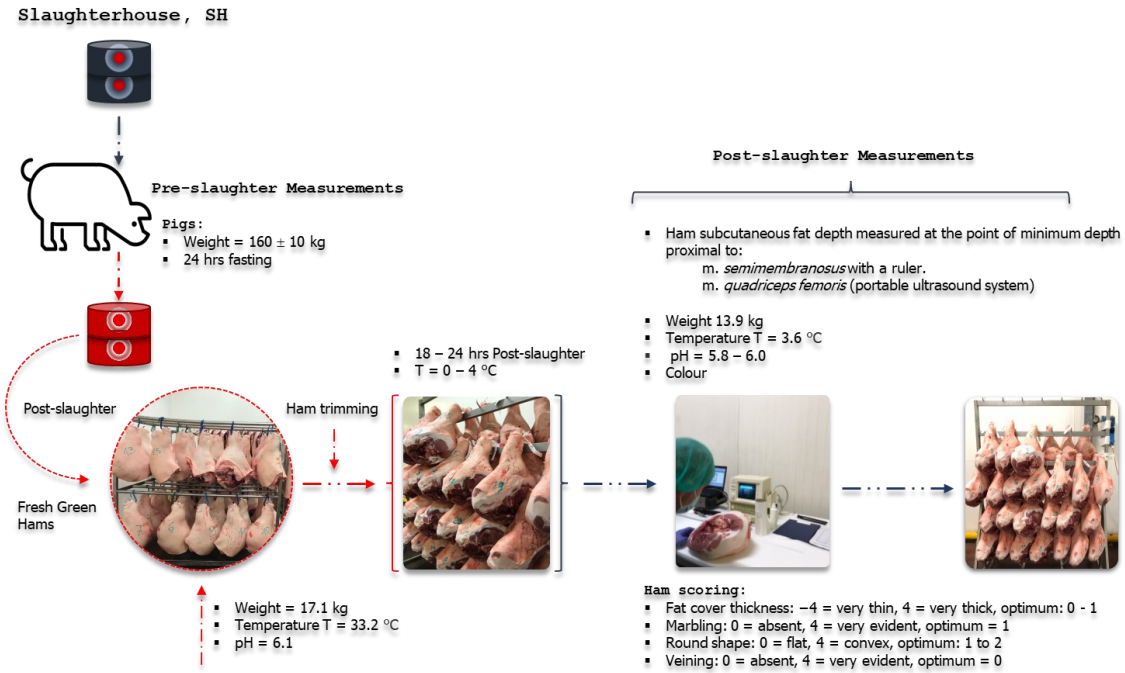

Figure 1. Schematic representation of ham processing at the slaughterhouse

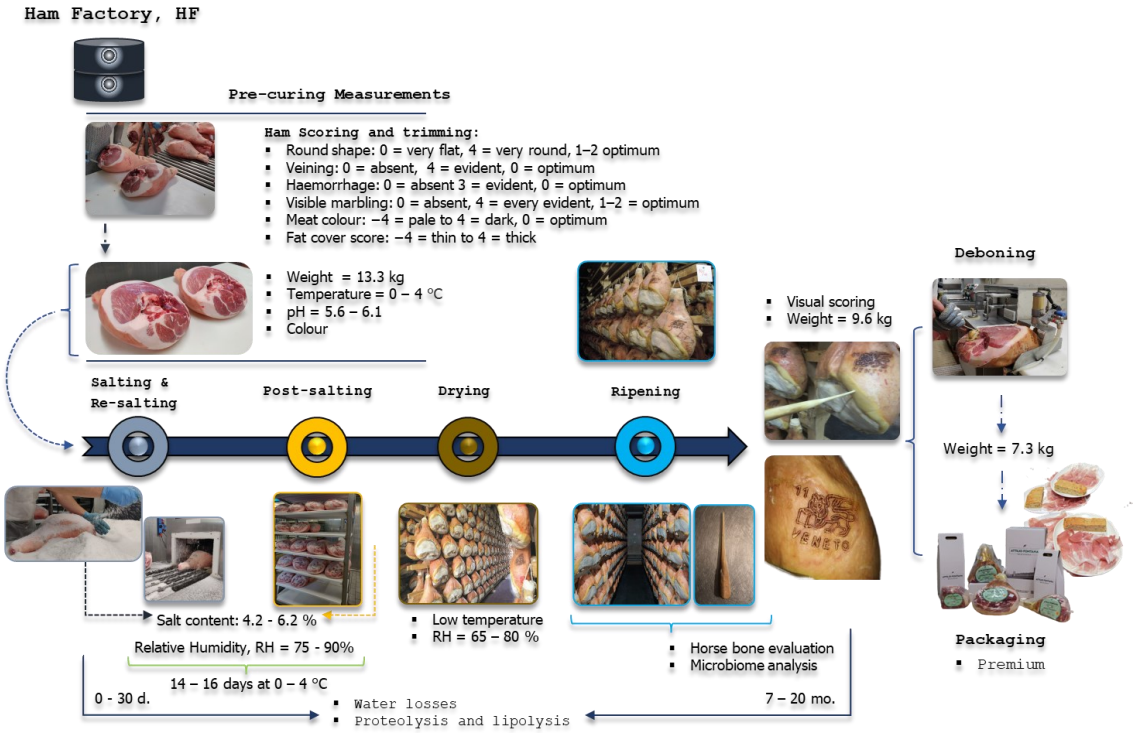

Figure 2. Schematic representation of the dry-curing and ageing process at the ham factory

The present thesis deals with feeding and rearing methods for heavy pigs, with an emphasis on how these practices affect growth performance, feed efficiency, energy portioning and protein utilisation, and the meat quality traits of the green and dry-cured ham. The topic has not been extensively studied and the production of dry-cured ham requires specific conditions for both the pig raising as well as the ham processing. There are some existential questions that nutritionists have yet to answer given the global challenge, such as the harmonization of quality aspects with efficiency and the environmental footprint of pork production, which forms the basis of the current thesis.

In accordance with the European law governing the product specifications of the Italian PDO dry-cured hams, pigs must be slaughtered at a minimum SA and SW of 9 months and 160 ± 16 kg, respectively [11,19]. Therefore, the average daily gain (ADG) from birth to slaughter must not exceed 0.60 - 0.70 kg/d in order to meet these minimal requirements (Chapter 1). For these reasons, diets with medium protein content and restricted feeding strategies have been used [21]. However, this practice has failed to meet the minimum quality requirement of the hams: the weight of the hams, the fat tissue quality traits (colour, thickness and texture), ham shape and the ham aptitude at processing and after dry curing. These characteristics influence the final product's flavour, the consumer's health, and the dry-cured ham industry's overall profitability Without doubt, a shift in the production and rearing strategies for pigs destined for dry-cured ham meat production is underway. The policy reforms that control the pig and dry-cured ham industries' investment choices are currently being revised. Therefore, it is practical to consider that an adjustment in SW or SA can result in higher carcass fatness and better ham characteristics [4,9,22], and compensate for the leanness of modern pig genotypes [23]. However, the lack of experimental reports regarding the effect of an increased SW or SA in heavy pigs exists [9,24]. To our knowledge, no prior studies have evaluated these factors or the effects of adopting potential alternative rearing strategies. For raising pigs under the best management circumstances (feeding, nutrition, and housing), a sustainable strategy must be sought. On the other hand, such a plan should guarantee the quality of the hams, human health, and the health of the environment. Nonetheless, nutritional plans and feeding strategies for pigs would yet differ with the region and the goals of the swine production practices [25]. With the recent proposal to the authorities (addressed in Chapter 1), expanding the range of carcass weight from 120 to 168 kg, corresponding to about 146 to 210 kg of SW is expected. With less body and carcass uniformity among pigs of the same batch, this expansion of the admitted SW range suggests the potential for using an *ad libitum* feeding strategy to better utilize the genetic potential of individual pigs for growth [26,27]. Interestingly, confounding effects between SW and age at slaughter have been noted in the existing literature on the impact of increased SW on growth performance, feed efficiency, carcass, and ham characteristics in such body weight (BW) intervals. Only occasionally have the two effects been separately evaluated, highlighting their varied implications [9,14,28-29].

It is also important to note that the NRC's most recent nutrient recommendations for pigs [8] were primarily focused on lean pig genotypes fed *ad libitum* up to 140 kg body weight (BW). Under the management practice(s) of heavy pig production systems for the dry-cured ham industry, this recommendation has limitations. Pigs are fed for these industries using a variety of feeding strategies designed to manipulate the pigs' age (SA) and weight at slaughter (SW) to improve the seasoning aptitude of the ham (addressed in Chapter 2). Assuming that pigs chosen for the production of dry-cured ham are slaughtered at roughly the same age, it can be hypothesized that those heavier at slaughter would be those with greater feed consumption, growth rate, and carcass and ham weight, as well as greater carcass adiposity, ham marbling, and ham fat covering. In addition, some loss in feed efficiency may occur, increasing the SW [6]. However, such responses would depend on the propension of the pig genotype for lean and fat deposition at heavyweights (Chapter 2).

Furthermore, since the concept of energy and nutrient utilization of heavy pigs under the dry-cured ham production systems has not been covered by existing literature, it remains uncertain if the recommended metabolizable energy (ME) requirement for maintenance (MEm = 1.03 MJ/kg in $BW^{0.60}$) by the NRC is applicable for pigs at heavier BWs [8]. It is of interest to explore the behaviour of their energy requirements under different rearing conditions and extended ranges of BW (Chapter 3). From a nutritional standpoint, it is crucial to assess the amino acid (AA) needs and partitioning of heavy pigs kept under various rearing strategies. Nutritionists have used ideal protein concepts extensively for many years as the foundation for estimating dietary AA requirements for maintenance and growth relative to the requirement for Lys (i.e., Lys = 100%) in pigs [30-37]. It is common knowledge that the requirements for AA should be constant when, in contrast to lysine, the other AA requirements are primarily controlled by protein synthesis [24]. Nevertheless, the influence of breed, sex, body weight (BW), and health status, as well as the existing environmental conditions like the climate, housing system, pig density, feed characteristics, etc., are important regulators of the current variation in feed intake, energy metabolism, and growth performance of pigs [7,8,30,38]. Therefore, it is necessary to investigate the knowledge of the pigs' energy and amino acid (AA) requirements and partitioning if we are to optimise their performance under such dry-cured ham production strategies that are soon to be adopted in practice [8,19]. This has been addressed in (Chapter 3).

In the final chapter of this thesis (Chapter 4), we extensively reviewed potential future aspects of how the knowledge of nutrition, genetics, biochemistry, and "omics"- based technologies published in the past ten years is extremely important in planning and designing nutrition interventions for efficient nutrient utilization, better growth performance, trait development, disease and health [39–47]. Nutritional genomics, also known as nutrigenetics and nutrigenomics, will soon be adopted as a tool in

the production and management of farm animals. In a nutshell, nutrigenetics is the branch of science that examines the impact(s) of genetic variation on dietary response, whereas nutrigenomics is the field that studies the function(s) of nutrients and bioactive food compounds in gene expression. These two nutritional disciplines are transforming and reshaping scientists' comprehension of the unyielding molecular mechanisms that control ageing, disease, fatness status, growth, health, and other traits in humans and animals. We concluded that nutrigenetics and nutrigenomics applications will be used to elucidate the underlying mechanisms of trait development in animals, including traits related to the quality of milk and meat, fat deposition and IMF accretion, health and disease resistance, and disease detection in the near future.

References

1. Sarmiento, F. (2005). Mercado internacional del jamón curado. Proceeding of 3th World Congress of Dry-Cured Ham, May, Teruel (Spain).
2. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* 2004. 3, 309–321. https://doi.org/10.4081/ijas.2004.309.
3. Krvavica, M.; Djugum, J. Prosciutto production worldwide and here. MEAT: The first Croatian meat journal [Internet]. **2006** [Accessed 02/28/2020]; VIII (6): 355-365. Available at: https://hrcak.srce.hr/22460.
4. Čandek-Potokar, M.; Škrlep, M. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal* **2012**, *6*, 327–338, DOI:10.1017/S1751731111001625.
5. Pagliarini, E.; Laureati, M.; Dinnella, C.; Monteleone, E.; Proserpio, C.; Piasentier, E. Influence of pig genetic type on sensory properties and consumer acceptance of Parma, San Daniele and Toscano dry-cured hams. *J. Sci. Food Agric.* **2016**, doi:10.1002/jsfa.7151.
6. Wu, F.; Vierck, K.R.; DeRouchey, J.M.; O'Quinn, T.G.; Tokach, M.D.; Goodband, R.D.; Dritz, S.S.; Woodworth, J.C. A review of heavyweight market pigs: Status of knowledge and future needs assessment. Transl. Anim. Sci. 2017, 1, 1-15. doi:10.2527/tas2016.0004.
7. Bikker, P. (1994). *Protein and lipid accretion in body components of growing pigs : effects of body weight and nutrient intake*. Bikker. https://edepot.wur.nl/201617.
8. National Research Council *Nutrient Requirements of Swine*; The National Academies Press, Ed.; Eleventh R.; National Academies Press: Washington, D.C., 2012; ISBN 978-0-309-22423-9.
9. Latorre, M.A.; Lázaro, R.; Valencia, D.G.; Medel, P.; Mateos, G.G. The effects of gender and slaughter weight on the growth performance, carcass traits, and meat quality characteristics of heavy pigs. *J. Anim. Sci.* **2004**, *82*, 526-533. doi:10.2527/2004.822526x.
10. Santos J. S., (2012). Production systems and sustainable management of pigs in the Mediterranean region. *Options Méditerranéennes. Séries A. Mediterranean Seminars*, *101*, 99–107. http://om.ciheam.org/om/pdf/a101/00006663.pdf.
11. EEC. 1992. Prosciutto di Parma. Protected Designation of Origin. General Rules, Dossier Pursuant to Article 4 of Council Regulation (EEC) No. 2081/92 of July, 14th 1992. *Off J Eur Communities.* L208:9–14.
12. Noblet, J.; van Milgen, J. Energy value of pig feeds: effect of pig body weight and energy evaluation system. J. Anim. Sci. 2004.
13. Lebret, B. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal* **2008**, *2*, 1548–1558, doi:10.1017/S1751731108002796.
14. Lebret, B.; Juin, H.; Noblet, J.; Bonneau, M. The effects of two methods of increasing age at slaughter on carcass and muscle traits and meat sensory quality in pigs. *Anim. Sci.* **2001**, *72*, 87-94. doi:10.1017/S1357729800055582.
15. Russo, V., and L. Nanni Costa. 1995. Suitability of pig meat for salting and the production ofquality processed products. Pig News Info. 16:17N–26N.
16. Virgili, R.; Degni, M.; Schivazappa, C.; Faeti, V.; Poletti, E.; Marchetto, G.; Pacchioli, M.T.; Mordenti, A. Effect of Age at Slaughter on Carcass Traits and Meat Quality of Italian Heavy Pigs. *J. Anim. Sci.* **2003**, doi:10.2527/2003.81102448x.
17. Cisneros, F.; Ellis, M.; McKeith, F.; McCaw, J.; Fernando, R. Influence of slaughter weight on growth and carcass characteristics, commercial cutting and curing yields, and meat quality of barrows and gilts from two genotypes. *J. Anim. Sci.* **1996**, *74*, 925–933. https://doi.org/10.2527/1996.745925x.
18. Toldrá F. Dry-cured meat products. Food and Nutrition press, Inc. Trimbull, Connecticut, USA. **2002**. pp. 54-59.
19. Gallo, L.; Dalla Bona, M.; Cecchinato, A.; Schiavon, S. Effect of growth rate on live performance, carcass and green thigh traits of finishing Italian heavy pigs. *Ital. J. Anim. Sci*. **2017**, *16*, 652–658, doi:10.1080/1828051X.2017.1318037.
20. Pugliese, C.; Franci, O.; Acciaioli, A.; Bozzi, R.; Campodoni, G.; Sirtori, F.; Gandini, G. Physical, Chemical and Technological Traits of Dry-Cured Ham of Cinta Senese Pigs Reared Outdoors and Indoors. *Ital. J. Anim. Sci.* **2006**, *5*, 265–276, doi:10.4081/ijas.2006.265.
21. Gallo, L.; Dalla Bona, M.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Effect of progressive reduction in crude protein and lysine of heavy pigs diets on some technological properties of green hams destined for PDO dry-cured ham production. *Meat Sci.* **2016**, *121*, 135-140. doi:10.1016/j.meatsci.2016.06.005.
22. Lo Fiego, D.P.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107-114. doi:10.1016/j.meatsci.2004.06.010.

23. Čandek-Potokar, M.; Žlender, B.; Lefaucheur, L.; Bonneau, M. Effects of age and/or weight at slaughter on *longissimus dorsi* muscle: Biochemical traits and sensory quality in pigs. *Meat Sci.* **1998**, *48*, 287-300. doi:10.1016/S0309-1740(97)00109-5.
24. Malgwi, I.H.; Gallo, L.; Halas, V.; Bonfatti, V.; Carc, G.; Sasso, C.P.; Carnier, P.; Schiavon, S. The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits. **2021**, *11*, 2447, doi: 10.3390/ani11082447.
25. Kyriazakis, I.; Whittemore, C.T. Whittemore's science and practice of pig production. Kyriazakis I., Whittemore, C.T., Blackwell Publishing, Oxford, UK; **2006**. pp. 417. https://doi.org/10.1002/9780470995624.ch13.
26. Noblet, J.; van Milgen, J. Energy value of pig feeds: effect of pig body weight and energy evaluation system. J. Anim. Sci. 2004.
27. Lebret, B. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal* **2008**, *2*, 1548–1558, doi:10.1017/S1751731108002796.
28. Gallo, L.; Dalla Montà, G.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Carcass quality and uniformity of heavy pigs fed restrictive diets with progressive reductions in crude protein and indispensable amino acids. *Livest. Sci.* **2015**, *172*, 50–58, doi:10.1016/j.livsci.2014.11.014.
29. Correa, J.A.; Faucitano, L.; Laforest, L.P.; Rivest, J.; Marcoux, M.; Gariépy, C. Effects of slaughter weight on carcass composition and meat quality in pigs of two different growth rates. *Meat Sci.* **2006,** *72*, 91-9, doi: 10.1016/j.meatsci.2005.06.006.
30. Van den Broeke,.A; Leen, F; Aluwé, M; Van Meensel, J; Millet, S. The effect of sex and slaughter weight on performance, carcass quality and gross margin, assessed on three commercial pig farms. *Animal*, **2020**, *14*, 1546-1554, doi: 10.1017/S1751731119003033.
31. Rodríguez-Sánchez, J.A.; Sanz, M.A.; Blanco, M.; Serrano, M.P.; Joy, M.; Latorre, M.A. The influence of dietary lysine restriction during the finishing period on growth performance and carcass, meat, and fat characteristics of barrows and gilts intended for dry-cured ham production. *J. Anim. Sci.* **2011**, *89*, 3651–3662, doi:10.2527/jas.2010-3791.
32. van Milgen, J.; Dourmad, J.Y. Concept and application of ideal protein for pigs. *J. Anim. Sci. Biotechnol.* 2015. 6:15, doi:10.1186/s40104-015-0016-1.
33. Izquierdo, O.A.; Wedekind, K.J.; Baker, D.H. Histidine requirement of the young pig. *J. Anim. Sci.* **1988**, *66*, 2886–2892, doi:10.2527/jas1988.66112886x.
34. Fuller, M.F.; McWilliam, R.; Wang, T.C.; Giles, L.R. The optimum dietary amino acid pattern for growing pigs. *Br. J. Nutr.* **1989**, *62*, 255–267, doi:10.1079/bjn19890028.
35. Wang, T.C.; Fuller, M.F. The optimum dietary amino acid pattern for growing pigs. *Br. J. Nutr.* **1989**, *62*, 77–89, doi:10.1079/bjn19890009.
36. Chung, T.K.; Baker, D.H. Ideal amino acid pattern for 10-kilogram pigs. *J. Anim. Sci.* **1992**, *70*, 3102–3111, doi:10.2527/1992.70103102x.
37. Chung, T.K.; Baker, D.H. Methionine requirement of pigs between 5 and 20 kilograms body weight. *J. Anim. Sci.* **1992**, *70*, 1857–1863, doi:10.2527/1992.7061857x.
38. Hahn, J.D.; Baker, D.H. Optimum ratio to lysine of threonine, tryptophan, and sulfur amino acids for finishing swine. *J. Anim. Sci.* **1995**, *73*, 482–489, doi:10.2527/1995.732482x.
39. Gaines, A.M.; Yi, G.F.; Ratliff, B.W.; Srichana, P.; Kendall, D.C.; Allee, G.L.; Knight, C.D.; Perryman, K.R. Estimation of the ideal ratio of true ileal digestible sulfur amino acids:lysine in 8- to 26-kg nursery pigs. *J. Anim. Sci.* **2005**, *83*, 2527–2534, doi:10.2527/2005.83112527x.
40. Puig-Oliveras, A.; Ramayo-Caldas, Y.; Corominas, J.; Estellé, J.; Pérez-Montarelo, D.; Hudson, N.J.; Casellas, J.; Folch, J.M.; Ballester, M. Differences in muscle transcriptome among pigs phenotypically extreme for fatty acid composition. *PLoS ONE* **2014**, *9*, e99720. https://doi.org/10.1371/journal.pone.0099720.
41. Muñoz, G.; Alves, E.; Fernández, A.; Óvilo, C.; Barragán, C.; Estellé, J.; Quintanilla, R.; Folch, J.M.; Silió, L.; Rodríguez, M.C.; Fernández, A.I. QTL detection on porcine chromosome 12 for fatty-acid composition and association analyses of the fatty acid synthase, gastric inhibitory polypeptide and acetyl-coenzyme A carboxylase alpha genes. *Anim. Genet.* **2007**, *38*, 639–646, doi:10.1111/j.1365-2052.2007.01668.x.
42. Latorre, M.A.; Lázaro, R.; Gracia, M.I.; Nieto, M.; Mateos, G.G. Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight. *Meat Sci.* **2003**, *65*, 1369–1377. https://doi.org/10.1016/S0309-1740(03)00059-7.
43. Wood, J.D.; Nute, G.R.; Richardson, R.I.; Whittington, F.M.; Southwood, O.; Plastow, G.; Mansbridge, R.; Da Costa, N.; Chang, K.C. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Sci.* **2004**, *67*, 651–667. https://doi.org/10.1016/j.meatsci.2004.01.007.
44. Hocquette, J.F.; Gondret, F.; Baza, E.; Mdale, F.; Jurie, C.; Pethick, D.W. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal* **2010**, *4*, 303–319. https://doi.org/10.1017/S1751731109991091.

45. Madeira, M.S.; Lopes, P.A.; Costa, P.; Coelho, D.; Alfaia, C.M.; Prates, J.A.M. Reduced protein diets increase intramuscular fat of psoas major, a red muscle, in lean and fatty pig genotypes. *Animal* **2017**, *11*, 2094–2102. https://doi.org/10.1017/S1751731117000921.
46. Ladeira, M.M.; Schoonmaker, J.P.; Swanson, K.C.; Duckett, S.K.; Gionbelli, M.P.; Rodrigues, L.M.; Teixeira, P.D. Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat. *Animal* **2018**, *12*, S282–S294. https://doi.org/10.1017/S1751731118001933.

Chapter 1

The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits

I. H. Malgwi [1,*], L. Gallo [1], V. Halas [2], V. Bonfatti [3], G. Carcò [1], C. P. Sasso [1], P. Carnier [3] and S. Schiavon [1]

[1] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell' Università 16, 35020 Legnaro (PD), Italy

[2] Department of Farm Animal Nutrition, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences, Guba Sándor Utca 40, 7400 Kaposvár, Hungary

[3] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Agripolis, Viale dell'Università 16, Legnaro, 35020 Padova, Italy

Published in: *Animals 2021, 11(8), 2447;* https://doi.org/10.3390/ani11082447

The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits

I. H. Malgwi [1,*], L. Gallo [1], V. Halas [2], V. Bonfatti [3], G. Carcò [1], C. P. Sasso [1], P. Carnier [3] and S. Schiavon [1]

Simple Summary:

Conventional rearing systems for heavy pigs intended for Italian dry-cured ham production require pigs to be slaughtered at 160 ± 16 kg and a minimum age of 9 months. With the current animal genetic trends providing progressively leaner animals, the conventional rearing system fails to provide pigs with optimal characteristics for the dry-cured ham industry. In this research, new combinations of age and weight at slaughter were explored, using different feeding conditions, as possible alternative rearing strategies for heavy pigs. Such alternative rearing strategies aimed to manipulate the growth rate of pigs, first allowing them to reach 160 ± 16 kg slaughter weight at a younger age; second, allowing pigs to maximize their slaughter weight at 9 months of age; and third, inducing slow growth in the pigs to reach the 160 ± 16 kg body weight at an older age. The first two strategies were the most promising alternatives as they improved the rate of gain, feed efficiency, and ham adiposity of the pigs. While the first strategy was the most economically convenient, the second produced the hams with the highest quality.

Abstract:

Italian dry-cured ham production requires pigs to be slaughtered at 160 ± 16 kg at 9 months of age (control, C). The study explored three alternatives, based on different feeding conditions: 1) allowing pigs to express their growth potential by letting them reach 160 ± 16 kg slaughter weight (SW) at younger slaughter age (SA) (younger Age, YA); 2) allowing pigs to express their growth potential by maximizing their SW at 9 months SA (greater weight, GW); 3) increasing the SA required to reach 160 ± 16 kg SW (older age, OA). Pigs (336 C21 Goland, 95 kg initial body weight) were slaughtered on average at 257, 230, 257, and 273 d SA and 172.7, 172.3, 192.9, and 169.3 SW kg for the four treatments, respectively. C pigs had an average daily gain (ADG) of 715 g/d and feed efficiency (FE) of 0.265 (gain to feed). Compared to C, YA pigs had higher ADG (+32%), FE (+7.5%), and better ham adiposity; GW pigs had higher carcass weight (+12%), ADG (+25%), trimmed ham weight (+10.9%), and better ham adiposity. OA treatment affected ADG (−16.4%), FE (−16.6%), and trimmed ham weight (−3.6%). YA and GW could be promising alternatives to C as they improved FE and ham quality traits.

Keywords: carcass quality; dry-cured ham; growth performance; pigs; slaughter age; slaughter weight

1. Introduction

Setting target slaughter weight (SW) and slaughter age (SA) is a management decision that impacts the productivity and profitability of pig production [1]. Over the past decades, the availability of lean pig lines has led to an increase in the SW in several countries, to minimize the cost of production per pig [1]. Globally, in the dry-cured ham pig production chain, heavy SW and advanced SA are required to ensure adequate ham size, ham fat covering depth, and lean tissue maturity [2,3]. Most often, SW and SA are mandatory criteria for quality assurance of hams and other pig products [4].

Product specifications of Italian Protected Designation of Origin (PDO) dry-cured hams set the minimum SA and SW to 9 months and 160 ± 16 kg, respectively [5,6]. To fulfil these requirements, the average daily gain (ADG) from birth to slaughter must be constrained to 0.60–0.70 kg/d. This leads to the adoption of restricted feeding strategies based on medium protein diets [7]. However, the supply of increasingly lean pig lines results in a growing proportion of hams that do not meet the industry quality standards [6,8]. An adjustment in SW and SA can result in higher carcass fatness and better ham characteristics [3,9]. Currently, the guidelines of the existing PDO product specifications are undergoing revision by the ham consortia. Given possible changes in the requirements for Italian heavy pig farming, valid alternatives to the current rearing strategy should be investigated. In addition, it can be observed

that due to animal welfare issues, feeding strategies that allow the pigs to eat ad libitum rather than restricted might be more appreciated by the consumers.

Different combinations of age and weight at slaughter can be attained by manipulating energy and dietary nutrient supplies [10,11], and this may affect not only raw ham properties [3] but also the growth performance and carcass traits of pigs. Therefore, this study was arranged to compare the current conventional production system to the following three alternative feeding and rearing strategies, representing different combinations of age and weight at slaughter: (i) allowing pigs to reach the conventional SW of 160 ± 16 kg at a younger age; (ii) allowing pigs to maximize their SW at the conventional SA of 9 months of age; and (iii) increasing the time required by the pigs to reach the conventional SW of 160 ± 16 kg SW.

Compared with the conventional system, both the first and the second strategies imply an increased growth rate obtained by increasing energy and dietary nutrient supplies. Conversely, the third strategy implies a reduction in the ADG, attainable through a reduced energy and protein supply, as this will stimulate a greater fat deposition at the expense of lean growth [11]. There are limited reports regarding the effect of an increased SW or SA in heavy pigs [2,9]. To our knowledge, prior studies have not considered such factors nor evaluated the consequences of the adoption of possible alternative strategies. This study aimed to evaluate the growth performance, feed efficiency, carcass traits, and green ham characteristics of heavy pigs raised under the three alternative rearing strategies outlined above, and to compare them with those obtained with the conventional production system.

2. Materials and Methods

2.1. Pig Housing, Rearing, and Slaughtering

The experiment involved 336 purebred Goland C21 pigs (Gorzagri, Fonzaso, Italy), barrows, and gilts (0.50:0.50), divided into 3 batches of 112 pigs each.

Pigs were members of 68 full-sibling families, generated by mating 13 boars to 67 sows. Besides growth and residual feed efficiency, the breeding goal of the Goland C21 pig line includes traits related to the quality of raw hams [12] and their suitability for dry-curing [13]. All the pigs from a given batch were born in the same week, raised on the same farm, and fed the same commercial diets till their transfer to the experimental station of the University of Padua at 95.0 ± 12.5 kg body weight (BW) and 149 ± 3 days of age. In each of the three batches, the 112 pigs were equally allocated to the four treatment groups, representing the control (C) and three alternative rearing strategies. The 3 batches of pigs entered the experimental period sequentially and experiments were conducted during different seasons (autumn, winter, and spring), avoiding the summer hot environmental temperatures. The duration of the experimental period ranged from 85 to 134 days, depending on the rearing strategy.

Pigs were housed in pens of 5.8 × 3.8 m with fully slatted floors (1.57 m^2/pig). Each pen was equipped with a single-space electronic feeder (Compident Pig–MLP, Schauer Agrotronic, Prambachkirchen, Austria) programmed to supply each pig with the planned daily amount of feed. The weighting system of each station was calibrated as described in [14]. For each visit and pig, the station recorded the time and date of the feeding event, the time spent eating, and the amount of feed consumed. For the current experiment, the daily feed intake of each pig was computed as the sum of the feed consumed during each visit in the day. Major details about feed distribution, consumption, and feeding behaviour measurement were reported previously [15]. Water was accessed freely from nipple drinkers within each pen. The average temperature in the housing rooms was set to 19–22 °C.

Pigs were weighed with an electronic scale at the start and the end of the trial, and at 120 kg BW, in correspondence with the change of feed. At each weighing, backfat depth (BF) was measured with an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco Corporation, Minneapolis, MN, USA). The BF measurements were taken at the last rib at approximately 5.5 to 8.0 cm from the midline, at an increasing distance with increasing BW [16]. The gain in BF depth was computed as a difference between the final and the starting BF depth. When pigs reached the average targeted BW or age, they were subject to fasting for 24 h before being transferred to a commercial abattoir and slaughtered following regulations for commercial practices.

During the trial, one pig died because of gastric torsion, and 10 pigs were moved to the infirmary because of lameness and their data were excluded from the study. A total of 325 records were available for analysis.

2.2. Experimental Design

The study, arranged as a split-plot design with treatments and sex within a pen, included 4 treatments, control (C) and 3 groups, representing 3 alternative rearing strategies. The characteristics of the 4 groups of pigs are summarized in Table 1. The C group corresponded to the traditional heavy pig farming system and included pigs fed restrictively medium protein (MP) feeds, with lysine as the first limiting indispensable amino acid (AA). Pigs were slaughtered at about 170 kg SW and 9 months SA.

The first alternative rearing strategy aimed to reach 170 kg of SW at the minimum age (younger age, YA). The pigs of this group were fed ad libitum high-protein (HP) feeds, not limiting for indispensable AA content. The second alternative strategy aimed to reach the maximum SW at 9 months SA (greater weight, GW). Pigs of this group were fed ad libitum the same HP feeds of the YA group and they were slaughtered at the same SW as the C pigs. The third alternative treatment (older age, OA) aimed to produce pigs of 170 kg SW with an increased age compared to C. These pigs were fed restrictively as the C group, but with low-protein (LP) feeds containing a low amount of lysine, as the first limiting AA.

In the experimental station, for each of the 3 batches, the 112 pigs were distributed in 8 pens (14 pigs/pen, 2 pens per treatment), with barrows and gilts equally mixed in the same pen. Means and standard deviations of initial BW were similar across the pens. Pigs assigned either to the C group or to one of the treatments (28 pigs per group) were housed in two pens. An across-batch rotation scheme was used to assign treatment groups to pens in different batches so that each treatment was assigned to every pen.

Table 1. Characteristics of the experimental groups rwere aised according to the traditional (control, C), and three alternative rearing strategies (younger age, YA; greater weight, GW; and older age, OA) [a].

	Rearing Strategy			
Item	**Control (C)**	**YA**	**GW**	**OA**
Weight on arrival, kg	95 ± 13	95 ± 13	95 ± 12	95 ± 12
Age on arrival, d	149 ± 3	149 ± 3	149 ± 3	149 ± 3
Target weight at slaughter, kg	170	170	>170	170
Target age at slaughter, d	270	<270	270	>270
Feeding regime	Restricted	Ad libitum	Ad libitum	Restricted
Protein content in early finishing feed [b]	Medium	High	High	Low
Protein content in late finishing feed [c]	Medium	High	High	Low

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg target SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg target SW. [b] Early finishing feed was administered from 90 to 120 kg. [c] Late finishing feed was administered from 120 kg onwad.

Feeds were manufactured (Progeo Feed Industry, Masone, Reggio Emilia, Italy) using the same batches of feed ingredients. The ingredient composition of early (90 to 120 kg average body weight) and late finishing feeds (over 120 kg body weight) is reported in Table 2.

Table 2. Ingredient composition (g/kg as-fed) of early (90 to 120 kg average body weight) and late finishing feeds (over 120 kg body weight).

Ingredient	Early Finishing Feeds			Late Finishing Feeds		
	High Protein	Medium Protein	Low Protein	High Protein	Medium Protein	Low Protein
Corn grain	361.3	350.0	390.0	398.3	398.3	398.8
Wheat grain	240.0	270.0	260.0	237.8	237.3	237.5
Barley grain	100.0	100.0	100.0	100.0	100.0	100.0
Soybean meal 48% (solv. ex.)	196.0	85.0	38.0	143.0	56.0	18.3
Wheat bran	26.5	87.5	85.3	7.5	57.5	62.5
Wheat middlings	-	20.0	30.0	40.0	67.5	90.0
Cane molasses	20.0	20.0	20.2	22.5	22.5	22.5
Lard	20.0	21.6	21.0	20.0	20.0	20.0
Dried sugar beet pulp	-	10.0	20.0	-	10.0	20.5
Calcium carbonate	15.0	15.0	15.0	13.0	13.0	13.0
Dicalcium phosphate	4.5	4.5	4.5	2.0	2.0	2.0
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0
Sodium bicarbonate	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin and mineral premix [a]	2.0	2.0	2.0	2.0	2.0	2.0
Grapeseed meal	7.5	7.5	7.5	7.5	7.5	7.5
Choline, liquid, 75% [b]	0.5	-	-	-	-	-
L-Lysine [c]	1.0	1.4	0.65	-	1.0	1.0
DL-Methionine [d]	0.2	-	-	-	-	-

[a] Providing per kilogram of feed: vitamin A, 8000 IU; vitamin D3, 1200 IU; vitamin E, 8 mg; Vitamin B7, 0.08 mg; vitamin B12, 0.012 mg; niacin, 16.0 mg; biotin, 8 mg; iron, 170 mg; zinc, 117 mg; copper, 14 mg; cobalt, 0.11 mg; iodine, 0.06 mg; manganese, 65 mg; magnesium, 0.14 mg; selenium 10 mg.
[b] Choline liquid 75% (Methodo Chemicals, 42017 Novellara, RE, Italy).
[c] L-Lysine Monoclohydrate, 98.5% pure, 78% L-Lysine (Methodo Chemicals, 42017 Novellara, RE, Italy).
[d] DL-Methionine, 98% pure min. (Methodo Chemicals, 42017 Novellara, RE, Italy).

Feed samples were collected and analyzed to evaluate, before their use in the experiment, the actual nutrient contents. Feed samples (10 samples for each feed collected online to achieve a 1-kg feed sample after pooling and mixing) were analyzed for proximate composition [17], starch content [18], and neutral detergent fibre content [19]. The nutrient composition of the feeds, including metabolizable and net energy, crude protein, and AA contents, was computed according to tabular data provided by NRC [20] and is reported in Table 3.

Table 3. Nutrient content (g/kg as-fed, unless otherwise indicated) of early (90 to 120 kg average body weight) and late finishing feeds (over 120 kg average body weight) [a].

Items	Early Finishing Feeds			Late Finishing Feeds		
	High Protein	Medium Protein	Low Protein	High Protein	Medium Protein	Low Protein
Analyzed nutrient composition						
DM	906	904	904	906	902	904
CP (N × 6.25)	162	128	113	138	119	104
Starch	413	460	488	483	470	490
Ether extract	43	46	44	48	50	48
NDF	131	138	141	118	132	134
Ash	48	47	48	42	41	41
Calculated nutrient composition [b]						
ME, MJ/kg	13.4	13.2	13.2	13.4	13.2	13.1
NE, MJ/kg	10.0	10.0	10.1	10.1	10.0	9.9
CP (N × 6.25)	162	128	109	142	116	103
Starch	424	449	470	454	470	477
Ether extract	44	47	47	46	47	47
Linoleic acid	14	15	16	15	16	17
Lysine	8.3	6.2	4.6	6.9	5.2	3.6
Methionine	2.7	2.0	1.9	2.2	1.9	1.7
Threonine	5.7	4.3	3.6	5.1	4.0	3.5
Tryptophan	2.0	1.5	1.3	1.6	1.3	1.0
Tyrosine	5.3	4.1	3.5	5.0	3.9	3.4

Analytical results are the average of 3 independent replications.
[b] Computed based on the NRC (2012) tabular values of each feed ingredient.

The early finishing HP feed, used in the groups' YA and GW from 90 to 120 kg BW, was designed to contain non-limiting amounts of indispensable standardized ileal digestible (SID) lysine, methionine, tryptophan, and threonine, according to the NRC recommendation for the 70–100 kg BW range [20]. The SID lysine content of the early-finishing MP feed, used in the C group, was 26% lower than that proposed by NRC [20] for the same BW range. Such feed was expected to guarantee an ADG of 0.7 kg/d, with lysine as the first limiting AA. The SID lysine content of the early finishing LP feed, used in the OA group, was limiting, consistent with an ADG of 0.650 kg/d, and was designed to be lower than that used in previous studies, where the limited dietary AA content did not influence growth performance and meat quality [15,21].

Within the treatment groups, the late-finishing HP, MP, and LP diets, fed from 120 kg BW on, were formulated to contain about 20–25% less indispensable SID AA than the corresponding HP, MP, and LP feed used in the early finishing period, with lysine as the first limiting AA.

Upon arrival at the experimental station, the amount of feed distributed was estimated based on the average initial BW, and the amount of feed was successively increased weekly without any further adjustment. The amount of feed provided to pigs fed restrictively was increased from 2.3 to 3.0 kg/d for the entire duration of the trial, corresponding to an increase of 57 to 82 g/kg$^{0.75}$ metabolic weight, as per common practice [15].

2.3. Slaughter and Evaluation of Carcass and Green Ham Quality

Slaughter and carcass dressing were carried out as described in Schiavon et al. [22]. Hot carcass weight was recorded online, and the lean percentage was estimated by image analysis of the left carcass side (CSB-Image-Meter®, CSB-System AG, Geilenkirchen, Germany) according to the EU guidelines [23,24]. Primary cuts (loin with ribs, shoulder, thigh, lard, and belly) were weighed using an electronic scale. Green hams were chilled (0–2 °C) for 24 h, trimmed to obtain the typical round ham shape, and weighed again. The ham subcutaneous fat depth was measured in the proximity of m. *biceps femoris* (P1) and m. *semimembranosus* (P2) using a calliper and a portable ultrasound system (Aloka SSD 500 equipped with UST-5512 7.5 MHz linear transducer probe, Hitachi Medical Systems S.p.A., Milan, Italy), respectively.

A trained operator scored all left hams as described in Schiavon et al. [15] for round shape (0 = low, to 4 = high, optimum: 1 to 2); visible marbling (0 = absent to 4 = very evident, optimum: 1); fat cover thickness (−4 = very thin to 4 = very thick, optimum: 0 to 1); lean colour intensity (−4 = very pale to 4 = very dark, optimum = 0); bicolor, indicating muscles with different colour (-4 = absent to 4 = very evident); and veining (0 = absent to 4 = very evident, optimum = 0). A similar scoring grid for these traits has also been reported by Magistrelli et al. [25], and comparable grids are used elsewhere [26,27].

2.4. Statistical Analysis

The data were analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) using the following linear model:

$$y_{ijklm} = \mu + RS_i + sex_j + (RS \times sex)_{ij} + batch_k + pen(RS \times batch)_{l:ik} + e_{ijklm} \quad (1)$$

where y_{ijklm} was the observed trait, μ was the overall intercept of the model, RS was the fixed effect of the i^{th} rearing strategy (i = 1, …, 4), sex was the fixed effect of the j^{th} sex (j: 1 = gilts, 2 = barrows), (RS × sex) was the interaction effect between rearing strategy and sex, batch was the random effect of the k^{th} batch (k = 1, …, 3), pen was the random effect of the l^{th} pen within the (batch × $RS)_{ik}$ interaction (l = 1, 2), and e_{ijklm} was the random residual.

The pen, the batch, and the residuals were assumed to be independently and normally distributed with a mean of zero and variance σ^2_k, σ^2_l, and σ^2_e, respectively. The effect of the rearing strategy was tested on the pen (RS × batch) variance, whereas sex and the rearing strategy × sex interaction were tested on the residual variance. The 3 degrees of freedom due to the rearing strategy were used to run orthogonal contrasts to test the effect of OA, YA, and GW with respect to C.

3. Results

3.1. Growth Performance

Pigs in the C group were slaughtered at 172 kg SW and 108 days on-feed, corresponding to 257 d SA (Table 4). The ADG was 715 g/d, the gain to feed ratio (feed efficiency) was 0.265, and the mean final backfat depth was 21.9 mm. The YA pigs were sacrificed at 81 days on feed (27 days earlier than the C). They exhibited greater daily feed intake ($p < 0.001$), ADG ($p < 0.001$), better feed efficiency ($p = 0.002$), higher final backfat depth ($p = 0.003$), and gain in backfat ($p < 0.001$) but lower cumulative feed consumption compared to C ($p < 0.001$).

The GW pigs had greater SW than C ($p < 0.001$) at the same SA. The GW pigs exhibited greater daily and cumulative feed consumption ($p < 0.001$), ADG ($p < 0.001$), final backfat depth ($p = 0.003$), and gain in backfat ($p < 0.001$) than C. Feed efficiency of GW tended to be higher, although not statistically different ($p = 0.09$), than that of C, despite the greater cumulative feed consumption.

Table 4. Growth performance of heavy pigs raised according to the traditional rearing system (control, C), and three alternative strategies (younger age, YA; greater weight, GW; and older age, OA) [a].

	Rearing Strategy					*p* Values		
Item	**C**	**YA**	**GW**	**OA**	**SEM [b]**	**C vs YA**	**C vs GW**	**C vs OA**
Animals, n	83	77	82	83	-	-	-	-
Days on feed [c], d	108	81	108	124	-	-	-	-
Initial bodyweight, kg	95.1	95.5	95.7	95.0	6.1	0.78	0.69	0.93
Slaughter weight, kg	172.7	172.3	192.9	169.3	1.5	0.81	<0.001	0.11
Daily feed consumption, g/d	2694	3310	3245	2697	42	<0.001	<0.001	0.96
Cumulative feed consumption, kg/pig	293	267	353	334	24	<0.001	<0.001	<0.001
Average daily gain, g/d	715	947	893	598	22	<0.001	<0.001	<0.001
Gain to feed ratio	0.265	0.285	0.275	0.221	0.007	0.002	0.09	<0.001
Backfat depth, mm								
initial	12.2	11.9	12.2	11.8	1.89	0.41	0.95	0.26
at slaughter	21.9	24.8	26.0	22.9	0.91	0.026	0.003	0.26
Gain in backfat depth, mm	9.7	12.9	13.9	11.1	1.68	0.004	<0.001	0.08

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg target SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg target SW.
[b] SEM: pooled standard error of the mean, n = 325. [c] At the start of the experiment, pigs were 149 ± 3 d old.

The OA pigs required additional 16 days on feed to reach the same SW as the C pigs. OA had lower ADG ($p < 0.001$), similar daily feed intakes ($p = 0.96$), and greater cumulative feed consumption ($p < 0.001$), resulting in a lower feed efficiency ($p < 0.001$) than the C. There was no significant difference in backfat depth and gain in backfat depth between C and OA at slaughter.

The GW pigs had greater SW than C ($p < 0.001$) at the same SA. The GW pigs exhibited greater daily and cumulative feed consumption ($p < 0.001$), ADG ($p < 0.001$), final backfat depth ($p = 0.003$), and gain in backfat ($p < 0.001$) than C. The feed efficiency of GW tended to be higher, although not statistically different ($p = 0.09$), than that of C, despite the greater cumulative feed consumption.

The OA pigs required an additional 16 days on feed to reach the same SW as C pigs. OA had lower ADG ($p < 0.001$), similar daily feed intakes ($p = 0.96$), and greater cumulative feed consumption ($p < 0.001$), resulting in a lower feed efficiency ($p < 0.001$) than the C. There was no significant difference in backfat depth and gain in backfat depth between C and OA at slaughter.

Sex had a significant influence on various traits: barrows had greater initial ($p = 0.007$) and final SW ($p = 0.034$) and a slightly greater daily ($p = 0.015$) and total ($p = 0.009$) feed consumption. Barrows and gilts had similar ADG ($p = 0.08$), but a greater feed efficiency ($p = 0.009$) and a lower final backfat depth ($p < 0.001$) was observed in gilts (Supplementary Materials Table S1). The treatment × sex interaction had minor influences on final SW and cumulative feed consumption (Supplementary Materials Figure S1).

Table S1. Growth performance of gilts and barrows raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA)[a].

	Sex			*P*-values	
Item	**Gilts**	**Barrows**	**SEM[a]**	**Sex**	**Sex × Rearing strategy**
Body weight, kg					
initial	94.0	96.7	6.1	0.007	0.37
final	175.3	178.3	1.0	0.034	0.030
Feed consumption, g/d	2,949	3,024	42	0.015	0.06
Cumulative feed consumption, kg	308	316	24.2	0.009	0.024
Average daily gain, g/d	787	789	16.3	0.85	0.08
Gain to feed ratio	0.265	0.259	0.006	0.009	0.64
Backfat depth, mm	23.3	24.5	0.78	0.002	0.75
Gain in backfat, mm	11.9	11.9	1.62	0.81	0.57

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg SW.
[b] SEM: pooled standard error of the mean, n = 325.

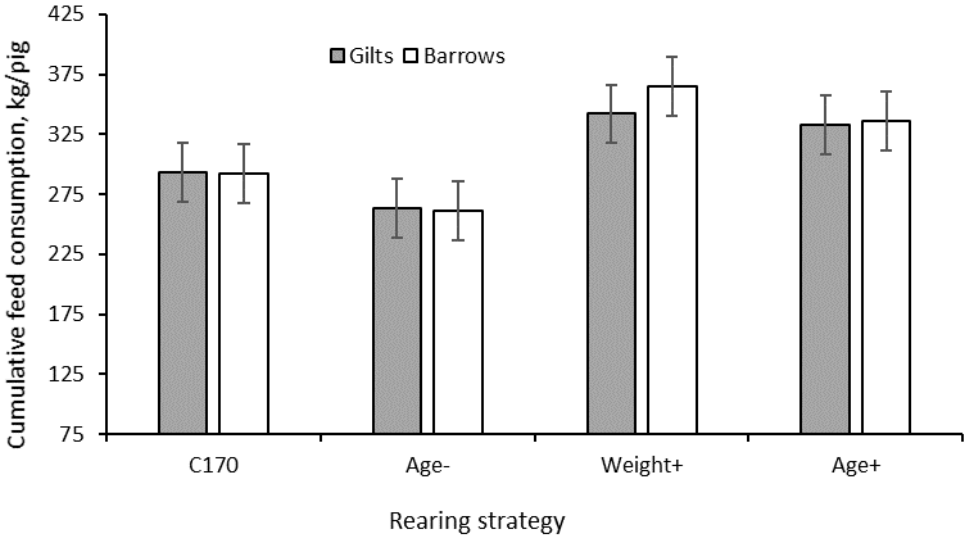

Figure S1. Influence of the sex × rearing strategy interaction (least-square means ± standard deviation; sex × rearing strategy interaction *P* =0.024) on cumulative feed consumption of heavy pigs raised according to the traditional rearing system (CONTROL, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA). [C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA (> 9 months) at 160 ± 16 kg SW; n = 325].

3.2. Carcass Traits

The pigs of the YA and C were slaughtered at the same SW, but the YA treatment influenced various carcass traits compared to C (Table 5). YA pigs had similar carcass weights and carcass yields but greater carcass backfat depth ($p < 0.035$) and lower meat percentage ($p = 0.010$) than C. The YA pigs also had greater fat cut yields ($p < 0.001$), namely backfat ($p < 0.001$) and lards ($p < 0.001$), and lower lean cut yields ($p = 0.011$), namely loin with ribs ($p = 0.009$) and shoulder ($p = 0.002$), compared to C. However, the YA treatment did not influence the green and trimmed hams yields nor the trimming losses.

GW treatment increased carcass weight ($p < 0.001$), carcass yield ($p < 0.001$), and carcass backfat depth ($p < 0.001$) but decreased carcass meat percentage ($p < 0.001$) compared to C. The yield of fat cuts increased ($p < 0.001$) for the contribution of both backfat ($p < 0.001$) and lards ($p < 0.001$), whereas the yield of the various lean cuts decreased ($p < 0.001$) compared to C, with the exception of the green hams ($p = 0.27$).

The OA treatment had little or no influence on the major carcass traits, such as weight, yield, backfat depth, and lean meat percentage, compared to C. OA slightly influenced the yield of commercial cuts, being associated with a reduction of the loin with ribs yield ($p = 0.025$).

Sex also had significant effects on carcass traits: gilts had lower carcass weight ($p = 0.007$), carcass yield ($p = 0.002$), and carcass meat percentage ($p < 0.001$) but similar carcass backfat depth, compared to barrows (Supplementary Materials Table S2). The carcass of the gilts also had a greater lean cut yield ($p < 0.001$), namely loins with ribs ($p < 0.001$), shoulders ($p = 0.050$), and green hams ($p < 0.001$), than barrows. The treatment × sex interaction had a limited influence on carcass traits, except for carcass yield ($p = 0.020$), as barrows had greater carcass yield than gilts, in particular in the YA group (Supplementary Materials Figure S2).

Table S2. Carcass traits of gilts and barrows raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA)[a].

	Sex			*P*-values	
Item	**Gilts**	**Barrows**	**SEM[b]**	**Sex**	**Sex × Rearing strategy**
Carcass weight, kg	143	146	1.0	0.007	0.07
Carcass yield, %	81.6	82.1	0.17	0.002	0.020
Backfat depth[c], mm	40.4	41.1	0.57	0.28	0.71
Lean meat, %	50.4	48.2	0.36	<0.001	0.27
Commercial cuts yield, g/kg carcass					
Fat cuts	199	202	1.6	0.04	0.24
Backfat	126	125	2.0	0.18	0.62
Lards	73	77	1.4	<0.001	0.14
Lean cuts	526	515	4.0	<0.001	0.14
Loin with ribs	152	146	1.4	<0.001	0.20
Shoulder	133	132	2.5	0.050	0.58
Green hams	240	237	0.87	<0.001	0.14
Trimmed hams [d]	196	192	1.57	<0.001	0.19
Trimming ham losses [e]	45	45	1.92	0.93	0.37

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg SW.
[b] SEM: pooled standard error of the mean, n = 325.
[c] Average of backfat depth measured with a calliper at the points of maximum depth at the shoulder and the loin.
[d] Trimming performed at the slaughterhouse the day after slaughtering.
[e] Trimming ham losses were computed as the difference between the green ham and the trimmed ham weights.

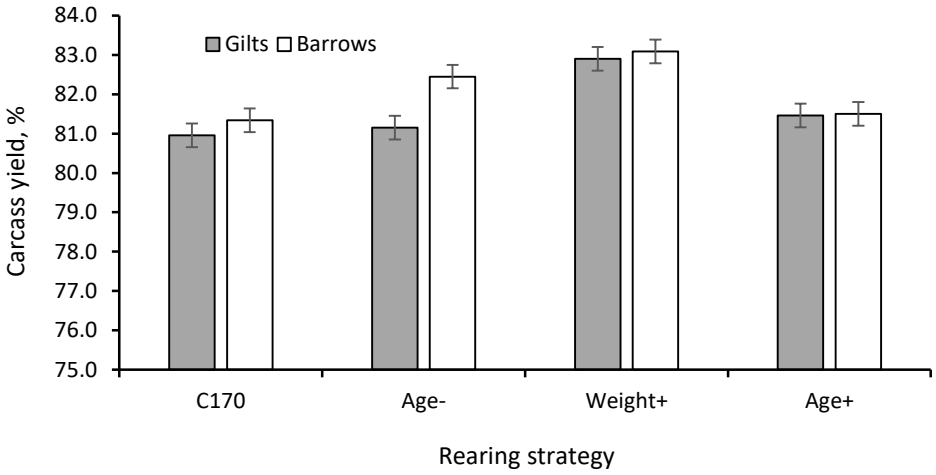

Figure S2. Influence of the sex × rearing strategy interaction (least-square means ± standard deviation; sex × rearing strategy interaction *P* =0.020) on carcass yield of heavy pigs raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA). [C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA (> 9 months) at 160 ± 16 kg SW; n = 325].

Table 5. Carcass traits of heavy pigs raised according to the traditional rearing system (control, C), and three alternative strategies (younger age, YA; greater weight, GW; and older age, OA) [a].

