Head of Office: University of Padova
Department of Agronomy, Food, Natural resources, Animals and Environment

Ph.D. Course in: Animal and Food Science
Series: XXXI

EFFECT OF REDUCTIONS IN FEED ALLOWANCE AND DIETARY AMINO ACIDS CONTENT ON FEEDING BEHAVIOUR, GROWTH PERFORMANCE, NUTRIENT EXCRETION AND MEAT QUALITY OF GROWING PIGS BELONGING TO DIFFERENT GENETIC TYPES

EFFETTO DELLA RIDUZIONE DELLA DISPONIBILITÀ DI ALIMENTO E DEL LIVELLO DI AMMINOACIDI NELLA DIETA SU COMPORTAMENTO ALIMENTARE, PRESTAZIONI PRODUTTIVE, ESCREZIONE DI NUTRIENTI E QUALITÀ DELLA CARNE DI MAIALI APPARTENENTI A DIVERSI TIPI GENETICI

Coordinator: Prof. Stefano Schiavon
Supervisor: Prof. Stefano Schiavon

Ph.D. student: Giuseppe Carcò

DECLARATION

I declare that the present thesis has not been previously submitted as an exercise for a degree at University of Padova, or any other University, and I further declare that work embodied is my own.

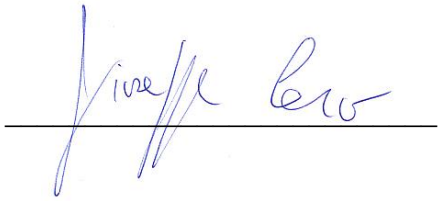


TABLE OF CONTENTS

ABSTRACT

Pig industry is required to reduce its environmental footprint, in order to satisfy the increasing demand of eco-friendly products. Moreover, the producers need to cut down with the feeding costs, by improving the pigs' feed efficiency. Despite the rich literature, knowledge about the effect of some feeding strategies on feeding behaviour, performance, and quality of meat and meat products seems still controversial or scarce.

With this background, the aims of this Ph.D. project were: i) to assess the influence of a reduction in dietary indispensable amino acids (AA) on feed intake, growth performance, N excretion and carcass and meat quality traits of fast growing pigs fed according to an ad libitum or a restricted feeding regime; ii) to study the feeding behaviour of group-housed pigs fed individually from single-space feeders and subjected to feed restriction and a reduction in the dietary indispensable AA content; iii) to explore the influence of feeding behaviour on growth performance, carcass and meat characteristics of pigs; iv) to investigate the influence of low-protein and AA diets on the characteristics of San Daniele like dry-cured hams obtained from two genetic groups of pigs with different lean growth potential.

In the 1st Chapter, 96 barrows were individually fed through automatic feeding stations, according to an ad libitum or restricted feeding regime and to a conventional or low-AA diet. Feed restriction decreased feed intake, average daily gain, carcass weight, backfat depth, but increased gain:feed ratio. The low-AA diets increased feed intake, carcass weight and the intramuscular fat content, with no effects on the feed efficiency and the estimated Pr. Nitrogen excretion was reduced by feed restriction and by the reduction of the dietary AA content.

In the 2nd Chapter, the data recorded by the automatic feeding stations were used to study the effects of the feeding regime and the dietary AA restriction on the feeding patterns of the pigs of the previous contribution. Feed restriction decreased daily feed intake, the number of visits and the time spent feeding, but increased feed consumption per visit and feeding rate. The low-AA diets increased daily feed intake, tended to increase feeding rate and interacted with feeding regime for the number

and duration of feeding visits. The pigs were able to adapt their feeding patterns to compensate for a reduction in feed allowance or nutrient restriction.

In the 3rd Chapter the phenotypic correlations among feeding patterns, growth performances and carcass traits were studied. The records of each pig were classified into feeding rate tertiles. Pigs eating faster showed greater final body weights, average daily gains, estimated protein gains, estimated lipid retention, carcass weights, weights of lean cuts, weights of fat cuts, proportions of fat in the carcass, and lower proportions of carcass lean cuts than pigs eating slowly. Manipulating the eating rate, through management or genetic strategies, could affect growth performances and carcass quality, with little influence on feed efficiency.

The 4th Chapter investigated the influence of diets lowered in protein and AA contents on the quality of 40 San Daniele like dry-cured hams produced by pigs of two genetic groups (Danbred and Anas) characterized by different potential for lean growth, eating conventional or low-protein diets. The Danbred fresh hams were heavier, but showed greater seasoning losses and thinner fat cover than Anas hams. Dry-cured hams from Danbred had higher protein content than the Anas ones. Dietary protein restriction had small influence on dry-cured ham quality. Due to its positive effects on sustainability of dry-cured ham chain by decreasing pig farm nitrogen excretion and feeding costs, the use of low-protein diets seems an advisable strategy for the feeding of traditional PDO heavy pigs.

RIASSUNTO

Il settore suinicolo deve oggi soddisfare la domanda di prodotti eco-friendly, ridurre i costi di produzione e migliorare l'efficienza alimentare dei maiali attraverso l'utilizzo di nuove strategie alimentari.

Gli obiettivi di questo progetto di dottorato sono: i) valutare l'influenza di una riduzione del contenuto di amminoacidi della dieta su consumo alimentare, prestazioni e qulità della carne di maiali alimentati secondo un regime alimentare ad libitum o razionato; ii) studiare il comportamento alimentare dei maiali sottoposti ad una restrizione alimentare e ad una riduzione del contenuto di amminoacidi essenziali dell'alimento; iii) conoscere l'influenza del comportamento alimentare sulle prestazioni e le caratteristiche della carcassa; iv) esaminare gli effetti di una riduzione del contenuto di proteina e amminoacidi sulla qualità di prosciutti crudi ottenuti da due linee genetiche caratterizzate da diversi potenziali di crescita magra e stagionati come prosciutti San Daniele DOP.

Nel I contributo, 96 maiali sono stati alimentati attraverso un regime alimentare ad libitum o una leggera restrizione alimentare utilizzando diete convenzionali o con basso contenuto di amminoacidi essenziali (LAA). La restrizione alimentare ha ridotto i consumi di alimento, gli accrescimenti, i pesi delle carcasse e gli spessori del grasso, ma ha aumentato l'efficienza alimentare. La dieta LAA ha aumentato i consumi, i pesi e il contenuto di grasso delle carcasse, senza influenzare l'efficienza alimentare e la crescita proteica. I due trattamenti alimentari hanno ridotto l'escrezione di azoto.

Nel II contributo, si sono valutati gli effetti del regime alimentare e della dieta LAA sul comportamento alimentare dei maiali. La restrizione alimentare ha ridotto l'ingestione di alimento, il numero di visite alla mangiatoia e il tempo utilizzato per mangiare, ma ha aumentato il consumo per visita, e la velocità d'ingestione. La dieta LAA ha aumentato i consumi alimentari, e ha provocato un leggero aumento della velocità d'ingestione. I maiali sono stati in grado di adattare il loro comportamento alimentare per superare i limiti dovuti alla restrizione alimentare e alla riduzione di nutrienti del mangime.

Nel III capitolo, si sono studiate le relazioni tra comportamento alimentare, prestazioni e caratteristiche della carcassa. I dati di ciascun maiale sono stati classificati secondo tre classi di velocità d'ingestione. I maiali che mangiavano più rapidamente avevano maggiori pesi, accrescimenti proteici e lipidici, pesi delle carcasse, dei tagli magri e dei tagli grassi, con una più elevata proporzione di tagli grassi, rispetto ai maiali più lenti. Manipolare il comportamento alimentare attraverso strategie manageriali e alimentari può avere effetti favorevoli sulle prestazioni produttive e la qualità della carcassa, senza alterare l'efficienza alimentare dei maiali.

Nel IV capitolo della tesi si è valutato l'effetto di una riduzione del contenuto di proteina e amminoacidi essenziali della dieta sulle caratteristiche di qualità di 40 prosciutti crudi lavorati come prosciutti San Daniele DOP e ottenuti da due linee genetiche con un diverso potenziale per la crescita magra alimentate con diete convenzionali o a basso tenore proteico. Le cosce rifilate dei Danbred erano più pesanti, ma mostravano maggiori perdite di stagionatura e minori spessori del grasso di copertura rispetto a quelle ottenute degli Anas. Inoltre, i prosciutti dei Danbred hanno avuto un maggior contenuto di proteina. Invece, la riduzione del contenuto proteico e amminoacidico della dieta ha influenzato poco le caratteristiche di qualità dei prosciutti crudi. Dunque, dati gli effetti favorevoli alla riduzione delle escrezioni di azoto e dei costi di alimentazione, l'uso di diete a basso tenore proteico può essere un'efficace soluzione per l'alimentazione del suino pesante italiano.

1. GENERAL INTRODUCTION

PIG PRODUCTION AND ENVIRONMENTAL IMPACT

In the last decades, the livestock production has received growing attention, being responsible for 14.5% of global greenhouse gas emissions [1, Figure 1]. Within the livestock production, pig industry is considered one of the most developed sectors, with a world production of about 118 million tons of meat in 2016 [2]. This sector is mainly based on intensive systems characterized by a huge increase in production and animal densities, high feed inputs and large volumes of manure produced. For these characteristics, current intensive pig production is often associated with environmental burdens [3], contributing to 9% of the greenhouse gas emissions produced by the livestock sector, with an estimated global emission around 668 million tonnes CO_2-eq [1].

In recent years, numerous studies have been developed in order to understand and quantify the impact of pig farms in the different Countries and environments [3-5]. The new systems for the evaluation of the environmental impact have identified feed production and manure storage and processing as the main sources of pollution from pig industry. In particular, feed production contributes to 48% of the emissions and is associated with a large use of artificial fertilizers and machinery for the cultivation and the transport of the grain [1,6; Figure 2]. In addition, we have to consider the phenomena of deforestation and the land-use changes caused by soybean expansion for feed production, which cause the 12.7% of the global emissions [1,6].

The intensity of the emissions changes according to the type of production, as a greater environmental impact is commonly observed in that systems where pigs show poorer feed conversion ratio [1].

Feed conversion ratio is the quantity of feed needed to promote 1 kg of body gain and represents the ability of the pig to convert the energy provided with the feed in energy for the growth. A high conversion ratio is associated with a scarce feed efficiency of the pigs, which results in increased feed costs, nutrient excretions and environmental impact of pig farms [7-9]. For the pig species, the optimal feed conversion ratio values ranged between 2.6 and 3.8, according to the

different production systems [10]. The lowest values are associated with the production of light pigs slaughtered at 90-110 kg of body weight (BW) and intended for fresh meat production. On the contrary, at higher slaughter weights the feed conversion ratio tends to increase, as the daily gain decreases with age and body weight. These aspects are very interesting for the Italian pig industry, which relies mostly on heavy pig intended for the production of Protected Denomination of Origin (PDO) dry cured hams and other typical products [11-12]. For this type of production, the PDO specifications require that the pigs are slaughtered at 165 kg BW and 9 months of age in order to obtain the meat with optimal characteristics for transformation [4,13]. Therefore, according to Bava et al. [4], the production of Italian heavy pigs would be responsible for a considerably higher environmental impact per pig produced, compared to that of standard pigs slaughtered at lower weights, but not necessarily per kg of meat produced [14].

For all these reasons, a renovation of pig industry is required to meet the social demand of eco-friendly productions able to respect the animal welfare and to guarantee the quality of pig products. Thus, in recent years, new feeding strategies have been developed with the aim to reduce nutrient excretions by improving growth performance, feed efficiency and economic sustainability of pig farms.

Figure 1. Global estimates of emissions by species [1].

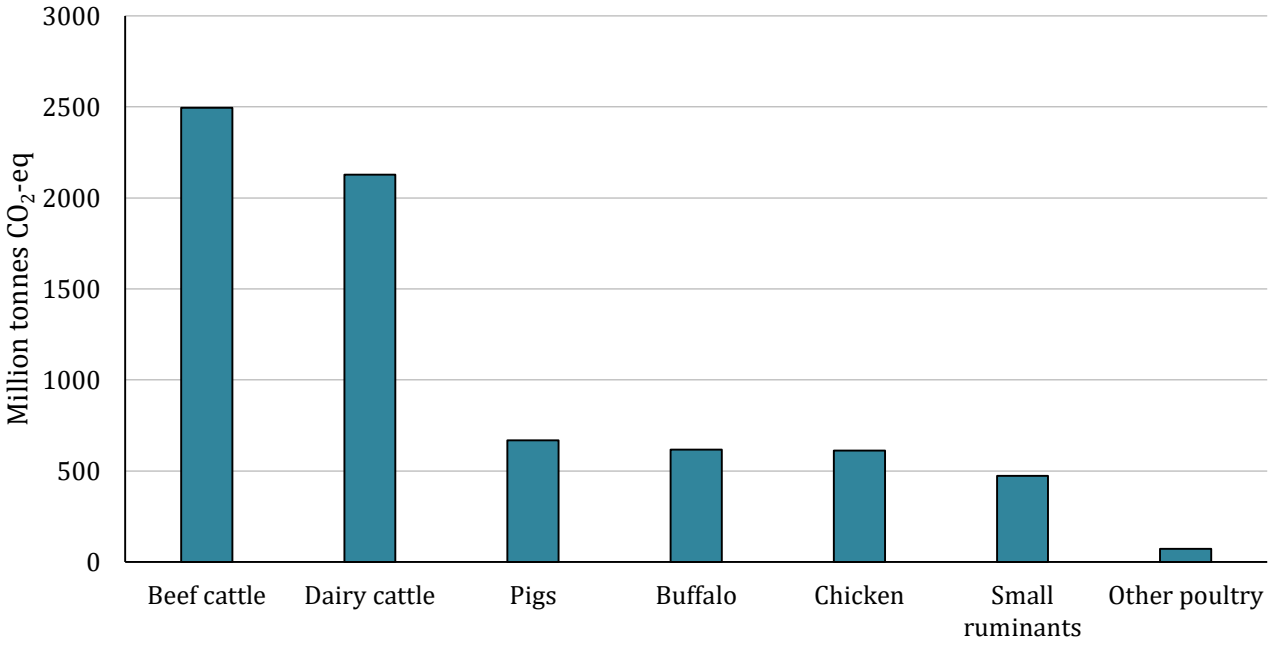
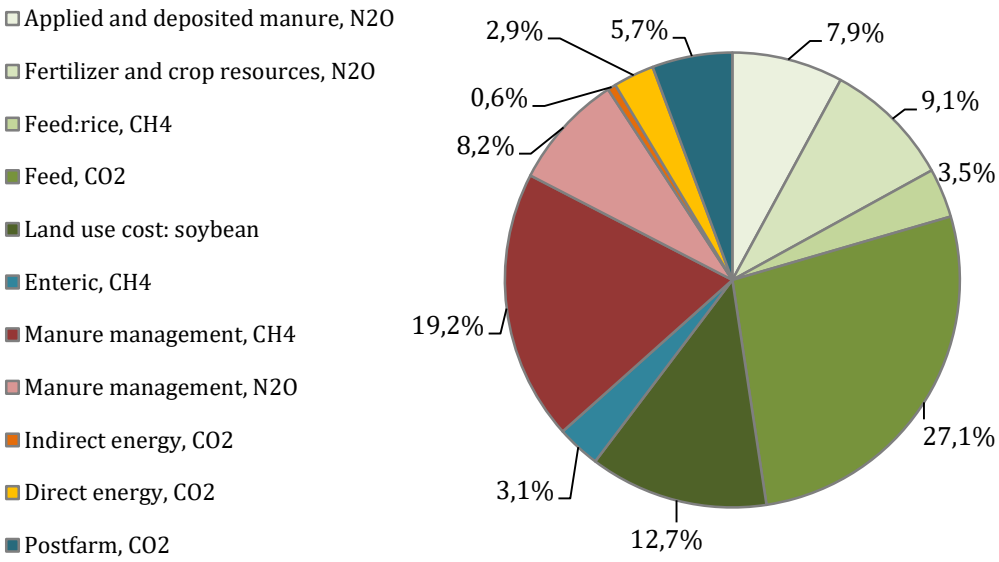


Figure 2. Global emissions from pig supply chain, by category of emission [1].



REDUCTION IN THE DIETARY CRUDE PROTEIN AND INDISPENSABLE AMINO ACIDS CONTENT

The modern pig industry is still based on the large use of high dietary protein sources to promote the animal growth. However, high levels of dietary protein correspond to a great excretion of nitrogen compounds, with a significant increase in the environmental impact of the piggeries.

Among the grains used in the feed industry, soybean represents the main protein source in the current concentrate feed formulations but its production is one of the major factors with high environmental impact. Hence, several authors have suggested to replace soybean with other protein sources [15]. However, this solution might present problems related to the alternative protein sources supply. Furthermore, some pig PDO products are often regulated by specifications that identify very restrictive limits to protein feed admitted in the diet of finishing pigs [4].

In recent years, the reduction of protein levels in the diet has been evaluated as one of the most promising strategy to reduce the environmental impact of pig farms. Numerous studies have shown a clear reduction in excretion and nitrogen emissions due to the use of low-protein diets [15-17]. Portejoie et al. [16] found a 67% reduction of ammonia emissions from slurry when dietary crude protein levels decreased from 20% to 12%. Moreover, in the recent study of Osada et al. [17], a 29% reduction in nitrogen excretion from the pigs fed low-protein diet determined the 39% reduction of GHG emissions.

Low protein diets can be obtained by reducing the protein content and supplying the synthetic indispensable amino acids, namely amino acids obtained by fermentation or chemical synthesis. The incorporation of these amino acids associated with a decrease in crude protein content of diets contributes to reduce the impacts of pig production on climate change, acidification and eutrophication [15]. However, the use of synthetic AAs might result in increase in production costs.

A second choice consists in reducing the dietary protein content and the level of indispensable amino acids. Several studies have already demonstrated the effects of this strategy on the physiology and feed intake of pigs. Kyriazakis and Whittemore [18] observed that, under not limiting conditions, an animal consumes the quantity of feed that satisfies its requirement for energy and nutrients. So,

according to the "*eating to meet requirement, subject to constraint*" theory, the pigs fed diets reduced in crude protein and indispensable amino acids content should increase their feed intake to cope with the amino acid deficiency, without significant changes in feed efficiency [19-20]. However, several studies have demonstrated that a reduction in dietary protein and indispensable amino acids content is not always associated with an increase in daily feed intake [21-22].

These disagreements may be reconciled by assuming that pigs fed low-protein and AA diets are able to respond to the indispensable amino acids deficiency by increasing their consumption, but the success of these attempts depends on several genetic, dietary and environmental factors. Genetics is one of the main variation factors of pig's feed intake, as some genetic types show greater feed intake than others. For example, Schiavon et al. [13] observed higher daily intake in traditional genetic types selected for dry-cured ham, in comparison with genetic lines with greater potential for lean growth. Instead, scarce is the knowledge on the effects of diets lowered in protein and AA content on genetic lines intended for the medium-heavy pig chain, a production system that has been recently diffused in Italy and based on pigs slaughtered at 140-145 kg, destined to cooked ham production [11].

Considering the carcass quality, an increase in feed intake might result in a greater body fat retention, with consequent changes in the quality of the carcass [23]. Moreover, several studies observed an increase in fat cover thickness and intramuscular fat of fresh hams, due to the use of diets lowered in protein and amino acids causes [13,24-25]. In raw hams destined to the dry-cured hams production, a thicker subcutaneous fat might improve the quality of the final products, by limiting the weight losses at seasoning [26]. However, since previous studies have focused on the influence of low-protein diets on the raw ham traits, knowledge on the effect of reducing the protein and amino acid content on the quality of dry-cured hams is still scarce.

REDUCTION IN THE FEED ALLOWANCE

Pig industry needs to cut down on feeding costs and to improve the feed efficiency of the pigs. In general, a low feed efficiency is associated with excesses of feed consumption and poor growth rates. In this context, a feed restriction might represent and advisable strategy to improve feed efficiency in pig farms. In commercial conditions, feed restriction is carried out by two different methods: by limiting the time of access to the feeder or by limiting the amount of feed offered per day [27]. In the second case, the levels of restriction might depend on the type of production. For medium-heavy pigs slaughtered at 145 kg BW, a mild feed restriction, close to the pigs feed requirements, is recommended to avoid an excessive reduction in the daily gain. On the contrary, in the heavy pig production, a more severe feed restriction is needed to comply with the target body weights and age at slaughter established by the PDO specifications. Feed restriction presents many advantages. Several studies have demonstrated that a slight reduction in the feed availability, despite a reduction in average daily gain, can increase feed efficiency [11,28] and reduce maintenance energy requirements [29]. In group-housing conditions, a restricted feeding regime reduces the variability in pigs feed intake, avoiding excessive feed consumptions from the pigs with the highest voluntary feed intake, and stimulating the pigs with the lowest motivation to eat. In this way, a greater uniformity of carcasses is obtained. Finally, feed restriction improves the carcass quality, by reducing the carcass leanness and increasing the proportion of unsaturated acids [30]. However, a higher incidence of lesions in carcasses of restricted fed pigs can indicate greater competition for feed and aggressive behaviour during the fattening period, which can be considered a negative aspect of the restriction [31].

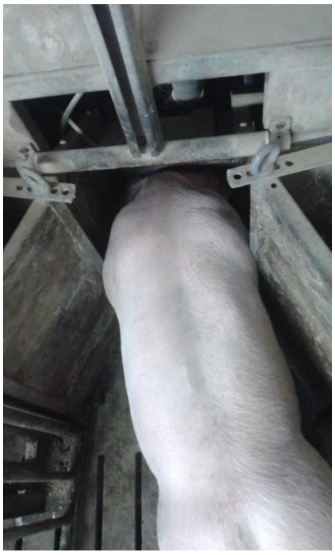
FEEDING STRATEGIES AND FEEDING BEHAVIOUR

Feeding behaviour has been defined as the strategy that a pig adopts to achieve its desired feed intake [21]. It has been extensively studied for more than 50 years, as it is considered a useful tool for understanding the mechanisms that regulate feeding [32]. In the past, there were numerous difficulties associated with measuring feeding behaviour, especially in group-housing conditions [33].

In these cases, to study the pig feeding patterns, each animal had to be identified by manual marking or simple observation [34]. Nowadays, these problems have been overcome with the diffusion of automatic feeding stations able to identify each animal through an antenna or a reader system and a magnetic transponder placed on the ear or the pig. There are various types of automatic stations on the market. In general, the most common are single feeding stations, equipped with a trough placed on a load cell, an automatic gate placed in front of the trough and lateral and rear barriers to limit the competition between the animals [32, Figure 3]. When a pig visits the manger, the station recognizes the ear transponder, opens the gate in front of the trough and releases the feed. Then the date and time of feeding, the time spent eating and the weights of the feed consumed are recorded.

The study of feeding behaviour is of interest as it allows to understand the effects of treatments and conditions on pigs' feed efficiency and motivation to eat [32-35]. Numerous studies have already demonstrated that feeding behaviour can be changed by managerial strategies and feeding treatments [36-38]. Indeed, a pig is able to modify its feeding behaviour when kept under limiting conditions. A reduction in nutrient content, for example, can modify feed intake and feeding patterns [39]. However, the pig's response to this reduction is controversial, as some authors found that the amino acids restriction reduced feed intake and feeding rate [39], whereas others did not observed variation in feeding patterns [40]. On the contrary, a reduction in the feed availability can cause permanent modifications in the feeding behaviour, for example by stimulating the eating rate [41]. This parameter has been identified as an indicator of the pigs' motivation to eat. Therefore, a pig with a greater motivation is expected to have greater feed intake and, as a consequence, better growth performances and carcass traits. Indeed, several studies have demonstrated the strong and positive relationship among feeding rate, growth rate [42] and carcass quality [43-43]. Therefore, all the strategies able to manipulate the feeding rate and feeding patterns might be a useful tool to improve the performance and the meat quality of growing pigs, without modifying their feed efficiency.

Figure 3. Example of single-space automatic feeding station. (Compident Pig – MLP; Schauer Agrotonic GmbH. Prambachkirchen. Austria)



REFERENCES

1. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO). Rome; 2013, pp. 35-37.
2. FAO, 2018. Available from: http://www.fao.org/faostat/en/#data/QL
3. Basset-Mens C, van der Werf HMG. Scenario-based environmental assessment of farming systems: the case of pig production in France. Agricul., Eco. and Env. 2005, 105: 127-144.
4. Bava L, Zucali M, Sandrucci A, Tamburini A. Environmental impact of typical heavy pig production in Italy. J. Clean. Prod. 2017, 140: 685-691.
5. Noya I, Villanueva-Rey P, González-García S, Fernandez MD, Rodriguez MR, Moreira MT. Life Cycle Assessment of pig production: A case of study in Galicia. J. Clean. Prod. 2017, 142: 4327-4338.
6. Cherubini E, Zanghelini GM, Freitas Alvarenga RA, Franco D, Soares SR. Life Cycle Assessment of swine production in Brazil: a comparison of four manure management systems. J. Clean. Prod. 2015, 87: 68-77.
7. Kingston C, Fry JM, Aumonier S. Life Cycle Assessment of Pork. Final Report. Environmental Resources Management, Agriculture and Horticulture Development Board Meat Services e AHDBMS. Available from: http://www.bpex.org.uk/prices-facts-figures/documents/LifeCycelAssmntofPorklaunchversion.pdf.
8. Schenck R. Life Cycle Assessment of USA Pork Production in 2004. Institute for Environmental Research and Education e IERE; 2016.
9. Williams AG, Audsley E, Sandars DL. Determining the environmental burdens and resource use in the production of agricultural and horticultural commodities. Cranfield Unverstity and Defra, Bedford; 2006 [online] Available from: www.silsoe.cranfield.ac.uk.

10. Santonja GG, Georgitzikis K, Scalet BM, Montobbio P, Roudier S, Delgado Sancho L. Best Available Techniques (BAT) Reference Document for the Intensive Rearing of Poultry or Pigs 2017; EUR 28674 EN

11. Dalla Bona M, Schiavon S, Carraro L, Gallo L. Growth performance, carcass traits and meat quality of growing pigs on different feeding regimes slaughtered at 145 kg –BW. Ital. J. Anim. Sci. 2016, 153: 419-427.

12. Gallo L, Dalla Bona M, Carraro L, Cecchinato A, Carnier P, Schiavon S. Effect of progressive reduction in crude protein and lysine of heavy pig diets on some technological properties of green hams destined for PDO dry-cured ham production. Meat Sci. 2016; 121: 135–140.

13. Schiavon S, Carraro L, Dalla Bona M, Cesaro G, Carnier P, Tagliapietra F, Sturaro E, Galassi G, Malagutti L, Trevisi E, Crovetto GM, Cecchinato A, Gallo L. 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.

14. Xiccato G, Schiavon S, Gallo L, Bailoni L, Bittante G. Nitrogen excretion in dairy cow, beef and veal cattle, pig, and rabbit farms in Northern Italy. Ital. J. Anim. Sci. 2005, 4: 103-111.

15. Garcia-Launay F, van der Werf HMG, Nguyen TTH, Le Tutour L, Dourmad JY. Evaluation of the environmental implications of the incorporation of feed-use amino acids in pig production using Life Cycle Assessment. Livest. Sci. 2014, 161:158-175.

16. Portejoie S, Dourmad JY, Martinez J, Lebreton Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. Livest. Prod. Sci. 2004, 91: 45–55.

17. Osada T, Takada R, Shinzato I. Potential reduction of greenhouse gas emission from swine manure by using a low-protein diet supplemented with synthetic amino acids. Anim. Feed. Sci. Technol. 2011, 166-67: 562-574.

18. Kyriazakis I, Whittemore CT. Whittemore's science and practice of pig production. Blackwell Publishing, Oxford, UK; 2006.

19. Emmans GC. Genetic components of potential and actual growth. In: British Society of Animal Production (Eds), Animal Breeding Opportunities. Occasional Publication no. 12; 1999, pp. 153-181.

20. Emmans G, Kyriazakis I. Consequences of genetic change in farm animals on food intake and feeding behaviour. Proc. Nutr. Soc. 2001, 60: 115–125.

21. Black JL. Models to predict feed intake. In: Tollardona D, Roura E, editors. Voluntary feed intake in pigs. Wageningen: Wageningen Academic Publishes; 1999, pp. 323-344.

22. Nyachoti CM, Zijlstra RT, de Lange CFM, Patience JF. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 2004, 84: 549–566.

23. Mordenti A, Bosi P, Corino C, Crovetto GM, Casa GD, Franci O, Piva A, Prandini A, Russo V, Schiavon S, Aspa commission' activity. A methodological approach to assess nutrient requirements of heavy pigs in Italy. Ital. J. Anim. Sci. 2003, 2: 73-87.

24. Suárez-Belloch J, Guada JA, Latorre MA. Effects of sex and dietary lysine on performances and serum and meat traits in finisher pigs. Anim. 2015, 9: 1731-1739.

25. Wood JD, Lambe NR, Walling GA, Whitney H, Jagger S, Fullarton PJ, 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.

26. Bosi P, Russo V. The production of the heavy pig for high quality processed products. Ital. J. Anim. Sci. 2004, 3: 309-321.

27. Ball R, Aherne FX. Effect of diet complexity and feed restriction on the incidence and severity of diarrhea in early-weaned pigs. Can. J. Anim. Sci. 1982, 62: 907-913.

28. Kim JS, Ingale SL, Lee SH, Choi YH, Kim EH, Lee DC, Kim YH, Chae BJ. Impact of dietary fat sources and feeding level on adipose fatty acids composition and lipid metabolism related gene expression in finisher pigs. Anim. Feed Sci. and Technol. 2014, 196: 60-67.

29. Garcia-Valverde R, Barea R, Lara L, Nieto R, Aguilera JF. The effects of feeding level upon protein and fat deposition in Iberian heavy pigs. Livest. Sci. 2008, 114: 263-273.

30. Candek-Potokar M, Skrlep M. Factors in pig production that impact the quality of dry-cured ham: a review. Animal 2012, 6: 327-338.

31. Batorek N, Skrlep M, Prunier I, Louveau J, Noblet M, Bonneau M, Candek-Potokar M. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. J. Anim. Sci. 2012, 90: 4593-4603.

32. Maselyne J, Saeys W, Van Nuffel A. Review: Quantifying animal feeding behaviour with a focus on pigs. Physiol. Behav. 2015, 138: 37–51.

33. Hyun Y, Ellis M, McKeith FK, Wilson ER. Feed intake pattern of group-housed growing-finishing pigs monitored using a computerized feed intake recording system. J. Anim. Sci. 1997, 75: 1443-1451.

34. Boumans IJMM, Bokkers EAM., Hofstede GJ, de Boer IJM. Understanding feeding patterns in growing pigs by modelling growth and motivation. Appl. Anim. Behav. Sci. 2015, 171: 69–80.

35. De Haer LCM., De Vries AG. Feed intake patterns of and feed digestibility in growing pigs housed individually or in groups. Livest. Prod. Sci. 1993, 33: 277-292.

36. Bornett HLI, Morgan CA, Lawrence AB, Mann J. The flexibility of feeding patterns in individually housed pigs. Anim. Sci. 2000, 40: 457-469.

37. Bornett HLI, Morgan CA, Lawrence AB, Mann J. The effect of group housing on feeding patterns and social behaviour of previously individually housed growing pigs. Appl. Anim. Behav. Sci. 2000, 70: 127–141.

38. Nielsen BL, Lawrence AB, Whittemore CT. Effect of group size on feeding behaviour, social behaviour, and performance of growing pigs using single-space feeders. Livest. Prod. Sci. 1995, 44: 73–85.

39. Montgomery GW, Flux DS, Carr JR. Feeding patterns in pigs: the effect of amino acids deficiency. Physiol. & Behav. 1978, 20:693-698.

40. Andretta I, Pomar C, Kipper M, Hauschild L, Rivest J. Feeding behaviour of growing – finishing pigs reared under precision feeding strategies 1. J. Anim. Sci. 2016, 94: 3042–3050.

41. Nielsen BL. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. Appl. Anim. Behav. Sci. 1999, 63: 79–91.

42. Labroue F, Guéblez R, Sellier P. Genetic parameters of feeding behaviour and performance traits in group-housed Large White and French Landrace growing pigs. Genet. Sel. Evol. 1997, 29: 451–468.

43. De Haer LCM, Luiting P, Aarts HLM. Relationship between individual (residual) feed intake and feed intake pattern in group housed growing pigs. Livest. Prod. Sci. 1993, 36: 233-253.

44. Rauw WM, Soler J, Tibau J, Reixach J, Raya LG. Feeding time and feeding rate and its relationship with feed intake, feed efficiency, growth rate, and rate of fat deposition in growing Duroc barrows. Am. Soc. Anim. Sci. 2006, 84: 3404–3409.

2. AIMS OF THE THESIS

The present thesis aimed to evaluate the effects of new feeding strategies on feeding behaviour, growth performance, N excretion, carcass traits and quality of meat and meat products of growing pigs belonging to different genetic types.

The specific aims for each contribution were:

i. To assess the influence of a reduction in dietary indispensable AA on feed intake, growth performance, N excretion and carcass and meat quality traits of fast growing pigs fed by automatic feeding stations according to an ad libitum or a restricted feeding regime;

ii. To study the feeding behaviour of group-housed pigs fed individually from single-space feeders and subjected to feed restriction alone or in combination with a reduction in the dietary indispensable AA content.;

iii. To explore the influence of feeding behaviour on growth performance, carcass and meat characteristics of pigs, using data collected from a previous experiment.;

iv. To investigate the influence of reduced dietary protein and AA contents on the characteristics of San Daniele like dry-cured hams obtained from two genetic groups of pigs with different lean growth potential.

3. CHAPTER 1st

Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs

Stefano Schiavon[1], Mirco Dalla Bona[1], Giuseppe Carcò[1*], Luca Carraro[1], Lutz Bunger[2], Luigi Gallo[1]

[1]*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Italy*
[2]*Scotland's Rural College (SRUC), Easter Bush, Edinburgh, Midlothian EH25 9RG, UK*

**Corresponding author*

E-mail: giuseppe.carco@phd.unipd.it

Published online in Plos ONE
DOI: 10.1371/journal.pone.019564

ABSTRACT

The hypothesis that pigs placed on diets with reduced indispensable amino acid (AA) content attempts to offset the reduction in the nutrient density with increased feed intake was tested. In the experiment, feeds with a high or a low AA content were administrated to pigs fed *ad-libitum* or restrictively according to a 2 × 2 factorial design. Ninety-six barrows were housed in 8 pens (12 pigs/pen) equipped with automatic feeders. Within pen, and from 47 body weight (BW) onwards, 6 pigs were fed *ad libitum*. The others pigs were allowed to consume, as a maximum, the feed amounts indicated by the breeding company feeding plane to optimize the feed efficiency. In early (86-118 kg BW) and late (118-145 kg BW) finishing, the pigs of 4 pens received feeds with high indispensable AA contents (8.1 and 7.5 g lysine/kg in the two periods, respectively). The other pigs received feeds with reduced indispensable AA contents (lysine, methionine, threonine and tryptophan) by 9 and 18% in early and late finishing, respectively. Body lipid and protein (Pr) retentions were estimated from BW and back-fat depth measures recorded at the beginning and the end of each period. Nitrogen excretion was estimated as actual intake minus estimated N-retention (Pr/6.25). Pigs were slaughtered at 144 kg BW. Restricted feeding decreased feed intake (-7%), daily gain (-5%), carcass weight (-2.6%) and back-fat depth (-8.0%) but increased gain:feed ratio (+2%). The AA restriction increased feed intake (+5.9%), carcass weight (+4.9%) and intramuscular fat (+17.6%), and reduced carcass weight variation (-36%), with no effects on the feed efficiency and the estimated Pr (142 g/d). N excreted was reduced by feed (-9%) and dietary AA (-15%) restrictions. Irrespectively of the feeding level, the pigs responded to a reduction of the dietary essential AA content by increasing their feed intake.

