

# INFLUENCE OF FEEDING RESTRICTION AND DIETARY PHOSPHORUS LEVELS ON BODY TISSUE COMPOSITION EVALUATED IN VIVO BY COMPUTED TOMOGRAPHY, BONE MINERALISATION AND SENSORY PROPERTIES OF THE MEAT FROM GILTS

**Xin Luo**

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DOCTORAL THESIS

**Influence of feeding restriction and dietary phosphorus levels  
on body tissue composition evaluated *in vivo* by computed  
tomography, bone mineralisation and sensory properties of the  
meat from gilts**



**Xin Luo**

**2019**



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Doctoral Programme in Technology

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Work submitted to the University of Girona in partial fulfilment of the requirements for the degree of Doctor of Philosophy





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I DECLARE:

That the thesis titles 'Influence of feeding restriction and dietary phosphorus levels on body tissue composition evaluated in vivo by computed tomography, bone mineralisation and sensory properties of the meat from gilts', presented by Xin Luo to obtain a doctoral degree, has been completed under my supervision.

For all intents and purposes, I hereby sign this document.



Maria Font i Furnols

Girona, 24<sup>th</sup> July 2019





千里之行，始于足下。

---老子 (中国古代哲学家)

*A journey of a thousand miles begins with a single step.*

--- Lao Zi (the ancient Chinese philosopher)

*Un viatge de mil quilòmetres comença amb un sol pas.*

--- Lao Zi (l'antic filòsof xinès)

*Un viaje de mil kilómetros comienza con un solo paso.*

--- Lao Zi (el antiguo filósofo chino)

*Un voyage de mille kilomètres commence par un seul pas.*

--- Lao Zi (l'ancien philosophe chinois)



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2. Sousa, R., Luo, X., Gispert, M., Brun, A., Pallisera, J., Dalmau, A., Soler, J., Esteve-Garcia, E., Lizardo, R., Font-i-Furnols, M. Influence of dietary P levels on bone tissue composition and mineralization in gilts evaluated in vivo by computed tomography. *Animal*, 2019 (submitted).

### **Technical paper derived from the PhD:**

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### **Congresses and Working day publications:**

1. Brun, A., Font-i-Furnols, M., Gispert, M., Luo, X., Soler, J., Esteve-Garcia, E., Lizardo, R. (2015). Influence of feeding restriction on body tissue composition of growing gilts evaluated in vivo by computed tomography. FAIM IV Conference of COST FA1102. Edinburgh, United Kingdom. 22nd-23rd September 2015.
2. Font-i-Furnols, M., Luo, X., Brun, A., Gispert, M., Soler, J., Esteve-Garcia, E., Lizardo, R. (2016). Influence de la conduite alimentaire sur la composition corporelle du porc en croissance évaluée in vivo par

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  5. Luo, X., Font-i-Furnols, M., Brun, A., Gispert, M., Soler, J., Esteve-Garcia, E., Lizardo, R. (2017). Effect of feeding restriction strategies on growth performance and meat quality of gilts. 68th EAAP Annual Meeting, Tallin, Estonia, 28th August – 1st September 2017.
  6. Font-i-Furnols, M., Luo, X., Brun, A., Gispert, M. (2019). Préférences d'achat de porc par le consommateur. 51<sup>e</sup> Journées de la Recherche Porcine, Paris, France, 5th - 6th February 2019, 355-356.

## List of Abbreviation

a\*: Redness

ADG: Average Daily Gain

AL: *Ad libitum* feeding

b\*: Yellowness

BW: Body weight (kg)

Ca: Calcium

CT: Computed tomography

FCR: Feed Conversion Ratio

GM: *Gluteus medius* muscle

HCW: Hot Carcass Weight

HU: Hounsfield Units

L\*: Luminosity

LMP: Lean Meat Percentage

LT: *Longissimus thoracis* muscle

Mg: Magnesium

P: Phosphorus

PCA: Principal Component Analysis

RV: Feeding restricted in volume

SM: *Semimembranosus* muscle

TBW: Target Body Weight





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## Abstract

Feeding strategy is very important for farmers to improve pig productive parameters and keep its competitive advantage in the market. The computed tomography (CT) technology can evaluate the effect of feeding strategy on the body/carcass tissue and bone composition of living pigs, since it allows the evaluation of the same animal at various moments of growth to avoid serial slaughtering of animals. This thesis at hand investigates the effect of different feeding restriction treatments on productive and carcass quality parameters of gilts using CT during growth and the effect of such treatments on meat quality, sensory properties and consumer preference (experiment 1) and the effect of different feeding strategies with different dietary P and phytases levels on density and the morphological characteristics of the bones of live gilts during growth and in their carcasses via CT images and the effect of these strategies on the chemical and physical characteristics of the bones, carcass and meat quality characteristics (experiment 2). Experiment 1 was performed on 36 Pietrain × (Large White × Landrace) gilts which were assigned to the following three feeding treatments: 1) ad libitum feeding (AL) during all fattening periods (AL-AL); 2) AL feeding between 30 and 70 kg target body weight (TBW) followed by volume restriction (84% of AL) until 120 kg TBW (AL-RV); and 3) volume restriction feeding (78% of AL) between 30 and 70 kg TBW followed by AL until 120 kg TBW (RV-AL). The animals were scanned by CT at 30, 70, 100 and 120kg and the information of carcass composition parameters were obtained from CT images. The loins were collected for trained panel evaluation and consumer tests after slaughtering these pigs (120 kg TBW). While the experiment 2 was performed on 48 gilts which were distributed in four feeding strategies: a positive control (C0), a negative control with low levels of P (P0) and two negative control diets supplemented with 500 (P1) and 1000 (P2) phytase units, these diets were maintained from the beginning of the study ( $48.5 \pm 2.7$  kg BW) until the slaughter of the animals ( $121 \pm 3.2$  kg BW). These gilts were scanned at 50, 75 and 120 kg TBW. At 120kg BW, the gilts were slaughtered, the carcasses were scanned, and the femur, tibia and metacarpus were extracted to determine area, length,

density, bending strength and chemical composition. Results of experiment 1 showed that a restriction of volume reduces the fat content during the period of the restriction and meat from the animals in AL-AL treatment was tougher than that from animals in the RV-AL and AL-RV treatments. However, there was no significant difference found in the carcass and cut composition and meat quality and scores for acceptability at the end of the experiment among the treatments. Results of experiment 2 showed no significant differences were found for the studied parameters between dietary treatments, possibly because the high daily feed intake compensated for the deficiency in P. These results indicate that, in the conditions of the present experiment, a low P diet such as P0 is enough to ensure correct bone mineralization and growth performance. Thus the results of these feeding strategies (experiment 1 and 2) should be considered to optimize cost and increase benefits for farmers. In the conditions of the present work it can be concluded that restriction in volume affects the tissue growth and fat deposition, but in the final product no effect can be seen due to compensatory growth. Also, meat and sensory quality is hardly affected by restriction strategies applied. Regarding restriction in P, it is possible to conclude that it is possible to reduce P of the diet using the present conditions to optimize the production costs without affecting productive and quality parameters.

## Resum (in Catalan)

Per als productors de porcí, la utilització d'estratègies alimentàries és fonamental per tal de millorar els paràmetres productius i mantenir els avantatges competitius al mercat. La tecnologia de la tomografia computeritzada (CT) pot usar-se per avaluar l'efecte de l'estratègia alimentària en la composició tissular i òssia tant en l'animal viu com en la canal, ja que permet l'avaluació del mateix animal en diferents moments del seu creixement evitant realitzar sacrificis seriatos dels animals. Aquesta tesi investiga l'efecte de diferents estratègies alimentàries sobre els paràmetres productius i de qualitat de la canal de porcs femelles utilitzant la CT durant el creixement i l'efecte dels tractaments sobre la qualitat de la carn, les propietats sensorials i les preferències dels consumidors (experiment 1). També s'ha estudiat l'efecte de diferents estratègies alimentàries amb diferents nivells de P a la dieta i nivells de fitases en la densitat i les característiques morfològiques dels ossos i en la canal de femelles durant el seu creixement a partir de imatges de CT i l'efecte d'aquestes estratègies en les característiques químiques i físiques dels ossos, i les característiques de qualitat de canal i de la carn (experiment 2). A l'experiment 1 es van utilitzar 36 femelles Pietrain × (Large White × Landrace) que es van assignar als tres següents tractaments alimentaris: 1) alimentació ad libitum (AL) durant tots els períodes d'engreix (AL-AL); 2) alimentació AL entre 30 i 70 kg de pes objectiu (TBW) seguida de restricció en volum (85% de l'AL) fins a 120 kg TBW (AL-RV); i 3) alimentació restringida en volum (78% de l'AL) entre 30 i 70 kg TBW seguida d'AL fins a 120 kg TBW (RV-AL). Els animals es van escanejar amb CT a 30, 70, 100 i 120 kg i de les imatges obtingudes se'n va treure informació sobre paràmetres de composició de la canal. Després del sacrifici dels porcs a 120 kg TBW, es van extreure els llocs que es van utilitzar per a l'avaluació sensorial mitjançant panel de degustadors entrenats i per a l'estudi de consumidors. En el segon experiment es van utilitzar 48 femelles que es van distribuir en quatre estratègies alimentàries: un control positiu (C0), un control negatiu amb nivells baixos de P (P0) i dues dietes com el control negatiu suplementades amb 500 (P1) i 1000 (P2) unitats de fitasa. Aquestes dietes es van mantenir des del principi de l'estudi ( $48.5 \pm 2.7$  kg BW) fins al sacrifici dels animals ( $121 \pm 3.2$  kg BW). Les

femelles es van escanejar a 50, 75 i 120 kg TBW. A 120 kg, les femelles es van sacrificar, les canals es van escanejar, i el fèmur, la tibia i el metacarpi es van extreure per determinar-ne l'àrea, llargada, densitat, força de flexió i composició química. Els resultats de l'experiment 1 mostren que la restricció en volum redueix el contingut de greix durant el període de restricció i que la carn dels animals del tractament AL-AL era més dura que la dels animals dels tractaments RV-AL i AL-RV. Ara bé, al final de l'experiment, no es van trobar diferències significatives entre tractaments en la composició de les canals i les peces, en la qualitat de la carn i en les puntuacions d'acceptabilitat. Per la seva banda, l'experiment 2 mostra que no es van trobar diferències significatives entre tractaments alimentaris pels paràmetres estudiats, possiblement a causa de l'elevat consum diari d'aliment que compensa la deficiència en P. Aquests resultats indiquen que, en les condicions del present experiment, una dieta baixa en P com la P0 és suficient per assegurar una correcta mineralització dels ossos i rendiment al creixement. Per tant, els resultats obtinguts aplicant aquestes estratègies alimentàries (experiments 1 i 2) haurien de considerar-se per optimitzar els costos i augmentar els beneficis dels ramaders. En les condicions del present treball, es pot concloure que la restricció en volum afecta el creixement tissular i la deposició de greix, però no es pot veure cap efecte en el producte final motivat per a un creixement compensatori. A més a més, les estratègies de restricció aplicades no van en detriment de la qualitat tecnològica i sensorial de la carn. Pel que fa a la restricció en P, es pot concloure que és possible reduir el P de la dieta utilitzant les condicions d'aquest experiment per tal d'optimitzar els costos de producció sense afectar els paràmetres productius i de qualitat.

## Resumen (in Spanish)

Para los productores de porcino, el uso de estrategias alimentarias es fundamental para mejorar los parámetros productivos y mantener las ventajas competitivas del mercado. La tecnología de la tomografía computarizada (CT) puede usarse para evaluar el efecto de la estrategia alimentaria en la composición tisular e ósea tanto en el animal vivo como en la canal, ya que permite la evaluación del mismo animal en diferentes momentos de su crecimiento evitando realizar sacrificios seriados de los animales. Esta tesis investiga el efecto de diferentes estrategias alimentarias sobre los parámetros productivos y de calidad de la canal de cerdos hembras usando la CT durante el crecimiento, así como el efecto de los tratamientos sobre la calidad de la carne, las propiedades sensoriales y las preferencias de los consumidores (experimento 1); también se ha estudiado el efecto de diferentes estrategias alimentarias con diferentes niveles de P de la dieta y niveles de fitasas en la densidad y las características morfológicas de los huesos y en la canal de hembras durante su crecimiento a partir de imágenes de CT y el efecto de estas estrategias en las características químicas y físicas de los huesos, y las características de calidad de la canal y de la carne (experimento 2). En el experimento 1 se utilizaron 36 hembras Pietrain × (Large White × Landrace) que se asignaron a los tres siguientes tratamientos alimentarios: 1) alimentación ad libitum (AL) durante todos los períodos de engorde (AL-AL); 2) alimentación AL entre 30 y 70 kg de peso objetivo (TBW) seguida de restricción en volumen (75% de la AL) hasta 120 kg TBW (AL-RV); y 3) alimentación restringida en volumen (78% de la AL) entre 30 y 70 kg TBW seguida de AL hasta 120 kg TBW (RV-AL). Los animales se escanearon con el CT a 30, 70, 100 y 120 kg y de las imágenes obtenidas se obtuvo información sobre parámetros de composición de la canal. Después del sacrificio de los cerdos a 120 kg TBW, se extrajeron los lomos que se utilizaron en la evaluación sensorial mediante un panel de catadores entrenados y para el estudio de consumidores. En el segundo experimento se utilizaron 48 hembras que se distribuyeron en cuatro estrategias alimentarias: un control positivo (C0), un control negativo en los niveles bajos de P (P0) y dos dietas como el control negativo suplementadas con 500 (P1) y 1000 (P2)