Item	Rearing Strategy					*p*-Values		
	C	YA	GW	OA	SEM [b]	C vs. YA	C vs. GW	C Vs. OA
Carcass weight, kg	140	141	160	138	1.4	0.65	<0.001	0.22
Carcass yield, %	81.2	81.8	82.9	81.5	0.27	0.10	<0.001	0.38
Backfat depth [c], mm	37.9	41.1	45.6	38.5	0.96	0.035	<0.001	0.71
Lean meat, %	50.8	48.8	47.4	50.4	0.50	0.010	<0.001	0.53
Commercial cuts yield, g/kg carcass								
Fat cuts	189	211	209	194	2.6	<0.001	<0.001	0.20
Backfat	122	129	130	123	2.2	<0.001	<0.001	0.57
Lard	67	82	79	71	2.5	<0.001	<0.004	0.28
Lean cuts	529	517	513	521	4.6	0.011	0.001	0.06
Loin with rib	154	148	145	149	1.7	0.009	<0.001	0.025
Shoulder	136	129	130	136	2.7	0.002	0.005	0.83
Green ham	240	240	238	236	1.4	0.86	0.27	0.05
Trimmed ham [d]	197	195	191	193	1.9	0.27	0.008	0.06
Trimming ham loss [d]	43.2	45.6	46.6	42.9	1.2	0.07	0.013	0.82

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg SW.
[b] SEM: pooled standard error of the mean, n = 325. [c] Average of backfat depth measured with a calliper at the points of maximum depth at the shoulder and the loin.
[d] Trimming was performed at the slaughterhouse the day after slaughtering.

The OA treatment had little or no influence on the major carcass traits, such as weight, yield, backfat depth, and lean meat percentage, compared to C. OA slightly influenced the yield of commercial cuts, being associated with a reduction of the loin with ribs yield (p = 0.025).
Sex also had significant effects on carcass traits: gilts had lower carcass weight (p = 0.007), carcass yield (p = 0.002), and carcass meat percentage (p < 0.001) but similar carcass backfat depth, compared to barrows (Supplementary Materials Table S2). The carcass of the gilts also had a greater lean cut yield (p < 0.001), namely loins with ribs (p < 0.001), shoulders (p = 0.050), and green hams (p < 0.001), than barrows.

The treatment × sex interaction had a limited influence on carcass traits, except for carcass yield (p = 0.020), as barrows had greater carcass yield than gilts, in particular in the YA group (Supplementary Materials Figure S2).

3.3. Green Ham Traits

The YA treatment did not alter the trimmed ham weight but increased the subcutaneous fat covering in the proximity of the *biceps femoris* muscle (p = 0.036), lean colour intensity (p = 0.030), bicolor scoring (p = 0.007), visible marbling (p = 0.041), and fat cover thickness score (p = 0.011) compared to C (Table 6). The trimmed hams of the GW pigs were heavier than those of C (p < 0.001). The hams of the GW pigs also had thicker subcutaneous fat depth in the proximity of the *biceps femoris* muscle (p= 0.04), and a greater round shape score (p = 0.038). The OA treatment was associated with a reduction in trimmed ham weight (p = 0.005), with an increased thickness of the subcutaneous fat depth in the proximity of the *semimembranosus* muscle (p = 0.002), and with an increased visible marbling score (p = 0.001) compared to C. Little or no effects associated with this treatment were observed on other traits.

Sex had little influence on quality traits (Supplementary Materials Table S3), except for the visible marbling score, which was markedly greater in barrows compared to gilts (0.985 vs. 0.636, p < 0.001).

Table 6. Green and trimmed ham characteristics of heavy pigs raised according to the traditional rearing system (control, C), and three alternative strategies (younger age, YA; greater weight, GW; and older age, OA) [a].

	Rearing Strategy					*p*-Values		
Item	**C**	**YA**	**GW**	**OA**	**SEM [b]**	**C vs YA**	**C vs GW**	**C vs OA**
Trimmed ham weight, kg	13.8	13.7	15.3	13.3	0.19	0.55	<0.001	0.005
Subcutaneous fat depth P1, mm [c]	28.2	32.4	32.3	28.7	2.57	0.036	0.040	0.78
Subcutaneous fat depth P2, mm [d]	6.4	6.8	6.8	7.2	0.3	0.08	0.06	0.002
Round shape (0 to 4) [e]	1.38	1.72	1.82	1.31	1.20	0.20	0.038	0.71
Visible marbling (0 to 4) [f]	0.57	0.87	0.73	1.08	0.38	0.041	0.26	0.001
Fat cover thickness (−4 to 4) [g]	−0.30	0.98	0.53	0.18	0.32	0.011	0.08	0.30
Lean colour intensity (−4 to 4) [h]	−1.32	−0.54	−0.73	−0.99	0.49	0.030	0.09	0.33
Bicolor (−4 to 4) [i]	1.08	2.28	1.38	1.56	0.33	0.007	0.40	0.29
Veining (0 to 4) [l]	1.13	1.57	1.48	1.15	0.13	0.14	0.36	0.16

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg SW.
[b] SEM: Standard error of means, n = 325.
[c] Measured in the proximity of m. *biceps femoris* (the higher the better).
[d] Point of minimum fat depth, measured in the proximity of m. *semimembranosus* (the higher the better);
[e] Round shape (0 = low, 4 = high, optimum: 1 to 2).
[f] Visible marbling (0 = absent, 4 = very evident, optimum = 1).
[g] Fat cover thickness (−4 = very thin, 4 = very thick, optimum: 0 to 1).
[h] Lean colour intensity (−4 = very pale, 4 = very dark, optimum = 0).
[i] Bicolor (0 = absent, 4 = very evident, optimum = 0). [l] Veining (0 = absent, 4 = very evident, optimum = 0).

4. Discussion

4.1. Traditional Rearing System

To fulfil the requirements of the current product specifications for Italian PDO dry-cured ham production (i.e., at least 9 months SA and 160 ± 16 kg SW) [6], farmers are forced to apply a restrictive medium-protein feeding regime [15]. However, the degree of restriction is heterogeneous across farms, as feed allowance adopted by the farmers largely depends on their own experience. In this study, we applied a feed restriction consistent with the recommendation of the major Italian companies supplying feed for heavy pigs intended for PDO dry-cured ham production [14,15]. Based on our results, the amount of feed administered to C pigs corresponded approximately to 79% of the average voluntary feed intake, similar to common practices in some regions of Spain [28]. Regarding the growth performance, feed efficiency, and carcass characteristics, our results are similar to those reported in other studies [8,14,21].

The quality of the dry-cured ham largely depends on the characteristics of the green ham before curing, provided the processing is standardized [29,30]. Previous studies suggested that the weight, depths of subcutaneous fat covering, and marbling of green hams are highly correlated with the final quality of the dry-cured product [8,29,31]. In Italy and other countries, the value of the green ham is determined at slaughter based on its weight, subcutaneous fat depths, fat colour, and other characteristics. For these reasons, besides growth performance and carcass traits, our study focused on the quality traits of green and trimmed hams.

For the C pigs, the average trimmed ham weight (13.8 kg), subcutaneous fat depth (proximal to the *biceps femoris* muscle, 28.2 mm), and round shape score (1.38, on a 0- to 4-point scale) were within the optimal range. However, their visible marbling (0.57, on a 0- to 4-point scale), fat cover thickness (−0.30, on a −4- to + 4-point scale), and colour intensity (−1.32, on a −4- to +4-point scale) were below the optimum. In their review, Čandek-Potokar and Škrlep [3] pointed out that these traits can be influenced by different SA and SW combinations. At the increase of days on feed and SA, pigs tend to become heavier and have larger hams with increased adiposity [3]. On the other hand, a prolonged on-feed period, especially under restricted feeding conditions, could result in increased energy requirements for maintenance, low feed efficiency, and increased nutrient excretion [15].

4.2. Decreasing Slaughter Age at Given Bodyweight (Younger Age, YA)

4.2.1. Growth Performance, Feed Efficiency, and Carcass Characteristics

When pigs are kept on diets low in essential AAs, they tend to increase their feed intake, in an attempt to meet the requirement for the deficient nutrients [22,32]. Under such conditions, the increased feed intake causes an extra amount of energy intake, which in turn results in extra fat deposition, also accompanied by a declining weight gain, so that pigs take longer to reach the target weights. Differently, under non-limiting energy and AAs supply, pigs can express their potential for growth rate and tissue deposition, provided there are no other environmental limiting factors. In our current experiment, the YA and GW groups' diets were formulated to be non-limiting in energy and AAs. Thus, it was expected that, under these treatments, the Goland C21 pigs would have approached their potential growth rate and protein and lipid deposition. Knap et al. [33] observed that, due to intensive selection for lean growth and feed efficiency, improved pig genotypes have a reduced potential for fat accretion and, consequently, a reduced voluntary feed intake. Therefore, lean pig genotypes with a low potential for fat accretion exhibit lower feed consumption than pig genotypes with a greater potential for fat accretion [33]. The pigs involved in our study, when exposed to an unlimited amount of feed, evidenced remarkable voluntary feed intake, ADG, and moderate carcass lean meat % (>3200 g/d, >890 g/d, <49%, respectively). The accretion rate (947 g/d) and the feed efficiency (0.285) of YA pigs were in line with those reported for lean pig genotypes of similar BW ranges [2,22,34]. This suggests that the Goland C21 pigs, genetically selected for green ham quality traits [13,27], have a good potential for both lean and fat tissue accretion.

The YA pigs reached the targeted SW 27 days earlier than the C pigs, at 230 days of age. They had higher daily feed consumption, growth rate, and feed efficiency (+23%, +32%, and +7.5%, respectively), and produced fatter carcasses than the C pigs, despite being sacrificed at the same SW. The greater feed efficiency of the YA pigs can be attributed to the shorter rearing period, requiring a lower energy expenditure for maintenance. However, this was partially compensated by greater energy costs due to the increased fat deposition of the YA pigs compared to C, as fat tissue accretion is expensive in energy terms [10]. The economic implication, in terms of feeding costs at the current

prices of the feed ingredients used, is that the cost per unit of BW gain of the YA strategy was 7.2% lower than that of the C groups (Table 7).

Table 7. Feeding costs of pigs raised according to the traditional (control, C), and three alternative strategies (younger age, YA; greater weight, GW; and older age, OA) [a].

	Rearing Strategy			
Item	**C**	**YA**	**GW**	**OA**
Feed price, euro/ton as fed	338	348	348	327
Feeding costs:				
Euro/pig produced	106.2	97.79	130.5	119.3
Euro/kg BW gain	1.369	1.273	1.343	1.606

[a] C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA at 160 ± 16 kg SW.

Lebret et al. [11] subjected pigs to restricted feeding regimes, to increase the SA while maintaining the same SW of the ad libitum control. They found that a 30-day increase in SA had a great influence on carcass and muscle chemical composition, with favourable or unfavourable influences depending on the feeding strategy applied to modify the growth rate. In the cited experiment of Lebret et al. [11], a voluntary feeding regime increased carcass yield, backfat depth, the proportion of fat cuts in the carcass, and intramuscular fat content of both *longissimus dorsi* and *biceps femoris* muscles and decreased the lean meat percentage and carcass proportions of lean cuts, including ham and loin, compared to a restrictive feeding regime between 30 and 110 kg BW. These responses were consistent with our experiment, despite the difference in the investigated SW range (30–110 kg).

4.2.2. Trimmed Ham Traits

Some authors suggested that it is preferable to slaughter pigs at older ages because of the positive impact of age on the final quality of the dry-cured ham [9]. However, the influence of increasing SA and SW and ham adiposity on the dry-curing aptitude of the ham was often confounded in the literature, as increasing ages were associated with increased BW and ham adiposity [3]. While a greater fat covering reduces dehydration during seasoning, improving ham quality, an earlier SA is thought to increase the dry-curing losses [3,9]. We found that the YA treatment did not change the trimmed ham weight but improved various measures of fat covering depth and the visible marbling score.

Hence, our results suggest that the YA strategy might be of interest, as it improves feed efficiency and the associated economic costs, requires less time to finish the pigs, and improves some ham quality traits, compared to the conventional practice. However, apart from effects associated with ham adiposity, improvements in the dry-curing aptitude of the ham due to increasing SA also result [3] in lowered moisture of hams, which could reduce the activity of hydrolytic enzymes, decrease the activity of proteolytic enzymes, and increasing the activity of exopeptidases and lipases, all aspects that can positively affect the quality of the dry-cured ham. Ultimately, it is not clear whether the negative influence exerted by an earlier SA on the dry-curing aptitude can be offset by the favourable increase in ham adiposity. More research on this aspect is needed.

4.3. Increasing Slaughter Weight at a Given Age (Greater Weight, GW)

4.3.1. Growth Performance, Feed Efficiency, and Carcass Characteristics

In general, heavy SW is undesired due to poor feed efficiency and increased production costs [1]. However, in dry-cured ham production systems, the decision to slaughter at a given BW is not limited to the feed efficiency of the pigs but is influenced by several factors, such as ham seasoning aptitude and differences in the curing process based on local practices. For this reason, pig growth performance, feed efficiency, and carcass characteristics are variable, making proper comparisons across studies more difficult to perform [35–38].

It was obvious that pigs subjected to the GW treatment had a greater feed consumption (+20.4%) and better ADG (+24.9%) compared to the C pigs. However, considering the magnitude of the difference between C and GW, it suggests that Goland C21 pigs can exploit remarkable ADG even when the SW is extended to more than 170 kg. Interestingly, the feed efficiency of the GW pigs was similar to that of pigs under the traditional rearing system. Thus, the feeding cost per unit of gained BW of the GW group proved to be equivalent to the traditional C group. Additionally, it was observed that 26% of the carcasses in the GW group were heavier than 168 kg. This corresponds to the new maximum threshold for carcass weight in the proposed revision of the product specifications. Therefore, depending on the pig genotype, it would be necessary to adopt mild feed restrictions to limit the full expression of the pig growth potential, while preserving the quality of the green hams.

4.3.2. Trimmed Ham Characteristics

Increased SW is thought to improve ham quality traits, such as weight, fat covering, and marbling [3]. Heavier hams are considered to have better seasoning aptitude, mainly because of lower seasoning losses. Čandek-Potokar and Škrlep [3] indicated that the reason for this is not directly related to ham weight, but it is ascribed to the greater adiposity of the heavier hams. The results of the current research were consistent with these expectations, as the GW treatment increased the ham weight (+10.9%) and the subcutaneous fat depth, in the proximity of the *biceps femoris* muscle (+14.5%), and it is therefore expected to lead to improvements in other qualitative traits of the ham.

We also observed that with the ad libitum feeding regime practised with the GW treatment, the uniformity of the dressed ham decreased when compared to C. The coefficient of variation for the dressed ham weight was 6.4 and 7.7% for C and GW, respectively. Uniformity commonly refers to the evenness of pig weights at the slaughterhouse but also the size and the weight of the carcass and the retailed cuts [21]. Uniformity is important for the dry-cured ham industry, as the amount of salt used, and the duration of salting must be adapted to the weight of the dressed hams [39]. Thus, the mild feed restriction that was suggested above to limit the occurrence of excessive SW is also expected to have some benefits in terms of carcass and ham uniformity, and to prevent excessive ham fat covering that might otherwise occur in some individuals [3,21].

4.4. Increased Slaughter Age at a Given BW (Older Age, OA)

The authors of [11,40,41] indicated that a protein restriction, in addition to energy restriction, is a strategy to increase the SA and yielded fatter carcasses, with greater marbling and better meat sensory quality. Such an approach would be interesting, as the use of low-protein diets is also indicated as a strategy to reduce N excretion and the potential environmental impact of pig farming [14]. Previously, studies have shown that low dietary indispensable AA diets fed restrictively to heavy pigs resulted in negligible influence on the ADG, carcass, and green ham quality traits in crossbred pigs [7,15]. Similarly, a dietary protein restriction had a small influence on the chemical profile of seasoned hams produced by pigs of two crossbreeds [41]. These findings suggest that, in those studies, the degree of AA restriction was not limiting for pig growth. Thus, in our present experiment, we applied a stronger reduction in the indispensable AA supply, to compel the pigs to use less energy for lean growth and more for fat accretion. This restriction significantly reduced the ADG of the OA pigs compared to the C pigs and confirms that the lysine supply was a limiting nutrient in the OA group.

Lebret et al. [11] found that a restricted-energy and lysine supply, between 30 and 110 kg BW, increased the feed efficiency, backfat depth, BW, and intramuscular fat content, and decreased the lean meat percentage but did not change ADG. In our study, the OA treatment strongly impaired ADG (−16%) and feed efficiency (−17%) compared to C. In addition, the OA treatment exerted small influences on carcass components, except for a slight, undesired, 3.7% reduction of the trimmed ham weight compared to C. However, the OA treatment was also positively associated with the greatest

increase in ham fat covering thickness in correspondence with the *semimembranosus* muscle (+12.5%) and the visible marbling score (+89%) compared to C, in agreement with previous reports [42,43].

The role of fat covering depth in correspondence with the *semimembranosus* muscle on ham quality has been scarcely investigated. In our experiment, the fat covering in this area is much thinner than that located close to the *biceps femoris* muscle. A sufficient subcutaneous fat layer is necessary to prevent rapid desiccation, which would cause the formation of a crust on the ham surface, and to reduce processing water losses [44,45]. Hams with thinner fat layers are also expected to have a greater NaCl content because of the negative correlation between fat thickness, seasoning losses, and salt content [3,46]. Nonetheless, despite being undesired due to health concerns, a high salt content in cured hams affects the product flavour, chemical, and biochemical processes, such as proteolysis, lipolysis, and lipid oxidation [3,44,46]. Thus, a greater thickness of the fat covering in this area is desired and the trait is included in the selection index of the Goland C21 sire line [13].

Overall, the OA treatment was inefficient, from both a nutritional and economic point of view. The decreased ADG and the increased time required to reach the target slaughter weight resulted in a marked reduction of feed efficiency with little benefits in terms of improved carcass and ham characteristics.

4.5. Sex and Treatment × Sex Interaction

Sex influenced growth performance and carcass characteristics. The effects of sex agreed with previous studies, as the barrows consumed more feed, were less efficient, their carcass was fatter, and their hams were characterized by greater marbling than gilts [2,46,47]. In our previous studies conducted on the same pig genetic line, kept under a restricted feeding regime, some differences due to the sex were observed but they were of a small magnitude [14]. With pigs kept on a voluntary feeding regime, we did expect greater differences between sexes, because of a better possibility to exploit the genetic propensity for the growth of various body components. Gilts had a carcass yield >81% only in the GW group, while barrows had a carcass yield >81% in all the *ad libitum* treatments YA and GW. This reflects a greater propensity for fat accretion at earlier ages of barrows compared to gilts. However, in the current study, the magnitude of the differences induced by the treatment × sex interaction was small, and the statistical level of significance was rarely reached. This result suggests that the gilts and barrows of the Goland C21 pig genotype have similar responses when exposed to the different treatments of the current study.

5. Conclusions

Despite the positive effect of the OA strategy on visible marbling and subcutaneous fat depth proximal to the *semimembranosus* muscle, this strategy was found to be inefficient as it impairs growth and feed efficiency and increases the production costs, with little influence on carcass composition, and with a reduction in ham size compared to the conventional practice.

The best rearing strategy, from an economic point of view, would be the YA strategy, as it permits anticipation of the slaughter by about 27 days earlier, with the highest improvements in ADG, feed efficiency, and ham adiposity. However, the use of this strategy should be applied with caution, as more research is required to clarify whether increased ham adiposity can compensate for the negative effects of younger slaughter age on dry-curing aptitude.

The GW strategy was associated with increased feed consumption, ADG, carcass, and ham weight, with an improvement in some ham quality indices compared to C. Due to its feed efficiency and competitive feeding costs, the GW strategy could be used in place of the conventional practice. However, the adoption of this strategy would be associated with the risk of an increased proportion of carcasses that weigh more than the maximum threshold indicated by the product specifications (168 kg). In such a case, to avoid a depreciation of the value of the carcass, mild feed restriction should be applied depending on the pigs' growth potential.

Supplementary Materials:

The following are available online at https://www.mdpi.com/article/10.3390/ani11082447/s1, Table S1: Growth performance of gilts and barrows raised according to the traditional rearing system (Control, C), and three alternative strategies (Age-, Weight+ and Age+) (Younger Age, YA; Greater Weight, GW; and Older Age, OA) [a], Table S2: Carcass traits of gilts and barrows raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA) [a], Table S3: Green ham characteristics of gilts and barrows raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA) [a], Figure S1: Influence of the sex × rearing strategy interaction (least square means ± standard deviation; sex × rearing Scheme 0. on cumulative feed consumption of heavy pigs raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA). [C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA (>9 months) at 160 ± 16 kg SW; n = 325], Figure S2: Influence of the sex × rearing strategy interaction (least-square means ± standard deviation; sex × rearing Scheme 0. on cumulative feed consumption of heavy pigs raised according to the traditional rearing system (Control, C), and three alternative strategies (Younger Age, YA; Greater Weight, GW; and Older Age, OA). [C system: 160 ± 16 kg slaughter weight (SW) and 9 months slaughter age (SA); YA = minimum SA at 160 ± 16 kg SW; GW = maximum SW at 9 months SA, and OA = increased SA (>9 months) at 160 ± 16 kg SW; n = 325].

Author Contributions:

Conceptualization, L.G., S.S., and P.C.; Methodology, L.G., S.S., G.C., and I.H.M.; Validation, V.H. and G.C.; Formal Analysis, I.H.M., S.S., and V.H.; Investigation, L.G., S.S., and P.C.; Resources, L.G., S.S., and P.C.; Data Curation, I.H.M., C.P.S., and G.C.; Writing—Original Draft Preparation, S.S., I.H.M., and V.H.; Writing—Review and Editing, I.H.M., L.G., V.H., V.B., G.C., C.P.S., P.C., and S.S.; Visualization, S.S. and I.H.M.; Supervision, S.S. and V.H.; Project Administration, P.C. and L.G.; Funding Acquisition, P.C., S.S., and L.G. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by the European Union Rural Development program 2014–2020, Reg. (CE) 1305/2013—PSR Veneto DGR n. 2175—December 23, 2016, interventions 16.1.1 and 16.2.2, code 3682902. The University of Padua also funded with Institutional funds (DOR2059255/20, DOR1990028/19, DOR1845849/18) and with a three-year PhD grant for Isaac Malgwi (Progetto di Eccellenza CASA).

Institutional Review Board Statement:

The experimental procedure was approved by the institutional animal care committee of the University of Padova. All procedures were conducted in compliance with European Union requirements and guidelines on the protection of animals used for scientific and educational purposes provided by the *"Organismo preposto per il Benessere Animale, OPBA"*, University of Padova (OPBA, approval document #36/2018).

Data Availability Statement:

The data supporting the findings of this study are available from Gorzagri s.s., but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available. Data are however available from the authors upon reasonable request and with permission of Gorzagri s.s.

Acknowledgements:

This research was supported by the University of Padova. The authors are indebted to the Progeo Feed industry for feed preparation and sampling, and Gorzagri s.s. for the provision of animals and technical support. The authors would like to thank Luca Carraro, Nadia Guzzo, Alberto Simonetto and all the herdsmen for their support in animal management and data recording. The authors also wish to thank Dott. Alessandro Ferretti for his help in managing the slaughtering operations at the slaughterhouse. Thanks also to dott. Giuseppe Tonin and dott. Massimo Pasqualotto for the evaluations carried out on the dressed hams the day after slaughtering.

Conflicts of Interest:

The authors confirm that there is no conflict of interest associated with the publication of this manuscript. The funding sources were not involved in the study design, collection, analysis, and interpretation of the data and the writing of the paper.

References

1. Wu, F.; Vierck, K.R.; DeRouchey, J.M.; O'Quinn, T.G.; Tokach, M.D.; Goodband, R.D.; Dritz, S.S.; Woodworth, J.C. A review of heavyweight market pigs: Status of knowledge and future needs assessment. Transl. Anim. Sci. 2017, 1, 1-15. doi:10.2527/tas2016.0004.
2. Latorre, M.A.; Lázaro, R.; Valencia, D.G.; Medel, P.; Mateos, G.G. The effects of gender and slaughter weight on the growth performance, carcass traits, and meat quality characteristics of heavy pigs. *J. Anim. Sci.* **2004**, *82*, 526-533. doi:10.2527/2004.822526x.
3. Čandek-Potokar, M.; Škrlep, M. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal* **2012**, *6*, 327-338. https://doi.org/10.1017/S1751731111001625.
4. Santos J. S., (2012). Production systems and sustainable management of pigs in the Mediterranean region. *Options Méditerranéennes. Séries A. Mediterranean Seminars*, *101*, 99–107. http://om.ciheam.org/om/pdf/a101/00006663.pdf
5. EEC. 1992. Prosciutto di Parma. Protected Designation of Origin. General Rules, Dossier Pursuant to Article 4 of Council Regulation (EEC) No. 2081/92 of July, 14th 1992. *Off J Eur Communities.* L208:9–14.
6. Gallo, L.; Dalla Bona, M.; Cecchinato, A.; Schiavon, S. Effect of growth rate on live performance, carcass and green thigh traits of finishing Italian heavy pigs. *Ital. J. Anim. Sci*. **2017**, *16*, 652–658, doi:10.1080/1828051X.2017.1318037.
7. Gallo, L.; Dalla Bona, M.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Effect of progressive reduction in crude protein and lysine of heavy pigs diets on some technological properties of green hams destined for PDO dry-cured ham production. *Meat Sci.* **2016**, *121*, 135-140. doi:10.1016/j.meatsci.2016.06.005.
8. Lo Fiego, D.P.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107-114. doi:10.1016/j.meatsci.2004.06.010.
9. Čandek-Potokar, M.; Žlender, B.; Lefaucheur, L.; Bonneau, M. Effects of age and/or weight at slaughter on *longissimus dorsi* muscle: Biochemical traits and sensory quality in pigs. *Meat Sci.* **1998**, *48*, 287-300. doi:10.1016/S0309-1740(97)00109-5.
10. Kyriazakis, I.; Whittemore, C.T. Whittemore's science and practice of pig production. Kyriazakis I., Whittemore, C.T., Blackwell Publishing, Oxford, UK; **2006**. pp. 417. https://doi.org/10.1002/9780470995624.ch13.
11. Lebret, B.; Juin, H.; Noblet, J.; Bonneau, M. The effects of two methods of increasing age at slaughter on carcass and muscle traits and meat sensory quality in pigs. *Anim. Sci.* **2001**, *72*, 87-94. doi:10.1017/S1357729800055582.
12. Rostellato, R.; Sartori, C.; Bonfatti, V.; Chiarot, G.; Carnier, P. Direct and social genetic effects on body weight at 270 days and carcass and ham quality traits in heavy pigs. *J. Anim. Sci.* **2015**, *93*, 1-10. doi:10.2527/jas.2014-8246.
13. Bonfatti, V.; Carnier, P. Prediction of dry-cured ham weight loss and prospects of use in a pig breeding program. *Animal* **2020**, *14*, 1128-1138. doi:10.1017/S1751731120000026.
14. Gallo, L.; Dalla Montà, G.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Growth performance of heavy pigs fed restrictively diets with decreasing crude protein and indispensable amino acids content. *Livest. Sci*. **2014**, *161*, 130-138. doi:10.1016/j.livsci.2013.12.027.
15. Schiavon, S.; Carraro, L.; Dalla Bona, M.; Cesaro, G.; Carnier, P.; Tagliapietra, F.; Sturaro, E.; Galassi, G.; Malagutti, L.; Trevisi, E.; et al. Growth performance, and carcass and raw ham quality of crossbred heavy pigs from four genetic groups fed low protein diets for dry-cured ham production. *Anim. Feed Sci. Technol.* **2015**, *208*, 170–181, doi:10.1016/j.anifeedsci.2015.07.009.
16. Schiavon, S.; Gallo, L.; Carnier, P.; Tagliapietra, F.; Ceolin, C.; Prandini, A.; Piva, A. Use of simple body measurements and allometry to predict the chemical growth and feed intake in pigs. *Ital. J. Anim. Sci.* **2007**, *6*, 27–44. https://doi.org/10.4081/ijas.2007.27.
17. AOAC. Official Methods of Analysis of the Association of Official Agricultural Chemists, 19th ed. **2012**; AOAC International, Gaithersburg, MD, USA.
18. Bouchard, J.; Chornet, E.; Overend, R.P. High-performance liquid chromatographic monitoring of carbohydrate fractions in partially hydrolyzed corn starch. *J. Agric. Food Chem.* **1988**, *36,* 1182-

1192. doi:10.1021/jf00084a016.
19. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* **1991**, *74*, 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2.
20. NRC. Nutrient Requirements of Swine, 11th ed.; National Academy Press: Washington, WA, USA, 2012; p. 278????.
21. Gallo, L.; Dalla Montà, G.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Carcass quality and uniformity of heavy pigs fed restrictive diets with progressive reductions in crude protein and indispensable amino acids. *Livest. Sci*. **2015,** *172*, 50–58. doi:10.1016/j.livsci.2014.11.014.
22. Schiavon, S.; Bona, M.D.; Carcò, G.; Carraro, L.; Bunger, L.; Gallo, L. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. *PLoS One* **2018**, *13*, 1–17. doi:10.1371/journal.pone.0195645.
23. EU, 2014a. Commission Implementing Decision of 24 January 2014 authorising methods for grading pig carcases in Italy [notified under document C (2014) 279]. O*ff. J.* L 23.
24. EU, 2014b. Corrigendum to Commission Implementing Decision 2014/38/EU of 24 January 2014 authorising methods for grading pig carcases in Italy (*Off. J.* L 23, 28.1.2014). *Off. J.* L 54.
25. Magistrelli, D.; Galassi, G.; Crovetto, G.M.; Rosi, F. Influenza della somministrazione di elevate quantità di polpe di bietola essiccate su parametri endocrino/metabolici, rilievi al macello e qualità del prosciutto nel suino pesante italiano. *Ital. J. Anim. Sci.* **2009**, *8*, 37–49. doi:10.4081/ijas.2009.37.
26. NPPC Pork composition and quality assessment procedures. In: Berg, E.P., ed.; National Pork Producers Council, Des Moines, Iowa, USA, **2000**; pp. 1-14.
27. Bonfatti V.; Rostellato R.; Carnier P. Estimation of additive and dominance genetic effects on body weight, carcass and ham quality in heavy pigs. *Animals,* **2021**, *11,* 481-499. https://doi.org/10.3390/ani11020481
28. Serrano, M.P.; Valencia, D.G.; Fuentetaja, A.; Lázaro, R.; Mateos, G.G. Influence of feed restriction and sex on growth performance and carcass and meat quality of Iberian pigs reared indoors. *J. Anim. Sci.* **2009**, *87*, 1676–1685. doi:10.2527/jas.2008-0989.
29. Pagliarini E, Laureati M, Dinnella C, Monteleone E, Proserpio C, Piasentier E, Influence of pig genetic type on sensory properties and consumer acceptance of Parma, San Daniele and Toscano dry-cured hams. *J. Sci. Food Agric.* **2016,** *96,* 798-806.
30. Gou, P.; Guerrero, L.; Arnau, J. Sex and Crossbreed Effects on the Characteristics of Dry-Cured Ham. *Meat Sci.* **1995**, *40*, 21–31, doi:10.1016/0309-1740(94)00021-X.
31. Peloso, J. V.; Lopes, P.S.; Gomide, L.A.M.; Guimarães, S.E.F.; Carneiro, P.L.S. Carcass and ham quality characteristics of heavy pigs from different genetic groups intended for the production of dry-cured hams. *Meat Sci*. **2010**, *86*, 371–376, doi:10.1016/j.meatsci.2010.05.017.
32. Carcò, G.; Dalla Bona, M.; Carraro, L.; Latorre, M.A.; Fondevila, M.; Gallo, L.; Schiavon, S. Influence of mild feed restriction and mild reduction in dietary amino acid content on feeding behaviour of group-housed growing pigs. *Appl. Anim. Behav. Sci.* **2018**, *198*, 27-35 doi:10.1016/j.applanim.2017.09.020.
33. Knap, P.W. Voluntary feed intake and pig breeding. In: *Voluntary Feed Intake in Pigs*, Torrallardona, D., Roura, E., eds.; Wageningen Academic Publishers, Wageningen, The Netherlands, 2009; pp. 13–35.
34. Fabian, J.; Chiba, L.I.; Kuhlers, D.L.; Frobish, L.T.; Nadarajah, K.; McElhenney, W.H. Growth performance, dry matter and nitrogen digestibilities, serum profile, and carcass and meat quality of pigs with distinct genotypes. *J. Anim. Sci.* **2003**, *81*, 1142-1149. doi:10.2527/2003.8151142x.
35. Toldrá F. Dry-cured meat products. Food and Nutrition press, Inc. Trimbull, Connecticut, USA. **2002**. pp. 54-59.
36. Virgili, R.; Degni, M.; Schivazappa, C.; Faeti, V.; Poletti, E.; Marchetto, G.; Pacchioli, M.T.; Mordenti, A. Effect of age at slaughter on carcass traits and meat quality of Italian heavy pigs. *J. Anim. Sci.* **2003**, *81*, 2448-2456. doi:10.2527/2003.81102448x.
37. Latorre, M.A.; García-Belenguer, E.; Ariño, L. The effects of sex and slaughter weight on growth performance and carcass traits of pigs intended for dry-cured ham from Teruel (Spain). *J. Anim. Sci*. **2008**, *86*, 1933−1942. doi:10.2527/jas.2007-0764.

38. Lowell, J.E.; Schunke, E.D.; Harsh, B.N.; Bryan, E.E.; Stahl, C.A.; Dilger, A.C.; Boler, D.D. Growth performance, carcass characteristics, fresh belly quality, and commercial bacon slicing yields of growing-finishing pigs from sire lines intended for different industry applications. *Meat Sci*. **2019**, *154*, 96–108, doi:10.1016/j.meatsci.2019.04.010.
39. Laureati, M.; Buratti, S.; Giovanelli, G.; Corazzin, M.; Lo Fiego, D.P.; Pagliarini, E. Characterization and differentiation of Italian Parma, San Daniele and Toscano dry-cured hams: A multi-disciplinary approach. *Meat Sci*. **2014**, *96*, 288-294. doi:10.1016/j.meatsci.2013.07.014.
40. Wood, J.D.; Lambe, N.R.; Walling, G.A.; Whitney, H.; Jagger, S.; Fullarton, P.J.; Bayntun, J.; Hallett, K.; Bünger, L. Effects of low protein diets on pigs with a lean genotype. 1. Carcass composition measured by dissection and muscle fatty acid composition. *Meat Sci.* **2013**, *95*, 123-128. doi:10.1016/j.meatsci.2013.03.001.
41. Carcò, G.; Schiavon, S.; Casiraghi, E.; Grassi, S.; Sturaro, E.; Dalla Bona, M.; Novelli, E.; Gallo, L. Influence of dietary protein content on the chemico-physical profile of dry-cured hams produced by pigs of two breeds. *Sci. Rep.* 2019, *9*, 19068. https://doi.org/10.1038/s41598-019-55760-0.
42. Grassi, S.; Casiraghi, E.; Benedetti, S.; Alamprese, C. Effect of low-protein diets in heavy pigs on dry-cured ham quality characteristics. *Meat Sci.* **2017**, *131*, 152–157. https://doi.org/10.1016/j.meatsci.2017.05.015
43. Cisneros, F.; Ellis, M.; Baker, D.H.; Easter, R.A.; McKeith, F.K. The influence of short-term feeding of amino acid-deficient diets and high dietary leucine levels on the intramuscular fat content of pig muscle. *Anim. Sci*. **1996**, *63*, 517-522. doi:10.1017/S1357729800015411.
44. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* **2004**, *3*, 309-321. doi:10.4081/ijas.2004.309.
45. Rodríguez-Sánchez, J.A.; Calvo, S.; Suárez-Beloch, J.; Latorre, M.A. Effect of pig slaughter weight on chemical and sensory characteristics of teruel dry-cured ham. *Ital. J. Food Sci.* **2014**. *26*, 420-426.
46. Tomažin, U.; Škrlep, M.; Prevolnik Povše, M.; Batorek Lukač, N.; Karolyi, D.; Červek, M.; Čandek-Potokar, M. The effect of salting time and sex on chemical and textural properties of dry cured ham. *Meat Sci.* **2020**, *161*, 107990. doi:10.1016/j.meatsci.2019.107990.
47. Latorre, M.A.; Olivares, A.; Callejo, A.; Rey, A.I.; Pérez-Ciria, L.; López Bote, C.J.; Daza, A. A comparison of female and castrate pigs slaughtered at weights above and below 120 kg on carcass traits, intramuscular fat and fatty acid composition of carcasses intended for dry-cured ham and shoulder production. *Anim. Prod. Sci.* **2019**, *59*, 1923–1930. doi:10.1071/AN18267.

Chapter 2

Influence of Slaughter Weight and Sex on Growth Performance, Carcass Characteristics and Ham Traits of Heavy Pigs Fed Ad-Libitum

I. H. Malgwi [1,*], D. Giannuzzi [1,*], L. Gallo [1], V. Halas [2], P. Carnier [3] and S. Schiavon [1]

[1] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell' Università 16, 35020 Legnaro (PD), Italy

[2] Department of Farm Animal Nutrition, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences, Guba Sándor Utca 40, 7400 Kaposvár, Hungary

[3] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Agripolis, Viale dell'Università 16, Legnaro, 35020 Padova, Italy

Published in: *Animals 2022, 12(2), 215;* https://doi.org/10.3390/ani12020215

Influence of Slaughter Weight and Sex on Growth Performance, Carcass Characteristics and Ham Traits of Heavy Pigs Fed Ad-Libitum

I. H. Malgwi [1,*], D. Giannuzzi [1,*], L. Gallo [1], V. Halas [2], P. Carnier [3] and S. Schiavon [1]

Simple Summary:

In recent years, pigs involved in the dry-cured ham production system have suffered from excessive leanness. This has led to the increase of slaughter weight (SW) to achieve greater carcass and ham fatness statuses to compensate for the loss in dry-curing aptitude. The production guidelines to produce Italian dry-cured ham are currently under revision and an extension of the range of carcass weights from 126 to 168 kg, corresponding to about 146 to 210 kg of SW, has been proposed. However, little is known about the influence of SW in the range of 140–200 kg on growth performance, feed efficiency, carcass quality and ham curing aptitude. We hypothesized that an increased SW could exert a positive influence on ham characteristics. Data from 159 pigs fed ad libitum with diets, un-limiting for nutrient contents, up to 8 or 9 months of age (140–200 kg SW) were used. Greater SWs were linearly and positively associated with the growth performance of the pigs and with better ham quality traits. Greater SW increased ham weight, muscularity, and greater fat covering, according to the dry-cured ham industry's expectations. Barrows produced hams with greater weight and marbling than gilts.

Abstract:

Slaughter weight (SW) is critical for dry-cured ham production systems with heavy pigs. A total of 159 C21 Goland pigs (gilts and barrows) at 95 ± 9.0 kg body weight (BW) from three batches were used to investigate the impact of ad libitum feeding on SW, growth performance, feed efficiency, and carcass and green ham characteristics. Diets contained 10 MJ/kg of net energy and 7.4 and 6.0 g/kg of SID-lysine. Slaughter weight classes (SWC) included <165, 165–180, 180–110 and >210 kg BW. In each batch, pigs were sacrificed at 230 or 258 d of age. Left hams were scored for round shape, fat cover thickness, marbling, lean colour, bicolour and veining. Data were analyzed with a model considering SWC, sex and SWC × Sex interactions as fixed factors and the batch as a random factor. The linear, quadratic and cubic effects of SWC were tested, but only linear effects were found. Results showed that pigs with greater SWC had greater average daily gain and feed consumption, with similar feed efficiency and better ham quality traits: greater ham weight, muscularity, and fat covering in correspondence of *semimembranosus* muscle. Barrows were heavier and produced hams with slightly better characteristics than gilts.

Keywords: pigs; slaughter weight; ham quality; feed efficiency; carcass quality; sex

1. Introduction

The major limitation of an increase in pig slaughter weights (SWs) is an increase in carcass adiposity and the worsening of feed efficiency with increasing SW [1]. However, in the last few decades, genetic improvements have determined a strong increase in feed efficiency and the production of very lean carcasses with a limited amount of fat. As a consequence, a progressive increase in pig SW has been observed in many countries [1]. Cisneros et al. [2] have indicated that modern, high-lean-gain genotypes have the potential to be slaughtered at heavier weights with less effect on carcass merit and (or) feed conversion efficiency compared with low-lean-gain genotypes. Indeed, they concluded that modern genotypes can be slaughtered at live weights up to 160 kg with limited impact on growth performance, commercial meat yields or meat quality characteristics [2].

For dry-cured ham production, adequate fat covering and marbling are required, so the pigs must be slaughtered at heavy weights, often greater than 130 kg [3,4]. In these production conditions, an increase in SW is considered a potential strategy to compensate for the increased leanness of modern pig genotypes [5]. In the Italian dry-cured ham production circuits, an SW of 160 ± 16 kg and a minimum age of nine months are indicated by the official production guidelines [6]. To comply with these prescriptions with modern pig genotypes, restricted feeding is required [7]. However, this is an

inefficient strategy, and a progressive increase in SW has also been observed in this production system INEQ [8]. Therefore, a revision of these guidelines is required and an extension of the range of carcass weights from 120 to 168 kg, corresponding to about 146 to 210 kg of SW, has been recently proposed to the authorities.

The extension of the admitted SW range implies the possibility of adopting an ad libitum feeding strategy that would better exploit the genetic potential of individual pigs for growth—although with a reduction of body and carcass uniformity among pigs of the same batch [9,10]. There are not many studies that have considered the influence of increased SW on growth performance, feed efficiency, and carcass and ham characteristics in such body weight (BW) intervals. In addition, previous studies that have considered the effect of SW [3,11,12] have shown confounded effects between SW and age at slaughter, and only sporadically have the two effects been separately evaluated, highlighting their diverse implications [13].

Assuming that pigs selected for dry-cured ham production are slaughtered at about the same age, it can be hypothesized that those heavier at slaughter would be those with greater feed consumption, growth rate and carcass and ham weight, but also with greater carcass adiposity, ham marbling and ham fat covering. In addition, some loss in feed efficiency may occur, increasing the SW [1]. However, such responses would depend on the propension of the pig genotype for lean and fat deposition at heavy weights.

This paper aimed to study, in groups of pigs fed ad libitum and selected for dry-cured ham production, the relationships between SW, growth performance, feed efficiency, and carcass and green ham characteristics.

2. Materials and Methods

2.1. Pig Housing, Rearing and Slaughtering

The data used in this research originated from a previous experiment that involved 336 pigs, from three batches of 112 pigs each [5]. Briefly, the pigs of Malgwi et al. [5] were divided into four experimental groups. The study, arranged as a split-plot design with sex within a pen, included four (4) feeding groups representing four (4) alternative rearing strategies. Only the two groups of pigs (for a total of 168 individuals) fed ad libitum high protein diets, not limiting for the indispensable amino acid content, were used for the purposes of current research. Such non-limiting conditions were applied to exploit the pigs' potential for protein and lipid deposition [14,15]. Among these two groups, the first represented a rearing strategy aimed at reaching 170 kg SW at the minimum possible age, which was in the order of 8 months (younger age, YA). The second represented a strategy aimed at reaching the maximum SW (>170 kg) at nine months' slaughter age (greater weight, GW). Pigs of this group were fed ad libitum the same high protein feeds of the YA group, and at slaughter, the pigs were about 190 kg SW. During the test, nine animals were moved to the infirmary and excluded for health problems, for a final number of 159 individuals.

Pigs were members of 68 full-sib families of the C21 Goland boar line (Gorzagri, Fonzaso, Italy), generated by mating 13 boars to 67 sows. Besides growth and residual feed efficiency, the breeding goal of the C21 Goland line includes traits related to the quality of raw ham [16] and its suitability for dry-curing [17]. All pigs were born in the same week, they were reared on the same farm and fed the same commercial diets until their transfer to the experimental station at 95 ± 9.0 kg BW. The pigs were housed in pens in groups of 14 pigs, with barrows and gilts mixed in equal proportion in the same pen. An across-batch rotation scheme was used to assign each treatment group to a given pen in different batches. Each pen (5.8 × 3.8 m, fully slatted floors) was equipped with a single-space electronic feeder (Compident Pig–MLP, Schauer Agrotronic, Prambachkirchen, Austria). The feeding station recorded, daily and on an individual basis, feed intake and other behaviour traits [18].

2.2. Diets and Feeding

In early (90 to 120 kg BW) and late (120 kg BW upwards) finishing periods, the pigs received cereal–soybean meal-based diets (Table 1). The feeds were formulated to contain 10 MJ/kg of net energy without limiting the indispensable amino acid content, with 7.4 and 6.0 g/kg of SID-lysine considered the first limiting amino acid [19]. Feeds were manufactured by the Progeo Feed Industry. Water was accessed freely from nipple drinkers within each pen. The major details of the nutritional characteristics of the feeds are given in [5].

Table 1. Ingredient composition (g/kg) of the high protein feeds used in early (90 to 120 kg BW) and late (>120 kg BW) finishing.

Ingredient	Early Finishing (90 to 120 kg Body Weight)	Late Finishing (120 kg Body Weight Upwards)
Corn grain	361.8	398.9
Wheat grain	240.0	238.0
Barley grain	100.0	100.0
Soybean meal 48% (solv. ex.)	196.0	143.0
Wheat bran	26.5	7.5
Wheat middlings	-	40.0
Cane molasses	20.0	22.5
Lard	20.0	20.0
Dried sugar beet pulp	-	-
Calcium carbonate	15.0	13.0
Dicalcium phosphate	4.5	2.0
Sodium chloride	3.0	3.0
Sodium bicarbonate	2.5	2.5
Vitamin and mineral premix[a]	2.0	2.0
Grapeseed meal	7.0	7.0
Choline, liquid, 75%[b]	0.5	0.3
L-Lysine[c]	1.0	0.3
DL-Methionine[d]	0.2	0.1
L-Thryptophan, 49%[e]	-	-

[a] Providing per kilogram of feed: vitamin A, 8000 IU; vitamin D3, 1200 IU; vitamin E, 8 mg; Vitamin B7, 0.08 mg; vitamin B12, 0.012 mg; niacin, 16.0 mg; biotin, 8 mg; iron, 170 mg; zinc, 117 mg; copper, 14 mg; cobalt, 0.11 mg; iodine, 0.06 mg; manganese, 65 mg; magnesium, 0.14 mg; selenium 10 mg.
[b] Choline liquid 75% (Methodo Chemicals, 42017 Novellara, RE, Italy).
[c] L-Lysine Monoclohydrate, 98.5% pure, 78% L-Lysine (Methodo Chemicals, 42017 Novellara, RE, Italy).
[d] DL-Methionine, 98% pure min. (Methodo Chemicals, 42017 Novellara, RE, Italy); [e] L-tryptophane, 50% L-Tryptophane (Methodo Chemicals, 42017 Novellara, RE, Italy).

At the start of the experiment, and the day before slaughtering, the pigs were weighed with a scale. The pigs of the YA and GW groups were reared in the same way, but they were slaughtered at different ages. These two groups had homoscedastic variances and ample variations in SW.

2.3. Slaughter and Evaluation of Carcass and Green Ham Traits

The pigs of the YA and GW groups were slaughtered, on average, after 85 or 116 days on feed—corresponding to almost 8 or 9 months of age. An extra month of feeding would increase the SW, the daily and cumulated feed consumption, the carcass and the ham fat covering, the ham size, and would reduce the average daily gain and feed efficiency. Slaughter and carcass dressing were carried out as described in [20].

Hot carcass weight was recorded online, and the lean percentage was estimated by image analysis of the left carcass side (CSB-Image-Meter, CSB-System AG, Geilenkirchen, Germany), as guided by [21,22]. Carcass weight was measured as the head-on weight, as is currently practised in Italy and Canada [23]. Loin with ribs, shoulder, thigh, lard and belly were weighed about 1 to 3 h after slaughter using an electronic scale. Green hams were chilled (0–2 °C) for 24 h, trimmed and weighed again. A trained operator scored all left hams as described in [24] for round shape (0 = low to 4 = high, optimum: 1 to 2), fat cover thickness (−4 = very thin to 4 = very thick, optimum: 0 to 1), marbling (0 = absent to 4 = very evident, optimum: 1), lean colour (−4 = very pale to 4 = very dark, optimum = 0), bicolor (0 = absent to 4 = very evident, optimum = 0) and veining (0 = absent to 4 = very evident, optimum = 0). A reference standard was used at the beginning of each of nine scoring sessions. The scoring sessions were performed by placing the hams on a table with a white plastic surface, all placed in the same room illuminated with artificial lamps. To limit the influence of personal subjective factors, a single operator with decades of experience in scoring ham for the genetic improvement of the Goland C21 pig line was involved. Comparable scoring grids for these traits have also been reported by others [25–27].

The subcutaneous fat depth of the green ham was measured in the proximity of the muscles *biceps femoris* (P1) and *semimembranosus* (P2) using a ruler or a portable ultrasound system (Aloka SSD 500 equipped with UST-5512 7.5 MHz linear transducer probe, Hitachi Medical Systems S.p.A., Milan, Italy), respectively. Hams were moved to the ham factory within two days after the slaughter, where they were trimmed again and weighted. The hams were trimmed to obtain the typical shape of Veneto ham, without the trotter.

2.4. Statistical Analysis

According to current guidelines, 160 kg ± 10% is the average weight of the batch. Accordingly, data were grouped into four SW classes (SWC), with about 20 kg SW of difference between one class and the following one. The first SWC represented pigs with lighter SW (<165 kg SW), which is still in agreement with current guidelines. The second SWC (165−180 kg SW) were somewhat heavier pigs, with SWs similar to those frequently found in practice. The third SWC represented pigs with SWs (>180, <210 kg SW) in agreement with the proposal of the guideline revisions, and the fourth SWC (>210 kg) represented pigs that were too heavy and would be discarded if the new production guidelines proposal is approved.

Carcass and ham trait data were analyzed using a GLM procedure in SAS (SAS Inst. Inc., Cary, NC, USA) using the following linear model:

$$y_{ijkl} = \mu + SWC_i + Sex_j + (SWC \times Sex)_{ij} + Batch_k + e_{ijkl} \quad (2)$$

where y_{ijklm} was the observed trait, μ was the overall intercept of the model, SWC was the fixed effect of the i^{th} class of SW (i = 1, …, 4), Sex was the fixed effect of the j^{th} sex (j: 1 = gilts, 2 = barrows), (SWC × sex) was the interaction effect between the SWC class and sex, Batch was the random effect of the k^{th} batch (k = 1,…,3), and e_{ijkl} was the random residual.

The Batch and the residuals were assumed to be independently and normally distributed, with a mean of zero and a variance of $\sigma^2_{\ l}$ and $\sigma^2_{\ e}$, respectively. SWC, Sex, and SWC × sex interaction effects were tested in relation to the residual variance (individual). Three degrees of freedom of SWC were used to test the linear, quadratic, and cubic effects of increasing SWC. As the quadratic and the cubic components were never significant, the *p*-values of these components were omitted from the tables.

Allometric relationships ($y = ax^b$) relating carcass weight to SW, and lean and fat masses to carcass weight were fitted using a spreadsheet.

3. Results

3.1. Growth Performance and Main Carcass Characteristics

As expected, the lighter SWC were represented in greater proportion by YA pigs, and the heavier SWC by the GW pigs. The most frequent class was the third, followed by the second, the first and the fourth (Table 2). Pigs with the lightest BW at the beginning of the experiment were those that attained the lightest SW. Indeed, initial BW, feed intake and average daily gain increased with increasing SWC ($p < 0.001$), but feed efficiency (gain: feed) did not ($p = 0.53$).

Consistently, with increasing SWC carcass weight ($p < 0.001$), carcass yield ($p < 0.001$) and carcass backfat depth ($p < 0.001$) linearly increased, whereas the lean meat percentage decreased ($p < 0.001$). The allometric coefficient relating carcass weight to SW was greater than 1.00 (1.046), as the increase in SW was associated with a more than proportional increase in carcass weight (Figure 1).

The weights of lean ($p < 0.001$) and fat cuts ($p < 0.001$) increased with increasing SWC, while the carcass yield of lean cuts decreased ($p < 0.001$), and that of fat cuts increased ($p < 0.001$). The relationships of the lean and the fat cuts on carcass weights evidenced allometric coefficients lower than one (b = 0.855) and greater than one (b = 1.342), respectively (Figure 2).

Sex had little influence on feed intake, average daily gain, SW, carcass weight, carcass yield, carcass backfat depth and total and lean cut weight. The feed efficiency of the barrows was somewhat lower than that of the gilts ($p = 0.018$). However, the barrows had greater SW ($p = 0.027$), carcass yield ($p = 0.039$), fat cuts weight ($p = 0.043$), and lower lean cuts yield ($p = 0.026$). The Sex $\times$ SW interaction had negligible influence on growth performance and major carcass traits.

Table 2. Influence of sex, slaughter weight class (SWC) and sex × SWC interactions on heavy pig growth performance and main carcass characteristics.