INTRODUCTION

Feed intake of an animal is influenced by its genotype (incl. sex, breed), health status, diet characteristics, various environmental factors and possible interactions. Several authors suggested that, under not limiting conditions, an animal will attempt to consume the amount of feed that will

satisfy its requirement for energy and nutrients, according to the principle of the first limiting resource [1]. This proposition implies that a dilution of the dietary content of energy or nutrients may encourage the animal to increase its feed intake. The achievement of a feed intake reflecting the requirement for the deficient nutrients will depend on the ability of the pig to cope with various productive circumstances [2]. Therefore, mathematical models to predict feed and water intake and growth from information about the pig genotype, the feed characteristics, the environment conditions and the initial body status have been developed on the basis of these assumptions [3-5].

In farm animals, the interest in using low protein diets to reduce N excretion and emission of volatile N compounds is increasing [6-8]. In the pig industry, the introduction of low impact strategies need to be evaluated considering the feeding costs, the qualitative and quantitative aspects of the production, and the environmental benefits. It can be hypothesized that a pig kept on a diet lowered in essential amino acids (AA) content might attempt to increase its feed intake to achieve its genetic potential for lean growth, or protein retention [2]. But this was not always the case [9, 10]. The increased feed intake might cause an extra amount of energy eaten, so that the pig might become fat, with consequent changes in carcass weight and value according to the payment schemes of the reference meat market [11].

Under *ad libitum* (AL) conditions, all the pigs in a group would increase their feed intake in response to a dietary nutrient dilution, according to their age and productive stage. In some fattening pig circumstances, a certain degree of feed control, or restriction, is practiced to increase the feed efficiency, and to improve carcass quality by reducing the fat content [1, 11, 12]. Under restricted feeding conditions, a dilution of the dietary nutrient density would stimulate the pigs with the lowest voluntary feed intake to increase the consumption, where the pigs with the highest desire for feed are forced to maintain the feed intake to the limit imposed by the feeder equipment. Automated feeding stations can be used for individual control of feed distribution, allowing for both *ad libitum* and restricted feeding of pigs reared in the same pen, and to evaluate possible feeding level × dietary AA interaction.

The aim of this experiment was to assess the influence of a reduction in dietary indispensable AA on feed intake, growth performance, and carcass and meat quality traits of fast growing pigs fed by automatic feeding stations according to an *ad libitum* or a restricted feeding regime.

MATERIAL AND METHODS

All the experimental procedures involving animals were approved by the "Ethical Committee for the Care and Use of Experimental Animals" of the University of Padua (CEASA, Legnaro, Italy).

Pigs and experimental design

The study involved 96 Topigs Talent × PIC barrows born within the same week. They arrived at the end of February and were slaughtered at the end of June, thereby avoiding hot ambient summer temperatures. The average temperature in the housing rooms ranged from 20 to 25°C, from the start to the end of the trial. At the start of the experiment, the pigs averaged 47.1 ± 3.3 kg body weight (BW). They were allotted to 8 pens, 12 pigs/pen. In each pen, 6 pigs were fed *ad libitum* (AL) while the others were given restricted amounts of feed throughout the entire growing phase. Each pig of the restricted feeding group was allowed to consume, as a maximum, the daily feed amounts indicated by the feeding plane for Topigs Talent barrows suggested by the breeding company [13], with minor modifications (Table 1). These amounts were chosen by the breeding company to optimize feed efficiency. This plane was formulated to prevent excessive feed consumption by the pigs with the highest appetite, which might be less efficient because of their propensity for fattening.

From 87 kg BW onwards, the pigs in 4 pens received feeds with high indispensable AA contents (HAA) [14], while the others received feeds low in indispensable AA (LAA).

Individual BW was measured weekly using electronic scales and, from 87 kg BW onwards, back-fat depth (BF) was measured every two weeks with an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco Corporation, Minneapolis, USA). The BF measure was taken above the last rib at approximately 5.5-8.0 cm from the midline, the distance increasing with increasing BW [15]. During

the experiment, 4 pigs died or were discarded because of injuries (1 pig in the AL-LAA, 2 pigs in the RF-HAA, and 1 in the RF-LAA group). The corresponding data were removed so that the final dataset was drawn from 92 pigs.

Feed distribution and control

Eight automated feeding stations (Compident Pig – MLP, Schauer Agrotronic, Austria), one per pen, were used to provide pigs with the designated amount of feed per day and to measure individual feed consumption. The pigs were allowed to visit the stations throughout the whole day. The stations were equipped with lateral barriers for limiting feed competition among pigs. When a pig visited the feeding station it was identified by an ear transponder, the automatic gate placed in front of the trough was opened and dry feed in form of pellets was released. The date and time of the feeding event, the time spent eating and the weights of feed eaten and leftovers were then recorded. The RF pigs had access to the station only if during their previous visit they had consumed less than the planned feed amount for that day [16]. The electronic feeder assigned the weight of eventual leftovers to the feed consumption of the pig of the following visit. As the feed was distributed by the station in doses of 200 g, some RF pigs may have been able to consume more than their planned feed amount (leftovers from the previous visit). Water was freely available from a nipple drinker placed in each pen, outside the feeding station.

Feed formulation, manufacturing and chemical analysis

The commercial feeds used during acclimation (36-47 kg BW) and the growing (47-86 kg BW) periods provided, respectively, 10.1 and 9.9 MJ/kg of net energy (NE), 164 and 161 g/kg crude protein (CP), and 11.2 and 9.0 g/kg standardized ileal digestible lysine (SID Lys). The dietary indispensable AA content was not reduced during the growing phase.

The pigs in four pens received HAA feeds formulated to contain 9.8 MJ/kg of NE, 158 g/kg of CP and 8.1 g/kg of SID Lys in early finishing (87-118 kg BW) and 9.8 MJ/kg of NE, 155 g/kg of CP and

7.5 g/kg of SID Lys in late finishing (119-145 kg BW). The HAA diets provided the amount of the 4 main indispensable AA recommended for the genetic line, in slight excess than those recommended by the NRC [14]. The LAA diets administered from 87 kg onward were formulated from the corresponding HAA feeds by replacing soybean meal with corn and wheat grain (Table 2) to contain almost the same amount of NE (9.8 MJ/kg of NE), and by adding small amounts of crystalline AA to equalize the contents of four indispensable AAs (lysine, methionine, threonine, tryptophan) per CP unit of the feeds (Table 3). Thus, the proportions of the various indispensable amino acids, for HAA and the corresponding LAA feeds, were almost identical when expressed per unit of CP. The resulting LAA feeds contained 143 g/kg of CP and 7.3 g/kg of SID Lys in early finishing, and 126 g/kg CP and 6.0 g/kg of SID Lys in late finishing. Based on the average feed allowance per pig given in table 1, in early (2.78 kg/d) and late finishing (2.80 kg/d), these concentrations provided 20.3 and 16.8 g/d of SID in the two periods, respectively.

The feeds were produced from the same batches of ingredients. Based on actual prices in the market of reference, the cost of the LAA feeds was 8 and 15 euros/1000 kg lower than the corresponding HAA feeds in early and late finishing, respectively. Ten samples of each feed were collected on-line during feed manufacturing. The samples were pooled and mixed to obtain a 1-kg feed sample and independent sub-samples were taken. The sub-samples were analyzed (3 replicates) for dry matter (DM: # 934.01), N (# 976.05), ether extract (EE: # 920.29), ash (# 942.05) [17] and neutral detergent fiber inclusive of the contents of residual ash with amylase treatment (aNDF) [18]. Starch was determined after hydrolysis to glucose by liquid chromatography [19].

The amino acid content of the feed samples (0.5 g/sample) was determined according to the Council of Europe (chapter ♯ 2.2.56) [20]. Amino acids were released from the protein molecules by acid hydrolysis with HCl 6 M (Method 1) [20] at 110°C for 24 h. For cysteine/cystine and methionine, oxidation with performic acid was carried out before protein hydrolysis (Method 4) [20]. Tryptophan was determined by protein hydrolysis with $Ba(OH)_2$ at 110° C for 20 h [21]. Pre-column derivatization was carried out using *o*-phthalaldeyde (OPA) for primary amino acids and 9-

fluorenylmethyl chloroformate (FMOC-Cl) for secondary amino acids (Methods 5 and 7) [20]. The amino acids were separated and quantified using an HPLC (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA). Separation was obtained on a Zorbax Eclipse-AAA (4.6 × 150 mm, 3.5 µm) operating at 40 °C and a flow rate of 2 mL/min. The mobile phase consisted of 40 mM NaH_2PO_4 pH 7.8 (A) and acetonitrile:methanol:water (45:45:10, v/v) with gradient elution. A diode-array detector (DAD) and a fluorescence detector were used to detect amino acids with the following parameters: UV: 338 nm for OPA amino acids and 262 nm for the 9-fluorenylmethyloxycarbonyl (FMOC) amino acids; FLD: excitation wavelength/emission wavelength 266/305 nm. Amino acids were quantified following calibration using four standards ranging from 10 pmol/µL to 1 nmol/µL.

Dietary ME, crude protein, SID amino acids and other nutrients were computed from the actual ingredient composition of feeds and the tabular values for each ingredient [14]. Differences between analyzed and theoretical amino acid contents of the feeds were negligible.

Body composition, energy and lysine utilization, and N balance estimates

Body composition, energy and lysine utilization, and N balance were estimated as described by Gallo et al. [16]. Briefly, empty BW (EBW) was estimated from BW at 86 and at 145 kg using the equation provided by Kloareg et al. [15] for barrows and gilts in the 85-150 kg BW range. Body lipid mass (BL, kg) was estimated from BF and BW [11]. Fat-free EBW mass (FFEBW) was computed as EBW minus BL. Body protein mass (BP, kg) was computed as $0.1353 \times FFEBW^{1.1175}$, according to NRC [14].

Metabolizable energy intake (ME intake) was computed from the measured feed intake and dietary ME content. Metabolizable energy for growth (ME_g) was computed assuming 44.35 MJ ME/kg for Pr and 52.30 MJ ME/kg for Lr [14]. The amount of ME used for maintenance (ME_m) was computed as ME intake - ME_g. The resulting value, expressed per unit of mean $BW^{0.60}$, was compared with the maintenance requirement recommended by [14]. SID Lys intake was computed from feed intake and the dietary SID Lys content, while the SID Lys requirement was computed from feed intake, BW,

maximum Pr and current Pr [14; eq. 8-42 and 8-43]. The results of the current experiment suggest a maximum of 167 g/d Pr for barrows.

Daily N excretion was determined as N intake - N retention, where N intake was calculated from feed intake and feed N content, and N retention was estimated from body Pr (Pr/6.25).

Slaughter and carcass data collection

All pigs were slaughtered on the same day in one batch. The day before slaughter, the pigs were weighed, BF was measured and feed distribution stopped. After 14 h of fasting, the pigs were weighed again and then moved to the slaughterhouse, where they were slaughtered after a further 10 h of fasting and 2 h of resting at the slaughter-house. Pigs were stunned by a high concentration of carbon dioxide, and killed by cutting the jugular vein and exsanguination, according to the slaughter house standard procedures. Carcasses were scalded, de-haired, eviscerated and split down the midline according to commercial slaughtering procedures. Hot carcass weight was individually recorded and dressing percentage was computed. Carcass lean percentage [22-23] was computed from back-fat thickness and loin depth, which were measured on the left half of each carcass between the 3rd and 4th ribs 8 cm off the midline using a FOM (Fat-O-Meat'er, Carometec, Soeborg, Denmark).

Hot carcasses were processed according to a standard commercial procedure to obtain the main lean cuts (loin with ribs, neck with bones but without skin and subcutaneous tissues, shoulder with bones and skin, and ham) and fat primal cuts (back-fat with skin and belly), which were weighed separately. A sample of *longissimus lumborum* (LL) including the last two lumbar vertebrae was collected from the left loin of each of the 92 carcasses, placed in individual plastic bags, refrigerated for 24 h, then vacuum-packed at -20 °C pending subsequent analyses. Thighs were deboned after 24 h of chilling and the deboned hams were weighed.

Meat quality assessment

All the samples of LL were collected and thawed in vacuum-packaged bags for 24 h at 4 °C, then removed from the packaging, blotted and weighed. Thawing losses were calculated to be the difference in weight between the fresh and thawed samples as a percentage of initial fresh weight. Cooking losses were determined on a 2.5 cm thick subsample of LL, which was weighed and sealed in a plastic bag, cooked in a water bath at 75° C for 50 min to reach a core temperature of 70° C, then cooled to room temperature, blotted and weighed. Cooking loss was calculated to be the difference between the pre- and post-cooked weights as a percentage of the pre-cooked weight. Shear force was measured on five cylindrical cores 1.00 cm in diameter from the same cooked sample sheared perpendicularly with a Lloyd® (Bognor Regis, UK) LS 5 series Warner-Bratzler shearing device (shearing speed 2 mm s^{-1}) using the NEXIGEN Plus 3 software. The data from each sample were averaged before statistical analyses. Another subsample of LL was ground, mixed and homogenized for 10 *s* at 4500 *g* (Grindomix GM200; Retsch, Haan, Düsseldorf, Germany) and analyzed for moisture (# 950.46), protein (# 981.10), lipids (#991.36) and ash (# 920.153) [17].

Statistical analysis

The SAS MIXED procedure (SAS Inst. Inc., Cary, NC) was used to analyze the data according to the following linear model:

$$y_{ijkl} = \mu + FL_i + AA_j + FL \times AA_{ij} + pen(AA)_{k:i} + e_{ijkl};$$

where y_{ijkl} is the observed trait; μ is the overall intercept of the model; FL_i is the fixed effect of the i_{th} feeding level (i = 1, 2); AA_j is the fixed effect of the j_{th} kind of feed with different amino acid contents (j = 1, 2); $pen_{k:j}$ is the random effect of the $k{:}j_{th}$ pen within AA (k = 1, …, 8); $FL \times AA_{ij}$ is the effect of the interaction between feeding level and kind of feed; and e_{ijkl} is the random residual. Pen within AA and the residuals were independently and normally distributed with a mean of zero and variances of σ_k^2 and σ_e^2, respectively. In line with the experimental design, the effect of AA was tested using pen within AA as the error line, where the effect of FL was tested on the residual (animal) as error

line, given that the pigs of both FL treatments were housed in the same pen. It is of note, that statistical analysis performed on estimated variables has to be considered with caution.

RESULTS

The feeding level × dietary AA density interaction was significant only exceptionally, thus the least square means for the main effects have been reported in the tables.

Growth performance and feed efficiency

Feeding level affected performance in the growing and finishing periods (Table 4). Compared with pigs fed AL, RF pigs consumed less feed in the growing ($P < 0.001$), finishing periods ($P = 0.002$) and overall ($P < 0.001$), and grew 7% less in the finishing period ($P = 0.033$) and 5% overall ($P = 0.014$), but were more efficient in the growing period ($P = 0.010$) and overall ($P = 0.050$). They were also 3% lighter at the end of the study ($P = 0.018$) and exhibited less weight loss after 14 h of pre-slaughter fasting ($P = 0.014$).

Dietary AA content affected performance in the finishing period only, given that pigs were fed the same feed in the growing phase. Regardless of the feeding level, in the finishing period pigs on the LAA diets ate 7% more feed ($P = 0.031$) and grew 9% faster ($P = 0.038$) than pigs on the HAA diets. Greater feed intake and better growth rate of pigs fed LAA diets during finishing period resulted also in greater feed intake and growth rate in the whole trial ($P < 0.05$).

The difference between actual feed intake and planned feed allowance of pigs fed AL averaged -15 g/d with HAA and 175 g/d with LAA, whereas the differences were -166 g/d (HAA) and -56 g/d (LAA) for RF-fed pigs. Thus, regardless of whether pigs were fed AL or RF, feed intake was higher with LAA than with HAA contents, the growth rate of pigs on LAA diets was greater than that of pigs on HAA diets, and this resulted in a heavier BW at the end of the trial (+4%, $P = 0.018$).

Gain:feed ratio during the finishing period was the only trait for which feeding level interacted with dietary AA content (P = 0.050; Figure 1), as the feed efficiency of pigs on the LAA diet was better than that of pigs on the HAA diet only when fed RF, but not when fed AL.

Estimated body composition, energy and SID lysine utilization

Estimated lipid (Lr) and protein (Pr) retentions were in the order of 257 and 142 g/d, respectively, with RF pigs having nearly 10% and 5% lower Lr (P = 0.13) and Pr (P = 0.050) than pigs fed AL, respectively (Table 5). The ME requirement for growth, taken as the sum of the ME requirements for Lr and Pr, was reduced with RF (-9%, P = 0.031) and increased as a consequence of the reduction in dietary AA (+11%, P = 0.043). The trend was similar to that observed for ME intake. The estimated ME required for maintenance was close to 1.00 MJ/kg $BW^{0.60}$ for pigs fed AL, and 0.95 MJ/kg $BW^{0.60}$ for RF pigs, with a significant effect of feeding level (P = 0.016) but not of AA content of the feeds. The estimated SID Lys requirement for maintenance and growth averaged 18.6 g/d and was not influenced by RF or by the reduction in dietary AA content in the early and late finishing periods. Lysine intake was reduced by RF (P = 0.002), and there was a tendency toward a reduction caused by the dietary AA content (P = 0.06). Therefore, the pigs on the LAA feeds were able to offset the large reduction in the dietary Lys content by increasing their feed intake. As a consequence, the Lys consumed surplus to the estimated requirement was close to zero for pigs on LAA diets and in the order of 2.2 to 2.5 g/d for those on the CAA diets, values significantly different from zero (P = 0.006). Feed restriction (P = 0.002) and the low AA dietary content (P = 0.024) reduced the amount of N intake, without affecting N retention. As a result, estimated N excretion was lowered by feeding restriction (-9%, P = 0.003) and by the reduction in dietary AA content (-15%, P = 0.008).

Carcass and meat quality

The carcass weight of pigs fed restrictively tended to be lower than that of pigs fed AL (P = 0.06), and pigs on LAA diets had considerably heavier carcasses than pigs on HAA diets (P = 0.012) (Table 6). The coefficient of variation of carcass weight was lower in the case of pigs fed restrictively on

LAA diets than of those on all the other treatments (4.5% vs. 7.0%, data not shown), probably as a consequence of the smaller daily variation in their feed intake as compared to that of other pigs (coefficients of variation: 17% vs. 26%, data not shown).

Barrows on RF had thinner back-fat than AL fed pigs ($P = 0.037$) and tended to yield carcasses with nearly 2% more lean meat ($P = 0.06$). Accordingly, the main fat cuts of RF fed pigs were nearly 5% lower in weight than those of AL fed pigs ($P = 0.047$). The AA contents of the diets also influenced the weight of some lean and fat cuts, but neither feeding level nor dietary AA contents affected lean and fat cuts when expressed as proportions of carcass weight.

The feeding level exerted no effect on meat quality traits, while chemical composition, water holding capacity and shear force were similar for LL samples from AL-fed and RF barrows. Conversely, the intramuscular fat content of the LL was greater in pigs on diets with lower AA contents than in pigs on HAA diets (+ 18%, $P = 0.037$), with no further effects of dietary AA content on physical meat traits. No differences in the water holding capacity due to the feeding treatments were observed. There was a tendency for greater thawing loss in AL compared to RF ($P = 0.06$), and a tendency for interaction between RF and AL supply in cooking losses ($P = 0.07$).

DISCUSSION

Effect of feeding level on feed intake, carcass quality and N excretion

Pigs raised in commercial conditions are normally penned and fed in groups, while in experimental studies they are frequently penned and fed individually [24]. Penning conditions affect competition for feed and the pigs' social interactions and stress levels. The feeding behavior and growth performance of pigs in pens may therefore differ according to whether they are fed in groups or individually. In the current experiment, the pigs were penned in groups, but they had individual access to the feeding stations. Caution must therefore be exercised in extending the present results to commercial conditions.

Our results provide evidence that the feed intake of pigs fed *ad libitum* on HAA diets was close to that expected from the theoretical feeding curve recommended by the breeding company, confirming the feed restriction applied was small. Nevertheless, the feed intake of barrows on restricted feeding was approximately 5% lower than that of barrows fed AL and showed also less variation (coefficients of variation: 19% vs 29%, respectively). This was because the treatment forced some of the pigs in the former group to consume less than their desired amount of feed over the course of the trial.

Across the world, many growing pigs are fed *ad libitum* through the fattening period up to slaughter, without a ration scale. The use of rationing scales is especially appropriate for pigs of unimproved genotypes or for those slaughtered at heavy weights, and/or where there is a desire for increased feed efficiency and lean carcasses [1]. Few comparisons have been made between pigs fed *ad libitum* or restrictively with pigs penned in groups. In the current experiment, moderate restriction of the feed allowance applied from 47 to 145 kg BW led to a 2% improvement in feed efficiency, but a 3.2% decrease in final BW and 2.6 % decrease in carcass weight. The lower influence of RF on carcass weight than on final BW was, at least partially, due to the 25% lower gut fill of RF than

AL fed pigs, as suggested by the notable differences in BW losses after 14 h of fasting. Feed restriction resulted in an 8% reduction in carcass back-fat thickness, consistent with the 5% reduction in the total amount of untrimmed carcass fat cuts, but it had no influence on the proportions of lean and fat primal cuts in the carcass. These results are in general agreement with previous studies [1, 25-26], which suggested that feed restriction would improve feed efficiency and increase the leanness of the carcass, but it might reduce growth rate compared with *ad libitum* feeding. Dalla Bona et al. [27] also reported that feed restriction reduced the feed intake, growth rate and carcass weight of pigs slaughtered at 145 kg BW, but it improved their feed efficiency compared to AL feeding. Therefore, despite the slight negative effect on growth rate, a mild feed restriction might be advisable in some production systems due to its positive effects on feed efficiency. Moreover, the carcass weights of pigs fed restrictively on LAA diets showed the lowest coefficient of variation, suggesting a greater uniformity of carcasses of pigs of this group, which may be relevant for pig production chains given the economic value of carcass uniformity [28-29]. Overall, the mild feed restriction applied in this experiment also caused a 9.4% reduction in N excretion, which is similar to the results by Schiavon et al. [30] in beef cattle. Such strong reduction of N excretion would have important consequences in terms of number of pigs and meat, which can be finished per unit of agricultural land in those areas where a fixed amount N/ha is stated by law. In relative terms, taking AL as comparison, the feed restriction would approximately increase by 7.0% the number of pigs produced per unit of land.

Effect of amino acid restriction on feed intake

Pigs on the LAA diets ate more feed than those on the HAA diets, regardless of the feeding level, and only a few interactions between the two factors were found. Although the SID lysine contents of the HAA and LAA diets differed by 9% in the BW interval between 87 and 118 kg and by 18%

in the 118 to 145 kg BW interval, lysine intake was similar for pigs on all treatments and met their estimated requirements. As a consequence, and in contrast to the reduction in carcass weight associated with feed restriction, the decrease in dietary AA contents was also associated with a 4.9% increase in carcass weight. The pigs responded to the decrease in the AA contents of the feeds by increasing their feed intake, even when kept on the RF diet. This was possible because many of the RF-LAA pigs were forced to consume more feed, so that their actual average feed intake was very close to the maximum planned feed allowance and was nearly 6% greater than that of the RF-HAA pigs. This would also explain why the feed intake and carcass weights of RF-LAA pigs had the lowest coefficients of variation of all treatments. In addition, feeding behavior data from the current experiment showed that the eating rate of pigs on the LAA diets tended to be higher than that of pigs on HAA (56.1 *vs* 49.3 g/min, respectively; $P = 0.07$), as well as the eating rate of the AL pigs was lower than that of the RF pigs (49.1 *vs* 56.3 g/min, respectively; $P = 0.016$) [31]. Despite the increased feed intake, the total feeding cost of the LAA treatment was still slightly lower compared to HAA (390 euros/1000 pigs), because of the lower costs of the LAA feeds.

The results of the current experiment are not consistent with data from some studies that found that voluntary feed intake decreased when pigs were placed on diets deficient in protein or indispensable AA, specifically tryptophan [6, 32, 33]. For example, Schiavon et al. [6] found that a notable reduction in dietary crude protein and indispensable AA did not alter feed intake, and decreased feed efficiency, but in slow growing pigs kept under a restricted feeding regime for the dry-cured ham production. However, the current experiment is consistent with several other studies that found mild deficiencies in protein, lysine or threonine to increase feed intake [10,34-35]. To this regard, it would be considered that it is commonly accepted that a reduction in dietary NE content leads to an increase in feed intake to maintain a constant net energy intake [36]. The influence of nutrient deficiencies on intake remains controversial, although Kyriazakis et al. [37]

clearly showed that pigs are able to control their protein intake when fed in different ways. These disagreements may be reconciled by assuming that an animal will eat sufficient feed to satisfy its genetically determined requirements for nutrients, specifically energy, although environmental and social factors relating to diet, climate, disease or housing may cause it to either increase or decrease feed intake from its potential, as proposed by several authors [3,10, 38-39]. Ferguson and Gous [2], for example, suggested that pigs on a low-protein diet would increase their feed intake to maintain their genetic potential for protein growth until the point at which the animals can no longer compensate, and feed intake will decline. The extent of compensation would also depend on the amount of heat the pigs need to lose. When the temperature rises to 20 to 24 °C, the need to dissipate heat makes pigs progressively less able to increase their feed intake [3-4, 40]. Therefore, the results of the current experiment are consistent with the idea that pigs respond to a reduction in dietary indispensable AA content by attempting to increase their feed intake. The success of these attempts would depend on the genetic, environmental and dietary constraints operating during the growth period. This issue requires further investigation.

Effect of amino acid restriction on carcass quality and N excretion

The small effect on feed efficiency, back-fat thickness, the proportions of lean and fat cuts in the carcass, and meat quality traits found when diets moderately decreased in their AA content were fed is in general agreement with findings of others [33, 41-42]. The greater intramuscular fat content of the *longissimus lumborum* found in pigs on LAA than in pigs on HAA is consistent with the results of Wood et al. [43], who observed a 43% increase in the intramuscular fat content of the LL of pigs fed on low protein - low lysine diets compared with control feeds, and with the findings of Suárez-Belloch et al. [42]. Schiavon et al. [6] also found that a sub-optimal protein and AA supply altered some quality traits of dressed hams by increasing the subcutaneous fat cover as well

as the marbling score, in heavy pigs destined to the dry-cured ham production. An increase in intramuscular fat content may improve the eating quality of the meat [43-45] and could represent a potential extra value when farmers are paid for intramuscular fat. This would be the case for medium-heavy pig systems oriented towards high quality cooked ham production in Italy [46].

In the current experiment, despite the 6% increase in feed intake, pigs on LAA diets evidenced a 7.5% lower N intake than those on HAA diets. As the estimated N retention was not influenced by the dietary AA level, the estimated N excretion was markedly lower (15%) in pigs fed LAA diets. Therefore, and in agreement with other authors, we suggest that the use of diets lowered in indispensable AA and in N contents would reduce N excretion but would have a small effect on weight gain and carcass characteristics [16, 41, 47].

CONCLUSIONS

Results from this experiment suggest that fast growing pigs respond to a reduction in dietary indispensable AA content by increasing their feed intake, under both *ad libitum* and restricted feeding conditions. This result supports the theory that feed intake would reflect the requirement for the deficient nutrients, depending by the ability of the pig to cope with the productive circumstances. Moreover, a mild feed restriction resulted in a decreased feed intake as well as in a slightly lower carcass weight, but also in greater feed efficiency and carcasses with thinner back-fat compared to the *ad libitum* feeding regime. Restriction of the dietary indispensable AA content does not necessarily result in reduced growth performance, as in the current experiment we found heavier final body and carcass weights, with an alteration of the carcass and meat quality. In addition, a reduction in the dietary CP, or N, content, alongside a reduction in AA contents, may be a useful strategy for reducing N excretion in pigs and for some lowering of the feeding costs, depending on the price of the protein sources and of the synthetic amino acids.

ACKNOWLEDGEMENTS

The authors would like to thank Veronesi SPA (Quinto di Valpantena, Verona, Italy) for financial support, Prof. Enrico Sturaro for technical support, and the Aldo Gini Foundation (Via Portello 15, Padua, Italy) for supporting the research activities of PhD student Giuseppe Carcò.

REFERENCES

1. Kyriazakis I, Whittemore CT. Whittemore's science and practice of pig production. Blackwell Publishing, Oxford, UK; 2006.

2. Ferguson NS, Gous RM. The influence of heat production on voluntary food intake in growing pigs given protein-deficient diets. Anim. Sci. 1997, 64: 365-378.

3. Ferguson NS, Arnold GA, Lavers G, Gous RM. The response of growing pigs to amino acids as influenced by environmental temperature. 1 Threonine. Anim. Sci. 2000, 70: 287-297.

4. Schiavon S, Emmans GC. A model to predict water intake of a pig growing in a known environment on a known diet. Brit. J. Nutr. 2000, 84: 873-883.

5. Mordenti A, Bosi P, Corino C, Crovetto GM, Casa GD, Franci O, Piva A, Prandini A, Russo V, Schiavon S, Aspa commission' activity. A methodological approach to assess nutrient requirements of heavy pigs in Italy. Ital. J. Anim. Sci. 2003, 2: 73-87.

6. Schiavon S, Carraro L, Dalla Bona M, Cesaro G, Carnier P, Tagliapietra F, Sturaro E, Galassi G, Malagutti L, Trevisi E, Crovetto GM, Cecchinato A, Gallo L. 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.

7. Schiavon, S, Cesaro, G, Tagliapietra, F, Gallo, L Influence of N shortage and conjugated linoleic acid supplementation on some productive, digestive, and metabolic parameters of lactating cows. Anim. Feed Sci. Technol. 2015, 208, 86-97.

8. Pierer M, Amon B, Winiwarter W. Adapting feeding methods for less nitrogen pollution from pig and dairy cattle farming: abatement costs and uncertainties. Nutr. Cycl. Agroecosyst. 2016, 104: 201-220.

9. Nyachoti CM, Zijlstra RT, de Lange CFM, Patience JF. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 2004, 84**:** 549–566.

10. Black JL. Models to predict feed intake. In: Tollardona D, Roura E, editors. Voluntary feed intake in pigs. Wageningen: Wageningen Academic Publishes; 1999, pp. 323-344.

11. Bosi P, Russo V. The production of the heavy pig for high quality processed products. Ital. J. Anim. Sci. 2004, 3: 309-321.

12. Patience JF, Rossoni-Serão MC, Gutiérrez NA. A review of feed efficiency in swine: biology and appplication. J. Anim. Sci. Biotechnol. 2015, 6: 33-42.

13. Topigs, 2012. Feeding manual Talent. Available from: https://varkens.nl/sites/default/files/Feeding%20Manual%20Talent%20progeny%202012.pdf. Accessed 18th February 2018.

14. NRC, 2012. Nutrient Requirements of Swine. 11th revised ed. Washington: National Academy Press.

15. Kloareg M, Noblet J, Van Milgen J. Estimation of whole lipid mass in finishing pigs. Anim. Sci. 2006, 82: 241-251.

16. 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, 16: 130-138.

17. AOAC, 2012. Official Methods of Analysis of the Association of Official Agricultural Chemists, 19th ed. AOAC International, Gaithersburg, MD, USA.

18. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition. J. Anim. Sci. 1991, 74: 3583–3597.
19. Bouchard J, Chornet E, Overend RP, High-performance liquid chromatographic monitoring carbohydrate fractions in partially hydrolyzed corn starch. J. Agric. Food Chem. 1988, 36: 1188-1192.
20. Council of Europe, 2005. Amino acid analysis. In: European Pharmacopoeia 5.0, 5th ed. Main volume 5.0, Strasbourg: Council of Europe; 2005, pp. 86-92.
21. Slump P, Flissendaalje TD, Haaksman IK. Tryptophan in food proteins: a comparison of two hydrolytic procedures. J. Sci. Food Agric. Chem. 1991, 55: 493-496.
22. EU, 2014a. Commission Implementing Decision of 24 January 2014 authorising methods for grading pig carcases in Italy [notified under document C (2014) 279]. Off. J. L 23.
23. 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.
24. Steyn WJ, Casey NH, Jansen van Rensburg C. Effects of different penning conditions, feeding regimens and season on growth and carcass attributes of boars of a selected genetic line. S. Afr. J. Anim. Sci. 2012, 42: 178-188.
25. Knap PW. Voluntary feed intake and pig breeding. In: Tollardona D, Roura E, editors. Voluntary feed intake in pigs. Wageningen Academic Publishes, Wageningen; 2009. pp. 13-31.
26. Lebret B. Effects of feeding and rearing systems on growth, carcass composition and meat quality of pigs. Anim. 2008. 2: 1548-1558.

27. Dalla Bona M, Schiavon S, Carraro L, Gallo L. Growth performance, carcass traits and meat quality of growing pigs on different feeding regimes slaughtered at 145 kg BW. Ital. J. Anim. Sci. 2016, 15: 419-427.

28. Alfonso L, Zudaire G, Sarries MV, Vigueraand J, Flamarique F. Investigation of uniformity in pig carcass and meat quality traits. Anim. 2010, 4: 1739-1745.

29. 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.

30. Schiavon S, Tagliapietra F, Cesaro G, Gallo L, Cecchinato A, Bittante G. Low crude protein diets and phase feeding for double-muscled crossbred young bulls and heifers. Livest. Sci. 2013, 157: 462-470.

31. Carcò G, Dalla Bona M, Carraro L, Latorre MA, Fondevila M, Gallo L, Schiavon S. Influence of mild feed restriction and mild reduction in dietary amino acid content on feeding behavior of group-housed growing pigs. Applied Animal Behavior Science 2018, 198: 27-35.

32. Tous N, Lizardo R, Vilà B, Gispert M, Font-I-Furnols M., Esteve-Garcia E. Effect of reducing dietary protein and lysine on growth performance, carcass characteristics, intramuscular fat, and fatty acid profile of finishing barrows. J. Anim. Sci. 2014, 92: 129-140.

33. Henry Y, Seve B, Colleaux Y, Ganier P, Saligaut C, Jego P. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs. J. Anim. Sci. 1992, 70: 1873-1887.

34. Henry Y. Effect of a dietary amino-acid deficiency or imbalance during the initial period of growth in pigs and subsequent performance at slaughter. Annal. Zootech. 1995, 44: 3-28.

35. Chiba LI, Kuhlers DL, Frobish LT, Jungst SB, Huff-Lonergan EJ, Lonergan SM, Cummins KA. Effect of dietary restrictions on growth performance and carcass quality of pigs selected for lean growth efficiency. Livest. Prod. Sci. 2002, 74: 93-102).
36. Càmara L, Berrocoso JD, Coma J, Lopez-Bote CJ, Mateos GG. Growth performance and carcass quality of crossbred pigs from two Pietrain sire lines fed isoproteic diets varying in energy concentration. Meat Sci. 2016, 114: 69-74.
37. Kyriazakis I, Emmans GC, Whittemore CT. The ability of pigs to control their protein intake when fed in three different ways. Physiol. Behav. 1991, 50: 1197-1203.
38. Emmans GC. Genetic components of potential and actual growth. In: Land RB, Bulfield G, Hill, W.G., editors. Animal Breeding Opportunities. British Society of Animal Production Occasional Publication no. 12, 1988 pp. 153-181.
39. Emmans GC, Kyriazakis I. Consequences of genetic change in farm animals on food intake and feeding behavior. Proc. Nutr. Soc. 2001, 60: 115-125.
40. Kouba M, Hermier D, Le Dividich J. Influence of a high ambient temperature on lipid metabolism in the growing pig. J. Anim. Sci. 2001, 79: 81-87.
41. Rodriguez-Sanchez JA, Sanz MA, Blanco M, Serrano MP, Joy M, Latorre MA. 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.
42. Suárez-Belloch J, Guada JA, Latorre MA. Effects of sex and dietary lysine on performances and serum and meat traits in finisher pigs. Anim. 2015, 9: 1731-1739.
43. Wood JD, Lambe NR, Walling GA, Whitney H, Jagger S, Fullarton PJ, 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.