unidades de fitasa. Estas dietas se mantuvieron desde el principio del estudio ( $48.5 \pm 2.7$  kg BW) hasta el sacrificio ( $121 \pm 3.2$  kg BW). Las hembras se escanearon a 50, 75 y 120 kg TBW. A 120 kg, las hembras se sacrificaron, las canales se escanearon, y el fémur, la tibia y el metacarpo se extrajeron para determina su área, longitud, densidad, fuerza de flexión y composición química. Los resultados del experimento 1 muestran que la restricción en volumen reduce el contenido en grasa durante el período de restricción y que la carne de los animales del tratamiento AL-AL era más dura que la de los animales de los tratamientos RV-AL y AL-RV. Sin embargo, al final del experimento, no se encontraron diferencias significativas entre tratamientos en la composición de las canales y de las piezas, en la calidad de la carne y en las puntuaciones de aceptabilidad. El experimento 2 muestra que no se encontraron diferencias significativas entre tratamientos alimentarios para los parámetros estudiados, posiblemente debido a que el elevado consumo diario de alimento compensó la deficiencia en P. Estos resultados indican que, en las condiciones del presente experimento, una dieta baja en P como la P0 es suficiente para asegurar una correcta mineralización de los huesos y rendimiento al crecimiento. Por tanto, los resultados obtenidos aplicando estas estrategias alimentarias (experimentos 1 y 2) deberían considerarse para optimizar los costes y aumentar los beneficios para los ganaderos. En las condiciones del presente trabajo, se puede concluir que la restricción en volumen afecta al crecimiento tisular y a la deposición de grasa, pero no se puede ver ningún efecto en el producto final debido a un crecimiento compensatorio. Además, las estrategias de restricción aplicadas no van en detrimento de la calidad tecnológica y sensorial de la carne. Por lo que respecta a la restricción en P se puede concluir que es posible reducir el P de la dieta utilizando las condiciones de este experimento para optimizar los costes de producción sin afectar a los parámetros productivos y de calidad.

## **INTRODUCTION**









# 1. Introduction

## 1.1. Importance of the pig sector

Pork has played a very important role in the market of global meat production because it is considered as good food source of nutrients need by a large number of people in the world. Figure 1 shows that the pork was the second highest world's meat production (35.8%) in 2016, only behind the poultry (36.5%) (FAOSTAT, 2017). Moreover, world's pork production has increased in the past decade, from 2006 to 2016 as can be seen in Figure 2 (FAOSTAT, 2017). Furthermore, Figure 3 shows the pork consumption in the 10 top world regions, which are: China, EU, USA, Russia, Brazil, Japan, Vietnam, Mexico, South Korea and Philippines (USDA, 2018a). The population of these countries corresponded, in 2017, in nearly 40% of the world population (United Nations, 2017). Thus, this huge demand in the global pork market has strengthened the development of swine industry and the research works on several subjects such as pork production, pork quality and marketing strategies, aiming to increase production benefits and to produce high quality value of pork to satisfy the market. Asia was the first world's biggest pork producer in 2016 followed by Europe (Figure 4). Because the population in Europe is far less than that in Asia, Europe can be considered a significant pork producer in the world. Regarding the main big pork producer countries in Europe (Figure 5), Germany is the first one, with more than 5 million tonnes and Spain is the second largest one with more than 3.5 million tonnes.

The swine sector highly developed in Spain and the pork export have played a very important role in Spanish food industry. The Global Agricultural Information Network Report (USDA, 2018b) showed that the Spanish pork production has increased in the last years although domestic pork consumption has not increased as much as the production, thus, this increase is mainly due to export. Now in the world pork market, Spanish pig producers have to face more competitors and they need to continue making more efforts to improve the quality of pork and to keep its competitive advantage in the world.

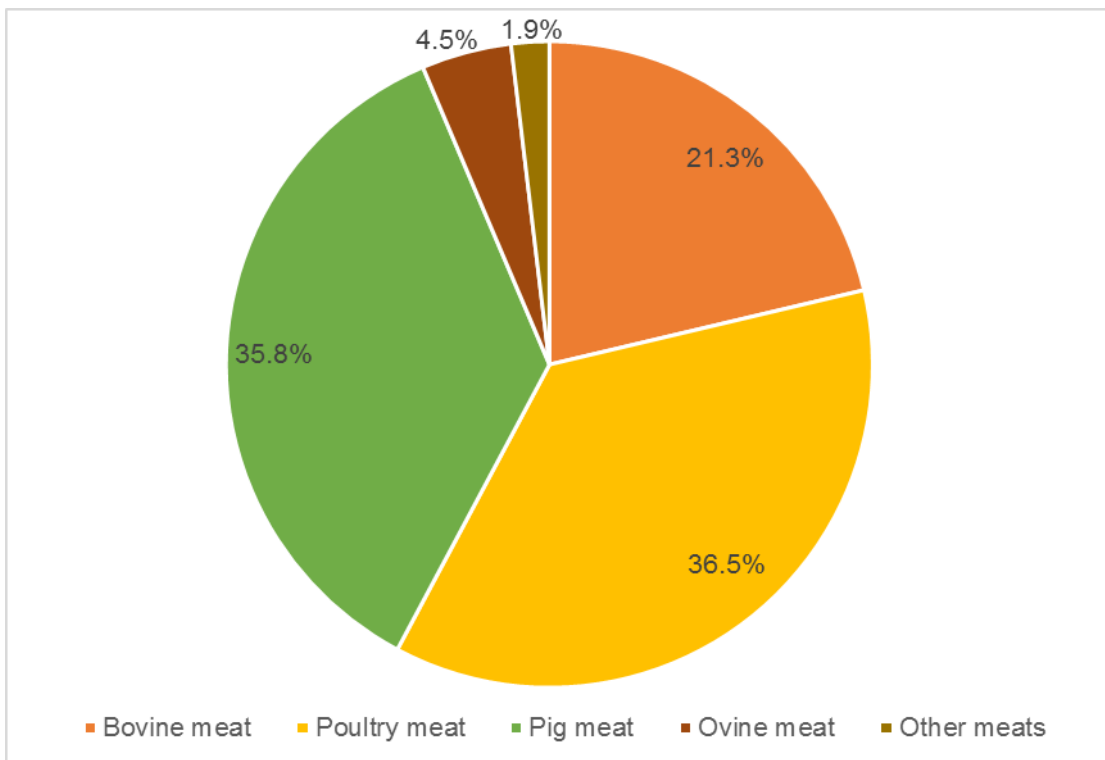


Figure 1. Relative production (%) of world's main different types of meat in 2016 (FAO, 2018).

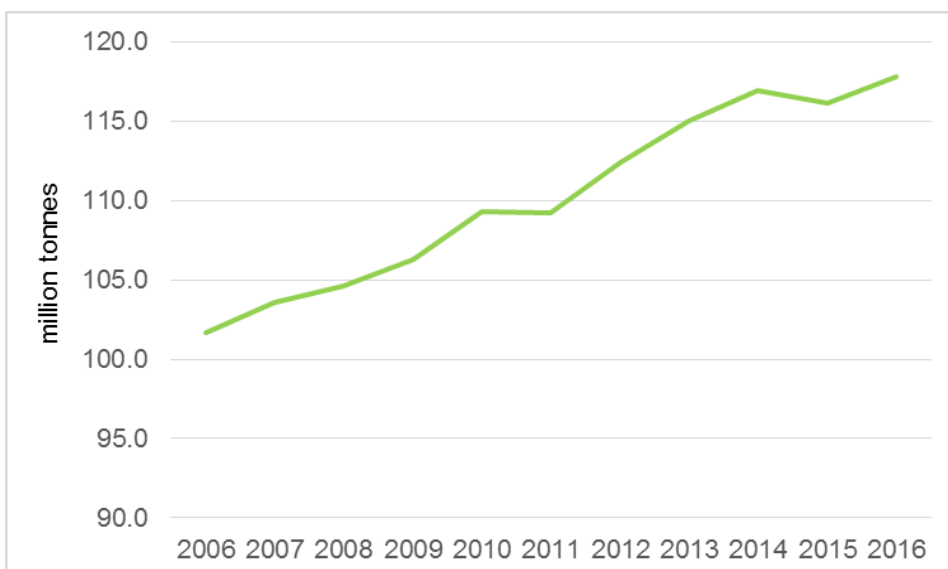


Figure 2. Global pork production from 2006 to 2016 (FAO, 2018).

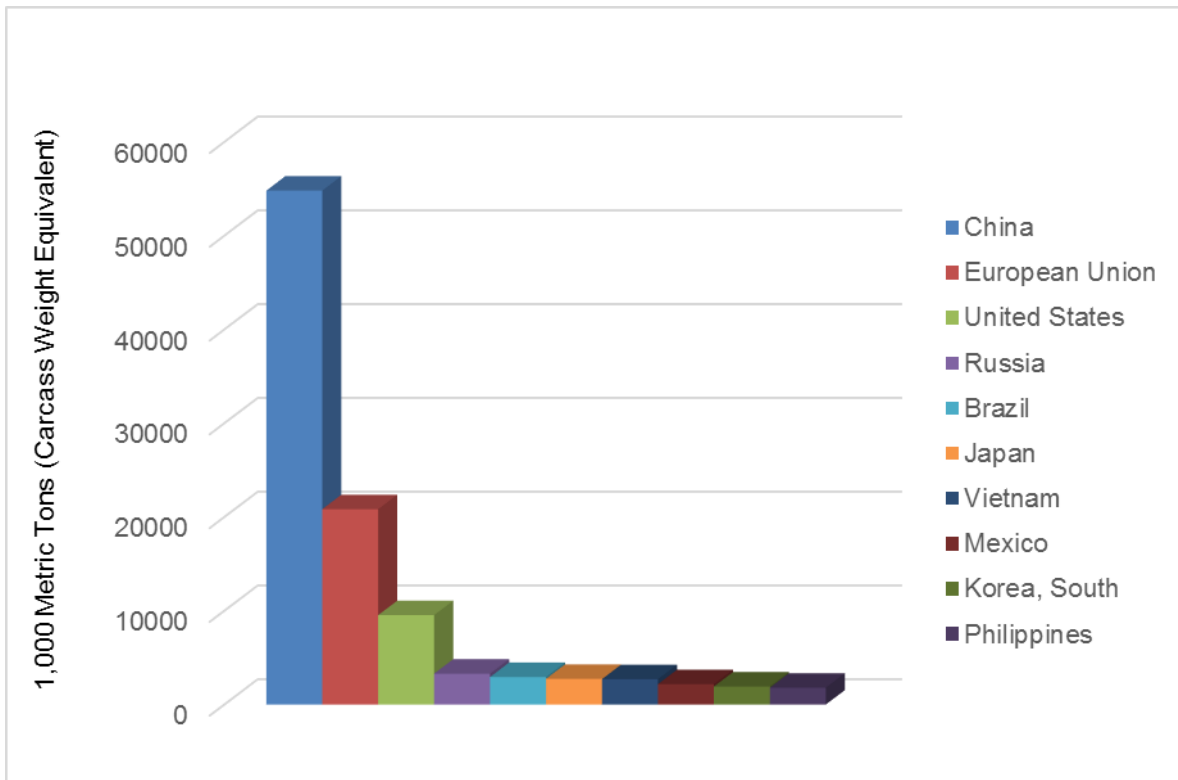


Figure 3. Pork consumption of the world's top 10 countries in 2017 (USDA, 2018a)

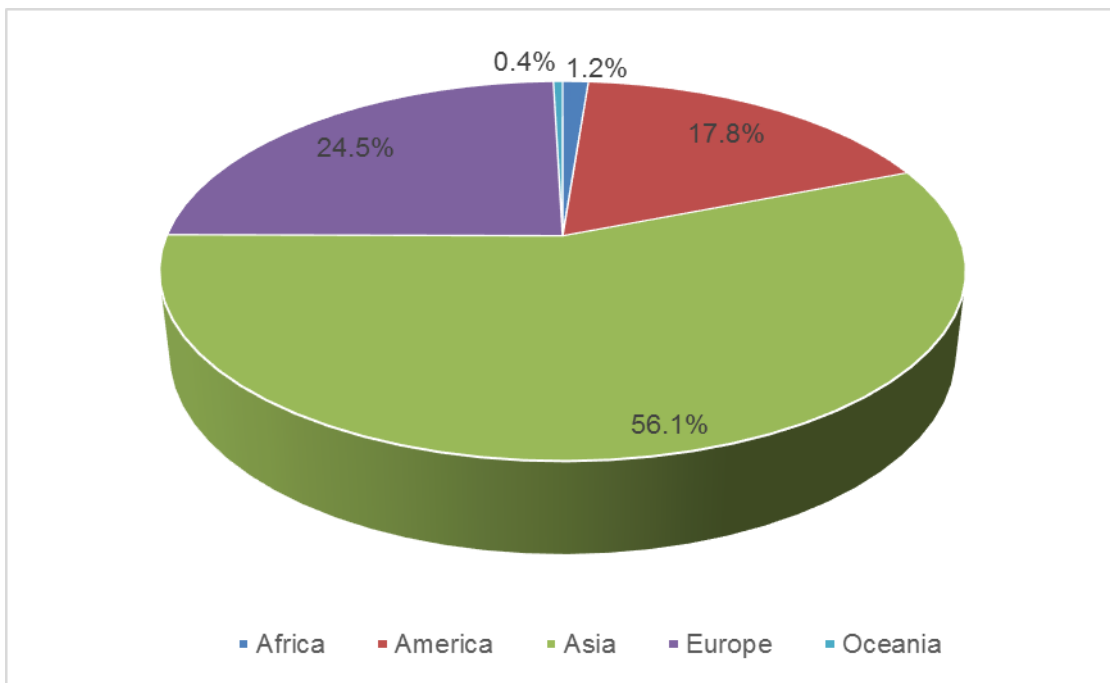


Figure 4. Relative pork production (%) in the five continents in 2016 (FAOSTAT, 2018)