	Class of Slaughter Weight (SWC), kg						Sex				Sex × SWC
Items	**<165**	**165 to 180**	**180 to 210**	**>210**	**SEM[1]**	***p-Linear*[2]**	**Gilts**	**Barrows**	**SEM[1]**	***p***	***p***
Pigs, *n*.	26	41	82	10	-	-	72	87	-	-	-
230 d-old pigs, *n*.	23	29	24	1	-	-	32	45	-	-	-
258 d-old pigs, *n*.	3	12	58	9	-	-	40	42	-	-	-
Average age at slaughter, d	235	238	249	262	-	-	246	244	-	-	-
Live performances:											
Initial body weight	86.0	95.4	97.7	105.0	3.4	<0.001	95.3	96.7	6.3	0.48	0.85
Slaughter weight (SW), kg	153.8	172.7	193.1	214.6	3.2	<0.001	182.9	184.3	5.7	0.027	0.94
Feed intake, g/d	2880	3130	3412	3835	130	<0.001	3287	3342	241	0.45	0.64
Average daily gain, g/d	821	874	959	1074	50	<0.001	944	920	96	0.42	0.18
Gain: feed	0.283	0.279	0.280	0.279	0.009	0.72	0.286	0.275	0.018	0.043	0.21
Post mortem performances:											
Carcass weight, kg	125.9	142.0	159.8	178.1	2.9	<0.001	150.3	152.6	5.4	0.16	0.97
Carcass yield, %	81.8	82.2	82.7	83.0	0.60	0.043	82.1	82.8	1.1	0.039	0.31
Carcass backfat depth[3], mm	36.2	40.6	46.3	50.4	2.3	<0.001	42.4	44.3	4.2	0.13	0.30
Lean meat g/kg	51.7	49.6	46.6	42.8	1.7	<0.001	48.0	47.4	3.1	0.53	0.023
Wholesale cuts weight, kg:											
Total cuts[4]	91.3	103.1	115.7	128.0	2.1	<0.001	108.9	110.2	3.8	0.25	0.73
Primal lean cuts	66.2	74.2	81.3	88.5	1.6	<0.001	77.7	77.5	3.0	0.84	0.62
Primal fat cuts	25.1	28.9	34.4	39.4	1.3	<0.001	31.2	32.7	2.5	0.043	0.89
Wholesale cuts yield, g/kg:											
Total cuts[4]	725	727	724	718	5.1	0.15	725	722	9.5	0.40	0.48
Lean cuts	526	523	509	497	7.6	<0.001	519	509	14.3	0.026	0.58
Fat cuts	199	204	215	221	7.3	0.001	206	213	13.7	0.08	0.64

[1] Standard error; Data were from 159 pigs: 72 gilts and 87 Barrows fed ad libitum, from 133.8 to 225.1 kg BW (*n* = 159); [2] As the quadratic and cubic components were never significant, the corresponding *p*-values were omitted;

[3] Average of two measures taken from the hot carcass at the points of minimum (lumbar) and maximum (shoulder) backfat depth;

[4] This measure corresponds to the sum of the weights of shoulders, hams, loins and ribs, belly and lard. Other minor cuts were not measured.

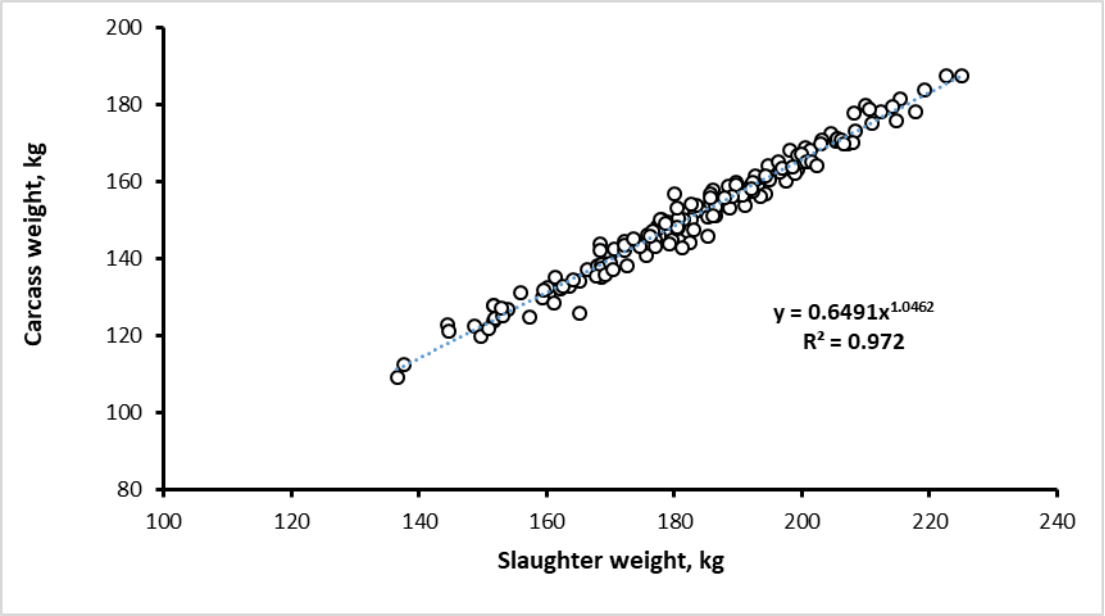

Figure 1. Allometric relationship between the slaughter and carcass weights of ad libitum-fed heavy pigs (*n* = 159).

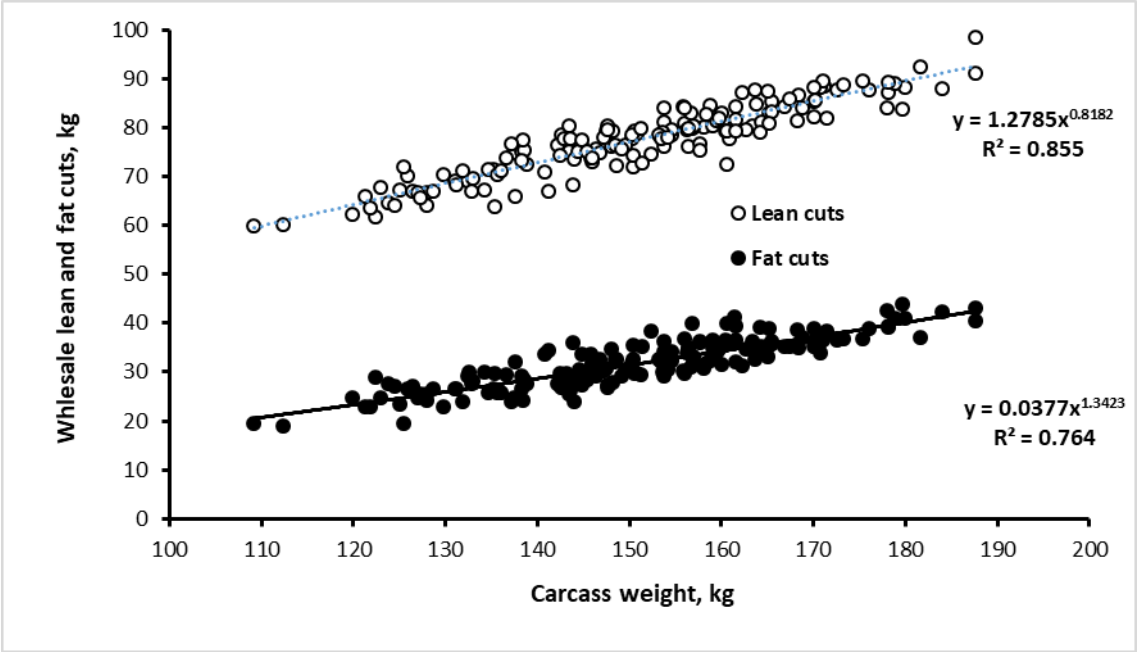

Figure 2. Allometric relationships between lean (shoulders, loins + ribs, and hams) fat (backfat and belly) cuts with carcass weight of ad libitum-fed heavy pigs (*n* = 159).

3.2. Wholesale Cuts Weights and Proportions

All the various wholesale cuts' weight increased linearly ($p < 0.001$) with increasing SWC (Table 3). However, the yields of all the various lean cuts, i.e., loins and ribs, shoulders, and green and trimmed hams, decreased ($p < 0001$), and those of the fat cuts, back fat and belly increased ($p < 0.001$). The trimming losses increased with increased SWC ($p < 0.001$), both in terms of weight and yield. The barrows had greater belly weight ($p < 0.001$) and yields ($p = 0.006$) than gilts, but lower yields of loins and ribs ($p = 0.003$).

Table 3. Influence of sex, slaughter weight and sex × SWC interactions on heavy pig commercial cut weights and yields.

	Class of Slaughter Weight (SWC), kg						Sex				Sex × SWC
Items	**<165**	**165 to 180**	**180 to 210**	**>210**	**SEM[1]**	***p-Linear*[2]**	**Gilts**	**Barrows**	**SEM[1]**	***p***	***p***
Wholesale cuts, kg:											
Loins and ribs	19.1	21.4	22.9	24.8	5.9	<0.001	22.4	21.8	1.1	0.06	0.98
Shoulders	16.7	18.6	20.5	22.3	0.5	<0.001	19.4	19.6	1.0	0.44	0.42
Green hams	30.5	34.1	37.9	41.4	0.8	<0.001	35.9	36.1	1.5	0.64	0.48
Trimmed hams[3]	24.8	27.8	30.4	32.9	0.7	<0.001	29.0	29.0	1.2	0.89	0.43
Trimming loss[3]	5.7	6.3	7.5	8.5	0.3	<0.001	6.9	7.1	0.6	0.37	0.93
Backfat	15.7	18.0	21.0	24.4	0.7	<0.001	19.7	19.9	1.3	0.66	0.94
Belly	9.4	10.9	13.4	15.0	0.8	<0.001	11.5	12.9	1.5	0.005	0.50
Wholesale Cut Yields, g/kg Carcass:											
Loins and ribs	152	151	143	140	3.7	<0.001	150	143	6.9	0.003	0.99
Shoulders	133	131	128	125	2.7	0.010	130	129	5.6	0.72	0.59
Green hams	242	240	237	232	3.5	0.003	239	237	6.5	0.16	0.26
Trimmed legs	197	196	190	184	3.3	<0.001	194	190	6.1	0.11	0.33
Ham trimming loss[3]	44.9	44.3	47.1	47.5	1.9	0.09	45.9	46.1	3.6	0.83	0.91
Backfat	124	127	131	137	3.8	<0.001	131	129	6.7	0.61	0.88
Belly	75	77	84	84	5.3	0.04	75.6	84.0	9.9	0.006	0.22

[1] Standard error; Data were from 159 pigs: 72 gilts and 87 Barrows fed ad libitum, from 133.8 to 225.1 kg BW.
[2] As the quadratic and cubic components were never significant, the corresponding *p*-values were omitted.
[3] Data are from ham trimming at the slaughterhouse (SH).

3.3. Green and Trimmed Ham Characteristics

The weights of trimmed ham at the slaughterhouse ($p < 0.001$), at the ham factory ($p < 0.001$), and the trimming losses at the ham factory ($p < 0.001$) linearly increased with increased SWC (Table 4). Pigs with greater SWC also had a more round shape ($p = 0.002$), and a greater subcutaneous fat depth in the P2 position ($p = 0.005$). However, the SWC class had little influence on other ham quality parameters.

Sex also had little influence on these ham characteristics, except for marbling and haemorrhage. Barrows had a lower hemorrhage score ($p = 0.037$) and greater marbling ($p = 0.011$) than gilts. The Sex × SWC interaction did not influence these ham traits.

Table 4. Influence of sex and slaughter weight on the characteristics of trimmed legs for dry-cured ham production.

	Class of Slaughter Weight (SWC), kg						Sex				Sex × SWC
Items	**<165**	**165 to 180**	**180 to 210**	**>210**	**SEM[1]**	***p-Linear*[2]**	**Gilts**	**Barrows**	**SEM[1]**	***p***	***p***
Trimmed ham, kg											
slaughter house (SH)	12.3	13.8	15.1	16.4	0.3	<0.001	14.4	14.4	0.6	0.96	0.44
ham factory (HF)[3]	11.9	13.2	14.4	15.7	0.3	<0.001	13.8	13.8	0.6	0.86	0.65
losses at the HF[3], kg	0.44	0.58	0.68	0.70	0.07	<0.001	0.58	0.62	0.14	0.33	0.21
losses at the HF[3], g/kg	35.6	42.0	44.5	43.2	0.49	0.11	40.1	42.5	0.93	0.41	0.39
Green ham quality traits:											
Round shape[4]	1.35	1.58	1.95	2.57	0.40	0.002	2.04	1.68	0.76	0.12	0.20
Veining[5]	1.73	1.64	1.43	1.12	0.41	0.12	1.40	1.57	0.76	0.45	0.95
Haemorrhage [6]	0.15	0.37	0.25	0.50	0.22	0.23	0.46	0.17	0.46	0.037	0.12
Visible marbling[7]	0.84	0.90	0.73	0.73	0.33	0.65	0.56	1.04	0.61	0.011	0.14
Meat colour[8]	−0.63	−0.69	−0.64	−0.56	0.61	0.88	−0.81	−0.44	1.14	0.29	0.28
Fat cover score [9]	0.20	0.41	1.09	1.20	0.69	0.09	0.71	0.74	1.28	0.94	0.52
Subcutaneous fat, mm:											
P1 position[10]	29.5	30.6	34.1	32.6	3.1	0.20	30.6	32.7	5.9	0.23	0.92
P2 position[11]	6.6	6.4	7.0	7.7	0.4	0.005	7.05	6.83	0.8	0.39	0.35

[1] Standard error; Data were from 159 pigs: 72 gilts and 87 Barrows fed ad libitum, from 133.8 to 225.1 kg BW.
[2] As the quadratic and cubic components were never significant, the corresponding *p*-values were omitted.
[3] At arrival at the ham factory (HF), the hams were trimmed again and weighted.
[4] Round shape (0 = very flat to 4 = very round; 1–2 optimum).
[5] Veining (0 = absent to 4 = evident, 0 = optimum).
[6] Haemorrhage (0 = absent 3 = evident, 0 = optimum).
[7] Visible marbling (0 = absent to 4 = every evident, 1–2 = optimum).
[8] Meat colour (−4 = pale to 4 = dark, 0 = optimum).
[9] Fat cover score (−4 = thin to 4 = thick).
[10] Ham subcutaneous fat depth measured at the point of minimum depth in the proximity of m. *biceps femoris* with a ruler.
[11] Ham subcutaneous fat depth measured in the proximity of m. *semimembranosus* with a portable ultrasound system (Aloka SSD 500 equipped with UST-5512 7.5 MHz linear transducer probe, Hitachi Medical Systems S.p.A., Milan, Italy).

4. Discussion

More than twenty years ago, Cisneros et al. [2] suggested that, for fresh meat production, lean pig genotypes can be slaughtered at live weights up to 160 kg with limited impact on growth performance, commercial meat yields or meat quality characteristics. These authors indicated that increases in SW were associated with increases in feed intake, backfat depth and loin eye area, with minimal changes in growth rate and gain: feed. However, for dry-cured ham production, pigs with greater adiposity compared to those intended for fresh meat consumption are required [1]. This kind of production is conducted according to a variety of systems and is influenced by different climatic environments, rearing and feeding practices, genetic resources, dry curing processes, and market demands and rules indicated by the disciplines of production [27]. In general, hams with insufficient fat covering are inadequate for the dry-curing process, as subcutaneous, intermuscular and intramuscular fat represents a barrier to salt penetration and water diffusion, so that leaner hams are expected to have higher salt contents and lower sensory quality [28]. On the contrary, high levels of fat infiltration were found to be related to softness and pastiness, due to water loss and salt penetration dynamics. Moreover, thick subcutaneous fat covering is undesirable to consumers [27].

The optimal SW and the degree of adiposity of the pigs for dry-cured ham production are strongly affected by the productive context. For instance, [3,29] concluded that an increase in SW up to 124 or 130 kg impairs growth performance and improves some aspects of carcass quality, with few benefits for the Teruel dry-cured ham industry. In Italy, the production guidelines established many decades ago indicate that pigs must be at least nine months old and have an SW of 160 ± 16 kg. Under such constraints, a restricted feeding practice is required with lean pig genotypes [10]. However, this results in low feed efficiencies, which are usually in the order of 0.28 ± 0.04 for pigs growing between 30 to 170 kg BW [24,30].

In recent times, such constraints have become progressively inadequate, and today over 15% of pigs at the age of 9 months are too lean for the needs of the ham industry [8]. Increased adiposity can be achieved in different ways—for example, with the use of pig genotypes with a high ability for fat deposition, with an increase in the dietary energy/protein ratio, the energy intake of the pigs and the SW [5,15]. The consortia for the protection of national dry-cured hams, under the domain of the Protected Denomination of Origin (PDO), proposed a revision of the guidelines permitting carcass weights in the range of 120–168 kg but still, the pigs must be nine months old at slaughter. The result of the current research raises the question of whether younger subjects with adequate fat covering could be suitable for high-quality dry-cured ham production [5]. In any case, it is expected that the production system will evolve towards an increase in SW.

4.1. Growth Performance and Feed Efficiency

In the current research, pigs were slaughtered at 230 d (7.7 months) and 258 d (8.6 months) of age, and the SW ranged from a minimum of 137 to a maximum of 225 kg. With increasing SWC, the frequency of pigs slaughtered at younger ages decreased, and that of pigs slaughtered at older ages increased. This partial confusion between age and SW was accepted, as it may become representative of the commercial conditions in the case of application of the innovative rearing strategies proposed in Malgwi et al [5]. Under current conditions, the age of slaughtered pigs is controlled by looking at the tattoo on the piglet's skin, applied within a week from birth. The tattoo reports only the month of birth so that piglets born towards the end of a month can be slaughtered at the beginning of the ninth month. In this way, the age at slaughter would be some days less than 270 d.

The results of the current experiment can be compared with others [10,31] achieved on heavy pigs fed restrictively in the same 90−170 kg BW interval. The pigs of these authors consumed on average 2.5−2.6 kg/d of feed, they grew on average 0.66−0.73 kg/d and the resulting gain: feed ratios were in the order of 0.253−0.284. The feed efficiency found by these authors was similar to that found in the current experiment, suggesting that there could be benefits from moving from a restricted to ad libitum feeding practice. This would result in pigs with greater SW and greater carcass and ham adiposity, without a loss of feed efficiency compared to conventional practice.

The first relevant finding of the current paper is that feed efficiency was not related to the increase in SW. This is in apparent contradiction with the literature, which reports that feed efficiency decreases with increasing physiological maturity [1]. These authors reviewed 25 studies involving pigs harvested at weights greater than 125 kg. They found that with increasing SW and age at slaughter, there was a linear decrease in feed efficiency (gain: feed). The magnitude of this change was −0.011 per 10 kg SW increase. [1] stated that the decrease in feed efficiency can be attributed to accelerated fat accretion, declining rates of water and protein deposition, and increased maintenance requirements

in heavy finishing pigs. In the current experiment, feed efficiency was not related to SW, because the heavier pigs were also those that attained greater feed intake, and a greater rate of growth. Pigs with greater SWs had greater energy and nutrient intake, so that a lower proportion of energy was partitioned towards the maintenance and a greater proportion toward the growth of the body's constituents. This result was consistent with the results of [7], where it was found that an increased growth rate was positively related to an increase in feed efficiency (gain: feed).

The pigs in the current research evidenced good potential for growth at heavy BWs, both for lean and fatty tissues. Besides growth and residual feed efficiency, the breeding goal of this line includes traits related to the quality of raw hams [16] and their suitability for dry-curing [17]. Considering the breeding goals and the results obtained here, it may be suggested that the pigs of this line have good aptitudes for lean gain over extended ranges of BW, but also fat accretion. However, it should be considered that positive or negative relationships between feed efficiency and SW could depend on the pig genotype, due to different energy partitioning among maintenance, protein and lipid accretion throughout growth.

4.2. Carcass Traits

The proposal of new guidelines for dry-cured ham production indicates that carcass weights must range between a minimum of 120 and a maximum of 168 kg. In our research, 10 out of 159 carcasses (6.2%) were heavier than the upper limit. This would suggest that, when fed ad libitum, some Goland C21 pigs would be heavier than the maximum indicated for dry-cured ham production. As an anticipation of the age at slaughter might be not permitted by the guidelines, this shortcoming would be resolved by introducing a mild feed restriction or practices of precision feeding, resulting in benefits in terms of uniformity (Figure 3) of the pigs at slaughter.

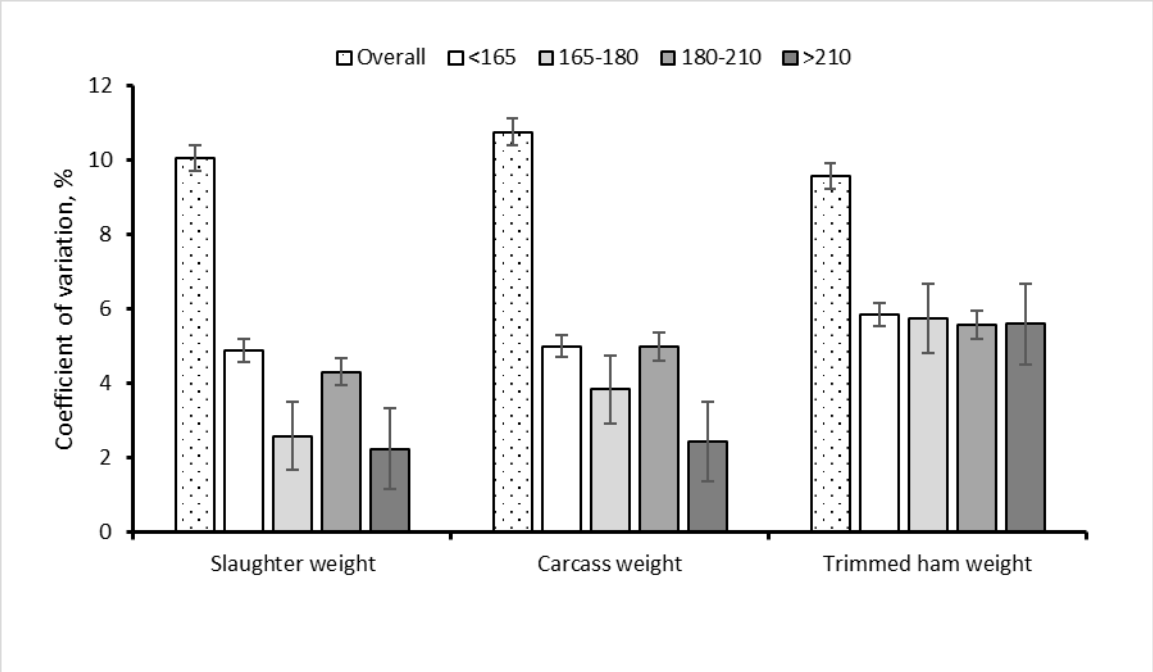

Figure 3. Coefficients of variation of slaughter weight, carcass weight and trimmed ham weight were computed for each class of slaughter weight (<165, 165–180, 180–210, >210 kg SBW) and overall.

In the current experiment, the coefficient of variation for carcass weight was 11%, whereas, in previous research, where pigs were kept on restricted feeding regimes, the coefficient of variation was in the order of 6−7% [10]. Herein, carcass yield ranged from 81.8 to 83.0%. These values are comparable with those frequently found in heavy pigs [32,33]. Several studies have found increases in carcass weight more than proportional to the increase in SW, resulting in increased carcass yield [31]. In the present research, carcass yield increased in the order of 0.20% per 10 kg increase in SW—a value lower, but comparable to that found by [1], who found an increase in carcass yield of an average of 0.40% per 10 kg of SW increase, but with an impressively large standard deviation (0.31%).

The correlation between carcass weight and carcass yield was appreciable and positive (r = 0.45; $p < 0.01$). Such an increase in carcass yield is due to the differential development of the carcass's fatty and lean tissues compared to the non-carcass parts [34]. In agreement with previous reports [13,34,35], the weights of the fatty cuts, namely those of back fat and belly, increased at a rate greater, while the weights of the lean tissues increased at a rate lower than that of the carcass weight. Due to the changes in the relative rate of fat and lean tissue accretion during the finishing period of the pigs, a substantial change in carcass composition occurred. The lean meat percentage, estimated from the CSB-system images, decreased linearly from 51.7 to 42.8%. The magnitude of this decrease was remarkable, as it averaged 1.47% for a 10 kg increase in carcass weight (r = 0.53; $p < 0.01$).

The guidelines for dry-cured ham production indicate that the lean meat percentage must range between 40 and 55% [6]. In the current dataset, it was found that despite the ad libitum feeding and the heavy SW, nine pigs (5.7%) still had a lean meat percentage >55%, being too lean for the needs of the ham industry. Five of these nine pigs were slaughtered at 230 d old (161 kg SLW, on average), and only four were slaughtered at 259 d of age (169 kg SW, on average). It was concluded that an increase in SW can be considered one of the most important ways to decrease the lean meat percentage.

4.3. Commercial Cuts

Information on the changes in the yields of primal cuts at different SWs is required for the analysis of pig production and the optimization of profits. The yields of the various cuts are difficult to compare with other research, because of the different dissection procedures at slaughter, different pig genotypes and different ranges of SW, according to market demand. However, it was observed that the yields of lean cuts in the current research were slightly lower than those of pigs slaughtered at the traditional 170 kg SW. In fact, in previous research, the yields of total lean cuts, shoulder and trimmed hams, averaged 521–630, 104–140 and 215–259 g/kg, respectively [10,24,36].

The lower yields of lean cuts were expected because of the heavier SW and the ad libitum feeding regime of the pigs in the current research compared to the traditional restrictively fed pigs. The weights and the yields of the fatty cuts increased with increasing SW, while the weights of the loins plus ribs, shoulder, and trimmed ham increased with increasing SW, but the corresponding yields decreased. The review of [1] suggests that the loin, shoulder, and ham yields decrease on average by 0.13, 0.16 and 0.17% per 10 kg of SW increase, while that of the belly increases by 0.32%. The magnitude of these trends in variation is comparable to that found for the pigs in the current research, where an increase of 10 kg of SW was associated with reductions of 0.218, 0.133, 0.164 and 0.223% of the loins plus ribs, shoulders, green hams, and trimmed hams yields, respectively.

4.4. Ham Traits

As expected, the weight of the ham, trimmed at the slaughterhouse or the ham factory, increased with increasing SW. The weight of the trimmed ham at the slaughterhouse ranged, on average, from 12.3 to 16.4 kg, within the 12.0−18.0 kg range indicated by the proposal of the new production guidelines. However, there were seven hams (4.4%) lighter than the minimum required to achieve the label. The weight of the trimmed ham was further reduced according to the additional trimming procedure conducted at the local ham factory.

The ham weight and size, together with the inter-and intramuscular fat content, the thickness of the subcutaneous fat and the lean meat content of the hind leg, represent the main factors that can also influence the aptitude of the ham to adsorb salt [37]. It is commonly assumed that heavier hams are characterized by better seasoning properties, because of lower seasoning losses [28]. However, previous experiments have found little or no correlation between ham weight and seasoning losses [4,38]. Thus, the greater seasoning aptitude of the heavier hams was attributed to the greater adiposity of the hams harvested from older and heavier pigs [27,28]. These authors suggested that the most relevant factor affecting seasoning losses is the fat thickness, which serves as a barrier to water evaporation during seasoning.

In the current experiment, the increased SW had little influence on the majority of the ham's quality traits, except on the subcutaneous fat depth, corresponding to the *semimembranosus* muscle, and on the roundness—a measure of muscularity. Interestingly, with increasing SW, the subcutaneous depth of the carcass increased, but the subcutaneous fat depth of the ham increased only in correspondence to the *semimembranosus* muscle, and not in correspondence to the *biceps femoris*. This seems to not be fully consistent with the results of [39], who found that the ultrasound fat thickness, measured in living pigs, was most correlated with the subcutaneous fat thickness in correspondence to the *biceps femoris* muscle (r = 0.53), rather than to the *semimembranosus* muscle (r = 0.18). In the current experiment, there was no correlation between the average carcass fat thickness

and the measures of subcutaneous fat thickness taken at the two positions, with simple correlation coefficients ranging from 0.06 to 0.02. However, in agreement with previous research [17,39], the subcutaneous fat depth in correspondence to the *semimembranosus* muscle was much thinner than that measured in correspondence to the *quadriceps femoris* muscle. As the thickness of subcutaneous fat influences salt penetration and water seasoning losses during seasoning [28], measurements taken at the *semimembranosus* muscle may exert a critical role in determining the dry-curing aptitudes of ham [17]. This result suggests that with an increase in SW, the seasoning aptitude of the ham might be improved without increasing the thickness of the fat layer in correspondence to the *biceps femoris* muscle, which may not be desired by the consumer and may limit the marketability of the ham [40].

The influence of a thicker round shape on the seasoning aptitude of the hams, or globosity, is poorly described in the literature. In practice, a greater ham roundness is frequently associated with excessive leanness, scarce subcutaneous fat covering, greater water content and salt absorption, greater seasoning losses and poor final quality of the seasoned ham [41]. In the case of the San Daniele Consortia, shortcomings associated with the roundness assume minor relevance because the tights are pressed [42]. In the current research, an increase in SW was associated with an increase in the roundness score. Considering that the optimal roundness is between one and two over a range from one to four, the number of pigs with a round shape score of three and four was notable; 33 (21%) and 6 (3.8%), respectively. It is not possible to indicate if this increase in roundness would result in greater difficulties in controlling the seasoning process, and therefore if this will require some adjustments in the manufacturing process. On the other hand, a greater roundness would have a less negative impact if associated with greater subcutaneous fat covering at the P2 position. This issue will merit future research efforts.

4.5. Sex Effects

In the Italian heavy pig production system, previous research has found little differences between gilts and barrows [43]. Such a finding could be attributed to the practice of feed restriction that could have reduced the exploitation of sexual dimorphism. In planning this research, and based on previous research [7,27,44], it was expected that the emersion of greater differences between gilts and barrows due to ad libitum feeding would permit better exploitation of inherent genetic differences. Such an expectation was confirmed, as barrows were 3.9% less efficient (gain: feed) and they had a 0.9% greater carcass yield, with a greater yield of fat and a lower yield of lean cuts. Such findings agree with those of previous research [3,29,45]. However, in the cited literature, the differences between barrows and gilts were more accentuated, as the barrows showed 16−17% greater feed intake, 8–13% greater average daily gain, 22−27% greater backfat depth, 3−5% lower gain: feed ratio and 3−5% lower ham yield than gilts.

Some differences between barrows and gilts were also found for some subjective scores, as barrows scored lower for haemorrhages and greater for visible marbling compared to gilts. However, the magnitude of these differences was modest. Therefore, it appears that no solid reasons can be given, at this point, to indicate that barrows are better than gilts when intended for Italian dry-cured ham production. This is dependent on the pig genetic line, as, in other productive contexts, barrows are better than gilts when intended for dry-cured ham production [24].

5. Conclusions

In conclusion, pigs with greater SWCs had greater average daily gain and feed consumption with similar feed efficiency, greater ham weight, muscularity and fat covering in correspondence to the *semimembranosus* muscle. Greater ham weight and fat covering in correspondence to the *semimembranosus* muscle are desired by the dry-cured ham industry for better curing aptitudes. Barrows produced hams with greater weight and marbling than gilts. A greater marbling is desired because of its positive influence on the flavour and the visual traits of green ham at the time of its selection for dry curing. These characteristics are evaluated by the dry-cured ham industry before the curing process for better profitability and consumer acceptability of the seasoned product. Data from this research also indicate that pigs of the Goland C21 genotype can reach the traditional weight of 160 ± 16 kg at only 8 months of age—one month less than the traditional age. New knowledge about the influence of slaughter age on the seasoning aptitude of the hams, not confounding SW with slaughter age, is desired.

Author Contributions:

Conceptualization, L.G., P.C. and S.S.; Methodology, I.H.M., D.G., L.G. and S.S.; Validation, V.H. and P.C.; Formal Analysis, I.H.M., D.G, S.S. and V.H.; Investigation, L.G., S.S. and P.C.; Resources, L.G., S.S., and P.C.; Data Curation, I.H.M. and D.G.; Writing—Original Draft Preparation, I.H.M., D.G. and S.S. and V.H.; Writing—Review and Editing, I.H.M., L.G., V.H., D.G., P.C. and S.S.; Visualization, I.H.M., D.G. and S.S.; Supervision, S.S. and V.H.; Project Administration, P.C. and L.G.; Funding Acquisition, L.G., P.C., and S.S. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by the European Union Rural Development program 2014–2020, Reg. (CE) 1305/2013—PSR Veneto DGR n. 2175—December 23, 2016, interventions 16.1.1 and 16.2.2, code 3682902. The University of Padua also provided funding with institutional funds (DOR2059255/20, DOR1990028/19, DOR1845849/18) and with a three-year PhD grant for Isaac Malgwi. The authors are indebted to the Progeo Feed industry for feed preparation and sampling, and Gorzagri s.s. for the provision of animals and technical support.

Institutional Review Board Statement:

The experimental procedures were approved by the institutional animal care committee of the University of Padova. All procedures were conducted in compliance with European Union requirements and guidelines on the protection of animals used for scientific and educational purposes provided by the *"Organismo preposto per il Benessere Animale, OPBA"*, University of Padova (OPBA, approval document #36/2018).

Data Availability Statement:

The data supporting the findings of this study are available from Gorzagri s.s., but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available. Data are however available from the authors upon reasonable request and with the permission of Gorzagri s.s.

Acknowledgements:

The authors would like to thank Luca Carraro, Nadia Guzzo, Alberto Simonetto and all the herdsmen for their support in animal management and data recording. The authors also wish to thank Alessandro Ferretti for his help in managing the slaughtering operations at the slaughterhouse. Thanks also to Giuseppe Tonin and Massimo Pasqualotto for the evaluations carried out on the dressed hams the day after slaughtering.

Conflicts of Interest:

The authors confirm that there is no conflict of interest associated with the publication of this manuscript. The funding sources were not involved in the study design, collection, analysis and interpretation of the data and in the writing of the paper.

References

1. Wu, F.; Vierck, K.R.; DeRouchey, J.M.; O'Quinn, T.G.; Tokach, M.D.; Goodband, R.D.; Dritz, S.S.; Woodworth, J.C. A review of heavy weight market pigs: Status of knowledge and future needs assessment. *Transl. Anim. Sci.* **2017**, *1*, 1–15. https://doi.org/10.2527/tas2016.0004.
2. Cisneros, F.; Ellis, M.; McKeith, F.; McCaw, J.; Fernando, R. Influence of slaughter weight on growth and carcass characteristics, commercial cutting and curing yields, and meat quality of barrows and gilts from two genotypes. *J. Anim. Sci.* **1996**, *74*, 925–933. https://doi.org/10.2527/1996.745925x.
3. Latorre, M.A.; Lázaro, R.; Valencia, D.G.; Medel, P.; Mateos, G.G. The effects of gender and slaughter weight on the growth performance, carcass traits, and meat quality characteristics of heavy pigs. *J. Anim. Sci.* **2004**, *82*, 526−533. https://doi.org/10.2527/2004.822526x.
4. Ramos, A.M.; Glenn, K.L.; Serenius, T.V.; Stalder, K.J.; Rothschild, M.F. Genetic markers for the production of US country hams. *J. Anim. Breed. Genet.* **2008**, *125*, 248–257. https://doi.org/10.1111/j.1439−0388.2007.00710.x.
5. Malgwi, I.H.; Gallo, L.; Halas, V.; Bonfatti, V.; Carc, G.; Sasso, C.P.; Carnier, P.; Schiavon, S. The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits. *Animals* **2021**, *11*, 2447. https://doi.org/10.3390/ani11082447.
6. EEC. Prosciutto di Parma. Protected Designation of Origin. General Rules, Dossier Pursuant to Article 4 of Council Regulation (EEC) No. 2081/92 of 14 July 1992. *Off. J. Eur. Communities* **1992**, *L208*, 9–14.
7. Gallo, L.; Dalla Bona, M.; Cecchinato, A.; Schiavon, S. Effect of growth rate on live performance, carcass and green thigh traits of finishing Italian heavy pigs. *Ital. J. Anim. Sci.* **2017**, *16*, 652–658. https://doi.org/10.1080/1828051X.2017.1318037.
8. INEQ (Istituto Nord-Est Qualità), 2015. Dossier 2014. Report on the control activity in the year 2014. Section 3. Protected Denomination Origin and Protected Geographic Indication, Products and productions. Istituto Nord-Est Qualità, San Daniele del Friuli, Udine, Italy.
9. Lebret, B. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal* **2008**, *2*, 1548–1558. https://doi.org/10.1017/S1751731108002796.
10. Gallo, L.; Dalla Montà, G.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Carcass quality and uniformity of heavy pigs fed restrictive diets with progressive reductions in crude protein and indispensable amino acids. *Livest. Sci.* **2015**, *172*, 50–58. https://doi.org/10.1016/j.livsci.2014.11.014. 18.
11. Correa, J.A.; Faucitano, L.; Laforest, L.P.; Rivest, J.; Marcoux, M.; Gariépy, C. Effects of slaughter weight on carcass composition and meat quality in pigs of two different growth rates. *Meat Sci.* **2006**, *72*, 91−99. https://doi.org/10.1016/j.meatsci.2005.06.006.
12. Van den Broeke, A.; Leen, F.; Aluwé, M.; Van Meensel, J.; Millet, S. The effect of sex and slaughter weight on performance, carcass quality and gross margin, assessed on three commercial pig farms. *Animal* **2020**, *14*, 1546−1554. https://doi.org/10.1017/S1751731119003033.
13. Čandek-Potokar, M.; Žlender, B.; Lefaucheur, L.; Bonneau, M. Effects of age and/or weight at slaughter on longissimus dorsi muscle: Biochemical traits and sensory quality in pigs. *Meat Sci*. **1998**, *48*, 287−300. https://doi.org/10.1016/s0309-1740(97)00109-5.
14. Ferguson, N.S.; Gous, R.M.; Emman, G.C. Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *S. Afr. J. Anim. Sci.* **1994**, *24*, 10–17. 1.
15. Kyriazakis, I.; Whittemore, C.T. (Eds.). *Whittemore's Science and Practice of Pig Production*; Blackwell Publishing: Oxford, UK, 2006; p. 417. https://doi.org/10.1002/9780470995624.ch13.
16. Rostellato, R.; Sartori, C.; Bonfatti, V.; Chiarot, G.; Carnier, P. Direct and social genetic effects on body weight at 270 days and carcass and ham quality traits in heavy pigs. *J. Anim. Sci.* **2015**, *93*, 1−10. https://doi.org/10.2527/jas.2014-8246.
17. Bonfatti, V.; Carnier, P. Prediction of dry-cured ham weight loss and prospects of use in a pig breeding program. *Animal* **2020**, *14*, 1128–1138. https://doi.org/10.1017/S1751731120000026.
18. Carcò, G.; Schiavon, S.; Casiraghi, E.; Grassi, S.; Sturaro, E.; Dalla Bona, M.; Novelli, E.; Gallo, L. Influence of dietary protein content on the chemico-physical profile of dry-cured hams produced by pigs of two breeds. *Sci. Rep.* **2019**, *9*, 1–12. https://doi.org/10.1038/s41598-019-55760-0.
19. NRC. *Nutrient Requirements of Swine,* 11th ed.; National Academy Press: Washington, DC, USA, 2012; p. 278.
20. Schiavon, S.; Bona, M.D.; Carcò, G.; Carraro, L.; Bunger, L.; Gallo, L. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. *PLoS ONE* **2018**, *13*, e0195645. https://doi.org/10.1371/journal.pone.0195645.

21. European Commission. Commission implementing decision of 24 January 2014 authorising methods for grading pig carcases in Italy [notified under document C (2014) 279]. *Off. J. Eur. Union* **2014**, *L23*.
22. European Commission. Corrigendum to commission implementing decision 2014/38/EU of 24 January 2014 authorising methods for grading pig carcases in Italy (Official Journal of the European Union L23 of 28 January 2014). *Off. J. Eur. Union* **2014**, *L54*.
23. Barducci, R.S.; Zhou, Z.Y.; Wormsbecher, L.; Roehrig, C.; Tulpan, D.; Bohrer, B.M. The relationship of pork carcass weight and leanness parameters in the Ontario commercial pork industry. *Transl. Anim. Sci.* **2021**, *4*, 331–338. https://doi.org/10.1093/TAS/TXZ169.
24. Schiavon, S.; Carraro, L.; Dalla Bona, M.; Cesaro, G.; Carnier, P.; Tagliapietra, F.; Sturaro, E.; Galassi, G.; Malagutti, L.; Trevisi, E.; et al. Growth performance, and carcass and raw ham quality of crossbred heavy pigs from four genetic groups fed low protein diets for dry-cured ham production. *Anim. Feed Sci. Technol.* **2015**, *208*, 170–181. https://doi.org/10.1016/j.anifeedsci.2015.07.009.
25. NPPC. *Pork Composition and Quality Assessment Procedures*; Berg, E.P., Ed.; National Pork Producers Council: Des Moines, IA, USA, 2000; pp. 1–42.
26. Magistrelli, D.; Galassi, G.; Crovetto, G.M.; Rosi, F. Influenza della somministrazione di elevate quantità di polpe di bietola essiccate su parametri endocrino/metabolici, rilievi al macello e qualità del prosciutto nel suino pesante italiano. *Ital. J. Anim. Sci.* **2009**, *8*, 37–49. https://doi.org/10.4081/ijas.2009.37.
27. Čandek-Potokar, M.; Škrlep, M. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal* **2012**, *6*, 327–338. https://doi.org/10.1017/S1751731111001625.
28. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* **2004**, *3*, 309–321. https://doi.org/10.4081/ijas.2004.309.
29. Latorre, M.A.; García-Belenguer, E.; Ariño, L. The effects of sex and slaughter weight on growth performance and carcass traits of pigs intended for dry-cured ham from Teruel (Spain). *J. Anim. Sci.* **2008**, *86*, 1933–1942. https://doi.org/10.2527/jas.2007-0764.
30. Fabro, C.; Sgorlon, S.; Guiatti, D.; Stefanon, B.; Susmel, P. Productive response of Duroc x Large white and commercial Hybrid x Large white crosses fed high and low protein diets. *Ital. J. Anim. Sci.* **2013**, *12*, 507–512. https://doi.org/10.4081/ijas.2013.e82.
31. Galassi, G.; Colombini, S.; Malagutti, L.; Crovetto, G.M.; Rapetti, L. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. *Anim. Feed Sci. Technol.* **2010**, *161*, 140–148. https://doi.org/10.1016/j.anifeedsci.2010.08.009.
32. Lo Fiego, D.P.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107–114. https://doi.org/10.1016/j.meatsci.2004.06.010.
33. Corino, C.; Musella, M.; Pastorelli, G.; Rossi, R.; Paolone, K.; Costanza, L.; Manchisi, A.; Maiorano, G. Influences of dietary conjugated linoleic acid (CLA) and total lysine content on growth, carcass characteristics and meat quality of heavy pigs. *Meat Sci.* **2008**, *79*, 307–316. https://doi.org/10.1016/j.meatsci.2007.10.001.
34. Fisher, A.V.; Green, D.M.; Whittemore, C.T.; Wood, J.D.; Schofield, C.P. Growth of carcass components and its relation with conformation in pigs of three types. *Meat Sci.* **2003**, *65*, 639–650. https://doi.org/10.1016/S0309-1740(02)00266-8.
35. Latorre, M.A.; Medel, P.; Fuentetaja, A.; Lázaro, R.; Mateos, G.G. Effect of gender, terminal sire line and age at slaughter on performance, carcass characteristics and meat quality of heavy pigs. *Anim. Sci.* **2003**, *77*, 33–45. https://doi.org/10.1017/s1357729800053625.
36. Minelli, G.; Macchioni, P.; Ielo, M.C.; Santoro, P.; lo Fiego, D.P. Effects of dietary level of pantothenic acid and sex on carcass, meat quality traits and fatty acid composition of thigh subcutaneous adipose tissue in Italian heavy pigs. *Ital. J. Anim. Sci.* **2013**, *12*, 329–336. https://doi.org/10.4081/ijas.2013.e52.
37. Zappaterra, M.; Zambonelli, P.; Schivazappa, C.; Simoncini, N.; Virgili, R.; Stefanon, B.; Davoli, R. Investigating the features of PDO green hams during salting: Insights for new markers and genomic regions in commercial hybrid pigs. *Animals* **2021**, *11*, 68. https://doi.org/10.3390/ani11010068.
38. Čandek-Potokar, M.; Monin, G.; Žlender, B. Pork quality, processing, and sensory characteristics of dry-cured hams as influenced by Duroc crossing and sex. *J. Anim. Sci.* **2002**, *80*, 988–996. https://doi.org/10.2527/2002.804988x.
39. Cecchinato, A.; Schiavon, S.; Tagliapietra, F.; Gallo, L. Relationships between in vivo Measurements of Backfat Thickness and Several Carcass and Ham Traits in Heavy Pigs. *Agric. Conspec. Sci.* **2013**, *78*, 255–258.

40. Rodríguez-Sánchez, J.A.; Calvo, S.; Suárez-Beloch, J.; Latorre, M.A. Effect of pig slaughter weight on chemical and sensory characteristics of teruel dry-cured ham. *Ital. J. Food Sci.* **2014**, *26*, 420–426.
41. Lo Fiego, D.P.; Comellini, M.; Ielo, M.C.; Ulrici, A.; Volpelli, L.A.; Tassone, F.; Costa, N. Preliminary investigation of the use of digital image analysis for raw ham evaluation. *Ital. J. Anim. Sci.* **2007**, *6*, 693–695. https://doi.org/10.4081/ijas.2007.1s.693.
42. Laureati, M.; Buratti, S.; Giovanelli, G.; Corazzin, M.; Lo Fiego, D.P.; Pagliarini, E. Characterization and differentiation of Italian Parma, San Daniele and Toscano dry-cured hams: A multi-disciplinary approach. *Meat Sci.* **2014**, *96*, 288–294. https://doi.org/10.1016/j.meatsci.2013.07.014.
43. Gallo, L.; Dalla Montà, G.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Growth performance of heavy pigs fed restrictively diets with decreasing crude protein and indispensable amino acids content. *Livest. Sci.* **2014**, *161*, 130–138. https://doi.org/10.1016/j.livsci.2013.12.027.
44. Quiniou, N.; Courboulay, V.; Salaün, Y.; Chevillon, P. Impact of the non-castration of male pigs on growth performance and behaviour- comparison with barrows and gilts. In Proceedings of the 61st Annual Meeting of the European Association for Animal Production, Heraklion, Greece, 23–27 August 2010; pp. 1–7
45. Auspurger, N.R.; Ellis, M.; Hamilton, D.N.; Wolter, B.F.; Beverly, J.L.; Wilson, E.R. The effect of sire line on the feeding patterns of grow-finish pigs. *Appl. Anim. Behav. Sci.* **2002**, *75*, 103–114. https://doi.org/10.1016/S0168-1591(01)00188-5.

Chapter 3

Impact of Rearing Strategies on the Metabolizable Energy and SID Lysine Partitioning in Pigs Growing from 90 to 200 kg in Body Weight

S. Schiavon [1], I. H. Malgwi [1,*], D. Giannuzzi [1,*], G. Galassi [2], L. Rapetti [2], P. Carnier [3], V. Halas [4] and L. Gallo [1]

[1] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell' Università 16, 35020 Legnaro (PD), Italy

[2] Department of Agricultural and Environmental Sciences—Production, Landscape, Agroenergy (DiSAA), University of Milan, Via G. Celoria 2, I-20133 Milan, Italy

[3] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Agripolis, Viale dell'Università 16, Legnaro, 35020 Padova, Italy

[4] Department of Farm Animal Nutrition, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences, Guba Sándor Utca 40, 7400 Kaposvár, Hungary

Published in: *Animals 2022, 12(2), 215;* https://doi.org/10.3390/ani12020215

Impact of Rearing Strategies on the Metabolizable Energy and SID Lysine Partitioning in Pigs Growing from 90 to 200 kg in Body Weight

S. Schiavon [1], I. H. Malgwi [1,*], D. Giannuzzi [1,*], G. Galassi [2], L. Rapetti [2], P. Carnier [3], V. Halas [4] and L. Gallo [1]

Simple Summary:

The nutritional recommendations for pigs largely focus on pigs with lean genotypes fed ad libitum until reaching up to 140 kg in body weight (BW). Under different rearing conditions, it is still unclear whether existing recommendations apply to pigs that weigh more than 140 kg in BW, especially in heavy pig production systems. In the current study, pigs growing from 90 to 200 kg in BW were raised with different feeding strategies. We observed that energy restriction had a negligible effect on pigs' estimated metabolizable energy requirements at heavier BW under different feeding conditions. Under energy and protein restrictions, a value of 0.73 could be assumed as the maximum marginal efficiency of standardized ileal digestible lysine (SID lysine) utilization for protein deposition irrespective of BW, which corresponds to 9.8 g of SID lysine per 100 g of protein deposition as a minimum requirement.

Abstract:

The current nutrient recommendations focus on pigs fed ad libitum up to 140 kg in body weight (BW). It remains unclear whether this applies to pigs weighing above 140 kg in BW under different rearing conditions. This study aimed to estimate protein (Pd) and lipid (Ld) depositions and the metabolizable energy (ME), standardized ileal digestible lysine (SID lysine) requirement and partitioning in 224 C21 Goland pigs (90–200 kg in BW). The control pigs (C) received diets limiting ME up to 170 kg in slaughter weight (SW) at 9 months of age (SA); older (OA) pigs had restricted diets limiting ME and SID lysine up to 170 kg in SW at >9 months SA; younger (YA) pigs were fed nonlimited amounts of ME and SID lysine up to 170 kg in SW at <9 months SA; and greater weight (GW) pigs were fed as the YA group, with 9 months SA at >170 kg in SW. The estimated MEm averaged 1.03 MJ/kg$^{0.60}$. An 11% increase in MEm was observed in OA pigs compared to the controls. Energy restriction had negligible effects on the estimated MEm. The marginal efficiency of SID lysine utilization for Pd averaged 0.725, corresponding to a SID lysine requirement of 9.8 g/100 g Pd.

Keywords: feed restriction; heavy pigs; nutrient partitioning; protein deposition; SID lysine

1. Introduction

Current nutrient recommendations for pigs by the NRC [1] focus on pigs with lean genotypes fed ad libitum until reaching up to 140 kg in body weight (BW). This recommendation has limitations under the management practice(s) of heavy pig production systems for the dry-cured ham industry. For this industry, pigs are fed according to a variety of feeding strategies aimed to manipulate the age (SA) and the weight at slaughter (SW) for the improvement of the ham seasoning aptitude. Such strategies include ad libitum or restricted feeding diets with different energy and amino acid content. A major challenge with these systems is the continuous increase in lean pig genotypes with inadequate ham adiposity for the ham industry, pushing them towards a progressive increase in SW and modifying the feeding strategies [2–4]. Recent studies have compared restricted medium-protein diets, restricted low-protein diets and ad libitum high-protein diets for Goland C21 heavy pigs sacrificed at 170 or even at 200 kg in SW, demonstrating that some of these strategies can improve the quality of the green hams [5,6]. However, to optimise the performance of the pigs under such conditions, knowledge of the pigs' energy and amino acid (AA) requirements and partitioning is important [7,8].

The energy and nutrient utilization of heavy pigs under the dry-cured ham production systems have not been covered by existing literature. It also remains uncertain if the recommended metabolizable energy (ME) requirements for maintenance (MEm = 1.03 MJ/kg in BW$^{0.60}$) by the NRC [1] apply to pigs at heavier BW. Additionally, the nutrient partitioning in heavy pigs kept on feeding strategies with limited or nonlimited energy and/or amino acid supply is yet to be fully addressed. A study by Labussière et al. [9], argued that MEm might not always be independent of ME intake. The

energy requirements are a function of feeding level for maintenance requirements and components associated with the BW gain of the pigs. For heavy pigs, whose incidence of MEm in total energy cost was reported to be greater than 45% [10], it is of interest to explore the behaviour of their energy requirements under different rearing conditions and extended ranges of BW. The evaluation of the AA requirements and partitioning of heavy pigs, when kept under different rearing strategies, is critical to nutritional viewpoints.

Generally, the AA requirement can be expressed in terms of standardized ileal digestible lysine (SID lysine) when lysine is the first-limiting AA [1]. The SID lysine requirement corresponds to the amount required to achieve the protein deposition (Pd), achievable when the pigs are kept under unlimited feeding and environmental conditions [11–13]. The knowledge of the marginal efficiency of SID lysine is necessary to estimate the SID lysine requirement for a given Pd [14]. To achieve this estimate, pigs must be supplied with SID lysine below their requirement for Pd. Additionally, it is crucial to define the potential Pd of the pigs, which can be achieved by not limiting the energy and amino acid supply. According to NRC [1], the marginal efficiency of SID lysine utilization for Pd in heavy pigs is expected to be low, as it would decline with increasing BW. However, this is different from the report of the InraPorc model, which considers the marginal efficiency for SID lysine (0.72) to be constant with increasing BW [15].

The current study aimed to investigate (i) the body protein and lipid accretions of Goland C21 heavy pigs between 90 and 200 kg in body weight (BW) when exposed to various rearing conditions; (ii) the ME and SID lysine partitioning for maintenance and growth and (iii) the marginal efficiency of SID lysine utilization for Pd.

2. Materials and Methods

2.1. Pig Housing and Rearing

Pigs weighing 95.0 ± 12.5 kg in BW and 149 ± 3 d of age, belonging to the Goland C21 breed (Gorzagri, Fonzaso, Italy) (n = 224, barrows and gilts), were divided into 2 batches of 112 pigs as described in [5]. The 2 batches of pigs entered the experimental period sequentially, and tests were conducted during different seasons (autumn-winter and winter-spring). The duration of the experimental period ranged from 85 to 134 d, depending on the rearing strategy. All the pigs from a given batch, born in the same week, were fed the same commercial diets till their transfer to the research pig station of the University of Padua.

An electronic feeding station in each pen (Compident Pig–MLP, Schauer Agrotronic, Prambachkirchen, Austria) was programmed to supply each pig with the planned amount of feed in each pen [16] with 14 pigs per pen (1.57 m^2/pig). Water was provided ad libitum from nipple drinkers, and the temperature within the room was between 19 and 22 ± 2 °C throughout the experiment. The amount of feed consumed per visit and other feeding behaviour traits were recorded for each pig. The daily dry matter intake (DMI) of each pig was computed from the amount of feed consumed during each visit on that day and its dry matter content.

2.2. Live and Postmortem Measurements

Pigs were weighed with an electronic scale at the start and the end of the trial. At each weighing, backfat (BF) depth was measured with an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco Corporation, Minneapolis, MN, USA). The BF measurements were taken from the last rib at approximately 5.5 to 8.0 cm from the midline, at an increasing distance with increasing BW [17]. When pigs reached the average targeted slaughter weight (SW) or age (SA), they were sacrificed following regulations for commercial practices [18].

2.3. Experimental Design

Means and standard deviations of the initial BW of the pigs were similar across the pens. A split-plot design with sex within a pen was used. A control group and three other treatment groups, representing 3 alternative rearing strategies, were used. The characteristics of the 4 treatment groups are given in Table 1, by Malgwi et al. [5]. A total of 28 pigs/treatment were assigned to each treatment and housed in two pens. An across-batch rotation scheme was used to assign treatment groups to pens in different batches so that each treatment was assigned to different pens. A description of this procedure is provided below:

(i) The control group (C) had pigs raised under the traditional heavy pig production system for dry-cured ham. Thus, feed restriction was applied, and pigs were fed a restricted medium-protein (MP) diet, with lysine as the first-limiting indispensable AA. They were slaughtered at 9 months SA and 170 kg in SW.