44. Cisneros F, Ellis M, Baker DH, Easter RA, McKeith FK. 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.

45. Teye GA, Sheard PR, Whittington FM, Nute GR, Stewart A, Wood JD. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. Meat Sci. 2006, 73: 157-165.

46. Rossi R, Ratti S, Pastorelli G, Crotti A, Corino C. The effect of dietary vitamin E and verbascoside on meat quality and oxidative stability of *longissimus dorsi* muscle in medium-heavy pigs. Food Res. Int. 2014, 65: 88-94.

47. He L, Wu L, Xu Z, Li T, Yao K, Cui Z, Yin Y, Wu G. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. Amino Acids. 2016, 48: 21-30.

Table 1. Planned feed allowances (kg/d) for restricted fed pigs.

Week	Initial BW	Final BW	Feed allowance[1]	Feeding phase
1	34	40	1.85	Acclimation
2	40	46	2.05	“
3	46	52	2.15	Growing
4	52	59	2.25	“
5	59	66	2.35	“
6	66	72	2.45	“
7	72	79	2.55	“
8	79	86	2.65	“
9	86	93	2.75	Early finishing
10	93	99	2.80	“
11	99	106	2.80	“
12	106	112	2.80	“
13	112	118	2.80	“
14	118	124	2.80	Late finishing
15	124	130	2.80	“
16	130	136	2.80	“
17	136	141	2.80	“
18	141	147	2.80	“

[1] The feed allowances are those suggested for Topigs Talent barrows, with some modification [13].

Table 2. Ingredient composition (g/kg) of the diets used in the various phases of growth.

	Acclimation (35 - 47 kg BW)	Growing (47 - 86 kg BW)	Early finishing (86 - 118 kg BW)		Late finishing (118 - 145 kg BW)	
			High amino acid (HAA)	Low amino acid (LAA)	High amino acid (HAA)	Low amino acid (LAA)
Corn	405.5	442.3	465.7	465.4	416.8	448
Soybean meal	175	170	160	120	145	70
Wheat grain	160	160	160	200	180	220
Wheat bran	65	70	90	90	95	100
Wheat middling	60	100	80	80	120	120
Barley	60	0	0	0	0	0
Beef tallow and pig lard (1:1)	32	24	19	19	19	18
Calcium carbonate	13	13.5	14	14	13.5	13
Dicalcium phosphate	5	2	0	0	0	0
Sodium chloride	4.5	4.5	4.5	4.5	4.5	4.5
Vitamin and mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
L-Lys HCl	6.4	3.5	2.6	2.9	2.2	2.6
L-Thr	2.7	1.3	0.8	0.9	0.8	0.8
DL-Met	2.4	1.1	0.6	0.5	0.5	0.4
L-Trp	0.5	0	0	0	0	0
Choline HCl	0.5	0.3	0.3	0.3	0.2	0.2
Liquid organic acids	5	5	0	0	0	0

[1]Providing per kg of diet: 9000 UI of vitamin A, 2000 UI of vitamin D_3, 1.5 mg of B_1, 4mg of vitamin B_2, 3 mg vitamin B_6, 20 □g of vitamin B_{12}, 30 mg of vitamin E, 2.1 mg of vitamin K_3, 22.5 mg of pantothenic acid, 25 mg of niacin, 0.3 mg of folic acid, 0.3 mg of biotin, 50 mg of Mn, 113 mg of Zn, 125 mg of Fe, 17.5 mg of Cu, 1.75 mg of J, 0.375 mg of Se.

Table 3. Chemical composition (g/kg) and energy content (MJ/kg) of the diets used during the various phases of growth

Item	Acclimation (35 - 47 kg BW)	Growing (47 – 86 kg BW)	Early finishing (86 - 118 kg BW)		Late finishing (118 -145 kg BW)	
			High amino acid (HAA)	Low amino acid (LAA)	High amino acid (HAA)	Low amino acid (LAA)
Analyzed composition[1]						
Dry Matter	900	893	891	891	895	894
Crude Protein (N × 6.25)	163	163	159	141	161	133
Starch	423	387	440	454	421	454
NDF	107	120	111	111	123	130
Ether Extract	58	46	42	45	44	42
Ash	42	41	42	40	42	41
Lysine	12.5	10.3	9.3	8.5	8.8	7.2
Methionine	4.8	3.5	3.1	2.7	3.0	2.5
Threonine	8.4	7.0	6.3	5.9	6.4	5.1
Tryptophan	2.3	2.2	2.0	1.8	1.9	1.6
Calculated composition[2]						
Dry Matter	882	879	879	878	878	877
Metabolizable energy	13.6	13.5	13.4	13.4	13.3	13.3
Net energy	10.1	9.9	9.8	9.8	9.7	9.8
Crude Protein	164	161	158	143	155	126
Starch	428	435	442	462	440	478
Lipid	56	50	46	46	45	45
Linoleic acid	16	16	16	16	16	16
Ca	7.2	6.5	6.4	6.3	5.8	5.8
P	5.0	4.6	4.3	4.2	4.5	4.3
Available P	3.7	3.2	2.9	2.9	2.9	2.9
Lysine	12.3	10.0	9.2	8.4	8.6	7.0
SID Lysine[3]	11.2	9.0	8.1	7.3	7.5	6.0
Methionine	4.7	3.5	3.0	2.7	2.9	2.4
SID Methionine[3]	4.4	3.2	2.7	2.4	2.6	2.1
Threonine	8.2	6.9	6.3	5.8	6.2	5.0
SID Threonine[3]	7.3	5.9	5.4	4.9	5.2	4.2
Tryptophan	2.4	2.0	1.9	1.7	1.9	1.5
SID Tryptophan[3]	2.1	1.6	1.6	1.4	1.6	1.3

[1]Analitical results as a mean from 3 independent replications.

[2] Computed from the ingredient composition according to NRC (2012).

[3] SID: standardized ileal digestible amino acid content.

Table 4. Growth performance of barrows fed *ad libitum* (AL) or restrictively (RF), feeds with high (HAA) or low (LAA) crude protein and indispensable AA contents. [1]

Item	Feeding level (FL)			Amino Acid level (AA)			*P values*		
	AL	RF	SEM	HAA	LAA	SEM	FL[2]	AA[3]	FL × AA
Body weight, kg:									
- Start of growing period[4]	47.0	47.2	0.49	46.7	47.6	0.49	0.78	0.22	0.81
- Start of finishing period[4]	86.0	85.3	0.90	84.9	86.3	1.06	0.48	0.41	0.85
- End of trial	145.8	141.2	1.35	140.4	146.6	1.35	0.018	0.018	0.82
- Weight loss for 14 h of fasting	3.85	2.88	0.28	3.37	3.36	0.28	0.014	0.98	0.67
Growth rate, kg/d:									
- Growing period	1.112	1.086	0.23	1.093	1.105	0.030	0.12	0.79	0.48
- Finishing period	0.881	0.823	0.020	0.817	0.887	0.020	0.033	0.038	0.77
- Overall	0.959	0.912	0.010	0.910	0.962	0.010	0.014	0.033	0.89
Feed intake, kg/d:									
- growing period	2.471	2.345	0.040	2.379	2.437	0.050	< 0.001	0.41	0.48
- finishing period	2.841	2.615	0.050	2.630	2.825	0.050	0.002	0.031	0.49
- overall	2.715	2.524	0.030	2.545	2.695	0.030	< 0.001	0.020	0.40
Actual feed intake – planned restricted feed allowance, kg/d[5]									
- growing period	0.121	-0.005	0.040	0.029	0.087	0.050	< 0.001	0.41	0.48
- finishing period	0.061	-0.165	0.050	-0.149	0.046	0.050	0.002	0.031	0.49
- overall	0.080	-0.111	0.030	-0.090	0.059	0.030	<0.001	0.020	0.40
Gain: feed:									
- growing period	0.451	0.463	0.005	0.460	0.454	0.005	0.010	0.52	0.94
- finishing period	0.307	0.311	0.003	0.306	0.312	0.003	0.49	0.28	0.050
- overall	0.352	0.359	0.003	0.355	0.356	0.003	0.050	0.93	0.09
Backfat thickness (P2)[6], mm:									
- start of finishing period	9.2	9.2	0.22	9.2	9.2	0.22	0.96	0.93	0.46
- End of trial	13.7	13.2	0.40	13.0	13.8	0.43	0.34	0.23	0.26

[1] Each data is the mean of 92 observations.

[2] FL is the within pen effect of restriction (RF) compared to the *ad libitum* (AL) feeding.

[3] AA is the effect of the dietary CP and AA content of the feeds.

[4] During the growing period (47-86 kg BW) all pigs received feeds with the same protein and amino acids content. In the following growing-finishing period (86 – 145 kg BW) feeds with different content of crude protein and amino acids were used.

[5] The planned restricted feed allowance is that suggested for Topigs Talent barrows [13], with minor modifications.
[6] Measurements of ultrasound backfat thickness were collect from 86 kg BW onward.

Table 5. Estimated body composition, energy, lysine and N balance of barrows fed *ad libitum* (AL) or restrictively (RF), feeds with high (HAA) or low (LAA) crude protein and indispensable amino acid contents.[1]

Item	Feeding level (FL)				Amino acid level (AA)			*P values*		
	AL	RF	SEM		HAA	LAA	SEM	FL[2]	AA[3]	FL × AA
Mean metabolic weight ($BW^{0.60}$)	16.0	15.4	0.29		15.6	15.9	0.34	0.05	0.58	0.09
Estimated body lipid[4]										
- initial, kg	16.0	15.9	0.25		15.8	16.1	0.26	0.92	0.38	0.67
- final, kg	34.4	32.4	0.85		32.1	34.7	0.95	0.06	0.11	0.54
- lipid retention (Lr), g/d	270.5	242.6	10.0		240.5	272.5	10.0	0.13	0.09	0.31
Estimated body protein[5]										
- initial, kg	14.5	14.3	0.17		14.3	14.5	0.20	0.46	0.67	0.86
- final, kg	24.4	23.7	0.26		23.6	24.5	0.25	0.06	0.046	0.46
- protein retention (Pr), g/d	145.8	138.5	3.00		137.4	146.9	3.00	0.050	0.10	0.42
ME requirement for Lr and Pr[6], MJ/d	20.6	18.8	0.65		18.7	20.8	0.65	0.031	0.043	0.65
ME intake, MJ/d	38.0	35.0	0.57		35.1	37.8	0.57	0.002	0.027	0.47
ME for maintenance[7], MJ/kg $BW^{0,60}$	1.00	0.95	0.02		0.97	0.98	0.02	0.016	0.66	0.46
SID lysine requirement [8], g/d	19.1	18.1	0.45		17.9	19.2	0.45	0.11	0.08	0.34
SID lysine intake (g/d)	20.5	18.9	0.36		20.3	19.1	0.36	0.002	0.06	0.63
SID lysine surplus [9], g/d	1.40	0.83	0.40		2.37	-0.15	0.43	0.26	0.006	0.10
N intake[10], g/d	65.9	60.7	1.16		65.7	60.8	1.16	0.002	0.024	0.66
N retention[10], g/d	23.3	22.2	0.54		22.0	23.5	0.54	0.13	0.09	0.31
N excretion[10], g/d	42.5	38. 6	1.04		43.8	37.4	1.16	0.003	0.008	0.28

[1] Each data is the mean of 92 observations.

[2] FL is the within pen effect of the restriction (RF) compared to the *ad libitum* (AL) feeding.

[3] Amino acid level is the effect of the dietary CP and AA content of the feeds.

[4] Computed from the empty BW (EBW) and the ultrasound backfat thickness measured at P2 level [15].

[5] Computed from the fat free empty BW (FFEBW) using relationships between body protein and body water and ash [14].

[6] Metabolizable energy (ME) computed assuming a requirement of 44.4 and 52.3 MJ/kg of protein and lipid retained, respectively [14].

[7] Metabolizable energy (ME) for maintenance computed as: (ME intake – ME requirement for growth)/average $BW^{0.60}$ [14].

[8] Calculated from BW, feed intake and protein retention according to NRC [14].

[9] Standardized ileal digestible (SID) lysine intake – SID lysine requirement.

[10] N intake was computed from feed intake and its N content, N retention was computed as estimated N retention/6.25, and N excretion as N intake- N retention.

Table 6. Carcass and meat quality of barrows fed *ad libitum* (AL) or restrictively (RF) feeds with high (HAA) or low (LAA) crude protein and indispensable AA contents.

Item	Feeding level (FL)			Amino acid level (AA)			*P values*		
	AL	RF	SEM	HAA	LAA	SEM	FL[2]	AA[3]	FL × AA
Carcass weight, kg	116.5	113.6	1.09	112.3	117.8	1.09	0.06	0.012	0.86
Carcass yield, %	79.9	80.4	0.23	80.0	80.4	0.23	0.13	0.31	0.94
Backfat thickness[1], mm	20.8	19.1	0.69	18.9	21.0	0.81	0.037	0.12	0.29
Loin depth[1], mm	64.3	65.2	0.68	64.8	64.7	0.68	0.38	0.88	0.51
Lean percentage (FOM)[4], %	56.4	57.3	0.39	57.4	56.3	0.44	0.06	0.12	0.40
Main untrimmed lean and fat cuts, kg:									
- loin with ribs	19.5	19.3	0.19	19.0	19.8	0.19	0.42	0.028	0.50
- neck	8.2	8.0	0.08	8.0	8.2	0.08	0.050	0.09	0.56
- shoulder	16.9	16.7	0.16	16.7	16.9	0.16	0.34	0.32	0.73
- ham	30.9	30.4	0.28	30.1	31.2	0.28	0.26	0.031	0.77
- deboned ham	19.2	19.1	0.17	18.8	19.4	0.17	0.61	0.050	0.60
- backfat	8.9	8.3	0.25	8.0	9.1	0.27	0.07	0.036	0.79
- belly	13.6	13.1	0.20	12.9	13.7	0.22	0.06	0.048	0.65
- total main lean cuts	75.5	74.3	0.64	73.8	76.0	0.64	0.17	0.047	0.85
- total main fat cuts	22.4	21.3	0.39	21.0	22.8	0.39	0.047	0.016	0.93
Yield of untrimmed lean and fat cuts, % of carcass:									
- total lean	64.8	65.5	0.36	65.7	64.6	0.43	0.13	0.10	0.97
- total fat	19.2	18.7	0.23	18.6	19.3	0.24	0.13	0.09	0.96
Yield of deboned ham, % of untrimmed ham	16.5	16.8	0.10	16.8	16.5	0.10	0.15	0.24	0.73
Longissimus lumborum (LL) muscle composition, %									
- moisture	70.8	70.9	0.15	71.1	70.6	0.16	0.42	0.06	0.17
- protein	23.5	23.6	0.10	23.6	23.5	0.10	0.63	0.28	0.19
- intramuscular fat	4.3	4.1	0.17	3.9	4.5	0.18	0.61	0.037	0.67
- ash	1.2	1.2	0.01	1.2	1.2	0.01	0.76	0.67	0.93
Water holding capacity of LL, %									
- thawing loss	10.5	10.4	0.42	10.6	10.3	0.42	0.06	0.60	0.55
- cooking loss	30.3	30.2	0.26	30.3	30.3	0.27	0.81	0.95	0.07
Warner-Bratzler shear force of LL, kg	2.3	2.2	0.08	2.3	2.2	0.10	0.86	0.23	0.96

[1]Assessed with a Fat-O-Meat'er between the third to fourth last ribs at 8 cm off the carcass midline.

[2] FL is the within pen effect of the restriction (RF) compared to the *ad libitum* (AL) feeding.
[3] AA is the effect of the indispensable AA content of the feed.
[4] Calculated from backfat thickness and loin depth taken between the third to fourth last ribs at 8 cm off the carcass midline [22-23].

S1 Table. Statistical descriptive. Growth performance of the experimental pigs.

	Mean	Standard deviation	Coefficient of variation (%)	Minimum	Maximum
Body weight, kg:					
- Start of growing period[1]	47.1	3.30	7.0	40.4	53.9
- Start of finishing period[1]	85.6	4.89	5.7	74.9	96.9
- End of trial	143.6	9.80	6.8	117.3	166.4
- Weight loss for 14 h of fasting	3.4	1.91	56.6	-3.2	7.6
Growth rate, kg/d:					
- Growing period	1.099	0.09	8.4	0.9	1.3
- Finishing period	0.852	0.13	15.6	0.4	1.1
- Overall	0.936	0.09	10.1	0.6	1.1
Feed intake, kg/d:					
- growing period	2.409	0.20	8.2	1.8	3.0
- finishing period	2.730	0.36	13.1	1.7	3.6
- overall	2.621	0.26	9.7	2.0	3.4
Actual feed intake - planned restricted feed allowance, kg/d[2]:					
- growing period	0.059	0.20	-	-0.5	0.7
- finishing period	-0.050	0.36	-	-1.0	0.8
- overall	-0.014	0.26	-	-0.7	0.8
Gain: feed:					
- growing period	0.457	0.02	5.4	0.4	0.5
- finishing period	0.309	0.02	7.6	0.2	0.4
- overall	0.355	0.02	5.3	0.3	0.4
Backfat thickness (P2)[3], mm:					
- start of finishing period	9.2	1.48	16.2	6.5	15.0
- End of trial	13.4	2.62	19.5	7.0	21.0

[1] During the growing period (47-86 kg BW) all pigs received feeds with the same protein and amino acids content. In the following growing-finishing period (86 – 145 kg BW) feeds with different content of crude protein and amino acids were used.
[2] The planned restricted feed allowance is that suggested for Topigs Talent barrows [13], with minor modifications.
[3] Measurements of ultrasound backfat thickness were collect from 86 kg BW onward.

S2 Table. Statistical descriptive. Estimated body composition, energy, lysine and N balance of the experimental pigs.

	Mean	Standard deviation	Coefficient of variation (%)	Minimum	Maximum
Mean metabolic weight ($BW^{0.60}$)	15.7	1.57	10.03	13.6	18.6
Estimated body lipid[1]					
- initial, kg	15.9	1.64	10.3	12.4	20.6
- final, kg	33.4	5.28	15.8	20.2	47.4
- lipid retention (Lr), g/d	256.5	70.4	27.4	64.0	426.2
Estimated body protein[2]					
- initial, kg	14.4	0.92	6.37	12.4	16.9
- final, kg	24.1	1.76	7.29	20.8	29.2
- protein retention (Pr), g/d	142.3	23.4	16.4	73.2	202.6
ME requirement for Lr and Pr[3], MJ/d	19.7	4.07	20.6	6.60	29.0
ME intake, MJ/d	36.5	4.77	13.1	23.3	48.4
ME for maintenance[4], MJ/kg $BW^{0,60}$	1.0	0.11	11.7	0.64	1.24
SID lysine requirement[5], g/d	18.6	3.12	16.8	9.49	26.9
SID lysine intake (g/d)	19.7	2.58	13.1	13.4	27.9
SID lysine surplus[6], g/d	1.10	2.79	-	-6.66	8.27
N intake[7], g/d	63.3	8.52	13.5	43.4	90.5
N retention[7], g/d	22.8	3.74	16.4	11.7	32.4
N excretion[7], g/d	40.6	7.38	18.2	24.2	66.5

[1] Computed from the empty BW (EBW) and the ultrasound backfat thickness measured at P2 level [15].

[2] Computed from the fat free empty BW (FFEBW) using relationships between body protein and body water and ash [14].

[3] Metabolizable energy (ME) computed assuming a requirement of 44.4 and 52.3 MJ/kg of protein and lipid retained, respectively [14].

[4] Metabolizable energy (ME) for maintenance computed as: (ME intake – ME requirement for growth)/average $BW^{0.60}$ [14].

[5] Calculated from BW, feed intake and protein retention according to NRC [14].

[6] Standardised ileal digestible (SID) lysine intake – SID lysine requirement.

[7] N intake was computed from feed intake and its N content, N retention was computed as estimated N retention/6.25, and N excretion as N intake- N retention.

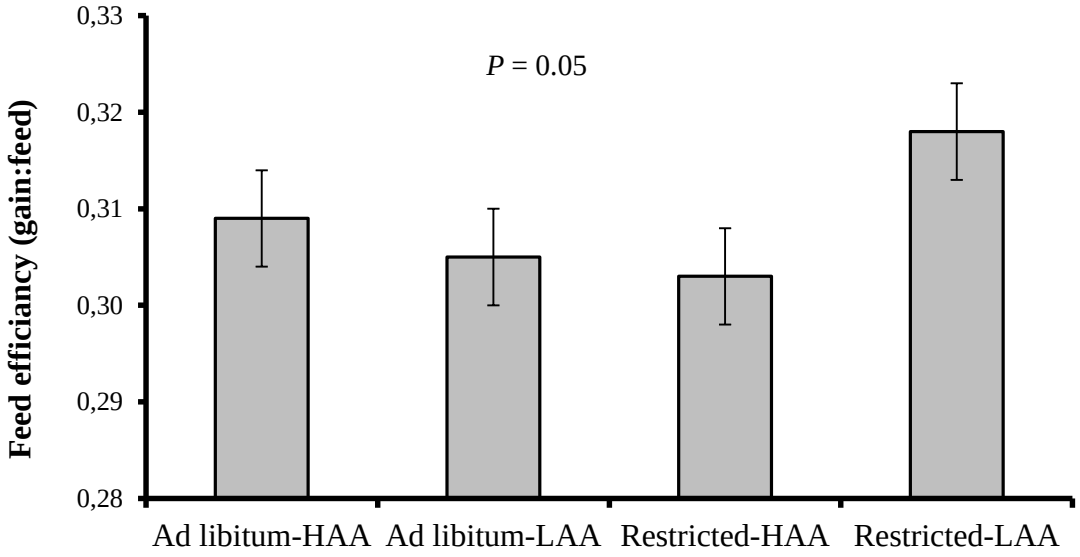
S3 Table. Statistical descriptive. Carcass and meat quality of the experimental pigs.

	Mean	Standard deviation	Coefficient of variation (%)	Minimum	Maximum
Carcass weight, kg	115.1	7.93	6.9	87.7	133.8
Carcass yield, %	0.8	0.02	2.0	0.7	0.8
Backfat thickness[1], mm	19.9	4.02	20.2	11.0	30.0
Loin depth[1], mm	64.8	4.50	6.9	42.0	74.0
Lean percentage (FOM)[2], %	56.8	2.35	4.1	50.3	62.9
Main untrimmed lean and fat cuts, kg:					
- loin with ribs	19.4	1.30	6.7	15.3	22.3
- neck	8.1	0.59	7.3	6.9	10.0
- shoulder	16.8	1.10	6.6	15.0	20.4
- ham	30.7	1.96	6.4	24.3	34.9
- deboned ham	19.1	1.15	6.0	15.5	20.7
- backfat	8.6	1.66	19.4	3.5	12.2
- belly	13.3	1.36	10.2	8.9	16.5
- total main lean cuts	74.9	4.42	5.9	61.5	86.7
- total main fat cuts	21.9	2.79	12.7	12.4	28.6
Yield of untrimmed lean and fat cuts, % of carcass:					
- total lean	65.2	2.11	3.2	59.5	70.5
- total fat	19.0	1.51	7.9	14.2	22.4
Yield of deboned ham, % of untrimmed ham	16.6	0.72	4.3	14.9	18.2
Longissimus lumborum (LL) muscle composition, %					
- moisture	70.8	1.05	1.5	68.5	74.2
- protein	23.5	0.65	2.8	21.5	25.5
- intramuscular fat	4.2	1.18	28.1	2.0	7.1
- ash	1.2	0.04	3.2	1.1	1.3
Water holding capacity of LL, %					
- thawing loss	10.5	2.82	27.0	4.0	17.5
- cooking loss	30.3	1.65	5.5	26.2	35.4
Warner-Bratzler shear force of LL, kg	2.3	0.38	16.9	1.4	3.1

[1]Assessed with a Fat-O-Meat'er between the third to fourth last ribs at 8 cm off the carcass midline.

[2] Calculated from backfat thickness and loin depth taken between the third to fourth last ribs at 8 cm off the carcass midline [22-23]

Figure 1. Influence of the interaction between feeding level (*ad libitum* or restricted) and dietary AA density [high amino acids feeds (HAA); low amino acids feeds (LAA)] on feed efficiency (gain:feed) of finishing pigs (118 to 145 kg of body weight; *P* = 0.05). Each bar is the least-squares mean from 23 observations and vertical bars indicate SEM.



4. CHAPTER 2nd

Influence of mild feed restriction and mild reduction in dietary amino acid content on feeding behaviour of group-housed growing pigs

Giuseppe Carcò[1], Mirco Dalla Bona[1], Luca Carraro[1], Maria Angeles Latorre[2], Manuel Fondevila[2], Luigi Gallo[1], Stefano Schiavon[1]

[1]*Dept. of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Italy*
[2]*Departamento de Producción Animal y Ciencia de los Alimentos, Instituto Agronómico de Aragón (IA2), Universidad de Zaragoza, Spain*

**Corresponding author*
E-mail: giuseppe.carco@phd.unipd.it

Published in Applied Animal Behaviour Science
DOI: 10.1016/j.applanim.2017.09.020

ABSTRACT

This study investigates changes in the feeding behaviour of pigs as a result of a restriction in their feed allowance and a reduction in dietary indispensable amino acid (AA) content. Ninety-six Topig Talent × PIC barrows were housed in 8 pens and individually fed either *ad libitum* (AL) or a restricted diet (RF) from 47 to 145 kg body weight (BW). The amount of feed given to RF pigs was close to their expected voluntary intake, but it was limited to proportions of 0.33, 0.66 and 1.00 of the estimated daily amount of feed in 3 time intervals, 00:01 to 8:00, 8:01 to 16:00 and 16:01 to 24:00 h, respectively. From 86 kg BW, the pigs in 4 of the pens were fed diets with conventional standardized ileal digestible AA content (CAA), while the pigs in the other pens received diets (LAA) in which the proportions of dietary indispensable AA were lowered with respect to CAA by 0.91 from 86 to 118 kg BW and by 0.82 from 118 to 145 kg BW. Automated feeders monitored individual feeding behaviour. Data were analysed by pig and feeding phase with a 2×2 factorial design. Over the whole experimental period, feed restriction resulted in a decrease in daily feed intake (7%, $P < 0.001$), the number of visits (27%, $P < 0.001$) and the time spent feeding (14%, $P < 0.001$), but an increase in feed consumption per visit (20%, $P = 0.001$) and feeding rate (10%, $P = 0.032$). The reduction in AA increased daily feed intake (7%, $P = 0.031$), tended to increase feeding rate (14%, $P = 0.07$) and interacted with feeding regime with respect to the number and duration of feeding visits. During growing and finishing, we observed high, negative, non-linear relationships between feed consumption per visit and visit frequency ($R^2 = 0.989$ to 0.876), between visit duration and visit frequency ($R^2 = 0.648$ to 0.695), and between feeding rate and time spent feeding in a day ($R^2 = 0.802$ to 0.707), and positive linear relationships between visit duration and feed consumption per visit ($R^2 = 0.614$ to 0.570). The individual feeding rate during growing was positively correlated with that during finishing ($R^2 = 0.458$). We conclude that pigs try to adapt their feeding pattern to compensate for a reduction in feed allowance or nutrient restriction by, for example, increasing their feeding rate, which may reflect increased feeding motivation.

Keywords: growing pigs; feeding behaviour; feeding phase; feed restriction; dietary AA reduction

Abbreviations

AA, amino acids; AL, *ad libitum* feeding; aNDF, neutral detergent fibre with amylase treatment and including the residual ash; BF, back fat depth; BW, body weight; CAA, conventional crude protein and amino acid diet; CP, crude protein; CV, coefficient of variation; DM, dry matter; EE, ether extract; FR, feeding regime; LAA, low-crude protein and amino acid diet; Lys, lysine; N, nitrogen; NE, net energy; RF, restricted feeding; SID, standardized ileal digestible; SD, standard deviation.

INTRODUCTION

Feeding behaviour may be thought of as the strategy pigs adopt to achieve their desired feed intake [1] and can basically be described in terms of the number, size and duration of feeding visits to the manger [2]. The feeding behaviour of individual pigs, usually measured by automated single- or multi-space feeding stations [3] is influenced by the number of animals, environmental and social factors related to the feed, and the production environment [4-5]. Studying animal feeding behaviour allows us to identify the effects of treatments and conditions in order to predict illness, and to understand the causes of variations in feed efficiency [6] and animals' feeding motivations [4-5]. In the case of feed or nutrient restriction, we would expect pigs to modify their feeding behaviour to compensate for the restriction, for example by increasing feeding speed or the time spent feeding. We recently investigated the effects of mild feed restriction alone or in combination with a reduction in the dietary indispensable amino acid (AA) content on the growth performance of pigs [7]. Feed restriction caused reductions in feed intake and average daily gain, but increased feed efficiency, whereas AA reduction increased feed intake and average daily gain but had no influence on feed efficiency. Although various studies have been carried out on the effects of feed restrictions and reductions in AA allowances on performance and carcass traits, few have looked at their influence on pig feeding behaviour. Our aim was to study the feeding behaviour of group-housed pigs fed individually from single-space feeders and subjected to feed restriction alone or in combination with a reduction in the dietary indispensable AA content.

MATERIAL AND METHODS

Pigs and experimental design

Readers are referred to Schiavon et al. [7] for details regarding the pigs and their diet, growth performance, and estimated N and energy balance. All experimental procedures were reviewed and approved by the University of Padua's Ethical Committee for the Care and Use of Experimental Animals.

Briefly, the experiment involved 96 Topigs Talent × PIC barrows of the same age, with an average body weight of 35.8 ± 2.8 kg. On arrival at the farm, the pigs were allotted to 8 pens (5.8 m × 3.8 m) at a density of 12 pigs/pen and were subjected to an acclimation period until about 47 kg BW. Each pen was equipped with an automated feeding station (Compident Pig – MLP, Schauer Agrotronic, Austria). From 47 to 145 kg BW, 6 pigs in each pen were fed *ad libitum* (AL), while the others were placed on the restricted feeding regime (RF). The RF allowance was established on a weekly basis in accordance with the feeding guidelines for TOPIGS Talent barrows [8]. Restriction was mild, with the planned feed allowances close to the expected voluntary feed intake to prevent excessive feed consumption by some pigs in this group, although we set a threshold quantity of feed to be consumed during the 3 daily feeding periods. From 86 kg BW onwards, the pigs in 4 pens were given feeds with conventional indispensable AA contents (CAA) close to the requirements estimated by NRC [9], while the others were given feeds low in indispensable AA (LAA).

Feed formulation and chemical analysis

During the growing period (47-86 kg BW), all the pigs received the same commercial feed, which had a CP content of 161 g/kg with SID lysine, methionine, threonine, and tryptophan contents of 56, 20, 37 and 10 g/kg CP, respectively. In the early (86 to 118 kg BW) and late (118 to 145 kg BW) finishing periods, the LAA feeds were formulated from the corresponding CAA feeds by replacing soybean meal with corn and wheat grain, and by adding a very small amount of crystalline AA so that the contents of the various AAs per unit of CP were similar.

During early finishing, the barrows of 4 pens were fed either a CAA diet with 158 g/kg of CP or an LAA diet with 143 g/kg of CP; both diets contained 51 g/kg CP of SID lysine, 17 of SID methionine, 34 of SID threonine and 10 of SID tryptophan. During late finishing, the CAA feed contained 155 g/kg of CP and the LAA 126 g/kg of CP, both containing 47 g/kg CP of SID lysine, 17 of SID methionine, 34 of SID threonine and 10 of SID tryptophan. The average net energy content of the various feeds, calculated according to NRC [9], was in the order of 10.4 MJ/kg.

The CAA and LAA feeds were composed from the same batches of ingredients. Feeds were sampled as described by Schiavon et al. [7] and analysed in triplicate for DM (# 934.01), N (# 976.05), EE (# 920.29), ash (# 942.05) [10], and neutral detergent fibre (aNDF), including residual ash, determined with amylase treatment [11]. Starch was determined by liquid chromatography after hydrolysis to glucose [12]. The AA content of the feed samples (0.5 g/sample) was determined in accordance with the Council of Europe (chapter ♯ 2.2.56) [13]. The chemical and nutritional characteristics of the diets are given in the supplementary material as Table S1.

Feed distribution and behaviour control

The feeding stations allowed the animals access to the feed throughout the whole day, while lateral barriers limited competition among the pigs during feeding. In each pen, 6 pigs were fed AL, and the other 6 were fed RF. The AL pigs were able to access the station and eat all day. The RF pigs had access to the station 24 h, but were allowed to eat only a portion of the feed ration for that day during specific periods of time: 0.33 from 00:01 to 08:00 h, 0.66 from 08:01 to 16:00 h and 1.00 from 16:01 to 24:00 h. When a pig visited the manger, the feeding station identified the ear transponder, opened the automatic gate placed in front of the trough and released the feed. The date and time of feeding, the time spent feeding and the weights of the feed consumed and left over were recorded. The leftovers were automatically weighed and assigned to the next pig visiting the station. Once the RF pig had consumed its ration for a given 8 h period, no further feed was provided until the next 8 h period. Since the feed was released by the station in doses of 200 g, it was possible for some RF pigs to consume more feed than their ration for that day. Nonetheless, the RF pigs always consumed less than their ration. All the pigs had free access to a nipple drinker placed in each pen. All feeding stations were calibrated at the start of the study and weekly thereafter using a 1-kg test weight.

Data editing

The dataset consisted of 107 249 records reporting animal identity code, date, entering and exiting times, and feed consumption per visit, which were collected throughout the experiment from the 8 feeding stations. The data were edited using the R software [14]. During the experiment, 4 pigs (one

pig in the AL-LAA group, two in RF-CAA, and one in RF-LAA) died or were discarded due to illness or injury and their data were removed from the database. The final dataset was compiled from 92 pigs.

The day, and not the single visit, was considered the proper temporal basis to describe the feeding behaviour traits of the pigs during growing and finishing, and thus problems of consistency due to not normal distributions of variables were partially avoided. Therefore, daily feed intake was computed as the sum of the feed consumed during all the visits in a given day by each pig. Visits to the feeding stations were counted and distinguished as feeding or sham visits, in other words, visits with or without feed consumption. Daily feeding time was calculated as the total duration of all feeding visits by a given pig in one day. The feeding rate of each pig was computed from its average feed intake per visit and the average duration of its feeding visits.

Secondarily, we also explored the within-day feeding behaviour of the pigs, but only the data regarding the finishing period were considered. In this case the temporal basis for the calculation of the various feeding behavioural traits, i.e. feed intake, number of feeding visits, time spent feeding, and feeding rate, was the within day time-interval.