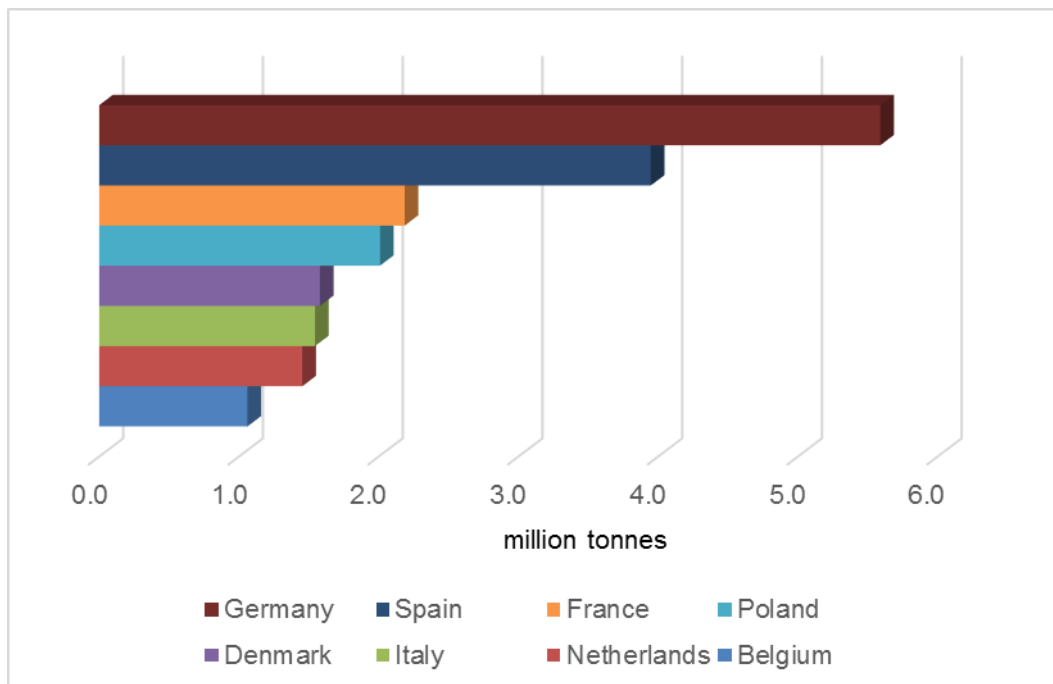


Figure 5. Pork production in EU countries in 2016 (FAOSTAT, 2018).

## 1.2. Feed restriction strategies

Regarding pig production, feeding is a very important sector, because it accounts for approximately 60-80% of the production costs (Abd Rahman et al. 2010; Chagwiza et al. 2016; Uyeh et al. 2018). Feed ration formulation and feed strategies influence at the total cost of the feed. Restriction strategies mainly mean reducing the amount of total feed, either quantitatively or qualitatively (energy, nutrients, crude protein, minerals, etc.), of the animals' diet in order to improve the feed efficiency (Giuseppe et al. 2018) and decrease the feeding costs (Manni et al. 2017). Thus, it is important to adjust the feed quantity and quality to reduce costs. Furthermore, if the feed oversupplies some nutrients, this may affect at the final product quality and/or modify the manure composition changing the environment balance and producing pollution and sustainability problems. Therefore, several type of restriction strategies can be applied to accomplish some objectives improving the production benefits, to improve growth

performance, carcass- and meat quality, as well as reducing cost of the feed or to reduce the excretion of contaminants improving environment and having a more sustainable production. This study mainly focusses on some of the most common restriction strategies used that are feeding restricted in volume and restriction of P.

### 1.2.1. Restriction in volume

Restriction of the volume of feed or nutrients during all the growth period or during some periods of the growth is a feeding strategy that has been investigated in order to optimize the cost of production maximizing gross margin and to obtain adequate carcass and pork meat quality (Alexander et al. 2006; Heyer and Lebret 2007). The effect of feeding strategies on pig performance, carcass and meat quality depends on feed intake or levels of restriction applied (Nyachoti et al. 2004; Patience and Li 2017), dietary composition (Ahmed et al. 2016; Patience and Li 2017) and feed pattern (periods of feed restriction) (Donker et al. 1986; Ramaekers et al. 1996; Daza et al. 2003; Heyer and Lebret 2007; Suarez-Belloch et al. 2013).

In the literature it is possible to find mainly three types of studies:

- Application of feed restriction only in the growing phase of growth (compensatory growth),
- Application of feed restriction only in the final phase of growth,
- Application of feed restriction in both growing and final phase of growth.

The duration and the level of restriction applied can be modified producing different effect on the growth of pigs, its carcass characteristics and meat quality.

#### 1.2.1.1. Effects of restriction in productive parameters

Feed restriction has an effect on productive parameters such as ADFI, ADG, FCR, total feed consumption and fat and muscle deposition.

The compensatory growth has been studied in several works. In general, it affects



positively not only at the overall efficiency of pig production but also lowers the waste of unused nutrients (Fabian et al. 2002). Prince et al.(1983) studied the restriction in the growing phase and found out that pigs fed 85% or 70% of AL for 2 or 4 weeks, respectively, followed by AL until 100 kg BW showed lower ADG during the restriction period and similar FCR than those non restricted. However, restricted pigs showed a compensatory growth since they presented higher ADG than the non-restricted ones in the post-restriction period, being the gain faster in 85% restricted pigs than in 70% non-restricted ones. The daily feed consumption was similar for all the treatments. The total ADG was not significantly different among these feeding treatments. Pigs restricted 85% of AL for 4 weeks had lower ratio feed per weight gain than those restricted for 2 weeks and similar ADG or daily feed consumption. Thus, the authors concluded that short term feed restriction produces a compensatory growth in ADG and FCR, but not in daily feed consumption, a result that suggests that restriction followed by AL produces a more efficient digestion or utilization of nutrients. Similar results were found by Heyer and Lebret (2007) who also demonstrated the compensatory growth, in this case, with pigs fed 65% of AL. They found that restricted pigs during growing period (30 to 70 kg of BW) had significantly less ADFI and ADG and similar efficiency than pigs fed AL during this period. However, during the AL period (from 70 to 110 kg) the ADG, ADFI and efficiency were higher in the previously restricted pigs. Lebret et al. (2007) also reported a compensatory growth when 70% restriction was applied during the growing period from 30 kg to 80 kg followed by AL feeding during the finishing period from 80 kg to 110 kg. Also Bee et al. (2007) showed a compensatory growth with 80% restriction. In another study, Chaosap et al. (2011a) found that pigs restricted 70% of AL for 40 days and then fed AL for 2 days had significantly less final BW and carcass weight than pigs fed AL for 42 days. In the same work, pigs restricted 70% of AL for 40 days and then fed AL for 42 days had no significant difference in final BW compared with the pigs fed AL for 82 days (Chaosap et al., 2011a). Higher final weight and better feed conversion efficiency were found in the pigs which were feed by the compensatory growth strategy applying a restriction of 70% of the control group's diet in the first growing period between nearly 17 and 25 kg (Valaja et al. 1992). However, with very short restrictions, it has been proved that the weight gain is due to the gains in water and/or the weight of the intestines. In

this case, the basis of the compensatory growth is the food intake rather than the improvement of the efficiency in the use of food (Lovatto et al. 2006). Therefore, this modification depends on the severity and time of the restriction, the genotype, and other factors that may be unnoticed (Mersmann and Hu 1987; Lovatto et al. 2006). If pigs can grow more quickly and/or more effectively in the compensatory phase, this can reduce the costs of food and the excretion of unused nutrients, such as nitrogen, being better from the environmental point of view (Fabian et al. 2004).

Cho et al. (2006) reported that restricted feeding from 70 kg to market weight (final phase of this treatment) has lower ADG and ADFI than those of AL during the whole growing period (from 50 kg to market weight).

Pigs fed restricted at 75% of the AL level during the whole growth period (from 30 kg to 110 kg) had no significant difference in food efficiency, but they had lower ADG and they needed to be slaughtered at an older age to reach the same final weight as pigs fed AL during all the growth period (Lebret et al. 2001). Similarly, when a feed restriction (ADG=650 g/d) was applied during the whole period, the total daily weight gain and days on feed were higher than when AL feeding was applied during the whole period (Madsen and Bee, 2015)

#### 1.2.1.2. Effects of restriction in carcass quality

Carcass characteristics and composition can be modified by means of feeding restriction periods.

In a longitudinal study performed in Denmark in the slaughter plants during one year, it was concluded that restricted feeding produce carcasses with significantly higher lean meat content than AL feeding, being the difference 60.6 vs 61.0% (Stege et al. 2011). This study considers all type of restrictions in volume performed in the country. Restriction in the final phase of growth, for example restricted feed from 65 kg weight until slaughter in two genotypes (Swiss Large White x Swiss Landrace and Duroc x Swiss Landrace), produces leaner carcasses than no restriction (Affentranger et al. 1996). Therefore, according to most of the works, the restriction in the final phase of the fattening would be a

good technique to reduce the fat content of the carcasses and adapt them to the desired characteristics. However, it would be interesting to see how the lean and fat content of the carcasses evolves once the restriction starts, since this would help to better determine the time and level of restriction required to achieve the desired type of carcass.

The results of Bee et al. (2007) showed that Swiss Large White pig restricted to 80% of AL during growth period results in increasing the lean percentage of carcass ( $P < 0.01$ ). Another work shows that Duroc x (Large White x Landrace) pigs fed a diet restricted up to 65% of the control diet during growing period reduced carcass fatness in terms of backfat thickness ( $P = 0.023$ ) and percentage ( $P = 0.004$ ) and also subcutaneous fat proportion at the ham level ( $P = 0.047$ ) (Heyer and Lebret 2007) and if they were restricted up to 70% of AL have thinner backfat ( $P < 0.05$ ) in carcass (Lebret et al. 2007). On the other side, Cho et al. (2006) reported little effect of feeding restriction on carcass characteristics like fat depth.

On the other hand, if the food restriction has occurred at the beginning of the fattening and has been completed with feeding AL, a compensatory effect can occur, in which the growth rate accelerates in the final phase, modifying rapidly the characteristics of the carcasses. Thus, in this type of restriction, deposition of fat at the end can become higher or similar than those that have not been restricted (Heyer and Lebret 2007).

Besides, there are also some studies which studied the effect of restricted feeding during both the growing and finishing period. For example the results of (Lebret et al. 2001) demonstrated that pigs fed restriction at 75 % during both the growing and finishing period (from 30 to 110 kg) had the carcass with higher lean meat content, ham and loin proportion, but lower mean back fat depth (mm), perirenal fat (kg) and backfat proportion (g/kg).

Consequently, different types of restrictions can be used as strategies to achieve the desired type of carcasses and to modify the weight at slaughter. Because of that, it is important to know the evolution of the deposition of fat and lean according to the diet pigs are subjected and throughout their growth. It is vital that this improvement in the characteristics of the carcasses does not have a negative effect on the quality of the meat.

### 1.2.1.3. Effect of restriction in meat quality

Feeding restriction has always received much attention in the porcine research and one of its aim is to improve the carcass and meat quality characteristics to satisfy consumer preferences.

Oksbjerg et al. (2002) found that lightness of meat, ultimate pH or drip loss were not changed by the feeding restriction during all growth period. Regarding the study of Danielsen et al. (2000), pigs fed with 70% of concentrate of clover grass or clover grass silage showed less tenderness and more hardness at first bite than the pigs fed AL (100% of concentrate). Madeira et al. (2013) demonstrated that the feeding restriction promoted the intramuscular fat deposition during the growing-finishing phase of pigs and a high marbling level which is in agreement with Kamalakar et al. (2009) who showed more marbling scores from restricted feeding treatment. Moreover, Kristensen et al. (2002) concluded that higher meat quality and less feed quantity for economical cost would be archived easily by using the compensatory growth strategy for feeding pigs.

Tenderness is considered an essential meat eating quality parameter (Becker et al. 1998; Moloney et al. 2001; Keady et al. 2017). Some works show that feeding strategies can modify the proteolysis and tenderness *post mortem* (Kristensen et al. 2002). Nevertheless, the effect is not clear because some studies showed that meat from animals with feeding constraints is slightly tougher than those from animals fed AL (Ellis et al. 1996; Bee et al. 2006) while other studies have found that there is no significant difference among the feeding treatments with restriction or not (Kristensen et al. 2004). Furthermore, other works show that pork from pigs with feed restriction in volume was not different in tenderness than those from pigs fed AL (Heyer and Lebret 2007; Chaosap et al. 2011b). Nevertheless, differences in tenderness depend on the muscle and the duration of the restriction (Kristensen et al. 2002; Kristensen et al. 2004; Therkildsen et al. 2008). Thus, probably because of these differences, effects of feeding restriction strategies on tenderness are contradictory and more work is needed to confirm the effect of the type of feed restriction strategies on the final quality of the product, both in terms of sensory and tenderness and in its intramuscular fat

content (Heyer and Lebret 2007). Regarding other species, for instance, the texture of samples meat from supraspinatus of bulls could be improved by a compensatory growth feeding strategy (Hansen et al. 2006).