(ii) The older pig (OA) strategy was based on a SID lysine restriction in addition to the feed or energy restriction to shift the pigs toward a greater lipid deposition (Ld) and lower Pd to improve the ham seasoning aptitude [19]. Thus, the OA pigs were fed as restrictively as the C pigs, but with feeds lower in SID lysine content (LP). The pigs were slaughtered at >9 months SA at 170 kg in SW. Information from this group of pigs was used to evaluate the ME partitioning and the marginal efficiency of SID lysine utilization for Pd.

(iii) The young pig (YA) rearing strategy set a 170 kg SW target for pigs younger than the minimum age. They were fed a high-protein (HP) diet ad libitum, not limiting indispensable AA content. Such unlimited conditions were applied to exploit the pig potential for Pd and Ld [20,21].

(iv) The third alternative strategy programmed pigs to reach the maximum SW (>170 kg) at 9 months SA (greater weight, GW). The pigs were given the same HP feeds, fed ad libitum, as the YA group and were slaughtered at the same SA (9 months) but at a greater SW (>170 kg) than the C pigs. A comparison between YA and GW was carried out for an evaluation of the effect of an increased SW and SA on energy and SID lysine needs and partitioning at the heavy BW range (170–200 kg in SW) of the pigs.

2.4. Feeds

The characteristics of the ingredients used in early diets of pigs 90 to 120 kg in BW and late-finishing diets of pigs >120 kg in BW are given in Table 1. The nutritional composition of the diets is given in Table 2.

Table 1. Composition of ingredients in g/kg DM of early diets (90 to 120 kg average BW) and late-finisher diets (over 120 kg in BW).

Ingredient	Early Finishing Feeds			Late-Finishing Feeds		
	High Protein	Medium Protein	Low Protein	High Protein	Medium Protein	Low Protein
Corn grain	350.9	342.0	381.7	388.7	390.2	391.1
Wheat grain	249.5	282.5	272.4	248.4	248.9	249.3
Barley grain	96.4	97.0	97.2	96.9	97.3	97.4
Soybean meal 48% (solv. ex.)	201.0	87.7	39.3	147.3	57.9	18.9
Wheat bran	25.5	84.6	82.6	7.2	55.8	60.7
Wheat middlings	0.0	19.6	29.4	39.1	66.3	88.5
Cane molasses	16.0	16.1	16.3	18.1	18.2	18.2
Lard	22.1	24.0	23.4	22.2	22.3	22.3
Dried-sugar beet pulp	-	9.9	19.8	0.0	9.9	20.4
Calcium carbonate	16.6	16.7	16.7	14.4	14.5	14.5
Dicalcium phosphate	4.8	4.9	4.9	2.2	2.2	2.2
Sodium chloride	3.3	3.3	3.3	3.3	3.3	3.3
Sodium bicarbonate	2.7	2.8	2.8	2.8	2.8	2.8
Vitamin and mineral premix [a]	2.0	-	2.0	2.0	2.0	2.0
Grapeseed meal	7.3	7.4	7.4	7.4	7.4	7.4
Choline, liquid, 75% [b]	0.6	0.0	-	-	-	-
L-Lysine [c]	1.1	1.6	0.7	-	1.1	1.1
DL-Methionine [d]	0.2	-	-	-	-	-

[a] Providing per kilogram of feed: vitamin A, 8000 IU; vitamin D_3, 1200 IU; vitamin E, 8 mg; vitamin B_7, 0.08 mg; vitamin B_{12}, 0.012 mg; niacin, 16.0 mg; biotin, 8 mg; iron, 170 mg; zinc, 117 mg; copper, 14 mg; cobalt, 0.11 mg; iodine, 0.06 mg; manganese, 65 mg; magnesium, 0.14 mg; selenium 10 mg.
[b] Choline liquid 75% (Methodo Chemicals, 42017 Novellara, RE, Italy).
[c] L-Lysine Monoclohydrate, 98.5% pure, 78% L-Lysine (Methodo Chemicals, 42017 Novellara, RE, Italy).
[d] DL Methionine, 98% pure min. (Methodo Chemicals, 42017 Novellara, RE, Italy).

The early finishing HP diets were fed to YA and GW pig groups from 90 to 120 kg in BW. The diet was designed to contain unlimited amounts of SID lysine, methionine, tryptophan and threonine, according to the NRC [1] recommendation for the 70–100 kg in BW range.

The SID lysine content of the early finishing MP feed fed to pigs in the C group was 26% lower than that proposed by NRC [1] for the same BW range. This diet was expected to result in an average daily gain of 0.700 kg/d, with lysine as the first-limiting AA. The SID lysine content of the diet was similar to that frequently used in practice [10].

The SID lysine content of the early finishing LP diet fed to the OA group was consistent, with an average daily gain of 0.650 kg/d. This was purposefully set at a lower amount than that used in previous studies where a shortage of dietary AA content did not influence growth performance, carcass or meat quality [22,23].

The late-finishing HP, MP and LP diets administered from 120 kg in BW onwards were formulated to contain about 20–25% less indispensable SID AA than the corresponding HP, MP and LP feed used in the early finishing period, with lysine as the first-limiting AA.

2.5. Feeding Regime

Feeds were administered using the feeding station for individual pigs in all the treatment groups. The restricted amount of feed distributed to the C and OA pig treatments was established based on the average initial BW. Thereafter, the quantity of feed was increased weekly without any further adjustment. The amount of feed administered to C and OA pig groups was increased from 2.3 to 3.0 kg/d throughout the trial, and this corresponds to an increase from 57 to 82 g/kg$^{0.75}$ in metabolic weight, a common practice in such a production system [22].

Table 2. Nutrient content (g/kg of DM unless otherwise indicated) of early diets (90 to 120 kg in average BW) and late-finisher diets (over 120 kg in average BW).

	Early Finishing Feeds [a]			Late-Finishing Feeds		
	HP	**MP**	**LP**	**HP**	**MP**	**LP**
Analyzed nutrient composition [b]						
DM, g/kg as fed	906	904	904	906	902	904
CP (N × 6.25)	178.8	141.6	125.0	152.3	131.9	115.0
Starch	455.8	508.8	539.8	533.1	521.1	542.0
Ether extract	47.5	50.9	48.7	53.0	55.4	53.1
aNDF-NDF	144.6	152.7	156.0	130.2	146.3	148.2
Ash	53.0	52.0	53.1	46.4	45.5	45.4
Lysine (Lys)	9.6	7.3	5.2	7.5	5.5	4.0
Methionine (Met)	3.0	2.4	2.1	2.8	2.2	2.0
Threonine (Thr)	7.2	5.0	4.8	5.5	4.8	3.9
Tryptophan (Trp)	2.0	1.7	1.3	1.4	1.2	1.1
Tyrosine (Tyr)	6.1	4.2	3.8	3.8	3.7	2.9
Calculated nutrient composition [c]						
ME, MJ/kg DM	14.8	14.6	14.6	14.8	14.6	14.5
NE, MJ/kg DM	11.0	11.1	11.2	11.1	11.1	11.0
CP (N × 6.25)	178.8	141.6	120.6	156.7	128.6	113.9
Digestible CP (DCP)	153.2	120.8	103.2	133.4	109.0	97.0
ME/Digestible CP, MJ/kg DCP	97	121	141	111	134	149
Starch	468.0	496.7	519.9	501.1	521.1	527.7
Linoleic acid	48.6	52.0	52.0	50.8	52.1	52.0
Lys	9.2	6.9	5.0	7.6	5.7	3.9
Met	3.0	2.2	2.0	2.4	2.1	1.9
Thr	6.3	4.8	4.0	5.4	4.2	3.8
Trp	2.2	1.7	1.3	1.9	1.4	1.2
Tyr	5.8	4.5	3.9	5.1	4.1	3.7
SID Lys	8.2	6.0	4.2	6.6	5.0	3.2
SID Met	2.8	2.0	1.8	2.3	1.9	1.8
SID Thr	5.5	4.0	3.2	4.9	3.7	3.1
SID Trp	1.8	1.3	1.0	1.5	1.2	1.0
SID Tyr	5.4	4.1	3.5	4.9	3.9	3.3
Ratios:						
Met/Lys (Optimum = 0.288)	0.34	0.33	0.42	0.35	0.38	0.55
Thr/Lys (Optimum = 0.672)	0.68	0.67	0.76	0.73	0.73	0.97
Trp/Lys (Optimum = 0.182)	0.22	0.22	0.24	0.23	0.24	0.31
Tyr/Lys (Optimum = 0.353)	0.66	0.69	0.84	0.73	0.78	1.03

[a] HP: high-protein diet, MP: medium-protein diet, and LP low-protein diet.
[b] Analytical results by averaging data from 4 independent replications.
[c] Computed according to NRC [1] from the ingredient composition of the feeds (2 batches); SID: standardized ileal digestible amino acid content; optimum ratios according to NRC [1].

2.6. Chemical Analysis

Feeds were manufactured by the Progeo Feed Industry (Masone, Reggio Emilia, Italy). Feed samples were collected from the production line and analyzed on the day after collection for evaluation before their use in the experiment to ensure the consistency between the theoretical and actual nutrient contents, with special regard to the AA content [16]. During feed manufacture in the trial and phase feeding, 10 samples of each feed were collected online, pooled, mixed and sampled to obtain a 1 kg feed sample from which independent subsamples were collected. The feed samples were analyzed with 3 independent replications for dry matter (DM: # 934.01; AOAC, 2003), N (# 976.05; AOAC, 2003), ether extract (EE: # 920.29; AOAC, 2003), ash (# 942.05, AOAC, 2003) and neutral detergent fibre with amylase treatment and expressed including residual ash (aNDF) contents [24]. Starch content was determined after hydrolysis of glucose [25] by liquid chromatography [26].

The amino acid content of the feed samples was determined according to the Council of Europe [27,28]. Dietary ME, crude protein, SID amino acid and other nutrient contents were computed from the actual ingredient composition of the feeds and the tabular values for each ingredient [1]. Differences between the analyzed and theoretical AA contents of the feeds were negligible.

2.7. Body Composition, Pd and Ld

Body chemical composition was estimated according to Gallo et al. [10], starting from the measurements of BW and BF taken at the start and the end of the experiment. Empty BW (EBW) was estimated from BW using an allometric equation developed for barrows and gilts in the range from 90 to 150 kg in BW, assuming that this equation holds for heavier BW [29]. Body lipid mass (BL) was estimated from BF and BW according to the equation developed by Schiavon et al. [17] using data from different datasets with pigs kept under different feeding conditions over an extended range of BW (12–207 kg in BW). Fat-free EBW mass (FFEBW) was computed as EBW minus BL. Based on the allometric relationships among body protein (BP), water and ash masses [1], BP (kg) was computed as $0.1353 \times FFEBW^{1.1175}$.

The Pd and Ld were calculated from the estimated protein and lipid body mass changes throughout the experiment for each treatment. As a control, the backfat and the belly weights collected and measured at slaughter from each pig were regressed against the estimated BL achieved from the BW and BF depth measures taken the day before slaughter.

2.8. Metabolizable Energy Partitioning

The energy and lysine partitioning were computed on an individual and daily basis from the estimated changes in body chemical composition and the measured feed intake over the finishing time intervals [10]. The daily ME intake was calculated from the average feed intake and the ME content of diets, adjusted for their actual dietary DM content. The ME used for growth was computed from the estimated Pd and Ld over the trial, assuming a requirement of 44.35 and 52.30 MJ ME/kg of retained protein and lipid, respectively [1]. The amount of ME used for maintenance (MEm) was estimated as ME intake—ME for growth. The resulting MEm value was scaled versus the mean metabolic weight computed as $BW^{0.60}$ [1].

2.9. SID Lysine Partitioning

The average SID lysine daily intake was computed from the measured feed intake and the dietary SID lysine content. The SID lysine maintenance requirement for pigs, including that for basal endogenous GIT and integument losses, was computed based on individual feed intake and the average metabolic weight over the testing period [1]. The individual SID lysine retention was computed taking Pd to contain 0.071 lysine, as suggested by the NRC [1]. The SID lysine consumed in excess or the deficit was computed as the difference between SID lysine intake and the estimated requirement for maintenance and Pd. The marginal (above maintenance) SID lysine intake was computed as SID lysine intake minus the SID lysine used for maintenance. The resulting value is expressed per day and per gram of estimated Pd.

Total lysine efficiency was estimated as: lysine retained divided by SID lysine intake, while the marginal efficiency of SID lysine utilization for Pd was computed as: lysine retained divided by SID lysine intake minus SID lysine required for maintenance.

2.10. Statistical Analysis

Data were analysed according to the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) using the following linear model:

$$y_{ijklm} = \mu + treatment_i + sex_j + (treatment \times sex)_{ij} + batch_k + pen(treatment \times batch)_{l:ik} + \varepsilon_{ijklm} \quad (1)$$

where y_{ijklm} is the observed trait; μ is the overall intercept of the model, treatment is the fixed effect of the i^{th} treatment (i = 1, …, 4); sex_j is the fixed effect of the j^{th} sex (j: 1 = barrow, 2 = gilt); $(treatment \times sex)_{ij}$ is the interaction effect between treatment and sex; $batch_k$ is the random effect of the k^{th} batch (k = 1, 2); $pen(treatment \times batch)_{l:ik}$ is the random effect of the l^{st} pen within the interaction treatment × batch, and ε_{ijklm} is the random residual error.

The batch, pen (treatment × batch) and residuals were assumed to be independently and normally distributed with a mean of zero and variances of σ^2_k, σ^2_l and σ^2_m, respectively. The effect of treatment was tested on the pen (treatment × batch) variance, whereas sex and the treatment × sex interaction were tested on the residual variance.

The 3 degrees of freedom due to the treatment were used to run orthogonal contrasts to test: (1) the C treatment versus the restricted low-protein feeding at the same SW and older SA (OA); (2) the C versus the ad libitum high-protein feeding at the same SW and early SA (YA); (3) the YA vs. GW, representing the influence of an increase in SW (170 vs. 200 kg) and SA (8 vs. 9 mo) under ad libitum high-protein feeding conditions.

3. Results

3.1. Dry Matter Intake and Growth Performance

The pigs of all the treatment groups, C, OA, YA and GW, were sacrificed after 116, 133, 85 and 116 d of feeding at 265, 282, 234 and 265 d SA, respectively (Table 3). Aside from the GW (185 kg in EBW) group, all pigs were sacrificed at about 164 kg in EBW.

The protein and energy restriction applied (OA vs. C) induced a reduction in the daily EBW gain (p = 0.007) without changing the daily DMI and increased the duration of feeding accompanied by increased cumulative DMI intake (p < 0.001). The OA strategy had a negligible impact on the final backfat depth.

The pigs receiving the YA diet had increased daily DMI intake (p < 0.001), an EBW gain (p < 0.001), final BF depth (p = 0.002) and reduced cumulative DMI intake (p = 0.006) because of the shorter duration of feeding for the target SW.

Extending the SW and SA (YA vs. GW), resulted in increased EBW, cumulative DMI and final BF depth (p < 0.001) with a reduced daily EBW gain (p = 0.021). There was no difference observed in daily DMI.

A greater cumulative feed intake (p = 0.027) and initial (p < 0.001) and final (p = 0.008) BF depth was observed in barrows compared to gilts. The sex × treatment interaction was significant for the final EBW (p = 0.008) and for the daily (p = 0.035) and cumulative DMI intake (p = 0.013). The nature of this interaction is such that barrows had a similar final EBW and cumulative DMI for treatment C, OA and YA, but a greater final EBW (Figure 1) and cumulative DMI (Figure 2) only when exposed to the GW treatment.

Table 3. Empty body weight (EBW), EBW gain, feed consumption and ultrasound backfat depth of the C21 Goland heavy pigs subjected to different rearing strategies [1].

Item	Treatment					*p* Values			Sex			*p* Values	
	C	OA	YA	GW	SEM [2]	C vs. OA	C vs. YA	YA vs. GW	Gilts	Barrows	SEM [2]	Sex	Sex × Treatment
Animals, n.	55	56	54	57	-	-	-	-	109	113	-	-	-
Days on feed	116 ± 4	133 ± 8	85 ± 4	116 ± 4	-	-	-	-	114 ± 17	112 ± 19	-	-	-
Empty body weight (EBW), kg													
Initial	84.6	84.1	84.7	85.2	1.31	0.82	0.93	0.80	83.7	85.7	0.96	0.12	0.17
Final [3]	164.2	162.5	164.7	185.4	1.74	0.52	0.85	<0.001	167.8	170.6	1.23	0.11	0.008
Daily EBW gain, kg/d	0.684	0.589	0.939	0.861	0.02	0.007	<0.001	0.021	0.766	0.770	0.01	0.77	0.20
Feed dry matter intake:													
daily, kg/d	2.42	2.43	3.05	2.96	45.3	0.77	<0.001	0.15	2.68	2.75	32.4	0.079	0.035
Cumulative [4], kg/pig	282	325	259	345	12.4	<0.001	0.006	<0.001	298	307	11.9	0.027	0.013
Backfat depth, mm													
initial	10.1	10.1	10.1	10.3	0.30	0.52	0.94	0.95	9.62	10.67	0.30	<0.001	0.85
final	20.8	22.4	24.8	25.9	1.24	0.14	0.002	<0.001	22.83	24.12	1.24	0.008	0.51

[1] The rearing strategies were as follows: C, pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW) (fed medium-protein feeds); OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW (fed low-protein feeds); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW (fed high-protein feeds); GW, pigs fed unlimited amounts of ME and protein ad libitum up to 9 months at slaughter age (about 200 kg in SW) (fed high-protein feeds).
[2] SEM: pooled standard error of the mean. [3] See Figure 1 for the form of the sex × treatment interaction. [4] See Figure 2 for the form of the sex × treatment interaction.

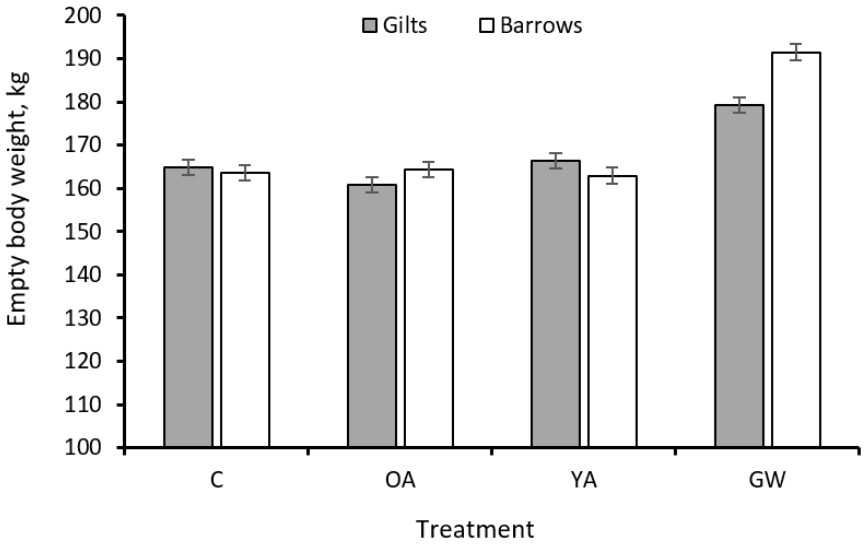

Figure 1. Final empty body weight of C21 Goland barrows and gilts according to different treatments (n = 224, sex × treatment interaction p = 0.008). C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW.

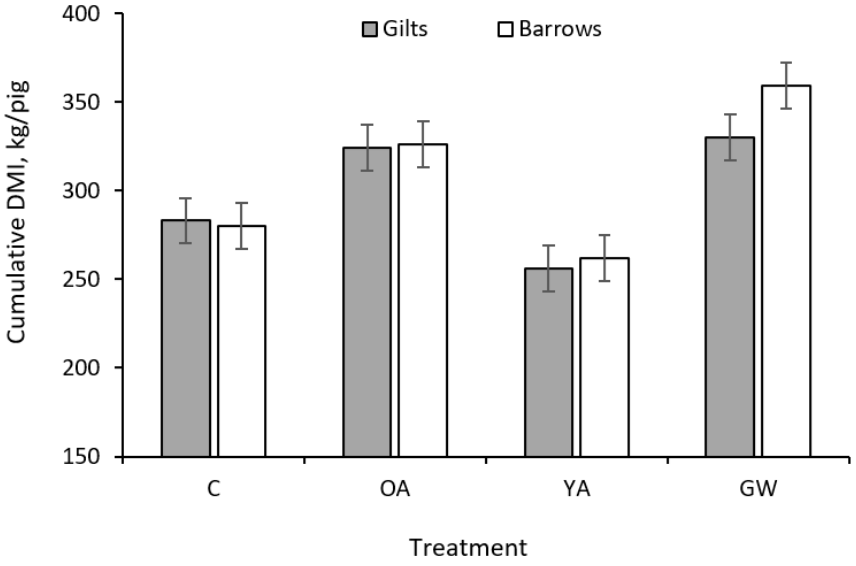

Figure 2. Cumulative dry matter intake of C21 Goland barrows and gilts according to different treatments (n = 224, sex × treatment interaction p = 0.013). C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW.

3.2. Body Composition Changes and ME Partitioning

The weight of the backfat and belly tissues collected at slaughter was linearly correlated (R^2 = 0.788) to the estimated final body lipid mass (Figure 3).

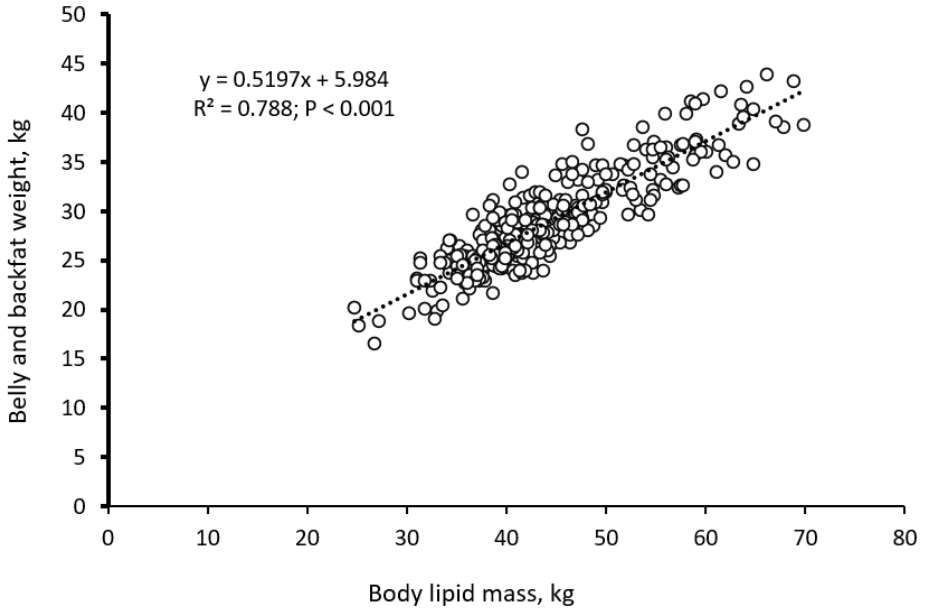

Figure 3. Relation between body lipid mass (x), estimated in vivo from body weight measurements and ultrasound backfat depth the day before slaughtering, and the belly plus backfat weights measured at slaughter (y; *n* = 224).

The slope of this relationship suggested the backfat plus the belly weight represented about 0.52 of whole-body lipid mass. The OA treatment had no influence on the estimated final body lipid mass and Ld, compared to C (Table 4), but it reduced Pd for growth compared to C by 7% ($p < 0.001$). Nevertheless, OA treatment resulted in a similar final body protein mass as that achieved from the C treatment because the OA pigs had a greater number of days on feed.

Despite the same ME intake, the OA pigs utilized 17% less ME for Pd ($p < 0.001$) and 11% more for maintenance ($p < 0.001$) than the pigs receiving a C diet.

The YA pigs presented a greater final body lipid mass (12%, $p = 0.010$), Ld (64%, $p < 0.001$) and Pd (24%, $p < 0.001$), but a 4.4% lower final whole-body protein mass ($p = 0.014$) compared to the C pigs. This lower estimated protein mass was due to earlier attainment of the targeted SW by the YA pigs.

The ME intake and ME utilized for growth (Pd and Ld) were 28 and 52% greater in YA compared to C pigs ($p < 0.001$), respectively. However, the ME for maintenance for a unit of MW remained unchanged for the pigs undergoing the two treatments.

The estimated final whole-body lipid and protein masses of GW pigs were greater ($p < 0.001$) than the corresponding masses of the YA pigs as a result of increased SW and SA. The Ld and Pd of the GW pigs tended to be 7–11% lower ($p < 0.07$ and 0.06, respectively) than the corresponding values of the YA pigs, reflecting the decline in the growth impulse with advancing SW and SA.

Compared to YA, the GW pigs had a similar daily ME intake with 10% less ME dedicated to growth, and the MEm remained the same, despite the remarkable increase in SW.

The estimated initial ($p = 0.005$) and final ($p = 0.012$) lipid masses were 5–7% greater in barrows compared to gilts, but no differences were observed for the initial and final protein masses. Barrows and gilts had similar ME partitioning between growth and maintenance.

Significant sex × treatment interactions were observed for the final estimated whole-body protein mass ($p = 0.009$; Figure 4) and the ME intake ($p = 0.035$) because the barrows responded differently from gilts only when subjected to the GW treatment. There was significant sex × treatment interaction for the estimated MEm ($p = 0.001$; Figure 5). However, the observed differences between the barrows and the gilts among all treatments for MEm were of small magnitude.

Table 4. Estimated body composition changes and metabolizable energy (ME) partitioning of the C21 Goland heavy pigs subjected to different rearing strategies [1].

Item	Rearing Strategy				SEM [2]	*p* Values			Sex		SEM	*p* Values	
	C	OA	YA	GW		C vs. OA	C vs. YA	YA vs. GW	Gilts	Barrows		Sex	Sex × Treatment
Estimated body lipid mass [3], kg													
initial	14.6	14.5	14.6	14.8	0.39	0.87	0.97	0.62	14.1	15.1	0.30	0.005	0.44
final	41.0	42.5	46.1	53.4	1.63	0.37	0.010	0.001	44.6	47.0	1.38	0.012	0.13
daily lipid deposition (Ld), g/d	226.4	209.8	370.1	331.4	14.2	0.40	<0.001	0.07	278.4	290.5	9.01	0.13	0.26
Estimated body protein mass [4], kg													
initial	15.6	15.5	15.6	15.7	0.24	0.80	0.80	0.88	15.5	15.7	0.17	0.30	0.11
final	29.4	28.5	28.1	31.7	0.46	0.08	0.014	<0.001	29.4	29.5	0.40	0.71	0.009
daily protein deposition (Pd), g/d	118.2	97.7	146.5	137.5	6.16	0.001	<0.001	0.06	0.13	0.12	5.67	0.39	0.33
Energy balance:													
ME intake [5], MJ/d	35.3	35.5	45.1	43.7	0.67	0.86	<0.001	0.15	39.4	40.4	0.47	0.08	0.035
ME for growth [6], MJ/d	17.0	15.2	25.9	23.4	0.74	0.12	<0.001	0.039	20.2	20.7	0.43	0.23	0.19
for Pd	5.2	4.3	6.5	6.1	0.27	<0.001	<0.001	0.06	5.6	5.5	0.25	0.39	0.34
for Ld	11.8	10.9	19.4	17.3	0.74	0.40	<0.001	0.07	14.6	15.2	0.47	0.13	0.26
ME for maintenance [7], MJ/kg$^{0.60}$	0.981	1.091	1.029	1.036	0.021	<0.001	0.08	0.79	1.02	1.04	0.015	0.34	0.001

[1] The rearing strategies were as follows: C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW.
[2] SEM: pooled standard error of the mean.
[3] Estimated from empty BW and backfat thickness according to Schiavon et al. [17].
[4] Estimated from fat-free empty BW (FFEBW = EBW - body lipid) using allometric relationships of body protein with body water and ash (NRC [1]). See Figure 4 for the form of the sex × treatment interaction for the final body protein mass.
[5] Computed from measured feed intake (FI) and tabulated ME content of feed ingredients (NRC [1]). The form of the sex × treatment interaction was similar to that of DMI given in Figure 1.
[6] Computed assuming a requirement of 44.4 and 52.3 MJ/kg of protein and lipid retained, respectively (NRC [1]).
[7] ME used for maintenance computed as (ME intake - ME for Ld - ME for Pd)/average metabolic weight.

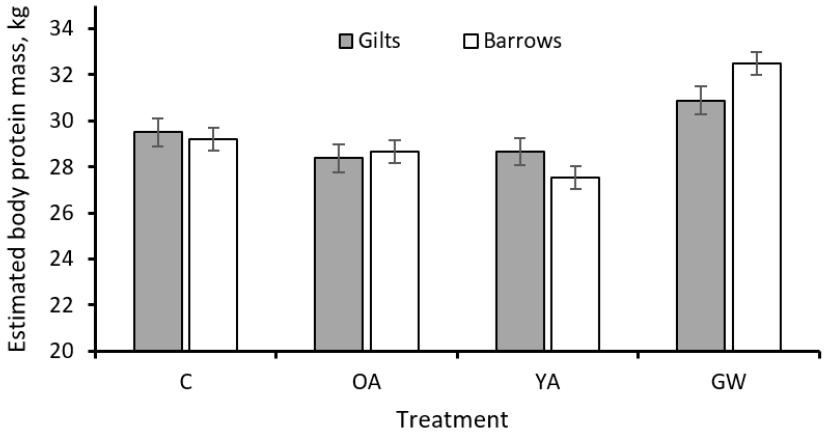

Figure 4. Estimated final body protein mass of C21 Goland barrows and gilts according to different treatments (n = 224, sex × treatment interaction p = 0.009). C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW.

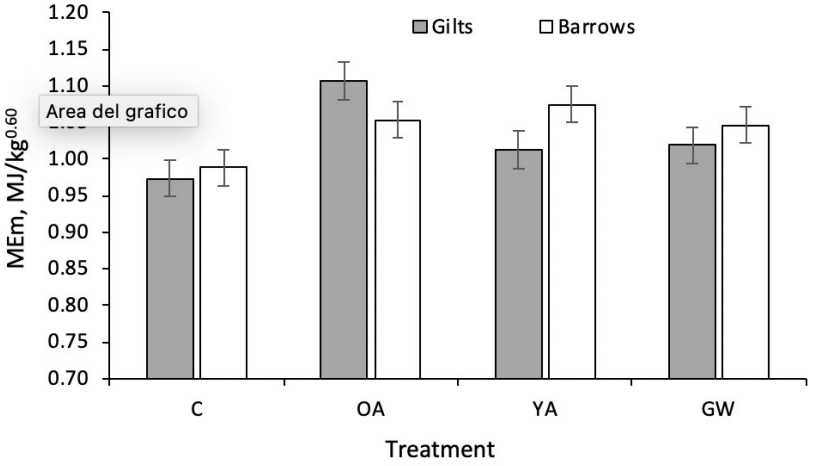

Figure 5. Estimated metabolizable energy used for maintenance (MEm) of C21 Goland barrows and gilts according to different treatments (n = 224, sex × treatment interaction p = 0.001). C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW.

3.3. SID Lysine Partitioning and Efficiencies of Utilization

Despite the DMI being the same between the OA and C treatments, the former reduced the total (p = 0.007) and the daily marginal SID lysine intake (p = 0.006) compared to the OA treatment due to the low level of dietary SID lysine in the LP feeds (Table 5). However, when expressed as per gram of estimated Pd, the marginal SID lysine intake of pigs fed OA was similar to that of the C pigs. All the pigs undergoing these two treatments had a SID lysine shortage compared to their estimated requirement. The estimated SID lysine requirement for Pd of the OA group was about 17% lower than that for the C group (p < 0.001) due to the SID lysine restriction in the LP diet. The marginal efficiency of SID lysine utilization for Pd increased significantly by 11% (p = 0.009) as a consequence of the SID lysine restriction in the OA compared to C treatment.

Table 5. Lysine partitioning and efficiencies of standardized ileal digestible (SID) lysine utilization of the C21 Goland heavy pigs subjected to different rearing strategies [1].

	Feeding Strategy					*p* Values			Sex			*p* Value	
Item	**C**	**OA**	**YA**	**GW**	**SEM [1]**	**C vs. OA**	**C vs. YA**	**YA vs. GW**	**Gilts**	**Barrows**	**SEM**	**Sex**	**Sex × Treatment**
SID lysine intake [2], g/d	14.6	11.3	24.3	23.0	0.70	0.007	<0.001	0.21	18.0	18.6	0.38	0.05	0.007
SID lysine marginal intake [2],													
per day, g/d	12.9	9.6	22.3	21.0	0.68	0.006	<0.001	0.05	16.2	16.7	0.37	0.049	0.041
per gram of protein deposited, g/g	0.110	0.099	0.154	0.155	0.009	0.14	<0.001	0.89	0.126	0.133	0.008	0.002	0.022
SID lysine consumed in excess [3], g/d	−1.48	−2.26	4.36	4.19	1.14	0.35	<0.001	0.84	0.85	1.55	1.03	0.002	0.21
Lysine losses and retention [4], g/d:													
basal GIT losses	1.08	1.09	1.36	1.32	0.02	0.77	<0.001	0.15	1.20	1.23	0.01	0.08	0.035
integumental losses	0.17	0.17	0.17	0.19	0.001	0.51	0.81	<0.001	0.18	0.18	0.001	0.07	0.51
retained	8.39	6.94	10.40	9.76	0.44	<0.001	<0.001	0.06	8.94	8.81	0.40	0.39	0.34
SID lysine requirement [5], g/d:													
maintenance	1.67	1.68	2.05	2.01	0.03	0.81	<0.001	0.31	1.83	1.87	0.02	0.07	0.029
protein deposition (Pd)	14.37	11.86	17.92	16.81	0.77	<0.001	<0.001	0.06	15.32	15.16	0.71	0.53	0.18
total	16.04	13.54	18.82	19.97	0.76	<0.001	<0.001	0.06	17.16	17.03	0.67	0.65	0.15
SID lysine efficiencies [6]:													
total efficiency	0.576	0.616	0.432	0.428	0.024	0.050	<0.001	0.83	0.522	0.504	0.02	0.007	0.81
marginal efficiency	0.650	0.725	0.472	0.469	0.025	0.009	<0.001	0.91	0.566	0.538	0.016	0.032	0.66

[1] The rearing strategies were as follows: C, control pigs fed restricted diets limiting ME supply up to 170 kg in slaughter weight (SW); YA, pigs fed unlimited amounts of ME and protein ad libitum up to 170 kg in SW; GW, pigs fed unlimited amounts of ME and protein ad libitum up to 200 kg in SW; OA, pigs fed restricted diets limiting ME and protein supply up to 170 kg in SW. SEM: pooled standard error of the mean.
[2] SID lysine computed from feed intake and dietary SID lysine content (NRC [1]). The form of the sex × treatment interaction was similar to that of DMI given in Figure 1. Marginal intakes were computed as SID lysine intake - SID lysine requirement for maintenance (NRC, 2012). The resulting amount was as expressed per day and per gram of estimated protein deposition.
[3] SID lysine consumed in excess of the requirement was computed as SID lysine intake - the SID lysine requirement for maintenance and protein deposition (NRC [1]).
[4] Basal gastrointestinal and integumental losses of lysine were estimated from dry matter intake and metabolic weight $BW^{0.75}$), as indicated by NRC [1]. The form of the sex × treatment interaction was similar to that of DMI given in Figure 1. However, the magnitude of the differences was negligible. SID lysine retained was assumed to be 0.071 of protein gain (NRC [1]).
[5] SID lysine requirements for maintenance and protein gain were computed according to NRC [1]. The form of the sex × treatment interaction was similar to that of DMI given in Figure 1. However, the magnitude of the differences was negligible. [6] Total efficiency was computed as lysine retained/SID lysine intake. Marginal efficiency was computed as lysine retained/SID lysine marginal intake.

Compared to the C treatment, total SID lysine and marginal intakes were higher ($p < 0.001$) in the YA pigs with SID lysine remarkably above the estimated requirements. Thus, the total and the marginal efficiencies of SID lysine utilization for Pd of the YA pigs were much lower (27%) compared to the C pigs ($p < 0.001$).

Differences between YA and GW pigs for the SID lysine partitioning were, in general, not significant or negligible, except for the daily SID lysine marginal intake ($p = 0.05$). The lower SID lysine marginal intake of the GW group was in part due to the reduction in the daily DMI during the last part of the finishing period (Figure 6) and to the increased average metabolic weight over the extended late-finishing period.

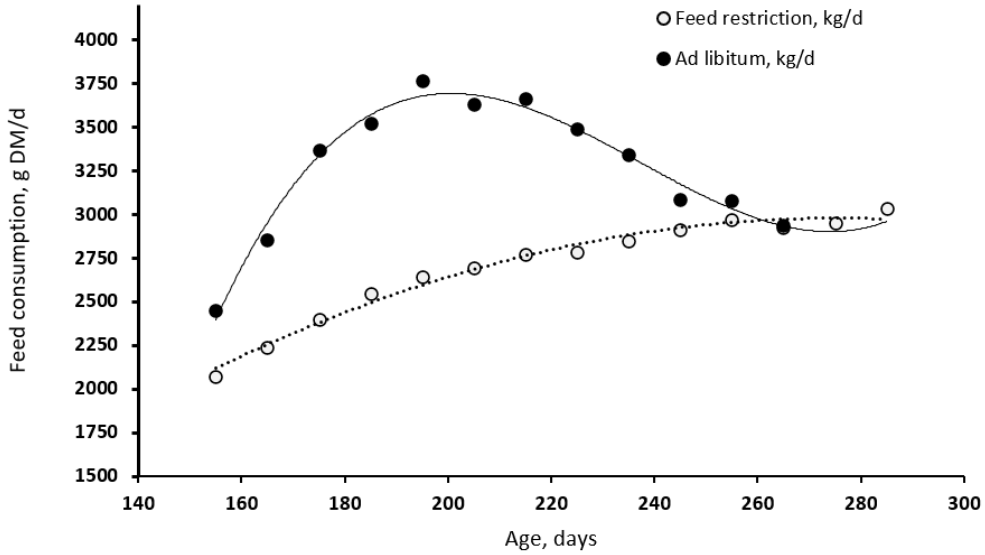

Figure 6. Daily feed DM consumption of the C21 Goland pigs fed ad libitum or restricted with increasing days of age. Each point represents a mean of 1176 to 1456 individual daily observations ($n = 224$).

Compared to the barrows, gilts had greater SID lysine marginal intake, both when expressed in absolute terms ($p = 0.049$) and per gram of Pd ($p = 0.002$). Conversely, the gilts showed a slightly lower total ($p = 0.007$) and the marginal efficiency of SID lysine utilization for Pd compared to barrows ($p = 0.032$). Significant sex × treatment interaction was observed for the SID lysine intakes ($p = 0.007$) and daily marginal intakes ($p = 0.041$), due to the higher DMI of the barrows in the GW treatment compared to gilts. The form of these interactions was similar to that observed for DMI (Figure 1), but the magnitude of the differences was negligible.

4. Discussion

4.1. Growth Performance and Dry Matter

We recently proposed that alternative rearing strategies could offer benefits in terms of growth performance and ham quality compared to the traditional C treatment [5]. The reader is invited to refer to this companion paper for a detailed discussion about the implication of the various rearing strategies on the growth performance, carcass and ham quality of the pigs under investigation [5]. However, it is important to mention in the current paper that:

(i) The range of BW studied is much heavier than that commonly practised in fresh meat production. Note, for instance, that nutrient recommendations for growing pigs heavier than 140 kg in BW are not currently available [1,30].

(ii) With the current C feeding regime, the degree of DMI or energy restriction was remarkable and in the order of 20%, similar to what is practised in some regions of Spain for dry-cured ham production [31].

(iii) A rearing practice based on protein restriction, in addition to the energy restriction, decreased the daily EBW gain and increased the duration of feeding for the target SW, but it had a small influence on the in vivo backfat depth compared to the C treatment.

(iv) C21 Goland pigs receiving the YA treatment evidenced a remarkable increase in EBW gain (0.939 kg/d) and backfat depth compared to the C treatment, despite the heavy range of BW (90–170 kg). Differences among individual pigs cannot be fully expressed when the pigs are kept on a restricted feeding regime, as the major factor limiting the growth is the energy and nutrient supply [32]. Therefore, the response of an EBW gain when shifting pigs from a restricted to ad libitum feeding strategy would largely depend on the growth characteristics of the pig genotype used [21]. This implies that moving from a restricted to voluntary feeding regime would lead to a greater heterogeneity among pigs intended for dry-cured ham production [23].

(v) Data from the current experiment evidenced that the voluntary DMI of the YA and GW pigs peaked at 3.750 kg/d at about 190 d of age, with a decline of 11.9 g/d (0.17 MJ/d of ME) thereafter. Assuming that voluntary feed intake is determined by the pig's attempt to fulfil its energy demands [33], the decline would represent the progressive decrease in the ME demand for growth with increasing maturity. However, this is partially counterbalanced by an increase in the demand for MEm.

(vi) Sex had some influence on growth performance. The barrows had greater cumulative DMI (+3.0%) and final backfat depth (+5.7%) but similar EBW gain compared to gilts. This suggests that barrows had a greater propensity for body fatness than gilts, in agreement with previous literature [34,35]. Similarly, few differences between barrows and gilts were observed in the same breed of pigs in a different study [10]. In our current work, we expected a greater difference between sex, because the ad libitum feeding regime would exploit the propensity for the growth of the various body parameters measured. However, this expectation was evidenced between barrows and gilts under the GW but not YA strategy. This is attributed to the effect of greater SW and SA resulting from the GW treatment, thus, suggesting that the differentiation between barrows and gilts would become more evident after 8 months of age and >170 kg in BW under unlimited feeding conditions.

4.2. Chemical Body Composition Estimates

The knowledge of energy and nutrient intake and body chemical composition changes is essential for estimating nutrient partitioning and requirements. Nutritional recommendations for maintenance are commonly based on the knowledge of metabolic weight or the body protein mass, while those for growth are based on Pd and Ld [1,21,36]. Recommendations have been developed based on comparative slaughter experiments with groups of pigs slaughtered at different ages [37,38]. Under practical conditions, the slaughtering of pigs is not feasible, for reasons such as time and costs. In pigs, reasonable estimates of body composition can be achieved from measurements of BW and BF depth [17,39]. This is achieved through repeated measurements on individual pigs from a given population. Earlier, it was proposed that the allometric relationships between body components (such as body water, body protein, body ash, etc.) are easily computed once the BL is estimated [1,20]. However, it is crucial to note that good estimates of body composition are dependent on the accuracy and precision of the equation that estimates the BL from BW and BF depth measurements. This is due to the variability between equations proposed by different researchers with respect to the difference in pig genotype, BW range, feeding conditions, environment and so on.

For instance, an equation for BL estimation proposed by Kloareg et al. [29] suggests that for each mm of BF depth increase, the BL percentage increases by 0.0113, while that of Schiavon et al. [17], suggests that the BL percentage increases by 0.007 for each mm increase in BF depth. Such discrepancies lead to strong differences in the estimation of body composition. In the current experiment, we used the equation proposed by Schiavon et al. [17] developed using data from comparative slaughter experiments conducted in the UK and Italy on pigs of different genotypes and sexes, with BW ranging from 12 to 200 kg fed ad libitum or with restricted feeds differing in nutrient contents. Our current results indicate that the estimated BL mass was linearly correlated to the weights of the fat tissues at slaughter (R^2 = 0.788). Additionally, the estimated MEm and the SID lysine maximum marginal efficiency of utilization for Pd obtained in the current paper are consistent with the values obtained by other authors [1,15]. Therefore, this suggests that the procedure adopted in the current paper for estimation of body composition changes was reliable for practical application.

Among treatments, the estimated final body protein mass ranged from 28.1 to 31.7 kg. A protein mass ranging from 32 to over 50 kg has been suggested for mature pigs, with the highest values for pigs belonging to the nucleus of improved genotypes [21,40]. Compared to the C treatment, the OA treatment had no impact on the estimated final body protein mass, despite the lower daily Pd, as the pigs of this treatment had more time to complete their protein growth. Conversely, when exposed to the YA conditions, the pigs accumulated 4.4% less final body protein mass compared to the C treatment, despite the greater daily Pd, because of the shorter duration on feed. Results also indicate that the C21 Goland pigs at 170 kg in SW and 8 months of age (YA) had not reached their mature body protein mass, as when kept on feed for one additional month (GW vs. YA), they demonstrated an increase in body protein mass of 13% and Pd in the order of 116 g/d.

The various treatments had a strong impact on the estimated final whole-body lipid mass, with values in the order of 41–43 kg for C and OA pigs and 46.1–53.4 kg for YA and GW pigs. The body fatness status, expressed as the ratio between the final lipid and protein masses, ranged between 1.4–1.5 and 1.6–1.7 for the C and OA and the YA and GW groups, respectively. For the ham-producing industry, the greater body fatness status of the latter groups is an indication that ad libitum feeding can increase their profitability and reduce the incidence of pigs that are too lean at slaughter, which does not meet the quality standards [2].

When provided with low-essential dietary AA contents, pigs balance their nutrient demands by increasing their feed intake to meet their potential for Pd [16]. Thus, an increased feed intake causes an extra amount of energy intake, which results in an extra fat deposition. However, this does not necessarily reflect the pigs' genotypic characteristics for fat deposition, but rather the interaction between the pigs' genotype, the energy density and the AA-to-energy ratio of the diet. In contrast, under unlimited energy and AA supply, pigs can express their potential for both Pd and Ld [11,12]. In our current experiment, the HP diets were formulated to be unlimited in energy and AA, and we intended to stimulate the Goland C21 pigs under YA and GW treatments to express their potential for both Pd and Ld, without the confounding effects related to the energy and AA densities of the feeds [18,21]. Thus, the voluntary DMI and the resulting final lipid-to-protein ratio achieved by the YA and GW pigs in the current experiment would represent the desire of the C21 Goland pigs to attain a given body fatness status, with modest influences due to the characteristics of the feeding resource [11,12].

4.3. Estimated Daily Protein Depositions

Experiments conducted with heavy pigs under conventional feeding regimes revealed estimated Pd in the order of 100 ± 20 g/d [41–43], in agreement with the estimates (118 g/d) achieved for the C treatment in the present study. Previous studies using heavy pigs (80–170 kg in BW) of various genotypes fed restricted diets have repeatedly investigated the effects of the reduction in SID lysine levels up to 4.8 and 3.5 g/kg when fed in early and late-finishing periods, respectively. These reductions were found not to influence growth performance, carcass quality or the dry curing aptitude of the fresh hams, and these studies failed to evidence an effect on Pd [10,22,44]. In the present study, the C dietary SID lysine densities were kept at 6.0 and 5.0 g/kg DM (5.4 and 4.5 g/kg as fed) in the early and late-finishing periods, respectively. This suggests that the SID lysine supplied in the C diet would have been adequate. On the other hand, the restricted feeding conditions could have limited the Pd of the pigs. Some of the literature indicated that even with an adequate AA supply, the partitioning of dietary ME between Pd and Ld could be influenced by the ME [37]. This issue is debated, and discrepancies remain about the actual influence of ME supply on Pd and Ld in the existing literature.

For instance, the model proposed by InraPorc assumes that the response of Pd to the ME supply follows a curvilinear plateau function [15], while NRC [1] suggested a linear plateau, with slopes that decrease with increasing BW. In our current experiment, we found that the C treatment induced a 19% reduction in Pd (119 g/d) compared to 147 g/d when YA was provided. In our C-fed pigs, the SID lysine marginal intake averaged 0.110 g/g Pd. This value was lower than the 0.125 g/g Pd requirement suggested by NRC [1] for pigs 120 kg in BW. This might suggest that in our current experiment the Pd of the C pigs was primarily reduced as a consequence of the inadequate SID lysine supply, but this does not exclude the possibility that a restricted ME intake could have limited Pd in pigs subjected to an energy restriction.

The OA pigs had similar DMI as C pigs with a remarkable reduction in Pd (119 to 97 g/d) which could be entirely attributed to the low SID lysine supply with the LP feeds (4.2 and 3.2 g/kg DM in the early and late-finishing period, respectively) compared to the MP. This restriction resulted in further marginal SID lysine intake reduction from 0.110 to 0.098 g/g Pd. This outcome is consistent with Wecke and Liebert [45] who reported a need for 0.100 g/g Pd of SID lysine marginal intake for pigs 15 to 110 kg in BW, despite the BW difference in our current investigation (90 to 170 kg in BW).

The estimated Pd of the YA pigs averaged 147 g/d, similar to previous studies conducted on modern pig genotypes, but for lighter BW ranges [21,45]. As the pigs were managed under unlimited, ambient feeding and health conditions, we could infer that the obtained value of 147 g/d might be close to the actual potential Pd of the C21 Goland genotype. According to NRC [1] and other studies [21,45], the point of maximum Pd, about 150 g/d, falls between 50 and 75 kg in BW, such that the pigs of our current experiment 90 to 200 kg in BW fell in the region of declining Pd. Extending the SA with GW, the final body protein mass increased by about 12.8%, but the daily Pd was slightly reduced (5.5%) compared to YA. Such a declining trend in Pd with increasing slaughter BW/age is consistent with pre-existing observations that the Pd rate decreases with increasing age after a point of maximum Pd, up to the attainment of mature-body protein mass [11,12,20].

Overall, the Pd estimates achieved in the present study suggest that the Goland C 21 pig genotype has good potential for Pd even at heavy BW, as is expected for modern pig genotypes.

4.4. Metabolizable Energy Requirements and Partitioning

The estimated MEm requirement of the pigs of our current study averaged 1.03 MJ/kg$^{0.60}$, in agreement with the value of 1.02 MJ/kg$^{0.60}$ proposed by Noblet et al. [46], although some variation across different genotypes and sexes were expected. Similarly, Milgen et al. [47] suggested that the fasting heat production of lean pigs is close to 0.962 MJ/kg$^{0.60}$, or slightly more because of physical activity and thermoregulation. NRC [1] suggests a standard maintenance requirement of 0.824 MJ/kg$^{0.60}$, with additional energy for thermogenesis, increased physical activity and genotype adjustment. Estimation of MEm, as a difference between ME intake and the ME used for growth, is influenced by the estimated Pd and Ld, and thus by the assumed ME efficiency for protein (kp) and lipid (kl) deposition. The values of kp and kl found by Noblet et al. [46] were 0.62 and 0.84, respectively. In the current study, the lower kp and kl values used, 0.53 and 0.75, were selected according to NRC [1].

The use of these coefficients implies that the calculated MEm values of the current research could have been greater than the values achievable using the Noblet et al. [46] partial efficiencies. On the other hand, NRC [1] also reported that kp and kl can considerably vary from 0.36 to 0.57 and 0.57 to 0.81, respectively. Therefore, despite the heavier weight of pigs in our present study, we achieved a

MEm estimate consistent with existing research in the literature. This suggests that our MEm estimates can be applied for heavier BW (>170 kg), which were not accounted for by the NRC [1].

Furthermore, there was an indication that MEm may increase under the condition of energy and protein restriction (OA compared to C). To our knowledge, no existing pieces of evidence reported that such a restriction strategy would increase the MEm requirement. However, such a result must be treated with caution, as the chemical body masses of the pigs in this current research were estimated from simple body measurements of BW and BF depth. Some evidence indicates that diets with insufficient indispensable AA contents increase intramuscular fat with little influence on the BF depth [48]. For this reason, a greater MEm utilization in the OA pigs in the current research could also be attributed to an increased intramuscular fat that was not entirely captured by the simple body measurements of BW and BF depth. On the other hand, our current research suggests that the need for MEm is not influenced by feed (energy) restriction or by increased SW. We can also infer that the NRC [1] MEm requirement of 1.02 MJ/kg$^{0.60}$ is applicable for pigs weighing more than 140 kg in BW regardless of the restricted feeding regime.

No relevant differences were evidenced between barrows and gilts in body composition changes in terms of Pd and ME intake and ME partitioning. This agreed with our previous studies [23], where little influence from sex or sex interaction on the partitioning of ME intake towards Pd, Ld and maintenance was observed.

4.5. SID Lysine Partitioning and Efficiency

According to NRC [1], deposited protein contains 7.1% lysine, and for maintenance, the efficiency of lysine utilization is 0.75 while the maximum efficiency of SID lysine utilization for Pd declines with BW from 0.682 (20 kg in BW) to 0.568 (120 kg in BW). These efficiencies are equivalent to a requirement of 10.4 and 12.5 g SID lysine per 100 g of Pd at 20 and 120 kg in BW, respectively. Dourmad et al. [49] investigated pigs fed ad libitum from 50 to 110 kg in BW and reported that the marginal efficiency of SID lysine utilization ranged between 0.65 and 0.70, which is equivalent to a requirement of 10.0–10.9 g/100 g Pd. Similarly, Wecke and Liebert [45] reported that 17–18 g/d SID lysine was required for 170 g/d Pd.

In our current study, the YA and the GW pigs consumed SID lysine well in excess compared to the estimated requirement based on the NRC [1] equation. Thus, the estimated total and marginal efficiency of SID lysine utilization for Pd are of little biological significance, and no comparison with the other treatments can be discussed. In contrast, it was estimated that the SID lysine intake of both the C and the OA pigs was below the estimated requirements. Under such conditions, the marginal efficiency of SID lysine utilization for Pd reflects the efficiency of the pig to utilize the SID lysine in protein accretion.

The C pigs fed a restricted medium-protein diet evidenced a marginal efficiency of SID lysine utilization of 0.650. This corresponds to a SID lysine requirement of about 10.9 g/100 g of Pd. Similarly, the marginal efficiency of SID lysine utilization for Pd of the OA pigs averaged 0.725, which corresponds to a requirement of 9.8 g/100 g Pd. This value is similar to that proposed by the InraPorc model, which is assumed to be constant throughout growth [15]. In contrast, NRC [1] reported that the marginal efficiency of SID lysine utilization for Pd declines with increasing BW. On the contrary, the results we obtained in the current experiment suggest that the marginal efficiency of SID lysine utilization for Pd does not change with increasing BW, in agreement with the InraPorc model [15]. This information is valuable in defining feeding strategies and formulating diets optimised in terms of AA supply according to the desired growth and the optimal body fatness statuses at slaughter for heavy pigs. Regarding the effect of sex, no relevant difference in the marginal efficiency of SID lysine utilization for Pd was observed.