Statistical analysis

Feeding behaviour data computed on daily basis were averaged by pig and by period, i.e. growing, finishing and overall, and each trait was analysed for deviation from normality. Descriptive statistics analyses were carried out with SAS software [15]. The MIXED procedure of SAS was used to analyse the daily-based feeding behavioural traits according to the following linear model:

$$y_{ijkl} = \mu + FR_i + AA_j + FR \times AA_{ij} + pen(AA)_{k:i} + e_{ijkl};$$

where y_{ijkl} is the observed trait, μ is the overall intercept of the model, FR_i is the fixed effect of the i_{th} feeding regime (I = 1, 2), AA_j is the fixed effect of the j_{th} kind of feed with differing amino acid contents (j = 1, 2), $pen_{k:j}$ is the random effect of the $k{:}j_{th}$ pen (k = 1, …, 8) within AA, FR × AA is the effect of the interaction between feeding regime and type of feed, and e_{ijkl} is the random residual. Pen within AA and residuals were independently and normally distributed with a mean of zero and variances of σ_k^2 and σ_e^2, respectively. In accordance with the experimental design, the effect of AA

was tested using pen within AA as the error line, where the effect of FR was tested on the residual (animal), given that each pen housed pigs of both FRs. Those variables that did not have a normal distribution, such as the numbers of feeding and sham visits, were log transformed. Because the experimental design was balanced, only one standard error of the least-squares means (SEM) is reported for each source of variation. The growing and the finishing periods were analysed separately as during the growing only the feed restriction, and not the amino acid restriction, was applied. The same model was used to analyse the feeding behavioural data computed on the within–day time interval basis, for the finishing period only.

The individual means obtained for the following pairs of variables, number of visits (x_i) vs. feed intake per visit (y_i), or the number of visits (x_i) vs. visit duration (y_i), or daily feeding time (x_i) vs. feeding rate (y_i), were plotted against each other to reveal mutual relationships, separately for the growing and finishing periods. For each of these comparisons the products $x_i \times y_i$ were computed with a spreadsheet. These values were averaged, and the resulting mean value "a" was used to compute a third variable "z_i" as a/x_i. The resulting z_i values of the isoline, representing all the combinations of x_i and z_i in the theoretical case of no influence of the dietary treatments, were plotted in each graph. The proportion of the y variance explained by each isoline (R^2) was computed.

Lastly, the R^2 of the linear relationship between visit duration and feed intake per visit was computed.

RESULTS

Descriptive statistics of feeding behaviour

Average feed intake was 2409 g/d with a coefficient of variation (CV) of 8.22 % during the growing stage, and 2730 g/d with a CV of 13.02% during finishing (Table 1). The pigs spent just over 60 minutes per day feeding at the growing stage and just under 60 minutes during finishing, with CVs ranging from 18.3 to 21.2%. The average number of individual feeding visits per day was 8.25 during

growing and 7.06 during finishing, while the average number of sham visits per day was 1.13 at growing and 1.34 at finishing. The CVs for these traits ranged from 35 to 144%, but the kurtosis and skewness values showed that the frequencies did not have a normal distribution. The number of feeding visits decreased from 5.90 to 4.28 with advancing stage of growth, with CVs for this trait ranging from 29.8 to 38.0%.

The average amount of feed consumed per feeding visit increased from 299 to 377 g with advancing growth, CVs ranging from 29.5 to 35.5%. The average individual feeding visit duration was 7.3 minutes over the entire trial, CV in the order of 30%. The average individual speed of feeding increased from 39 to 53 g/min with advancing stage of growth, CVs ranging from 18.5 to 27.9%.

The within-pig standard deviations for the various feeding behaviour traits were similar in magnitude to the among-pigs SDs; while those during growing were weakly correlated with those during finishing (data not shown).

Relationships among feeding behaviour traits

The average individual feed intakes per visit during growing and finishing were highly negatively related (R^2 = 0.989 and 0.876, respectively) to the number of feeding visits (Figure 1a,b). The curved isolines denote all the combinations of feeding visits and feed intakes per feeding visit, resulting in mean daily feed intakes of 2409 g/d during growing and 2730 g/d during finishing. These curves explained large proportions of the total variance of feed intake per feeding visit. Similarly, the mean individual duration of feeding visits was negatively and non-linearly related to the number of feeding visits, and the proportion of variance explained by the isolines ranged from 0.648 to 0.695 (Figure 1c,d). Consequently, the mean individual feed intake per visit was positively (and linearly) related to the average duration of the feeding visits (Figure 2a,b), and the strength of the relationships decreased from growing (R^2 = 0.614) to finishing (R^2 = 0.570). The slopes of these regressions, which represent the average rates of feeding in the two periods, were 37.7 g/min during growing and 48.9 g/min during finishing.

There were also high, non-linear negative relationships between the average individual feeding rate and the time spent feeding (Figure 3a,b). The isolines, representing all the combinations between the y and the x axes, which revealed daily feed intake to be 2409 min/d during growing and 2730 min/d during finishing, absorbed a large part of the total variance in the feeding rate.

Finally, a linear relationship between the average individual feeding rates during growing and finishing. The overall relationship had a value of $R^2 = 0.45$ (data not shown). However, the strength of these relationships was greater for the two groups of pigs fed AL (R^2 ranging from 0.625 to 0.682) than for the pigs fed restrictively (R^2 ranging from 0.386 to 0.304).

Influence of dietary variations on feeding behaviour during growing and finishing

The RF diet had many significant effects on the pigs' feeding behaviour (Table 2). It resulted in a 7% reduction in feed intake, a 14% reduction in the time spent feeding per day, halved the number of sham visits, and reduced by 27% the number of feeding visits. It also caused a 20% increase in the amount of feed consumed per feeding visit, a 10 % increase in feed intake per visit, and a 10% increase in the rate of feeding.

Reducing the dietary AA content only during the finishing period resulted in a 7.4% increase in feed intake, along with a tendency to shorten the visit duration by 14% ($P = 0.09$) and a corresponding 14% increase in the speed of feeding ($P = 0.07$). The effects of this dietary reduction on the various feeding behaviour traits were often non- significant. However, during finishing there was a tendency towards an FR×AA interaction with respect to the number of feeding visits ($P = 0.07$), as the LAA diet increased the number of feeding visits under AL conditions by 35%, compared with an 8% decrease under RF conditions. The FR×AA interaction, therefore, also tended to influence the mean quantity of feed consumed per feeding visit ($P = 0.09$), which was 7.5% lower under dietary AA reduction and AL conditions, compared with an increase of 14.4% under AA reduction and RF conditions.

During the finishing period (Table 3), feed restriction increased feed intake by 41% during the first part of the day, and lowered it by 23% during the middle part and by 15% during the last part of the

day. During the first 8 h of the day, feed restriction increased the time spent feeding (by 23%), the feed intake per feeding visit (by 35%) and the feeding rate (by 16%), but not the number of feeding visits. During the rest of the day, on the other hand, feed restriction caused a reduction in the time spent feeding and the number of feeding visits, but some increase in the feeding rate, so that there was a weak or no influence on feed intake per feeding visit.

The reduction in dietary AA content during the finishing period had little influence on feeding behaviour during the various daily time intervals. The most important change was that the feeding rate increased by about 14% in all three time intervals, and the FR×AA interaction was sometimes significant for the number of feeding visits.

DISCUSSION

General pattern

The magnitude and trends of the results regarding feeding behaviour are in general agreement with those reported in previous studies conducted with group-housed pigs fed individually from single-spaced automated feed dispensers [3,16]. In the present experiment, feeding activity was predominantly diurnal. The proportions of feed consumed during the 0.01-8.00, 8.01-16.00, and 16.01-24.00 periods were 0.19, 0.42 and 0.39 of total daily intake, respectively, reflecting the influence of the circadian rhythm [17]. On average, each pig spent 57.8 min/d feeding, so that the mean individual daily occupation of the feeder was in the order of 0.48 (57.8 min/60 min × 12 pigs/24 h). However, taking the circadian distribution into account, average feeder occupancies were 0.36, 0.60, and 0.49 during the three consecutive daily time intervals, respectively.

Individual feeding behaviour

Few studies have reported data on the feeding behaviour of individual pigs. In the present experiment, feeding behaviour varied greatly, among and within animals. The individual mean number of feeding visits, feed intakes per visit and visit durations are the major factors influencing flexibility in feeding behaviour patterns and we found negative relationships between them. Variation

in the actual or desired feed intake could, therefore, be achieved by a combination of modifications, even small ones, to these three variables. The within-animal standard deviation for the various traits confirms the existence of strict relationships between behavioural traits, as the pigs with high variation for one trait tended also to have high variation for the other traits. As previously noticed by Nielsen et al. [18], the average individual feeding rate was negatively and non-linearly related to the time spent feeding, although this result might be an artefact of the calculation method as the feeding rate was calculated from feed intake per visit and visit duration. Nonetheless, there was a good correlation between the individual feeding rates of the pigs at growing and at finishing. This supports the view that feeding rate might be an intrinsic characteristic of the pig and its previous feeding experience, for example, its feeding motivation, since faster rates would suggest greater feeding motivation [2,19]. Labroue et al. [20] found that among the feeding behaviour criteria of Large White growing pigs, the rate of feed intake had a high genetic correlation with daily feed intake (around 0.5) and average daily gain (around 0.4), while Young and Lawrence [3] found an increase in the feeding rate and number of visits, and a consequent decrease in consumption per visit, when there was high competition among the animals. In our experiment, the strongest correlation between feeding rate at growing and at finishing was found with the pigs fed AL, the weakest by the pigs fed restrictively. This suggests that the feed restriction interfered with feeding motivation and altered the pigs' feeding patterns. Nielsen [2] provided evidence to suggest that a period of environmental constraint may have long term consequences on feeding rate due to the increased feeding rate of pigs previously subjected to feed restriction.

Influence of feed restriction on feeding patterns

Most studies on the feeding behaviour of pigs have been carried out under *ad libitum* conditions, so there is very little literature on the effects of feed restriction on feeding patterns [5]. In the present study, feed restriction altered the feeding pattern, in that there was a reduction in the number and duration of feeding visits, but an increase in feed intake per visit and feeding rate. Feed restriction also resulted in increased consumption during the night, with a consequent reduction in intake during

the rest of the day. The magnitude and trend of these results are in agreement with previous studies showing the flexibility of pigs in being able to modify their feeding patterns when under limiting conditions. In Nielsen et al.'s [18] study, pigs kept in groups of 20 per pen made fewer visits to the trough but increased their feed intake per visit and feeding rate compared with pigs raised in smaller groups. Bornett et al. [21-22] found similar changes in feeding behaviour when individually-housed pigs were switched from AL conditions to a regime restricting the amount of time they had access to the trough, or were switched from individual to group housings. In our experiment, the RF pigs' feed allowance was almost identical to the average actual feed consumption of the AL-CAA fed pigs (2620 g/d). The RF-CAA pigs consumed about 6.4% less feed than their allowance, and the actual feed consumption of the RF-LAA pigs was only 2% less than the planned feed amounts (data not shown). This would suggest that the RF pigs were reluctant to consume feed during the night, generally preferring to maintain their feed intake during the diurnal period through a better combination of visit frequency, feeding rate and time spent per visit [3,16-17], but the inadequate nutrient provision forced them to increase their feed consumption during the night.

However, in the present study the feed restriction was mild and close to the expected voluntary feed intake as we wanted to prevent excessive feed consumption by some pigs in the group. The pigs on restricted feed did not consume more than the established threshold throughout the growing and finishing periods. This regime caused a reduction in feed intake and growth rate, but an increase in average feed efficiency (2%) and carcass leanness (2%), as reported in detail by Schiavon et al (2017). The observed 10% increase in feeding speed may reflect an increase in the feeding motivation of those RF pigs that were more severely underfed in comparison with their desired feed intake. That restricted feeding of pigs alters their feeding motivation is also suggested by the greater CV in the feeding rate of the RF-fed pigs (11.8%) compared with AL-fed pigs (8.5%). The lower correlation between the feeding rates of growing and finishing RF pigs (R^2=0.304-0.386) compared with that of *ad libitum* fed pigs (R^2 = 0.625-0.682) is also indicative of the effect of feed restriction on the pigs' feeding motivation.

Influence of dietary amino acid reduction on feed intake and feeding behaviour

Short- and long-term regulation of feed intake is under physiological control, although the underlying mechanisms are not fully understood [23]. A useful working hypothesis is that feed intake reflects the pig's desire for nutrients and may be constrained by animal, diet and environmental factors [24]. Conceptually, "this implies that pigs eat because they desire to grow, not that pigs grow because they eat" [23]. Numerous studies have shown the effects of dietary energy concentration and AA level on pigs' voluntary feed intake [25]. It is commonly accepted that a reduction in dietary net energy (NE) content leads to an increase in feed intake to keep the NE intake constant [26], although the influence of nutrient deficiencies, particularly of indispensable AA, is controversial. Some authors found that voluntary feed intake decreased when pigs were placed on diets that were deficient in protein or indispensable AA [27-29], especially tryptophan [30]. Other studies found that mild deficiencies in protein, lysine or threonine resulted in increased feed intake [31-33]. Moreover, Kyriazakis et al. [34] showed that pigs were able to control their protein intake when they had free access to feeds with differing protein contents. In this regard, reduced levels of tryptophan in the diet increased the feeding activities of pigs under AL conditions [35], and a similar effect was observed by Jensen et al. [36] as a consequence of dietary CP restriction. However, Andretta et al. [17] found that dietary crude protein and SID lysine contents had no correlation with various feeding behavior variables, and Montgomery et al. [37] found total feed intake and feeding rate decreased when dietary tryptophan was reduced. Some of the above inconsistencies may be explained by assuming that a pig always tries to eat enough to meet its nutrient requirements, but may be hampered by social and environmental factors related to diet, climate, disease or housing [38-40].

In the present experiment, reduction in the dietary indispensable AA content during finishing increased feed intake, body weight at slaughter, and carcass weight and fat content [7]. The feeding pattern of pigs was altered, and the most evident effect, though only in terms of a tendency, was an increase in the feeding rate in both feeding regimes. This supports Nielsen's [2] suggestion that the feeding rate may be considered an index that reflects the pig's feeding motivation. This might be

useful in identifying sub-optimal dietary nutrient allowances, even in conditions where there can be no compensatory increase in daily feed intake because this is constrained.

CONCLUSIONS

We conclude that pigs try to adapt their feeding pattern to compensate for a reduced feed allowance or nutrient restriction, for example by increasing their feeding rate, which may reflect the pigs' increased feeding motivation. However, the results of present experiment show that a change of feeding rate is not the only strategy that pigs adopt to reach their desired feed intake. Depending on the context, they may also modify the number of visits and the time spent feeding per visit, so we can expect considerable variation in feeding patterns among individual pigs and across experiments. This suggests we should be cautious in generalising the results obtained across experimental conditions, or even individuals.

ACKOWLEDGEMENTS

This research was funded by the University of Padua under grants CPDR143385/2014 and DOR1655405/2016, and Regione Veneto under grant P.O.R. - F.S.E. n. 2105-41- 2121-2015. The authors would like to thank Prof. Enrico Sturaro for the technical support, Veronesi SPA (Quinto di Valpantena, Verona, Italy) for financial and technical support, and the Aldo Gini Foundation (Via Portello 15, Padua, Italy) for supporting the research activities of PhD student Giuseppe Carcò, during his study period at the University of Zaragoza.

REFERENCES

1. Emmans G, Kyriazakis I. Consequences of genetic change in farm animals on food intake and feeding behaviour. Proc. Nutr. Soc. 2001, 60: 115–125.

2. Nielsen BL. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. Appl. Anim. Behav. Sci. 1999, 63: 79–91.

3. Young RJ, Lawrence AB. Feeding behaviour of pigs in groups monitored by a computerized feeding system. Anim. Prod. 1994, 58: 145–152.

4. Boumans IJMM, Bokkers EAM, Hofstede GJ, de Boer IJM. Understanding feeding patterns in growing pigs by modelling growth and motivation. Appl. Anim. Behav. Sci. 2015, 171: 69–80.

5. Maselyne J, Saeys W, Van Nuffel A. Review: Quantifying animal feeding behaviour with a focus on pigs. Physiol. Behav. 2015, 138: 37–51.

6. De Haer LCM & De Vries AG. Feed intake patterns of and feed digestibility in growing pigs housed individually or in groups. Livestock Production Science 1993, 33: 277-292.

7. Schiavon S, Dalla Bona M, Carcò G, Carraro L, Bunger L, Gallo L. Effects of feed and indispensable amino acid restrictions on feed intake, growth performance and carcass characteristics of growing pigs. PLoS ONE 2018, https://doi.org/10.1371/journal.pone.0195645.

8. Topigs, 2012. Feeding manual Talent. Available from: http://www.varkens.nl/dier/ voeren-vleesvarkens.

9. NRC, 2012. Nutrient Requirements of Swine. 11th revised ed. Washington: National Academy Press.

10. AOAC, 2012. Official Methods of Analysis of the Association of Official Agricultural Chemists, 19th ed. AOAC International, Gaithersburg, MD, USA.

11. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and no starch polysaccharides in relation to animal nutrition. J. Anim. Sci. 1991, 74: 3583–3597.

12. Bouchard J, Chornet E, Overend RP. High-performance liquid chromatographic monitoring carbohydrate fractions in partially hydrolyzed corn starch. J. Agric. Food Chem. 1988, 36: 1188–1192.

13. Council of Europe, 2005. Amino acid analysis. In: European Pharmacopoeia 5.0, 5th ed. Main volume 5.0, Strasbourg: Council of Europe, pp. 86-92.

14. R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. Vienna, Austria.

15. SAS Institute, Inc., 2009. SAS/STAT®. 9.4. Cary, NC.

16. De Haer LCM. Relevance of feeding pattern for selection of growing pigs. Wageningen University 1992, The Netherlands.

17. Andretta I, Pomar C, Kipper M, Hauschild L, Rivest J. Feeding behavior of growing – finishing pigs reared under precision feeding strategies 1. J. Anim. Sci. 2016, 94: 3042–3050.

18. Nielsen BL, Lawrence AB, Whittemore CT. Effect of group size on feeding behaviour, social behaviour, and performance of growing pigs using single-space feeders. Livest. Prod. Sci. 1995, 44: 73–85.

19. Colpoys JD, Johnson AK, Gabler NK. Daily feeding regimen impacts pig growth and behavior. Physiol. Behav. 2016, 159: 27–32.

20. Labroue F, Guéblez R, Sellier P. Genetic parameters of feeding behaviour and performance traits in group-housed Large White and French Landrace growing pigs. Genet. Sel. Evol.1997, 29: 451–468.

21. Bornett HLI, Morgan CA, Lawrence AB, Mann J. The flexibility of feeding patterns in individually housed pigs. Anim. Sci. 2000, 40: 457-469.

22. Bornett HLI, Morgan CA, Lawrence AB, Mann J. The effect of group housing on feeding patterns and social behaviour of previously individually housed growing pigs. Appl. Anim. Behav. Sci. 2000, 70: 127–141.

23. Nyachoti CM, Zijlstra RT, de Lange CFM, Patience JF. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 2004, 84: 549–566.

24. Whittemore EC, Green DM. Technical review of the energy and protein requirements of growing pigs: food intake. Anim. Sci. 2001, 73: 3–17.

25. Li Q, Patience JF. Factors involved in th eregulation of feed and energy intake of pigs. Anim. Feed. Sci. Technol. 2016 http://dx.doi.org/10.1016/j.anifeedsci.2016.01.001

26. Cámara L, Berrocoso JD, Fuentetaja A, Lopez-Bote CJ, De Blas C, Mateos GG. Regrouping of pigs by body weight at weaning does not affect growth performance, carcass quality or uniformity at slaughter of heavy weight pigs. Anim. Sci. J. 2016, 87: 134–142.

27. 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.

28. Schiavon S, Carraro L, Dalla Bona M, Cesaro G, Carnier P, Tagliapietra F, Sturaro E, Galassi G, Malagutti L, Trevisi E, Crovetto GM, Cecchinato A, Gallo L. 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.

29. Suárez-Belloch J, Guada JA, Latorre MA. Effects of sex and dietary lysine on performances and serum and meat traits in finisher pigs. Animal 2015, 9: 1731–1739.

30. Henry Y, Seve B, Colleaux Y, Ganier P, Saligaut C, Jego P, Saligautt C, Jegot P. Interactive effects of dietary levels of tryptophan and protein on voluntary feed intake and growth performance in pigs, in relation to plasma free amino acids and hypothalamic serotonin. J. Anim. Sci. 1992, 70: 1873–1887.

31. Ferguson NS, Gous RM. The influence of heat production on voluntary food intake in growing pigs given protein-deficient diets. Anim. Sci. 1997, 64: 365-378.

32. Henry Y. Effect of a dietary amino-acid deficiency or imbalance during the initial period of growth in pigs and subsequent performance at slaughter. Annal Zootech. 1995, 44: 3-28.

33. Chiba LI, Kuhlers DL, Frobish LT, Jungst SB, Huff-Lonergan EJ, Lonergan SM, Cummins KA. Effect of dietary restrictions on growth performance and carcass quality of pigs selected for lean growth efficiency. Livest. Prod. Sci. 2002, 74: 93-102.

34. Kyriazakis I, Emmans GC, Whittemore CT. The ability of pigs to control their protein intake when fed in three different ways. Physiol. Behav. 1191, 50: 1197-1203.

35. Dalcin Castilha L, Pagliari Sangali C, Costa Esteves LA, Muniz CF, Furlan AC, Souza Vasconcellos R, Pozza PC. Day-night behaviour and performance of barrows and gilts (70–100 kg) fed low protein diets with different levels of tryptophan and B6 vitamin. Appl. Anim. Behav. Sci. 2016, 180: 35–42.

36. Jensen MB, Kyriazakis I, Lawrence AB. The activity and straw directed behaviour of pigs offered foods with different crude protein content. Applied Animal Behaviour Science 1993, 37: 211-221.

37. Montgomery GW, Flux DS, Carr JR. Feeding patterns in pigs: the effect of amino acids deficiency. Physiol. & Behav. 1978, 20:693-698.

38. Black JL. Models to predict feed intake. In: Tollardona D, Roura E, editors. Voluntary feed intake in pigs. Wageningen: Wageningen Academic Publishes; 1999, pp. 323-344.

39. Emmans GC. Genetic components of potential and actual growth. In: British Society of Animal Production (Eds), Animal Breeding Opportunities. Occasional Publication no. 12; 1999, pp. 153-181.

40. Ferguson NS, Arnold GA, Lavers G, Gous RM. The response of growing pigs to amino acids as influenced by environmental temperature. 1 Threonine. Anim. Sci. 2000, 70: 287-297.

Table 1. Statistical descriptive of growth performance and behavioral traits[1].

	Mean	Median	SD	CV	Min	Max	Kurtosis	Skewness
Feed intake								
growing (47 -86 kg BW)	2409	2405	198	8.22	1825	3041	0.987	0.265
finishing (86-145 kg BW)	2730	2749	356	13.02	1750	3623	0.672	-0.183
Overall	2621	2633	255	9.74	1966	3427	0.938	0.095
Feeding time, min/d								
growing (47 -86 kg BW)	64.0	63.2	11.84	18.5	39.8	93.3	-0.231	0.3245
finishing (86-145 kg BW)	54.6	54.6	11.56	21.2	29.2	83.5	-0.233	-0.007
Overall	57.8	57.5	10.60	18.3	32.8	86.9	-0.134	0.033
Sham visits, n								
growing (47 -86 kg BW)	1.13	0.50	1.62	144	0.00	10.0	12.59	3.124
finishing (86-145 kg BW)	1.34	1.01	1.35	101	0.00	7.13	3.565	1.726
Overall	1.27	0.88	1.13	89.2	0.00	4.75	1.253	1.156
Feeding visit, n								
growing (47 -86 kg BW)	8.87	8.25	3.16	35.6	4.07	22.5	3.410	1.554
finishing (86-145 kg BW)	8.80	7.06	5.80	65.9	3.77	47.0	20.638	3.839
Overall	8.83	7.56	4.40	49.9	4.18	35.3	15.041	3.266
Feed intake per feeding visit, g								
growing (47-86 kg BW)	299	288	88.0	29.5	105	496	-0.333	0.316
finishing (86-145 kg BW)	378	384	134	35.5	53.0	612	-0.674	-0.364
Overall	338	337	102	30.3	71.9	536	-0.412	-0.313
Feeding visit duration, min								
growing (47-86 kg BW)	7.81	7.79	2.29	29.4	3.72	16.2	1.208	0.758
finishing (86-145 kg BW)	7.36	7.20	2.59	35.2	1.33	14.7	-0.028	0.150
Overall	7.32	7.21	2.18	29.7	2.04	13.6	0.054	0.111
Feeding rate, g/min								
growing (47-86 kg BW)	38.8	38.0	7.15	18.5	25.7	59.1	0.671	0.375
finishing (86-145 kg BW)	52.5	51.8	14.8	27.9	26.8	100.7	0.856	1.033
Overall	46.9	45.7	10.42	22.2	29.6	83.6	0.854	0.768

[1] Data were computed from the means of 92 pigs (n=92).

Table 2. Feeding behaviour of pigs over growing[1], finishing[1] and overall period as influenced by the feeding regime [FR, *ad libitum* (AL-) or restricted (RF-)], and by the indispensable amino acid content of the diets [AA, conventional (CAA) or low (LAA) contents][2].

	Feeding treatment						*P* values[3]		
	AL- CAA	**AL-LAA**	**RF -CAA**	**RF-LAA**	**SEM**		**FR**	**AA**	**FR×AA**
Feed intake, kg/d									
growing (47 -86 kg BW)	2.429	2.513	2.329	2.361	0.052		< 0.001	0.41	0.48
finishing (86-145 kg BW)	2.719	2.962	2.541	2.689	0.069		0.002	0.031	0.49
Overall	2.620	2.810	2.470	2.579	0.048		< 0.001	0.020	0.40
Feeding time, min/d									
growing (47 -86 kg BW)	65.7	69.6	61.1	59.4	2.37		0.003	0.66	0.24
finishing (86-145 kg BW)	59.4	59.3	52.2	47.4	2.20		<0.001	0.31	0.29
Overall	61.6	62.8	55.2	51.5	2.02		<0.001	0.56	0.22
Sham visits[2], n									
growing (47 -86 kg BW)	2.00	0.92	1.15	0.33	0.49		0.002	0.16	0.81
finishing (86-145 kg BW)	2.06	1.58	0.89	0.75	0.32		<0.001	0.26	0.47
Overall	2.04	1.35	0.98	0.60	0.29		<0.001	0.15	0.80
Feeding visits[2], n									
growing (47 -86 kg BW)	9.96	10.40	8.31	6.74	0.74		<0.001	0.48	0.043
finishing (86-145 kg BW)	8.66	11.71	7.68	7.08	1.29		0.001	0.45	0.07
Overall	9.10	11.27	7.89	6.96	0.95		<0.001	0.74	0.043
Feed intake per feeding visit, g									
growing (47-86 kg BW)	270	264	304	360	19.6		<0.001	0.31	0.06
finishing (86-145 kg BW)	369	324	389	433	32.9		0.016	1.00	0.09
Overall	320	296	346	396	26.5		0.001	0.70	0.06
Feeding visit duration, min									
growing (47-86 kg BW)	7.16	7.26	7.89	8.97	0.51		0.009	0.33	0.29
finishing (86-145 kg BW)	8.00	6.34	7.89	7.24	0.61		0.45	0.14	0.33
Overall	7.45	6.49	7.69	7.71	0.53		0.10	0.47	0.26
Feeding rate, g/min									
growing (47-86 kg BW)	37.9	37.1	39.3	40.8	1.49		0.09	0.85	0.43
finishing (86-145 kg BW)	46.7	51.5	51.9	60.8	2.99		0.016	0.07	0.50
Overall	43.5	46.0	46.6	52.1	2.12		0.032	0.11	0.49

[1] During the growing period all pigs received feeds with the same amino acids content, so that a P-value > 0.05 was expected for the AA treatment. In the finishing period pigs were subjected to different FR and fed diets with different amino acid content.

[2] Data were computed from the means of 92 pigs (n=92).

[3] *P*-values computed on logarithm transformed values.

Table 3. The within-day feeding behaviour of pigs during finishing as influenced by the feeding regime [FR, *ad libitum* (AL-) or restricted (RF-)] and by the dietary indispensable amino acid contents [AA, conventional (CAA) or low (LAA) contents]. [1]

	Feeding treatment						*P* values[2]		
	AL- CAA	**AL-LAA**	**RF-CAA**	**RF-LAA**	**SEM**		**FR**	**AA**	**FR×AA**
Feed intake, kg/d									
00:00-08:00 h	509	573	718	809	45.7		<0.001	0.14	0.77
08:01-16:00 h	1161	1336	953	972	49.2		<0.001	0.10	0.12
16:01-23:59 h	1047	1046	874	909	49.1		0.002	0.75	0.71
Feeding time, min/d									
00:00-08:00 h	11.73	12.00	14.60	14.58	0.93		0.004	0.90	0.87
08:01-16:00 h	25.97	26.98	19.91	17.40	1.40		<0.001	0.61	0.21
16:01-23:59 h	21.72	20.36	17.66	15.40	1.09		<0.001	0.15	0.68
Sham visits[2], n									
00:00-08:00 h	0.42	0.33	0.21	0.15	0.07		<0.001	0.30	0.74
08:01-16:00 h	1.18	0.84	0.43	0.43	0.18		<0.001	0.31	0.20
16:01-23:59 h	0.46	0.41	0.25	0.17	0.09		0.005	0.32	0.95
Feeding visits[2], n									
00:00-08:00 h	1.78	2.37	2.12	1.89	0.26		0.96	0.22	0.11
08:01-16:00 h	4.15	5.92	3.12	3.02	0.78		<0.001	0.39	0.10
16:01-23:59 h	2.73	3.43	2.45	2.17	0.33		0.013	0.59	0.09
Feed intake per feeding visit, g									
00:00-08:00 h	342	301	399	467	33.9		0.001	0.71	0.10
08:01-16:00 h	335	296	358	390	33.1		<0.001	0.72	0.030
16:01-23:59 h	441	384	415	464	35.6		0.010	0.45	0.20
Feeding visit duration, min									
00:00-08:00 h	7.83	6.15	8.31	8.08	0.64		0.054	0.20	0.25
08:01-16:00 h	7.35	5.84	7.29	6.49	0.60		0.53	0.15	0.44
16:01-23:59 h	9.23	7.29	8.20	7.54	0.65		0.39	0.92	0.54
Feeding rate, g/min									
00:00-08:00 h	43.8	49.1	49.9	58.5	2.87		0.008	0.053	0.56
08:01-16:00 h	46.4	50.9	51.8	60.6	3.02		0.011	0.07	0.47
16:01-23:59 h	48.3	53.3	53.7	63.0	3.27		0.017	0.08	0.48

[1] Data were computed from the means of 92 pigs (n=92).

[2] *P*-values computed on log values.

S1 Table. Chemical composition (g/kg) and energy content (MJ/kg) of the diets.

Item	Growing (47 - 86 kg BW)	Early finishing (86 - 118 kg BW)		Late finishing (118 -145 kg BW)	
		Conventional amino acid (CAA)	Low amino acid (LAA)	Conventional amino acid (CAA)	Low amino acid (LAA)
Analyzed composition[1]					
Dry Matter	893	891	891	895	894
Crude Protein (N × 6.25)	163	159	141	161	133
Lysine	10.3	9.3	8.5	8.8	7.2
Methionine	3.5	3.1	2.7	3.0	2.5
Threonine	7.0	6.3	5.9	6.4	5.1
Tryptophan	2.2	2.0	1.8	1.9	1.6
Starch	387	440	454	421	454
NDF	120	111	111	123	130
Ether Extract	46	42	45	44	42
Ash	41	42	40	42	41
Calculated composition[2]					
Dry Matter	879	879	878	878	877
Net Energy	10.4	10.4	10.4	10.3	10.4
Crude Protein (CP)	161	158	143	155	126
SID Lysine[3], g/kg CP	56	51	52	47	48
SID Methionine[3], g/kg CP	20	17	17	17	17
SID Threonine[3], g/kg CP	37	34	34	34	33
SID Tryptophan[3], g/kg CP	10	10	10	10	10

[1]Analitical results as a mean from 3 independent replications.

[2] Computed from the ingredient composition according to NRC (2012).

[3] SID: standardized ileal digestible amino acid content

Figure 1. Relationships of feeding visits with feed intake per visit or occupation time per visit of pigs fed, *ad libitum* or restrictively, conventional (CAA) or low (LAA) amino acids feed, during growing (a, c), and finishing (b, d). The isolines denote all combinations of feeding visits and feed intake per visit resulting in 2409 and 2730 g/d mean daily feed intake and all combinations of feeding visits and occupation time per visit resulting in 64.0 and 54.6 min/d mean daily feeding times, during growing and finishing, respectively [each single point represents a pig, n = 92].

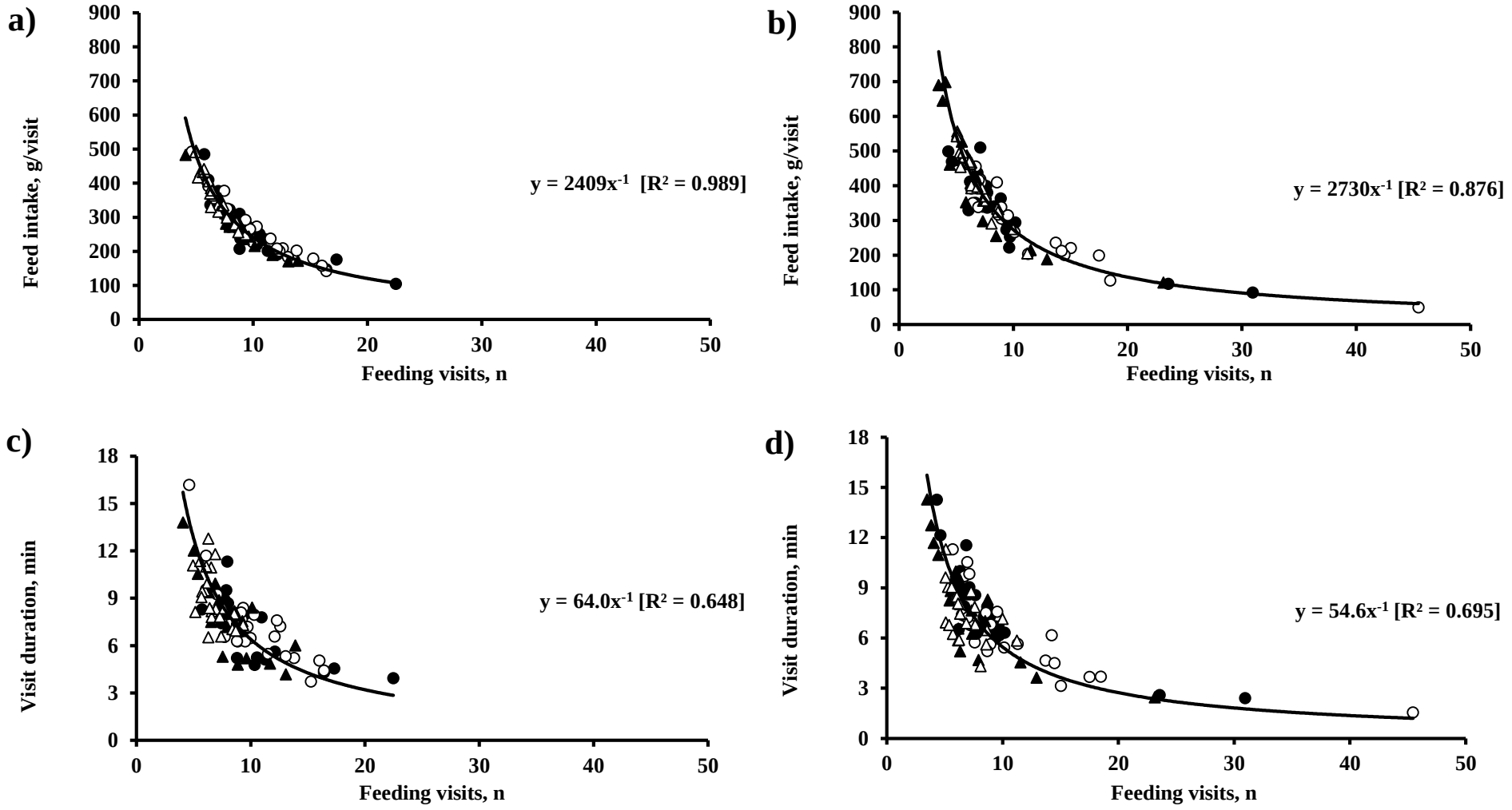


Figure 2. Relationship between individual visit duration and feed intake per visit of pigs fed *ad libitum* or restrictively, conventional (CAA) or low (LAA) amino acids feed, during a growing (a), and a finishing (b) periods [each single point represents a pig, n = 92].