Besides, some other parameters of meat quality such as juiciness, cooking loss and colour were also evaluated in several works and no significant differences between feeding restriction strategies, diet composition or phase were found (Lebret et al. 2001; Heyer and Lebret 2007; Kristensen et al. 2002; Apple et al. 2004; Hinson et al. 2011; Suarez-Belloch et al. 2013). While some studies reported that pigs under restriction feeding produced less juicy meat (Ellis et al. 1996), meat with higher cooking losses (Bee et al. 2007), and meat with lower  $a^*$  if volume restriction is in the first phase (Kristensen et al. 2002), or higher  $L^*$  if it is in the second phase (Becker et al. 1998).

### 1.2.2. Restriction in Phosphorus

Bone can be considered both tissue and structure since it ensures physiological and mechanical functions; it controls the metabolism of Ca, P and Mg, supports the organism and protects its organs (Guede et al., 2013). Phosphorus is an essential nutrient due to its role in energy regulation, enzyme activity, bone development, and other metabolic functions for the growth of the animal (Eastwood 2003; Partanen et al. 2010; Oster et al. 2016). It is reported that 80% of P is concentrated within the animal bone (Breves and Schröder 1991). Regarding the economic benefice of swine business, the research on the content of P in the formulations of diets is very important for developing the feeding strategy since the cost of dietary P is high (Teixeira et al. 2016). In fact, P together with carbohydrates (energy) and protein are considered the three most expensive nutrients in the swine feeding industry (Symeou et al. 2014).

P is present in the ingredients of swine feed (i.e. grains of maize, rye, triticale, soybeans, etc) mostly in the form of myoinositol hexakisphosphate (phytate). If the phytate of the fed is not hydrolysed, it is unabsorbed and is excreted with the manure. This excretion, if excessive, can produce environmental problems, like eutrophication (Vats et al. 2005; Létourneau-Montminy et al. 2011; Pomar et al.

2007). The intensive production of swine generates huge amounts of manure that results in nutrient accumulation in the soil, constituting a risk to the environment (Kornegay et al. 1997). Therefore, it is important to adjust the amount of P in the diet without harming bone mineralization or animal productivity. Thus in the last years, interest in minimizing P in finishing pig diets increased in order to reduce nutrient excretion and feed costs (Shaw et al. 2002; Shaw et al. 2006). Also, one modelling approach was developed for investigating the P excretion by quantifying the consequences of different feeding strategies (Symeou et al. 2016). Regarding feeding strategies to reduce excreted P, one option is to group pigs according to BW to homogenize the nutritional requirements (Symeou et al. 2016) or to apply the precise feeding to adjust the nutritional content of the feed to satisfy the feeding requirements by means of multiphase feeding systems or precise feeding (Pomar et al. 2014). It is also an option the inclusion of the enzyme phytase (Selle and Ravindran 2008). Phytase is the enzyme capable of breaking the bond of inositol phosphate, releasing the molecule of P.

Pomar et al. (2007) reported a system for reducing the excreted P by modifying the amount of phytases in the diet and the rest of the food formulation. Food conversion ratio and bone mineralization can be improved if low P diet is applied if there is a higher inclusion of phytase in it (Varley et al. 2011). Phytase enzyme catalyses the hydrolysis of phytate in the upper digestive tract making orthophosphate available for absorption. One way for meeting the P needs of swine without supplementing inorganic P is the use of microbial phytases on pig's meals (Baxter et al. 2003; Selle and Ravindran 2008). Previous studies have proven that the supplementation of low-P diets with phytase restores pigs' growth performance, affects bone breaking strength, improves the use of phytate P from 25 to 30% and reduces the amount of excreted P between 30 and 40% (Cromwell et al. 1993; Cromwell et al. 1995; Kornegay and Qian 1996; Harper et al. 1997; Radcliffe and Kornegay 1998).

The dietary P concentrations necessary to maximize growth performance in growing-finishing pigs are well defined (Council National Research, 1998).

It is important that the pigs' supply of dietary P is adequate to maximize growth performance without compromising bone development (Partanen et al. 2010), presence of aplomb problems and lameness (Cromwell et al. 1995).

#### 1.2.2.1. P and bone characteristics

One of the problems of P deficiency in the diet is the decrease in bone strength although it depends on the level of reduction and the time of application (Ryan, et al., 2011a; Varley et al. 2011; Fabà et al., 2019). The increased available P in the diets could continue increasing the percentage of bone ash and bending moment as Hastad et al. (2004) reported. The needs of P that optimize the growth of the pig are not equivalent to those that optimize the bone resistance and this can have consequences when differentiated feeds are not supplied to fattening pigs and future breeding females. The latter should not only have greater bone strength, but also have more mineral reserves to respond to the mechanism of depletion-repletion that they will suffer throughout their reproductive life. On the other hand, an adult sow at the end of pregnancy can weight more than 350 kg, thus it is imperative to have an adequate bone structure, without deformation of the column or aplomb's to have a long productive life. As a result of animal welfare legislation, pregnant sows cannot be kept in boxes and, therefore, they are housed in group. Consequence or not, the reality is that there is a very high percentage of sows with lameness, problems in the hooves or weakness of aplomb's. Having good aplomb is an essential feature in the selection of females as breeders since it allows to increase the longevity of animals (Fukawa and Kusuhara 2001), improve reproductive capacity and produce healthier piglets (Quinn et al. 2013). In this sense, diets low in P, inadequate Ca: P ratio and/or low in vitamin D may be the base of the aplomb and lameness problems due to a decrease in bone strength (Crenshaw 1981; Cromwell et al.1995). The use of diets low in P (often bordering on the deficiency limit) or the risk of possible interactions with other nutrients in the diet (bivalent ions, such as Ca or Zn) can lead to a decrease in yield, quality of meat (Kongsro and Gjerlaug-Enger 2013) or negatively affect bone mineralization (Varley et al. 2011), especially if the females are destined to be future breeders.

There are some studies that showed the effect of P deficiency on the characteristics of bones. Furthermore, higher P in the diet of pigs could increase the retention and excretion of P without having any effect on the bone measures

(Sørensen et al. 2018). Ryan et al. (2011a) found that there is no compensatory effect on the bone mineral density when the pigs were fed with low P diets in the first period of growth and followed by high P fed in the following period. However, Varley et al. (2011) concluded that pigs with initial temporary reduction of P can compensate for growth, P and Ca of the bones when the P increased in the final diet, although this cannot reach a similar level of bone mineral density than those with high P diet during the whole growth period. Nevertheless, some studies showed that the amount of P does not significantly affect the growth performance of pigs (Crenshaw et al. 1981; Ryan et al. 2011a).

The mechanical properties, geometrical measurements and percentage of ash of bones were increased with the higher levels of Ca and P in the diets (Crenshaw 1981). Cromwell et al. (1995) found that the addition of phytase increases the hardness of the bones, since it can enhance the utilization of the P of the food and the increase of this utilization of P is significant in diets with low P. One recent study also suggest that the low P and Ca levels in feeding treatment can have a positive effect on the improvement of resource efficiency and the reduction of phosphorus waste (Oster et al. 2018).

#### 1.2.2.2. Lameness

Lameness, which is a result of leg weakness, constitutes one of the major problems in the pig industry. It has been observed that the weakness of the bones of the posterior limbs is positively correlated with the appearance of lameness (Jørgensen 1995). Lameness is usually assessed visually although (Kongsro 2013) developed an automatic vision system to detect it. Lameness leads to the premature slaughter of a large number of gilts and sows, which results in economic losses since sows only become profitable after the third litter (Aasmundstad et al. 2013; Quinn 2014; Fukawa and Kusuhara 2001). Consequently, sow lameness should be reduced for decreasing the economic loss since it is the main reason that affects the sow longevity negatively in swine breeding herds (Anil et al., 2009). It is important that the gilts destined for breeding animals do not have the problems of lameness, because they could



harm their reproductive capacity and the health of the offspring. Lameness is a very serious problem since it affects around 10-15% of gilts in Western Europe, and it results in hoof problems to 50 or 100% of gilts according to the report from van Riet et al. (2013). There is one study that shows that lameness is one of main health problems in Irish pig farms and it has a negative effect on the sow longevity, number of pigs and expenses of farm in the view of economics (Quinn et al., 2013).

Therefore, in the production of females and in their selection, it is vital to consider the aplomb's and lameness, especially if the P levels of the diets used are not considered.

### 1.2.2.3. Phytase supplementation

Phytase was considered to improve the bioavailability of phytate P effectively in the diet of pigs (Cromwell et al. 1993). The pigs were found to have higher P digestibility and P retention in the feeding treatment with phytase addition than those in the treatment without phytase supplementation (Varley et al., 2011). There is interest in supplementation with exogenous phytases, because phytases allow the use of a good part of the phytic P of the raw materials (Cromwell et al. 1995; Létourneau-Montminy et al. 2011), reducing the contribution of inorganic phosphates. The benefit of the use of phytases is multiple and leads to a significant reduction in P levels of the diet without harming the health and animal productivity (Létourneau-Montminy et al. 2011), because the phytases can strengthen P utilization and minimize the faecal P excretion (Adhikari et al., 2016). Phytases are highly available, but they are expensive to produce, so it seems very important to find out a proper quantity phytase supplementation added in the diets to gain a better profit for the swine industry.

### 1.2.2.4. Effect of P restriction on productive parameters

There have been some works tried to decrease the P levels of diets without negative effects on the productive characteristics of pig. Kyriazakis et al. (2013)

showed that it is possible to restrict the P dietary levels in the feeding treatment safely without affecting the performance of growing or finishing pigs. Crenshaw (1981) showed that the growth rate of pigs at any age period and different sex were not affected by the P level in the diets, and Nimmo et al. (1980) also didn't detect any difference in ADG, ADFI and ratio gain:feed these parameters among the pigs fed the different dietary levels of P. This is similar to the study of Varley et al. (2011) which measured the effect of P concentration on weaner and finisher pig performance and also the study of Ryan et al. (2011a) presented the compensatory effect of dietary P on performance results of growing pigs. In the study of Alexander (2008), the overall ADG and ADFI were not affected by the dietary P levels, but the PIC337-sired pigs (lighter-boned genetic line) showed higher ratio gain:feed in the diet with P adequate than the diet with P deficient. Another study demonstrate that the increasing available P had no effect on growth performance of pigs during day 0 to day 28, while the diets without supplemental P could cause their ADG and ratio gain:feed decreased during the finishing period of pigs (>88kg) (Hastad et al. 2004). Regarding the carcass and meat quality, Cromwell (1972) found that the diet which was lack with P lead to the reduction of efficient gains, fatter carcass, less ash content in the metacarpals and also the ash content of turbinates decreased significantly. In the study of Alexander (2008), live weight, HCW and carcass fat-free lean percentage was significantly different between the two dietary P level treatments (P-adequate diet and a 20% P-deficient diet). Other works show a decrease in daily gain when diets with low P levels are supplied, although it depends on the type of P added (Cromwell et al. 1995).

### 1.3. Computed tomography

#### 1.3.1. The technology

Computed tomography was invented by the engineer Godfrey Hounsfield in 1972 (Hounsfield 1973). The CT equipment consists of a gantry in which the X-ray source and detectors are placed, an electric generator and a workstation were images are reconstructed and analysed.

The X-ray source emits X-ray that go through the object or subject along the 360° and reach the detectors more or less attenuated, depending on the density of the object/subject. The attenuation is measured in HU. The matrix of attenuation values allows to create images in grey tones after applying reconstruction algorithms. The images are a visualization of the inner part of the body in two dimensions although each image has a thickness and it represents, in fact, a three-dimension (3D) image. Each image is composed by several voxels (3D-pixel) and the number of voxels depends on the size of the image. Images are in a grey scale being the lighter the densest tissues (higher Hounsfield –HU values) and the darker the less dense tissues (lower HU values) (Font i Furnols and Marina, 2009). HU value of 0 correspond to water, HU values higher than 0 correspond to tissues with higher density than water, *i.e.* muscle and bone while HU lower than 0 correspond to tissues with less density than water, *i.e.* fat. The limit for fat and lean vary slightly between studies as well as the limit between lean and bone. According to its HU value, each voxel can be classified as lean, fat or bone, and jointing several images it is possible to determine a volume, either of all the whole image or of some of the tissues. Nevertheless, some voxels present the partial volume effect, *i.e.* they have more than one type of tissue and they are difficult to be classified. This technology opens new opportunities in the study of the effect of diets on body/carcass tissue composition, since it is not destructive, it allows the evaluation of the same animal at various moments of growth without the need to slaughter the animals and, therefore, to monitor the same animal to subjected to the feed strategy.

### 1.3.2. Use of computed tomography in livestock

Computed tomography is not only used as a medical instrument for the human beings. It has also been applied in the study for the internal quality inspection of many agricultural products and livestock. Scholz et al. (2015) summarized the applications of main non-invasive techniques for body or carcass composition measurements of livestock and reported that CT is the most accurate one. Carabús et al. (2016) also review the use of non destructive technologies, including CT, to evaluate live pigs.