5. Conclusions

The modelling approach, based on repeated BW and BF measurements, used in the current experiment could have a practical application in estimating the ME and the amino acid requirement of growing pigs through extended BW and feeding conditions. We found that the energy restriction had little or no influence on the estimated MEm. This study also confirmed that a MEm value of 1.02 MJ/kg$^{0.60}$ is applicable for pigs weighing 90 to 200 kg in BW, irrespective of the feeding regime. Our results suggest that under energy and protein restriction, the maximum marginal efficiency of SID lysine utilization for protein deposition (Pd) was 0.73. This corresponds to 9.8 g of SID lysine per 100 g of Pd, as a minimum requirement, irrespective of body weight (BW).

Author Contributions:

Conceptualization, S.S., I.H.M., P.C. and L.G.; methodology, S.S., I.H.M., D.G., P.C. and L.G.; software, S.S., I.H.M. and D.G.; validation, V.H.; formal analysis, S.S., D.G., G.G., L.R., V.H. and L.G.; investigation, S.S., D.G., P.C. and L.G.; resources, S.S. and L.G.; data curation, S.S., I.H.M., G.G. and L.R.; writing—original draft preparation, S.S., I.H.M. and D.G.; writing—review and editing, S.S., I.H.M., D.G., G.G., L.R., P.C., V.H. and L.G.; visualization, S.S. and I.H.M.; supervision, S.S., P.C., V.H. and L.G.; project administration, P.C. and L.G.; funding acquisition, P.C. and L.G. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by the European Union Rural Development program 2014-2020, Reg (CE) under grant numbers 1305/2013—PSR Veneto DGR 2175—December 23, 2016, interventions 16.1.1 and 16.2.2, code 3682902. The University of Padua also provided institutional funds (DOR2059255/20, DOR2120795/21, DOR1990028/19) and a three-year PhD grant for Isaac Malgwi.

Institutional Review Board Statement:

The experimental procedure was approved by the institutional animal care committee of the University of Padova. All procedures were conducted in compliance with European Union requirements, and guidelines on the protection of animals used for scientific and educational purposes were provided by the “Organismo preposto per il Benessere Animale, OPBA”, University of Padova (OPBA, approval document #36/2018).

Informed Consent Statement: Not applicable.

Data Availability Statement:

The data supporting the findings of this study are available from Gorzagri s.s., but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from Gorzagri s.s.

Acknowledgements:

This research was supported by the University of Padova. The authors are indebted to the Progeo Feed industry for feed preparation and sampling, and Gorzagri s.s. for the provision of animals and technical support. The authors would like to thank Luca Carraro, Nadia Guzzo, Alberto Simonetto and all the herdsmen for their support in animal management and data recording. The authors also wish to thank Alessandro Ferretti for his help in managing the slaughtering operations at the slaughterhouse. Thanks also to Giuseppe Tonin and Massimo Pasqualotto for the evaluations carried out on the dressed hams the day after slaughtering.

Conflicts of Interest:

The authors declare no conflict of interest.

References

1. National Research Council. *Nutrient Requirements of Swine*, 11th ed.; National Academies Press: Washington, DC, USA, 2012; ISBN 978-0-309-22423-9.
2. Fiego, D.L.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107–114. https://doi.org/10.1016/j.meatsci.2004.06.010.
3. Latorre, M.A.; García-Belenguer, E.; Ariño, L. The effects of sex and slaughter weight on growth performance and carcass traits of pigs intended for dry-cured ham from Teruel (Spain). *J. Anim. Sci.* **2008**, *86*, 1933–1942. https://doi.org/10.2527/jas.2007-0764.
4. Gallo, L.; Bona, M.D.; Cecchinato, A.; Schiavon, S. Effect of growth rate on live performance, carcass and green thigh traits of finishing Italian heavy pigs. *Ital. J. Anim. Sci.* **2017**, *16*, 652–658. https://doi.org/10.1080/1828051x.2017.1318037.
5. Malgwi, I.H.; Gallo, L.; Halas, V.; Bonfatti, V.; Carcò, G.; Sasso, C.P.; Carnier, P.; Schiavon, S. The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits. *Animals* **2021**, *11*, 2447. https://doi.org/10.3390/ani11082447.
6. Malgwi, I.H.; Giannuzzi, D.; Gallo, L.; Halas, V.; Carnier, P.; Schiavon, S. Influence of Slaughter Weight and Sex on Growth Performance, Carcass Characteristics and Ham Traits of Heavy Pigs Fed Ad-Libitum. *Animals* **2022**, *12*, 215. https://doi.org/10.3390/ani12020215.
7. Rodríguez-Sánchez, J.A.; Sanz, M.A.; Blanco, M.; Serrano, M.P.; Joy, M.; Latorre, M.A. The influence of dietary lysine restriction during the finishing period on growth performance and carcass, meat, and fat characteristics of barrows and gilts intended for dry-cured ham production. *J. Anim. Sci.* **2011**, *89*, 3651–3662. https://doi.org/10.2527/jas.2010-3791.
8. Gallo, L.; Bona, M.D.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Effect of progressive reduction in crude protein and lysine of heavy pigs diets on some technological properties of green hams destined for PDO dry-cured ham production. *Meat Sci.* **2016**, *121*, 135–140. https://doi.org/10.1016/j.meatsci.2016.06.005.
9. Labussière, E.; van Milgen, J.; De Lange, C.F.; Noblet, J. Maintenance Energy Requirements of Growing Pigs and Calves Are Influenced by Feeding Level. *J. Nutr.* **2011**, *141*, 1855–1861. https://doi.org/10.3945/jn.111.141291.
10. Gallo, L.; Montà, G.D.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Growth performance of heavy pigs fed restrictively diets with decreasing crude protein and indispensable amino acids content. *Livest. Sci.* **2014**, *161*, 130–138. https://doi.org/10.1016/j.livsci.2013.12.027.
11. Ferguson, N.S.; Gous, R.M. Evaluation of pig genotypes Theoretical aspects of measuring genetic parameters. *Anim. Sci.* **1993**, *56*, 233–243. https://doi.org/10.1017/s0003356100021310.
12. Ferguson, N.S.; Gous, R.M. Evaluation of pig genotypes: Testing experimental procedure. *Anim. Sci.* **1993**, *56*, 245–249. https://doi.org/10.1017/s0003356100021322.
13. Wellock, I.J.; Emmans, G.C.; Kyriazakis, I. Describing and predicting potential growth in the pig. *Anim. Sci.* **2004**, *78*, 379–388. https://doi.org/10.1017/s1357729800058781.
14. Van Milgen, J.; Dourmad, J.-Y. Concept and application of ideal protein for pigs. *J. Anim. Sci. Biotechnol.* **2015**, *6*, 15. https://doi.org/10.1186/s40104-015-0016-1.
15. van Milgen, J.; Valancogne, A.; Dubois, S.; Dourmad, J.-Y.; Sève, B.; Noblet, J. InraPorc: A model and decision support tool for the nutrition of growing pigs. *Anim. Feed Sci. Technol.* **2008**, *143*, 387–405. https://doi.org/10.1016/j.anifeedsci.2007.05.020.
16. Schiavon, S.; Bona, M.D.; Carcò, G.; Carraro, L.; Bünger, L.; Gallo, L. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. *PLoS ONE* **2018**, *13*, e0195645. https://doi.org/10.1371/journal.pone.0195645.
17. Schiavon, S.; Gallo, L.; Carnier, P.; Tagliapietra, F.; Ceolin, C.; Prandini, A.; Piva, A. Use of simple body measurements and allometry to predict the chemical growth and feed intake in pigs. *Ital. J. Anim. Sci.* **2007**, *6*, 27–44. https://doi.org/10.4081/ijas.2007.27.
18. Carcò, G.; Gallo, L.; Bona, M.D.; Latorre, M.A.; Fondevila, M.; Schiavon, S. The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs. *PLoS ONE* **2018**, *13*, e0205572. https://doi.org/10.1371/journal.pone.0205572.
19. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* **2004**, *3*, 309–321. https://doi.org/10.4081/ijas.2004.309.

20. Ferguson, N.S.; Gous, R.M.; Emmans, G.C. Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *S. Afr. J. Anim. Sci.* **1994**, *24*, 10–17.
21. Kyriazakis, I.; Whittemore, C.T. *Whittemore's Science and Practice of Pig Production*; Kyriazakis, I., Whittemore, C.T., Eds.; Blackwell Publishing Ltd: Oxford, UK, 2006; ISBN 9780470995624.
22. Schiavon, S.; Carraro, L.; Bona, M.D.; Cesaro, G.; Carnier, P.; Tagliapietra, F.; Sturaro, E.; Galassi, G.; Malagutti, L.; Trevisi, E.; et al. Growth performance, and carcass and raw ham quality of crossbred heavy pigs from four genetic groups fed low protein diets for dry-cured ham production. *Anim. Feed Sci. Technol.* **2015**, *208*, 170–181. https://doi.org/10.1016/j.anifeedsci.2015.07.009.
23. Gallo, L.; Montà, G.D.; Carraro, L.; Cecchinato, A.; Carnier, P.; Schiavon, S. Carcass quality and uniformity of heavy pigs fed restrictive diets with progressive reductions in crude protein and indispensable amino acids. *Livest. Sci.* **2015**, *172*, 50–58. https://doi.org/10.1016/j.livsci.2014.11.014.
24. Van Soest, J.P.; Robertson, J.B.; Lewis, B.A. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *71*, 1587–1597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.
25. AOAC International. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2003.
26. Bouchard, J.; Chornet, E.; Overend, R.P. High-performance liquid chromatographic monitoring of carbohydrate fractions in partially hydrolyzed corn starch. *J. Agric. Food Chem.* **1988**, *36*, 1188–1192. https://doi.org/10.1021/jf00084a016.
27. 27. Council of Europe. Amino acid analysis. In *European Pharmacopoeia 5.0*; Humana Press: Strasbourg, France, 2005; Volume 5.0, pp. 86–92.
28. Slump, P.; Flissebaalje, T.D.; Haaksman, I.K. Tryptophan in food proteins: A comparison of two hydrolytic procedures. *J. Sci. Food Agric.* **1991**, *55*, 493–496. https://doi.org/10.1002/jsfa.2740550318.
29. Kloareg, M.; Noblet, J.; Van Milgen, J. Estimation of whole body lipid mass in finishing pigs. *Anim. Sci.* **2006**, *82*, 241–251. https://doi.org/10.1079/asc200529.
30. Wu, F.; Vierck, K.R.; DeRouchey, J.M.; O'Quinn, T.G.; Tokach, M.D.; Goodband, R.D.; Dritz, S.S.; Woodworth, J.C. A review of heavy weight market pigs: Status of knowledge and future needs assessment. *Transl. Anim. Sci.* **2017**, *1*, 1–15. https://doi.org/10.2527/tas2016.0004.
31. Serrano, M.P.; Valencia, D.G.; Fuentetaja, A.; Lázaro, R.; Mateos, G.G. Influence of feed restriction and sex on growth performance and carcass and meat quality of Iberian pigs reared indoors. *J. Anim. Sci.* **2009**, *87*, 1676–1685. https://doi.org/10.2527/jas.2008-0989.
32. Schiavon, S.; Carcò, G.; Sturaro, E.; Gallo, L.; Bona, M.D. Responses of Pigs of Different Genotypes to a Variation in the Dietary Indispensable Amino Acid Content in Terms of Their Growth and Carcass and Meat Quality Traits. *Animals* **2019**, *9*, 508. https://doi.org/10.3390/ani9080508.
33. Knap, P.W. *Voluntary Feed Intake in Pigs*; Torrallardona, D., Roura, E., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2009; ISBN 978-90-8686-096-8.
34. Latorre, M.A.; Lázaro, R.; Valencia, D.G.; Medel, P.; Mateos, G.G. The effects of gender and slaughter weight on the growth performance, carcass traits, and meat quality characteristics of heavy pigs. *J. Anim. Sci.* **2004**, *82*, 526–533. https://doi.org/10.2527/2004.822526x.
35. Latorre, M.A.; Olivares, A.; Callejo, A.; Rey, A.; Pérez-Ciria, L.; Bote, C.J.L.; Daza, A. A comparison of female and castrate pigs slaughtered at weights above and below 120 kg on carcass traits, intramuscular fat and fatty acid composition of carcasses intended for dry-cured ham and shoulder production. *Anim. Prod. Sci.* **2019**, *59*, 1923–1930. https://doi.org/10.1071/an18267.
36. Naatjes, M.; Susenbeth, A. Energy requirement of growing pigs under commercial housing conditions. *Arch. Anim. Nutr.* **2014**, *68*, 93–110. https://doi.org/10.1080/1745039x.2014.887814.
37. Quiniou, N.; Noblet, J.; Dourmad, J.-Y. Effect of energy intake on the performance of different types of pig from 45 to 100 kg body weight. 2. Tissue gain. *Anim. Sci.* **1996**, *63*, 289–296. https://doi.org/10.1017/s1357729800014843.
38. Garcia-Valverde, R.; Barea, R.; Lara, L.; Nieto, R.; Aguilera, J. The effects of feeding level upon protein and fat deposition in Iberian heavy pigs. *Livest. Sci.* **2008**, *114*, 263–273. https://doi.org/10.1016/j.livsci.2007.05.005.

39. Kloareg, M.; Noblet, J.; van Milgen, J. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *Br. J. Nutr.* **2007**, *97*, 35–44. https://doi.org/10.1017/s0007114507205793.
40. Ferguson, N.; Kyriazis, S. Evaluation of the growth parameters of six commercial crossbred pig genotypes Under commercial housing conditions in individual pens. *South Afr. J. Anim. Sci.* **2003**, *33*, 11–20. https://doi.org/10.4314/sajas.v33i1.3732.
41. Manini, R.; Piva, A.; Prandini, A.; Mordenti, A.; Piva, G.; Dourmad, J. Protein retention in Italian heavy pigs: Development of a factorial approach for the determination of lysine requirement. *Livest. Prod. Sci.* **1997**, *47*, 253–259. https://doi.org/10.1016/s0301-6226(96)01413-3.
42. Tagliapietra, F.; Ceolin, C.; Schiavon, S. On-farm estimation of pig growth parameters from longitudinal data of live weight and feed consumption and the use of a mathematical model. *Ital. J. Anim. Sci.* **2005**, *4*, 116–118. https://doi.org/10.4081/ijas.2005.3s.116.
43. Galassi, G.; Colombini, S.; Malagutti, L.; Crovetto, G.; Rapetti, L. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. *Anim. Feed Sci. Technol.* **2010**, *161*, 140–148. https://doi.org/10.1016/j.anifeedsci.2010.08.009.
44. Galassi, G.; Malagutti, L.; Colombini, S.; Rapetti, L.; Gallo, L.; Schiavon, S.; Tagliapietra, F.; Crovetto, G.M. Nitrogen and Energy Partitioning in Two Genetic Groups of Pigs Fed Low-Protein Diets at 130 kg Body Weight. *Ital. J. Anim. Sci.* **2015**, *14*, 293–298. https://doi.org/10.4081/ijas.2015.4012.
45. Wecke, C.; Liebert, F. Lysine requirement studies in modern genotype barrows dependent on age, protein deposition and dietary lysine efficiency. *J. Anim. Physiol. Anim. Nutr.* **2009**, *93*, 295–304. https://doi.org/10.1111/j.1439-0396.2009.00923.x.
46. Noblet, J.; Karege, C.; Dubois, S.; Van Milgen, J. Metabolic utilization of energy and maintenance requirements in growing pigs: Effects of sex and genotype. *J. Anim. Sci.* **1999**, *77*, 1208–1216. https://doi.org/10.2527/1999.7751208x.
47. Van Milgen, J.; Bernier, J.F.; Lecozler, Y.; Dubois, S.; Noblet, J. Major determinants of fasting heat production and energetic cost of activity in growing pigs of different body weight and breed/castration combination. *Br. J. Nutr.* **1998**, *79*, 509–517. https://doi.org/10.1079/bjn19980089.
48. Wood, J.; Lambe, N.; Walling, G.; Whitney, H.; Jagger, S.; Fullarton, P.; Bayntun, J.; Hallett, K.; Bunger, L. Effects of low protein diets on pigs with a lean genotype. Carcass composition measured by dissection and muscle fatty acid composition. *Meat Sci.* **2013**, *95*, 123–128. https://doi.org/10.1016/j.meatsci.2013.03.001.
49. Dourmad, J.; Guillou, D.; Sève, B.; Henry, Y. Response to dietary lysine supply during the finishing period in pigs. *Livest. Prod. Sci.* **1996**, *45*, 179–186. https://doi.org/10.1016/0301-6226(96)00004-8.

Chapter 4

Genes Related to Fat Metabolism in Pigs and Intramuscular Fat Content of Pork: A Focus on Nutrigenetics and Nutri-genomics

I. H. Malgwi [1,*], V. Halas [2], P. Grünvald [2], S. Schiavon [1] and I. Jócsák [3]

[1] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell' Università 16, 35020 Legnaro (PD), Italy

[2] Department of Farm Animal Nutrition, Kaposvár Campus, Hungarian University of Agricultuthe re and Life Sciences, Guba Sándor Utca 40, 7400 Kaposvár, Hungary

[3] Institute of Agronomy, Kaposvár Campus, Hungarian University of Agriculture and Life Sciences, Guba Sándor Utca 40, 7400 Kaposvár, Hungary

Published in: *Animals* 2022, *12*(2), 150; https://doi.org/10.3390/ani12020150

Genes Related to Fat Metabolism in Pigs and Intramuscular Fat Content of Pork: A Focus on Nutrigenetics and Nutrigenomics

I. H. Malgwi [1,*], V. Halas [2], P. Grünvald [2], S. Schiavon [1] and I. Jócsák [3]

Simple Summary:

The intramuscular fat (IMF) or marbling is an essential pork sensory quality that influences the preference of the consumers and premiums for pork. IMF is the streak of visible fat intermixed with the lean within a muscle fibre and determines sensorial qualities of pork such as flavour, tenderness, and juiciness. Fat metabolism and IMF development are controlled by dietary nutrients, genes, and their metabolic pathways in the pig. Nutrigenetics explains how the genetic make-up of an individual pig influences the pig's response to dietary nutrient intake. Differently, nutrigenomics is the analysis of how the entire genome of an individual pig is affected by dietary nutrient intake. The knowledge of nutrigenetics and nutrigenomics, when harmonised, is a powerful tool in estimating nutrient requirements for swine and programming dietary nutrient supply according to an individual pig's genetic make-up. The current paper aimed to highlight the roles of nutrigenetics and nutrigenomics in elucidating the underlying mechanisms of fat metabolism and IMF deposition in pigs. This knowledge is essential in redefining nutritional intervention for swine production and the improvement of some economically important traits such as growth performance, backfat thickness, IMF accretion, disease resistance etc., in animals.

Abstract:

Fat metabolism and intramuscular fat (IMF) are qualitative traits in pigs whose development is influenced by several genes and metabolic pathways. Nutrigenetics and nutrigenomics offer prospects for estimating nutrients required by a pig. The application of these emerging fields in nutritional science provides an opportunity for matching nutrients based on the genetic make-up of the pig for trait improvements. Today, integration of high throughput "omics" technologies into nutritional genomics research has revealed many quantitative trait loci (QTLs) and single nucleotide polymorphisms (SNPs) for the mutation(s) of key genes directly or indirectly involved in fat metabolism and IMF deposition in pigs. Nutrient–gene interaction and the underlying molecular mechanisms involved in fatty acid synthesis and marbling in pigs are difficult to unravel. While existing knowledge on QTLs and SNPs of genes related to fat metabolism and IMF development is yet to be harmonised, the scientific explanations behind the nature of the existing correlation between the nutrients, the genes and the environment remain unclear, being inconclusive or lacking precision. This paper aimed to: (1) discuss nutrigenetics, nutrigenomics and epigenetic mechanisms controlling fat metabolism and IMF accretion in pigs; (2) highlight the potentials of these concepts in pig nutritional programming and research.

Keywords: epigenetics; fat metabolism; genes; intramuscular fat; nutrigenetics; nutrigenomics; pigs

1. Introduction

The intramuscular fat (IMF) or marbling is an essential pork sensory quality that influences the preference of the consumers and premiums for pork. Marbling is the streak of visible fat intermixed with the lean within a muscle fibre which varies with the breed (genetics), age, sex, nutrition, muscle type and muscle location [1,2]. From an economic viewpoint, the pork industry is faced with increasing lean pig genotypes characterized by reduced IMF content which has a minimum range between 2.2% and 3.4% [3]. As such, strategies to optimise fat deposition traits in pigs have been extensively researched [4–8]. Improving the quality of the fatty acid profile and IMF content of pork is a major interest to swine nutritionists, breeders and geneticists for health and economic reasons [9]. This remains critical to the industry. Fat metabolism and marbling are multiplex traits regulated by several genes which are directly or indirectly involved in fatty acid metabolism, cell proliferation and differentiation [10–12]. An approach to unwinding the expression pattern of lipid metabolism genes and the molecular mechanisms behind IMF deposition is being researched [13–17].

Nutrigenetics and nutrigenomics are distinct fields providing a holistic approach to unravelling how nutrient intake affects the entire genome response and molecular mechanisms involved in fat deposition [18–20]. Nutrigenetics and nutrigenomics as fields of nutritional genomics research integrate computational systems biology (bioinformatics) with high-throughput functional genomic technologies (transcriptomics, proteomics, metabolomics, and muscle biochemistry) in understanding how the cellular pathways and the entire genome respond to nutritional programming in farm animals [7,21–24]. Several factors such as the genetic make-up of the pig, sex, age, dietary micronutrients, etc., and environmental conditions, influence fat metabolism and phenotypic responses in pigs [15–17]. For instance, studies have evidenced that the combined effects of nutrients in the diet and environmental conditions could result in up-regulation/down-regulation of one gene which will then sway the response of other genes, and in turn, alter the expression of these genes [25]. Additionally, the relationship between mRNA expression of lipid metabolism genes and nutrient availability during transcription could be linear or quadratic and also depends on the ability of carrier proteins to recognize only one substance or group of similar substances in diets [25–28]. Furthermore, nutrients in the diet may be assembled at secondary metabolic pathways to alter substrate concentrations or act as ligands for transcription factors for genes involved in fatty acid metabolism [29,30]. Literature has suggested the existence of a genetic correlation between dietary nutrient intake and fat metabolism genes in pigs. [14–17]. In pigs, epigenetic mechanisms (DNA methylation and histone modification) are intermediaries influencing mechanisms of fat deposition and are sensitive to environmental factors and dietary nutrients [31,32]. Today, studies are evincing patterns of epigenetic mechanisms and molecular pathways that regulate gene expression (switching transcription on and off) in offspring, and the regulatory effects of messenger ribonucleic acids RNAs (mRNAs) and microRNAs (miRNAs) in fat and IMF depositions in pigs [31–35].

The underlying molecular mechanisms involved in fatty acid synthesis and marbling in pigs are difficult to unravel. Existing quantitative trait loci (QTL) for genes and their mutations in lipogenesis, disease susceptibility and the development of other traits in pigs are yet to be harmonised. Studies on the role(s) of epigenetic mechanisms in transgenerational effects of nutrition and environment in adipocyte differentiation and development of traits in pigs are lacking. To date, these gaps still exist in the literature. The scientific explanations behind the nature of the existing correlation between the nutrients in the diet and genes remain unclear, being inconclusive or lacking precision. This review aimed to: (1) discuss the roles of nutrigenetics, nutrigenomics and epigenetic mechanisms controlling fat metabolism and IMF accretion in pigs; (2) highlight the potential application of these concepts in pig nutritional research in nutritional intervention for swine production and the improvement of economically important traits in animals.

2. Introduction to Nutrigenetics and Nutrigenomics

It is important to clearly distinguish between nutrigenetics and nutrigenomics as these two distinct terms are often confused. For the purpose of intelligibility of scientific communication and reports in these domains, it is important to define certain words used herein. “Nutri” or nutrient refers to chemical compounds in a diet needed for cellular functions. Genetics is the study of individual genes, whereas genomics is the study of the entire genome (the whole of an organism’s genes, their interactions, and how they are affected by the environment). Therefore, we could infer that a common relationship between nutrigenetics and nutrigenomics is diet–gene interaction.

Verbatim definitions of nutrigenetics and nutrigenomics as expressed by different authors are quoted below:

“Nutrigenetics is concerned with how genetic variation affects the interaction between these bioactive dietary components and the health and disease potential of individual persons while nutrigenomics is concerned with the effects of bioactive dietary components on the genome, proteome (the sum total of all proteins), and metabolome (the sum of all metabolites)” [36]. “Nutrigenetics focuses on the potential effects of single-nucleotide polymorphisms, copy number variants, epigenetic marks, and other genomic markers on the biological and behavioural responses to micronutrients, macronutrients, and calories whereas nutrigenomics has evolved to signify the field concerned by the investigation of the effects of nutrients on gene expression and related downstream molecular and biological events. Nutrigenomics will increasingly incorporate transcriptomics, proteomics, and metabolomics” [37]. “Nutrigenomics has evolved to signify the field concerned by the investigation of the effects of nutrients on gene expression and related downstream molecular and biological events while nutrigenomics will increasingly incorporate transcriptomics, proteomics, and metabolomics” [38]. “Nutrigenetics aims to understand how the genetic makeup of an individual coordinates the response to a diet while nutrigenomics offers a powerful and exciting approach to unravelling the effects of diet on health” [39]. “The term nutrigenetics refers to the impact of inherited traits on the response to a specific

dietary pattern, functional food or supplement on a specific health outcome while the term nutrigenomics refers to the effect of diet on gene expression" [40]. "Nutrigenetics includes the study of individual differences at the genetic level that sways individual responses to diet. These individual differences may be at the level of single nucleotide polymorphisms rather than at the gene level while nutrigenomics comprises the analysis of the effect of nutrient intake on the whole genome (complete genetic make-up, including epigenetic changes), the proteome (the sum total of all proteins), and the metabolome (the sum of all metabolites)" [41]. "Nutrigenetics studies the influence of the genetic variations in the body promoted by the nutrients while nutrigenomics studies the influence of the nutrients on gene expression" [42].

Each definition provided by the cited authors presents nutrigenetics and nutrigenomics as the science which integrates "omics" tools in providing insights into the nature of the interaction between inherited genes and nutrients in the diet. The importance of the application of nutrigenetics and nutrigenomics has since been utilized in human nutrition for understanding disease onsets and has been used to birth treatment options based on the concept of "individualized nutrition" [26]. In pigs, the combined effect of diets, genes, sex, age, environment, etc., on disease susceptibility, growth performance, fat metabolism and meat quality traits are starting to emerge. It could be hypothesized from Fench et al. [25] that just as in humans, the existence of differences in inherited genes affects nutrient bioavailability and metabolism in pigs regardless of breed differences.

3. Genes Involved in Fat Metabolism and IMF Accretion in Pigs

The post-genomic era has advanced the knowledge of genes that are associated with the molecular and genetic basis for fat deposition and IMF development in pigs. Studies have shown that most fat metabolism-related genes indirectly influence the IMF content of pork. However, their effects have shown variability with regards to muscle location and mechanisms of lipogenesis and adipogenesis [24]. Local pig breeds (such as Italian Landrace, local Basque, local Wujin, Mangalitsa, Meishan, etc.) present higher IMF content and better meat quality traits compared to modern breeds (e.g., Duoc–Iberian crosses, Large White breed, etc.). Higher expressions of genes and enzymes involved in fatty acid synthesis and lipid metabolism have shown to be the key drivers of the observable increase in IMF content of such local pig breeds [14,24].

Genes which are mostly implicated for their active role(s) in lipid metabolism and fatty acid synthesis in pigs and other animal species include: acetyl-CoA carboxylase alpha (*ACACA*), acyl-CoA oxidase 1 (*ACOX1*), acyl-CoA synthetase long-chain family member 3 (*ACSL3*), acyl-CoA synthetase short-chain family member 2 (*ACSS2*), adiponectin (*ADIPOQ*), adiponectin receptor 1 (*ADIPOR1*), 1-acylglycerol-3-phosphate o-acyltransferase 1 (*AGPAT1*), CCAAT/enhancer-binding proteins (*C/EBP*), alpha (*CEBPα*), CCAAT/enhancer-binding proteins (*C/EBP*), beta (*CEBPβ*), Catalase (*CAT*), diacylglycerol acyltransferase 1 (*DGAT1*), diacylglycerol acyltransferase 2 (*DGAT2*), fatty-acid-binding protein 3, muscle and heart (*FABP3* and *H-FABP*), fatty-acid-binding protein 4, adipocyte (*FABP4* and *A-FABP*), fatty acid synthase (*FASN*), leptin (*LEP*), leptin receptor (*LEPR*), lipase, hormone-sensitive (*LIPE* and *HSL*), lipoprotein lipase (*LPL*), peroxisome proliferator-activated receptor alpha and gamma (*PPARα* and *PPARγ*), retinoid X receptor gamma (*RXRγ*), solute carrier family 2 (facilitated glucose transporter) member 4 (*SLC2A4* and *GLUT4*) and sterol regulatory element-binding transcription factor 1 (*SREBF1* and *SREBP-1C*) [25].

3.1. Adipogenesis and Lipogenesis

Adipogenesis is a cell differentiation process where fibroblast-like preadipocytes develop into mature adipocytes regulated by the *PPARγ* gene, while the process of fatty acid and triglyceride synthesis is called lipogenesis. Both processes are regulated by different adipogenic and lipogenic genes, respectively [43,44]. Many authors have described the mechanisms controlling growth (increase in number and size; hyperplasia and hypertrophy, respectively), adipogenesis and lipogenesis [43–46]. For a polygenic trait such as fat metabolism, during transcription and adipogenesis, transcription factors bind specifically to the promoter region of their target genes and control their expression in different metabolic pathways [26]. In pigs, the determination and terminal differentiation stages of adipocyte differentiation occur in the adipose tissue. Conversely, in poultry, these stages of adipogenesis occur in the liver [9,43]. Adipogenesis is a consequence of the interaction between *PPARγ* with several different co-regulators involved in the control of the differentiation of fibroblast cells. At the determination stage, increased *CEBPβ* and *CEBPδ* activate *CEBPα* and *PPARγ*. *CEBPα* induces *PPARγ* expression as well as its expression. This cycle of interaction between *PPARγ* and *CEBPα* maintains increased levels of *PPARγ* and *CEBPα* and subsequently results in the start of adipocyte differentiation [43]. From

examined literature [26,43–46], a simplified schematic representation of the process of adipose tissue development is presented in Figure 1.

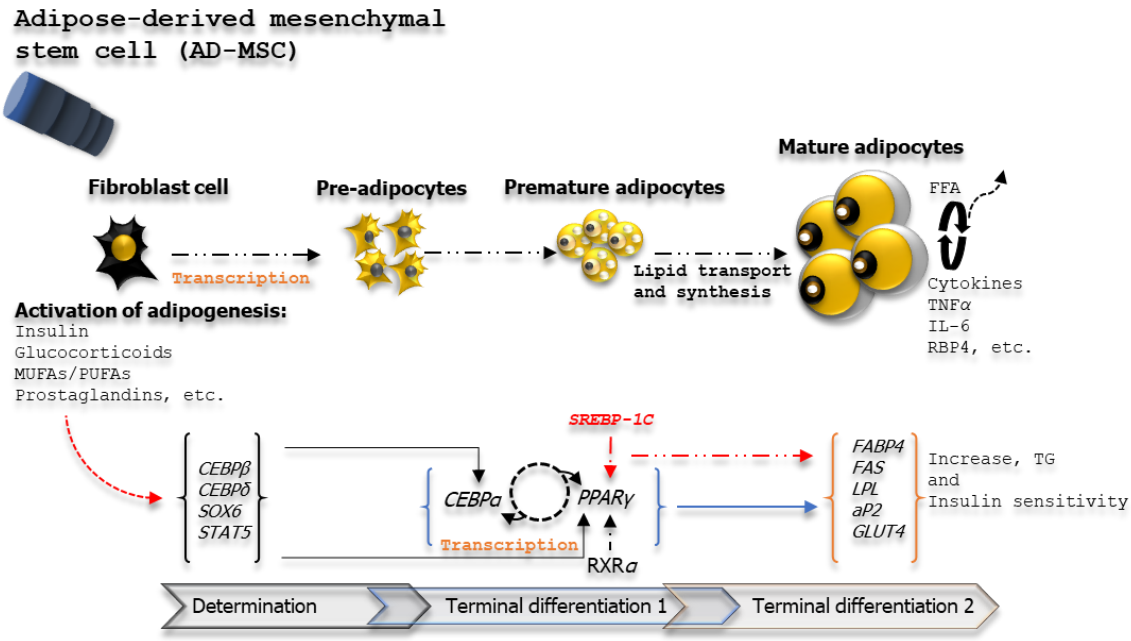

Figure 1. Schematic representation of adipocyte differentiation during adipogenesis.

Adipocyte protein 2 = *aP2*; CCAAT/enhancer-binding protein = *CEBPβ* and *CEBPδ*; fatty-acid-binding protein = *FABP4*; glucose transporter type-4 = *GLUT4*; lipoprotein lipase = *LPL*; peroxisome proliferator-activated receptor gamma = *PPARγ*; retinoic X-receptor = *RXRα*; sterol regulatory element-binding protein-1c = *SREBP-1c*; tumor necrosis factor-alpha = *TNFα*.

3.2. The de novo Fatty Acid (FA) Synthesis

During lipogenesis in the adipose tissue, glucose is converted into triglycerides through glycolysis and tricarboxylic acid (TCA) cycle, generating the energy required by the pig for metabolic activities [43–45]. However, this process varies between different breeds, fat depots and between the sexes. When glycolysis is initiated as a response mechanism to an increase in glucose or insulin, citrate is formed from the TCA cycle and used for *de novo* lipogenesis (de novo fatty acid synthesis). In response to carbohydrate intake, glucose is taken by adipocytes through insulin-stimulated *GLUT4* (see Figure 2). There are several published schematic representations of the pathways involved in *de novo* fatty acid synthesis [43–49]. A simplified pathway is shown in Figure 2.

Figure 2 shows the conversion of glucose to pyruvate through the cytosol of the cell tissue and transported into the mitochondria for further oxidation in the TCA cycle to produce citrate. In response to insulin secretion, the expression of *SREBP-1c* is initiated for adipocyte lipogenesis. The citrate generated from the TCA cycle is then exported back into the cytosol as a substrate for de novo lipogenesis which subsequently results in the release of acetyl-CoA by ACLY. *FASN* then converts malonyl-CoA to palmitate which becomes elongated to produce oleic, stearic, and palmitic acid. The activation of *ChREBP-α* by glucose metabolites (generated during glycolysis) binds to promoter regions of *ACLY, ACC1, FASN, SCD1,* and *ChREBP-β* coding genes. Fatty acid synthesis is then promoted by the *ChREBP-β* sequel to activation of its target genes. However, fat intake blocks the expression of *ChREBP-β* and suppresses de novo lipogenesis [43–45].

Poklukar et al. [46] published a detailed review on the transcriptomic networks, hormones and enzymes modulating transcriptional regulation of adipogenesis in local and modern pig genotypes. Additionally, other studies have also revealed putative IMF accretion and fat metabolism-related genes [45–49], hormones, enzymes, transcription factors, and miRNAs [50–52] and their interaction with dietary nutrients [2,12,53,54] in pigs. Other findings evinced the possible association of genes influencing fat deposition and IMF accretion to the mitogen-activated protein kinase (MAPK) pathway regulating adipogenesis and lipogenesis [55,56]. However, studies on such mechanisms related to fat metabolism and pork quality traits, including IMF, are limited while existing few investigations remain elusive.

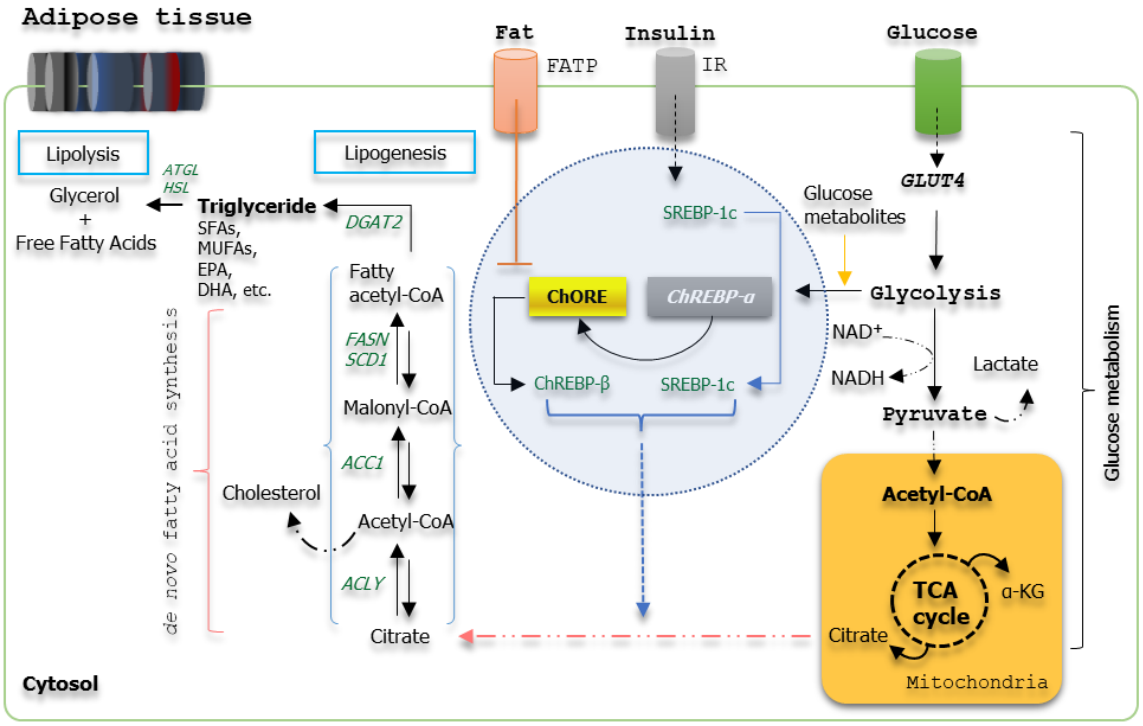

Figure 2. Schematic representation of de novo fatty acid (FA) synthesis from adipose tissue.

ATP-citrate lyase = *ACLY;* acetyl-CoA carboxylases 1 = *ACC1*; carbohydrate response element-binding protein *α* and *βl* = *ChREBP-α* and *ChREBP-βl*; fatty acid transport protein-1 = FATP; fatty acid synthase = *FASN*; stearoyl-CoA desaturase-1 = *SCD1*; lipogenic transcription factor sterol regulatory element-binding protein-1 = SREBP-1; diacylglycerol O-acyltransferase homolog 2 = *DGAT2*; insulin receptor = IR; short-chain fatty acids = SFA; monounsaturated fatty acids = MUFAs; docosahexaenoic acid = DHA; Eicosapentaenoic acid = EPA;.

Active enzymes and their functional roles in fat metabolism and IMF include: hormone-sensitive lipase (LIPE) involved in IMF hydrolysis [57], acetyl-CoA carboxylase (ACC) which regulates the irreversible formation of malonyl-CoA from acetyl-CoA, fatty acid synthase (FAS) which regulates the synthesis of palmitate from acetyl-CoA and malonyl-CoA, stearoyl-CoA desaturase (SCD) that controls the transformation of monounsaturated fatty acids (MUFAs) from short-chain fatty acids (SFAs), and glucose-6-phosphate DH (G6PDH) and malic enzyme (ME) which generate nicotinamide adenine dinucleotide phosphate NADPH for reductive biosynthesis of fatty acids [46,58]. Main hormones such as insulin and glucocorticoids are reported to be involved in the regulation and initiation of adipocyte differentiation [59], depending on the existence of differentially methylated sites for genes involved in lipid metabolism and their associated pathways, as well as the muscle tissue location [46,60].

Some studies indicate the genes that could be considered as functional genetic markers and nutritional targets for individual nutrient-matching and dietary nutrient-based trait improvement strategies in pigs. These studies have shown how promising applications of “omics” based technologies are in nutritional genomics. A summary of the genes which are directly or indirectly involved in fat metabolism and IMF accretion in pigs is presented in Table 1.

Table 1. A list of genes related to fat metabolism and IMF deposition in pigs.

Study	Gene Name	Breed	Tissue	Sampling Age (d) or Body Weight (kg)	Trait
[60]	*FABP4, FASN*	Chinese local and Large White	LD, L	150 d	IMF
[61]	*ADIPOQ, PPARG, LIPE, CIDEC, PLIN1, CIDEA,* and *FABP4*	Purebred Duroc	LD	108 kg	IMF
[62]	*ATGL, FAS, HSL, CPT-1B, SREBP-1c, SCD, A-FABP* and *H-FABP*	Wujin and Landrace	LD	100 kg	IMF
[63]	*RAD9A, IGF2R, SCAP, TCAP, SMYD1, PFKM, DGAT1, GPS2, IGF1, MAPK8, FABP, FABP5, LEPR, UCP3, APOF,* and *FASN*	Landrace and Songliao Black sows	SF, LD, L	100 kg	Fat deposition
[64]	*H-FABP* and *LEPR*	Duroc, Pietrain, Puławska, Polish Large White (PLW), and Polish Landrace (PL)	LD, SMM, L	Slaughter at 6 age groups 60-, 90-, 120-, 150-, 180- and 210-d-old pig	Fat deposition and IMF
[65]	*FABP3* and *LEPR*	Duroc, Pietrain, Puławska, Polish Large White (PLW) and Polish Landrace (PL)	LD	100 kg	Fatty acid metabolism and IMF levels
[66]	*FABP3* and *LEPR*	Korean native pig and Yorkshire crossed animals.	LD	90–100 kg	IMF
[67]	*H-FABP* and *MASTR*	Large White	BL	95– 105 kg	IMF
[68]	*PRKAG3*	Large White X Duroc X Pietrain	SM	110 kg	IMF
[69]	*EEF1A2, FABP3, LDLR, OBSCN, PDHB, TRDN* and *RYR1*	Landrace X Large White X Pietrain	LD	30, 60, 90 and 120 kg	IMF
[70]	*IGF2*	Large White, Polish Landrace and Puławska pigs	BL	100 kg	IMF
[71]	*PPARG* and *ADRP*	Laiwu, Lulai Black, and Large Whites	LD	114 kg	Fat deposition and IMF
[72]	*PPARA, PPARG, SCD* and *PCK2*	Shanzhu X Duroc commercial crossbreds	LD	90 kg	Lipid deposition and IMF
[73]	*BMPER* promoter	Duroc X Large White X Yorkshire	LD	-	IMF
[74]	*FABP3* promoter	Large White X Landrace background X Pietrain	LTL, SMM, BL	-	IMF
[75]	*SCD* and *LEPR*	Duroc	GM, LD	128 kg	IMF and fatty acid composition
[76]	*FASN* and *LIPE*	Jinhua and Landrace	SA	Slaughtered at 35, 80 and 125 days of age	IMF
[77]	*CAV2, MYOZ2, FRZB,FASN, SCD, ESR1,* and *ADORA1,*	Chinese Diannan Small-ear pig, Tibetan, Landrace and Yorkshire	LD	-	Lipid deposition and muscle growth
[78]	*SCD, ACACA,* and *FASN*	Puławska, Polish Large White and Polish Landrace	LD, BL	100 kg	IMF and lipid metabolism
[79]	*MSTN*	MSTN-knockout (KO) cloned Meishan	SF, BL	70 kg	Fatty acid metabolism
[80]	*FGF2*	Italian Large White	SMM	150 kg	IMF
[81]	*FABP3, LIPE, IGF1, IGF2, LEP, LEPR, MC4R, PHKG1, RETN, RYR1, SCD,* and *UBE3C*	Chinese Shuai pigs	LD	80–90 kg	IMF
[82]	*FASN, SCD, ELOVL6, DGAT2, PLIN1, CIDEC,* and *ADIPOQ*	Iberian	LD	165 kg	Lipid metabolism and higher content of IMF

BL = blood; GM = gluteus medius; L = liver; LD = longissimus dorsi; SA = subcutaneous adipose; SF = subcutaneous fat; SM = skeletal muscle; SMM = *semimembranosus* muscle; LTL = longissimus thoracis et lumborum.

3.3. Most Implicated Genes in Fat Metabolism and IMF Deposition in Pigs

Different studies have reported many genes that are associated with fat metabolism and IMF content in pig breeds. Nonetheless, when the whole-body fat depots of the pig are considered, it has been observed that variations exist between each fat depot and pig breed [62]. The genes that are mostly studied as key actors in adipogenesis, lipogenesis and IMF accretion in pigs are discussed below.

PPAR genes: Mainly, *PPARα* and *PPARγ* are a sub-family of the nuclear hormone receptor (*NHR*) super-family associated with metabolic pathways that are related to fat adipogenesis, lipogenesis, and gluconeogenesis [82–84]. *PPARα* and *PPARγ* are the most studied and implicated isoforms of the *PPARs* related to fat metabolism in pigs [71,85]. While *PPARα* is an important regulator for the transcription of genes that are involved in lipid metabolism, *PPARγ* principally regulates adipogenesis and promotes adipocyte differentiation and glucose homeostasis [86]. In newborn piglets, *PPARγ* expression is regulated by several transcription factors; however, its differential expression among piglets is yet to be established [85]. The gamma factor of the *PPARγ* is essential in the differentiation and maturation of preadipocytes and adipocytes, respectively, and it also induces the activation of fat cells through the *PPAR* transcription factor [71]. Higher concentrations of *PPARα* are found mainly in organs such as the liver while *PPARγ* is more concentrated in the adipose tissue of the longissimus dorsi muscle [86]. Interestingly, *PPARs* are activated by polyunsaturated fatty acids and their expressions vary between lean and fat pig genotypes [87].

FABP genes: Adipocyte and heart fatty-acid-binding proteins (*A-FABP* and *H-FABP*) are involved in fat metabolism and carry out intracellular transport of fatty acids from the cell membrane to sites of fatty acid oxidation [64,88]. The *H-FABP* (*FABP3*) gene is expressed predominantly in heart and skeletal muscle cells, while *A-FABP* (*FABP4*) is expressed almost exclusively in adipocytes [89]. Their expression tends to increase with the maturation of the longissimus dorsi muscle, thus affecting the expression of lipogenic genes [53,89]. Under the *FABP* class of genes, the *FABP3* and *FABP4* types are found to be associated with the marbling and IMF content of pork [65]. Studies have shown *FABP3* to be a strong genetic marker for IMF deposition and could independently influence IMF content and fatness traits in pigs [74,90]. In another study, *FABP3* expression was shown to be reduced in pigs with higher IMF and it is more strongly associated with the accretion of backfat when diets with low-fat contents are fed to pigs [66]. The expression of the porcine *A-FABP* (*FABP4*) gene varies between breeds. For example, its role in cell differentiation and IMF accretion is found to be more in Duroc pigs than in Meishan pigs [88]. The study of Chen et al. [89] reported a positive correlation between the *A-FABP* mRNA expression level and IMF content in Laiwu and Lulai Black pig populations. Despite this variability observed between breeds, *FABP4* has been proposed as a candidate gene in pig nutrigenomics applications due to its functional role in adipogenesis and increased IMF content [89,91].

SCD gene: Stearoyl-coenzyme A desaturase gene (*SCD*) is a functional gene that encodes an important enzyme stearoyl-CoA desaturase necessary for the conversion of saturated fatty acids (SFAs) into monounsaturated fatty acids (MUFAs) [92]. The *SCD* gene has been associated with the fatty acid composition of porcine longissimus dorsi muscle [79] and acts as an important regulator of the genetic mechanism of lipid deposition and fatty acid synthesis in pigs [77,82,92]. Additionally, it is involved in the *PPAR* signalling pathway and is important for meat quality traits in pigs [72]. The downward regulation in the expression of the *SCD* gene was reported to be accompanied by an increase in the saturated fatty acid level in the adipose tissue [93], while up-regulation of *SCD* gene expression showed an increase in IMF content [72].

LEP (*LEPR*) gene: Porcine leptin and its receptor, *LEPR,* are known to be involved in food intake and energy homeostasis, and strongly affect the rate of IMF accretion. Its expression level tends to increase with age in pigs [67]. Generally, fatness is associated with leptin production and plasma level, thus, an increased expression of the *LEP* gene is expected in animals with increased fat deposition as has been observed in the fatty pig breeds [75]. *LEPR* is a candidate gene involved in fat metabolism, influencing not only IMF content but other pork quality traits such as moisture, cholesterol and flavour [66]. It has been recognized as one of the most functional genetic markers influencing growth and fat deposition in pigs [94]. As the IMF content tends to increase, Ros-Freixedes et al. [75] observed that the ratio of saturated fatty acids to polyunsaturated fatty acids (SFA: PUFA) tends to increase with more saturated fatty acids (SFA) and less polyunsaturated fatty acids (PUFA) in the porcine muscle [75]. *LEPR* gene expression controls the rate of IMF content and alters the fatty acid profile of the longissimus dorsi muscle.

ACACA and *FASN* genes: Acetyl-CoA carboxylase-α (*ACACA*) is a protein-coding gene while fatty acid synthase (*FAS*) is an enzyme encoded by the *FASN* genes. Both genes regulate the de novo synthesis of fatty acids from acetyl-coenzyme A and malonyl-co-enzyme A in the presence of NADPH [78,95]. Their expression levels also vary across breeds of pigs [78,95]. *ACACA* and *FASN* initiate the synthesis of fatty acids and saturated fatty acids during the early stages of lipid metabolism [46,78]. Studies have shown that the *FASN* gene is associated with IMF content and lipid metabolism pathways and is a candidate gene influencing fat traits in pigs [95,96]. However, Piórkowska et al. [78] recently reported that IMF content in Polish Landrace and Polish Large White pigs was influenced by a mutated *ACACA* gene. Zhao et al. [62] suggested that the mechanism of an increased rate of IMF deposition is related to a decrease in the rate of lipolysis and an increased rate of lipogenesis in fatty pigs. Such a mechanism is found to regulate the activity of *FASN* gene during anabolism, catabolism, and fatty acid transportation [62]. The effect of *FASN* gene expression in IMF deposition in the porcine longissimus muscle is not clear; however, it was suggested to have a functional role as an enzyme of fat storage with several effects in subcutaneous adipose tissue and intramuscular fat tissue [62]. In Polish Large White pig breeds, the effect of the *FASN* gene is not largely detected on fat metabolism and IMF content [94]. Nonetheless, a recent longissimus dorsi transcriptome analysis confirmed that the *FASN* gene is key in lipid metabolism and highly associated with high IMF content in pigs [25,82].

MSTN or *GDF8* gene: The myostatin or growth differentiation factor 8 (*MSTN* or *GDF8*) gene belongs to the transforming growth factor-beta (*TGF-β*) super-family. It is responsible for double muscling in cattle and Belgian domestic pig breeds, as well as in *MSTN*-knockout pigs [97]. Although naturally occurring *MSTN* mutation is yet to be established in pigs [98], it is reported to be associated with reduced fat metabolism [79], and significantly lower IMF content in *MSTN* mutant mouse lines [99,100]. Inducing *MSTN* mutation in pigs could result in an increase in longissimus dorsi muscle area, better lean meat yield, and reduced backfat and carcass fat content in pigs [100]. Despite its involvement in muscle development and pork quality characteristics, there is limited scientific evidence on the functional role of the porcine *GDF8* gene in fat metabolism and IMF accretion in pigs. This gap necessitates further research to understand how it influences pork fat metabolism, IMF deposition and other meat quality traits. A study [101] shows that *MSTN* knockout using CRISPR/Cas9-mediated genome editing with subsequent somatic cell nuclear transfer offers a promising possibility for genetic improvement of economically important traits in pigs. Ren et al. [79] demonstrated the active potential of *MSTN* in inhibiting the growth of muscles (double muscling) and acts via *myogenic transcription factor 2C* (*MEF2C*) which binds to the miR-222 promoter and suppresses the translation of *SCD5* to affect fat deposition [79].

SREBF-1 (*SREBP-1c*) gene: Sterol regulatory element-binding transcription factor-1c (*SREBF-1c*) was suggested to be an important lipogenic gene that has a critical role in the gene transcription mechanism and regulation of muscle fat deposition [62,102]. The role of *SREBF-1* in fat metabolism and IMF accretion remains contradictory between studies and could be breed-dependent. The role of *SREBP-1c* in increasing lipogenesis and accompanied reduction of lipolysis in Wujin pigs is associated with increased adipocyte diameter, polyunsaturated fatty acid levels and IMF content [62]. Due to its regulatory role in muscle fat deposition during post-natal growth, it could be targeted as a gene marker for the genetic improvement of IMF in pigs [103]. While Chen et al. [103] reported a positive correlation between the expression of *SREBF-1* mRNA and IMF accretion in the longissimus dorsi muscle of pigs [103], Stachowiak et al. [104] found no association between *SREBF-1* gene transcript levels and fatty acid compositions in longissimus dorsi muscle and adipose tissue. Such differences require more investigation to understand the clear role of the *SREBF-1* gene in porcine fat metabolism and marbling.

4. QTL Regions and SNPs for Fat Metabolism and IMF Accretion in Pigs

Genome-wide association study (GWAS) has uncovered many key single nucleotide polymorphisms (SNPs or mutations) for genes and their quantitative trait loci (QTLs), sphingolipid signalling pathways, and enzyme co-factors related to fatness traits in pigs, [105–108]. However, it is yet unknown the gene (s) controlling mechanisms of IMF deposition in pigs. Pieces of literature have strongly suggested a difference in the gene expression and heritability (below 0.5%) for IMF deposition during muscle adipogenesis, myogenesis, lipogenesis and lipolysis, occurring at different stages of growth and development [69,107–111]. Certain genes are found to affect IMF deposition independent of backfat in pigs. For instance, Zhang et al [112], revealed that QTL located on *Sus Scrofa* (SSC) 1 (167938652, 166363826, 164829874 and 167171587) and transducin-like enhancer of split 3 (*TLE3*), SMAD family member 6 (*SMAD6*), progestin and adipoQ receptor family member 5 (*PAQR5*) and integrin subunit alpha 11 (*ITGA11*) genes are associated with IMF content accretion without affecting backfat in Duroc

pigs. Such molecular markers are important in pig breeding programs targeted at IMF content improvement in pigs. Also, the applications of biological and dietary markers in marker-assisted selection for better fat deposition and IMF content are useful in pig nutrigenetic intervention [111].