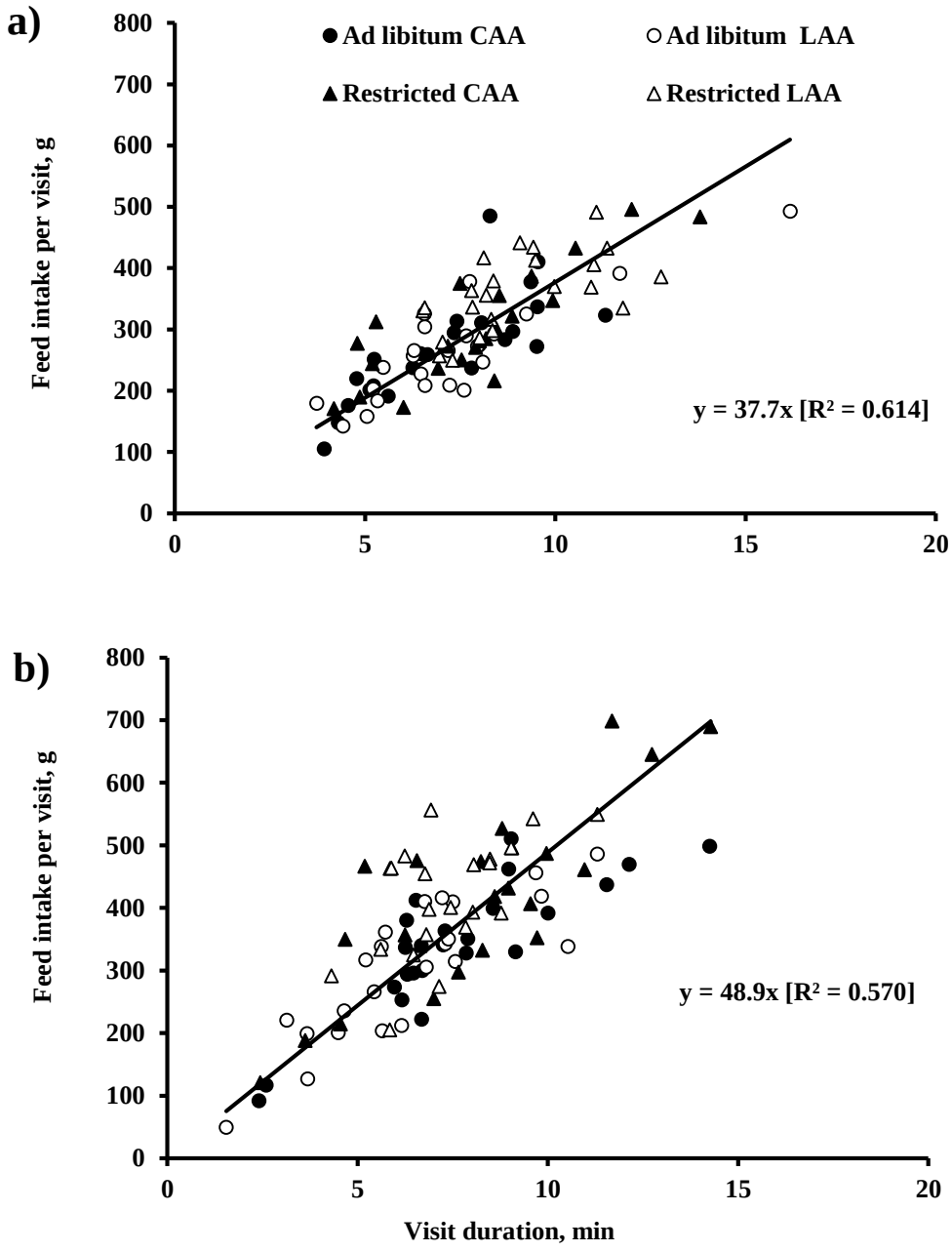
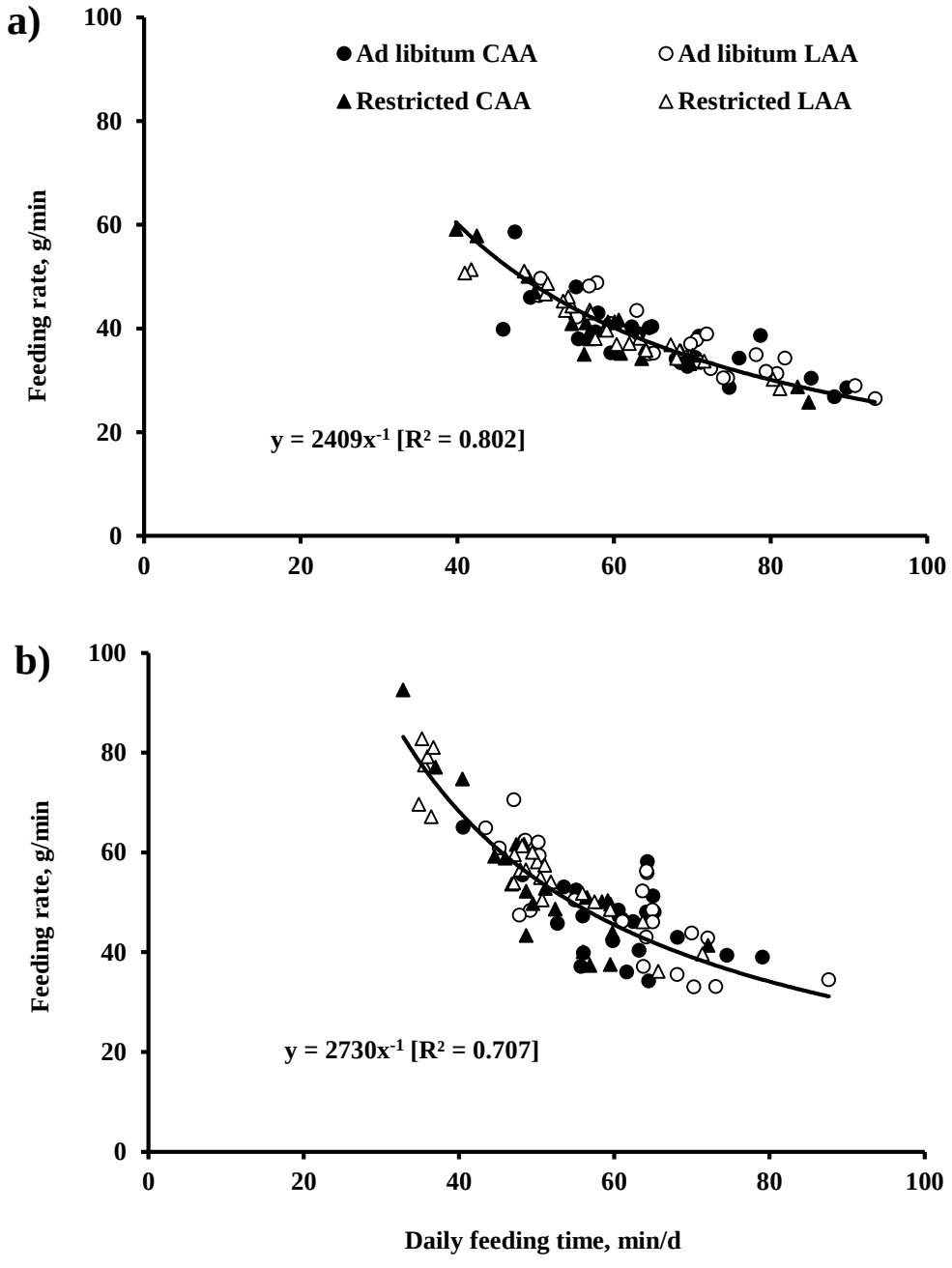


Figure 3. Relationship between individual daily feeding time and feeding rate of pigs fed, *ad libitum* or restrictively, conventional (CAA) or low (LAA) amino acids feed, during a growing (a) and a finishing (b) period. The isolines denote all combinations of daily feeding time and feeding rate which result in the actual mean daily intakes of 2409 and 2730 g/d for growing and finishing, respectively [each single point represents a pig, n = 92].



.

5. CHAPTER 3rd

The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs

Giuseppe Carcò[1*], Luigi Gallo[1], Mirco Dalla Bona[1], Maria Angeles Latorre[2], Manuel Fondevila[2], Stefano Schiavon[1]

[1]*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Italy*
[2]*Department of Animal Production and Food Sciences, IA2 CITA-University of Zaragoza, Spain*

**Corresponding author*
E-mail: giuseppe.carco@phd.unipd.it

Published online in Plos ONE
DOI: 10.1371/journal.pone.0205572

ABSTRACT

This study investigated the effect of the feeding behaviour on growth performance, and carcass and meat characteristics of 96 barrows fed *ad libitum* or restrictively with high or low amino acids (AA) diets according to a 2 × 2 factorial design. The feeding behaviour traits were measured with automated feeders. From 86 kg BW, half of the pigs were given feeds with high indispensable (AA) contents, while the other half received feeds with indispensable AA contents reduced by 9% in early finishing (86-118 kg BW) and by 18% in late finishing (118-145 kg BW). Body lipid and protein retentions were estimated from BW and backfat depth measures recorded at the beginning and end of each period. Pigs were slaughtered at 145 kg BW and carcass and meat quality data were recorded. Phenotypic correlations among feeding behaviours, growth performances, and carcass and meat traits were computed from all the data after adjustment for the effects of feeding treatments. As feeding rate was the behavioural trait most highly correlated with performance and carcass traits, the records of each pig were classified into feeding rate tertiles. Then, the data were statistically analysed using a mixed model, which included feed restriction (FR), AA reduction (AAR), the FR × AAR interaction and the feeding rate tertile as fixed factors, and pen as a random factor. Pigs eating faster (52.1 to 118.9 g/min) had significantly greater final body weights (16%), average daily weight gains (27%), estimated protein gains (22%), estimated lipid retention (46%), carcass weights (16%), weights of lean cuts (14%), weights of fat cuts (21%), proportions of fat in the carcass (14%), and 4% lower proportions of carcass lean cuts than pigs eating slowly (12.6 to 38.2 g/min). Manipulating the eating rate, through management or genetic strategies, could affect feed intake and subsequent growth performance, hence carcass quality, but have little influence on feed efficiency.

Keywords: Growing pigs, Feeding behaviour, Feeding rate, Growth performance, Carcass traits

INTRODUCTION

The availability of automated feeding stations enabled the measurement of the feeding behaviour in growing pigs [1]. Feeding behaviour can be defined by criteria such as the time spent eating per day, feed consumption per day, number of feeding visits, time spent eating per visit and feeding rate [2]. A better knowledge of pig feeding behaviours can help to clarify the role played by factors influencing feed intake, growth performance, feed utilisation efficiency, the quality of the products and the social interrelationships among pigs [3]. Previous studies have explored the phenotypic and genetic relationships among feeding behaviour traits, growth performances and feed efficiency [2,4-7]. Alterations of feeding patterns, such as those imposed by the farmer through feed distribution, have been found to affect feed efficiency and body composition [8-10]. De Haer et al. [2] showed that meal size can negatively affect feed digestibility, and that rate of feed intake and meal size are the factors most commonly associated with growth performance, whereas daily eating time and eating frequency are associated with the residual feed intake. Similarly, Andretta et al. [11] found that feeding rate and number of meals per day were the variables most closely related to performance results. They also found that feed efficiency was negatively correlated with the amount of feed consumed per meal and feeding rate, and that feeding rate was negatively correlated with protein utilisation efficiency.

However, inconsistencies have been found in studies of the mutual influences among feeding behaviour, growth performance, and feed utilisation efficiency [3], which may be due to the very large variability among individuals with respect to these behavioural traits. In general, among-pig variation in behavioural traits is much greater than feed intake variation [12]. A useful way to interpret this huge variability is to consider feeding behaviour as a flexible strategy the pig follows to reach its desired feed intake when kept in a given social and productive environment [1,

12-14]. The desired feed intake, which is the amount of feed required for maintenance and growth, mainly depends on the pig's genotype and physiological state [15,16], although nutrient imbalances [17,18], and climatic [18,20] and social [4] conditions may also influence the nutritional motivation of the pigs and their desired, and hence actual, feed intake. De Haer et al. [2] found that pigs with different nutritional motivations and feed intake patterns would also have different carcass and meat quality characteristics. In that study, the pigs with the lowest rate of feed intake and the lowest feed consumption per meal had the lowest daily weight gain and the highest estimated carcass lean percentage; the authors suggest that these pigs may have been the subordinate ones in the pen, chased away from the mangers. As a consequence, pigs with the fastest feeding rates or highest feed consumption per visit might also be those with the greatest feed intake, growth rates and carcass fatness, which would in turn affect the lipid content and hence quality of the meat. However, very few studies have examined the effect of the feeding behaviour on carcass traits and meat quality.

Thus, the aim of the current paper is to explore the influence of feeding behaviour on growth performance, carcass and meat characteristics of pigs, using data collected from a previous experiment.

MATERIAL AND METHODS

Pigs and experimental design

All experimental procedures were reviewed and approved by the University of Padova's Ethical Committee for the Care and Use of Experimental Animals (Prot. #147683). The data were taken from a previous experiment aimed at investigating the influence of mild restrictions to the feed

allowance and dietary amino acid content on the growth performance [17] and feeding behaviour of growing pigs [12].

Briefly, the experiment involved 96 Topigs Talent × PIC barrows born within the same week. They arrived at the experimental station of the University of Padova at the end of February and were slaughtered at the end of June, thereby avoiding hot ambient summer temperatures. The average temperature in the housing rooms ranged from 20 to 25°C, from the start to the end of the trial. The pigs were allotted to 8 pens (5.8 × 3.8 m), at an average body weight (BW) of 35.8 ± 2.82 kg, with 12 pigs/pen. Each pen was equipped with an automated feeding station (Compident Pig – MLP, Schauer Agrotronic, Austria). After an acclimation period of 12 days, 6 pigs in each pen were fed *ad libitum* (AL), while the other 6 were subjected to a moderate restricted feeding regime (RF) from 47 to 145 kg BW. Each pig of the RF group was allowed to consume, as a maximum, the daily feed amount suggested by the breeding company for Topigs Talent barrows [21], and daily feed allowance ranged between 2.15 and 2.80 kg at the start and the end of the trial, respectively. The RF plane aimed to prevent excessive feed consumption by the greedier pigs, and resulted in a 7% lower average feed intake with respect to AL pigs, according to Schiavon et al. [17]. From 86 kg BW upwards, the pigs of 4 pens were given feeds with high indispensable AA contents (HAA), in slight excess of NRC recommendations [22], while the pigs of the other 4 pens were given feeds with indispensable AA (LAA) reduced by 9% in early finishing (86-118 kg BW) and by 18% in late finishing (118-145 kg BW), with respect to the HAA diet. The dietary composition is given in Table 1, and major details about the experimental conditions are given in Schiavon et al. [17].

The feeding stations of pens allowed the pigs access to the feed throughout the whole day. The AL pigs were able to access the station and eat as much as they wished all day, whereas the RF

pigs had 24 h access to the station, but were allowed to eat up to 0.33, up to 0.66 and up to 1.00 portions of the daily planned feed ration during the 0.01-8.00 h, 8.01-16.00 h, and 16.01-24.00 h time intervals, respectively. Lateral barriers restrained competition among the pigs during eating. A gate placed in front of the trough was opened only after the pig identification and avoided that other pigs could steal the feed. The date and time of feeding, the time spent eating and the weights of the feed consumed and left over by each individual pig were recorded. The leftovers were weighed and assigned to the next pig visiting the station. All the pigs had free access to a nipple drinker placed in each pen.

Individual BW was measured weekly using an electronic scale, and backfat depth (BF) was measured every two weeks with an A-mode ultrasonic device (Renco Lean-Meater series 12, Renco Corporation, Minneapolis, USA) from 86 kg BW upwards. The BF measure was taken above the last rib at approximately 5.5–8.0 cm from the midline, the distance increasing with increasing BW [23].

Slaughter and assessment of carcass data and meat quality

All pigs were slaughtered on the same day in one batch after 24 h of fasting. They were stunned with carbon dioxide, and killed by exsanguination after cutting the jugular vein, according to standard slaughter house procedures. Carcasses were scalded, de-haired, eviscerated and split down the midline, according to commercial slaughtering procedures. Individual hot carcass weights were recorded and the dressing percentages calculated. Carcass lean percentage [24-25] was calculated from BF and loin depth measurements taken on the left side of each carcass between the 3rd and 4th ribs 8 cm off the midline using a FOM (Fat-O-Meat'er, Carometec, Soeborg, Denmark).

Hot carcasses were processed according to the standard commercial procedure to obtain the main lean cuts (loin with ribs, neck with bones but without skin and subcutaneous tissues, shoulder

with bones and skin, and ham) and fat (backfat and belly) primal cuts, which were separately weighed.

A sample of the *Longissimus lumborum* (LL) including the last two lumbar vertebrae was collected from the left loin of each carcass, and each sample placed in individual plastic bags, refrigerated for 24 h, then vacuum-packed at -20 °C for subsequent analyses. After 24 h of chilling, the thighs were deboned, and then weighed.

The LL samples were thawed in vacuum-packaged bags for 24 h at 4 °C, then removed from the packaging and blotted for 15 min and weighed. Thawing losses were calculated as the difference in weight between the fresh and thawed samples expressed as a percentage of the initial fresh weight.

Cooking losses were determined on a subsample of LL 2.5 cm in thickness, which was weighed, sealed in a plastic bag, cooked in a water bath at 75° C until it reached a core temperature of 70° C, then cooled to room temperature, blotted and weighed. Cooking loss percentages were calculated by dividing the difference between the pre- and post-cooked weights by the pre-cooked weight.

Shear force was measured on five cylindrical cores 1.00 cm in diameter taken from the same cooked sample sheared perpendicularly with a Lloyd® (Bognor Regis, UK) LS 5 series Warner-Bratzler shearing device (shearing speed 2 mm s^{-1}) managed by the NEXIGEN Plus 3 software. The measurements from each sample were averaged before statistical analyses.

Another subsample of LL was ground, mixed and homogenised for 10 s at 4500 g in a Grindomix GM200 (Retsch, Haan, Düsseldorf, Germany) then analysed in duplicate for moisture (# 950.46), protein (# 981.10), lipids (#991.36) and ash (# 920.153) [26].

Data editing

During the experiment, 4 pigs died or were discarded due to illness or injury and their data were removed from the database, thus the final dataset consisted of data from 92 pigs. Six behavioural traits were analysed from the data recorded by the feeding stations after excluding visits where feed consumption was zero (Table 2). The main details are given in Carcò et al. [12].

Protein (Pr) and lipid (Lr) retentions were estimated from BW and ultrasound BF measurements recorded at the beginning and end of the trial, as described in Schiavon et al. (2018) [17]. Residual metabolizable energy intake (REI, MJ/d) was determined for each pig as the difference between metabolizable energy (ME) intake and ME used for maintenance (MEm) and growth (MEg). ME intake was calculated as the total feed intake × ME of the diet; MEm as 0.845 MJ × the average BW of the period$^{0.6}$; and MEg as 44.35 × Pr + 52.30 × Lr, in accordance with the NRC [22].

Statistical analysis

Individual day-by-day patterns of variation in each behavioural trait were averaged for all the pigs of the experiment, edited, plotted and regressed against the days on feed in a spreadsheet. The SAS PROC CORR [27] was carried out to investigate the correlations between the behavioural traits and the days on feed, in order to test the magnitude and the significance of each trend.

The data regarding feeding behaviour and growth performance averaged by pig, and carcass and meat quality traits were analysed for deviation from normality in SAS [27].

To adjust the data for the effects of the feeding treatments, a preliminary analysis of the individual means of each trait was carried out using SAS PROC GLM [15] and a model that included feed restriction (FR), amino acid restriction (AAR), and the FR × AAR interaction as fixed effects, and pen within AAR as a random effect. The residuals were analysed using SAS PROC CORR [27], and the partial correlation coefficients among variables were computed. As feeding rate was found to be the behavioural trait most frequently and highly correlated with the

carcass characteristics, the records of each pig were classified according to feeding rate tertiles computed on the residuals of the previous model. Data were analysed by SAS PROC MIXED [27] using the model described above with the further inclusion of the feeding rate tertile as a fixed factor and its interaction with FR and AAR. As these interactions were never significant, they were excluded from the final model. The pig was considered to be the experimental unit to test the influence of the feeding rate. Two of the three degrees of freedom of the feeding rate tertile were used to evaluate the significance of the linear and quadratic components.

RESULTS

Patterns in the feeding behaviour traits

Readers are referred to Carcò et al. [12] and Schiavon et al. [17] for details on the effect of feed allowance and AA level on feeding behaviour, growth performance and carcass and meat quality.

The average daily feed intake increased and the time spent eating decreased with the number of days on feed ($R^2 = 0.60$, $P < 0.001$, Fig 1A; and $R^2 = 0.50$, $P < 0.001$, Fig 1B, respectively), and there were only small variations in the number of visits to the manger ($R^2 = 0.08$, $P = 0.028$, Fig 2A). The standard deviation among individuals with respect to all these variables was large. The amount of feed consumed per visit increased quadratically ($R^2 = 0.75$, $P < 0.001$, Fig 2B) and there was a small change in the time spent eating per visit ($R^2 = 0.20$, $P < 0.001$, Fig 3A) with increasing days on feed. The feeding rate changed quadratically ($R^2 = 0.83$, $P < 0.001$, Fig 3B) and the standard deviation, in the order of 50% of the mean, increased notably towards the end of the period of observation.

Partial correlations between feeding behaviour and performance traits

The partial phenotypic correlations among the feeding behaviour traits are given in Table S1 of the supplementary material.

Daily feed intake was positively correlated with final BW (r = 0.821, $P < 0.001$), growth rate (r = 0.847, $P < 0.001$), Pr (r = 0.230, $P < 0.05$) and REI (r = 0.267, $P < 0.05$) (Table 3). Time spent eating was negatively correlated with final BW (r = -0.247, $P < 0.05$), growth rate (r = -0.253, $P < 0.01$), the gain:feed ratio (r = -0.224, $P < 0.05$) and Lr (r = -0.360, $P < 0.001$), but it did not affect feed intake and REI.

The number of feeding visits and the feeding time per visit were not correlated with growth performance and feed efficiency, whereas feed intake per visit was positively related to final BW (r = 0.268, $P <0.01$), growth rate (r = 0.251, $P < 0.05$), Lr (r = 0.260, $P < 0.05$) and daily feed intake (r = 0.203, $P <0.05$). The time spent per visit did not affect growth performance.

Feeding rate was the behavioural trait most highly correlated with final BW (r = 0.522, $P < 0.001$), growth rate (r = 0.539, $P < 0.001$), and both Pr (r = 0.408, $P < 0.001$) and Lr (r = 0.430, $P < 0.001$). Also, feeding rate was positively correlated with daily feed intake (r = 0.506, $P < 0.001$), but not with feed or energy efficiency traits.

Partial correlations between feeding behaviour, carcass traits and meat quality traits

Feed intake and feeding rate were the variables most highly correlated with the various carcass traits (Table 4). Feed intake was positively related to carcass weight (r = 0.840, $P < 0.001$), backfat thickness (r = 0.595, $P < 0.001$), the weights of all the untrimmed lean and fat cuts ($P < 0.001$) and the proportions of carcass fat components (r = 0.663, $P < 0.001$), but was negatively correlated with the proportions of lean cuts (r = -0.677, $P < 0.001$). Feed intake was also negatively related to the moisture content of the muscle (r = -0.249, $P < 0.05$) and positively related to the lipids content (r = 0. 265, $P < 0.01$).

Feeding rate was positively related to carcass weight (r = 0.535, $P < 0.001$), BF (r = 0.269, $P < 0.05$), the weights of all the separated lean and fat cuts ($P < 0.001$), and the proportions of carcass fatty tissues (r = 0.419, $P < 0.001$), and negatively related to the proportions of carcass lean cuts (r = -0.344, $P < 0.01$). We found no significant relation between feeding rate and the chemical and physical characteristics of the LL muscle.

Time spent eating was negatively related to carcass weight (r = -0.256, $P < 0.05$) and the weights of some lean and fat cuts. Feed intake per visit also was also related to carcass characteristics as it was positively related to carcass weight (r = 0.272, $P < 0.01$), the weights of some cuts, like the ham (r = 0.32, $P < 0.01$), belly (r = 0.288, $P < 0.01$) and total lean and fat cuts ($P < 0.05$).

The number of visits was poorly related to carcass and meat characteristics, although this trait was positively related to the moisture content of the *Longissimus lumborum* muscle (r = 0.303, $P < 0.01$) and negatively related to its protein content (r = 0.307, $P < 0.01$). The average time spent eating per visit was positively related only to the protein content of the *Longissimus lumborum* muscle (r = 0.268, $P < 0.01$).

Feeding rate, growth performance, carcass traits and meat quality

The distribution of RF and AL and HAA and LAA pigs in the classes of feeding rate was homogenous. The class of feeding rate had a linear influence on final BW ($P < 0.001$), growth rate ($P < 0.001$), Pr ($P < 0.001$), Lr ($P < 0.001$) and feed intake ($P < 0.001$) (Table 5), with values that increased moving from the first (12.6 to 38.2 g/min), to the second (38.3 to 51.6 g/min) and the third (52.1 to 118.9 g/min) tertiles. Moreover, it was consistently linearly related to carcass weight ($P < 0.001$), but not to carcass yield, BF thickness, loin depth and lean percentage (Table 6). Feeding rate was also positively linearly related to the weights of all the lean and fat cuts, the linear decrease in the carcass lean percentage ($P = 0.014$), and the linear increase in carcass fat content

($P < 0.001$). Since no correlation were found between feeding rate and meat quality traits, the class of feeding rate had no influence on the meat quality parameters.

DISCUSSION

Feeding rate, feed intake and growth performance

The current literature provides evidence that feeding rate could reflect the pig's feeding motivation, with faster rates associated with greater feeding motivation [3,12-13].

Firstly, greater feeding motivation may reflect a greater desire for the nutrients required for maintenance and for protein and lipid growth [13]. This would, in turn, result in greater feed intake, and different carcass and meat characteristics. In the companion paper to the current study, a reduction in the essential amino acid content of the diet was found to increase the feeding rate, feed intake, growth rate, carcass yield and carcass fat content [17]. Labroue et al. [5] found that the feeding rate had a high genetic correlation with daily feed intake (around 0.5) and average daily gain (around 0.4). In the current study, feeding rate was the variable most highly correlated with the estimated daily gains in protein and lipids, while the variation in feeding rate only partially explained the variation in daily feed intake ($r = 0.51$), due to the contextual variation in the number and duration of the feeding visits. The phenotypic relationship between feeding rate and daily feed intake was slightly stronger than that observed by de Haer & Merks [28], Labroue et al. [29] and Hyun et al. [30] in pigs penned in groups (r values ranging 0.17 to 0.41), but lower than that observed by de Haer & Merks [28] in individually penned pigs ($r = 0.81$).

Secondly, pigs have frequently been found to respond to a feeding constraint by increasing their feeding rate [31-32]. For example, recent experiments found that a feeding restriction increased the rate of feed consumption [12,17]. In this regard, Nielsen [13] suggested that feeding rate could be used as an indicator of social constraint. Young & Lawrence [33] found that where there was strong

competition among pigs for feed, there was an increase in the feeding rate and number of visits, with a consequent reduction in feed consumption per visit. Similarly, pigs housed in groups notably increased their feeding rate compared with pigs housed individually [4]. Nielsen [13] also reported that in a given social context the pig's feeding behaviour is influenced by the desire to eat at the same time as its conspecifics.

For the current study, the data presented in the companion papers of Schiavon et al. [17] and Carcò et al. [12] were statistically adjusted for the effects of the experimental treatments. Nevertheless, there was wide among-pig variation in feeding rate, which was about three times greater for the pigs in the third tertile than those in the first. The pigs in the third tertile - those that ate faster - had 16% heavier final body weights, 27% greater average daily weight gains, 22% greater estimated protein gains, and 46% greater estimated lipid retention than the pigs in the first tertile (13 to 38 g/min). The magnitude of these differences may be due to individual variations in the desired nutrient intake, which might be reflected in different body constituent growth rates, and/or to the social hierarchy, which impacts on the feeding strategy followed by each pig to reach its preferred, or constrained, feed intake. It should be borne in mind that in the current study the feeding station in each pen gave access to only one pig at a time, and it is not clear whether this restriction had an impact on the feeding motivation of the other pigs accessing it later. The role of the social environment in pig feeding behaviour and its impact on performance needs to be further clarified.

Feeding rate, and carcass and meat characteristics

Surprisingly, we found few studies on relationships between carcass and meat characteristics and feeding behaviour traits, despite the economic importance of this issue. De Haer et al. [2] found that pigs that consumed larger amounts of feed per visit and at faster rates of eating exhibited

greater growth rates, thicker carcass backfat depths and lower lean percentages. Colpoys et al. [3] did not find any significant correlations among feeding rate, daily feed intake, daily weight gain, and tissue accretion of protein, lean and fat estimated with dual X-ray tomography, but they only studied a small number of gilts fed either *ad libitum* or twice a day.

In the current study, the feeding rate had a strong influence on carcass characteristics. Compared to the group of pigs eating slowly, the pigs of the third tertile, eating at a rate of 52-119 g/min, had greater carcass weight (16%, without change in carcass yields), weight of lean cuts (14%), weight of fat cuts (21%), proportion of fat in carcass (14%), and a corresponding 4% decrease of the proportion of carcass lean cuts. Data measured on the carcass were quantitatively consistent with the *in vivo* estimates of Pr and Lr. Interestingly, the feeding rate had almost no effect on feed efficiency and meat quality traits. These results are similar to the findings of de Haer et al. [2], and to those of Rauw et al. [7] who found that the pigs that ate faster also ate more and grew faster and became fatter, but with the same residual feed intake. In some productive situations, strategies to increase the feeding rate may be based on dietary imbalances in some nutrients. For example, Schiavon et al. [17] found that a small reduction in dietary amino acid stimulated the pigs to increase their feed intake to compensate for this reduction, but in doing so they consumed more energy, and increased growth and fat accretion. The dataset we used in the current study was not suitable for estimating the heritability and the genetic correlations. However, previous studies of Von Felde et al. [34] and Schulze et al. [35] have found high heritability (h^2 = 0.42-0.51) for several feeding behaviour traits. This information is needed to assess whether the rate of feed intake, or any other feeding behaviour trait, could be included in the selection objectives.

CONCLUSIONS

The results of the current study support the idea that the feeding rate reflects the pig's feeding motivation, with faster rates associated with increased feeding motivation. Feeding behaviour traits were highly correlated with growth performance and carcass quality. Namely, growth rate, final body weight and carcass traits were positively related to feed intake and feeding rate, but negatively related to the time spent eating. Among the behavioural traits, feeding rate was the one most frequently and highly correlated with daily feed intake, growth rate, protein and fat retention and many carcass traits. Manipulating the eating rate would affect feed intake and subsequently growth performance and carcass quality, but would have little influence on feed efficiency.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Enrico Sturaro for the technical support, Veronesi SPA (Quinto di Valpantena, Verona, Italy) for financial and technical support, and the Aldo Gini Foundation (Via Portello 15, Padua, Italy) for supporting the research activities of PhD student Giuseppe Carcò, during his study period at the University of Zaragoza (Spain).

REFERENCES

1. Maselyne J, Saeys W, Van Nuffel A. Review: Quantifying animal feeding behaviour with a focus on pigs. Physiol. Behav. 2015, 138: 37–51.
2. de Haer LCM, Luiting P, Aarts HLM. Relationship between individual (residual) feed intake and feed intake pattern in group housed growing pigs. Livest. Prod. Sci. 1993, 36: 233-253.
3. Colpoys JD, Johnson AK, Gabler NK. Daily feeding regimen impacts pig growth and behavior. Physiol. & Behav. 2016, 159: 27-32.

4. de Haer LCM, de Vries AG. Feed intake patterns of and feed digestibility in growing pigs housed individually or in groups. Livest. Prod. Sci. 1993, 33: 277-292.
5. Labroue F, Guéblez R, Sellier P, Meunier-Salaün MC. Feeding behaviour of group-housed Large White and Landrace pigs in French central test stations. Livest. Prod. Sci. 1994, 40: 303–312.
6. Meunier-Salaun MC, Guérin C, Billon Y, Sellier P, Noblet J, Gilbert H. Divergent selection for residual feed intake in group-housed growing pigs: characteristics of physical and behavioural acitivity according to line and sex. Anim. 2014, 8: 1898-1906.
7. Rauw WM, Soler J, Tibau J, Reixach J, Raya LG. Feeding time and feeding rate and its relationship with feed intake, feed efficiency, growth rate, and rate of fat deposition in growing Duroc barrows. Am. Soc. Anim. Sci.2006, 84: 3404–3409. doi:10.2527/jas.2006-209.
8. Le Naou T, Le Floc'h N, Louveau I, van Milgen J, Gondret F. Meal frequency changes the basal and time-course profiles of plasma nutrient concentrations and affects feed efficiency in young growing pigs, J. Anim. Sci. 2014, 92: 2008–2016.
9. Newman RE, Downing JA, Thomson PC, Collins CL, Henman DJ, Wilkinson SJ. Insulin secretion, body composition and pig performance are altered by feeding pattern. Anim. Prod. Sci. 2014, 54: 319–328.
10. Schneider JD, Tokach MD, Goodband RD, Nelssen JL, Dritz SS, DeRouchey JM, et al. Effects of restricted feed intake on finishing pigs weighing between 68 and 114 kilograms fed twice or 6 times daily, J. Anim. Sci. 2011, 89: 3326–3333.
11. Andretta I, Pomar C, Kipper M, Hauschild L, Rivest J. Feeding behavior of growing – finishing pigs reared under precision feeding strategies. J. Anim. Sci. 2016, 94: 3042–3050. doi:10.2527/jas2016-0392.