Computed tomography scanning technology has a high potential for valuing the physical and chemical body composition of pigs (Arthur et al. 2011). It can be used to scan live pigs at different moments of their live allowing to quantify and mathematically describe the growth of pigs and of their body components (Giles et al. 2009; Barchia et al. 2010; Lambe et al. 2013; Carabús et al. 2014; Carabús et al. 2015). Kolstad (2001) used CT to quantify the subcutaneous, inter/intra muscular and internal fat depots of pigs at different weights and different genetic groups, demonstrated that CT is valuable for studying the growth and development of pigs. More details related to body composition of pig can be gained from CT than the dissection methods (Gjerlaug-Enger et al. 2012). Computed tomography has been used in livestock to analyse the adiposity of minipigs fed different caloric diets in order to use pigs as a model for human obesity (Val-Laillet et al. 2010). Computed tomography has also been used to measure and predict the intramuscular fat with more or less accuracy in muscle LT of cattle (Anderson et al. 2018; Font-i-Furnols et al 2014 ), in Texel lamb loins (Clelland et al. 2014), in live pigs (Lambe et al., 2013; Kongsro 2013; Font-i-Furnols et al., 2019) and in pork loins (Font-i-Furnols et al. 2012). Also McEvoy et al. (2009) explored subcutaneous adipose tissue as an indicator of body composition by using the CT equipment.

From all the images obtained of the scan of a pig it is possible to study the volume associated to each HU value and obtain prediction equations to estimate several tissues or body composition characteristics (Font-i-Furnols et al. 2015; Zomeño et al. 2016). Thus, CT can be used to determine body tissue composition at one moment of the growth or at several moments during growth avoiding (serial) slaughters when different feeding strategies are applied or depending on the sex or the genotype of the pigs (Lambe et al. 2013; Carabús et al. 2014; Font-i-Furnols et al. 2015). Because of that, it is worthwhile to investigate the effect of different feeding strategies considering combinations of AL and RV restriction feeding periods on the tissue growth and carcass quality of gilts at different stages of growth by means of CT images to evaluate this effect and to confirm that CT is a suitable tool for this purpose in this type of studies. Some studies have shown the suitability of CT to study the effect of weight maintenance feeding (Kolstad et

al 1996; Kolstad and Vangen 1996) and different type of diets (Lambe et al. 2013) on the characteristics of body.

Computed tomography would be a novel and highly interesting technology for nutritionists, geneticists, producers and industrialists of the pig, since it would allow to know the body composition throughout the growth and, therefore, see how it can be optimized to reach the desired final product in terms of quantity of fat and muscle.

Ryan et al. (2011a) analysed the density of the bones by means of the dual X-ray equipment (DXA) by scanning the bones extracted from the sacrificed animal. Using CT, Bertaud du Chazaud et al. (2015), demonstrated the effect of the diet with different P levels on the characteristics of the bones. However, there are no known studies that evaluate the effect of dietary P levels on the characteristics of the bones of live animals by CT at various times of their growth. This technology allows determining the density of the bones as well as their dimensions without slaughtering the animal.

## **OBJECTIVES**



## 2. Objectives

The general purpose of this PhD work was to assess the influence of different type of feeding restriction strategies and dietary P levels on body tissue composition evaluated *in vivo* with CT, bone mineralisation and sensory properties of the meat from gilts, and to determine the suitability of CT to study the effect of the nutrition on the evolution of body characteristics.

These objectives will be achieved with two experiments with the following specific objectives:

*Experiment 1:* Feeding restriction: AL and RV in the growing or finishing phase.

- To determine the effect of the feeding strategy on the productive parameters of the pig during growth.
- Evolution of body tissue composition during growth depending on feed restriction by means of CT images.
- To determine the effect of the feeding restriction strategies on technological and sensory quality of the meat and on the acceptability of meat by consumers.

*Experiment 2:* Feeding restriction: low P, two diets with two levels of phytases and control.

- To evaluate the influence of levels of P in the diet on lameness, bone resistance and composition during pig growth
- To determine the effect of different levels of P on the morphological and chemical characteristics of bones.
- Evolution of body tissue deposition depending on feed restriction and level of dietary P.





## **MATERIALS & METHODS**



### 3. Materials and methods

The work consisted in two separate experiments:

#### 3.1. Experiment 1

##### 3.1.1. Animals and Diets

Thirty-six Pietrain x (Large White x Landrace) gilts were distributed into 4 groups and assigned to the following 3 feeding strategies: 1) AL feeding during all fattening period (AL-AL); 2) AL feeding between 30 and 70 kg TBW followed by RV (84% of AL) until 120 kg TBW (AL-RV); and 3) RV (78% of AL) between 30 and 70 kg TBW followed by AL until 120 kg TBW (RV-AL). The composition and nutritional value of the diets are presented in Table 1.

Pigs were reared in individual pens, and they were weighed every two weeks. Feed restriction was calculated every two weeks based on the BW and ADFI of the AL pigs. Feed consumption was controlled individually for each pig. Additionally, at the end of each period (growth and finishing), the fat depth and muscle thickness were measured with the Piglog 105 ultrasound device (Frontmatec A/S, Herlev, DK) at the last rib level and at 4-6 cm from the midline. All the animals were used in the following analysis.

Table 1. Composition of the experimental diets for different feeding strategies

Ingredient, %	Growing diet	Finishing diet
Wheat	30.00	25.64
Maize	25.00	25.00
Barley	12.32	13.95
Triticale	1.50	11.11
Soybean meal	13.38	7.17
Rapeseed meal	6.00	6.00
Wheat middling's	---	---
Biscuit meal	4.56	3.20
Rice bran	1.50	1.60
Peas	---	1.50
Molasses	1.00	1.00
Fat 3/5 Grefacsa	1.24	0.76
L-Lysine HCl	0.68	0.60
DL-Methionine	0.09	0.08
L-Threonine	0.16	0.13
L-Tryptophan	0.19	0.03
Dicalcium phosphate	0.66	---
Limestone	0.68	1.22
Salt	0.34	0.30
Vitamin and mineral premix <sup>1</sup>	0.20	0.20
<b>Chemical composition<sup>2</sup></b>		
Gross energy, Mcal/kg	3.904	3.923
Net energy, Mcal/kg	2.264	2.275
Ether extract, g/kg	50.4	35.7
Crude fibre, g/kg	26.7	28.7
Crude protein, g/kg	175.4	148.1
Total lysine, g/kg	9.80	7.70
Total threonine, g/kg	6.40	5.80
Total methionine, g/kg	3.50	2.80
Total Met+Cys, g/kg	6.20	5.10

<sup>1</sup> Provided per kg feed: vitamin A (E 672), 5500 UI; vitamin D3 (E 671), 1100 UI; vitamin E (alfa tocopherol), 7 mg; vitamin B1, 0.5 mg; vitamin B2, 1.4 mg; vitamin B6, 1 mg; vitamin B12, 8 µg; vitamin K3, 0.5 mg; calcium panthotenate, 5.6 mg; nicotinic acid, 8 mg; choline, 120 mg; Fe (E 1) (from FeSO<sub>4</sub>·7H<sub>2</sub>O), 80 mg; I (E 2) (from Ca(IO<sub>3</sub>)<sub>2</sub>), 0.5 mg; Co (E 3) (from 2CoCO<sub>3</sub>·3Co(OH)<sub>2</sub>·H<sub>2</sub>O), 0.4 mg; Cu (E 4) (from CuSO<sub>4</sub>·5H<sub>2</sub>O), 5 mg; Cu (E 4) (from the amino acid quelate), 5 mg; Mn (E 5) (from MnO), 40 mg; Zn (E 6) (from ZnO), 100 mg; and Se (E 8) (from Na<sub>2</sub>SeO<sub>3</sub>), 0.25 mg.

<sup>2</sup> Analysed nutrient contents

### 3.1.2. CT Scanning and Image Analysis

The pigs were CT scanned when they reached 30, 70, 100 and 120 kg TBW (Figure 6). When the pigs reached the desired TBW, they were fasted for eight hours and then transported to the CT facility. Intramuscular sedation with azaperone (0.1 mg/kg body weight) and ketamine (0.2 mg/kg body weight) and intravenous sedation with propofol (0.22 mg/kg body weight) for 100 and 120 kg TBW were applied to anaesthetize the pigs before scanning with a General Electric HiSpeed Zx/I CT scanner (GE Healthcare, Madrid, Spain). The acquisition conditions were as follows: 140 kW; 145 mA; 512x512 matrix; axial; 7 mm thickness (30 kg TBW) and 10 mm thickness (70,100 and 120 kg TBW); 350 to 460 mm displayed field of view; and STD+ reconstruction algorithm. After scanning, the pigs were returned to the experimental farm to continue the study. One pig from the RV-AL treatment died during the experiment after scanning at TBW of 120 kg.

CT images were analysed using the software *VisualPork* (Bardera et al. 2012; Boada et al. 2009). Based on previous works (Carabús et al. 2014; Carabús et al. 2015), three images (tomograms) were selected for the analysis at the following anatomical location: between the 11<sup>th</sup> and 12<sup>th</sup> ribs, between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae, and at the ham level in the joint between the femur and the pubis bones. The measures performed for each image were the loin area and perimeter in the loin cuts, total area and perimeter in the ham, and subcutaneous fat area and perimeter (Figure 7). The distribution of the volume associated with each Hounsfield value was also determined and used to determine the lean meat content of the carcass and pieces as well as the weight of the pieces according to the equations developed by Font-i-Furnols et al. (2015). Additionally, the ash, moisture, protein and fat contents from the carcass were calculated according to the equations developed by Zomeño et al. (2016).



Figure 6. Computed tomography scanning of a live pig.)

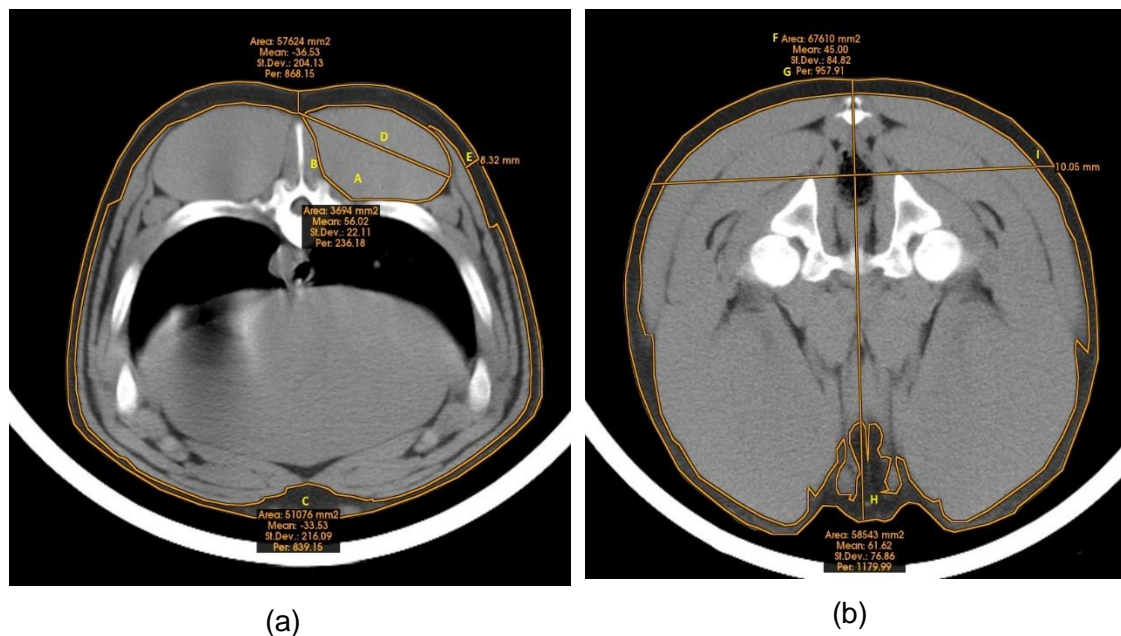


Figure 7. Anatomical measures obtained from the tomograms obtained at the 11<sup>th</sup>-12<sup>th</sup> rib and 3<sup>rd</sup>-4<sup>th</sup> lumbar vertebrae levels (a) (A: loin eye area; B: loin eye perimeter; C: subcutaneous fat area; D: maximum width of the longissimus area; E: lateral fat thickness at the edge of D perpendicular to the skin) and at the ham in the joint of the femur and pelvis bones (b) (F: area of the whole ham; G: perimeter of the whole ham image; H: subcutaneous fat area; I: lateral fat thickness at the upper part of the bones level)

### 3.1.3. Slaughter, Quality Measurements and Sampling

After the last CT scan of the 120 kg TBW pigs, the animals were sent back to the farm for  $13 \pm 4$  d. Then, after approximately 20 h fasting, the pigs were transported to an experimental abattoir placed at IRTA (Monells) for slaughter after CO<sub>2</sub> stunning on 4 different days. The live weight and warm carcass weight were recorded, and the yield was calculated. The back fat thickness and muscle depth were measured at 6 cm from the midline at the intercostal space between the 3<sup>rd</sup> and 4<sup>th</sup> last ribs via a Fat-O-Meat'er (FOM) (Frontmatec A/S, Herlev, DK). These two measures were used to determine the carcass lean meat percentage (LMP) using the Spanish official equation for FOM:  $LMP = 66.91 - 0.895 \times LR_{3/4}FOM + 0.144 \times MFOM$  (Font i Furnols and Gispert, 2009; Commission Decision 2009/11/EC) (Figure 8).