Few QTLs associated with the *Sus Scrofa* chromosomes (SSC) 4, 6, 8, 13 and 14 have been reported to be more often involved with IMF deposition and fatty acid (SFAs and MUFAs) profiles in pigs [24]. The pig SSC14 and SSC6 QTLs have known regions for lipid metabolism and are related to *LEPR* and *SCD* genes with mutations or quantitative trait nucleotide (QTN) [93,106]. Earlier, QTL located on chromosome 4 (SSC4) was found to be responsible for the difference in fat deposition [106,113]. Today, about 778 QTLs related to different traits have been identified and documented in the pig QTL database, pigQTLdb (see https://www.animalgenome.org/cgi-bin/QTLdb/SS/index, accessed on 23 December 2021). Studies by Harper and Pethick [102] reported that the onset of marbling is located at chromosomal regions for QTL on chromosome 5 (SSC5), which is responsible for muscle growth and fat deposition. This QTL was genetically related to the *RARγ* gene which is involved in the transcription and expression of many other genes [114]. Later on, candidate genes associated with QTL on chromosome 6 (SSC6) were used to establish the functional role of the *RARγ* gene in fat deposition and marbling in pigs [115].

SNPs in pigs' fat mass and obesity (*FTO*) gene are strongly associated with backfat and marbling and regulate average daily gain and lipid deposition [116]. Findings by Meadus and co-workers [117] revealed sire variability in terms of the IMF content of pork using SNP markers on chromosomes 5, 7, and 16. This implies that every sire is unique in terms of marbling genes [117]. Several chromosomal regions (QTLs) and molecular markers (SNPs) are now providing insights into specific candidate gene(s) controlling growth, nutrient uptake, disease resistance, meat quality traits and fat metabolism [93,105]. However, it remains a major challenge to nutritionally sway existing differentially methylated sites where genes involved in lipid metabolism are found [118].

Transcriptome analysis has deepened our scientific knowledge of the molecular pathways and genetic basis of fat metabolism and IMF accretion in pigs [12,94,119]. To this end, there is clear evidence that the use of nutrient-gene biomarkers is a crucial fingerprint for accurately elucidating the genetic and nutritional regulation of fat metabolism. Potential QTLs of complex traits and functional genes related to muscle growth, fat and IMF deposition, and many putative genes involved in the mechanism of fat distribution and marbling in pigs are becoming available [47,114,120,121]. Despite the far-reaching pieces of evidence from literature, the application of DNA-specific markers in simultaneously enhancing fat deposition and IMF content of pork without altering other carcass traits remains difficult to achieve. In addition, the precision of mapping the existing gene markers in terms of selection across breed populations for genetic variation remains limited [75,117].

5. Epigenetic Mechanisms: Role of mRNAs, miRNAs, DNA Methylation and Histone Modification in Fat Metabolism

Genome-wide high throughput DNA analysis was recently developed to profile the human and animal genomes [122,123]. Literature is starting to evince significant epigenetic responses associated with fat deposition, mainly the role of DNA methylation in the regulation of gene activities, and how genes are expressed in pigs and other species (cow, chicken, etc.) [31–33]. Also, epigenetic memory is reported to be associated with some DNA methylation patterns which results in heritable phenotypic responses [124]. Epigenetics is the basis for heritable changes in gene expression without altering the original genetic code or DNA sequence itself [125]. It is the beginning of cell differentiation processes through which genes are turned "on" and "off" or silenced [33] and is influenced by environment and nutrition [34], whereas epigenomics is the analysis of epigenetic responses of genes in the entire epigenome chemical compounds and proteins that can attach to DNA during gene expression [117].

The effects of epigenetic mechanisms in the fat metabolism process are controlled by the transcriptional roles of miRNAs in binding to protein-coding genes, DNA methylation, and histone modification [124,125]. Epigenetic studies have revealed variability in differential DNA methylation patterns of lean and fat pigs [32]. Many genes regulated by differentially methylated promoters were implicated in lipid metabolism, sensory and olfactory processes, and ATPase activity [32]. In addition, polygenic trait effects related to IMF deposition and fat metabolism as well as their degree of heritability are controlled/regulated by epigenetic modifications [119,126]. The role of epigenetics in fat metabolism is becoming clearer as studies are uncovering the underlying pattern of expression of coding and non-coding genes as well as the functional role(s) of mRNA and miRNA during adipocyte and myocyte cell differentiation [125]. Thus, it is relevant to take into cognizance the important roles that epigenetics is playing in how pigs express phenotypic traits in response to nutrient intake.

5.1. Role of Messenger and Micro RNAs (mRNAs and miRNAs)

During DNA transcription and translation, the enzyme RNA polymerase catalyzes DNA base-pairing, which is regulated by miRNAs to produce a pre-mRNA transcript that is further processed into an mRNA molecule (a single-stranded copy of the gene). The mRNA is “read” based on the genetic code which relates the DNA sequence to the amino acid sequence in proteins (polypeptides) encoded by the original gene [128,129]. miRNA-mediated events include: translational repression, mRNA decay, RNA-binding protein inactivation, protein synthesis [127] and fatty acid metabolism through related pathways [62]. The literature suggests the indispensable role of miRNA in fat deposition and adipocyte differentiation [130,131]. Additionally, the use of miRNA sequence in investigating IMF content-related genes is uncovering differentially expressed genes (DEGs) associated with muscle growth and lipid deposition in pigs [56]. MiRNAs have the potential to down-regulate gene expression by blocking mRNA translation of certain genes. Their structure, synthesis, and action in adipogenesis and their strong regulatory roles in animals have been extensively reviewed [127–131]. Mobuchon et al. [132] reported two miRNAs (miR-142-5p and miR-20a-5p) associated with *PPARα, PPARγ, ELOVL6* and *ACATI1* genes which are involved in nutrient-gene regulation mechanisms of cell proliferation, cell differentiation and lipid metabolism [77,132]. Furthermore, miRNAs in adipose and muscle tissue whose target genes are associated mainly with signalling pathways rather than metabolic and biosynthetic processes have been detected in various pig breeds [133,134]. While the behaviour of miRNAs tends to be dissimilar between breeds, their expression pattern also varies with age [133] and cell differentiation, such as osteogenesis, myogenesis, adipogenesis, etc. [133–138].

It has been established that even when isolated from the same tissue but different animal breeds, miRNAs’ differentially expressed gene profiles tend to be breed-specific [139]. Many studies have confirmed their involvement in myogenesis and adipogenesis by altering the expression of their target genes and proteins [52,131,140,141]. Wang et al. [77] reported the mechanism of lipid deposition from a transcriptome profile of pig muscle tissues. Their results revealed *CAV2*, *MYOZ2*, *FRZB*, miR-29b, miR-122, miR-145-5p and miR-let-7c as key genes and miRNAs, respectively, regulating muscle growth while *FASN*, *SCD*, *ADORA1*, miR-4332, miR-182, miR-92b-3p, miR-let-7a and miR-let-7e were key genes and miRNAs, respectively, involved in the regulation of lipid deposition in pigs. miRNAs’ involvement with mitogen-activated protein kinase (MAPK) cascades, a key signalling pathway that regulates a wide variety of cellular processes including cell proliferation, differentiation, apoptosis, and stress responses, have been documented [77]. The knowledge of the potential transcriptomic roles of such ribonucleic acids is changing approaches to trait improvement and is providing more information on epigenomic modifications associated with phenotypic variability in pigs [142,143].

5.2. DNA Methylation and Histone Modification in Fat Metabolism

DNA methylation is a biochemical gene modification process that determines gene expression patterns or “gene silencing" (regulating the turning “on” and “off” of some genes) related to the metabolic synthesis of fats. Histone modification involves histone acetylation, regulated by histone acetyltransferases (HATs), and deacetylation, on specific lysine residues regulated by histone deacetylases (HDACs) [144]. Gene expression involving the interaction of HATs, HDACs and histones can activate or repress gene transcription such that histone acetylation unlocks and activates chromatin, while chromatin becomes transcriptionally silent through deacetylation of histones and DNA methylation [144]. However, it is yet to be proven the clear role of DNA methylation and histone modification mechanisms in fat metabolism.

Nutrition and environmental factors have a significant effect on DNA methylation, leading to an increase in the expression of genes related to production performance, disease, and meat quality traits. DNA methylation is regulated by DNA-methyl-transferase enzymes (DNMTs) and methyl-CpG-binding domain proteins (MBDs) during gene expression in mammals [145–147]. Specifically, DNMT1 maintains DNA replication and cell division while DNMT3A and DNMT3B maintain de novo methylation during early development. A diagram showing the pathway involved in DNA methylation and histone modification is shown in Figure 3.

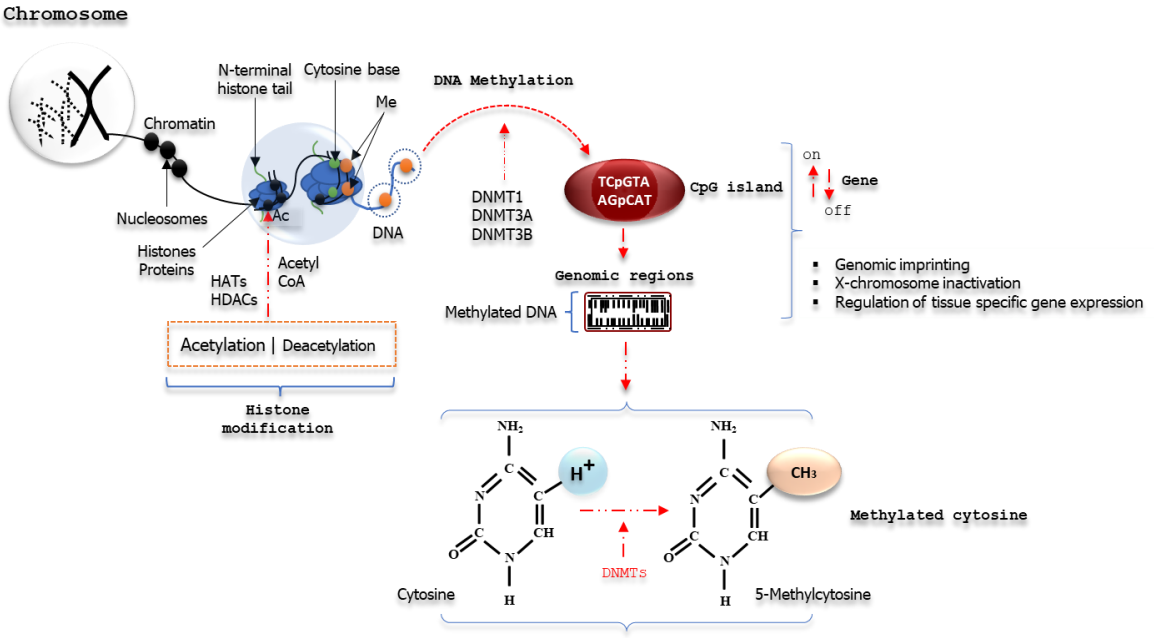

Figure 3. Epigenetic modifications of chromatin by histone modification and DNA methylation of cytosine nucleotides on the 5th carbon of the cytosine base at the CpG site.

Histone modification alters gene expression through mechanisms of HATs' and HDACs' functions during acetylation of histones at their lysine residue sites. Histone modification begins with the addition of an acetyl group (Ac) by acetyl CoA followed by HATs-regulated acetylation. HDACs serve as catalysts for the hydrolytic removal of the acetyl groups from histone (Figure 3). When this mechanism is altered, mutation and disease or trait progression are observed. DNMT1, DNMT3A and DNMT3B initiate and maintain CpG methylation across the genome by either blocking or allowing the binding of proteins associated with methyl-CPG-binding sites [148]. Such sites are genomic regions where cytosine is separated from guanine by just a phosphate group (CpG islands) in a linear sequence of a base in the direction of 5' → 3' [149–151]. The effects of cytosine methylation within the base sequence of a gene include processes involving genomic imprinting, X-chromosome inactivation, suppression of repetitive elements, lipogenesis, and carcinogenesis [148]. DNMT1 has a significant regulatory effect on genes at the CpG-binding sites. Studies have shown that when it binds at CpG to the *SREBP1* gene, it down-regulates the activity of *SREBP1* while an unmethylated promoter exerts an opposite effect by up-regulating the activity of the *SREB1* gene during adipogenesis [152]. Another mode of action of DNMT1 shows that it regulates adipogenesis by promoting differentiation at an early stage while inhibiting lipogenesis at the late stage of preadipocyte differentiation [153].

Studies have shown that methylating dietary micronutrients elicited differential expressions of genes involved in lipid metabolism, and later, gene repression of certain housekeeping genes [23]. Qimuge and others [119] demonstrated that DNMT3A increased proliferation and inhibited the differentiation of intramuscular preadipocytes by decreasing the expression of cyclin-dependent kinase inhibitor 1A (*p21* also known as *CDKN1A*), and down-regulated the levels of *PPARγ*, *SREBP-1c*, and *FABP4* through the methylation of *PPARγ* promoter [119]. The study of Stachecka et al. [153] showed that the onset of adipogenesis elicited an increase in transcript level of the *DNMT1* gene followed by a decrease, while *DNMT3A* and *DNMT3B* gene transcripts increase during the in vitro differentiation. This in vitro investigation of the differentiation of mesenchymal stem cells (AD-MSC) into adipocytes established how the expression of DNMT transcripts proceeds in the AD-MSC and bone marrow tissue (BM-MSC) [153]. Today, chromatin regulators can be targeted to regulate and control gene expression [147]. When combined with other nanobodies, DNMT3A have the potential to enhance gene silencing speed and epigenetic memory [147].

6. Nutritional Genomics in Pigs

6.1. Nutrigenetics and Nutrigenomics

While nutrigenetics shows the variation in DNA sequence in response to dietary nutrients, nutrigenomics deals with the roles of dietary nutrients in gene expression and/or structure [154]. Nutrigenetics deals with how the genetic predisposition of an individual pig controls its responses to dietary nutrients, whereas nutrigenomics deals with the effect of nutrient intake on the whole genome (complete genetic make-up, including epigenetic changes), transcriptomics (RNA transcripts that are produced by the genome), proteomics (proteins produced in an organism which changes from cell to cell and changes over time), and the metabolome (detailed characterization of metabolic phenotypes) of the pig [28,41]. Both nutrigenetics and nutrigenomics encompass the tenets of nutritional genomics. The inter-relationship between nutrigenetics, nutrigenomics and epigenetics is presented in Figure 4.

Since the completion of the human genome project, nutritional genomics emerged as a nutritional science that deals with nutrition, genome, and health in understanding the genetic and nutritional basis of disease and ageing in humans [26,30]. Today, it has found enormous applicability in the field of animal nutrition research as well. Nutritional genomics offers the possibility to elucidate complex mechanisms of gene–nutrient interaction and the environment on the entire genome. The use of high-throughput DNA-based “omics” technologies with system biology is defining a new post-genomic era in nutritional genomics of animals (Figure 4). Nutrients can be matched more accurately with inherited genes to harmonize metabolic functions and improve health and economically important traits in animals [26]. Loor et al. [155] reported a summary of how the application of nutrigenetics and nutrigenomics in animal nutrition is promising in disentangling the complexities associated with interactions between nutrients, physiological status and cellular functions of dairy cows, pigs, and poultry. In addition, biological and nutritional pathways related mainly to fat metabolism have confirmed that matching nutriome (nutrient intake combination) in pigs to enhance cellular metabolic functions and desired genetic responses in pigs can be successful [45,59,60].

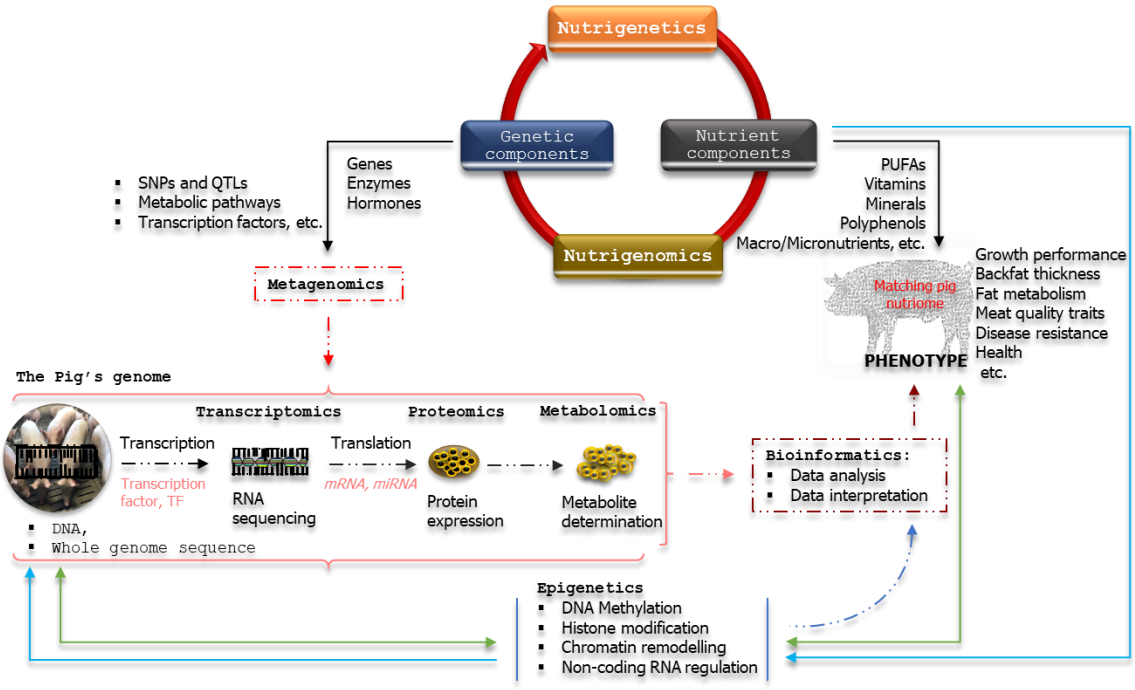

Figure 4. The schematic workflow chart in nutrigenetics, nutrigenomics, and epigenetics science.

The main goals of nutritional genomics as summarized by Kaput and Rodriguez [30] include:

(i) Nutrients in the diet can alter the genome, either directly or indirectly.

(ii) Dietary nutrients and bioactive compounds have the potential to be “risk factors” for disease.

(iii) Some diet-regulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of diseases.

(iv) The degree to which diet influences the balance between health and disease states may depend on an individual’s genetic makeup, and

(v) Diseases can be cured or treated through a dietary intervention based on knowledge of nutritional requirements, nutritional status, and genotype (i.e., “individualized nutrition”).

Translating these five goals into disease and trait improvements in pigs has a wide range of applications in swine nutrition and could result in better phenotypic responses in a breeding program.

6.2. Impact of Dietary Nutrient Supply on Some Genes Related to Fat Metabolism and IMF Deposition in Pigs

The functional role of amino acids in muscle or adipose tissue content and gene expression have high applicability during nutrient intake combination. The impact of reduced feed intake resulted in an increased expression of *GLUT1* and *GLUT4* mRNA in the skeletal muscle of growing pigs [45]. Studies have shown that amino acids such as methionine, lysine, histidine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and valine are essential in several metabolic pathways [35,156,157]. However, establishing their individual effects on gene responses remains a challenge due to data limitations and the complex variability between pigs’ genetics, environment and the quality and quantity of the nutrients in a given diet [17].

6.2.1. Impact of Dietary Crude Protein Supply

Protein, fat and micro/macro-nutrient supplementation have been proposed as nutritional interventions applied during different growth and developmental stages of the animal (prenatal, neonatal, or post-natal) [158,159]. To elucidate the regulatory mechanisms of dietary protein levels on gene expression related to lipid metabolism, the study conducted by Zhao et al. [53] showed that a high dietary protein supply at 18% CP significantly reduced expressions of mRNA, enzyme activities and expression levels of key fat and marbling genes in pigs. They demonstrated the effect of increasing body weight from 30 kg to 60 kg to 100 kg by feeding pigs high or low protein diets. In the same study, gene expression was reduced at 60 kg and 100 kg with high protein dietary feeding. *ACC, FAS, SREBP-1c* and *PPARγ* expressions and enzyme activities of *A-FABP, LPL*, carnitine palmitoyltransferase 1B (*CPT-1B*), *PPARγ* and *SREBP-1c*, were promoted at 60 kg [53].

To achieve a significant effect on growth, body composition and gene expression patterns in the skeletal muscle of pig offspring, the best stage for applying nutritional intervention is suggested to be at gestation period and early life [160,161,163]. However, caution is needed as reducing protein supply in diets of gestating sows could impair fetal development as well as piglets’ life post-partum. Another study showed that dietary supplementation with alpha-ketoglutarate (AKG) increased the expression level of mRNA of *FABP4* and *FASN* genes during low dietary protein feeding of growing pigs at 44 ± 1 d of age (11.96 ± 0.18 kg BW) [162]. The number of adipocytes in longissimus dorsi and IMF content tends to increase following energy and protein feed restriction during the suckling stage in young piglets [162].

A

6.2.2. Effect of Lysine, Methionine, Vitamin A, Micro/Macro-Nutrients

Lysine is an essential amino acid in pigs. A low supply of lysine in the diet of heavy finishing pigs alters the functional role of transcription factors such as *PPARγ*, *SREBF1* and adipocyte *FABP-4* [45]. Earlier studies by Katsumata et al. [163] have shown that reduced intake of lysine promotes the IMF deposition in the longissimus dorsi of finishing gilts by up-regulating the expression of the *PPARγ* gene [163]. Similarly, when six (6) week old pigs were fed the diet of three (3) week old piglets, *PPARγ* and *GLUT4* mRNA expression were upregulated following low dietary lysine supply in the longissimus dorsi and muscle rhomboideus of the pigs [164,165]. The mRNA expression of *GLUT4* was found to be higher in longissimus dorsi muscle of pigs the fed a low dietary threonine [166].

In general, altering the level of dietary lysine regardless of the physiological status of the pig could have a huge nutrigenetic impact. Studies showed that a 0.78% lysine supply resulted in higher IMF content in growing pigs [167]. Methionine (formyl-methionine), arginine and lysine are the first three amino acids incorporated into any new protein during gene sequence determination [168–170]. Other nutrients such as α-linolenic acid have been shown to influence and alter expressions of *SREBP-1c* in the liver and 2,4-dienoyl CoA reductase 2 (*DECR2*) gene in the longissimus dorsi muscle [171]. Conversely, dietary lysine restriction (diets low in lysine: energy ratio) evinced better marbling and fat deposition rate during the growing-finishing period in lean pig genotypes [172,173]. The results of Schiavon et al. [173] indicated that reduced dietary crude protein supply resulted in better IMF content and fatty acid composition in heavy pigs [173,174]. Studies on the excess supply of lysine are scarce and this necessitates more studies to find out the effect of excess lysine supply on gene expression in pigs.

In the case of vitamin A (retinoid) supplementation, the effect of nutrient–nutrient interaction with vitamin A and its impact on nutrient bioavailability (absorption and utilization) related to fat metabolism and IMF accretion is still unclear. However, activation of the *PPARs* signalling pathway, *RAR* and *RXR*, using vitamin A (retinoid) promotes the process of fat metabolism [101]. When included in diet at 100,000 IU/kg, retinoid increased IMF content [21,168]. On the other hand, when retinoid was not added to the diet (at 0 IU/kg), no effect on IMF or fat content of the longissimus dorsi muscle was observed but a reduction in the expression of *PPARα* gene occurred [22].

Micronutrients influence the pattern of expression of several genes in pigs. They can modulate signalling pathways of genes and their regulatory elements during growth and development [161,175,176]. Additionally, dietary fatty acids have a vital regulatory effect on DNA receptors and enzymes during DNA transcription and translation [177,178]. Wang et al. [178] opined that when pigs are fed a low protein diet at growth-finishing stages, a direct relationship with higher expression of intramuscular lipogenic genes and decline in expression of a lipolytic gene is achieved. Another study by Kloareg et al. [179] showed the impact of feeding pigs with a diet containing 15 g/kg soyabean oil and 44 g/kg fat on the body fat distribution of pigs. The pigs in the experiment were serially sacrificed between 90 and 150 kg. These pigs evidenced that 0.31 and 0.40 of the digested n-6 and n-3 FA were deposited, respectively, while about 1/3 of the n-3 supply that was deposited resulted from the conversion of 18:3 to other metabolites (i.e., EPA, docosapentaenoic acid and DHA). The study indicated that lipogenic and lipolytic activities change with increasing body weight, while in another study, the average whole-body fatty acid composition varies with tissue but remains constant during the finishing period of pigs [179].

The application of nutritional genomics in fine-tuning dietary nutrients to alter gene expression in pigs would no doubt lead to improvements in economically heritable traits, production performance, health and disease management [58,160]. Scanning an entire genome for the regions of increased or decreased copy number, or differentially methylated sequence will offer animal nutritionists unlimited possibilities to optimise feeding and meat quality traits (as IMF) in pigs. It can also mitigate pet and livestock diseases. In addition to understanding the nature of gene–nutrient and environment interaction, research in the future could consider these unanswered questions:

(i) How can nutrients be matched to an individual pig's genetic predisposition, especially when dealing with the same genes controlling desired/undesired phenotypic traits in pigs?

(ii) How can we quantitatively define nutrient requirements in swine using an individual gene or whole-genome data to initiate an optimal metabolic or trait response?

(iii) How can we fine-tune nutrients and bioactive compounds in a diet to ensure the heritability of genes related to production performance (meat and milk quality), metabolism and genome stability?

(iv) How do we deal with genes capable of controlling different traits that are functionally interdependent such that altering one could lead to a responsive effect in another one?

(v) How can we harmonize nutritional genomic information in modulating genes and their transcriptional factors and subsequently match them with reference dietary nutrients to alter epigenetic response in pigs?

Thus far, from the literature, we can accurately map the genetic, physiological, and nutritional regulatory pathways involved in many cellular functions such as molecular mechanisms of fat and IMF accretion in pigs. This has made the impact of individual dietary nutrients on the whole genome less elusive. Soon, harmonizing the existing knowledge of nutritional genomics might be the major tool for precise estimations of nutrient requirements of pigs with different physiological statuses, age, sex and breed for fat metabolism and other trait improvements (such as growth performance, backfat thickness, IMF accretion, disease resistance, etc.) in pigs and other livestock species.

7. Conclusions

Different studies have reported and confirmed a number of QTLs, SNPs, and mRNAs and miRNAs involved in molecular mechanisms of fat metabolism and IMF deposition in pigs. The main focus earlier was on the identification of single genes involved in the regulation of fatty acid synthesis and IMF deposition in pigs, but later, it was revealed that epigenetic factors and processes are also influential in this field. This might provide more significance to external factors, such as nutritional properties of feed, nutrients, and dietary bioactive substances whose levels in the diet can be difficult to control, in addition to environmental factors.

The science of nutrigenetics, nutrigenomics and epigenetic mechanisms are efficient and precise in defining changes in gene sequences that predispose individual pig breeds to respond in a certain way in terms of performance, meat, and milk quality as well as health and disease detection. As a result, it is possible to measure nutritional effects towards fine-tuning gene expressions and regulating genome responses in pigs, to optimise growth performance, backfat thickness, IMF deposition, disease resistance and meat quality traits. However, the question remains: how prepared are we to integrate this science as a tool in animal nutrition and swine feeding?

Author Contributions:

Conceptualization, V.H., I.J., S.S. and I.H.M; Writing—Original Draft Preparation, I.H.M., V.H., S.S. and I.J.; Writing—Review and Editing, I.H.M., V.H., P.G., I.J. and S.S.; Funding Acquisition, V.H. and S.S. All authors have read and agreed to the published version of the manuscript.

Funding:

This work was funded and supported by: i) The European Union Rural Development Program 2014–2020, Reg. (CE) 1305/2013-PSR Veneto DGR n. 2175—23 December 2016, interventions 16.1.1 and 16.2.2, code 3682902.; ii) The University of Padua also funded with Institutional funds (DOR2059255/20, DOR1990028/19, DOR1845849/18) and with a three-year grant for Isaac Malgwi.; iii) The EFOP-3.6.3-VEKOP-16-2017-00005 project. The project is co-financed by the European Union and the European Social Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest:

The authors confirm that there is no conflict of interest associated with the publication of this review. The funding sources were not involved in the design and writing of the review.

References

1. Wang, Y.; Zhou, J.; Wang, G.; Cai, S.; Zeng, X.; Qiao, S. Advances in low-protein diets for swine. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 60. doi:10.1186/s40104-018-0276-7.
2. Benítez, R.; Trakooljul, N.; Núñez, Y.; Isabel, B.; Murani, E.; De Mercado, E.; Gómez-Izquierdo, E.; García-Casco, J.; López-Bote, C.; Wimmers, K.; et al. Breed, diet, and interaction effects on adipose tissue transcriptome in iberian and duroc pigs fed different energy sources. *Genes* **2019**, *10*, 589. https://doi.org/10.3390/genes10080589.
3. Font-i-Furnols, M.; Tous, N.; Esteve-Garcia, E.; Gispert, M. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. *Meat Sci.* **2012**, *91*, 448–453. https://doi.org/10.1016/j.meatsci.2012.02.030.
4. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* **2004**, *3*, 309–321. https://doi.org/10.4081/ijas.2004.309.
5. Knap, P.W. *Voluntary Feed Intake and Pig Breeding*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2009; pp. 13–35.
6. Čandek-Potokar, M.; Škrlep, M. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal* **2012**, *6*, 327–338.
7. Bertol, T.M.; de Campos, R.M.L.D.; Ludke, J.V.; Terra, N.N.; de Figueiredo, E.A.P.; Coldebella, A.; dos Santos Filho, J.I.; Kawski, V.L.; Lehr, N.M. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. *Meat Sci.* **2013**, *93*, 507–516. https://doi.org/10.1016/j.meatsci.2012.11.012.
8. Schiavon, S.; Dalla Bona, M.; Carcò, G.; Sturaro, E.; Gallo, L. Responses of pigs of different genotypes to a variation in the dietary indispensable amino acid content in terms of their growth and carcass and meat quality traits. *Animals* **2019**, *9*, 508. https://doi.org/10.3390/ani9080508.
9. Wood, J.D.; Enser, M.; Fisher, A.V.; Nute, G.R.; Sheard, P.R.; Richardson, R.I.; Hughes, S.I.; Whittington, F.M. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* **2008**, *4*, 343–358. https://doi.org/10.1016/j.meatsci.2007.07.019.
10. Plotnikov, A.; Zehorai, E.; Procaccia, S.; Seger, R. The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim. Biophys. Acta-Mol. Cell Res.* **2011**, *9*, 1619–1633. https://doi.org/10.1016/j.bbamcr.2010.12.012.
11. Miller, R.K.; Moeller, S.J.; Goodwin, R.N.; Lorenzen, C.L.; Savell, J.W. Consistency in meat quality. *Int. Congr. Meat Sci. Technol.* **2000**, *46*, 566–580.
12. Davoli, R.; Catillo, G.; Serra, A.; Zappaterra, M.; Zambonelli, P.; Zilio, D.M.; Steri, R.; Mele, M.; Buttazzoni, L.; Russo, V. Genetic parameters of backfat fatty acids and carcass traits in large white pigs. *Animal* **2019**, *13*, 924–932. https://doi.org/10.1017/S1751731118002082.
13. Puig-Oliveras, A.; Ramayo-Caldas, Y.; Corominas, J.; Estellé, J.; Pérez-Montarelo, D.; Hudson, N.J.; Casellas, J.; Folch, J.M.; Ballester, M. Differences in muscle transcriptome among pigs phenotypically extreme for fatty acid composition. *PLoS ONE* **2014**, *9*, e99720. https://doi.org/10.1371/journal.pone.0099720.
14. Muñoz, G.; Alves, E.; Fernández, A.; Óvilo, C.; Barragán, C.; Estellé, J.; Quintanilla, R.; Folch, J.M.; Silió, L.; Rodríguez, M.C.; Fernández, A.I. QTL detection on porcine chromosome 12 for fatty-acid composition and association analyses of the fatty acid synthase, gastric inhibitory polypeptide and acetyl-coenzyme A carboxylase alpha genes. *Anim. Genet.* **2007**, *38*, 639–646, doi:10.1111/j.1365-2052.2007.01668.x.
15. Latorre, M.A.; Lázaro, R.; Gracia, M.I.; Nieto, M.; Mateos, G.G. Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight. *Meat Sci.* **2003**, *65*, 1369–1377. https://doi.org/10.1016/S0309-1740(03)00059-7.
16. Wood, J.D.; Nute, G.R.; Richardson, R.I.; Whittington, F.M.; Southwood, O.; Plastow, G.; Mansbridge, R.; Da Costa, N.; Chang, K.C. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Sci.* **2004**, *67*, 651–667. https://doi.org/10.1016/j.meatsci.2004.01.007.
17. Hocquette, J.F.; Gondret, F.; Baza, E.; Mdale, F.; Jurie, C.; Pethick, D.W. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal* **2010**, *4*, 303–319. https://doi.org/10.1017/S1751731109991091.
18. Madeira, M.S.; Lopes, P.A.; Costa, P.; Coelho, D.; Alfaia, C.M.; Prates, J.A.M. Reduced protein diets increase intramuscular fat of psoas major, a red muscle, in lean and fatty pig genotypes. *Animal* **2017**, *11*, 2094–2102. https://doi.org/10.1017/S1751731117000921.
19. Ladeira, M.M.; Schoonmaker, J.P.; Swanson, K.C.; Duckett, S.K.; Gionbelli, M.P.; Rodrigues, L.M.; Teixeira, P.D. Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat. *Animal* **2018**, *12*, S282–S294. https://doi.org/10.1017/S1751731118001933.

20. Núñez, Y.; Radović, Č.; Savić, R.; García-casco, J.M.; Čandek-Potokar, M.; Benítez, R.; Radojković, D.; Lukić, M.; Gogić, M.; Muñoz, M.; et al. Muscle transcriptome analysis reveals molecular pathways related to oxidative phosphorylation, antioxidant defense, fatness and growth in mangalitsa and moravka pigs. *Animals* **2021**, *11*, 844. https://doi.org/10.3390/ani11030844.
21. Olivares, A.; Daza, A.; Rey, A.I.; Lopez-Bote, C.J. Interactions between genotype, dietary fat saturation and vitamin A concentration on intramuscular fat content and fatty acid composition in pigs. *Meat Sci.* **2009**, *82*, 6–12. https://doi.org/10.1016/j.meatsci.2008.11.006.
22. Tous, N.; Lizardo, R.; Theil, P.K.; Vilà, B.; Gispert, M.; Font-i-Furnols, M.; Esteve-Garcia, E. Effect of vitamin A depletion on fat deposition in finishing pigs, intramuscular fat content and gene expression in the longissimus muscle. *Livest. Sci.* **2014**, *167*, 392–399. https://doi.org/10.1016/j.livsci.2014.05.025.
23. Braunschweig, M.; Jagannathan, V.; Gutzwiller, A.; Bee, G. Investigations on transgenerational epigenetic response down the male line in F2 pigs. *PLoS ONE* **2012**, *7*, e30583. https://doi.org/10.1371/journal.pone.0030583.
24. Natacha Pena, R.; Ros-Freixedes, R.; Tor, M.; Estany, J. Genetic marker discovery in complex traits: A field example on fat content and composition in pigs. *Int. J. Mol. Sci.* **2016**, *17*, 2100. https://doi.org/10.3390/ijms17122100.
25. Wang, H.; Wang, J.; Yang, D.D.; Liu, Z.L.; Zeng, Y.Q.; Chen, W. Expression of lipid metabolism genes provides new insights into intramuscular fat deposition in Laiwu pigs. *Asian-Australas. J. Anim. Sci.* **2020**, *33*, 390–397. https://doi.org/10.5713/ajas.18.0225.
26. Fenech, M.; El-Sohemy, A.; Cahill, L.; Ferguson, L.R.; French, T.A.C.; Tai, E.S.; Milner, J.; Koh, W.P.; Xie, L.; Zucker, M.; et al. Nutrigenetics and nutrigenomics: Viewpoints on the current status and applications in nutrition research and practice. *J. Nutrigenet. Nutrigenom.* **2011**, *4*, 69–89. https://doi.org/10.1159/000327772.
27. Wang, L.; Lu, H.; Wang, Y.; Yang, S.; Xu, H.; Cheng, K.; Zhao, Y.; Tian, B.; Hua, Y. An Improved Method for Identifying Specific DNA-Protein-Binding Sites In Vitro. *Mol. Biotechnol.* **2017**, *59*, 59–65. https://doi.org/10.1007/s12033-017-9993-y.
28. Ghormade, V. Nutrigenomics and its Applications in Animal Science. *Vet. Res. Forum* **2011**, *2*, 147–155.
29. Dauncey, M.J.; White, P.; Burton, K.A.; Katsumata, M. Nutrition–hormone receptor–gene interactions: Implications for development and disease. *Proc. Nutr. Soc.* **2001**, *60*, 63–72. https://doi.org/10.1079/pns200071.
30. Kaput, J.; Rodriguez, R.L. Nutritional genomics: The next frontier in the postgenomic era. *Physiol. Genom.* **2004**, *16*, 166–177. https://doi.org/10.1152/physiolgenomics.00107.2003.
31. Huang, Y.Z.; Sun, J.J.; Zhang, L.Z.; Li, C.J.; Womack, J.E.; Li, Z.J.; Lan, X.Y.; Lei, C.Z.; Zhang, C.L.; Zhao, X.; et al. Genome-wide DNA methylation profiles and their relationships with mRNA and the microRNA transcriptome in bovine muscle tissue (Bos taurine). *Sci. Rep.* **2014**, *3*, 1–17. https://doi.org/10.1038/srep06546.
32. Zhang, S.; Shen, L.; Xia, Y.; Yang, Q.; Li, X.; Tang, G.; Jiang, Y.; Wang, J.; Li, M.; Zhu, L. DNA methylation landscape of fat deposits and fatty acid composition in obese and lean pigs. *Sci. Rep.* **2016**, *6*, 1–10. https://doi.org/10.1038/srep35063.
33. Cedar, H. DNA methylation and gene activity. *Cell* **1988**, *53*, 3–4. https://doi.org/10.1016/0092-8674(88)90479-5.
34. Li, X.J.; Liu, L.Q.; Dong, H.; Yang, J.J.; Wang, W.W.; Zhang, Q.; Wang, C.L.; Zhou, J.; Cheng, H.Q. Comparative genome-wide methylation analysis of *longissimus dorsi* muscles in Yorkshire and Wannanhua pigs. *Anim. Genet.* **2020**, *52*, 78-89. https://doi.org/10.1111/age.13029.
35. Marín-garcía, P.J.; Llobat, L. How does protein nutrition affect the epigenetic changes in pig? A review. *Animals* **2021**, *11*, 544. https://doi.org/10.3390/ani11020544.
36. Debusk, R.M.; Fogarty, C.P.; Ordovas, J.M.; Kornman, K.S. Nutritional genomics in practice: Where do we begin? *J. Am. Diet. Assoc.* **2005**, *105*, 589–598. https://doi.org/10.1016/j.jada.2005.01.002.
37. Ordovas, J.M.; Corella, D. Nutritional genomics. *Annu. Rev. Genom. Hum. Genet.* **2004**, *5*, 71–118. https://doi.org/10.1146/annurev.genom.5.061903.180008.
38. Bouchard, C; Ordovas, J.M. Fundamentals of nutrigenetics and nutrigenomics. *Prog Mol Biol Transl Sci*. **2012**, *108*, 1–15. https://doi.org/10.1016/B978-0-12-398397-8.00001-0.
39. Mutch, D.M.; Wahli, W.; Williamson, G. Nutrigenomics and nutrigenetics: The emerging faces of nutrition. *FASEB J.* **2005**, *19*, 1602–1616. https://doi.org/10.1096/fj.05-3911rev.
40. Fenech, M. Genome health nutrigenomics and nutrigenetics—Diagnosis and nutritional treatment of genome damage on an individual basis. *Food Chem. Toxicol.* **2008**, *46*, 1365–1370. https://doi.org/10.1016/j.fct.2007.06.035.

41. Peña-Romero, A.C.; Navas-Carrillo, D.; Marín, F.; Orenes-Piñero, E. The future of nutrition: Nutrigenomics and nutrigenetics in obesity and cardiovascular diseases. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 3030–3041. https://doi.org/10.1080/10408398.2017.1349731.
42. Sharma, P.; Dwivedi, S. Nutrigenomics and Nutrigenetics: New Insight in Disease Prevention and Cure. *Indian J. Clin. Biochem.* **2017**, *32*, 371–373. https://doi.org/10.1007/s12291-017-0699-5.
43. Urrutia, O.; Alfonso, L.; Mendizabal, J.A. Cellularity Description of Adipose Depots in Domesticated Animals. *Adipose Tissue* **2018**, *23*, 73–90. https://doi.org/10.5772/intechopen.74109.
44. Bourgeois, C.; Gorwood, J.; Barrail-Tran, A.; Lagathu, C.; Capeau, J.; Desjardins, D.; Le Grand, R.; Damouche, A.; Béréziat, V.; Lambotte, O. Specific Biological Features of Adipose Tissue, and Their Impact on HIV Persistence. *Front. Microbiol.* **2019**, *10*, 2837. https://doi.org/10.3389/fmicb.2019.02837.
45. Katsumata, M. Promotion of intramuscular fat accumulation in porcine muscle by nutritional regulation. *Anim. Sci. J.* **2011**, *82*, 17–25. https://doi.org/10.1111/j.1740-0929.2010.00844.x.
46. Poklukar, K.; Čandek-Potokar, M.; Lukač, N.B.; Tomažin, U.; Škrlep, M. Lipid deposition and metabolism in local and modern pig breeds: A review. *Animals* **2020**, *10*, 424. https://doi.org/10.3390/ani10030424.
47. Szydlowski, M.; Buszka, A.; Mackowski, M.; Lechniak, D.; Switonski, M. Polymorphism of genes encoding cytokines IL6 and TNF is associated with pig fatness. *Livest. Sci.* **2011**, *136*, 150–156. https://doi.org/10.1016/j.livsci.2010.08.008.
48. Hamill, R.M.; McBryan, J.; McGee, C.; Mullen, A.M.; Sweeney, T.; Talbot, A.; Cairns, M.T.; Davey, G.C. Functional analysis of muscle gene expression profiles associated with tenderness and intramuscular fat content in pork. *Meat Sci.* **2012**, *92*, 440–450. https://doi.org/10.1016/j.meatsci.2012.05.007.
49. Albuquerque, A.; Óvilo, C.; Núñez, Y.; Benítez, R.; López-Garcia, A.; García, F.; Félix, M.D.R.; Laranjo, M.; Charneca, R.; Martins, J.M. Transcriptomic Profiling of Skeletal Muscle Reveals Candidate Genes Influencing Muscle Growth and Associated Lipid Composition in Portuguese Local Pig Breeds. *Animals* **2021**, *11*, 1423. https://doi.org/10.3390/ani11051423.
50. Huang, W.; Zhang, X.; Li, A.; Xie, L.; Miao, X. Genome-wide analysis of mRNAs and lncRNAs of intramuscular fat related to lipid metabolism in two pig breeds. *Cell. Physiol. Biochem.* **2018**, *50*, 2406–2422. https://doi.org/10.1159/000495101.
51. González-Prendes, R.; Quintanilla, R.; Mármol-Sánchez, E.; Pena, R.N.; Ballester, M.; Cardoso, T.F.; Manunza, A.; Casellas, J.; Cánovas, Á.; Díaz, I.; et al. Comparing the mRNA expression profile and the genetic determinism of intramuscular fat traits in the porcine gluteus medius and *longissimus dorsi* muscles. *BMC Genom.* **2019**, *20*, 1–18. https://doi.org/10.1186/s12864-019-5557-9.
52. Wang, Y.; Ma, C.; Sun, Y.; Li, Y.; Kang, L.; Jiang, Y. Dynamic transcriptome and DNA methylome analyses on *longissimus dorsi* to identify genes underlying intramuscular fat content in pigs. *BMC Genom.* **2017**, *18*, 1–18. https://doi.org/10.1186/s12864-017-4201-9.
53. Zhao, S.; Wang, J.; Song, X.; Zhang, X.; Ge, C.; Gao, S. Impact of dietary protein on lipid metabolism-related gene expression in porcine adipose tissue. *Nutr. Metab.* **2010**, *7*, 1–13. https://doi.org/10.1186/1743-7075-7-6.
54. McNamara, J.P. *Nutrigenomics for improved reproduction*; Wiley-Blackwell: Ames, IA, USA, 2010; pp. 413–435.
55. Won, S.; Jung, J.; Park, E.; Kim, H. Identification of genes related to intramuscular fat content of pigs using genome-wide association study. *Asian-Australas. J. Anim. Sci.* **2018**, *31*, 157–162. https://doi.org/10.5713/ajas.17.0218.
56. Zappaterra, M.; Gioiosa, S.; Chillemi, G.; Zambonelli, P.; Davoli, R. Dissecting the Gene Expression Networks Associated with Variations in the Major Components of the Fatty Acid *Semimembranosus* Muscle Profile in Large White Heavy Pigs. *Animals* **2021**, *11*, 628. https://doi.org/10.3390/ani11030628.
57. Zappaterra, M.; Deserti, M.; Mazza, R.; Braglia, S.; Zambonelli, P.; Davoli, R. A gene and protein expression study on four porcine genes related to intramuscular fat deposition. *Meat Sci.* **2016**, *121*, 27–32. https://doi.org/10.1016/j.meatsci.2016.05.007.
58. Duran-Montgé, P.; Theil, P.K.; Lauridsen, C.; Esteve-Garcia, E. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. *Animal* **2009**, *3*, 535–542. https://doi.org/10.1017/S1751731108003686.
59. Baumgard, L.H.; Hausman, G.J.; Sanz Fernandez, M.V. Insulin: Pancreatic secretion and adipocyte regulation. *Domest. Anim. Endocrinol.* **2016**, *54*, 76–84. https://doi.org/10.1016/j.domaniend.2015.07.001.
60. Gao, Y.; Zhang, Y.H.; Jiang, H.; Xiao, S.Q.; Wang, S.; Ma, Q.; Sun, G.J.; Li, F.J.; Deng, Q.; Dai, L.S.; et al. Detection of differentially expressed genes in the *longissimus dorsi* of northeastern

indigenous and large white pigs. *Genet. Mol. Res.* **2011**, *10*, 779–791. https://doi.org/10.4238/vol10-2gmr1170.
61. Zhao, X.; Hu, H.; Lin, H.; Wang, C.; Wang, Y.; Wang, J. Muscle Transcriptome Analysis Reveals Potential Candidate Genes and Pathways Affecting Intramuscular Fat Content in Pigs. *Front. Genet.* **2020**, *11*, 877. https://doi.org/10.3389/fgene.2020.00877.
62. Zhao, S.M.; Ren, L.J.; Chen, L.; Zhang, X.; Cheng, M.L.; Li, W.Z.; Zhang, Y.Y.; Gao, S.Z. Differential expression of lipid metabolism related genes in porcine muscle tissue leading to different intramuscular fat deposition. *Lipids* **2009**, *44*, 1029–1037. https://doi.org/10.1007/s11745-009-3356-9.
63. Zhao, X.; Mo, D.; Li, A.; Gong, W.; Xiao, S.; Zhang, Y.; Qin, L.; Niu, Y.; Guo, Y.; Liu, X.; et al. Comparative analyses by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness. *PLoS ONE* **2011**, *6*, e19774. https://doi.org/10.1371/journal.pone.0019774.
64. Tyra, M.; Ropka-Molik, K.; Eckert, R.; Piórkowska, K.; Oczkowicz, M. H-FABP and LEPR gene expression profile in skeletal muscles and liver during ontogenesis in various breeds of pigs. *Domest. Anim. Endocrinol.* **2011**, *40*, 147–154. https://doi.org/10.1016/j.domaniend.2010.10.001.
65. Tyra, M.; Ropka-Molik, K. Effect of the FABP3 and LEPR gene polymorphisms and expression levels on intramuscular fat (IMF) content and fat cover degree in pigs. *Livest. Sci.* **2011**, *142*, 114–120. https://doi.org/10.1016/j.livsci.2011.07.003.
66. Li, X.; Kim, S.W.; Choi, J.S.; Lee, Y.M.; Lee, C.K.; Choi, B.H.; Kim, T.H.; Choi, Y.I.; Kim, J.J.; Kim, K.S. Investigation of porcine FABP3 and LEPR gene polymorphisms and mRNA expression for variation in intramuscular fat content. *Mol. Biol. Rep.* **2010**, *37*, 3931–3939. https://doi.org/10.1007/s11033-010-0050-1.
67. Han, X.; Jiang, T.; Yang, H.; Zhang, Q.; Wang, W.; Fan, B.; Liu, B. Investigation of four porcine candidate genes (H-FABP, MYOD1, UCP3 and MASTR) for meat quality traits in Large White pigs. *Mol. Biol. Rep.* **2012**, *39*, 6599–6605. https://doi.org/10.1007/s11033-012-1490-6.
68. Ryan, M.T.; Hamill, R.M.; O'Halloran, A.M.; Davey, G.C.; McBryan, J.; Mullen, A.M.; McGee, C.; Gispert, M.; Southwood, O.I.; Sweeney, T. SNP variation in the promoter of the PRKAG3 gene and association with meat quality traits in pig. *BMC Genet.* **2012**, *13*, 66. https://doi.org/10.1186/1471-2156-13-66.
69. Serão, N.V.L.; Veroneze, R.; Ribeiro, A.M.F.; Verardo, L.L.; Braccini Neto, J.; Gasparino, E.; Campos, C.F.; Lopes, P.S.; Guimarães, S.E.F. Candidate gene expression and intramuscular fat content in pigs. *J. Anim. Breed. Genet.* **2011**, *128*, 28–34. https://doi.org/10.1111/j.1439-0388.2010.00887.x.
70. Oczkowicz, M.; Tyra, M.; Ropka-Molik, K.; Mucha, A.; Zukowski, K. Effect of IGF2 intron3-g.3072G>A on intramuscular fat (IMF) content in pigs raised in Poland. *Livest. Sci.* **2012**, *149*, 301–304. https://doi.org/10.1016/j.livsci.2012.06.021.
71. Cui, J.; Chen, W.; Liu, J.; Xu, T.; Zeng, Y. Study on quantitative expression of PPARγ and ADRP in muscle and its association with intramuscular fat deposition of pig. *Springerplus* **2016**, *5*, 1501. The https://doi.org/10.1186/s40064-016-3187-0.
72. Wang, W.; Xue, W.; Xu, X.; Jin, B.; Zhang, X. Correlations of genes expression in PPAR signalling pathway with porcine meat quality traits. *Czech J. Anim. Sci.* **2016**, *7*, 333–339. https://doi.org/10.17221/85/2015-CJAS.
73. Liu, Z.; Sun, W.; Zhao, Y.; Xu, C.; Fu, Y.; Li, Y.; Chen, J. The effect of variants in the promoter of BMPER on the intramuscular fat deposition in *longissimus dorsi* muscle of pigs. *Gene* **2014**, *542*, 168–172. https://doi.org/10.1016/j.gene.2014.03.038.
74. Sweeney, T.; O'Halloran, A.M.; Hamill, R.M.; Davey, G.C.; Gil, M.; Southwood, O.I.; Ryan, M.T. Novel variation in the FABP3 promoter and its association with fatness traits in pigs. *Meat Sci.* **2015**, *100*, 32–40. https://doi.org/10.1016/j.meatsci.2014.09.014.
75. Ros-Freixedes, R.; Gol, S.; Pena, R.N.; Tor, M.; Ibáñez-Escriche, N.; Dekkers, J.C.M.; Estany, J. Genome-wide association study singles out SCD and LEPR as the two main loci influencing intramuscular fat content and fatty acid composition in duroc pigs. *PLoS ONE* **2016**, *11*, e0152496. https://doi.org/10.1371/journal.pone.0152496.
76. Miao, Z.; Zhu, F.; Zhang, H.; Chan, H.; Xie, X.; Zhang, J.; Xu, Z. Developmental patterns of FASN and LIPE mRNA expression in adipose tissue of growing jinhua and landrace gilts. *Czech J. Anim. Sci.* **2010**, *55*, 557–564. https://doi.org/10.17221/2514-cjas.
77. Wang, Z.; Li, Q.; Chamba, Y.; Zhang, B.; Shang, P.; Zhang, H.; Wu, C. Identification of genes related to growth and lipid deposition from transcriptome profiles of pig muscle tissue. *PLoS ONE* **2015**, *10*, e0141138. https://doi.org/10.1371/journal.pone.0141138.
78. Piórkowska, K.; Małopolska, M.; Ropka-Molik, K.; Nędza, M.S.; Wiechniak, A.; Żukowski, K.; Lambert, B.; Tyra, M. Evaluation of scd, acaca and fasn mutations: Effects on pork quality and

other production traits in pigs selected based on rna-seq results. *Animals* **2020**, *10*, 123. https://doi.org/10.3390/ani10010123.
79. Ren, H.; Xiao, W.; Qin, X.; Cai, G.; Chen, H.; Hua, Z.; Cheng, C.; Li, X.; Hua, W.; Xiao, H.; et al. Myostatin regulates fatty acid desaturation and fat deposition through MEF2C/miR222/SCD5 cascade in pigs. *Commun. Biol.* **2020**, *3,* 612. https://doi.org/10.1038/s42003-020-01348-8.
80. Zappaterra, M.; Gioiosa, S.; Chillemi, G.; Zambonelli, P.; Davoli, R. Muscle transcriptome analysis identifies genes involved in ciliogenesis and the molecular cascade associated with intramuscular fat content in Large White heavy pigs. *PLoS ONE* **2020**, *15*, e0233372. https://doi.org/10.1371/journal.pone.0233372.
81. Wang, B.; Li, P.; Zhou, W.; Gao, C.; Liu, H.; Li, H.; Niu, P.; Zhang, Z.; Li, Q.; Zhou, J.; et al. Association of twelve candidate gene polymorphisms with the intramuscular fat content and average backfat thickness of chinese suhuai pigs. *Animals* **2019**, *9*, 858. https://doi.org/10.3390/ani9110858.
82. Muñoz, M.; García-Casco, J.M.; Caraballo, C.; Fernández-Barroso, M.Á.; Sánchez-Esquiliche, F.; Gómez, F.; Rodríguez, M.C.; Silió, L. Identification of Candidate Genes and Regulatory Factors Underlying Intramuscular Fat Content Through *Longissimus dorsi* Transcriptome Analyses in Heavy Iberian Pigs. *Front. Genet.* **2018**, *9*, 608. https://doi.org/10.3389/fgene.2018.00608.
83. Kersten, S.; Desvergne, B.; Wahli, W. Roles of PPARS in health and disease. *Nature* **2000**, *405*, 421–424. https://doi.org/10.1038/35013000.
84. Rosen, E.D.; Spiegelman, B.M. PPARγ: A Nuclear Regulator of Metabolism, Differentiation, and Cell Growth. *J. Biol. Chem.* **2001**, *276*, 37731–37734. https://doi.org/10.1074/jbc.R100034200.
85. Ayuso, M.; Fernández, A.; Núñez, Y.; Benitez, R.; Isabel, B.; Barragán, C.; Fernández, A.I.; Rey, A.I.; Medrano, J.F.; Cánovas, Á.; et al. Comparative analysis of muscle transcriptome between pig genotypes identifies genes and regulatory mechanisms associated to growth, Fatness and metabolism. *PLoS ONE* **2015**, *10*, e0145162. https://doi.org/10.1371/journal.pone.0145162.
86. Michalik, L.; Auwerx, J.; Berger, J.P.; Chatterjee, V.K.; Glass, C.K.; Gonzalez, F.J.; Grimaldi, P.A.; Kadowaki, T.; Lazar, M.A.; Rahilly, S.O.; et al. International Union of Pharmacology. LXI. Peroxisome Proliferator-Activated Receptors. *Pharmacol. Rev.* **2006**, *58*, 726–741. https://doi.org/10.1124/pr.58.4.5.(NR1C1).
87. Keller, H.; Dreyer, C.; Medin, J.; Mahfoudi, A.; Ozato, K.; Wahli, W. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2160–2164. https://doi.org/10.1073/pnas.90.6.2160.
88. Gerbens, F.; Verburg, F.J.; Van Moerkerk, H.T.B.; Engel, B.; Buist, W.; Veerkamp, J.H.; Te Pas, M.F.W. Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. *J. Anim. Sci.* **2001**, *79*, 347–354. https://doi.org/10.2527/2001.792347x.
89. Chen, Q.M.; Wang, H.; Zeng, Y.Q.; Chen, W. Developmental changes and effect on intramuscular fat content of H-FAthe BP and A-FABP mRNA expression in pigs. *J. Appl. Genet.* **2013**, *54*, 119–123. https://doi.org/10.1007/s13353-012-0122-0.
90. Cho, K.H.; Kim, M.J.; Jeon, G.J.; Chung, H.Y. Association of genetic variants for FABP3 gene with back fat thickness and intramuscular fat content in pig. *Mol. Biol. Rep.* **2011**, *38*, 2161–2166. https://doi.org/10.1007/s11033-010-0344-3.
91. Gerbens, F.; Van Erp, A.J.M.; Harders, F.L.; Verburg, F.J.; Meuwissen, T.H.E.; Veerkamp, J.H.; Te Pas, M.F.W. Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. *J. Anim. Sci.* **1999**, *77*, 846–852, https://doi.org/10.2527/1999.774846x.
92. Catillo, G.; Zappaterra, M.; Zambonelli, P.; Buttazzoni, L.; Steri, R.; Minelli, G.; Davoli, R. Genome-wide association study identifies quantitative trait loci regions involved in muscle acidic profile in large white heavy pigs. *Animal* **2020**, *14*, 1342–1350. https://doi.org/10.1017/S1751731120000099.
93. Rossi, R.; Pastorelli, G.; Cannata, S.; Corino, C. Recent advances in the use of fatty acids as supplements in pig diets: A review. *Anim. Feed Sci. Technol.* **2010**, *162*, 1–11. https://doi.org/10.1016/j.anifeedsci.2010.08.013.
94. Hirose, K.; Ito, T.; Fukawa, K.; Arakawa, A.; Mikawa, S.; Hayashi, Y.; Tanaka, K. Evaluation of effects of multiple candidate genes (LEP, LEPR, MC4R, PIK3C3, and VRTN) on production traits in Duroc pigs. *Anim. Sci. J.* **2014**, *85*, 198–206. https://doi.org/10.1111/asj.12134.
95. Ponsuksili, S.; Murani, E.; Walz, C.; Schwerin, M.; Wimmers, K. Pre- and postnatal hepatic gene expression profiles of two pig breeds differing in body composition: Insight into pathways of metabolic regulation. *Physiol. Genom.* **2007**, *29*, 267–279. https://doi.org/10.1152/physiolgenomics.00178.2006.