12. Carcò G, Dalla Bona M, Carraro L, Latorre MA, Fondevila M, Gallo L et al. Influence of mild feed restriction and mild reduction in dietary amino acid content on feeding behavior of group-housed growing pigs. Appl. Anim. Behav. Sci. 2018, 198: 27-35.
13. Nielsen BL. On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. Appl. Anim. Behav. Sci. 1999, 63: 79–91.
14. Emmans GC, Kyriazakis I. Consequences of genetic changes in farm animals on food intake and feeding behaviour. Proc.Nutr. Soc. 2001, 60: 115-125.
15. Emmans GC. A Method to predict the food intake of domestic animals from birth to maturity as a function of time. J. Theor. Biol. 1997, 186: 189-199.
16. Nyachoti CM, Zijlstra, RT, de Lange CFM., Patience JF. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can. J. Anim. Sci. 2004, 84: 549–566.
17. Schiavon S, Dalla Bona M, Carcò G, Carraro L, Bunger L, Gallo L. Effects of feed and indispensable amino acid restrictions on feed intake, growth performance and carcass characteristics of growing pigs. PLoS ONE 2018, https://doi.org/10.1371/journal.pone.0195645.
18. Mordenti A, Bosi P, Corino C, Crovetto GM, Casa GD, Franci O, et al. A methodological approach to assess nutrient requirements of heavy pigs in Italy. Ital. J. Anim. Sci. 2003, 2: 73-87.
19. Ferguson NS, Arnold GA, Lavers G, Gous RM. The response of growing pigs to amino acids as influenced by environmental temperature. 1 Threonine. Anim. Sci. 2000, 70: 287-297.
20. Schiavon S, Emmans GC. A model to predict water intake of a pig growing in a known environment on a known diet. Brit. J. Nutr. 2000, 84: 873-883.

21. Topigs, 2012. Feeding manual Talent.
Available from:
https://varkens.nl/sites/default/files/Feeding%20Manual%20Talent%20progeny%202012.pdf.
Accessed 05th April 2018.
22. NRC. Nutrient Requirements of Swine. 11th revised ed. Washington: National Academy Press; 2012.
23. Kloareg M, Noblet J, Van Milgen J. Estimation of whole lipid mass in finishing pigs. Anim. Sci. 2006, 82: 241-251.
24. EU. Commission Implementing Decision of 24 January 2014 authorising methods for grading pig carcases in Italy [notified under document C (2014) 279]. Off. J. L 23.
25. EU. 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.
26. AOAC. Official Methods of Analysis of the Association of Official Agricultural Chemists, 19th ed. AOAC International, 2012.
27. SAS. SAS Institute, SAS/STAT®. 9.4. Cary, NC. 2009
28. de Haer LCM., Merks JWM. Patterns of daily food intake in growing pigs. Anim. Sci. 1992, 54: 95-104.
29. Labroue F, Guéblez R, Sellier P. Genetic parameters of feeding behaviour and performance traits in group-housed Large White and French Landrace growing pigs. Genet. Sel. Evol. 1997, 29: 451–468.
30. Hyun Y, Ellis M, Mckeith FK, Wilson ER. Feed intake pattern of group-housed growing-finishing pigs monitored using a computerized feed intake recording system. J. Anim. Sci. 1997, 75: 1443–1451.

31. Bornett HLI, Morgan CA, Lawrence AB, Mann J. The flexibility of feeding patterns in individually housed pigs. Anim. Sci. 2000, 40: 457-469.
32. Nielsen BL, Lawrence AB, Whittemore CT. Effect of group size on feeding behaviour, social behaviour, and performance of growing pigs using single-space feeders. Livest. Prod. Sci. 1995, 44: 73–85.
33. Young RJ, Lawrence AB. Feeding behaviour of pigs in groups monitored by a computerized feeding system. Anim. Prod. 1994, 58: 145–152.
34. von Felde A, Roehe R, Looft H, Kalm E. Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars. Livest. Prod. Sci. 1996, 47: 11–22.
35. Schulze V, Roehe R, Bermejo JL, Looft H, Kalm E. The influence of feeding behaviour on feed intake curve parameters and performance traits of station-tested boars. Livest. Prod. Sci. 2003, 82: 105-116.

Table 1. Chemical composition (g/kg) and energy content (MJ/kg) of the diets.

Item	Growing (47 - 86 kg BW)	Early finishing (86 - 118 kg BW)		Late finishing (118 -145 kg BW)	
		High amino acid (HAA)	Low amino acid (LAA)	High amino acid (HAA)	Low amino acid (LAA)
Analyzed composition[1]					
Dry Matter	893	891	891	895	894
Crude Protein (N × 6.25)	163	159	141	161	133
Lysine	10.3	9.3	8.5	8.8	7.2
Methionine	3.5	3.1	2.7	3.0	2.5
Threonine	7.0	6.3	5.9	6.4	5.1
Tryptophan	2.2	2.0	1.8	1.9	1.6
Starch	387	440	454	421	454
NDF	120	111	111	123	130
Ether Extract	46	42	45	44	42
Ash	41	42	40	42	41
Calculated composition[2]					
Dry Matter	879	879	878	878	877
Net Energy	9.9	9.8	9.8	9.7	9.8
Crude Protein (CP)	161	158	143	155	126
SID Lysine[3], g/kg CP	56	51	52	47	48
SID Methionine[3], g/kg CP	20	17	17	17	17
SID Threonine[3], g/kg CP	37	34	34	34	33
SID Tryptophan[3], g/kg CP	10	10	10	10	10

[1]Analitical results as a mean from 3 independent replications.
[2] Computed from the ingredient composition according to NRC [22].
[3] SID: standardized ileal digestible amino acid content

Table 2. Individual feeding behaviour parameters and the criteria used to compute them.

Parameter	Criterion
Feed intake (g/d)	feed consumed in a given day by a pig
Time spent eating (min/d)	total duration of the visits in a given day by a pig
Feeding visits (n/d)	visit with feed intake > 0 g by a pig
Feed intake per visit (g/visit)	average amount of feed consumed per visit by a pig
Feeding time per visit (min/visit)	the time spent eating per visit by a pig
Feeding rate, g/min	feed intake per visit / visit duration by a pig

Table 3. Partial correlations between feeding behaviour traits and growth performance (n = 92)[1].

Item	Feed intake	Time spent eating	Feeding visits	Feed intake per visit	Feeding time per visit	Feeding rate
Initial body weight, kg	-0.083	0.019	-0.110	0.046	0.140	-0.054
Final body weight, kg	0.821***	-0.247*	-0.106	0.268**	-0.121	0.522***
Feed intake, kg/d	-	-0.143	-0.003	0.203*	-0.190	0.506***
Growth rate, kg/d	0.847***	-0.253**	-0.067	0.251*	-0.170	0.539***
Protein retention (Pr), g/d[2]	0.230*	0.167	-0.044	0.147	0.173	0.408***
Lipid retention (Lr), g/d[3]	-0.152	-0.360***	-0.069	0.260*	-0.070	0.430***
Feed efficiency (Gain:feed)	-0.096	-0.224*	-0.113	0.121	0.001	0.150
Residual energy intake[4]	0.267*	0.133	0.174	-0.168	-0.179	-0.001

[1] *, **, and *** stand for $P < 0.05$, $P < 0.01$ and $P < 0.001$.

[2] Estimated from body protein mass changes (kg), from 47 to 145 kg BW. Body protein mass was estimated as $0.1353 \times FFEBW^{1.1175}$ (NRC, 2012), where FFEBW is the fat free empty body weight.

[3] Estimated from body lipid mass changes (kg), from 47 to 145 kg BW. Body lipid mass was estimated from backfat and body weight (BW) according to Kloareg et al., [11].

[4] Computed as: metabolizable energy (ME) intake - (ME req. for maintenance + ME req. for growth), where ME req. for maintenance = $BW^{0.60} \times 0.845$ MJ, and ME req. for growth = $44.35 \times Pr + 52.30 \times Lr$ [10].

Table 4. Partial correlations between feeding behaviour traits and carcass and meat quality (n = 92)[1].

Item	Feed intake	Time spent eating	Feeding visits	Feed intake per visit	Feeding time per visit	Feeding rate
Carcass weight, kg	0.840***	-0.256*	-0.102	0.272**	-0.124	0.535***
Carcass yield, %	0.111	-0.057	0.002	0.039	-0.017	0.079
Backfat thickness[2], mm	0.595***	-0.050	0.064	0.093	-0.098	0.269*
Loin depth[2], mm	0.039	-0.012	-0.010	0.076	0.033	-0.028
Lean percentage (FOM)[3], %	-0.071	0.056	0.043	-0.052	-0.002	-0.061
Main untrimmed lean and fat cuts, kg:						
- loin with ribs	0.543***	-0.222*	-0.009	0.163	-0.136	0.387***
- neck	0.476***	0.118	-0.089	0.171	-0.011	0.249*
- shoulder	0.465***	-0.123	-0.045	0.114	-0.094	0.280**
- ham	0.625***	-0.305**	-0.219*	0.320**	-0.029	0.485***
- deboned ham	0.532***	-0.285**	-0.130	0.259*	-0.059	0.439***
- backfat	0.812***	-0.137	0.016	0.161	-0.175	0.452***
- belly	0.771***	-0.306**	0.096	0.288**	-0.121	0.557***
- total main lean cuts	0.613***	-0.256*	-0.116	0.225*	-0.101	0.440***
- total main fat cuts	0.857***	-0.230*	-0.037	0.236*	-0.163	0.539***
Yield of untrimmed lean and fat cuts, % of carcass:						
- total lean	-0.677***	0.086	0.002	-0.177	0.085	-0.344**
- total fat	0.663***	-0.165	0.027	0.158	-0.158	0.419***
Yield of deboned ham, % of untrimmed ham	-0.293**	0.086	0.239*	-0.183	-0.066	-0.168
Longissimus lumborum (LL) muscle composition, %						
- moisture	-0.249*	0.143	0.303**	-0.326**	-0.175	-0.191
- protein	-0.082	-0.047	-0.307**	0.204	0.268**	0.018
- lipids	0.265**	-0.099	-0.085	0.167	-0.004	0.157
- ash	0.029	0.033	-0.066	0.018	0.037	0.019
Water holding capacity of LL, %						
- thawing loss	0.099	-0.026	0.093	-0.055	-0.114	0.032
- cooking loss	-0.197	0.016	0.031	-0.046	0.008	-0.087
Warner-Bratzler shear force of LL, kg	-0.090	-0.012	-0.204	0.082	0.123	-0.047

[1] *, **, and *** stand for $P < 0.05$, $P < 0.01$ and $P < 0.001$.

[2] Assessed with a Fat-O-Meat'er between the third to fourth last ribs at 8 cm off the carcass midline.

[3] Calculated from backfat thickness and loin depth taken between the third to fourth last ribs at 8 cm off the carcass midline.

Table 5. Influence of feeding rate on the growth performance of barrows (n = 92).

Item	Class of feeding rate					*P values*[1]	
	12.6 to 38.2 g/min	38.3 to 51.6 g/min	52.1 to 118.9 g/min	SEM[2]		L	Q
Initial body weight, kg	48.5	45.7	47.3	1.19		0.46	0.14
Final body weight, kg	131.6	146.1	152.4	2.91		<0.001	0.24
Feed intake, kg/d	2.296	2.707	2.845	0.07		<0.001	0.11
Growth rate, kg/d	0.807	0.975	1.021	0.03		<0.001	0.07
Gain:feed ratio	0.352	0.360	0.360	0.01		0.39	0.65
Residual energy intake[3]	1.97	1.98	2.63	0.53		0.39	0.62
Protein retention (Pr), g/d[4]	143	164	174	5.00		<0.001	0.38
Lipid retention (Lr), g/d[5]	192	270	280	20.0		<0.001	0.08

[1] L = linear component, Q = quadratic component.

[2] Standard error of the means.

[3] Computed as: metabolizable energy (ME) intake - (ME requirement for maintenance + ME requirement for growth), where ME req. for maintenance = $BW^{0.60}$× 0.845 MJ (NRC, 2012) and ME req. for growth = 44.35 × Pr + 52.30 × Lr [10].

[4] Computed from body protein mass changes (kg), from 47 to 145 kg BW. Body protein mass was calculated as 0.1353 × fat free empty body $weight^{1.1175}$ [10].

[5] Computed from body lipid mass changes (kg), from 47 to 145 kg BW. Body lipid mass was estimated from backfat thickness and body weight [11].

Table 6. Influence of feeding rate on the carcass and meat quality traits of barrows (n = 92)

Item	Class of feeding rate					*P values*[1]	
	12.6 to 38.2 g/min	38.3 to 51.6 g/min	52.1 to 118.9 g/min	SEM[2]		L	Q
Carcass weight, kg	105.1	117.2	122.5	2.33		<0.001	0.25
Carcass yield, %	0.80	0.80	0.81	0.01		0.45	0.93
Backfat thickness[3], mm	16.5	20.9	20.4	1.41		0.05	0.16
Loin depth[3], mm	62.4	63.9	61.5	2.92		0.82	0.56
Lean percentage (FOM)[4], %	56.6	53.9	54.3	2.26		0.42	0.51
Main untrimmed lean and fat cuts, kg:							
- loin with ribs	18.4	19.5	20.4	0.44		0.002	0.79
- neck	7.69	8.10	8.42	0.20		0.013	0.86
- shoulder	15.9	17.1	17.4	0.38		0.007	0.33
- ham	28.2	31.3	32.4	0.61		<0.001	0.17
- deboned ham	17.9	19.4	20.1	0.38		<0.001	0.42
- backfat	7.03	8.78	9.81	0.52		<0.001	0.56
- belly	11.5	13.7	14.7	0.39		<0.001	0.27
- total main lean cuts	68.8	76.1	78.6	1.41		<0.001	0.28
- total main fat cuts	18.5	22.4	24.5	0.82		<0.001	0.37
Yield of untrimmed lean and fat cuts, % of carcass:							
- total lean	66.5	64.9	64.1	0.68		0.014	0.58
- total fat	17.6	19.2	20.1	0.49		<0.001	0.51
Yield of deboned ham, % of untrimmed ham	63.4	61.8	62.0	0.55		0.07	0.20
Longissimus lumborum (LL) muscle composition, %							
- moisture	71.6	70.8	70.9	0.36		0.15	0.29
- protein	23.5	23.7	23.4	0.24		0.87	0.34
- lipids	3.74	4.31	4.53	0.40		0.18	0.72
- ash	1.18	1.18	1.18	0.01		0.90	0.98
Water holding capacity of LL, %							
- thawing loss	10.2	9.59	11.6	1.01		0.33	0.30
- cooking loss	31.3	29.4	30.2	0.57		0.19	0.07
Warner-Bratzler shear force of LL, kg	2.19	2.41	2.16	0.124		0.84	0.12

[1] L = linear component, Q = quadratic component.

[2] Standard error of the means.

[3] Assessed with a Fat-O-Meat'er between the third to fourth last ribs at 8 cm off the carcass midline.

[4] Calculated from backfat thickness and loin depth taken between the third to fourth last ribs at 8 cm off the carcass midline [12,13].

Table S1. Partial correlations among feeding behavior traits (n = 92)[1].

Item	Time spent eating	Feeding visits	Feed intake per visit	Feeding time per visit	Feeding rate
Feed intake	-0.143	-0.003	0.203*	-0.190	0.506***
Feeding time	-	0.336**	-0.410***	0.226*	-0.892***
Feeding visits		-	-0.808***	-0.694***	-0.245*
Feed intake per visit			-	0.727***	0.394***
Feeding time per visit				-	-0.318**

[1] *, **, and *** stand for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Fig 1. Growing pigs' individual patterns in feed intake (A) and time spent eating (B) with increasing days on feeding regimes (n=92, Mean = thick line; mean ± standard deviation = dotted line, trend = thin line; the experiment started on the 13th day after the pigs' arrival).

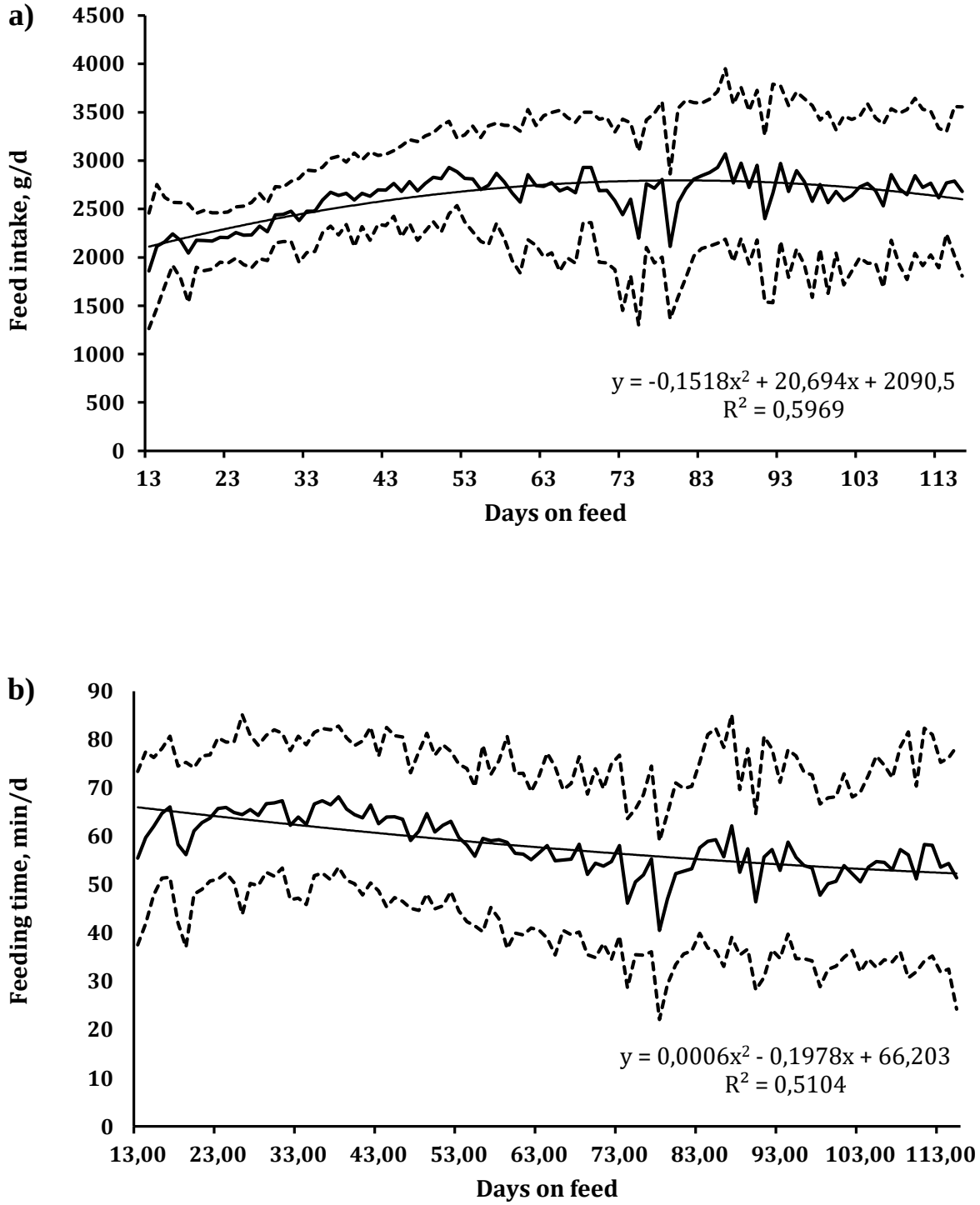
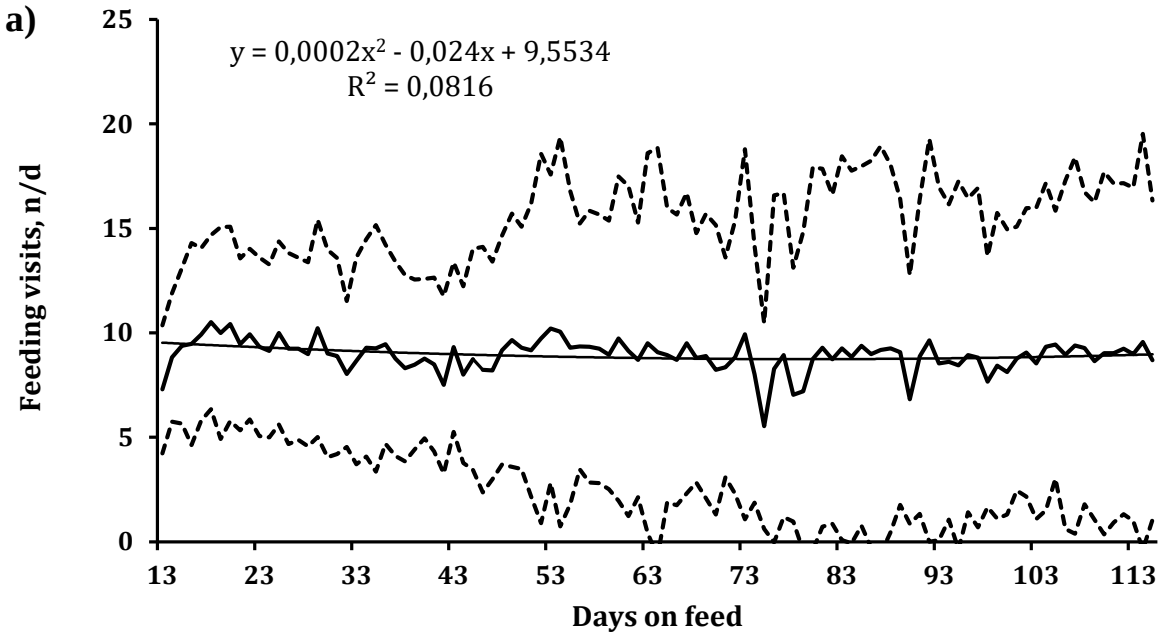


Fig 2. Growing pigs' individual patterns in the number of feeding visits (A) and feed intake per visit (B) with increasing days on feeding regimes (n=92, Mean = thick line; mean ± standard deviation = dotted line, trend = thin line; the experiment started on the 13th day after the pigs' arrival).



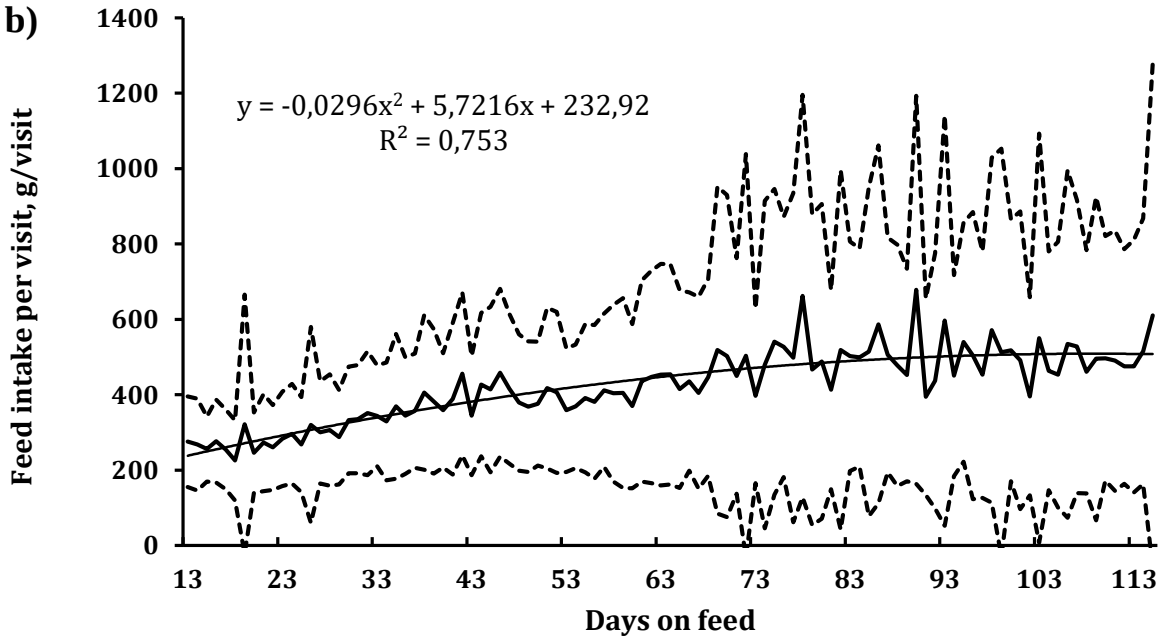
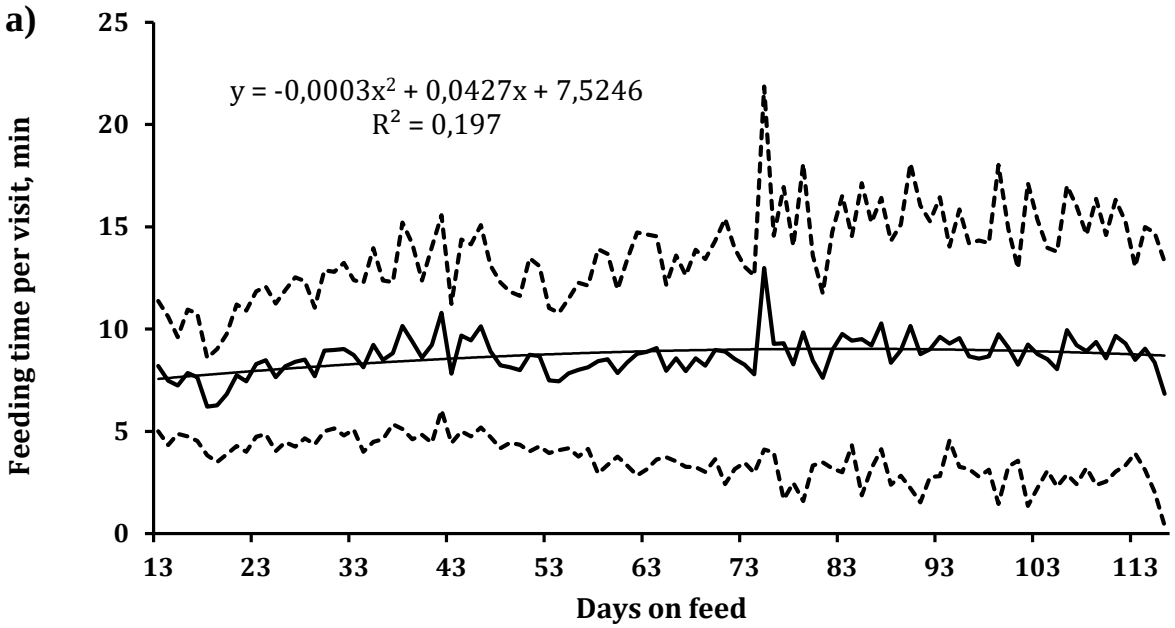
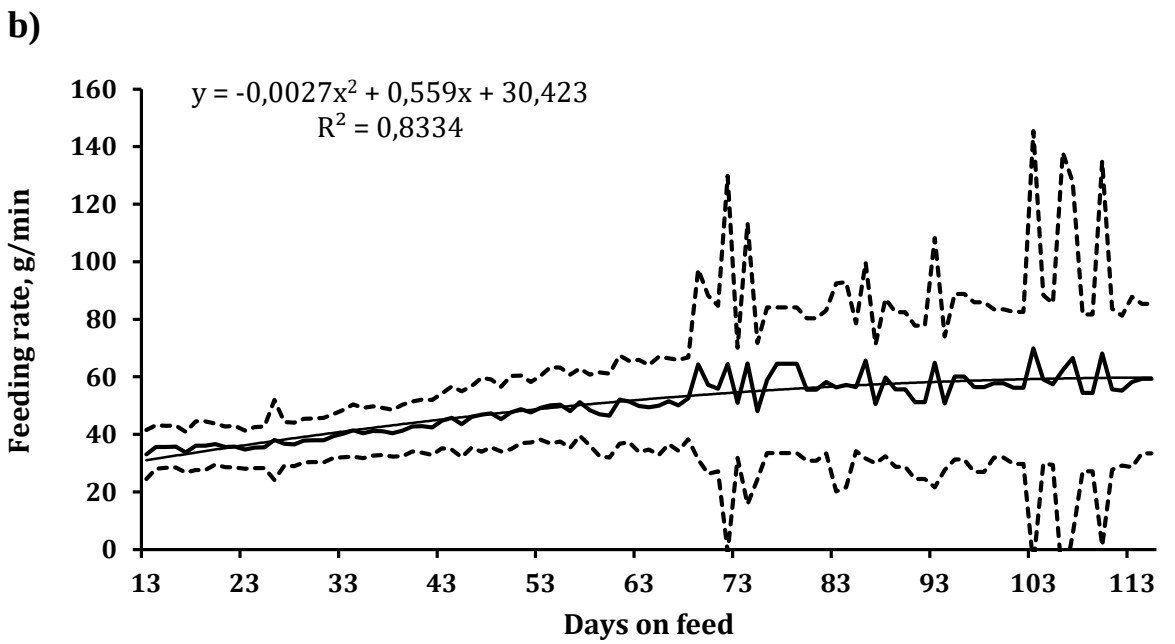


Fig 3. Growing pigs' individual patterns in duration of feeding time per visit (A) and feeding rate (B) with increasing days on feeding regime (n=92, Mean = thick line; mean ± standard deviation = dotted line, trend = thin line; the experiment started on the 13th day after the pigs' arrival).





6. CHAPTER 4th

Influence of the dietary crude protein content on the quality of San Daniele like dry-cured hams of two genetic group of pigs

Giuseppe Carcò[1]*, Stefano Schiavon[1], Ernestina Casiraghi[2], Silvia Grassi[2], Enrico Sturaro[1], Mirco Dalla Bona[1], Enrico Novelli[3], Luigi Gallo[1]

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

[2] *Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, via G. Celoria 2, 20133 Milan, Italy*

[3] *Department of Comparative Biomedicine and Food Science, University of Padov, Viale dell'Università 16, 35020 Legnaro (PD), Italy*

**Corresponding author*

E-mail: giuseppe.carco@phd.unipd.it

Submitted to Plos ONE

ABSTRACT

This study aimed to investigate the influence of low protein diets and two genetic groups of pigs with different lean growth potential on the quality traits of San Daniele like dry-cured hams. We analysed 40 left dry-cured hams produced by 96 pigs of four genetic groups fed conventional or reduced protein diets. The conventional feed was representative of those commonly distributed to heavy pig in Italy, while low-protein feed had a crude protein content reduced by 20% with respect to the conventional one. The selected hams were representative of the two genetic groups [Duroc-Danbred (Danbred) and Duroc × Large White (Anas)], the two dietary treatments and the two sexes. The Danbred fresh hams were heavier (P = 0.008) and had a thinner fat cover (P = 0.003) and greater seasoning losses (P = 0.002) than the Anas hams. The Danbred dry-cured hams had a higher protein content than the Anas ones (P = 0.039), but there were small differences between them in other quality traits. Salt content of the dry-cured ham was weakly related to ham fat cover, but it was related to water activity, seasoning losses and proteolytic index. Dietary protein restriction had small effects on dry-cured ham quality. Due to its positive effects on decreasing pig farm nitrogen excretion and feeding costs, low protein diets may be used to improve the environmental sustainability of the dry-cured ham industry.

Keywords: heavy pig; dry-cured ham; genetic group; low-protein diet; ham quality

INTRODUCTION

Dry-cured ham is a traditional product in many Mediterranean areas [1]. At present, European Union quality control schemes recognise over 30 types of dry-cured ham, roughly half of which are classified Protected Designation of Origin (PDO) and half Protected Geographical Indication [2].

It is generally agreed that ham quality depends on a combination of factors, including the pig genotype, feeding and management practices, and curing procedures [3-4]. Producers of Parma, San Daniele and Veneto PDO hams, for example, must comply with specific requirements regarding pig genotype and feeding practices [5-6]. Previous studies of the effects of pig genotype and feed composition on the quality of raw hams [7-8] have assumed the weight, back-fat cover and marbling of the raw material to be highly correlated with the final quality of the dry-cured hams [9-10]. When processing is standardised, it is reasonable to assume that the quality of the final product largely depends on the characteristics of the ham before curing [6], but this has yet to be verified.

The use of low protein and low amino acid feeds has recently emerged as one of the best strategies to reduce the environmental release of N compounds from pig farms. In many experiments, low-protein diets have been found to increase fat cover thickness and intramuscular fat [11-12]. Greater fat cover thickness would have a positive effect on seasoning losses and the final quality of the product [13-14]. In contrast, a lean pig genotype is assumed to negatively affect the quality of dry-cured hams, because of inadequate subcutaneous fat cover and “immature” meat [5]. These assumptions are reflected in the current regulations for the production of San Daniele and Parma PDO hams, which stipulate that they must come from at least 9 month-old pigs with a body weight of 165 kg and progeny of boars belonging to the Italian pig genotypes selected for PDO dry-cured ham production or to other genotypes yielding raw hams compliant with PDO requirements [15].

The aim of this study was to investigate the influence of reduced dietary protein and AA contents on the characteristics of San Daniele like dry-cured hams obtained from two genetic groups of pigs with different lean growth potential.

MATERIAL AND METHODS

Animals and experimental design

All the experimental procedures were approved by the "Ethical Committee for the Care and Use of Experimental Animals - CEASA" of the University of Padua, Italy. For this study, we randomly selected and analysed 40 of the 192 dry-cured hams used in a previous experiment [8]; details of the pigs, diets, growth performances, and meat and raw ham characteristics can be found in this paper. Briefly, that experiment involved 96 pigs with an average body weight (BW) of 38 kg ± 7.0 kg, allotted to 8 pens (12 pigs/pen) on the basis of their genetic group, sex and BW. These pigs belonged to four genetic groups, but for the current study we selected the pigs from only two of the groups. The first was a traditional cross between Italian Duroc boars (D) and Italian Large White (LW) sows selected by the Italian Pig Breeders Association national breeding programme (ANAS). D × LW (Anas) pigs are specifically intended for traditional heavy pig production and are genetically selected for carcass and ham quality traits [16-17]. The second group consisted of pig progeny of commercial Danish Duroc (Danbred) boars mated to crossbred sows of their parent sow lines, selected as an example of a lean pig genotype, but one that the regulations do not currently permit for the production of PDO dry-cured ham in Italy. The semen of five different boars for each genetic group was mixed and used to produce the pigs to be used in the experiment through heterospermic insemination. Twenty-four piglets for each genetic group, 12 gilts and 12 barrows, were randomly selected to be used in the *in vivo* experiment, and 6 gilts and 6 barrows for each genetic group were assigned to each dietary treatment.

All the animals were fed via automatic feeding stations and according to the restricted feeding regime, increased on weekly from 2.3 to 3.2 kg/d during the trial. From 89 kg BW onward,

the pigs in four pens were given conventional feeds (CONV), while the others received low-protein feeds (LP). The conventional feeds contained, in the early (89-120 kg BW) and late (121-165 kg BW) finishing periods, respectively, 13.1 and 13.2 MJ/kg of metabolisable energy (ME), 147 and 132 g/kg of crude protein (CP) and 6.0 and 4.4 g/kg of standardized ileal digestible (SID) lysine (Lys). The low-protein diets contained only 119 and 103 g/kg of CP and 4.8 and 3.5 g/kg of SID Lys in the early and late finishing periods, respectively. At about 165 kg BW and after 24 h of fasting the pigs were sent to the slaughterhouse, where they were electrically stunned and killed by exsanguination.

Fresh ham collection and measurements

Raw hams were separated from each carcass, refrigerated for 24 hours, trimmed and weighed. The final pH of each left ham was measured with a pH meter (HI-8314, Delta Ohm, Padova, Italy) equipped with a glass electrode and a temperature probe and calibrated at pH 7 and pH 4. Fat cover thickness was measured with a ruler at the level of the *Biceps femoris* muscle below the femur head of the ham. The fresh hams were scored for visual quality traits [8] then sent to the "Casa del Prosciutto" ham factory (Udine, Italy) to be processed into dry-cured hams in accordance with San Daniele procedures [18].