Figure 8. Fat and muscle thickness and lean meat content determined with Fat-O-Meat'er.

At 45 min after slaughter, the pH values of the *longissimus thoracis* (LT) muscle at the last rib level and the *semimembranosus* muscle of the ham were measured with a Crison tool with a Xerolyt electrode (Crison, Barcelona, Spain) (Figure 9).





Figure 9. pH measured in the longissimus muscle.

The minimum fat thickness (plus skin) (F-ZP) was measured perpendicularly to the skin surface of the carcass over the *gluteus medius* (GM) muscle, and muscle depth was measured between the medular canal and the cranial end of the *gluteus medius* muscle (M-ZP). Additionally, the backfat thickness was measured in the midline at the level of the last rib.

Subsequently, the carcasses were kept in a chilling room for 24 h at 2°C. At 24 h *post mortem*, the cold left carcass was weighed. At this time *post mortem*, ultimate pH measurements were also performed in the *LT* and *SM* muscles. In the same muscles, electrical conductivity was measured using a Pork Quality Meter (PQM-Kombi, Aichach, Germany).

Furthermore, the loin muscle (from 3rd-4th last ribs to caudal direction) was sampled for further analysis: trained panel and consumer test (4 slices of 1.5-cm thick each), cooking losses and texture (2.5 cm), marbling, colour and drip losses (2 cm) and moisture and intramuscular fat (4 cm). Samples were vacuum packed and stored at -20°C until its use except those for marbling, colour and drip losses since analyses were performed immediately.

For cooking losses, the loin was cooked in an oven (FAGOR Innovation Class A; Fagor Electrodomésticos, S. Coop., Mondragón, Spain) at 200°C until reach the internal temperature of 71°C. Cooking losses were obtained by weight difference. The same cut, when cold, was used for texture analysis. Warner-Bratzler test was

performed in the texturometer TA.XT2 (Stable Micro Systems Ltd., Godalming, UK).

Marbling was determined by a trained technician using the National Pork Producers Council (NPPC, 1999) standards, which range from 1 (devoid of marbling) to 10 (abundantly marbled). At the same position, colour was determined after 15 min of blooming with the colorimeter Minolta CR 400 to obtain the  $L^*$ ,  $a^*$  and  $b^*$  variables (CIE 1976), and the Japanese Scale of Colour from 1 (pale) to 6 (dark colour) was applied (Nakai et al. 1975) (Figure 10). From the same loin two samples of 2.7 cm of diameter were used to determine drip losses by means of Rasmussen (Rasmussen and Andersson 1996) method (Figure 11). Intramuscular fat was measured by near an infrared FoodScan system (Foss Analytical, Denmark) at wavelengths between 850 nm and 1050 nm.



Figure 10. Colour measurement with the colorimeter Minolta (left) and the Japanese Scale of Colour (right).



Figure 11. Drip loss determination.

### 3.1.4. Trained panel test

The trained panel test was performed in a sensory room at IRTA-Monells prepared according to the ISO standards ISO (8589:2007). The evaluation was carried out by eight trained panellists (Figure 12). Four training sessions were performed initially to establish the final attributes to be evaluated and fix the measurement scale. The final descriptors, which were obtained by consensus in these sessions, were odour attributes (pork, pig and abnormal), flavour attributes (pork, pig, abnormal, acid, sweet and metallic) and texture attributes (hardness, juiciness after first chewing, juiciness during chewing, tenderness, fibrosity and chewiness) (Table 2). The attributes were evaluated via a numerical intensity scale ranging from 1 (low/weak) to 10 (high/strong). A total of 10 sessions, with 3 samples per session (one of each dietary treatment), were carried out. Sample preparation was similar to that reported by Font-i-Furnols et al. (2008). In brief, meat slices (1.5-cm thick) were cook in a pre-heated oven (at 200°C) until reaching 72°C internal temperature. After cooking, the slices were cut into 4 pieces each, wrapped in aluminium foil with a 3-digit code, and kept warm until they were distributed to the panellists monadically and following a designed order to avoid the first sample and carry-over effect (MacFie et al. 1989).



Figure 12. Sensory evaluation of loins by trained panellists.

Table 2. Codes and description of the sensory attributes evaluated by the trained panellists.

Code	Attribute <sup>1</sup>	Definition
ODOUR		
O_Meat	Pork meat	Intensity of boiled pork with normal smell
O_Pig	Pig	Intensity of live pig smell
O_Abnor	Abnormal	Intensity of boiled pork with different and not normal smell
FLAVOR		
F_Meat	Pork meat	Intensity of boiled pork with normal flavour during chewing
F_Pig	Pig	Intensity of live pig flavour
F_Abnor	Abnormal	Intensity of boiled pork with different and not normal flavour during chewing / residual
F_Acid	Acid	Ref: Citric acid
F_Sweet	Sweet	Ref: Sugar
F_Met	Metallic	Ref: Blood
TEXTURA		
T_Hard	Hardness	Force required to compress meat between molars and first bite
T_Juic1	Juiciness at first bite	Amount of water released from first bite
T_Juic	Juiciness during chewing (5 bites)	Amount of water released during chewing (after 5 bites)
T_Tend	Tenderness	Ease at which meat is divided into small particles when chewing.
T_Fibro	Fibrosity	Amount of fibres during chewing (ref. asparagus)
T_Chew	Chewiness	Amount of required bites before swallowing the meat

<sup>1</sup>Scored from 0: low/weak to 10: high/strong

### 3.1.5. Consumer study

A total of 120 consumers were randomly selected in Barcelona trying to follow the Spanish national distribution for age and gender (Table 3). Ten sessions were carried out in 2 d, with 12 consumers per session. Samples preparation was the same as those used in the trained panel sensory evaluation. After cooking, the slices were cut into 4 pieces each, wrapped in aluminium foil with a 3-digit code and kept warm until being served to the consumers (Figure 13).

Each consumer evaluated in blind conditions three pieces of meat, one from each feeding treatment. Samples were served monadically to the consumers and in a different order to avoid the first sample and carry-over effect (MacFie et al. 1989). Consumers' were asked to eat unsalted toast and drink water before evaluating each sample and also before evaluating the first one. For each sample, the consumers were asked to score the overall acceptability, tenderness, odour and flavour according to a 9-point scale (from 1 'like extremely' to 9 'dislike extremely'). To obtain a more specific response from consumers (Guerrero 1999), the intermediate point corresponding to 5 'neither like nor dislike' was not included. In addition, demographic information and habits of consumption were also recorded. Finally, the consumers were asked to rank by purchasing preference several meat characteristics (from entire males, without exudate, colour, less marbling, less subcutaneous fat, certain brand or genetic, organic rearing and best price).



Figure 13. Preparation of the samples for the consumer study (left) and consumers performing the sensory evaluation (right).

Table 3. Socio-demographic characteristics of the participants in the consumer study.

	Women	Men	Total
<b>Participants</b>			
Total (n)	68	52	120
Total%	56.7	43.3	100.0
<b>Age group (%)</b>			
<26 years old	8.8	11.5	10.0
26-40 years old	19.1	30.8	24.2
41-60 years old	38.2	30.8	35.0
>61 years old	33.8	26.9	30.8
<b>Education level (%)</b>			
Primary	13.2	17.3	15.0
Secondary	51.5	50.0	50.8
University	35.3	32.7	34.2
<b>Do you decide on/perform the purchasing of meat at home? (%)</b>			
Yes	92.7	63.5	80.0
No	0.0	21.2	9.2
Only decide	4.4	13.5	8.3
Only purchase	2.9	1.9	2.5
<b>Where do you buy meat? (multiple choice answer)</b>			
Traditional butcher	43.9	35.5	40.4
Supermarket/hypermarket /butchery	28.0	34.2	30.6
Packed meat in Super/hypermarket	25.2	28.9	26.8
Others	2.8	1.3	2.2

### 3.1.6. Statistical Analysis

All the statistical analyses were performed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA), and individual animals were considered the experimental unit. An analysis of variance was performed using the MIXED procedure. For productive parameters, the model included treatment as a fixed effect. Additionally, the BW at the beginning of each feeding phase was included as a covariate. For the carcass quality variables, the same model was applied but carcass weight was included as a covariate; and for the meat quality variables, slaughter day was included as a blocking effect. Regarding the CT variables, the model considered repeated measures and included feeding strategy, TBW and its interactions as fixed effects. In this analysis, a weighted least squared approach was applied to address the heteroscedasticity of variance due to the differences of weight, *i.e.*, at each TBW, the dependent variables were weighed by the inverse of the standard deviation of the residuals. The level of significance was established at a *P* value lower than 0.5.

For trained sensory data the model was applied to the previously standardized data to correct for differences in the use of the scale between panellists, and the feeding treatment and panellists within session were included as fixed effects while the session was included as a blocking effect. Standardization (mean and standard deviation) was performed for the samples.

The model for the consumer study data included feeding treatment as a fixed effect and the consumer as a random effect. The ranking of purchasing preference was analysed in the model using the meat characteristic as a fixed effect. In all analyses, Tukey's test was used to determine significant ( $P < 0.05$ ) differences between feeding treatments.

A PCA was performed using the factor procedure of SAS, and the variables included certain carcass characteristics, meat quality characteristics and an average by treatment of the consumer and sensory data.

## 3.2. Experiment 2

### 3.2.1. Animals and dietary treatments

A total of 48 gilts (Large White x Landrace) with an average initial body live weight of  $48.5 \pm 2.7$  kg were used in this study. The pigs were assigned randomly, avoiding assigning animals from the same litter to the same treatment and balancing the average weight per lot. The study was conducted at IRTA-Monells experimental farm, where the pigs were kept in individual pens and fed AL for the duration of the experiment. The diets were isoproteic and isoenergetic, differing only in P, Ca, phytic P, non-phytic P, digestible P and phytase contents. Twelve pigs were assigned to each of four distinct diet-treatments: a control diet with recommended levels of P (positive-control - C0) (de Blas et al. 2013), a negative-control diet with low levels of P (negative-control - P0) and two negative-control diets supplemented with 500 phytase units (Phyzyme XP 5000, Danisco Animal Nutrition, Copenhagen, Denmark) (P1) and 1000 phytase units (P2) (Table 4). These diets were maintained from the beginning of the study ( $48.5 \pm 2.7$  kg BW) until the slaughter of the animals ( $121 \pm 3.2$  kg BW).

Fat and loin depths were measured at the farm at the last rib, 4 cm from the midline, on the left and right sides using Piglog 105 ultrasound equipment (Frontmatec A/S, Herlev, DK), and the weight was taken before each scan.



Table 4. Composition of gilt diets by dietary treatment.

Ingredient, g/kg	Growing diet		Finishing diet	
	CO	P0	CO	P0
Maize	429.2	425.0	478.9	453.2
Barley	319.9	330.4	275.7	304.9
Corn dry distiller grains	100.0	100.0	100.0	100.0
Soybean meal 44%	105.2	108.7	74.5	57.2
Rapeseed meal	-	-	30.8	50.0
Fat 3/5 Grefacsa	15.0	15.0	15.0	15.0
L-Lysine HCl	3.9	3.9	2.4	2.5
DL-Methionine	0.4	0.3	-	-
L-Threonine	0.9	0.9	-	-
L-Tryptophane	0.4	0.4	0.1	0.1
Dicalcium phosphate	10.4	3.9	8.6	0.8
Limestone	5.8	7.5	5.0	7.2
Na bicarbonate	2.3	2.3	2.7	2.9
Salt	1.9	1.9	1.6	1.4
Vitamin and mineral premix <sup>(1)</sup>	4.6	4.6	4.6	4.6
Etoxyquin 66%	0.2	0.2	0.2	0.2
Phyzyme XP 5000 <sup>(2)</sup>	-	0/0.1/0.2	-	0/0.1/0.2
Chemical composition				
Net energy, MJ/kg <sup>(3)</sup>	9.850	9.869	9.835	9.836
Organic matter, g/kg <sup>(4)</sup>	851.9	851.9	844.2	851.8
Crude protein, g/kg <sup>(4)</sup>	149.7	148.9	140.0	144.1
Total lysine, g/kg <sup>(5)</sup>	9.00	9.00	7.50	7.50
Sid lysine, g/kg <sup>(5)</sup>	7.87	7.87	6.34	6.30
Ash <sup>(4)</sup>	51.0	44.3	45.2	40.0
Calcium <sup>(4)</sup>	7.84	5.49	6.10	4.82
Phosphorus <sup>(4)</sup>	6.76	4.95	6.37	4.49
Phytic phosphorus <sup>(5)</sup>	3.40	2.71	3.36	2.70
Non-phytic phosphorus <sup>(5)</sup>	3.36	2.24	3.01	1.79
Digestible phosphorus <sup>(5)</sup>	2.50	1.63	2.30	1.30
ratio Ca:P	1.16	1.11	0.96	1.07

(1) Provides per kg feed: vitamin A (E 672) 5500 UI; vitamin D3 (E 671) 1100 UI; vitamin E (alfa tocopherol) 7 mg; vitamin B1 0.5 mg; vitamin B2 1.4 mg; vitamin B6 1 mg; vitamin B12 8 µg; vitamin K3 0.5 mg; calcium panthotenate 5.6 mg; nicotinic acid 8 mg; choline 120 mg; Fe (E 1) (from FeSO4·7H2O) 80 mg; I (E 2) (from Ca(IO3)2) 0.5 mg; Co (E 3) (from 2CoCO3·3Co(OH)2·H2O) 0.4 mg; Cu (E 4) (from CuSO4·5H2O) 75 mg; Cu (E 4) (from aminoacids quelate) 5 mg; Mn (E 5) (from MnO) 40 mg; Zn (E 6) (from ZnO) 100 mg; Se (E 8) (from Na2SeO3) 0.25 mg.