96. Crespo-Piazuelo, D.; Criado-Mesas, L.; Revilla, M.; Castelló, A.; Noguera, J.L.; Fernández, A.I.; Ballester, M.; Folch, J.M. Identification of strong candidate genes for backfat and intramuscular fatty acid composition in three crosses based on the Iberian pig. *Sci. Rep.* **2020**, *10*. https://doi.org/10.1038/s41598-020-70894-2.
97. Stinckens, A.; Luyten, T.; Bijttebier, J.; Van Den Maagdenberg, K.; Dieltiens, D.; Janssens, S.; De Smet, S.; Georges, M.; Buys, N. Characterization of the complete porcine MSTN gene and expression levels in pig breeds differing in muscularity. *Anim. Genet.* **2008**, *39*, 586–596. https://doi.org/10.1111/j.1365-2052.2008.01774.x.
98. Zou, Y.; Long, L.; Yuan, Z.; Zou, J.Y.; Hao, H.; Yang, H.; Xiang, J.; Li, N.; Li, Y.Q. Generation of pigs with a Belgian Blue mutation in MSTN using CRISPR/Cpf1-assisted ssODN-mediated homologous recombination. *J. Integr. Agric.* **2019**, *18*, 1329–1336. https://doi.org/10.1016/S2095-3119(19)62694-8.
99. Kärst, S.; Strucken, E.M.; Schmitt, A.O.; Weyrich, A.; de Villena, F.P.M.; Yang, H.; Brockmann, G.A. Effect of the myostatin locus on muscle mass and intramuscular fat content in a cross between mouse lines selected for hypermuscularity. *BMC Genom.* **2013**, *14*, 16. https://doi.org/10.1186/1471-2164-14-16.
100. Li, W.; Li, R.; Wei, Y.; Meng, X.; Wang, B.; Zhang, Z.; Wu, W.; Liu, H. Effect of mstn mutation on growth and carcass performance in duroc x meishan hybrid populationDuroc*mals* **2020**, *10*, 932. https://doi.org/10.3390/ani10060932.
101. Zhu, X.X.; Zhan, Q.M.; Wei, Y.Y.; Yan, A.F.; Feng, J.; Liu, L.; Lu, S.S.; Tang, D.S. CRISPR/Cas9-mediated MSTN disruption accelerates the growth of Chinese Bama pigs. *Reprod. Domest. Anim.* **2020**, *55*, 1314–1327. https://doi.org/10.1111/rda.13775.
102. Xing, K.; Zhao, X.; Liu, Y.; Zhang, F.; Tan, Z.; Qi, X.; Wang, X.; Ni, H.; Guo, Y.; Sheng, X.; et al. Identification of differentially expressed micrornas and their potential tarmicroRNAs in adipose tissue from pigs with highly divergent backfat thickness. *Animals* **2020**, *10*, 624. https://doi.org/10.3390/ani10040624.
103. Chen, J.; Yang, X.J.; Xia, D.; Chen, J.; Wegner, J.; Jiang, Z.; Zhao, R.Q. Sterol regulatory element binding transcription factor 1 expression and genetic polymorphism significantly affect intramuscular fat deposition in the longissimus muscle of Erhualian and Sutai pigs. *J. Anim. Sci.* **2008**, *86*, 57–63. https://doi.org/10.2527/jas.2007-0066.
104. Stachowiak, M.; Nowacka-Woszuk, J.; Szydlowski, M.; Switonski, M. The ACACA and SREBF1 genes are promising markers for pig carcass and performance traits, but not for fatty acid content in the *longissimus dorsi* muscle and adipose tissue. *Meat Sci.* **2013**, *95*, 64–71. https://doi.org/10.1016/j.meatsci.2013.04.021.
105. Badke, Y.M.; Bates, R.O.; Ernst, C.W.; Schwab, C.; Steibel, J.P. Estimation of linkage disequilibrium in four US pig breeds. *BMC Genom.* **2012**, *13*, 24. https://doi.org/10.1186/1471-2164-13-24.
106. Ernst, C.W.; Steibel, J.P. Molecular advances in QTL discovery and application in pig breeding. *Trends Genet.* **2013**, *29*, 215–224. https://doi.org/10.1016/j.tig.2013.02.002.
107. Ding, R.; Yang, M.; Quan, J.; Li, S.; Zhuang, Z.; Zhou, S.; Zheng, E.; Hong, L.; Li, Z.; Cai, G.; et al. Single-locus and multi-locus genome-wide association studies for intramuscular fat in Duroc pigs. The *Front. Genet.* **2019**, *10*, 619. https://doi.org/10.3389/fgene.2019.00619.
108. Dalla Costa, O.A.; de Tavernari, F.C.; dos Lopes, L.S.; Dalla Costa, F.A.; Feddern, V.; de Lima, G.J.M.M. Performance, carcass and meat quality of pigs submitted to immunocastration and different feeding programs. *Res. Vet. Sci.* **2020**, *131*, 137–145. https://doi.org/10.1016/j.rvsc.2020.04.015.
109. Wimmers, K.; Murani, E.; Te Pas, M.F.W.; Chang, K.C.; Davoli, R.; Merks, J.W.M.; Henne, H.; Muraniova, M.; Da Costa, N.; Harlizius, B.; et al. Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition. *Anim. Genet.* **2007**, *38*, 474–484. https://doi.org/10.1111/j.1365-2052.2007.01639.x.
110. Wang, X.; Chen, J.; Liu, H.; Xu, Y.; Wang, X.; Xue, C.; Yu, D.; Jiang, Z. The pig p160 co-activator family: Full length cDNA cloning, expressFull-lengthects on intramuscular fat content in *Longissimus dorsi* muscle. *Domest. Anim. Endocrinol.* **2008**, *35*, 208–216. https://doi.org/10.1016/j.domaniend.2008.05.006.
111. Zhao, C.; Chen, X.; Wu, W.; Wang, W.; Pang, W.; Yang, G. MAT2B promotes adipogenesis by modulating SAMe levels and activating AKT/ERK pathway during porcine intramuscular preadipocyte differentiation. *Exp. Cell Res.* **2016**, *344*, 11–21. https://doi.org/10.1016/j.yexcr.2016.02.019.

112. Zhang, Z.; Zhang, Z.; Oyelami, F.O.; Sun, H.; Xu, Z.; Ma, P.; Wang, Q.; Pan, Y. Identification of genes related to intramuscular fat independent of backfat thickness in Duroc pigs using single-step genome-wide association. *Anim. Genet.* **2020**, *52*, 108–113. https://doi.org/10.1111/age.13012.
113. Andersson, L.; Haley, C.S.; Ellegren, H.; Knott, S.A.; Johansson, M.; Andersson, K.; Andersson-Eklund, L.; Edfors-Lilja, I.; Fredholm, M.; Hansson, I.; et al. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* **1994**, *263*, 1771–1774. https://doi.org/10.1126/science.8134840.
114. Harper, G.S.; Pethick, D.W. How might marbling begin? *Aust. J. Exp. Agric.* **2004**, *44*, 653–662. https://doi.org/10.1071/EA02114.
115. Lee, K.T.; Park, E.W.; Moon, S.; Park, H.S.; Kim, H.Y.; Jang, G.W.; Choi, B.H.; Chung, H.Y.; Lee, J.W.; Cheong, I.C.; et al. Genomic sequence analysis of a potential QTL region for fat trait on pig chromosome 6. *Genomics* **2006**, *87*, 218–224. https://doi.org/10.1016/j.ygeno.2005.09.002.
116. Switonski, M.; Stachowiak, M.; Cieslak, J.; Bartz, M.; Grzes, M. Knowledge on the genetic background of fat tissue accumulation is important in livestock production. *J. Appl. Genet.* **2010**, *51*, 153–168. https://doi.org/10.1007/BF03195724.
117. Ponsuksili, S.; Trakooljul, N.; Basavaraj, S.; Hadlich, F.; Murani, E.; Wimmers, K. Epigenome-wide skeletal muscle DNA methylation profiles at the background of distinct metabolic types and ryanodine receptor variation in pigs. *BMC Genom.* **2019**, *20*, 1–16. https://doi.org/10.1186/s12864-019-5880-1.
118. Meadus, W.J.; Duff, P.; Juarez, M.; Roberts, J.C.; Zantinge, J.L. Identification of marbling gene loci in commercial pigs in Canadian herds. *Agriculture* **2018**, *8*, 122. https://doi.org/10.3390/agriculture8080122.
119. Qimuge, N.; He, Z.; Qin, J.; Sun, Y.; Wang, X.; Yu, T.; Dong, W.; Yang, G.; Pang, W. Overexpression of DNMT3A promotes proliferation and inhibits differentiation of porcine intramuscular preadipocytes by methylating p21 and PPARg promoters. *Gene* **2019**, *696*, 54–62. https://doi.org/10.1016/j.gene.2019.02.029.
120. Hamill, R.M.; Aslan, O.; Mullen, A.M.; O'Doherty, J.V.; McBryan, J.; Morris, D.G.; Sweeney, T. Transcriptome analysis of porcine M. *semimembranosus* divergent in intramuscular fat as a consequence of dietary protein restriction. *BMC Genom.* **2013**, *14*, 453. https://doi.org/10.1186/1471-2164-14-453.
121. Xiong, Q.; Chai, J.; Deng, C.; Jiang, S.; Liu, Y.; Huang, T.; Suo, X.; Zhang, N.; Li, X.; Yang, Q.; et al. Characterization of porcine SKIP gene in skeletal muscle development: Polymorphisms, association analysis, expression and regulation of cell growth in C2C12 cells. *Meat Sci.* **2012**, *92*, 490–497. https://doi.org/10.1016/j.meatsci.2012.05.016.
122. Bibikova, M.; Barnes, B.; Tsan, C.; Ho, V.; Klotzle, B.; Le, J.M.; Delano, D.; Zhang, L.; Schroth, G.P.; Gunderson, K.L.; et al. High density DNA methylation array with single CpG site resolution. *Genomics* **2011**, *98*, 288-295. https://doi.org/10.1016/j.ygeno.2011.07.007.
123. Schachtschneider, K.M.; Madsen, O.; Park, C.; Rund, L.A.; Groenen, M.A.M.; Schook, L.B. Adult porcine genome-wide DNA methylation patterns support pigs as a biomedical model. *BMC Genom.* **2015**, *16*, 743. https://doi.org/10.1186/s12864-015-1938-x.
124. Kim, M.; Costello, J. DNA methylation: An epigenetic mark of cellular memory. *Exp. Mol. Med.* **2017**, *49*, e322. https://doi.org/10.1038/emm.2017.10.
125. Bannister, A.J.; Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res.* **2011**, *21*, 381–395. https://doi.org/10.1038/cr.2011.22.
126. Zappaterra, M.; Catillo, G.; Belmonte, A.M.; Lo Fiego, D.P.; Zambonelli, P.; Steri, R.; Buttazzoni, L.; Davoli, R. Genetic parameters of muscle fatty acid profile in a purebred Large White heavy pig population. *Meat Sci.* **2020**, *163*, 108057. https://doi.org/10.1016/j.meatsci.2020.108057.
127. Friedman, R.C.; Farh, K.K.H.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **2009**, *19*, 92–105. https://doi.org/10.1101/gr.082701.108.
128. Clancy, S.; Brown, W. Translation : DNA to mRNA to Protein. *Nat. Educ.* **2008**, *1*, 101. Available online: https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393/ (accessed on 7 November 2021).
129. Fabian, M.R.; Sonenberg, N.; Filipowicz, W. Regulation of mRNA translation and stability by microRNAs. *Annu. Rev. Biochem.* **2010**, *9*, 351–379. https://doi.org/10.1146/annurev-biochem-060308-103103.
130. Ortega, F.J.; Moreno-Navarrete, J.M.; Pardo, G.; Sabater, M.; Hummel, M.; Ferrer, A.; Rodriguez-Hermosa, J.I.; Ruiz, B.; Ricart, W.; Peral, B.; et al. MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. *PLoS ONE* **2010**, *5*, e9022. https://doi.org/10.1371/journal.pone.0009022.
131. Hilton, C.; Neville, M.J.; Karpe, F. MicroRNAs in adipose tissue: Their role in adipogenesis and obesity. *Int. J. Obes.* **2013**, *37*, 325–332. https://doi.org/10.1038/ijo.2012.59.

132. Mobuchon, L.; Le Guillou, S.; Marthey, S.; Laubier, J.; Laloë, D.; Bes, S.; Le Provost, F.; Leroux, C. Sunflower oil supplementation affects the expression of miR-20a-5p and miR-142-5p in the lactating bovine mammary gland. *PLoS ONE* **2017**, *12*, e0185511. https://doi.org/10.1371/journal.pone.0185511.
133. Moon, J.K.; Kim, K.S.; Kim, J.J.; Choi, B.H.; Cho, B.W.; Kim, T.H.; Lee, C.K. Differentially expressed transcripts in adipose tissue between Korean native pig and Yorkshire breeds. *Anim. Genet.* **2009**, *40*, 115–118. https://doi.org/10.1111/j.1365-2052.2008.01798.x.
134. Liang, Y.; Wang, Y.; Ma, L.; Zhong, Z.; Yang, X.; Tao, X.; Chen, X.; He, Z.; Yang, Y.; Zeng, K.; et al. Comparison of microRNAs in adipose and muscle tissue from seven indigenous Chinese breeds and Yorkshire pigs. *Anim. Genet.* **2019**, *50*, 439–448. https://doi.org/10.1111/age.12826.
135. Tyra, M.; Ropka-Molik, K.; Terman, A.; Piórkowska, K.; Oczkowicz, M.; Bereta, A. Association between subcutaneous and intramuscular fat content in porcine ham and loin depending on age, breed and FABP3 and LEPR genes transcript abundance. *Mol. Biol. Rep.* **2013**, *40*, 2301–2308. https://doi.org/10.1007/s11033-012-2311-7.
136. Schiavina, S.; Colombo, M.; Hedegaard, J.; Hornshøj, H.; Davoli, R.; Fontanesi, L.; Stella, A.; Nanni Costa, L.; Bendixen, C.; Russo, V. Analysis of skeletal muscle tissue expression profiles in pig to identify genes involved in meat quality traits: Effect of stress conditions before slaughtering in different pig breeds. *Ital. J. Anim. Sci.* **2007**, *6* (Suppl. 1), 205.
137. Martin, E.C.; Qureshi, A.T.; Dasa, V.; Freitas, M.A.; Gimble, J.M.; Davis, T.A. MicroRNA regulation of stem cell differentiation and diseases of the bone and adipose tissue: Perspectives on miRNA biogenesis and cellular transcriptome. *Biochimie* **2016**, *124*, 98–111. https://doi.org/10.1016/j.biochi.2015.02.012.
138. Miao, Z.; Wang, S.; Wang, Y.; Wei, P.; Khan, M.A.; Zhang, J.; Guo, L.; Liu, D. Comparison of microRNAs in the intramuscular adipose tissue from Jinhua and Landrace pigs. *J. Cell. Biochem.* **2019**, *120*, 192–200. https://doi.org/10.1002/jcb.27298.
139. Timoneda, O.; Balcells, I.; Núñez, J.I.; Egea, R.; Vera, G.; Castelló, A.; Tomàs, A.; Sánchez, A. miRNA Expression Profile Analysis in Kidney of Different Porcine Breeds. *PLoS ONE* **2013**, *8*, e55402. *https://doi.org/*10.1371/journal.pone.0055402.
140. Li, H.Y.; Xi, Q.Y.; Xiong, Y.Y.; Liu, X.L.; Cheng, X.; Shu, G.; Wang, S.B.; Wang, L.N.; Gao, P.; Zhu, X.T.; et al. Identification and comparison of microRNAs from skeletal muscle and adipose tissues from two porcine breeds. *Anim. Genet.* **2012**, *43*, 704–713. https://doi.org/10.1111/j.1365-2052.2012.02332.x.
141. Li, X.J.; Zhou, J.; Liu, L.Q.; Qian, K.; Wang, C.L. Identification of genes in *longissimus dorsi* muscle differentially expressed between Wannanhua and Yorkshire pigs using RNA-sequencing. *Anim. Genet.* **2016**, *47*, 324–333. https://doi.org/10.1111/age.12421.
142. Mariman, E.C.M. Nutrigenomics and nutrigenetics: The 'omics' revolution in nutritional science. *Biotechnol. Appl. Biochem.* **2006**, *44*, 119. https://doi.org/10.1042/ba20050112.
143. Guo, B.; Dalrymple, B.P. Transcriptomics of Meat Quality. *New Asp. Meat Qual.* **2017**, *11*, 259–320. https://doi.org/10.1016/B978-0-08-100593-4.00012-6.
144. Ferrari, A.; Longo, R.; Peri, C.; Coppi, L.; Caruso, D.; Mai, A.; Mitro, N.; De Fabiani, E.; Crestani, M. Inhibition of class I HDACs imprints adipogenesis toward oxidative and brown-like phenotype. *Biochim. Biophys. Acta-Mol. Cell Biol. Lipids* **2020**, *1865*, 158594. https://doi.org/10.1016/j.bbalip.2019.158594.
145. Baubec, T.; Colombo, D.F.; Wirbelauer, C.; Schmidt, J.; Burger, L.; Krebs, A.R.; Akalin, A.; Schübeler, D. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **2015**, *520*, 243–247. https://doi.org/10.1038/nature14176.
146. Schneider, J.W.; Oommen, S.; Qureshi, M.Y.; Goetsch, S.C.; Pease, D.R.; Sundsbak, R.S.; Guo, W.; Sun, M.; Sun, H.; Kuroyanagi, H.; et al. Dysregulated ribonucleoprotein granules promote cardiomyopathy in RBM20 gene-edited pigs. *Nat. Med.* **2020**, *26*, 1788–1800. https://doi.org/10.1038/s41591-020-1087-x.
147. Van, M.V.; Fujimori, T.; Bintu, L. Nanobody-mediated control of gene expression and epigenetic memory. *Nat. Commun.* **2020**, *12*, 537. https://doi.org/10.1038/s41467-020-20757-1.
148. Gujar, H.; Weisenberger, D.J.; Liang, G. The roles of human DNA methyltransferases and their isoforms in shaping the epigenome. *Genes* **2019**, *10*, 172. https://doi.org/10.3390/genes10020172.
149. Jabbari, K.; Bernardi, G. Cytosine methylation and CpG, TpG (CpA) and TpA frequencies. *Gene* **2004**, *333*, 143–149. https://doi.org/10.1016/j.gene.2004.02.043.
150. Moore, L.D.; Le, T.; Fan, G. DNA methylation and its basic function. *Neuropsychopharmacology* **2013**, *38*, 23–38. https://doi.org/10.1038/npp.2012.112.
151. Pistek, V.L.; Fürst, R.W.; Kliem, H.; Bauersachs, S.; Meyer, H.H.D.; Ulbrich, S.E. HOXA10 mRNA expression and promoter DNA methylation in female pig offspring after in utero estradiol-17β

exposure. *J. Steroid Biochem. Mol. Biol.* **2013**, *138*, 435–444. https://doi.org/10.1016/j.jsbmb.2013.09.006.
152. Yang, X.; Wu, R.; Shan, W.; Yu, L.; Xue, B.; Shi, H. DNA methylation biphasically regulates 3T3-L1 preadipocyte differentiation. *Mol. Endocrinol.* **2016**, *30*, 677–687. https://doi.org/10.1210/me.2015-1135.
153. Stachecka, J.; Lemanska, W.; Noak, M.; Szczerbal, I. Expression of key genes involved in DNA methylation during in vitro differentiation of porcine mesenchymal stem cells (MSCs) into adipocytes. *Biochem. Biophys. Res. Commun.* **2020**, *522*, 811–818. https://doi.org/10.1016/j.bbrc.2019.11.175.
154. Ferguson, L.R. Nutrigenomics Approaches to Functional Foods. *J. Am. Diet. Assoc.* **2009**, *109*, 452–458. https://doi.org/10.1016/j.jada.2008.11.024.
155. Loor, J.J.; Vailati-Riboni, M.; McCann, J.C.; Zhou, Z.; Bionaz, M. Triennial Lactation symposium: Nutrigenomics in livestock: Systems biology meets nutrition. *J. Anim. Sci.* **2015**, *93*, 5554–5574. https://doi.org/10.2527/jas.2015-9225.
156. Katsumata, M.; Kaji, Y.; Takada, R.; Dauncey, M.J. Nutritional regulation of GLUT expression, glucose metabolism, and intramuscular fat content in porcine muscle. *Asian-Australas. J. Anim. Sci.* **2007**, *20*, 1297–1304. https://doi.org/10.5713/ajas.2007.1297.
157. Li, F.; Duan, Y.; Li, Y.; Tang, Y.; Geng, M.; Oladele, O.A.; Kim, S.W.; Yin, Y. Effects of dietary n-6:n-3 PUFA ratio on fatty acid composition, free amino acid profile and gene expression of transporters in finishing pigs. *Br. J. Nutr.* **2015**, *113*, 739–748. https://doi.org/10.1017/S0007114514004346.
158. Zglejc-Waszak, K.; Waszkiewicz, E.M.; Franczak, A. Periconceptional undernutrition affects the levels of DNA methylation in the peri-implantation pig endometrium and in embryos. *Theriogenology* **2019**, *123*, 185–193. https://doi.org/10.1016/j.theriogenology.2018.10.002.
159. Nowacka-Woszuk, J. Nutrigenomics in livestock—recent advances. *J. Appl. Genet.* **2020**, *61*, 93–103. https://doi.org/10.1007/s13353-019-00522-x.
160. McNamara, L.B.; Giblin, L.; Markham, T.; Stickland, N.C.; Berry, D.P.; O'Reilly, J.J.; Lynch, P.B.; Kerry, J.P.; Lawlor, P.G. Nutritional intervention during gestation alters growth, body composition and gene expression patterns in skeletal muscle of pig offspring. *Animal* **2011**, *5*, 1195–1206. https://doi.org/10.1017/S1751731111000176.
161. Rehfeldt, C.; Stabenow, B.; Pfuhl, R.; Block, J.; Nurnberg, G.; Otten, W.; Metges, C.C.; Kalbe, C. Effects of limited and excess protein intakes of pregnant gilts on carcass quality and cellular properties of skeletal muscle and subcutaneous adipose tissue in fattening pigs. *J. Anim. Sci.* **2012**, *90*, 184–196. https://doi.org/10.2527/jas.2011-4234.
162. Chen, J.; Zhang, H.; Gao, H.; Kang, B.; Chen, F.; Li, Y.; Fu, C.; Yao, K. Effects of Dietary Supplementation of Alpha-Ketoglutarate in a Low-Protein Diet on Fatty Acid Composition and Lipid Metabolism Related Gene Expression in Muscles of Growing Pigs. *Animals* **2019**, *9*, 838. https://doi.org/10.3390/ani9100838.
163. Katsumata, M.; Kobayashi, S.I.; Matsumoto, M.; Tsuneishi, E.; Kaji, Y. Reduced intake of dietary lysine promotes accumulation of intramuscular fat in the Longissimus dorsi muscles of finishing gilts. *Anim. Sci. J.* **2005**, *76*, 237–244. https://doi.org/10.1111/j.1740-0929.2005.00261.x.
164. Katsumata, M.; Kawakami, S.; Kaji, Y.; Takada, R.; Dauncey, M.J. Low lysine diet selectively up-regulates muscle GLUT4 gene expression during postnatal development. In *Energy Metabolism in Animals*; Chwalibog, A., Jakobsen, K., Eds.; EAAP Publication no. 103; Wageningen Pers: Wageningen, The Netherlands, 2001; pp. 237–239.
165. Katsumata, M.; Matsumoto, M.; Kobayashi, S.I.; Kaji, Y. Reduced dietary lysine enhances proportion of oxidative fibers in porcine skeletal muscle. *Anim. Sci. J.* **2008**, *79*, 347–353. https://doi.org/10.1111/j.1740-0929.2008.00536.x.
166. Katsumata, M.; Kawakami, S.; Kaji, Y.; Takada, R. Circulating levels of insulin-like growth factor-1 and associated binding proteins in plasma and mRNA expression in tissues of growing pigs on a low threonine diet. *Anim. Sci.* **2004**, *79*, 85–92. https://doi.org/10.1017/s1357729800054552.
167. Katsumata, M.; Kobayashi, H.; Ashihara, A.; Ishida, A. Effects of dietary lysine levels and lighting conditions on intramuscular fat accumulation in growing pigs. *Anim. Sci. J.* **2018**, *89*, 988–993. https://doi.org/10.1111/asj.13019.
168. Flinta, C.; Persson, B.; Jörnvall, H.; Heijne, G. von Sequence determinants of cytosolic N-terminal protein processing. *Eur. J. Biochem.* **1986**, *154*, 193–196. https://doi.org/10.1111/j.1432-1033.1986.tb09378.x.
169. Bushati, N.; Cohen, S.M. MicroRNA functions. *Annu. Rev. Cell Dev. Biol.* **2007**, *23*, 175–205. https://doi.org/10.1146/annurev.cellbio.23.090506.123406.
170. Bartel, D.P. MicroRNAs: Target Recognition and Regulatory Functions. *Cell* **2009**, *136*, 215–233. https://doi.org/10.1016/j.cell.2009.01.002.

171. De Tonnac, A.; Labussière, E.; Vincent, A.; Mourot, J. Effect of α-linolenic acid and DHA intake on lipogenesis and gene expression involved in fatty acid metabolism in growing-finishing pigs. *Br. J. Nutr.* **2016**, *116*, 7–18. https://doi.org/10.1017/S0007114516001392.
172. Madeira, M.S.; Costa, P.; Alfaia, C.M.; Lopes, P.A.; Bessa, R.J.B.; Lemos, J.P.C.; Prates, J.A.M. The increased intramuscular fat promoted by dietary lysine restriction in lean but not in fatty pig genotypes improves pork sensory attributes. *J. Anim. Sci.* **2013**, *91*, 3177–3187. https://doi.org/10.2527/jas.2012-5424.
173. Schiavon, S.; Carraro, L.; Dalla Bona, M.; Cesaro, G.; Carnier, P.; Tagliapietra, F.; Sturaro, E.; Galassi, G.; Malagutti, L.; Trevisi, E.; et al. Growth performance, and carcass and raw ham quality of crossbred heavy pigs from four genetic groups fed low protein diets for dry-cured ham production. *Anim. Feed Sci. Technol.* **2015**, *208*, 170–181. https://doi.org/10.1016/j.anifeedsci.2015.07.009.
174. Carcò, G.; Schiavon, S.; Casiraghi, E.; Grassi, S.; Sturaro, E.; Dalla Bona, M.; Novelli, E.; Gallo, L. Influence of dietary protein content on the chemico-physical profile of dry-cured hams produced by pigs of two breeds. *Sci. Rep.* **2019**, *9*, 1–12. https://doi.org/10.1038/s41598-019-55760-0.
175. Olivares, A.; Rey, A.I.; Daza, A.; López-Bote, C.J. Low levels of dietary vitamin A increase intramuscular fat content and polyunsaturated fatty acid proportion in liver from lean pigs. *Livest. Sci.* **2011**, *137*, 31–36. https://doi.org/10.1016/j.livsci.2010.09.023.
176. Maloney, C.A.; Rees, W.D. Gene-nutrient interactions during fetal development. *Reproduction* **2005**, *130*, 401–410. https://doi.org/10.1530/rep.1.00523.
177. Yin, J.; Li, D. Nutrigenomics approach-a strategy for identification of nutrition responsive genes influencing meat edible quality traits In swine. *Asian-Australas. J. Anim. Sci.* **2009**, *22*, 605–610. https://doi.org/10.5713/ajas.2009.r.05.
178. Wang, J.; Zhao, S.M.; Song, X.L.; Pan, H.B.; Li, W.Z.; Zhang, Y.Y.; Gao, S.Z.; Chen, D.W. Low protein diet up-regulate intramuscular lipogenic gene expression and down-regulate lipolytic gene expression in growth-finishing pigs. *Livest. Sci.* **2012**, *148*, 119–128. https://doi.org/10.1016/j.livsci.2012.05.018.
179. Kloareg, M.; Noblet, J.; van Milgen, J. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *Br. J. Nutr.* **2007**, *97*, 35–44. https://doi.org/10.1017/S0007114507205793.

General Discussion

General Discussion

The present pig rearing, nutrition, and feeding management strategies are unsustainable for heavy pigs intended for the production of pork and dry-cured ham. Additionally, the animal genetic trends that make it difficult for pigs to produce the best ham and pork for the dry-cured ham industry are causing the swine production industry to deal with increasingly leaner pigs. Therefore, management choice that ensures the productivity and profitability of pig production by targeting adjustments in slaughter weight (SW) and slaughter age (SA) could be promising [1], as was covered in Chapter 1. This can be achieved through different combinations of age and weight at slaughter by optimising energy and dietary nutrient supplies [2,3]. The qualities of the green ham before curing are well known to have a significant impact on the quality of the dry-cured ham, provided that the processing is standardized [4,5]. Previous research suggested that the weight, depths of subcutaneous fat covering, and marbling of green hams are highly correlated with the dry-cured product's final quality [4,6,7].

In Italy and other nations, the value of the green ham is decided at the time of slaughter based on the animal's weight, subcutaneous fat depths, fat colour, and other characteristics. If pigs selected for the production of dry-cured hams are slaughtered at roughly the same age, it is possible to assume that the heavier pigs had consumed more feed, grew faster, had heavier carcasses and hams, as well as more marbling, fat covering, and carcass adiposity, than the lighter pigs at the time of the slaughter. However, these responses would depend on the genetic ability of the pig breed for lean and fat deposition at heavy weights. Thus, according to the current thesis, pigs with higher SWs had greater energy and nutrients such that a lower proportion of energy was partitioned towards the maintenance and a greater proportion toward the growth of the body’s constituents. This was consistent with other studies that reported that an increased growth rate was positively related to an increase in feed efficiency (gain: feed) [8] (see chapter 2). Furthermore, while studies have indicated that slaughtering the pigs at older ages has a positive impact on the quality traits of the dry-cured ham product [9], the influence of increasing SA and SW and ham adiposity on the dry-curing aptitude of the ham is often confounded in existing literature, as increasing ages were associated with increased BW and ham adiposity [10]. Moreover, a greater fat covering reduces dehydration during seasoning, improving ham quality, an earlier SA is thought to increase the dry-curing losses [9,10]. Interestingly, when the pigs in the current thesis were given a treatment (Younger Age, YA) to obtain pigs with a reduced slaughter age at a given body weight, such a strategy did not change the weight of trimmed ham but instead improved various measures of fat covering depth and the visible marbling score. This also ensures finishing pigs with better ham quality traits than under conventional conditions with increased feed efficiency and reduced associated economic costs (see Chapter 1). On the other hand, by increasing the slaughter weight at a given age by raising the pigs under a greater weight (GW) treatment, the feed efficiency of the pigs was similar to that of pigs under the conventional rearing system. As a result, it was discovered that the GW group's feeding expense per unit of gained BW was equal to that of the conventional C group. Furthermore, 26% of the carcasses in the GW group were heavier than 168 kg, which corresponds to the new upper limit for carcass weight in the currently under consideration revision to the new product specification guideline. Therefore, depending on the pig genotype, it would be necessary to adopt mild feed restrictions to limit the full expression of the pig growth potential, while preserving the quality of the green hams (see Chapter 1).

The impact of sex on the quality of pork has been studied in the literature. In the current thesis, we investigated how SW and sex affected the ham traits of the pig genotype under investigation as well as their growth performance and traits. The results demonstrated that higher SWs were linearly and positively correlated with the pigs' growth performance and with better ham quality traits (Chapter 2). The main variables that can also affect the ham's ability to absorb salt are its weight and size, inter- and intramuscular fat content, subcutaneous fat thickness, and the amount of lean meat in the hind leg [11]. It is commonly assumed that heavier hams are characterized by better seasoning properties, because of lower seasoning losses [28]. However, earlier studies [12,13] found either little or no correlation between ham weight and seasoning losses. Therefore, the greater adiposity of the hams harvested from older and heavier pigs was attributed to the greater seasoning aptitude of the heavier hams [10,14]. These authors proposed that fat thickness, which acts as a barrier to water evaporation during seasoning, is the most important factor affecting seasoning losses. Except for the subcutaneous fat depth, which corresponds to the *semimembranosus* muscle, and the roundness - a measure of muscularity, our study found that increased SW had little effect on the majority of the ham's quality traits. It's interesting to note that as SW increased, the subcutaneous depth of the carcass also increased, but the ham's subcutaneous fat depth only increased in correlation with the *semimembranosus* muscle and not with the *biceps femoris*. Additionally, there were some differences between barrows and gilts for some subjective scores, with barrows scoring higher for visible marbling

and lower for haemorrhages than gilts. These differences were only of a small magnitude, though. Therefore, it seems that no compelling evidence can be provided at this time to suggest that barrows are superior to gilts when intended for the production of Italian dry-cured ham (see Chapter 2).

Considerable research progress in the development of mathematical models for growing and fattening pigs has been made [15–18]. Therefore, it is possible to predict the distribution of nutrients and energy among farm animals using these models [15]. The growth rate as well as the chemical and anatomical compositions of the body are predicted by these models to aid in decision-making [15, 16]. Therefore, Understanding the energy and amino acid (AA) requirements and partitioning of the C21 Goland pigs, as well as how they respond to the various nutritional and feeding methods discussed in Chapters 1 and 2, is crucial. Therefore, this was discussed and addressed using a similar model in Chapter 3. As stated earlier, the emphasis of the nutrition guidelines currently being used in practice is on pigs fed ad libitum up to 140 kg in body weight (BW). This recommendation does not take into account the effects of keeping pigs at an extended BW. The applicability of this recommendation for pigs weighing more than 140 kg in BW under various rearing circumstances is still up for debate. Furthermore, there is currently no literature that examines how heavy pigs use energy and nutrients in dry-cured ham production systems. The issue of nutrient partitioning in heavy pigs maintained on feeding regimens with limited or unlimited energy and/or amino acid supplies needs more attention. Again, it is uncertain whether pigs with a higher BW will still require the suggested metabolizable energy (ME) requirements for maintenance (MEm = 1.03 MJ/kg in $BW^{0.60}$) listed by the NRC [19]. In general, dietary recommendations for maintenance are frequently based on knowledge of metabolic weight or body protein mass, whereas growth is typically based on Pd and Ld [2,19,20]. These unanswered questions are the basis of the study in Chapter 3. In order to develop recommendations, groups of pigs were slaughtered at various ages in comparative experiments [37,38]. Under practical conditions, the slaughter of pigs is not feasible, for reasons such as time and costs. Measurements of BW and BF depth in pigs can be used to estimate body composition reasonably [21,22]. This is achieved through repeated measurements of individual pigs from a given population. It was previously suggested that once the BL is estimated, the allometric relationships between body components (such as body water, body protein, body ash, etc.) are simple to compute [19,23]. However, it is important to note that accurate body composition estimates depend on the precision and accuracy of the equation that calculates the BL from BW and BF depth measurements. There is existing disparity in equations proposed in various studies regarding the variation in pig genotype, BW range, feeding conditions, environment, and so forth (see Chapter 3). To fill this gap, the current thesis' modelling approach used repeated BW and BF measurements to calculate the ME and amino acid requirements of growing pigs under extended BW and feeding conditions. When calculating an estimate of protein (Pd) and lipid (Ld) depositions, metabolizable energy (ME), standardized ileal digestible lysine (SID lysine) requirement, and partitioning of the C21 Goland pig genotype (90-200 kg in BW), under investigation (Chapter 3), such a method offers a practical application. We concluded and confirmed that, regardless of the feeding regimen, pigs weighing 90 to 200 kg in BW apply to the MEm value of 1.02 MJ/kg0.60. The outcome we obtained thus suggests that the maximum marginal efficiency of SID lysine utilization for protein deposition (Pd) under energy and protein restriction was 0.73. As a minimum requirement, regardless of body weight (BW), this equates to 9.8 g of SID lysine per 100 g of Pd (Chapter 3).

The production and management of farm animals would soon adopt nutritional genomics research (nutrigenetics and nutrigenomics). These two nutritional disciplines are changing and reshaping the understanding of scientists on the undelaying molecular mechanisms regulating growth, ageing, fatness statuses, health, disease, etc in humans and animals. In contrast to nutrigenomics, which studies how nutrients and bioactive food substances affect gene expression, nutrigenetics is the branch of science that deals with the study of the effect (s) of genetic variation on the dietary response (addressed in Chapter 4). One needs a thorough understanding of nutrition, genetics, biochemistry, and "omics" technologies to plan and design nutrition interventions that optimise growth performance, trait development, and health. The quality of meat and meat products is largely influenced by the animal's diet, cellular metabolic processes, and genetic predisposition, according to existing literature (see Chapter 4). We presented in-depth knowledge on how nutritional genomics is used to understand the genes involved in fat metabolism and the development of the intramuscular fat content of pork. Intramuscular fat (IMF), also known as marbling, is a crucial sensory characteristic of pork that affects consumer preference and price premiums. Marbling, which varies depending on the breed (genetics), age, sex, nutrition, muscle type, and muscle location, is the streak of visible fat mixed in with the lean within a muscle fibre [24,25]. From an economic viewpoint, the pork industry is faced with increasing lean pig genotypes characterized by reduced IMF content, which has a minimum range between 2.2% and 3.4% [26]. Therefore, methods to improve the traits of pigs that deposit fat have been received significant attention [27–30]. For both health and financial reasons, swine nutritionists, breeders, and geneticists are very interested in improving the quality of the fatty acid profile and IMF content of pork

[31] and this remains critical to the industry. However, fat metabolism and marbling are multiplex traits regulated by several genes which are directly or indirectly involved in fatty acid metabolism, cell proliferation, and differentiation [32–34]. As such, the molecular mechanisms underlying IMF deposition as well as the pattern of gene expression for lipid metabolism remain a subject for research [35–39]. Through nutrigenetics, we can observe how a pig's unique genetic makeup affects the pig's potential response to dietary nutrient intake. In contrast, nutrigenomics allows us to examine how dietary nutrient intake affects a single pig's entire genome. When these two pieces of information are harmonised, they form a potent tool for determining the nutritional needs of swine and tailoring the supply of nutrients in the diet to the genetic makeup of specific pigs. The application (s) of nutrigenetics and nutrigenomics in illuminating the underlying mechanisms of trait development (milk and meat quality traits, fat deposition and IMF accretion, health and disease resistance and detection, and so on) in animals will determine the future of animal production and breeding programs for superior traits in farm animals. This knowledge holds the potentials for the improvement and the redefinition of nutritional intervention for environmentally friendly and sustainable livestock production.

References

1. Wu, F.; Vierck, K.R.; DeRouchey, J.M.; O'Quinn, T.G.; Tokach, M.D.; Goodband, R.D.; Dritz, S.S.; Woodworth, J.C. A Review of Heavy Weight Market Pigs: Status of Knowledge and Future Needs Assessment. *Transl. Anim. Sci.* **2017**, doi:10.2527/tas2016.0004.
2. Kyriazakis, I.; Whittemore, C.T. Whittemore's science and practice of pig production. Kyriazakis I., Whittemore, C.T., Blackwell Publishing, Oxford, UK; **2006**. pp. 417. https://doi.org/10.1002/9780470995624.ch13.
3. Lebret, B.; Juin, H.; Noblet, J.; Bonneau, M. The Effects of Two Methods of Increasing Age at Slaughter on Carcass and Muscle Traits and Meat Sensory Quality in Pigs. *Anim. Sci.* **2001**, doi:10.1017/S1357729800055582.
4. Pagliarini E, Laureati M, Dinnella C, Monteleone E, Proserpio C, Piasentier E, Influence of pig genetic type on sensory properties and consumer acceptance of Parma, San Daniele and Toscano dry-cured hams. *J. Sci. Food Agric.* **2016,** *96,* 798-806.
5. Gou, P.; Guerrero, L.; Arnau, J. Sex and Crossbreed Effects on the Characteristics of Dry-Cured Ham. *Meat Sci.* **1995**, *40*, 21–31, doi:10.1016/0309-1740(94)00021-X.
6. Lo Fiego, D.P.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid compothe sition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107-114. doi:10.1016/j.meatsci.2004.06.010.
7. Lowell, J.E.; Schunke, E.D.; Harsh, B.N.; Bryan, E.E.; Stahl, C.A.; Dilger, A.C.; Boler, D.D. Growth performance, carcass characteristics, fresh belly quality, and commercial bacon slicing yields of growing-finishing pigs from sire lines intended for different industry applications. *Meat Sci*. **2019**, *154*, 96–108, doi:10.1016/j.meatsci.2019.04.010.
8. Lo Fiego, D.P.; Santoro, P.; Macchioni, P.; De Leonibus, E. Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid compothe sition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Sci.* **2005**, *69*, 107-114. doi:10.1016/j.meatsci.2004.06.010.
9. Čandek-Potokar, M.; Žlender, B.; Lefaucheur, L.; Bonneau, M. Effects of age and/or weight at slaughter on *longissimus dorsi* muscle: Biochemical traits and sensory quality in pigs. *Meat Sci.* **1998**, *48*, 287-300. doi:10.1016/S0309-1740(97)00109-5.
10. Čandek-Potokar, M.; Škrlep, M. Factors in pig production that impact the quality of dry-cured ham: A review. *Animal* **2012**, *6*, 327-338. doi:10.1017/S1751731111001625.
11. Zappaterra, M.; Zambonelli, P.; Schivazappa, C.; Simoncini, N.; Virgili, R.; Stefanon, B.; Davoli, R. Investigating the features of PDO green hams during salting: Insights for new markers and genomic regions in commercial hybrid pigs. *Animals* **2021**, *11*, 68. doi:10.3390/ani11010068.
12. Ramos, A.M.; Glenn, K.L.; Serenius, T.V.; Stalder, K.J.; Rothschild, M.F. Genetic markers for the production of US country hams. *J. Anim. Breed. Genet.* **2008**, *125*, 248–257. doi:10.1111/j.1439−0388.2007.00710.x.
13. Čandek-Potokar, M.; Monin, G.; Žlender, B. Pork quality, processing, and sensory characteristics of dry-cured hams as influenced by Duroc crossing and sex. *J. Anim. Sci.* **2002**, *80*, 988–996. doi:10.2527/2002.804988x.
14. Bosi, P.; Russo, V. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Sci.* **2004**, *3*, 309–321. doi:10.4081/ijas.2004.309.
15. Halas, V., Dijkstra, J., Babinszky, L., Verstegen, M. W. A., & Gerrits, W. J. J. (2004). Modelling of nutrient partitioning in growing pigs to predict their anatomical body composition. 1. Model description. *British Journal of Nutrition*. https://doi.org/10.1079/bjn20041237.
16. van Milgen, J.; Dourmad, J.Y. Concept and application of ideal protein for pigs. *J. Anim. Sci. Biotechnol.* 2015. 6:15, doi:10.1186/s40104-015-0016-1.
17. Izquierdo, O.A.; Wedekind, K.J.; Baker, D.H. Histidine requirement of the young pig. *J. Anim. Sci.* **1988**, *66*, 2886–2892, doi:10.2527/jas1988.66112886x.
18. Fuller, M.F.; McWilliam, R.; Wang, T.C.; Giles, L.R. The optimum dietary amino acid pattern for growing pigs. *Br. J. Nutr.* **1989**, *62*, 255–267, doi:10.1079/bjn19890028.
19. National Research Council. *Nutrient Requirements of Swine*, 11th ed.; National Academies Press: Washington, DC, USA, 2012; ISBN 978-0-309-22423-9.
20. Naatjes, M.; Susenbeth, A. Energy requiremeThe energygrowing pigs under commercial housing conditions. *Arch. Anim. Nutr.* **2014**, *68*, 93–110. doi:10.1080/1745039x.2014.887814.
21. Schiavon, S.; Gallo, L.; Carnier, P.; Tagliapietra, F.; Ceolin, C.; Prandini, A.; Piva, A. Use of simple body measurements and allometry to predict the chemical growth and feed intake in pigs. *Ital. J. Anim. Sci.* **2007**, *6*, 27–44. doi:10.4081/ijas.2007.27.

22. Kloareg, M.; Noblet, J.; van Milgen, J. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. *Br. J. Nutr.* **2007**, *97*, 35–44. doi:10.1017/s0007114507205793.
23. Ferguson, N.S.; Gous, R.M.; Emmans, G.C. Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *S. Afr. J. Anim. Sci.* **1994**, *24*, 10–17.
24. Wang, Y.; Zhou, J.; Wang, G.; Cai, S.; Zeng, X.; Qiao, S. Advances in low-protein diets for swine. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 60. doi:10.1186/s40104-018-0276-7.
25. Benítez, R.; Trakooljul, N.; Núñez, Y.; Isabel, B.; Murani, E.; De Mercado, E.; Gómez-Izquierdo, E.; García-Casco, J.; López-Bote, C.; Wimmers, K.; et al. Breed, diet, and interaction effects on adipose tissue transcriptome in iberian and duroc pigs fed different energy sources. *Genes* **2019**, *10*, 589. doi:10.3390/genes10080589.
26. Font-i-Furnols, M.; Tous, N.; Esteve-Garcia, E.; Gispert, M. Do all the consumers accept marbling in the same way? The relationship between eating and visual acceptability of pork with different intramuscular fat content. *Meat Sci.* **2012**, *91*, 448–453. doi:10.1016/j.meatsci.2012.02.030.
27. Knap, P.W. *Voluntary Feed Intake and Pig Breeding*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2009; pp. 13–35.
28. Čandek-Potokar, M.; Škrlep, M. Factors in Pig Production That Impact the Quality of Dry-Cured Ham: A Review. *Animal* **2012**, *6*, 327–338, doi:10.1017/S1751731111001625.
29. Bertol, T.M.; de Campos, R.M.L.D.; Ludke, J.V.; Terra, N.N.; de Figueiredo, E.A.P.; Coldebella, A.; dos Santos Filho, J.I.; Kawski, V.L.; Lehr, N.M. Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. *Meat Sci.* **2013**, *93*, 507–516. doi:10.1016/j.meatsci.2012.11.012.
30. Schiavon, S.; Dalla Bona, M.; Carcò, G.; Sturaro, E.; Gallo, L. Responses of pigs of different genotypes to a variation in the dietary indispensable amino acid content in terms of their growth and carcass and meat quality traits. *Animals* **2019**, *9*, 508. https://doi.org/10.3390/ani9080508.
31. Wood, J.D.; Enser, M.; Fisher, A.V.; Nute, G.R.; Sheard, P.R.; Richardson, R.I.; Hughes, S.I.; Whittington, F.M. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* **2008**, *4*, 343–358. doi:10.1016/j.meatsci.2007.07.019.
32. Plotnikov, A.; Zehorai, E.; Procaccia, S.; Seger, R. The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim. Biophys. Acta-Mol. Cell Res.* **2011**, *9*, 1619–1633. doi:10.1016/j.bbamcr.2010.12.012.
33. Miller, R.K.; Moeller, S.J.; Goodwin, R.N.; Lorenzen, C.L.; Savell, J.W. Consistency in meat quality. *Int. Congr. Meat Sci. Technol.* **2000**, *46*, 566–580.
34. Davoli, R.; Catillo, G.; Serra, A.; Zappaterra, M.; Zambonelli, P.; Zilio, D.M.; Steri, R.; Mele, M.; Buttazzoni, L.; Russo, V. Genetic parameters of backfat fatty acids and carcass traits in large white pigs. *Animal* **2019**, *13*, 924–932. doi:10.1017/S1751731118002082.
35. Puig-Oliveras, A.; Ramayo-Caldas, Y.; Corominas, J.; Estellé, J.; Pérez-Montarelo, D.; Hudson, N.J.; Casellas, J.; Folch, J.M.; Ballester, M. Differences in muscle transcriptome among pigs phenotypically extreme for fatty acid composition. *PLoS ONE* **2014**, *9*, e99720. doi:10.1371/journal.pone.0099720.
36. Muñoz, G.; Alves, E.; Fernández, A.; Óvilo, C.; Barragán, C.; Estellé, J.; Quintanilla, R.; Folch, J.M.; Silió, L.; Rodríguez, M.C.; Fernández, A.I. QTL detection on porcine chromosome 12 for fatty-acid composition and association analyses of the fatty acid synthase, gastric inhibitory polypeptide and acetyl-coenzyme A carboxylase alpha genes. *Anim. Genet.* **2007**, *38*, 639–646, doi:10.1111/j.1365-2052.2007.01668.x.
37. Latorre, M.A.; Lázaro, R.; Gracia, M.I.; Nieto, M.; Mateos, G.G. Effect of sex and terminal sire genotype on performance, carcass characteristics, and meat quality of pigs slaughtered at 117 kg body weight. *Meat Sci.* **2003**, *65*, 1369–1377. doi:10.1016/S0309-1740(03)00059-7.
38. Wood, J.D.; Nute, G.R.; Richardson, R.I.; Whittington, F.M.; Southwood, O.; Plastow, G.; Mansbridge, R.; Da Costa, N.; Chang, K.C. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Sci.* **2004**, *67*, 651–667. doi:10.1016/j.meatsci.2004.01.007.
39. Hocquette, J.F.; Gondret, F.; Baza, E.; Mdale, F.; Jurie, C.; Pethick, D.W. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal* **2010**, *4*, 303–319. doi:10.1017/S1751731109991091.

Main Conclusions Drawn from the Thesis

Main Conclusions from the Thesis

From the present thesis, the following conclusions can be drawn:

1. For the heavy pig intended for dry-cured ham production, a new feeding and rearing strategy (younger age, YA) has been developed, allowing anticipation of the slaughter to be about 27 days earlier with better improvements in ADG, feed efficiency, and ham adiposity (Chapter 1).

2. Applying the younger age (YA) rearing strategy should be done with caution as more study is needed to determine whether increased ham adiposity can offset the negative effects of younger slaughter age on dry-curing aptitude (Chapter 1).

3. Although the older age (OA) strategy has a favourable effect on the subcutaneous fat depth and visible marbling close to the *semimembranosus* muscle, this strategy was found to be ineffective because it impairs growth and feed efficiency, increases production costs, has little effect on the composition of the carcass, and reduces ham size (Chapter 1).

4. The greater weight (GW) strategy of keeping pigs over an extended body weight was linked to higher feed consumption, ADG, carcass and ham weight, as well as an improvement in some ham quality indices in comparison to C. Adopting this strategy, however, carries the risk of an increased percentage of carcasses weighing more than the upper limit specified by the product specifications (168 kg). Nevertheless, depending on the pigs' potential for growth, it might be supplemented with a slight feed restriction (Chapter 1).