Dry-curing

The trimmed hams collected from each pig were salted with sea salt and stored at 2-3°C for the number of days corresponding to the weight in kg of each fresh ham [19]. After salting, the hams were weighed and salting losses calculated. The hams were then pressed for 48 hours to obtain the typical San Daniele guitar-like shape, and rested for 90 days at 4-6 °C and 70-80% relative air moisture. The hams were then rinsed with cold water, dried for one week, greased with a natural mixture made from lard, salt and cereal meal, ripened in a naturally ventilated room for 15 months, weighed, deboned and weighed again. Seasoning and deboning losses were measured.

Ham selection and sampling

We selected forty left hams, which were representative of two of the four genetic groups, the two dietary protein contents and the two sexes. For each genetic group and each dietary treatment, the left dry cured hams resulting from 5 barrows and 5 gilts were collected and analysed, according to a 2 genetic groups × 2 dietary treatments × 2 sexes × 5 replications factorial design. The Anas and Duroc-Danbred breeds were chosen on the basis of Schiavon et al. [8] results, because they showed the largest reciprocal differences between them in final back-fat thickness (20.2 *vs*. 15.8 mm, respectively), carcass lean yields (52.1 *vs*. 54.4%, respectively), dressed ham weights (14.2 *vs*. 15.1 kg, respectively) and fat cover thickness of the ham (24.8 *vs*. 19.4 mm, respectively), traits which reflected different aptitudes for lean growth.

The hams were cut below the head of the femur to obtain three slices of different thicknesses. The first slice (15 mm thick) was used to evaluate water activity and thiobarbituric acid reactive substances (TBARS), reflecting secondary lipid oxidation products. The second slice (14 mm thick) was used for physical and texture analyses, after which the lean part was separated from the fat with a knife, minced and analysed for proximate composition, Na content and fatty acid profile. The separated subcutaneous fat was analysed for fatty acid composition. The third slice (3 mm thick) was ground and analysed for proximate composition of the whole slice including subcutaneous fat.

Analyses of water activity and secondary lipid oxidation products (TBARS)

The water activity in the lean part of the ham was measured with a dew-point hygrometer (AquaLab 4 TEV, Decagon Devices, Pullman, WA, USA) on squared-shaped samples (15 × 15 mm) taken from the part closest to the *Biceps femoris* muscle.

The lean part and the subcutaneous fat of the slice were separated with a knife and minced, then 2-gram samples of each part were analysed for TBARS [20]. After adding 5 ml of n-heptane and 8 ml of trichloroacetic acid 5%, the samples were homogenized for 30 s at minimum speed using a rod homogenizer (T25 Digital Ultra-Turrax, Ika, Staufen, Germany). Following centrifugation

(2834 g for 3 min at 4 °C) the supernatant was removed and 2.5 ml of the lower layer was filtered and mixed with 2.5 ml of thiobarbituric acid (0.02M) in Pyrex test tubes. The solution was incubated in a Falc SB24 thermostatically-controlled water bath (Falc Instruments, Treviglio, Bergamo, Italy) at 95 °C for 35 min then cooled with running water. The absorbance of the chromatic complex was measured at 532 nm using a UV–Vis spectrophotometer (V-750, Jasco Europe, Cremella, Lecco, Italy) and expressed as milligrams of malondialdehyde/kg of sample using a calibration curve made with solutions of 1,1,3,3-Tetramethoxypropane at scalar concentrations (Sigma-Aldrich, Saint Louis, Missouri, USA).

Physical and texture analyses

Physical and texture analyses were performed on the four muscles of each slice (*Biceps femoris, bf*; *Quadriceps femoris, qf*; *Semimembranosus, sm*; and *Semitendinosus, st*; Fig 1). The lightness (L*), green-red (a*) and blue-yellow (b*) components of each muscle were assessed with a Minolta CM-500 colorimeter (Minolta, Osaka, Japan) with 10° standard observer, D65 illuminant and an aperture of 8 mm, according to CIE [21]. The pH was measured with a Crison Basic 20 pH meter (Crison SpA, Carpi, Modena, Italy).

The texture profile (TPA) was analysed with a TA.XT plus Texture Analyser (Stable Micro Systems, London, UK) at 15 °C with a 500 N load cell and a 20 mm compression probe. Each of the four muscles was compressed twice to 30% of its original thickness (14 mm) at a speed of 2 mm/s. The Texture Exponent software (Stable Micro System, London, UK) was used to compute the hardness, cohesiveness, adhesiveness, springiness and chewiness of each muscle from the two compression curves, according to Tabilo et al. [22]. Hardness (N) was defined as the maximum peak force, which is the force needed to obtain deformation. Cohesiveness (dimensionless) was defined as the ratio between the area under the second curve and the area under the first curve. Adhesiveness (N×s) was the negative area between the two curves, which represents the work needed to overcome the attractive forces between the compression device and the muscle surface. Springiness (dimensionless) was the ratio of the time recorded between the start of the second area

and the second probe reversal to the time recorded between the start of the first area and the first probe reversal, which represents the elasticity of the muscle. Chewiness (N) was calculated as hardness × cohesiveness × springiness.

Shear force was measured with a Warner-Bratzler texture analyser (LS5, Ametek Lloyd Instruments, Fareham, UK) equipped with an inverted V-shaped shear blade. Five 1-cm^3 prisms per muscle were obtained from each sample and cut with a force of 500 N and a speed of 2 mm/s. Shear force was then calculated with the NEXIGEN Plus 3 software (Bognor Regis, UK).

Chemical analyses

The proximate compositions of the minced whole slice and the lean part of it were determined for moisture (# 950.46), total protein N×6.25 (# 981.10), lipids (#991.36) and ash (# 920.153), according to AOAC [23]. On the lean part of the slice the soluble N was determined in trichloroacetic acid 10% solution [24]. Soluble protein was calculated as soluble N × 6.25. The proteolysis index was calculated as the percentage ratio between the soluble and total protein of the lean part.

Na was determined with an inductively coupled plasma - optical emissions spectrometer (ICP-OES; Ciros Vision EOP, Spectro Analytical Instruments GmbH, Kleve, Germany) using an aliquot of 1 g of the minced lean part of the slice, which was mixed with 7 ml of 67% nitric acid and 2 ml of 30% hydrogen peroxide and mineralized at 200 °C for 15-18 min in a microwave digestion system (Milestone Start, Sorisole, Bergamo, Italy). The samples were cooled to 35 °C and made up to volume with distilled water. Salt was calculated as Na × 2.50 [25].

Fat was extracted from the samples according to Schiavon et al. [26]. The lean and the subcutaneous fat were ground separately and homogenized for 10 s at 4500 g (Grindomix GM200; Retsch, Haan, Düsseldorf, Germany). A sub-sample of 20 to 30 g was stored at -20 °C until analysis. After thawing at ambient temperature, the fat was extracted from a 4.0 g subsample of each part mixed with 15 g of anhydrous sodium sulphate. The mixture was homogenized with a Hydromatrix (Phenomenex, Castel Maggiore, Bologna, Italy) and transferred to 15-mL stainless

steel extraction cells for accelerated solvent extraction (ASE, Thermo Fisher Scientific Inc., Waltham, MA, USA) with petroleum ether as the solvent. The extraction conditions were: temperature, 120 °C; pressure, 10 MPa; three static cycles of 1 min each; rinse, 100%; purge, 60 s using 8 mL/sample of fresh solvent [27]. The solvent was evaporated using a rotary film evaporator (Rotavapor® R-205, Buchi Italia s.r.l., Cornaredo, Italy) and samples were placed in an oven at 60 °C for 15 min before being weighed. An aliquot of 40 mg of extracted fat was collected to be methylated according to Christie [28], with minor modifications. Fat samples were transferred to a test tube fitted with a condenser, to which was added 2 mL of 2% sulphuric acid in methanol. The mixture was left overnight in a stoppered tube at 50 °C, then 2 ml of n-heptane and water (4 mL) containing potassium bicarbonate (2%) was added. Samples were centrifuged at 2834 g for 10 min, the supernatant was collected with a micropipette and transferred to a vial for gas chromatographic (GC) analysis.

The fatty acid methyl ester contents were determined with an Agilent 7820 gas chromatography system (Agilent, Palo Alto, CA, USA) equipped with a flame-ionization detector and an Omegawax 250 capillary column (Omegawax 250, Supelco, Bellefonte, PA, USA; 30 m, 0.25mm i.d.; film thickness 0.25 μm). The carrier gas was hydrogen at a flow rate of 1 mL min-1. A split/splitless injector with a split ratio of 1:80 was used to inject an aliquot of the sample into the GC system under the following conditions: initial oven temperature 60 °C held for 1 min, then increased to 173 °C at a rate of 2 °C/min and held for 30 min, then increased to 185 °C at 1 °C/min and held for 5 min, and finally increased to 220 °C at a rate of 3 °C/min and held for 19 min. The injector temperature was set at 270 °C and the detector temperature at 300 °C. Individual fatty acid methyl esters were identified by comparison with a standard mixture (18918-1AMP 595 N, Supelco, Bellefonte, PA, USA). Fatty acid methyl esters were quantified using methyl 12-tridecenoate as internal standard, and the area of each peak was corrected using flame ionization detector (FID) relative response factors. These response factors were determined using calibrations

obtained from five serial dilutions for each standard fatty acid [26]. All calibrations were linear and all R^2 were > 0.998. The FA composition was expressed as grams per 100 g of total FAs.

Statistical analysis

The SAS MIXED procedure (SAS Inst. Inc., Cary, NC) was used to analyse the data according to the following linear model:

$$y_{ijklm} = \mu + GG_i + diet_j + tissue_k + sex_l + GG \times diet_{ij} + GG \times tissue_{ik} + diet \times tissue_{jk} + GG \times diet \times tissue_{ijk} + ham(GG \times diet \times sex)_{m:ijl} + e_{ijklm};$$

where y_{ijklm} is the observed trait; μ is the overall intercept of the model; GG_i is the fixed effect of the ith genetic group (i = 1, 2); $diet_j$ is the fixed effect of the jth feeding treatment (j = 1, 2); $tissue_k$ is the fixed effect of the kth muscle (k = 1,…, 4) or part of slice (k = 1, 2); sex_l is the fixed effect of the lth gender (l = 1,2; [1 = barrow, 2 = gilt]); $GG \times diet_{ij}$ is the effect of the interaction between genetic group and diet; $GG \times tissue_{ik}$ is the effect of the interaction between genetic group and muscle or part of slice; $diet \times tissue_{jk}$ is the effect of the interaction between diet and muscle or part of slice; $GG \times diet \times tissue_{ijk}$ is the effect of the interactions among genetic group, diet and muscle or part of slice; $ham_{m:ijl}$ is the random effect of the m^{th} ham (m = 1,…, 40) within genetic group, diet and sex; e_{ijklm} is the random residual.

Ham within genetic group, diet and sex, and residuals were assumed to be independently and normally distributed with a mean of zero and variances of σ_{ham}^2 and σ_e^2, respectively. In line with the experimental design, the effects of genetic group, diet, sex and GG × diet were tested using ham within the GG × diet × sex interaction as the error line, whereas the effects of tissue and its interactions were tested on the random residual.

The 4 degrees of freedom of the $GG \times tissue_{ik}$ interaction were used to test the significances of the differences due to GG in the chemical compositions of both the whole slice and the lean part of it. The same was done with the $diet \times tissue_{ik}$ interaction to test the differences due to diet. Differences among muscles for pH, colour and the texture variables were compared using the Bonferroni correction for multiple testing.

Statistical analyses were carried out on the data regarding the weights and weight losses of hams, and the variables regarding the lean part of the slice only (salt, soluble protein, proteolysis index, water activity, TBARS) using a model without the effects of tissue and its interactions.

RESULTS

Weight changes

The trimmed Duroc-Danbred hams had nearly 24% less fat cover thickness ($P = 0.003$) than the Anas hams, but were heavier both before salting (5.6%, $P = 0.008$) and after (5.7%, $P = 0.004$) (Table 1). However, they also had greater weight losses at seasoning (10.1%, $P = 0.002$) and deboning (9.3%, $P = 0.001$), so that, despite their greater initial weight, they had similar weights to the Anas hams at the end of seasoning and deboning ($P = 0.08$ and 0.29, respectively). Diet and the GG × diet interaction had little or no influence on the weights and weight losses of the hams. Hams from barrows exhibited greater weight loss at salting (+34.2%, $P = 0.047$) but lower losses at deboning (-5.2%, $P = 0.048$) than those from gilts.

Chemical composition

The hams from the Duroc-Danbred pigs had a greater protein content (2.5%, $P = 0.039$) than those from Anas, and the hams from pigs fed the low protein diet had a greater lipid content (+9.8%, $P = 0.05$) and a lower protein content (-3.1%, $P = 0.003$) and protein:lipid ratio (-10.5%, $P = 0.008$) than those from pigs fed conventional diets (Table 2). However, diet interacted with GG for lipids ($P = 0.045$, Fig 2), as the dietary protein reduction increased the lipid content and lowered the protein content of the Anas hams compared with little variation in the Duroc-Danbred hams, and there was consistent variation in the protein:lipid ratio ($P = 0.023$). Genetic group and diet had no influence on the salt content of the lean part of the ham. The salt content was negatively correlated with water activity and the proteolysis index, and positively correlated with seasoning losses (Fig 3), but there was only weak correlation between cover fat thickness of the fresh ham and the salt content (Fig 4).

The lean part of the slice had greater water (10.4%, $P < 0.001$), protein (4.7%; $P < 0.001$) and ash (10.9%, $P < 0.001$) contents, a higher protein:lipid ratio (68.9%, $P < 0.001$) and a lower lipid content (-39.7%, $P < 0.001$) than the whole slice. However, a GG × tissue interaction was found for protein ($P = 0.038$) and lipid ($P = 0.003$) contents and for the protein:lipid ratio ($P = 0.004$). In fact, the whole slice from the Duroc-Danbred hams had a 4.4% greater protein content and a 9.1% lower fat content than Anas hams, but the composition of the lean part was similar in the hams from the two genetic groups (Fig 5).

Physical and textural traits

Genetic group and diet had little or no influence on pH, water activity, the colour variables and the textural traits (Table 3). However, almost all traits were markedly influenced by muscle and there was evidence for a significant D × T interaction for shear force and for the GG × T interaction for springiness (Fig 6). The shear force was 99% greater in the *semimembranosus* muscle than in the *biceps femoris*. The hardness and chewiness values of the *biceps femoris* and the *quadriceps femoris* were almost twice those of the *semitendinosus* and *semimembranosus* muscles ($P < 0.001$). The *biceps femoris* had the greatest adhesiveness and the lowest cohesiveness.

Fatty acid composition

The fatty acid profiles of the ham tissues were influenced by genetic group (Table 4). The Duroc-Danbred hams had a greater polyunsaturated fatty acid content ($P < 0.001$), mainly because of the 18:2 *cis*-9, *cis*-12 content ($P < 0.001$), and slightly lower amounts of some other monounsaturated and saturated fatty acids compared with Anas hams. The reduction in the dietary protein level had no significant influence on the fatty acid composition of the ham tissues. The intramuscular and the subcutaneous fatty acid profiles differed greatly, the former having a greater polyunsaturated fatty acid content ($P < 0.001$), mainly due to the proportions of 18:2 *cis*-9, *cis*-12 ($P < 0.001$) and 20:4 cis-5, cis-8, cis-11, cis-14, and a lower monounsaturated fatty acid content ($P < 0.001$), due to 18:1 *cis*-9 ($P < 0.001$).

DISCUSSION

Dietary protein restriction

The use of low protein diets is an effective strategy for reducing the environmental release of N and its potential polluting effects [29]. Previous experiments in the Italian heavy pig industry have found that diets formulated to contain a reduction from 146 to 117 g/kg of crude protein and from 7.3 to 5.8 g/kg of total lysine in early finishing (90 to 130 kg BW) and 133 to 108 g/kg of crude protein and 5.7 to 4.7 g/kg of total lysine in late finishing (130 to 165 kg BW) had negligible influence on growth performance [30], weight of carcass and primary cuts, and yields of the dressed hams [31], but greatly reduced N excretion [30]. Interestingly, Gallo et al. [13] found that reducing the 14% crude protein content in conventional diets to 11% increased the subcutaneous fat thickness, decreased linoleic and polyunsaturated fatty acid in fat depots, and reduced seasoning losses in fresh hams destined for PDO dry-cured ham production.

In a companion paper to the current experiment, Schiavon et al. [8] found that the reduction in crude protein had little impact on ham quality traits, but increased fat cover and marbling. The current experiment covers a gap in knowledge, as previous researches have looked only at the impact of low protein diets on the quality traits of fresh hams, and not at the true impact on the quality of dry-cured hams. The current experiment suggests that a low protein diet has little overall influence on the quality of dry-cured ham, although the protein content and the protein:lipid ratio of the whole slice were lower, and there was a tendency towards an increase in the lipid content. Previous work has already demonstrated the positive effects of LP diets on the proportion of fat in the carcass and in the meat [32,33]. These increases would depend on replacing the protein source with carbohydrates, which are more easily converted to fat [34]. Dietary CP reduction also tended to reduce the soluble protein content, but not the proteolysis index, and the values of the TBARS measured on the subcutaneous fat. The effect of these differences on the eating quality of the ham is unknown, and major research is required to clarify this. In any case, and in agreement with Grassi et al. [35], the differences in ham quality resulting from the use of low protein diets were

very small, thereby confirming them as a valid strategy for sustainable production of dry-cured ham.

Pig genetics

Pig genetics and feed characteristics are considered to be the most important factors affecting the quality of dry-cured hams [4-5]. Most of the literature in this area has focussed on the influence of genetics and feeding on the quality of the raw ham [7,10,13] and less attention is paid to the quality of the dry-cured ham [36]. Furthermore, few experiments have compared the quality traits of dry-cured hams obtained from pigs of different genetic groups and fed on different diets under the same rearing conditions [5].

Pigs with different genetic backgrounds differ in growth rates, carcass composition, lean/fat ratios and adipose tissues characteristics [4]. As discussed in a companion paper [8], the Duroc-Danbred pigs used in the current experiment and fed restrictively exhibited greater feed efficiency (gain:feed, 0.271 *vs*. 0.269) and total carcass lean cuts (54.4 *vs*. 52.1 kg/100 kg carcass), and thinner carcass back-fat cover (30.2 *vs*. 34.1 mm) than D × LW Anas pigs, but had the same average daily gain (0.703 *vs.* 0.700 kg/d). Furthermore, the fresh hams obtained from the Duroc-Danbred pigs were 5.6% heavier with 24% less fat cover thickness and a 42% greater marbling score than the D × LW Anas pigs [8]. These data show that when kept under the same feeding and rearing conditions the two genetic lines have different levels of leanness. The results were consistent with previous studies in which Danish lines derived from Duroc were compared with the traditional lines used to produce Italian dry-cured hams [7, 37]. Vitale et al. [37] found that the thighs from Duroc-Danbred were heavier but had lower fat thickness and seasoning aptitude, with losses greater than 30%, compared with other traditional or commercial genetic lines.

It is generally agreed that raw hams from lean pig genotypes are less suitable for the production of dry-cured hams because leaner carcasses and thinner subcutaneous fat cover are frequently associated with high seasoning losses, high salt absorption, increased dehydration and hardening of the meat, and the development of a salty flavour [38]. For these reasons, the consortia

for the protection of Parma and San Daniele hams restrict the breeds that can be used as boar line in the crossbreeding schemes aimed to originate heavy pigs for traditional dry-cured ham production. Italian Large White, Italian Landrace and Italian Duroc boars are always compliant with PDO dry-cured ham production, while other breeds can be used as sire lines only if they originate from selection schemes having purposes consistent with those of this type of production [5]. The Duroc breed, which was the sire breed of both genetic groups used in the current experiment, is allowed only as a component of a crossbreed because it has a greater intramuscular fat content than other breeds, and this is acknowledged to have a positive effect on the sensory quality of meat products [39].

High fat cover and high intramuscular fat content of the ham are a barrier to water diffusion and salt penetration [4,40]. Seasoning losses in ham are known to be inversely related to the depth of fat cover [9], which, in turn, is related to the depth of back-fat at the loin [41]. Rapid desiccation can also cause a crust to form on the surface, and once this has occurred, further diffusion of water is difficult so that the inner part of the ham becomes soft [1]. In the current experiment, and in line with expectations, seasoning losses were 4% higher in the Duroc-Danbred than in the D × LW Anas hams, reflecting the leaner characteristics of the former breed. Despite the initial weight differences, at the end of seasoning the ham weight of the two genetic group did not differ. In addition, at the end of seasoning, the lipid and the protein content of the ham lean part did not differ in the two genetic groups, but the Duroc-Danbred ham still tended to be 5% richer in protein and 10% poorer in lipid than that from the D × LW Anas pigs, reflecting the different fat cover of the two genetic groups hams. This result was consistent with the values of fat thickness measured on the fresh hams. The fatty acid profiles of the various fatty depots in the ham showed there to be an 8% greater proportion of polyunsaturated fatty acid in the Duroc-Danbred than in the D × LW Anas hams, consistent with the observation that a reduction in back-fat thickness is associated with an increase in the proportion of polyunsaturated fatty acid [7].

Salt confers a salty flavour to the meat and diminishes the health properties of the ham [42-43]. The negative relationship between meat salt content and the proteolysis index found in the current experiment confirmed the anti-proteolytic properties of salt [44]. The salt content of the lean was also negatively related to water activity and positively related to seasoning losses.

Surprisingly, despite notable differences in fresh ham weight, the quantity and quality of fat, and seasoning losses, there were only small differences between the hams of the two genetic groups in other quality traits, such as salt content, soluble protein, proteolysis index, TBARS measured in the muscle and the adipose tissue, and physical and texture characteristics. The relationships between ham fat thickness and these quality traits, including the salt content, were weak. The small correlation between fresh ham fat thickness, salt content and the other quality traits may be partly due to the small number of hams examined in the current experiment and the high degree of variation in some variables. Further experiments with a greater number of dry-cured hams are, therefore, needed.

CONCLUSIONS

There is potential to use low protein diets in the production of San Daniele dry-cured PDO hams as it reduces the release of N into the environment but has little influence on the various quality traits of the hams. This study also provides evidence that pig genotypes, namely Duroc-Danbred and Italian Duroc x LW, with different potentials for lean growth present notable differences in the initial ham weight, fat cover and seasoning losses. Differences in the various chemical, physical and textural quality traits of the dry-cured hams due to genetic group were less evident, and further research with a greater number of hams is required.

ACKONOWLEDGEMENTS

Supported by: Progetto AGER (Italy), Grant 2011-0280, and University of Padova, DOR1655405/16. The authors greatly appreciate the technical assistance of A. Simonetto and L.

Carraro, all the herdsmen, and all the laboratory staff. Our grateful thanks to the Agricola Tre Valli of the Gruppo Veronesi for excellent collaboration and technical assistance. The Authors also thank the Aldo Gini Foundation (Via Portello 15, Padua, Italy) for supporting the research activities of PhD student Giuseppe Carcò, M. Pasqualotto and G. Tonin for their support in the assessment of raw hams, and G. Cadel and M. Bassi (Regional Breeders Association of Friuli Venezia Giulia, AAFVG) for their support in pig retrieval.

REFERENCES

1. Toldrá F, Aristoy MC. (2010). Dry cured ham. In: F. Toldrá editor. Handbook of meat processing. Ames, Iowa: Blackwell Publishing; 2010. pp. 351-362.
2. EC, 2015. http://ec.europa.eu/agriculture/quality/door/list.html (accessed 15/05/2018)
3. Bermúdez R, Franco D, Carballo J, Lorenzo JM. Physicochemical changes during manufacture and final sensory characteristics of dry-cured Celta ham. Effect of muscle type. Food Contr. 2014; 43: 263–269.
4. Čandek-Potokar M, Škrlep M. Factors in pig production that impact the quality of dry-cured ham: a review. Anim. 2012; 6: 327-338. http://doi.org/10.1017/S1751731111001625
5. Bosi P, Russo V. The production of the heavy pig for high quality processed products. Ital. J. Anim. Sci. 2004; 3: 309–32145.
6. 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.
7. Lo Fiego DP, 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.
8. Schiavon S, Carraro L, Dalla Bona M, Cesaro G, Carnier P, Tagliapietra F, Sturaro E, Galassi G, Malagutti L, Trevisi E, Crovetto GM, Cecchinato A, Gallo L. 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.

9. Gou P, Guerrero L, Arnau, J. Sex and crossbreed effects on the characteristics of dry-cured ham. Meat Sci. 1995; 40: 21–31.
10. Peloso JV, Lopes PS, Gomide LAM, Guimarães SEF, Carneiro PLS. 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.
11. Rodríguez-Sánchez JA, Sanz MA, Blanco M, Serrano MP, Joy M, Latorre MA. 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.
12. Schiavon S, Dalla Bona M, 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; https://doi.org/10.1371/journal.pone.0195645.
13. 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.
14. Suárez-Belloch J, Latorre MA, Guada JA. The effect of protein restriction during the growing period on carcass, meat and fat quality of heavy barrows and gilts. Meat Sci. 2016; 112: 16–23.
15. EC, 2006. Prosciutto di San Daniele. Protected Designation of Origin. Publication pursuant to article 18/(2) of the commission regulation (EC) No. 1898/2006, in accordance with the article 17 of Council Regulation (EEC) No. 2081/92 of July, 14th 1992. Off. J. Eur. Commun. C188, 24–27
16. Fontanesi L, Schiavo G, Galimberti G, Calò DG, Scotti E, Martelli PL, Buttazzoni L, Casadio R, Russo, V. A genome wide association study for backfat thickness in Italian Large White

pigs highlights new regions affecting fat deposition including neuronal genes. BMC Genom. 2012; 13: 583.

17. Sturaro E, Gallo L, Noventa M, Carnier P. The genetic relationship between enzymatic activity of cathepsin B and firmness of dry-cured hams. Meat Sci 2008; 79: 375–381.
18. Laureati M, Buratti S, Giovanelli G, Corazzin M, Lo Fiego DP, 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.
19. Gaspardo B, Procida G, Toso B, Stefanon B. Determination of volatile compounds in San Daniele ham using headspace GC–MS. Meat Sci. 2008; 80: 204–209.
20. Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ, Trakatellis, AG. Rapid, Sensitive, and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animal Tissue, Food, and Feedstuff Samples. J. Agric. Food Chem. 1994; 42: 1931-1937.
21. CIE, 1978. Recommendations on uniform color spaces-color difference equations psychometric color terms. Paris: Commission International de L'Eclairage Supplement No. 2 to CIE Publication No. 15 (E-1.3.1) 1971/(TC-1.3).
22. Tabilo G, Flores M, Fiszman SM, Toldrá F. Postmortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham. Meat Sci. 1999; 51: 255–260.
23. AOAC (2003). Official Methods of Analysis of the Association of Official Agricultural Chemists, 19th ed. AOAC International, Gaithersburg, MD, USA.
24. Careri M, Mangia A, Barbieri G, Bolzoni L, Virgili R, Parolari G. Sensory property relationships to chemical data of Italian-type dry-cured ham. J. Food Sci. 1993, 58, 968–972.
25. EU, 2011. Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011. Official Journal of the European Union 22/11/2011.
26. Schiavon S, Pellattiero E, Cecchinato A, Tagliapietra F, Dannenberger D, Nuernberg K, Nuemberg G, Bittante G. The influence of different sample preparation procedures on the determination of fatty acid profiles of beef subcutaneous fat, liver and muscle by gas

chromatography. J. Food Comp. Analysis 2016; 50: 10–18.

27. Schäfer K. Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. Analytica Chim. Acta, 1998; 358: 69–77.
28. Christie WW. Preparation of ester derivatives of fatty acids for chromatographic analysis. In: Christie WW editor. Advances in Lipid Methodology – Two. Dundee, Scotland, United Kingdom: Oily Press; 1993. pp. 69-111
29. Canh TT, Aarnik AJA, Schutte JB, Sutton A, Langhout DJ, Verstegen, MWA. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing–finishing pigs. Livest. Prod. Sci. 1998; 56: 181-191.
30. 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. Livestock Sci. 2014; 161: 130–138.
31. Gallo L, Dalla Bona M, 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.
32. Lambe NR, Wood JD, McLean KA, Walling GA, Whitney H, Jagger S, Fullarton P, Bayntun J, Bünger L Effects of low protein diets on pigs with a lean genotype 2. Compositional traits measured with computed tomography (CT). Meat Sci. 2013; 95: 129–136.
33. Wood JD, Lambe NR, Walling GA, Whitney H, Jagger S, Fullarton PJ, 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.
34. Tous N, Lizardo R, Vilà B, Gispert M. Effect of reducing dietary protein and lysine on growth performance, carcass characteristics, intramuscular fat, and fatty acid profile of finishing barrows 1. J. Anim. Sci. 2014; 92: 129–140.
35. 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.

36. Carrapiso AI, García C. Effect of the Iberian pig line on dry-cured ham characteristics. Meat Sci. 2008; 80: 529–53416.

37. Vitale M, Corazzin M, Favotto S, Saccà E, Piasentier E. Variability in the characteristics of fresh meat and thighs in relationship to genetic type of the heavy pig. Ital. J. Anim. Sci. 2008; 8: 561–563.

38. Ruiz-Carrascal J, Ventanas J, Cava R, Andrés AI, García C. Texture and appearance of dry cured ham as affected by fat content and fatty acid composition. Food Res. Intern. 2000; 33: 91–95.

39. Cilla I, Altarriba J, Guerrero L, Gispert M, Martínez L, Moreno C, Beltrán JA, Guàrdia MD, Diestre A, Arnau J, Roncalés P. Effect of different Duroc line sires on carcass composition, meat quality and dry-cured ham acceptability. Meat Sci. 2006; 72: 252–260.

40. Capraro D, Buccioni A, Piasentier E, Spanghero M. Feeding finishing heavy pigs with corn silages: effects on backfat fatty acid composition and ham weight losses during seasoning. Ital. J. Anim. Sci. 2017; 16: 588–592.

41. 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.

42. Jiménez-Colmenero F, Ventanas J, Toldrá F. Nutritional composition of dry-cured ham and its role in a healthy diet. Meat Sci. 2010; 84: 585–593.

43. Ruusunen M, Puolanne E. Reducing sodium intake from meat products - a review. Meat Sci. 2005; 70:531-541.

44. Sárraga C. Meat proteinases and their relation with curing. In: Smulders FJM, Toldrá F, Flores J, Prieto M. editors. New technologies for meat and meat products. Nijmegen, The Netherlands: Audet Tijdschriften BV; 1992. pp. 233–246.

Table 1. Weights and losses of dry-cured hams obtained from pigs of different genetic groups and sex fed on conventional (CONV) or low protein (LP) diets.

	Diet (D)				Genetic group (GG)				D×GG
	CONV	**LP**	**SEM**	***P***	**Anas**	**Danbred**	**SEM**	***P***	***P***
Raw ham fat thickness[1], mm	19.8	21.9	1.21	0.23	23.7	18.1	1.20	0.003	0.72
Hams weight, kg:									
raw (trimmed)	14.7	14.8	0.21	0.70	14.4	15.2	0.21	0.008	0.36
after salting	14.4	14.4	0.18	0.92	14.0	14.8	0.18	0.004	0.29
after seasoning	10.1	10.1	0.15	0.72	9.92	10.3	0.15	0.08	0.37
after deboning	7.59	7.65	0.13	0.72	7.52	7.72	0.13	0.29	0.66
Weight losses, kg									
after salting[2]	0.37	0.46	0.04	0.15	0.39	0.43	0.04	0.48	0.97
after seasoning	4.65	4.69	0.10	0.77	4.44	4.89	0.10	0.002	0.55
after deboning[3]	7.13	7.18	0.13	0.78	6.84	7.47	0.13	0.001	0.29
Weight losses, %									
after salting	2.45	3.03	0.26	0.12	2.70	2.78	0.26	0.82	0.86
after seasoning	31.6	31.7	0.42	0.96	30.9	32.2	0.42	0.041	0.99
after deboning	48.4	48.4	0.49	0.93	47.6	49.2	0.49	0.030	0.56

[1] External fat cover thickness was measured with a ruler on the *Biceps femoris* muscle below the head of the femur of the fresh trimmed ham.
[2] Weight losses after salting were 0.47 kg for barrows and 0.35 kg for gilts ($P = 0.047$; SEM = 0.003).
[3] Weight losses after deboning were 6.97 kg for barrows and 7.33 kg for gilts ($P = 0.048$; SEM = 0.13).

Table 2. Chemical characteristics of the dry-cured hams obtained from pigs of different genetic groups and sex[1] fed on conventional (CONV) or low protein (LP) diets.

	Diet (D)				Genetic group (GG)				Tissue (T)				GG ×D
	CONV	**LP**	**SEM**	***P***	**Anas**	**Danbred**	**SEM**	***P***	**Slice, whole**	**Slice, lean part**	**SEM**	***P***	***P***
Chemical composition, g/kg:													
Water	507	505	3.3	0.67	508	504	3.3	0.39	481	531	3.2	<0.001	0.06
Protein[2]	289	280	2.1	0.003	281	288	2.1	0.039	278	291	1.7	<0.001	0.36
Ash	70.6	69.3	1.2	0.48	68.6	71.3	1.2	0.12	66.3	73.5	0.9	<0.001	0.49
Lipid[3]	133	146	4.5	0.05	142	137	4.5	0.44	174	105	4.2	<0.001	0.045
Protein:Lipid[4]	2.37	2.12	0.06	0.008	2.25	2.25	0.06	0.99	1.67	2.82	0.06	<0.001	0.023
Soluble protein[5]	81.5	78.9	1.0	0.07	80.3	80.2	1.0	0.98	-	-	-	-	0.54
Salt[5]	53.1	51.9	0.9	0.35	51.6	53.4	0.9	0.18	-	-	-	-	0.32
Proteolysis index[5]	0.277	0.275	0.004	0.68	0.276	0.276	0.004	0.92	-	-	-	-	0.69
TBARS, mg/kg:[6]													
Biceps femoris muscle	0.54	0.55	0.03	0.85	0.56	0.52	0.03	0.44	-	-	-	-	0.12
subcutaneous fat	0.56	0.48	0.03	0.07	0.52	0.53	0.03	0.77	-	-	-	-	0.11

[1] The fixed effect of sex was not significant.
[2] The least square means of the GG ×D interaction are given in Figure 2, the *P* value of the GG × T interaction was 0.038 (Figure 3).
[3] The least square means of the GG ×D interaction are given in Figure 2, the *P* value of the GG × T interaction was 0.003 (Figure 3).
[4] The least square means of the GG ×D interaction are given in Figure 2, the *P* value of the GG × T interaction was 0.004 (Figure 3).
[5] Measured on the lean part of the slice
[6] TBARS: thiobarbituric acid reactive substances.

Table 3. Physical characteristics of the different muscles of dry-cured hams obtained from pigs of different genetic groups and sex[1] fed on conventional (CONV) or low protein (LP) diets.