(2) Phytase (Phyzyme XP 5000) was included at 100 and 200 g/MT in treatment P1 and P2, respectively.

(3) Net energy content calculated as 0.58 of analysed gross energy contents, according to INRA (2002).

(4) Analysed nutrient contents.

(5) Values obtained by feed formulation.

### 3.2.2. Lameness evaluation

The lameness of pigs was evaluated 3 times during growth: at the beginning of the experiment at approximately three months (50 kg BW), after five weeks (75 kg BW) and after five more weeks (120 kg BW). The evaluation was performed using a 5-point scale adapted from the Welfare Quality® protocol:

- 1: no lameness,
- 2: slight lameness,
- 3: severe lameness,
- 4: highly severe lameness,
- 5: extreme lameness.

The evaluation protocol was applied to pigs that had been standing up or walking at least one minute before the evaluation. All lameness evaluations were carried out by a trained technician.

### 3.2.3. Computed tomography scanning

#### 3.2.3.1. Scanning of live pigs

At the beginning of the study, a subsample of three randomly selected pigs per treatment were scanned at the live weight of 50 kg to verify the lack of differences between the different lots of animals. All pigs were then scanned *in vivo* at two TBWs: 75 kg and 120 kg. Before scanning, the animals were fasted for a minimum of 8 h and anaesthetized with azaperone (0.1 ml/kg) and ketamine (0.2 ml/kg) administered intramuscularly. Additionally, in pigs with target BWs of 75 and 120 kg, propofol (0.22 ml/kg) was applied intravenously to minimize possible disturbances in the CT images due to breathing movement.

The scan was performed in a General Electric HiSpeed Zx/I tomograph (GE Healthcare, Boston, Massachusetts, USA) following the protocol detailed at Font-i-Furnols et al. (2015) with axial acquisition, 140 kV and 145 mA, 512x512 matrix, 10 mm-thick images, a displayed field of view (DFOV) of 410mm for 50 and 75 kg animals, and a DFOV of 460 mm for 120 kg animals. After scanning, the

animals were returned to the IRTA experimental farm until the end of the experiment. The IRTA's ethical committee approved this experiment.

### 3.2.3.2. Slaughtering, carcass and meat quality measurements

Next,  $11.9 \pm 4.8$  days after the last scan at 120 kg, the animals were stunned with CO<sub>2</sub> and slaughtered at the IRTA slaughter plant 512 x 512 matrix, 10 mm-thick images and a DFOV of 460 mm.

Fat (F34FOM) and muscle (M34FOM) thickness were measured with Fat-O-Meat'er (FOM; Frontmatec A/S, Herlev, DK) between the 3<sup>rd</sup> and 4<sup>th</sup> ribs 6 cm from the midline (Figure 8). The lean meat percentage (LMP) was estimated using the official Spanish equation for FOM (Font i Furnols and Gispert, 2009). The ham fat thickness (minimum fat thickness over the *gluteus medius* muscle) and muscle fat thickness (minimum distance from the medullar channel to the cranial edge of the *gluteus medius* muscle) were determined with a ruler at the midline of the left half of the carcass. Measurements of the carcass length (from the anterior part of the symphysis pubis to the recess of the first rib) and loin length (length from the atlas to the first lumbar vertebrae) were taken. The pH and electrical conductivity of the *semimembranosus* (SM) and *longissimus thoracis-lumborum* (LT) muscles were taken 24 h post mortem using a KNICK portable pH-meter (Knick, Berlin, Germany) equipped with a Xerolyt electrode and a Pork Quality Meter (PQM-Kombi, Aichach, Germany) (Figure 9).

The colour of the meat was evaluated in the LT muscle, at the level of the last rib, after 15 minutes of blooming. Objective meat colour was measured using a CM-600D Minolta colorimeter (Konica Minolta Sensing Americas Inc., Ramsey, NJ, US), and luminosity (L\*), redness (a\*) and yellowness (b\*) were recorded (CIE, 1976) (Figure 10). Additionally, a subjective colour score was obtained using the Japanese colour scale (1: extremely pale to 6: extremely dark) (Nakai et al., 1975). Two experienced technicians determined this measurement, and the mean of their scores was calculated.

Water-holding capacity was evaluated by means of drip loss assessed with the EZ drip loss method. A piece of LT was placed in an appropriate closeable

container and stored at 4° C during 24h (Rasmussen and Andersson, 1996) (Figure 11). Then it was reweighted and drip losses were obtained.

### 3.2.3.3. Scanning of carcasses

Then, 24 h *post mortem*, the left carcass was CT scanned following the protocol detailed at Font-i-Furnols et al. (2009a). In brief, the acquisition conditions were as follows: helical, 140 kV, 145 mA, the tibia and the hand of each pig were extracted for new scanning (helical acquisition, 140 kV, 145 mA, 512 x 512 matrix, 10 mm-thick image and 200 mm of displayed field of view) and further analysis (Figure 15).



Figure 14. Computed tomography scanning of a pig carcass.

### 3.2.3.4. Scanning of bones

Once the carcasses were scanned, the femur, the tibia and the hand of each pig were extracted for new scanning (helical acquisition, 140 kV, 145 mA, 512 x 512 matrix, 10 mm-thick image and 200 mm of DFOV) and further analysis.

### 3.2.3.5. Texture analysis

Texture analysis was performed on the metacarpus III and metacarpus IV from the left hand of the animals.

Metacarpus breaking strength was measured in an MTS Alliance RT/5 material testing system (MTS Systems Corp., Eden Prairie, MN, USA) with a force of 5 kN by the three-point bend test (Sharir et al, 2008). The metacarpus was positioned on two base supports (separated by 3.6 mm), with the midpoint directly under the cross-head (Figure 15). The cross-head was lowered at a constant speed of 1 mm/s until fracture occurred. The test was carried out at the metacarpus midpoint. Using the TestWork4S software (MTS Systems Corporation, U.S), the maximum load, total energy and modulus were calculated from the load-displacement curve.

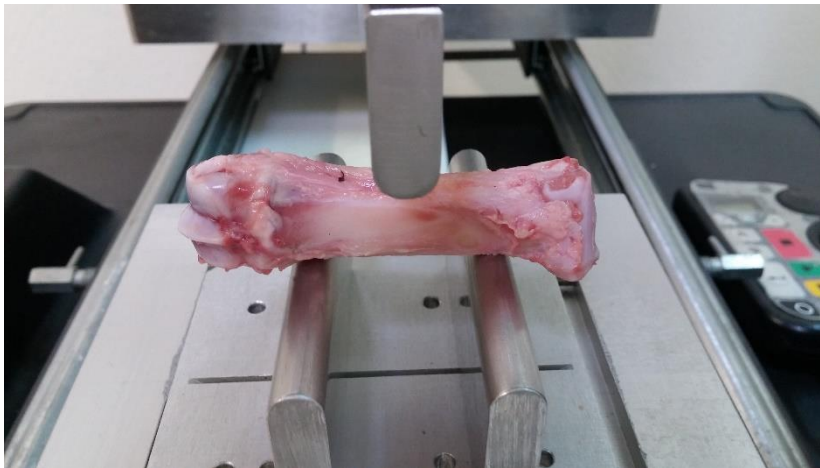


Figure 15. Texture analysis of gilt bones determined by break-tess or triple-point tests.

### 3.2.3.6. Dry matter and metacarpus ashes

The percentage of dry matter from metacarpus III and IV was determined by weight difference between fresh bone and dried bone after 24h in an incubator at  $103 \pm 2^\circ\text{C}$ . Once the dry matter was determined, the bones were burned by placement in a muffle oven at  $550^\circ\text{C}$  for 60h, after which the bones were re-weighed to calculate the ash percentage.

### 3.2.4. Bone quality measurements

Texture analysis was performed on the metacarpus III and metacarpus IV from the left hand of the animals.

Metacarpus breaking strength was measured in a MTS Alliance RT/5 material testing system (MTS Systems Corp., Eden Prairie, MN, USA) with a force of 5 kN, by the three-point bend test (Alexander 2008). Metacarpus was positioned on two base supports (separated 3.6 mm), with the midpoint directly under the cross-head (Figure 16). The cross-head was lowered at a constant speed of 1 mm/s until fracture occurred. The test was carried out at the metacarpus midpoint, and using the TestWork4S software (MTS Systems Corporation, U.S) the maximum load, total energy and the modulus were calculated from the load-displacement curve.

Furthermore, the percentage of dry matter from metacarpus III and IV was determined by weight difference between fresh bone and dried bone after 24h in the incubator at  $103 \pm 2^\circ\text{C}$ . Once determined the dry matter, the bones were burned and placed in a muffle oven at  $550^\circ\text{C}$  for 60h. After this period, the bones were re-weighed to calculate the percentage of ashes.

### 3.2.5. Image analysis

Computed tomography image analysis was performed using VisualPork (Girona, Spain) (Bardera et al. 2012; Boada et al. 2009) and HOROS (GNU Lesser General Public License, Version 3 (LGPL-3.0) software.

From the images of live pigs and carcasses, density, volume and the proportion of bone were determined. The number of voxels associated with each HU value was obtained. The number of voxels with HU values between +141 and +1400 were converted into bone volume by means of the matrix size, image thickness and DFOV values (Font i Furnols et al. 2009a). Additionally, several ranges of Hounsfield values were considered, from less dense to denser (between +141 to +500, between +501 and +1000 and between 1001 and 1400). The relative

volume of bones with respect to the total volume of the bone was calculated. Carcass density was obtained applying the formula described by (Picouet et al. 2010) ( $\text{density} = 0.997649 + \text{HU} \times 0.001413$ ). Density was obtained for each HU value, and this density was weighted by the frequency of each HU value. All these weighted density values were summed and divided by the total number of voxels, obtaining an averaged density of the carcass.

Measures of total bone, marrow and cortical bone areas of the femur, tibia and metacarpus were taken from the images of bones and hands (Figure 16).

The density of each bone was calculated. A region of interest (ROI) was obtained by delimiting the central part of each bone. From this ROI, the number of voxels (frequency) associated with each HU value was determined and used to calculate an average bone density as follows: the formula described by (Picouet et al. 2010) was obtained for each HU value and the density obtained was weighted by the frequency of each HU value. All these weighted density values were summed and divided by the total number of voxels of the ROI, obtaining an averaged density of the ROI, *i.e.* of the central part of the bone. From the ROI, the average HU value and its SD were obtained.

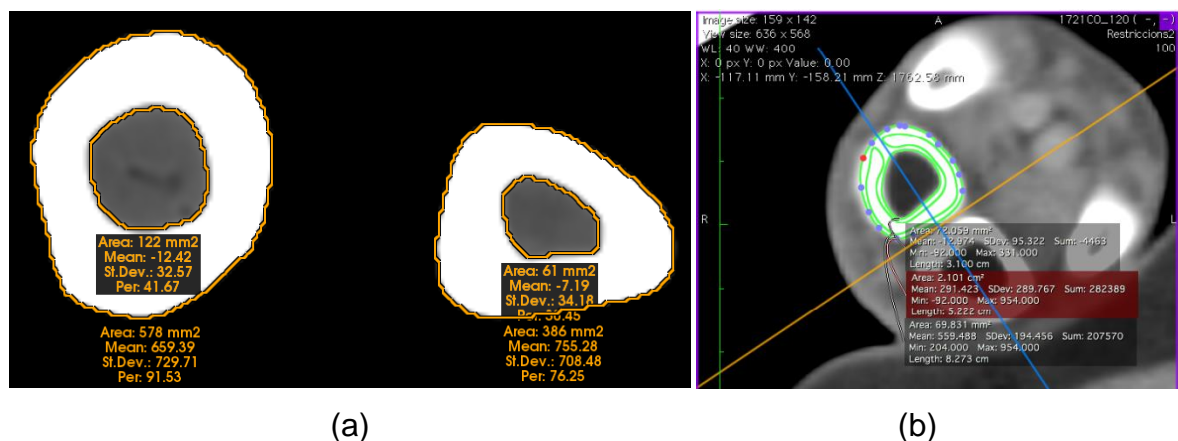


Figure 16. Femur and tibia areas of gilts measured with VisualPork software (a) and metacarpus measures with Horus software(b).

### 3.2.6. Statistical analysis

All the statistical analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, US). The experimental unit was one animal. For the

productive and quality parameters, the mixed procedure was used. For productivity data, the model included the treatment as fixed effect and live weight at the beginning of each phase as covariate. For carcass quality variables, the model included the treatment as fixed effect and the carcass weight as covariate. For meat quality variables, the model included treatment and slaughter day as fixed and blocking effects, respectively. Regarding texture, dry matter, ashes and CT image analysis parameters, the model included the treatment as a fixed effect. Significant differences ( $P < 0.05$ ) between least squared means were determined after applying the Tukey test.

Lameness was analysed using the genmod procedure of SAS. The model included the treatment as fixed effect. Multinomial distribution was considered, and pairwise comparisons were performed.