5. The current slaughter weight classes taken into consideration in this study proved that pigs with higher slaughter weight classes (SWC) have greater average daily gains and feed consumption with comparable feed efficiency, greater ham weight, and muscularity and fat covering in correspondence to the *semimembranosus* muscle (Chapter 2).

6. Barrows produced hams with greater weight and marbling than gilts, which are desired because they have a positive effect on the flavour and visual characteristics of green ham at the time of its selection for dry-curing (Chapter 2).

7. The present model based on repeated body weight (BW) and backfat (BF) measurements provided reliable estimates for metabolizable energy (ME) and the amino acid requirement of growing pigs through extended BW and feeding conditions (Chapter 3).

8. Energy restriction had little or no effect on the estimated MEm, which was confirmed to be 1.02 $MJ/kg^{0.60}$ for pigs weighing 90 to 200 kg in BW, regardless of the feeding regime (Chapter 3 and Discussion).

9. The maximum marginal efficiency of SID lysine utilization for protein deposition (Pd) under energy and protein restriction was 0.73. This translates to a minimum requirement of 9.8 g of SID lysine per 100 g of Pd, irrespective of body weight (BW) (Chapter 3).

10. Numerous QTLs, SNPs, mRNAs, and miRNAs regulate the molecular mechanisms of pigs' fat metabolism and IMF deposition. The nutritional qualities of feed, nutrients, and dietary bioactive substances, whose levels in the diet as well as environmental factors can be difficult to control. The study of nutrigenetics, nutrigenomics, and epigenetic mechanisms are effective and precise in locating changes in gene sequences that predispose specific pig breeds to respond in a particular way in terms of performance, meat and milk quality, health, and disease detection (Chapter 4 and Discussion).

11. As a result, nutritional effects can be measured in order to regulate genome responses and fine-tune gene expressions in pigs to improve growth performance, backfat thickness, IMF deposition, disease resistance, and meat quality traits. How well prepared are we to use this science as a tool in swine feeding and animal nutrition, though, is still an open question. (Chapter 4)

New Scientific Results

New Scientific Results from the Thesis

1. A new feeding and rearing method (younger age, YA) for the heavy pig intended for the production of dry-cured ham has been developed. This approach enables anticipation of the slaughter to occur approximately 27 days earlier with better improvements in ADG, feed efficiency, and ham adiposity (Chapter 1).

2. Although the older age (OA) strategy increases the subcutaneous fat depth and visible marbling near the *semimembranosus* muscle, it is ineffective because it decreases growth and feed efficiency, increases production costs, has little impact on carcass composition, and produces smaller hams (Chapter 1).

3. Greater ham weight, muscularity, and fat covering in correspondence with the *semimembranosus* muscle are all characteristics of pigs with greater slaughter weight class (SWC) than other pigs (Chapter 2).

4. The effect of sex on green ham quality traits was confirmed, as barrows produced hams with more weight and marbling than gilts. These characteristics are desired due to their favourable impact on the flavour and aesthetic characteristics of green ham at the time of its selection for dry-curing (Chapter 2).

5. A mechanistic model based on repeated measurements of body weight (BW) and backfat (BF) was used to estimate metabolizable energy (ME) and SID lysine partitioning in pigs growing from 90 to 200 kg in body weight (Chapter 3).

6. Energy restriction had little or no influence on the estimated Mem. It was established that pigs weighing 90 to 200 kg in BW should have a MEm value of 1.02 MJ/kg$^{0.60}$ regardless of the feeding regimen. (Discussion and Chapter 3).

7. Under restrictions on both energy and protein, the maximum marginal efficiency of SID lysine utilization for protein deposition (Pd) was 0.73. This translates to a minimum requirement of 9.8 g of SID lysine per 100 g of Pd, regardless of body weight (BW) (Chapter 3).

8. The field of nutritional genomics, which includes both nutrigenetics and nutrigenomics, provides a method for unwinding how genes are expressed and the molecular processes that underlie animal trait development. It may be possible to measure the effects of nutrition on regulating the genome's responses and fine-tuning gene expressions in pigs in order to improve growth performance, backfat thickness, IMF deposition, disease resistance, and meat quality traits (Chapter 4).

Summaries

English Summary

Pigs must be slaughtered at 160 ±16 kg and a minimum age of 9 months under the traditional rearing systems for heavy pigs intended for the production of Italian dry-cured hams. Because current animal genetic trends are producing animals that are getting increasingly leaner, the conventional rearing system is unable to produce pigs with the ideal characteristics for the dry-cured ham industry. In this research, C21 Goland pigs (gilts and barrows) were at 95–9.0 kg body weight (BW). We investigated potential alternative rearing strategies for heavy pigs using new age and slaughter weight combinations. These alternative rearing methods included: 1) letting pigs express their growth potential by reaching 160 ± 16 kg slaughter weight (SW) at younger slaughter age (SA) (younger age, YA); 2) maximizing their SW at 9 months SA (greater weight, GW); and 3) lengthening the SA necessary to reach 160 ± 16 kg SW (older age, OA). For the four treatments, the average slaughter age (SA) and slaughter weight (SW) were 257, 230, 257, and 273 days and 172.7, 172.3, 192.9, and 169.3 kg, respectively. Pigs in group C had a feed efficiency (FE) of 0.265 (gain to feed) and an average daily gain (ADG) of 715 g/d. In comparison to C, YA pigs had higher FE (+7.5%), ADG (+32%), and ham adiposity, while GW pigs had higher carcass weight (+12%), ADG (+25%), and trimmed ham weight (+10.9%). Treatment with OA affected the ADG (16.4%), FE (16.6%), and trimmed ham weight (3.6%). Given that YA and GW improved FE and ham quality traits, they might be promising alternatives to C (Chapter 1). It was determined that the first two (1 and 2) strategies were the most effective options because they increased the pigs' rate of gain, feed efficiency, and ham adiposity. While the first method was the most practical from an economic standpoint, the second method resulted in the best hams (Chapter 1).

The major limitation of an increase in pig slaughter weights (SWs) is an increase in carcass adiposity and poor feed efficiency associated with increasing SW. The extension of the admitted SW range suggests the possibility of implementing an ad libitum feeding strategy that would better exploit the genetic potential of individual pigs for growth with decreased body and carcass uniformity among pigs of the same batch. Ad libitum feeding's effects on SW, growth performance, feed efficiency, and carcass and green ham characteristics were studied (Chapter 2). Dietary SID-lysine concentrations were 7.4 and 6.0 g/kg, respectively, and net energy content was 10 MJ/kg. There were four different slaughter weight classes (SWC): 165, 165-180, 180-210, and >210 kg BW. Pigs were slaughtered in each batch when they were 230 or 258 days old. Scores were given for the roundness, fat cover thickness, marbling, lean colour, bicolour, and veining of the left hams. The model used to analyze the data taken into account SWC, sex, and SWC × Sex interactions as fixed factors and the batch as a random factor. We tested the linear, quadratic, and cubic effects of SWC, but only linear effects were discovered. Results showed that pigs with greater SWC had greater average daily gain and feed consumption, with similar feed efficiency and better ham quality traits: greater ham weight, muscularity, and fat covering in correspondence of *semimembranosus* muscle. Compared to gilts, hams from barrows had slightly better qualities due to their weight (Chapter 2).

It is critical to evaluate the AA requirements and partitioning of heavy pigs raised using various rearing methods from a nutritional standpoint. The NRC's current nutrient recommendations for pigs, however, are focused on pigs with lean genotypes fed ad libitum until they reach a body weight (BW) of up to 140 kg. This recommendation has restrictions when applied to heavy pig production systems for the dry-cured ham industry. The utilization of energy and nutrients by heavy pigs in dry-cured ham production systems is not covered in the existing literature. The NRC's recommended metabolizable energy (ME) requirements for maintenance (MEm = 1.03 MJ/kg in $BW^{0.60}$) for pigs with heavier BWs remain an open question. Furthermore, the problem of nutrient partitioning in heavy pigs kept on feeding schedules with finite or infinite supplies of energy and/or amino acids has not yet been fully solved. Energy requirements are a function of feeding level for maintenance needs and factors related to the pigs' BW gain. For heavy pigs, whose incidence of MEm in total energy cost was reported to be greater than 45% (Chapter 3), it is of scientific interest to investigate the behaviour of their energy requirements under various rearing conditions and extended BW ranges. In the current study, a modelling approach based on repeated body weight (BW) and backfat (BF) measurements were used to investigate (i) the body protein and lipid accretions of Goland C21 heavy pigs between 90 and 200 kg BW when exposed to different rearing conditions; (ii) the metabolizable energy (ME) and (iii) standardized ileal digestible lysine (SID lysine) requirement and partitioning for maintenance and growth. The younger (YA) and older (OA) pigs received non-limited amounts of ME and SID lysine up to 170 kg in SW at 9 months SA, while the greater weight (GW) pigs were fed as the YA group, with 9 months SA at >170 kg in SW. The diets given to the control pigs (C) were limiting for ME up to 170 kg

of slaughter weight (SW) at 9 months of age (SA). 1.03 MJ/kg$^{0.60}$ was the average estimated MEm value. In OA pigs compared to C, MEm increased by 11%. Energy restriction had negligible effects on the estimated MEm. The marginal efficiency of SID lysine utilization for Pd was on average 0.725, translating to a 9.8 g/100 g Pd SID lysine requirement.

Nutritional genomics (nutrigenetics and nutrigenomics), which provides new information, explains the intricate molecular mechanisms underlying fatty acid synthesis and marbling in pigs. Nutrigenetics is the branch of science that focuses on the investigation of the effects of genetic variation on dietary response, as opposed to nutrigenomics, which studies how nutrients and bioactive food compounds influence gene expression (Chapter 4). Epigenetic mechanisms (DNA methylation and histone modification), which are intermediaries affecting the mechanisms of fat deposition, are also sensitive to environmental factors and dietary nutrients. The patterns of epigenetic mechanisms and molecular pathways that control gene expression (switching transcription on and off) in offspring, as well as the regulatory effects of fat and IMF depositions in farm animals, are now better understood. Nutritional genomics allows for the elucidation of complex mechanisms of gene-nutrient interaction and the environment across the entire genome. In animal nutritional genomics, a new post-genomic era is being defined by the application of high-throughput DNA-based "omics" technologies and system biology (Chapter 4). Animal metabolic processes can be better synchronized with inherited genes to improve health and economically significant traits. For instance, existing quantitative trait loci (QTL) for genes involved in lipogenesis, disease susceptibility, and the emergence of other traits in pigs have not yet been synchronized. There has been little research into the role(s) of epigenetic mechanisms in the transgenerational effects of nutrition and environment on adipocyte differentiation and trait development in pigs. We can precisely map the genetic, physiological, and dietary regulatory pathways involved in many cellular functions, such as the molecular mechanisms of fat and IMF accretion in pigs. This has made the effect of specific dietary nutrients on the entire genome less elusive. In the near future, combining the existing nutritional genomics knowledge may serve as the primary tool for accurate estimations of the nutrient requirements of pigs with various physiological statuses, ages, sex, and breed for fat metabolism and other trait improvements (such as growth performance, backfat thickness, IMF accretion, disease resistance, etc.) in pigs and farm animals.

The following conclusions were drawn from the present thesis:

1. For the heavy pig intended for dry-cured ham production, a new feeding and rearing strategy (younger age, YA) has been developed, allowing anticipation of the slaughter to be about 27 days earlier with better improvements in ADG, feed efficiency, and ham adiposity (Chapter 1).

2. Applying the younger age (YA) rearing strategy should be done with caution as more study is needed to determine whether increased ham adiposity can offset the negative effects of younger slaughter age on dry-curing aptitude (Chapter 1).

3. Although the older age (OA) strategy has a favourable effect on the subcutaneous fat depth and visible marbling close to the *semimembranosus* muscle, this strategy was found to be ineffective because it impairs growth and feed efficiency, increases production costs, has little effect on the composition of the carcass, and reduces ham size (Chapter 1).

4. The greater weight (GW) strategy of keeping pigs over an extended body weight was linked to higher feed consumption, ADG, carcass and ham weight, as well as an improvement in some ham quality indices in comparison to C. Adopting this strategy, however, carries the risk of an increased percentage of carcasses weighing more than the upper limit specified by the product specifications (168 kg). Nevertheless, depending on the pigs' potential for growth, it might be supplemented with a slight feed restriction (Chapter 1).

5. The current slaughter weight classes taken into consideration in this study proved that pigs with higher slaughter weight classes (SWC) have greater average daily gains and feed consumption with comparable feed efficiency, greater ham weight, and muscularity and fat covering in correspondence to the *semimembranosus* muscle (Chapter 2).

6. Barrows produced hams with greater weight and marbling than gilts, which are desired because they have a positive effect on the flavour and visual characteristics of green ham at the time of its selection for dry-curing (Chapter 2).

7. The present model based on repeated body weight (BW) and backfat (BF) measurements provided reliable estimates for metabolizable energy (ME) and the amino acid requirement of growing pigs through extended BW and feeding conditions (Chapter 3).

8. Energy restriction had little or no effect on the estimated MEm, which was confirmed to be 1.02 $MJ/kg^{0.60}$ for pigs weighing 90 to 200 kg in BW, regardless of the feeding regime (Chapter 3 and Discussion).

9. The maximum marginal efficiency of SID lysine utilization for protein deposition (Pd) under energy and protein restriction was 0.73. This translates to a minimum requirement of 9.8 g of SID lysine per 100 g of Pd, irrespective of body weight (BW) (Chapter 3).

10. Numerous QTLs, SNPs, mRNAs, and miRNAs regulate the molecular mechanisms of pigs' fat metabolism and IMF deposition. The nutritional qualities of feed, nutrients, and dietary bioactive substances, whose levels in the diet as well as environmental factors can be difficult to control. The study of nutrigenetics, nutrigenomics, and epigenetic mechanisms are effective and precise in locating changes in gene sequences that predispose specific pig breeds to respond in a particular way in terms of performance, meat and milk quality, health, and disease detection (Chapter 4 and Discussion).

11. As a result, nutritional effects can be measured in order to regulate genome responses and fine-tune gene expressions in pigs in order to improve growth performance, backfat thickness, IMF deposition, disease resistance, and meat quality traits. How well prepared are we to use this science as a tool in swine feeding and animal nutrition, though, is still an open question. (Chapter 4).

Italian Summary

I sistemi di allevamento convenzionali dei suini pesanti destinati alla produzione del prosciutto crudo italiano prevedono che i suini siano macellati a 160 ± 16 kg e un'età minima di 9 mesi. Con le attuali tendenze genetiche animali che forniscono animali progressivamente più magri, il sistema di allevamento convenzionale non riesce a fornire suini con caratteristiche ottimali per l'industria del prosciutto crudo. In questa ricerca, suini C21 Goland (scrofette e maschi castrati) a 95 ± 9,0 kg di peso corporeo (PC). Sono state studiate nuove combinazioni di età e peso alla macellazione, utilizzando diverse condizioni di alimentazione, come possibili strategie di allevamento alternative per suini pesanti. Tali strategie di allevamento alternative miravano a manipolare il tasso di crescita dei suini: 1) consentire ai suini di esprimere il loro potenziale di crescita consentendo loro di raggiungere 160 ± 16 kg di peso al macello (SW) all'età di macellazione più giovane (SA) (età più giovane, YA); 2) consentire ai suini di esprimere il loro potenziale di crescita massimizzando il loro SW a 9 mesi SA (maggiore peso, GW); 3) aumentando la SA richiesta per raggiungere 160 ± 16 kg SW (età avanzata, OA). I suini sono stati macellati in media rispettivamente a 257, 230, 257 e 273 d SA e 172,7, 172,3, 192,9 e 169,3 SW kg per i quattro trattamenti. I suini C hanno avuto un accrescimento medio giornaliero (ADG) di 715 g/d e un'efficienza del mangime (FE) di 0,265 (accrescimento/consumo di mangime). Rispetto a C, i maiali YA avevano ADG più elevato (+32%), FE (+7,5%) e una migliore adiposità del prosciutto; I suini GW avevano un peso carcassa maggiore (+12%), ADG (+25%), peso del prosciutto rifilato (+10,9%) e una migliore adiposità del prosciutto. Il trattamento con OA ha influenzato l'ADG (-16,4%), FE (-16,6%) e il peso del prosciutto rifilato (-3,6%). YA e GW potrebbero essere alternative promettenti a C poiché migliorano i tratti di qualità di FE e prosciutto (Capitolo 1). È stato dimostrato che le prime due (1 e 2) strategie erano le alternative più promettenti in quanto miglioravano il tasso di guadagno, l'efficienza del mangime e l'adiposità del prosciutto dei suini. Mentre la prima strategia era la più conveniente dal punto di vista economico, la seconda produceva i prosciutti con la massima qualità (Capitolo 1).

Il principale limite per un aumento dei pesi di macellazione dei suini (SW) è un aumento dell'adiposità della carcassa e una scarsa efficienza dell'alimentazione associata all'aumento del SW. L'estensione del range di SW ammesso implica la possibilità di adottare una strategia di alimentazione ad libitum che sfrutti al meglio il potenziale genetico dei singoli suini di accrescimento anche se questo può ridurre il grado di uniformità tra suini dello stesso lotto. È stata condotta un'indagine sull'impatto dell'alimentazione ad libitum su SW, sulle prestazioni di crescita, sull'efficienza del mangime e sulle caratteristiche della carcassa e del prosciutto crudo (Capitolo 2). Le diete contenevano 10 MJ/kg di energia netta e 7,4 e 6,0 g/kg di SID-lisina. Le classi di peso della macellazione (SWC) includevano <165, 165–180, 180–210 e >210 kg di peso corporeo. In ogni lotto, i maiali sono stati sacrificati a 230 o 258 giorni di età. I prosciutti di sinistra sono stati valutati per forma rotonda, spessore della copertura del grasso, marezzatura, colore magro, bicolore e venature. I dati sono stati analizzati con un modello che considera SWC, sesso e l'interazione SWC x sesso come fattori fissi e il batch come fattore casuale. Sono stati testati gli effetti lineari, quadratici e cubici di SWC, ma solo gli effetti lineari sono risultati significativi. I risultati hanno mostrato che i suini con SWC maggiore avevano accrescimenti medi giornalieri e consumi di mangime maggiori, con un'efficienza del mangime simile e migliori caratteristiche di qualità del prosciutto: maggiore peso del prosciutto, muscolosità e copertura di grasso in corrispondenza del muscolo semimembranoso. I maschi castrati erano più pesanti e producevano prosciutti con caratteristiche leggermente migliori rispetto alle scrofette (Capitolo 2).

La valutazione del fabbisogno di AA e la partizione dei nutrienti conseguenti a diverse strategie di allevamento, sono informazioni necessarie nel suino pesante. Le attuali raccomandazioni nutrizionali per suini da parte dell'NRC si concentrano sui suini con genotipi magri alimentati a volontà fino a raggiungere i 140 kg di peso corporeo (PC). Queste raccomandazioni presentano limiti evidenti nelle pratiche di gestione dei sistemi di produzione di suini pesanti per l'industria del prosciutto crudo. L'utilizzo dell'energia e dei nutrienti dei suini pesanti nell'ambito dei sistemi di produzione del prosciutto crudo non è stato trattato dalla letteratura esistente. Rimane inoltre incerto se i fabbisogni di energia metabolizzabile (ME) per il mantenimento (MEm = 1,03 MJ/kg in $BW^{0,60}$) suggeriti dall'NRC si possano applicare al suino pesante. Inoltre, la partizione dei nutrienti nei suini pesanti soggetti a strategie di alimentazione con apporti limitanti o meno di energia e/o di aminoacidi deve ancora essere chiarita. Il fabbisogno energetico è una funzione del livello di alimentazione per i requisiti di mantenimento e dei componenti associati all'aumento di peso corporeo dei suini. Per i suini pesanti, in cui incidenza di MEm nel costo totale dell'energia è risultata superiore al 45%, è necessario verificare i fabbisogni energetici in diverse condizioni di allevamento e di intervalli estesi di BW (Capitolo 3). Nel presente studio, è stato

utilizzato un approccio di modellizzazione basato su misurazioni ripetute del peso corporeo (BW) e del grasso dorsale (BF) fornite per studiare (i) le proteine corporee e gli accrescimenti lipidici dei suini pesanti Goland C21 tra 90 e 200 kg di peso corporeo (BW) se esposto a diverse condizioni di allevamento; (ii) il fabbisogno di energia metabolizzabile (ME) e lisina ileale digeribile standardizzata (lisina SID) e la ripartizione per il mantenimento e la crescita e (iii) l'efficienza marginale dell'utilizzo della lisina SID per Pd. I suini di controllo (C) hanno ricevuto diete limitanti la ME fino a 170 kg di peso al macello (SW) a 9 mesi di età (SA); i suini più anziani (OA) avevano diete limitate limitando la lisina ME e SID fino a 170 kg in SW a >9 mesi SA; i maiali più giovani (YA) sono stati alimentati con quantità non limitanti di lisina ME e SID fino a 170 kg in SW a <9 mesi SA; e i suini di peso maggiore (GW) sono stati alimentati come il gruppo YA, con 9 mesi SA e > 170 kg a SW. Il MEm stimato è stato in media di 1,03 MJ/kg^{0,60}. È stato osservato un aumento dell'11% di MEm nei suini OA rispetto al controllo. La restrizione energetica ha avuto effetti trascurabili sul MEm stimato. L'efficienza marginale dell'utilizzo della lisina SID per Pd è stata in media pari a 0,725, corrispondente a un fabbisogno di lisina SID di 9,8 g/100 g Pd.

La genomica nutrizionale (nutrigenetica e nutrigenomica) fornisce informazioni sui meccanismi molecolari sottostanti coinvolti nella sintesi degli acidi grassi e lo stato di ingrassamento nei suini è un tema complicato. La nutrigenetica è la scienza che si occupa dello studio dell'effetto (i) della variazione genetica sulla risposta nutrizionale mentre la nutrigenomica è la disciplina scientifica che indaga il ruolo dei nutrienti e dei composti alimentari bioattivi nell'espressione genica (Capitolo 4). Inoltre, i meccanismi epigenetici (metilazione del DNA e modifica dell'istone) sono intermediari che influenzano i meccanismi di deposizione di grasso e sono sensibili ai fattori ambientali e ai nutrienti apportati con la dieta. Oggi si stanno svelando i meccanismi epigenetici e i percorsi molecolari che regolano l'espressione genica (attivando e disattivando la trascrizione) e gli effetti regolatori degli acidi ribonucleici messaggeri RNA (mRNA) e microRNA (miRNA) nei depositi di grasso e IMF negli animali in produzione. La nutrigenomica offre la possibilità di chiarire meccanismi complessi di interazione gene-nutriente e l'ambiente sull'intero genoma. L'uso di tecnologie "omiche" basate sul DNA e della biologia dei sistemi sta definendo una nuova era post-genomica nella genomica nutrizionale degli animali (Capitolo 4). I nutrienti possono essere abbinati in modo più accurato con i geni ereditari per armonizzare le funzioni metaboliche e migliorare la salute e le caratteristiche economicamente importanti negli animali. Ad esempio, i loci dei tratti quantitativi (QTL) esistenti per i geni e le loro mutazioni nella lipogenesi, la suscettibilità alle malattie e lo sviluppo di altri tratti nei suini devono ancora essere armonizzati. Mancano studi sul ruolo dei meccanismi epigenetici negli effetti transgenerazionali della nutrizione e dell'ambiente nella differenziazione degli adipociti e nello sviluppo dei caratteristiche dei suini. E' possibile ora mappare accuratamente i percorsi regolatori genetici, fisiologici e nutrizionali coinvolti in molte funzioni cellulari come i meccanismi molecolari di accrescimento di grasso e IMF nei suini. Ciò ha reso meno elusivo l'impatto dei singoli nutrienti dietetici sull'intero genoma. Presto, l'armonizzazione delle conoscenze esistenti sulla genomica nutrizionale potrebbe diventare lo strumento principale per stime precise del fabbisogno nutritivo di suini con diversi stati fisiologici, età, sesso e razza per il metabolismo dei grassi e altri miglioramenti dei tratti (come prestazioni di crescita, spessore del grasso dorsale, accrescimento del FMI, resistenza alle malattie, ecc.) nei suini e negli altri animali in produzione.

Dalla presente tesi sono state tratte le seguenti conclusioni:

1. È stata sviluppata una nuova strategia di alimentazione e allevamento (giovane età, YA) per il suino pesante destinato alla produzione di prosciutto crudo. Questa strategia consente di anticipare la macellazione di circa 27 giorni prima con migliori miglioramenti in ADG, efficienza del mangime e adiposità del prosciutto (Capitolo 1).

2. La strategia di allevamento in età più giovane (YA) dovrebbe essere applicata con cautela, poiché sono necessarie ulteriori ricerche per chiarire se l'aumento dell'adiposità del prosciutto può compensare gli effetti negativi dell'età della macellazione più giovane sull'attitudine alla stagionatura (Capitolo 1).

3. Sebbene la strategia dell'età avanzata (OA) abbia un effetto positivo sulla marezzatura visibile e sulo spessore del grasso sottocutaneo prossimale al muscolo semimembranoso, questa strategia è risultata inefficiente, compromette la crescita e l'efficienza dell'alimentazione e

aumenta i costi di produzione, con poca influenza sulla composizione della carcassa e con una riduzione delle dimensioni del prosciutto (Capitolo 1).

4. La strategia di mantenere i suini con un peso corporeo esteso (peso maggiore, GW) è stata associata a un aumento del consumo di mangime, ADG, carcassa e peso del prosciutto, con un miglioramento di alcuni indici di qualità del prosciutto rispetto a C. Tuttavia, l'adozione di questa strategia è connessa al rischio di un aumento della quota di carcasse di peso superiore alla soglia massima indicata dal disciplinare di produzione (168 kg). Tuttavia, potrebbe essere aumentata con una lieve restrizione del mangime a seconda del potenziale di crescita dei suini (Capitolo 1).

5. Le attuali classi di peso al macello considerate nel presente studio hanno confermato che i suini con una maggiore classe di peso al macello (SWC) hanno un maggiore accrescimento medio giornaliero e consumo di mangime con efficienza alimentare simile, maggiore peso del prosciutto, muscolosità e copertura di grasso in corrispondenza del muscolo semimembranoso (Capitolo 2).

6. L'effetto del sesso sui prosciutti freschi ha confermato che i maschi castrati producevano prosciutti di peso e marezzatura maggiori rispetto alle scrofette. Queste caratteristiche sono desiderate per il loro effetto positivo sul sapore e sulle caratteristiche visive del prosciutto crudo al momento della sua selezione per la stagionatura (Capitolo 2).

7. Il presente modello basato su misurazioni ripetute del peso corporeo (BW) e del grasso dorsale (BF) ha fornito stime affidabili per l'energia metabolizzabile (ME) e il fabbisogno di aminoacidi dei suini in accrescimento per range estesi di BW e varie condizioni di alimentazione (Capitolo 3).

8. È stato confermato che un valore MEm di 1,02 MJ/kg^{0,60} è applicabile per suini di peso corporeo compreso tra 90 e 200 kg, indipendentemente dal regime di alimentazione. La restrizione energetica ha avuto poca o nessuna influenza sul MEm stimato (Capitolo 3 e Discussione).

9. In condizioni di restrizione energetica e proteica, l'efficienza marginale massima dell'utilizzo della lisina SID per la deposizione proteica (Pd) è stata pari a 0,73. Ciò corrisponde a un fabbisogno di 9,8 g di lisina SID per 100 g di Pd, come requisito minimo, indipendentemente dal peso corporeo (PC) (Capitolo 3).

10. Diversi QTL, SNP, mRNA e miRNA sono coinvolti nei meccanismi molecolari del metabolismo dei grassi e nella deposizione di IMF nei suini. Proprietà nutrizionali dei mangimi, nutrienti e sostanze bioattive dietetiche i cui livelli nella dieta possono essere difficili da controllare, oltre ai fattori ambientali. La scienza della nutrigenetica, della nutrigenomica e dei meccanismi epigenetici è efficiente e precisa nel definire i cambiamenti nelle sequenze geniche che predispongono le singole razze suine a rispondere in un certo modo in termini di prestazioni, qualità della carne e del latte, nonché rilevamento della salute e delle malattie (Capitolo 4 e Discussione).

11. Di conseguenza, è possibile misurare gli effetti nutrizionali sulle espressioni geniche e la regolazione delle risposte del genoma nei suini, per ottimizzare le prestazioni di crescita, lo spessore del grasso dorsale, la deposizione di FMI, la resistenza alle malattie e le caratteristiche qualitative della carne. Tuttavia, la domanda rimane: quanto siamo preparati a integrare questa scienza come strumento nell'alimentazione animale e nell'alimentazione dei suini? (Capitolo 4).

Hungarian Summary

Az olasz szárazon pácolt sonkaelőállításra szánt nehéz sertések hagyományos tenyésztési rendszerei megkövetelik, hogy a sertéseket 160 ± 16 kg-os testtömeggel és legalább 9 hónapos korban vágják. A jelenlegi tenyészcélok miatt a sertések egyre nagyobb izomtömeggel és kisebb zsírtartalommal rendelkeznek, így a hagyományos nagyüzemi tenyésztés nem képes optimális tulajdonságokkal rendelkező sertéseket biztosítani a szárított sonka gyártás ipara számára. Kutatásunkban 95 ± 9,0 kg testtömegű (BW) C21 Goland sertéseket használtunk (emséket és ártányokat). Különböző takarmányozási körülmények között különböző életkor és vágáskori súly kombinációkat vizsgáltunk, mint lehetséges alternatív nevelési stratégiákat nagysúlyú sertések esetében. Az alternatív tartási stratégiák a sertések növekedési ütemének befolyásolását célozták: 1) lehetővé tettük, hogy a sertések genetikailag meghatározott növekedési potenciáljuknak megfelelően, de fiatalabb vágási korban (fiatalabb korban, YA) érjék el a 160 ± 16 kg vágási súlyt; 2) lehetővé tettük, hogy a sertések növekedési potenciáljuknak megfelelően fejlődjenek és a vágási súlyt nem tekintve korlátnak, 9 hónapos korban vágtuk (nagyobb súly, GW); 3) a 160 ± 16 kg vágósúly (idősebb kor, OA) eléréséhez szükséges életkort növeltük azáltal, hogy az állatok gyarapodását visszafogtuk. A sertéseket átlagosan 257, 230, 257 és 273 nap életkorban, illetve 172,7, 172,3, 192,9 és 169,3 kg élősúlyban vágták le a négy kezelés során. A kontroll sertések átlagos napi gyarapodása (ADG) 715 g/d volt, a takarmányértékesítésük (FE) pedig 0,265 (gyarapodás/takarmány fogyasztás). A kontroll csoporthoz képest a fiatalabb korban vágott (YA) sertéseknél magasabb volt az ADG (+32%), FE (+7,5%), és jobb volt a sonka zsírosodása; A nagyobb súlyú (GW) sertéseknél nagyobb volt a hasított testtömeg (+12%), az ADG (+25%), a sonka tömege (+10,9%), és jobb volt a sonka zsírral való borítottsága a kontrollhoz képest. Az idősebb korban való vágás (OA-kezelés) befolyásolta az ADG-t (-16,4%), az FE-t (-16,6%) és a sonka tömegét (-3,6%). Mind a korábbi életkorban való vágás (YA) mind a nagyobb élősúlyra való hízlalás (GW) ígéretes alternatívák lehetnek a hagyományos takarmányozási stratégia (C) helyett, mivel javították az FE és a sonka minőségi tulajdonságait (1. fejezet). Kimutattuk, hogy az első két (1 és 2) stratégia volt a legígéretesebb alternatíva, mivel javította a sertések növekedési ütemét, a takarmányozás hatékonyságát és a sonka zsírral való borítottságát. Míg az első stratégia volt gazdaságilag a legelőnyösebb, addig a második stratégiát alkalmazva lehetett előállítani a legjobb minőségű sonkákat (1. fejezet).

A sertés vágósúlyának növelésének fő korlátja a hasított test zsírosodásának növekedése és a takarmányozási hatékonyság romlása. A sonkatermelés szempontjából engedélyezett vágósúly tartomány kiterjesztése egy olyan *ad libitum* takarmányozási stratégia elfogadásának lehetőségét jelenti, amely jobban kiaknázza a sertések genetikai potenciálját, de csökkenti az állatok és a hasított test uniformitását az azonos tételhez tartozó sertések között. Vizsgáltam az *ad libitum* takarmányozás hatását a vágósúlyra, a növekedési teljesítményre, a takarmányozási hatékonyságra, valamint a hasított test és a friss sonka jellemzőire (2. fejezet). Az etetett takarmány 10 MJ/kg nettó energiát és 7,4 valamint 6,0 g/kg SID lizint tartalmazott. A vágási súlykategóriák az alábbiak voltak: <165, 165–180, 180–210 és >210 kg. A vizsgálatba vont sertéseket 230 vagy 258 napos korukban leöltük. A bal oldali sonkákat a kerek forma, a zsírréteg vastagsága, a márványosság, a hús szín és az erezettség alapján pontozták. Az adatokat olyan statisztikai modellel elemeztük, amelyben fix hatásnak tekinettük a súlykategóriát vágáskor, az ivart és a két hatás interakcióját, az egyes tételek véletlen hatásként szerepeltek a modellben. Az súlykatagória lineáris, kvadratikus és köbös hatásait teszteltük, de csak lineáris hatást találtunk. Az eredmények azt mutatták, hogy a nagyobb súlykategóriájú sertések átlagos napi gyarapodása és takarmányfogyasztása nagyobb volt, de hasonló takarmány értékesítéssel és jobb sonkaminőségi tulajdonságokkal rendelkeztek: nagyobb sonkatömeg, jobb izmoltság és zsírborítás a *semimembranosus* izomzaton. Az ártányok nehezebbek voltak, és valamivel jobb tulajdonságokkal rendelkeztek, mint a kocasüldők (2. fejezet).

A különböző tenyésztési stratégiákon nevelt nagysúlyú sertések aminosav (AS) szükségletének értékelése és az AS-ak megoszlása különösen fontos takarmányozási szempontból. Az NRC sertésekre vonatkozó jelenlegi táplálóanyag-ajánlásai azonban csak a hústípusú sertésekre taralmaznak ajánlást. Az ajánlás szerint összeállított keverékeket *ad libitum* etetik amíg a sertések el nem érik a 140 kg-os testsúlyt (BW). Az ajánlás nem feltétlenül adaptálható olyan sertésekre, melyeket szárazon pácolt sonka gyártására nevelnek nagy súlyig. A nagysúlyú sertések energia- és tápanyag-hasznosításáról szakirodalom nem lelhető fel, pedig ezek az állatok adják a szárazon pácolt sonka előállítás alapanyagát. Az is bizonytalan, hogy az NRC által javasolt létfenntartó metabolizálható energia (ME) szükséglet (MEm = 1,03 MJ/kg $BW^{0,60}$) érvényes-e a nagysúlyú sertésekre. Ezen

túlmenően a korlátozott vagy korlátlan energia- és/vagy aminosav-ellátást biztosító takarmányozási stratégia szerint etetett nagy súlyra hízlalt sertések tápanyag-megoszlását sem ismerjük pontosan. A takarmányozási szint és az aktuális energia ellátás a létfenntartás (MEm) és a gyarapodás összetevőinek beépüléséhez szükséges energia szükségletnek a függvénye. Nagysúlyú sertések esetében, amelyeknél az MEm részaránya a teljes energiaszükségletben az idevonatkozó vizsgálatok szerint meghaladja a 45%-ot, különösen érdekes az energiaigény mértékének és megoszlásánk ismerete különböző tenyésztési körülmények és szélsőséges testtömeg-tartományok mellett (3. fejezet). A jelen kutatásban alkalmazott modell a rendszeresen mért testtömeg- (BW) és hátszalonna vastagság (BF) adatok felhasználásával becsülte (i) a 90 és 200 kg közötti testtömegű Goland C21 sertések testfehérje és testzsír beépülését különböző nevelési körülmények mellett; (ii) a metabolizálható energia (ME) és a standardizált ileális emészthető lizin (SID lizin) szükségletet és megoszlását a létfenntartás és a növekedés szükségletén belül, valamint (iii) a SID lizin értékesülésének marginális hatékonyságát a fehérjebeépülés esetében. A kontroll sertések (C) 9 hónapos korukban, 170 kg élősúlyban kerültek levágásra, hízlalásuk során korlátozott energia ellátásban részesültek; a későbbi korig tartott (OA) sertések korlátozott takarmányozásban részesültek, 170 kg élősúlyig tartott a hízlalás korlátozott ME és SID lizin ellátással, >9 hónapos korban történt a vágás; fiatalabb (YA) sertéseket korlátlan takarmányfelvétel mellett, a szükségleteknek megfelelő ME és SID lizin ellátást biztosítva 170 kg-ig kevesebb, mint 9 hónapos korig tartott a hízlalás; valamint nagyobb súlyra hízlalt (GW) sertéseket használtunk YA csoportként, 9 hónapos vágási kort és >170 kg testsúlyt elérve vágáskor. A becsült létfenntartó metabolizálható energy szükséglet (MEm) átlagosan 1,03 MJ/kg^{0,60}. Az OA sertéseknél a MEm 11%-os növekedését tapasztaltuk a kontrollokhoz képest. Az energiafelvétel korlátozása elhanyagolható hatással volt a becsült MEm-re. A SID lizin értékesülésének marginális hatékonysága a fehérjebeépülés (Pd) során átlagosan 0,725 volt, ami 9,8 g/100 g Pd SID lizin szükségletnek felel meg.

A táplálkozási/takarmányozási genomika (nutrigenetika és nutrigenomika) betekintést nyújt többek között a zsírsavszintézis hátterében meghúzódó molekuláris mechanizmusokba, melyek a sertéshús márványozottságát eredményezik, és amiket más módon nehéz megfejteni. A nutrigenetika az a tudomány, ami a takarmányozásra adott válaszreakciók genetikai varianciájával foglalkozik, míg a nutrigenomika a tápanyagok és a bioaktív élelmiszer-vegyületek szerepét vizsgálja a génexpresszióban (4. fejezet). Ezenkívül ismert, hogy az epigenetikai mechanizmusokban (DNS-metiláció és hisztonmódosítás) pl. a zsírlerakódás mechanizmusait befolyásoló közvetítők érzékenyek a környezeti tényezőkre és a takarmányból származó tápanyagokra. Ma már világos, hogy az utódokban a génexpressziót (transzkripció be- és kikapcsolását) szabályozó epigenetikai mechanizmusok és molekuláris útvonalak mintázata, valamint a hírvivő ribonukleinsavak (mRNS-ek) és mikroRNS-ek (miRNS-ek) szabályozó hatásúak a zsír- és az intramuszkuláris zsír (IMF) lerakódásában. haszonállatokban. A nutrigenomika lehetőséget kínál a gén-táplálóanyag kölcsönhatás és a környezet komplex mechanizmusainak feltárására a teljes genom ismerete alapján. A nagy áteresztőképességű DNS-alapú „omika” technológiák és a rendszerbiológia alkalmazása egy új posztgenomikus korszakot nyitott meg a takarmányozási kutatások genomikával való összekapcsolásában (4. fejezet). A megszerzett ismeret a tápanyagok szerepéről az öröklött gének kifejeződésében segítségünkre lesz az anyagcsere-funkciók harmonizálásában és az állatok egészségének és gazdaságilag fontos tulajdonságainak javításában. Például a táplálóanyagoknak a lipogenezisben szerepet játszó gének kifjeződésére, illetve más a sertések egyéb tulajdonságainak kifejlődéséért felelős génekben bekövetkező mutációikra vonatkozó meglévő kvantitatív tulajdonságlókuszok (QTL) harmonizálása még várat magára. Hiányosak az ismereteink az epigenetikai mechanizmusok szerepéről a táplálkozás és a környezet transzgenerációs hatásaiban, a zsírsejtek differenciálódásában és a sertések tulajdonságainak kialakulásában. Fel kell térképeznünk számos sejtfunkcióban szerepet játszó genetikai, fiziológiai és táplálkozási szabályozási útvonalat, mint például a zsír molekuláris mechanizmusait és az intramuszkuláris zsír beépülését a sertésekben. A rendszer megismerésével az egyes tákarmányból származó táplálóanyagok hatása a teljes genomra már kevésbé lesz megfoghatatlan. Hamarosan a nutrigenomikai ismeretek harmonizálása lehet a fő eszköz a különböző fiziológiai állapotú, korú, ivarú és fajtájú sertések táplálóanyagszükségletének pontos becslésére a zsíranyagcsere és egyéb tulajdonságok (például növekedési teljesítmény, hátzsír vastagsága, IMF-felhalmozódás) szempontjából.

Jelen dolgozatból az alábbi következtetéseket vontuk le:

1. Új takarmányozási és nevelési stratégiát (fiatalabb életkorig való hízlalás) dolgoztunk ki a szárazon pácolt sonkatermelésre szánt nehézsertés számára. Ez a stratégia lehetővé teszi, hogy a vágás körülbelül 27 nappal korábban történjen, miközben számítani lehet az átlagos napi súlygyarapodás, a takarmány értékesítés és a sonka zsírtartalmának és zsírral való borítottságának kedvezőbb alakulásával (1. fejezet).

2. A fiatalabb korig való nevelési stratégiát óvatosan kell alkalmazni, mivel további kutatásra van szükség annak tisztázására, hogy a sonka job zsírtartalma és -eloszlása kompenzálhatja-e a fiatalabb vágási kor negatív hatását a szárításra való alkalmasság tekintetében (1. fejezet).

3. Bár az idősebb korig való hízlalás stratégiája pozitív hatással van a *semimembranosus* combizomhoz közel látható márványosodásra és a bőr alatti zsírvastagságra, ez a stratégia kevésbé hatékony, rontja a növekedést és a takarmányozás hatékonyságát, valamint növeli a termelési költségeket, befolyásolhatja a hasított test összetételét, és csökkenti a sonka méretét (1. fejezet).

4. A sertések nagyobb testtömegig való nevelése a takarmányfogyasztás, az álagos napi súlygyarapodás, a hasított test és a sonka tömegének növekedésével járt együtt, néhány sonkaminőségi index javulásával a kontrollnak tekintett hagyományos neveléshez képest. Ennek a stratégiának az elfogadása azonban nem elégíti ki a termékleírásban megjelölt maximális vágósúlyt (168 kg), alkalmazása esetén a küszöbértéket meghaladó, nagyobb súlyú hasított testek aránya nő. A sertések növekedési potenciáljától függően azonban a starégia enyhe takarmánykorlátozással kiegészíthető (1. fejezet).

5. A kutatásunkban vizsgált vágási súlykategóriákban kapott eredményeink megerősítik, hogy a nagyobb vágási súlykategóriában tartozó sertések átlagos napi gyarapodása és takarmányfogyasztása nagyobb, de azonos takarmányértékesítéssel, nagyobb sonkatömeggel, jobb izomoltsággal és zsírborítással rendelkeznek a *semimembranosus* izom esetében (2. fejezet).

6. Az ivar hatással van a nyerssonkák minőségére, az ártányok esetében a sonkák nagyobb súlyúak és márványozottabbak, mint az emsék sonkái. Ezek a tulajdonságok azért kívánatosak, mert pozitív hatást gyakorolnak a nyerssonka ízére és vizuális tulajdonságaira a szárazon pácolásra való kiválasztás idején (2. fejezet).

7. A folyamatos testtömeg és hátszalonna vastagság mérések figyelembevételével olyan modellt alakíottunk ki, ami megbízhatóan képes becsülni a széles testtömeghatárok és takarmányozási feltételek mellett hízósertések metabolizálható energia (ME) és aminosavszükségletét (3. fejezet).

8. Megerősítést nyert, hogy az 1,02 MJ/kg^{0,60} MEm érték alkalmazható a 90-200 kg testtömegű sertések esetében, függetlenül a takarmányozási rendszertől. Az energiakorlátozásnak alig vagy egyáltalán nem volt hatása a becsült MEm-re (3. fejezet és Diszkusszió).

9. Restriktív energia és fehérje ellátás mellett a SID lizin értékesülése a fehérdepozícióban (Pd) maximálisan 0,73 volt. Ez azt jelenti, hogy 100 g fehérjebépüléshez 9,8 g SID lizin szükséges legalább testtömegtől függetlenül (3. fejezet).

10. Számos QTL, SNP, mRNS és miRNS vesz részt a zsíranyagcsere és az intramuszkuláris zsír (IMF) beépülésének molekuláris mechanizmusaiban sertésekben. A környezeti tényezők mellett a takarmány táplálóértéke, a tápanyagok és az étrendi bioaktív anyagok egyértelműen hozzájárulnak e mechanizmusok szabályozásához. A nutrigenetika, a nutrigenomika és az epigenetikai mechanizmusok ismeretében hatékonyan és pontosan meghatározhatók a génszekvenciák változásai, amelyek következtében az adott sertésfajta sertében egy adott teljesítmény, a hús és a tej minőség kialakuljon (4. fejezet és Diszkusszió).

11. Ezek ismeretében lehetőség nyílik a sertések génexpressziójának finomhangolására és a genomválaszok szabályozására irányuló táplálkozási hatások mérésére, a növekedési teljesítmény, a hátszalonna vastagság, a hús inramuszkuláris zsírtartlama és egyéb húsminőségi jellemzők optimalizálására. A kérdés azonban továbbra is fennáll: mennyire vagyunk felkészültek arra, hogy ezt a tudományt eszközként integráljuk az gazdasági állatok takarmányozásába, különösen a sertéstakarmányozásba? (4. fejezet).

Publications and Presentations

Papers Related to the Thesis

1. **Malgwi, I.H.;** Gallo, L.; Halas, V.; Bonfatti, V.; Carcò, G.; Sasso, C.P.; Carnier, P.; Schiavon, S. The Implications of Changing Age and Weight at Slaughter of Heavy Pigs on Carcass and Green Ham Quality Traits. *Animals* **2021**, *11*, 1–16, doi:10.3390/ani11082447.

2. **Malgwi, I.H.;** Giannuzzi, D.; Gallo, L.; Halas, V.; Carnier, P.; Schiavon, S. Influence of Slaughter Weight and Sex on Growth Performance, Carcass Characteristics and Ham Traits of Heavy Pigs Fed Ad-Libitum. *Animals* **2022**, *12*, 215, doi:10.3390/ani12020215.

3. Schiavon, S.; **Malgwi, I.H.;** Giannuzzi, D.; Galassi, G.; Rapetti, L.; Carnier, P.; Halas, V.; Gallo, L. Impact of Rearing Strategies on the Metabolizable Energy and SID Lysine Partitioning in Pigs Growing from 90 to 200 Kg in Body Weight. *Animals* **2022**, *12*, 1–21, doi:10.3390/ani12060689.

4. **Malgwi, I.H.;** Halas, V.; Grünvald, P.; Schiavon, S.; Ildikó I.; Genes Related to Fat Metabolism in Pigs and Intramuscular Fat Content of Pork: A Focus on Nutrigenetics and Nutrigenomics. *Animals* **2022**, *12*, doi:10.3390/ani12020150.

5. Schiavon, S.; **Malgwi, I.H.;** Carnier, P.; Carcò, G.; Sasso, C.P.; Gallo, L. Prestazioni produttive e caratteristiche di carcasse e cosce conseguenti a differenti strategie di allevamento del suino pesante da prosciuttificio. 40 *Suinicoltura* – n. *6* giugno 2021 La registrazione integrale dell'evento è disponibile al link: https://bit.ly/2RKxQOx.

6. Grünvald, P.; Jócsák, I.; **Malgwi, I.H.;** János, T.; Gergő, S.; Halas, V. Effect of different dietary lysine on the functioning of genes participating in the build-up the of intramuscular fat in pork. (Submitted to *ACTA Agraria Kaposváriensis*).

"…errors are not in the art but in the artificers".

— Isaac Newton.

Oral Presentations

1. **Malgwi, I.H.;** Carnier, P.; Sasso, C.P.; Halas, V.; Schiavon, S. Innovative Feeding Strategies for the Heavy Pigs: Effects on Technological and Quality Traits of Dry-Cured Hams. In: Mantovani, R.; Cecchinato, A. ASPA 24th Congress Book of Abstract. *Ital. J. Anim. Sci.* **2021**, *20*, 1–236, doi:10.1080/1828051x.2021.1968170.

2. **Malgwi, I.H.;** Grünvald, P; Jócsák, I; Halas, V. Genes related to fat metabolism in pigs and intramuscular fat content of pork. In: Mantovani, R.; Cecchinato, A. ASPA 24th Congress Book of Abstract. *Ital. J. Anim. Sci.* **2021**, *20*, 1–236, doi:10.1080/1828051x.2021.1968170.

3. **Malgwi, I.H.;** Gallo, L.; Halas, V.; Bonfatti, V.; Carcò, G.; Sasso, C.P.; Carnier, P.; Schiavon, S. Methods of Changing Slaughter Age at the Same Bodyweight: Effects on Feed Efficiency and Green Ham Traits of Heavy-Pigs. The Hungarian University of Agriculture and Life science. *29th international symposium animal science days*, Gödöllő – Hungary, 13th – 17th September 2021.

4. **Malgwi, I.H.;** Broccanello, C; Stevanato, P; Squartini, A; Halas, V; Gallo, L; Schiavon, S. Microbiome Analysis in Dry-Cured Ham Samples with Different Alteration Levels. 'University of Padova. *International Metagenomics Workshop*, San Servolo, Venice - Italy, 22nd – 24th November 2022.

5. **Malgwi, I.H.;** Patel, N.; Giannuzzi, D.; Toscano A.; Yakubu, H.G.; Bazar, G.; Gallo, L.; and Schiavon, S. Evaluating Chemical Composition of Pig Faeces Based on Different Diets Using Near-Infrared Reflectance Spectroscopy (NIRS). The Hungarian University of Agriculture and Life science. *29th International Symposium on Animal Nutrition,* Kaposvár - Hungary, 29th September – 2nd October 2022.

"...the increase of known truths stimulates the investigation, establishment, and growth of the arts; not their diminution or destruction."

— Galileo Galilei, 1623.

Curriculum Vitae

Dott. Isaac Hyeladi Malgwi was born in Maiduguri, Nigeria, on August 8, 1987. In 2005, he completed his high school studies at the University of Maiduguri Staff Secondary School. From 2006 to 2012, he attended the University of Maiduguri in Nigeria, where he received a five-year BSc in general agriculture with honours in animal science.

The Governments of Hungary and Nigeria, respectively, awarded him the Stipendium Hungaricum Scholarship and the Bilateral Education Agreement (BEA) Scholarship for a master's degree in Hungary in 2016 at the Kaposvár University, now Hungarian University of Agriculture and Life Sciences (MATE) - Kaposvár Campus. In June 2018, Isaac completed the MSc in Animal Nutrition and Feed Safety Engineering with First Class Honours. He started working full-time as a research assistant in the molecular biology laboratory of the Kaposvár University's Institute of Physiology and Animal Nutrition in October 2018 and continued through October 2019.

Following a selection procedure for a PhD in Animal and Food Science XXXV Cycle 2019-2022) in the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), at the world's oldest University, the University of Padua, established in 1222 - Italy, Isaac was awarded the 2019 DAFNAE "di Dipartimenti di eccellenza—Progetto CASA" - Department Excellence Scholarship. This makes Isaac the first Nigerian and the second African student to be admitted to the PhD programme in Animal and Food Science Course in the 35th cycle (35° ciclo) at the 800-year-old University of Padua. During the 3 years of the PhD programme, Isaac was able to spend a total of twelve (12) months, working on PhD-related research at the Hungarian University of Agriculture and Life Sciences (MATE) - Kaposvár Campus, from October 2021–October 2022 with his co-supervisor Assoc. Professor Veronika Halas. He has taught, mentored and co-supervised BSc and MSc students both at the University of Padua and the MATE – Kaposvár Campus.

Dott. Isaac have co-authored and wrote scientific papers published in high-impact factor (Q1, peer-reviewed) journals, as well as presented papers and participated in scientific conferences and meetings in Hungary, Italy, Switzerland, and the United States.

Galileo Galilei's letter in sunspots from Villa delle Selve to Mark Wesler, December 1, 1612, in which Venus, the Moon, and the Medicean Planets are also discussed, and new appearances of Saturn are revealed, is Dott. Isaac's most famous scientific remark. It is quoted from Stillman Drake's Galileo's Discoveries and Opinions (1957), pp. 134-135.

"…sì perché l'autorità dell'opinione di mille nelle scienze non val per una scintilla di ragione di un solo, sì perché le presenti osservazioni spogliano d'autorità i decreti de' passati scrittori, i quali se vedute l'avessero, avrebbono diversamente determinato."

"…for in the sciences, the authority of thousands of opinions is not worth as much as one tiny spark of reason in an individual man. Besides, the modern observations deprive all former writers of any authority, since if they had seen what we see, they would have judged as we judge."

— Galileo Galilei, 1612.

Degree achievement notice board

Student

First name	ISAAC HYELADI
Surname	MALGWI
Freshman	1219516
Course of Study	ANIMAL AND FOOD SCIENCE

Summary of application for graduation

Session/call detail

Question status	Presented
Session	Esame finale 35° ciclo | Final exam 35th cycle
Appeal / Appello	Appello PROROGA 3 MESI EX D.L. n. 41/2021
	Appeal EXTENSION 3 MONTHS PURSUANT TO DL n. 41/2021
Appeal start date	01/12/2022

Thesis summary

Thesis detail

Thesis type

NORMAL

Thesis title

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, Feed Efficiency, and Dry-Cured Ham Meat Quality Traits

Thesis title in English

Feeding and Rearing Strategies for Heavy Pigs: Effects on Growth Performance, Feed Efficiency, and Dry-Cured Ham Meat Quality Traits

Thesis language

English

How your thesis can be accessed

Libera consultazione

Valutazione tesi | Thesis evaluation

Minor revision richiesta | Minor revision required.

Revisioni tesi | Thesis revision

Revisioni effettuata | Revision Done.

“La filosofia è scritta in questo grandissimo libro, che continuamente ci sta aperto innanzi agli occhi (io dico l'Universo), ma non si può intendere, se prima non il sapere a intender la lingua, e conoscer i caratteri ne quali è scritto.

“Philosophy is written in this grand book, the universe, which stands continually open to our gaze. But the book can not be understood unless one first learns to comprehend the language and read the letters in which it is composed.”

— Galileo Galilei, 1623.

“...Eppur si muove.”

“...still it moves.”

— Galileo Galilei, 1633.