	Diet (D)				Genetic group (GG)				Tissue (T)[2]						GG×D
	CONV	**LP**	**SEM**	***P***	**Anas**	**Danbred**	**SEM**	***P***	**BF**	**QF**	**SM**	**ST**	**SEM**	***P***	***P***
pH	5.49	5.52	0.01	0.07	5.51	5.50	0.01	0.39	5.51^{bc}	5.53^{c}	5.50^{b}	5.48^{a}	0.01	<0.001	0.49
Water activity[3]	0.90	0.90	0.002	0.76	0.90	0.89	0.002	0.36	-	-	-	-	-	-	0.84
Colour:															
Lightness (L*)	37.5	37.3	0.29	0.64	37.7	37.2	0.29	0.30	38.1^{c}	35.6^{b}	33.5^{a}	42.7^{d}	0.34	<0.001	0.37
Green-red (a*)	7.00	6.92	0.18	0.78	7.05	6.87	0.18	0.46	6.86^{b}	8.28^{d}	7.53^{c}	5.18^{a}	0.19	<0.001	0.77
Blue-yellow (b*)	8.24	8.30	0.12	0.75	8.26	8.28	0.12	0.92	7.47^{a}	8.61^{b}	7.02^{a}	9.97^{c}	0.14	<0.001	0.80
Texture:															
Shear force, N[4]	32.8	33.6	1.10	0.59	33.0	33.4	1.10	0.81	21.4^{a}	36.2^{b}	42.5^{c}	32.7^{b}	1.56	<0.001	0.22
Hardness (30%), N	20.7	19.3	1.09	0.36	19.1	21.0	1.09	0.22	26.3^{b}	24.0^{b}	13.6^{a}	16.2^{a}	1.07	<0.001	0.30
Adhesiveness, N×s	-1.73	-1.73	0.08	1.00	-1.81	-1.66	0.08	0.20	-2.00^{c}	-1.48^{a}	-1.84^{bc}	-1.62^{ab}	0.09	<0.001	0.88
Cohesiveness	0.54	0.55	0.01	0.25	0.55	0.54	0.01	0.56	0.53^{a}	0.60^{b}	0.53^{a}	0.52^{a}	0.01	<0.001	0.51
Springiness[5]	0.73	0.73	0.01	0.81	0.73	0.73	0.01	0.81	0.74	0.72	0.72	0.72	0.01	0.34	0.10
Chewiness, N	8.42	7.92	0.52	0.50	7.76	8.58	0.52	0.27	10.6^{b}	10.6^{b}	5.33^{a}	6.18^{a}	0.54	<0.001	0.65

[1] The fixed effect of sex was not significant.

[2] BF: *Biceps femoris*; QF: *Quadriceps femoris*; SM: *Semimembranosus muscle*; ST: *Semitendinosus muscle.*

[3] Measured on square samples (15 × 15 mm) taken from close to the *Biceps femoris* muscle.

[4] The *P* value of the D × T interaction was 0.014 (Figure 4a).

[5] The *P* value of the GG × T interaction was 0.046 (Figure 4b).

Table 4. Fatty acid compositions of the intramuscular (IM) and subcutaneous (SC) fat of dry-cured hams obtained from pigs of different genetic groups and sex fed on conventional (CONV) or low protein (LP) diets.

	Diet (D)				Genetic group (GG)				Tissue (T)				GG×D
	CONV	**LP**	**SEM**	***P***	**Anas**	**Danbred**	**SEM**	***P***	**IM**	**SC**	**SEM**	***P***	***P***
Satured fatty acids (SFA) %													
10:0	0.17	0.17	0.004	0.37	0.18	0.16	0.004	0.015	0.19	0.14	0.004	<0.001	0.93
12:0 [1]	0.13	0.12	0.005	0.24	0.13	0.12	0.003	0.010	0.12	0.12	0.003	0.020	0.35
14:0 [2]	1.57	1.51	0.003	0.19	1.61	1.47	0.03	0.11	1.52	1.56	0.02	<0.001	0.63
16:0	21.4	21.5	0.28	0.99	21.9	20.9	0.21	0.69	20.8	21.4	0.16	<0.001	0.80
17:0	0.23	0.22	0.01	0.08	0.20	0.25	0.01	<0.001	0.23	0.22	0.01	0.63	0.06
18:0	8.79	9.09	0.12	0.06	8.96	8.88	0.12	0.64	9.18	8.66	0.12	0.003	0.009
20:0	0.10	0.10	0.003	0.62	0.11	0.10	0.003	0.012	0.11	0.10	0.003	0.007	0.08
Total SFA	30.8	33.1	0.71	0.19	32.6	32.1	0.72	0.52	32.5	32.2	0.44	0.52	0.69
Monounsatured fatty acids (MUFA) %													
16:1 cis-7 [3]	0.39	0.38	0.01	0.43	0.35	0.42	0.01	<0.001	0.38	0.39	0.01	0.29	0.95
16:1 cis-9	2.96	2.78	0.07	0.09	2.86	2.87	0.07	0.88	3.05	2.69	0.05	<0.001	0.51
17:1 cis-10 [4]	0.26	0.24	0.01	0.10	0.23	0.27	0.01	0.009	0.21	0.29	0.001	<0.001	0.54
18:1 cis-9	41.9	42.0	0.33	0.74	42.4	41.5	0.33	0.12	40.9	43.1	0.33	<0.001	0.82
18:1 cis-11	4.18	4.11	0.09	0.57	4.09	4.20	0.09	0.37	4.11	4.18	0.09	0.59	0.07
18:1 isomers [5]	0.32	0.31	0.01	0.60	0.32	0.32	0.01	0.96	0.28	0.35	0.01	<0.001	0.44
20:1 trans-11 [6]	0.80	0.76	0.03	0.46	0.81	0.76	0.03	0.39	0.70	0.87	0.03	0.001	0.38
Total MUFA	51.0	50.8	0.33	0.75	51.4	50.4	0.33	0.12	49.9	52.0	0.34	<0.001	0.53
Polyunsatured fatty acids (PUFA) %													
18:2 cis-9, cis-12	13.8	13.5	0.19	0.33	13.1	14.2	0.19	<0.001	14.0	13.2	0.16	<0.001	0.83
18:2 trans-9, trans-12	0.14	0.14	0.02	0.90	0.13	0.16	0.02	0.21	0.22	0.06	0.02	<0.001	0.74
18:2 other isomers [7]	0.18	0.17	0.01	0.15	0.18	0.17	0.01	0.75	0.22	0.14	0.01	<0.001	0.21
18:3 cis-9, cis-12, cis-15	0.69	0.67	0.01	0.14	0.64	0.72	0.01	<0.001	0.65	0.71	0.01	<0.001	0.79
CLA sum	0.12	0.12	0.004	0.43	0.11	0.13	0.005	0.028	0.13	0.11	0.01	<0.001	0.26
20:2 cis-11, cis-14	0.65	0.62	0.02	0.41	0.61	0.66	0.02	0.13	0.59	0.68	0.004	<0.001	0.23
20:3 cis-8, cis-11, cis-14	0.18	0.17	0.01	0.32	0.18	0.17	0.01	0.95	0.24	0.11	0.02	<0.001	0.34
20:3 cis-11, cis-14, cis-17	0.14	0.12	0.01	0.07	0.11	0.13	0.01	0.64	0.13	0.13	0.01	0.77	0.92
20:4 cis-5, cis-8, cis-11, cis-14[8]	0.78	0.76	0.02	0.64	0.76	0.78	0.02	0.53	1.36	0.18	0.02	<0.001	0.51
Total PUFA	16.8	16.4	0.22	0.21	15.9	17.2	0.22	<0.001	17.7	15.4	0.18	<0.001	0.95
n-3 fatty acids [9]	1.54	1.49	0.03	0.21	1.47	1.55	0.03	0.09	2.08	0.95	0.03	<0.001	0.93
n-6 fatty acids	15.0	14.7	0.16	0.25	14.2	15.5	0.20	<0.001	15.4	14.4	0.16	<0.001	0.97
n-6/n-3	11.1	11.5	0.14	0.14	11.1	11.5	0.25	0.10	7.46	15.1	0.14	<0.001	0.78
Minor fatty acids [10]	0.86	0.78	0.03	0.09	0.80	0.84	0.03	0.37	0.85	0.79	0.03	0.047	0.64

[1] C12:0 was 0.12 for barrows and 0.13 for gilts (P = 0.035; SEM = 0.03).
[2] The P value of the GG × T interaction was 0.019.
[3] C16:1 cis-7 was 0.37 for barrows and 0.40 for gilts (P < 0.001; SEM = 0.008).
[4] The P value of the D × T interaction was 0.032.
[5] The P value of the D × T interaction was 0.033.
[6] C20:1 trans-11 was 0.72 for barrows and 0.85 for gilts (P = 0.014; SEM = 0.03).
[7] The P value of the D × T interaction was 0.013.
[8] C20:4 cis-5, cis-11, cis-14 was 0.83 for barrows and 0.72 for gilts (P = 0.002; SEM = 0.02).
[9] *n-3* fatty acids were 1.56 for barrows and 1.47 for gilts (P = 0.002; SEM = 0.03).
[10] Minor fatty acids include: C6:0; C8:0; C10:1 *cis*-9; C11:0; C13:0; C14:1 *cis*-9; C15:0; C15:1 *cis*-10; C16:0 iso; C16:0 anteiso; C17:0 iso; C17:0 anteiso; C18:0 iso; C18:0 anteiso; C18:3 *cis*-6, *cis*-9, *cis*-12; C19:0; C21:0; C20:5 *n-3*; C22:0; C22:1 *trans*-13; C22:2 cis-13, cis-16, C23:0, C24:0; C24:1 *cis*-15, C22:6 *cis*-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19

Fig 1. Slice of deboned dry-cured ham. bf: *biceps femoris*; qf: *quadriceps femoris*; sm: *semimembranosus muscle*; st: *semitendinosus muscle*. a: Bone area; b: Fatty area; c: Subcutaneous fat.

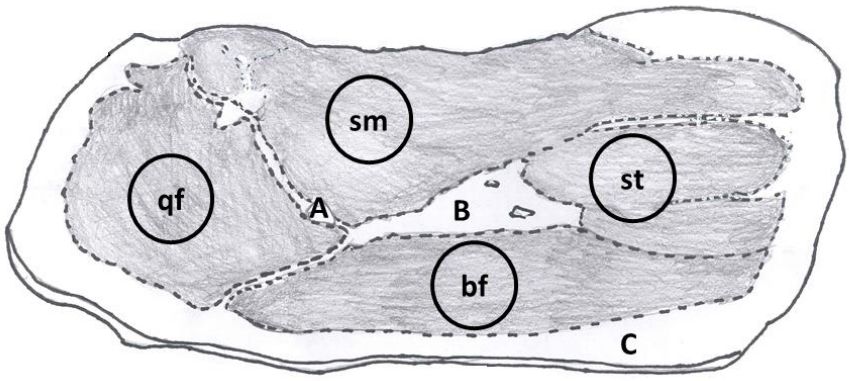


Fig 2. Influence of the genetic group × diet interaction on: a) the protein content (*P* = 0.36), b) the lipid content (*P* = 0.045), and c) the protein:lipid ratio (*P* = 0.023) of the dry-cured hams. Contrasts were run to evidence differences between the GGs on conventional (CONV) and on low protein (LP) diets (n = 10, vertical bars indicate standard errors of the means).

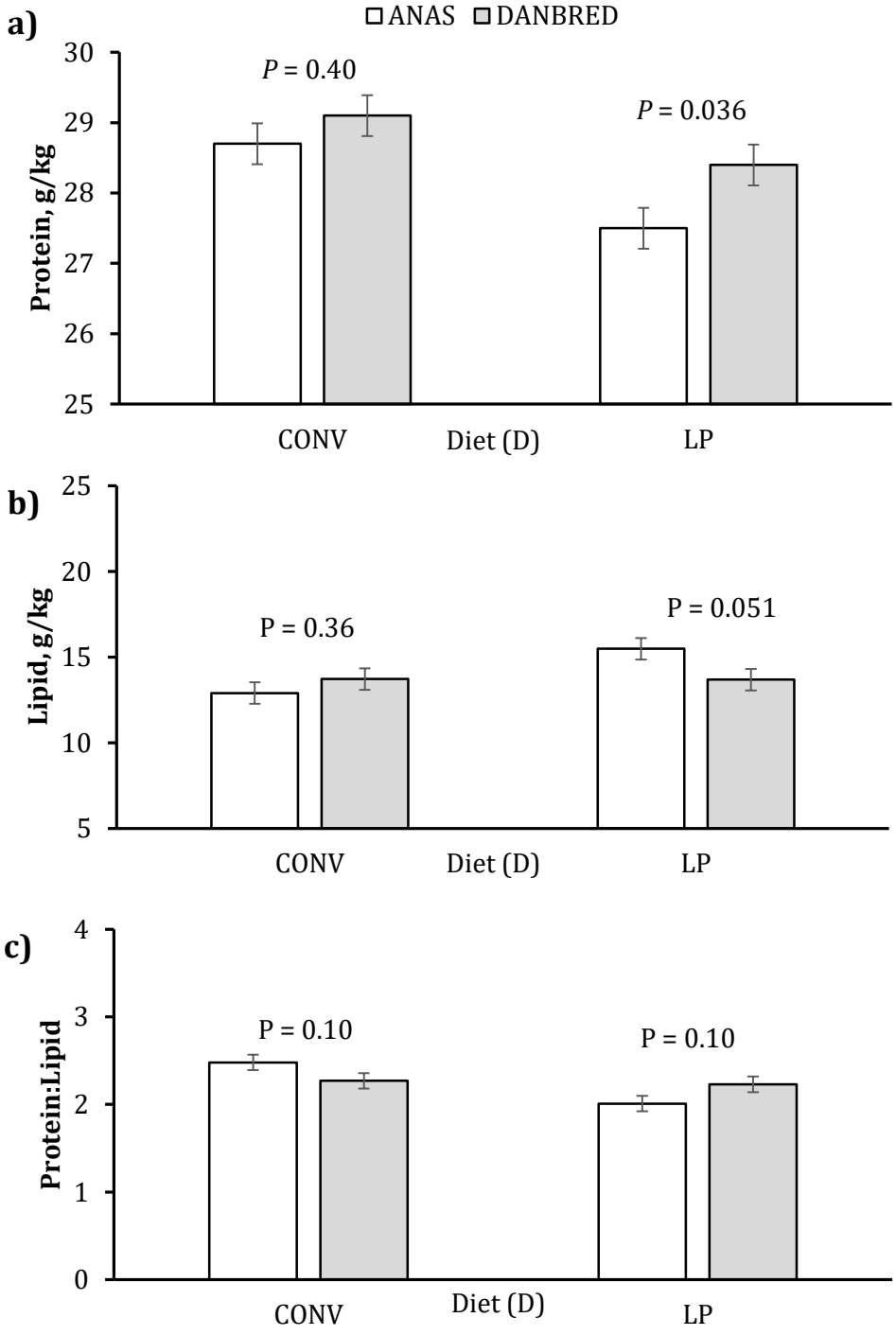


Fig 3. Relationships between salt content and a) water activity in the lean part of the ham slice, b) seasoning and deboning losses, and c) proteolysis index.

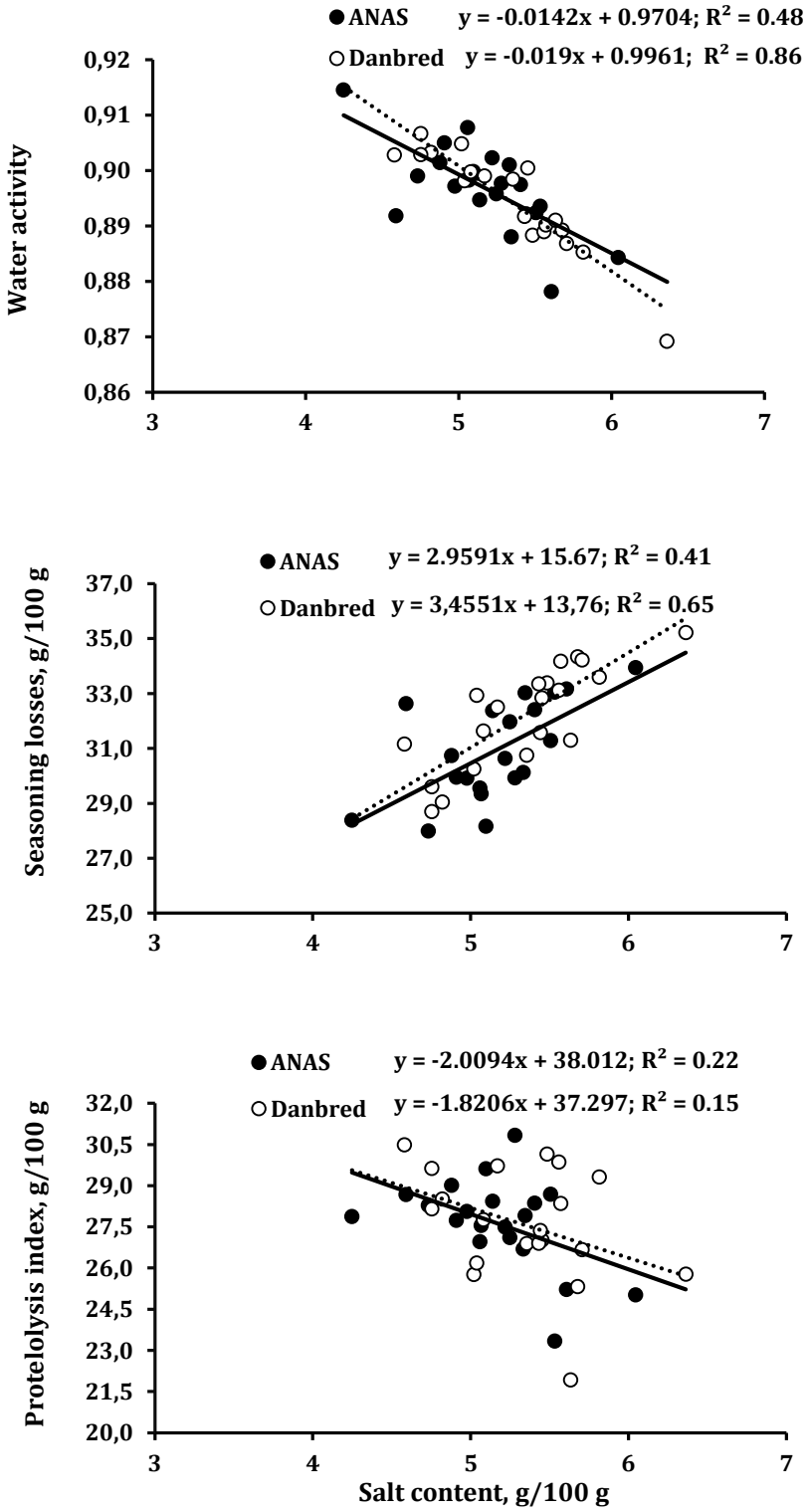


Fig 4. Relationship between the subcutaneous fat depth of the raw ham and the salt content of the lean part of the slice.

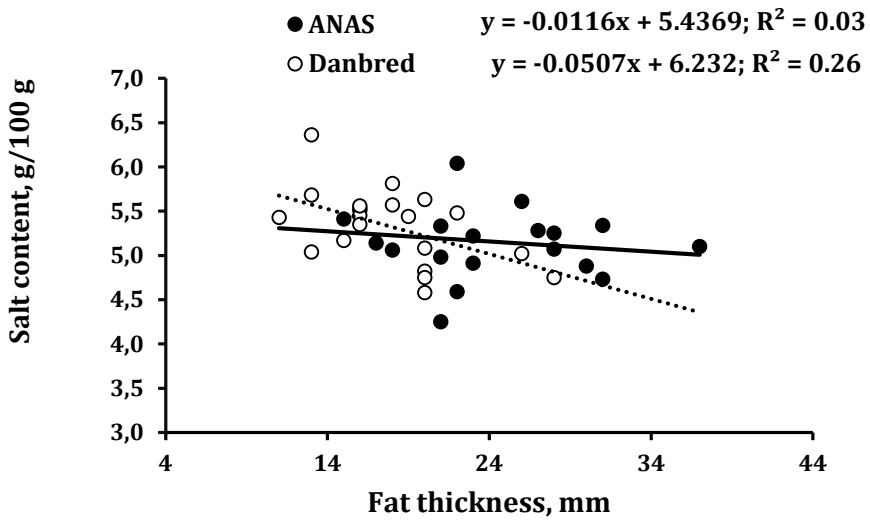


Fig 5. Influence of the genetic group × tissue interaction on: a) the protein content (*P* = 0.038), b) the lipid content (*P* = 0.003), and c) the protein:lipid ratio (*P* = 0.004) of the whole and the lean part of the ham slice. Contrasts were run to evidence differences between the GGs in the constituent contents of the whole and of the lean part of the slice (n = 10, vertical bars indicate SE of the means).

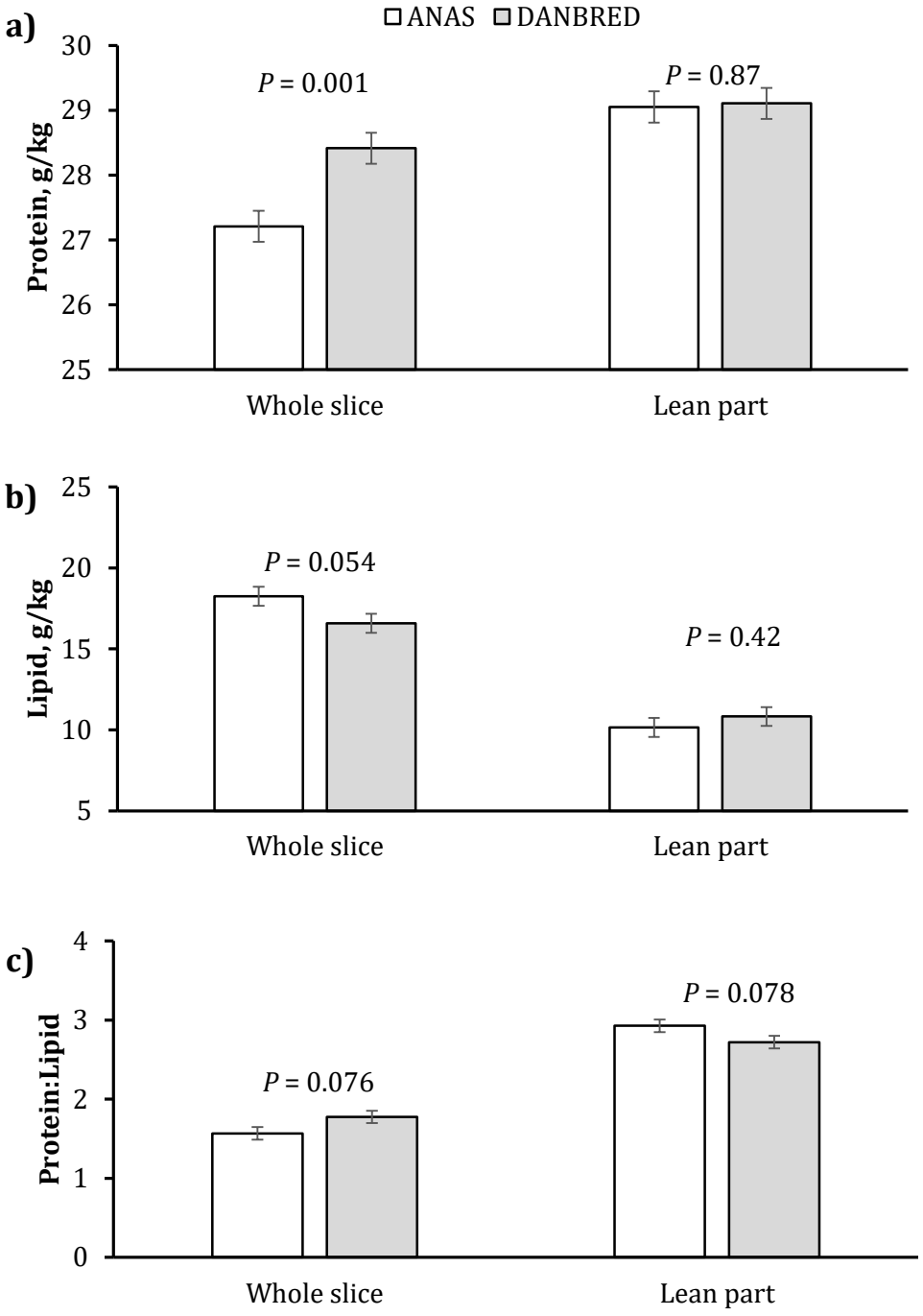
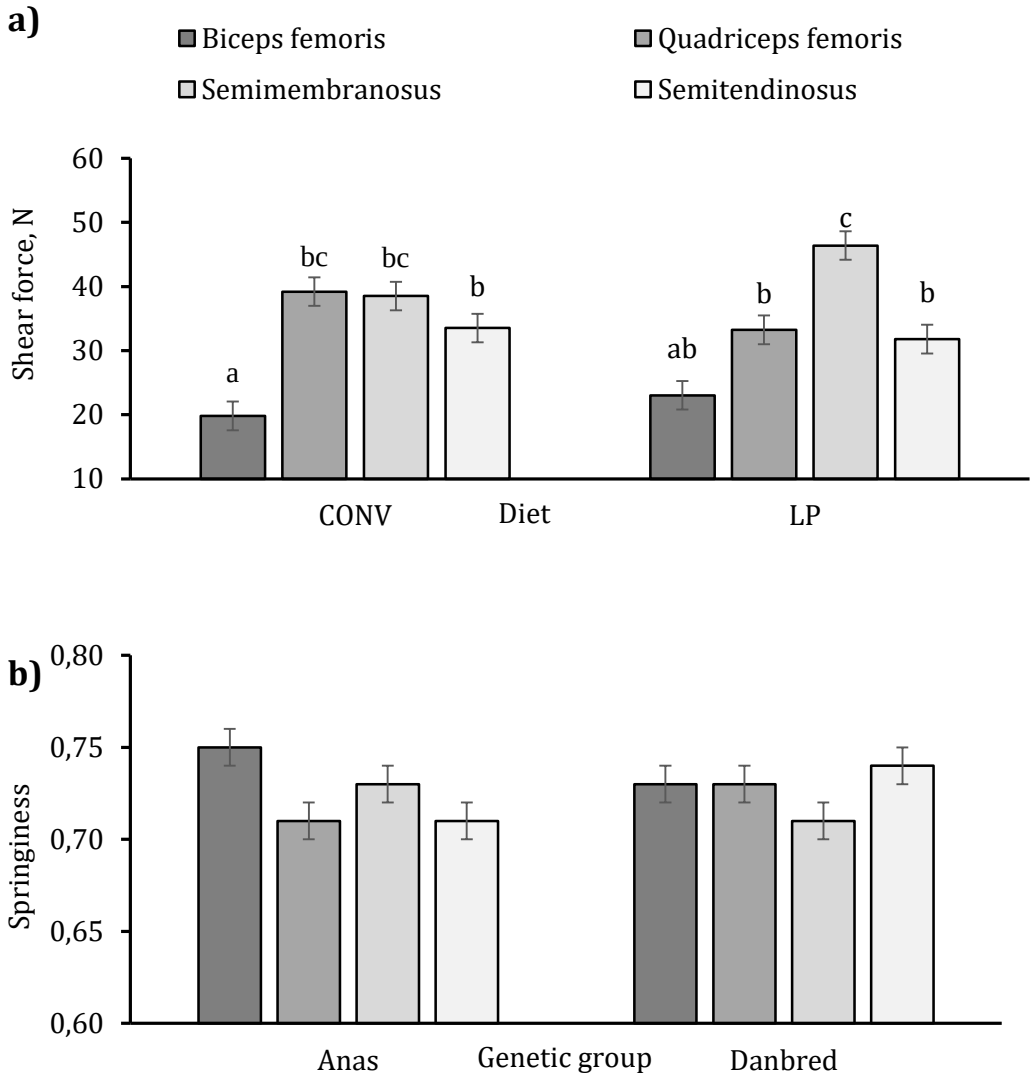


Fig 6. Influence of: a) the diet × tissue interaction on the shear force (*P* = 0.014), and b) the genetic group × tissue interaction on the springiness (*P* = 0.046) of the four muscles of the dry cured-hams obtained from Anas and Danbred genotypes fed on conventional (CONV) and low protein (LP) diets.



8. GENERAL CONCLUSIONS

The main objective of this PhD thesis was to investigate the effects of some feeding strategies on the feeding behaviour, growth performances, carcass traits and meat and meat-products quality of Italian medium-heavy and heavy pigs, which represent two pig production systems still poorly studied.

The results obtained from the trials exhibited that:

I. A mild feed restriction resulted in a 7% reduction in feed intake but also in a 2% increase in feed efficiency. The reduced feed allowance increased by 36% the uniformity among the carcasses, as the restricted feed pigs showed similar feed intakes and final body weights. Moreover, feed restriction lowered by 9% the N excretion. Restricted fed animals were able to modify their feeding behaviour to cope with feed restriction, reducing by 27% the number of visits to the manger and by 14% the time spent eating, but increasing by 20% and by 10% their feed consumption per visit and feeding rate, respectively. These variables were considered as indicators of a greater pigs' motivation to eat and were associated with better performance and carcass characteristics.

II. Reducing the protein and indispensable AA content, by 9% and 18%, in early and late finishing, respectively, increased the pigs feed intake (+7%) the carcass weight (+4.9%) and the fat content of the carcasses (+17.6%). Considering the environmental impact, the pigs fed LAA diets showed a 15% reduction in nitrogen excretion, confirming the results of the previous studies. Finally, the low-protein and AA diets had little effect on the feeding behaviour of pigs. However, a tendency to increase the feeding rate may suggest a greater pigs' motivation to eat, which is in in line with the

theory that a pig fed diet lowered in the nutrient contents, would show a greater desire for feeding to compensate for this reduction.

III. The feeding behaviour of the pigs was very adaptable, as the pigs kept on restricted feeding regime and low-AA diets were able to modify their feeding patterns to cope with these restrictions. Moreover, feeding patterns resulted highly correlated with growth performances and carcass quality traits, and the animals with a greater feeding rate showed also higher growth rates (+16%), and heavier carcasses (+16%) with higher fat content (+14%). It was concluded that the strategies aimed at modifying the feeding behaviour of pigs may influence their performance and the quality of their products, according to the productive circumstances, without alterations in feed efficiency.

IV. A mild reduction in the protein and amino acid content of the diet increased by 10% the fat content of the dry-cured from pigs slaughtered at 165 kg body weights. This increase was more evident in the genetic group showing a lower potential for lean growth, suggesting that the response to the dietary AA restriction would be closely related to the genetic type of the pigs. The low-protein and AA diets did not promote large modifications in the other chemical and physical characteristics of the dry-cured hams. However, this might depend on the small sample size used in the study. Thus, further research involving a larger number of hams is requested to assess the positive effects of a mild reduction in dietary protein and indispensable AA content on the quality of the dry-cured hams.

In conclusion, basing on the three years of research and on the results obtained in the different experiments, we can state that the new feeding strategies have a great impact on the pigs' performances and feeding behaviour and on the quality of their meat and meat products. Moreover, a potential in the use of a reduction in the feed allowance and in dietary protein and AA content as effective strategies to reduce the environmental impact and the N excretion of the Italian pig industry is evidenced.

9. LIST OF PUBLICATIONS

Publications:

- **Carcò G.**, Gallo L., Dalla Bona M., Latorre M.A., Fondevila M., Schiavon S. The influence of feeding behaviour on growth performance, carcass and meat characteristics of growing pigs. Plos ONE, DOI: 10.1371/journal.pone.0205572.
- Schiavon S., Dalla Bona M., **Carcò G.**, Carraro L., Bunger L., Gallo L. 2018. Effects of feed allowance and indispensable amino acid reduction on feed intake, growth performance and carcass characteristics of growing pigs. Plos ONE, DOI: 10.1371/journal.pone.019564.
- **Carcò G.**, Grajewzki B., Cassandro M., Lisowski M., Szwaczkowski T. 2018. Genetic variability of some Italian and Polish duck breeds. DOI: 10.1080/1828051X.2018.1436006.
- **Carcò G.**, Dalla Bona M., Carraro L., Latorre M.A., Fondevila M., Gallo L., Schiavon S. 2018. Influence of mild feed restriction and mild reduction in dietary amino acid content on feeding behavior of group-housed growing pigs. Applied Animal Behaviour Science, 198: 27-35.
- Pérez-Ciria L., **Carcò G.**, Latorre M.A. Key aspect in hyper-prolific sow nutrition. Albéitar, 202:12-13, January-February 2017.
- Pérez-Ciria L., **Carcò G.**, Schiavon S., Latorre M.A. How to improve the feeding of the suckling piglet. Suis, 141: 8-14, October 2017.

Forthcoming publications:

- **Carcò G**., Schiavon S., Casiraghi E., Grassi S., Sturaro E., Dalla Bona M., Novelli E., Gallo L. Influence of the dietary crude protein content on the quality of San Daniele like dry-cured hams of two genetic group of pigs. *Submitted to Plos ONE in date 26th June 2018.*

Oral communications:

- Dalla Bona M., **Carcò G.**, Gallo L., Schiavon S. Feeding behaviour of group-housed growing pigs under feeding and crude protein constraints, using single space automated feeding stations. I DAFNAE Postgraduate Scientists Meeting, Legnaro, Italy, 22-23/09/2016.
- Dalla Bona M., **Carcò G.**, Fiore E., Schiavon S., Carraro L., Morgante M., Gallo L. 2017.Effects of dietary protein and lysine content on growth performances, carcass traits and estimated nitrogen input-output flow of growing pigs. Italian Journal of Animal Science, 16: 60.
- **Carcò G**., Dalla Bona M., Carraro L., Latorre M.A., Fondevila M., Gallo L., Schiavon S. 2017. The daily feeding behaviour of growing pigs subjected to a feed restriction and to a reduction of dietary indispensable amino acid contents. Italian Journal of Animal Science, 16: 93-94.
- Pérez-Ciria L., **Carcò G**., Miana-Mena F.J., Mitjana O., Abadías, J.I., Latorre M.A. Impact of oestrus suppression in carcass quality of gilts intended for Teruel dry-cured ham production. X European Symposium of Porcine Health Management (ESPHM), Barcelona, Spain, 9 - 11th May 2018.
- **Carcò G.**, Dalla Bona M., Carraro L., Latorre M.A., Fondevila M., Gallo L., Schiavon S. 2018. Quality attributes of PDO dry-cured hams as affected by low-protein diets and genetic line of pigs. Book of Abstracts of the 69th Annual Meeting of the European Federation of Animal Science, Dubrovnik, Croatia, 27-31 August 2018. Vol 24, pp. 278. ISBN: 978-90-8686-323-5; doi: 10.3920/978-90-8686-871-1.

Posters:

- Latorre M.A., Pérez-Ciria L., **Carcò G.**, Garitano Iriondo, I., Marín Alcalá, M. Impacto de la dieta de finalización en los rendimientos productivos y la calidad de la canal de cerdas destinadas a Jamón DOP Teruel. IX Congreso Mundial del Jamón Curado, Toledo, Spain, 07 - 09 June 2017. http://hdl.handle.net/10532/3683.
- Pérez-Ciria L., **Carcò G.**, Ripoll G., Marín C., Alvarez-Rodriguez J., Latorre M.A. Improving pork quality from pigs intended for Spanish dry-cured ham by pig diet. LXIII International Congress of Meat Science and Technologies (ICOMST), Cork, Ireland, 13-18 August 2017.
- Suárez-Belloch J., Pérez-Ciria L., **Carcò G.**, Latorre M.A. Effect of freezing and its storage length on physicochemical characteristics of meat from heavy pigs. LXVI International Congress of Meat Science and Technologies (ICOMST), Melbourne, Australia, 12-17 August 2018;
- Pérez-Ciria L., **Carcò G**., Miana-Mena F.J., Mitjana O., Ripoll G., Abadías, J.I., Latorre M.A. The protocol of immunocastration can affect carcass fatness, meat quality and fat composition of heavy gilts. LXVI International Congress of Meat Science and Technologies (ICOMST), Melbourne, Australia, 12-17 August 2018.