## **RESULTS & DISCUSSION**



## 4. Results and Discussion

### 4.1. Experiment 1

#### 4.1.1. Productive parameters by feeding strategies

The performance results based on feeding strategy are shown in Table 5. Differences in BW were not observed because this was imposed by the experimental design. It is important to remark that the between 30 and 70 kg period there were not differences between AL-AL and AL-RV, indicating that the pigs from both treatments responded similarly, which make sense since both have *ad libitum* feeding. As expected, when the pigs were fed a RV, they required more days to achieve the TBW. Even if this effect was common for the growing phase RV-AL (30-70 kg) and the finishing phase AL-RV (70-120 kg), the impact was greater during the growing phase RV-AL (133% vs.117%). Overall, the restricted pigs needed more than 10 d to achieve the same body weight as the AL-AL pigs although the total feed intake was similar for all the pigs. Even there were a compensatory growth, this was not enough to reach the same final weight in the same number of days. Maybe the consume capacity during the compensatory period is maximum and does not allow a higher growth to compensate the restriction period.

Weight gain in the RV pigs was significantly lower in both phases and tended to be lower overall. However, the RV pigs during the growing phase showed increased average daily weight gains during the finishing phase relatively to the AL pigs. Thus, the RV pigs appeared to exhibit compensatory growth as a consequence of nutrient deficiency during growth.

The restriction in volume applied for the RV-AL feeding treatment in the present experiment was slightly lower than that applied by Heyer and Lebret (2007) in the growing period (78% vs 65% of AL). However, during finishing, the results were similar since the ADFI of RV-AL pigs was 108% of that of the AL-AL pigs in both works, thus indicating a compensatory growth effect. In the growing period, pigs subjected to a restriction in volume (RV-AL 78%) presented 76% and 80% of the ADG and ADFI of the AL pigs, respectively, and these percentages were 65%

and 66% in Heyer and Lebret (2007), respectively. In the pigs subjected to a restricted in volume in the finishing period (AL-RV 84%), the ADG and ADFI were higher at 89 and 87%, respectively. Differences in reduction of ADG and ADFI based on the phase at which the restricted feeding was applied may be due to the different weights of the pigs during application and different levels of restriction. Considering the total growth of the pigs, the ADG and ADFI in AL-RV pigs were 94% and 93% and in RV-AL pigs were 93% and 91% of those of the AL-AL pigs, respectively. This information must be considered when formulating diets and determining the feeding strategy to obtain the maximum economic benefit without affecting the quality of the final product. Although numerical differences were observed, significant differences were not observed in the feed conversion ratio in separate phases or overall. This is probably related with the fact that the restriction is moderate and the feed consumption when restricted, although it was numerically higher, it was not significantly higher. This result is consistent with the work of Daza et al. (2003).

The fat thickness of the restricted pigs during the growth phase (RV-AL) was 84% of that of the AL-AL pigs ( $P < 0.05$ ). Heyer and Lebret (2007), Lebret et al. (2007) and Ellis et al. (1996) also reported a decrease in fat depth during the restriction in volume phase. In addition, the loin thickness was not affected by the feeding treatment from the initial live weight (body weight initial) to the final live weight (body weight final) in this experiment. This finding is consistent with the results reported by Lebret et al. (2007), who found no significant differences in the loin among different feeding strategies.

Table 5 Productive parameters by feeding strategy during the growing and finishing periods<sup>+</sup>

	Feeding strategy <sup>1</sup>			RMSE	P-value
	AL-AL	AL-RV	RV-AL		
<i>Growing 30-70 kg</i>					
Days	46.3 <sup>b</sup>	46.3 <sup>b</sup>	61.6 <sup>a</sup>	6.6	<.0001
BW initial, kg	33.13	33.88	32.67	2.23	0.432
ADG, g/d	867 <sup>a</sup>	879 <sup>a</sup>	657 <sup>b</sup>	80	<.0001
ADFI, g/d	2109 <sup>a</sup>	2128 <sup>a</sup>	1677 <sup>b</sup>	118	<.0001
FCR, kg/kg	2.45	2.43	2.59	0.25	0.245
Fat thickness <sup>1</sup> , mm	9.1 <sup>a</sup>	8.3 <sup>ab</sup>	7.6 <sup>b</sup>	1.0	0.003
Muscle depth <sup>1</sup> , mm	47.1	47.9	49.6	2.5	0.073
<i>Finishing 70-120 kg</i>					
Days	49.4 <sup>b</sup>	57.8 <sup>a</sup>	45.2 <sup>b</sup>	6.6	0.0003
ADG, g/d	955 <sup>b</sup>	852 <sup>c</sup>	1116 <sup>a</sup>	97	<.0001
ADFI, g/d	2799 <sup>a</sup>	2436 <sup>b</sup>	3016 <sup>a</sup>	232	<.0001
FCR, kg/kg	2.94	2.90	2.71	0.28	0.137
Fat thickness <sup>1</sup> , mm	12.9	11.5	12.8	1.5	0.059
Muscle depth <sup>1</sup> , mm	60.5	58.7	60.4	4.1	0.478
<i>Total 30-120 kg</i>					
Days	95.7 <sup>b</sup>	104.1 <sup>ab</sup>	106.8 <sup>a</sup>	9.4	0.020
BW final, kg	120.13	121.83	123.41	3.48	0.093
ADG, g/d	911	856	852	65.9	0.063
ADFI, g/d	2463 <sup>a</sup>	2287 <sup>b</sup>	2244 <sup>b</sup>	123	0.0003
FCR, kg/kg	2.71	2.69	2.64	0.21	0.731

ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion

+Different letters within a row indicate significant (P <0.05) differences between feeding strategies (ratio).

<sup>1</sup>AL-AL: Feeding ad libitum (AL) during all period of growth; AL-RV: Feeding AL and then volume limited to 84% until slaughter; RV-AL: Feeding volume limited to 78% of AL and then AL until slaughter.

<sup>2</sup>Fat thickness and muscle depth measured on the P2 point in live pigs with an ultrasonic device (Piglog, Frontmatec A/S, DK).

#### 4.1.2. Carcass composition by feeding strategy during growth

##### 4.1.2.1. Morphometric measures

The evolution of morphometric measures during growth evaluated from the CT images is shown for the loin image between the 3<sup>rd</sup> and 4<sup>th</sup> *lumbar vertebrae* in Figure 17. The loin area and perimeter were similar for all dietary treatments during growth except at 70 kg TBW. At this weight, the loin area was significantly higher in the RV-AL pigs compared with the AL-AL pigs and the loin perimeter was also significantly higher in the RV-AL and AL-AL pigs compared with the AL-RV pigs. Note that in the loin between the 11<sup>th</sup> and 12<sup>th</sup> ribs, no differences in loin area and perimeter were found at any weight (Figure 18). Madsen and Bee (2015) also did not find differences in loin area between RV-AL (RV: ADG=650 g/d) and AL-AL pigs at a carcass weight of approximately 80 kg. The RV-AL pigs presented lower increases in loin subcutaneous fat area and fat thickness compared with the other treatments during the restriction period (growing phase), although they showed greater increases in these traits with respect to the other treatments during the AL period (finishing phase) both, between 3<sup>rd</sup> and 4<sup>th</sup> *lumbar vertebrae* and between 11<sup>th</sup> and 12<sup>th</sup> ribs of the loin. However, at the end of the finishing phase, subcutaneous fat area was significantly ( $P<0.05$ ) higher in AL-AL than RV-AL while AL-RV was in between. When the subcutaneous fat thickness is considered, at the end of the finishing phase, there were not significant differences between treatments between the 3<sup>rd</sup> and 4<sup>th</sup> *lumbar vertebrae* and only small differences ( $P<0.10$ ) between AV-AL and AL-RV between the 11<sup>th</sup> and 12<sup>th</sup> ribs. Thus, fat thickness of the loin was not significantly different among the treatments at the end of the finishing phase, which is consistent with the results of Madsen and Bee (2015). This lack of differences observed at the end of the finishing phase between AL-AL and RV-AL indicate that compensatory growth occurred because fat deposition was greater than lean deposition after a restriction in the volume of feeding (Heyer and Lebret 2007).

Animals from the AL-RV feeding strategy showed a significantly lower subcutaneous fat area than those from the AL-AL at TBW 100 kg in the loin images (Figure 17 and Figure 18), which was expected because the daily feed

intake also decreased significantly. As explained before, this difference was also visible and significant at 120 kg in the loin area between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar *vertebrae*, and the 11<sup>th</sup> and 12<sup>th</sup> ribs. This is in disagreement with Madsen and Bee (2015) who did not find significant differences in backfat thickness at the end of the experiment for pigs fed AL-AL and RV-AL strategies (restriction considered as ADG=650 g/d).

In the ham region (Figure 19), as well as in the loin, no differences in total area and perimeter were found between treatments. Regarding subcutaneous fat area and thickness they were not significantly different between animals from any treatment at 120 kg (Figure 19), although it was lower at 70 kg for the RV-AL pigs compared with the AL-AL pigs. At 100 kg, the RV-AL and AL-RV fat thickness of the ham was numerically, but not significantly, lower than that from animals fed AL-AL. The fat area at this weight tended to be higher ( $P < 0.10$ ) in pigs fed AL-AL than those fed AL-RV. The lack of differences in fat area and thickness at the end of the finishing phase showed that RV feeding at the growing phase (and also in some locations at the finishing phase) can increase the fat content of the ham, to make it similar to AL feeding during all growth (compensatory growth) and can lead to the desired final product. Thus, in the case of the ham, all of these strategies are suitable for producing the same type of product; thus, the easiest and cheapest strategy for the farmer should be utilized. Furthermore, modifications of these strategies (e.g., changing the level of RV applied or the time and length of application) should be studied to obtain economical improvements in the production of ham in the pork industry.



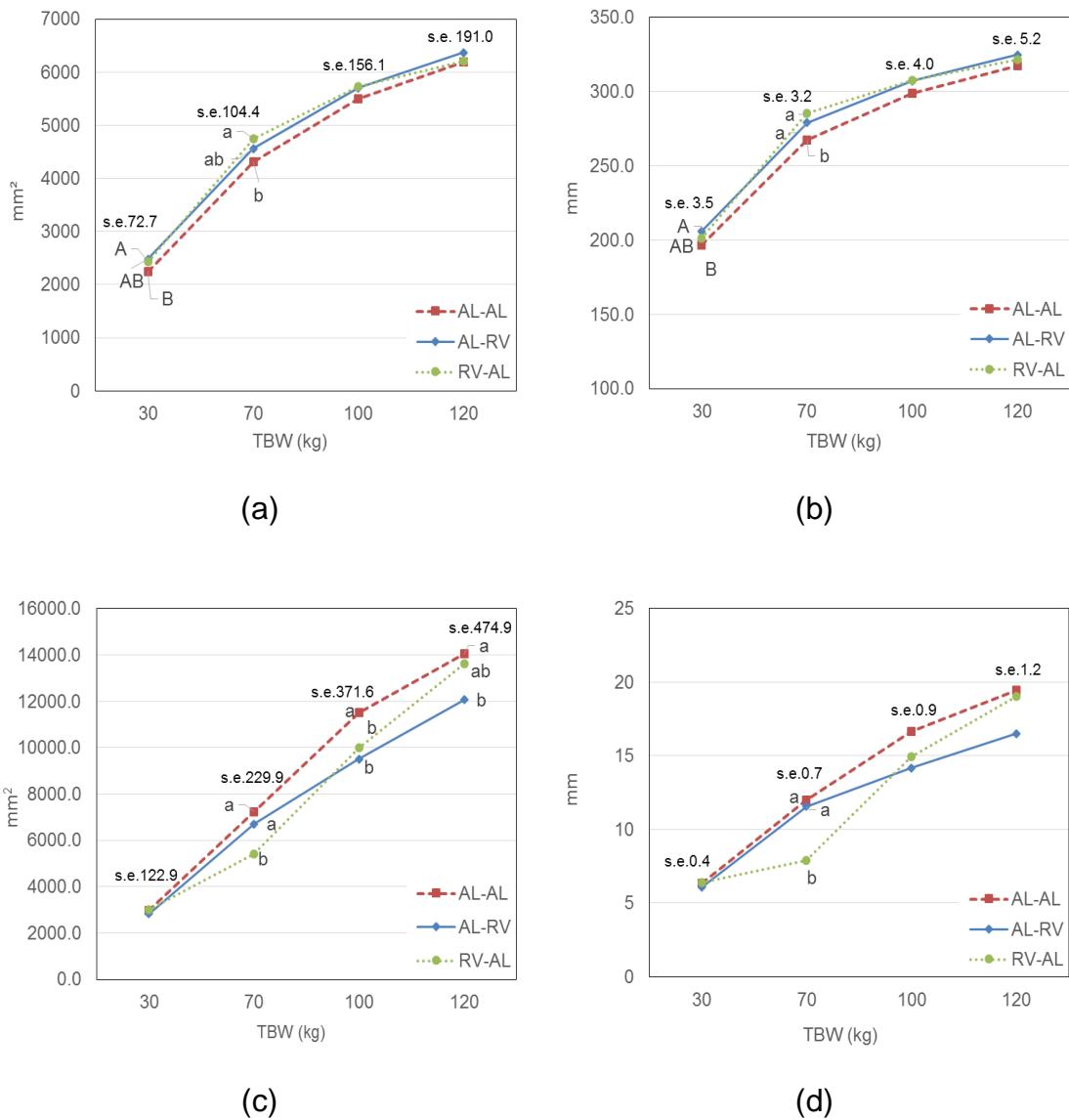
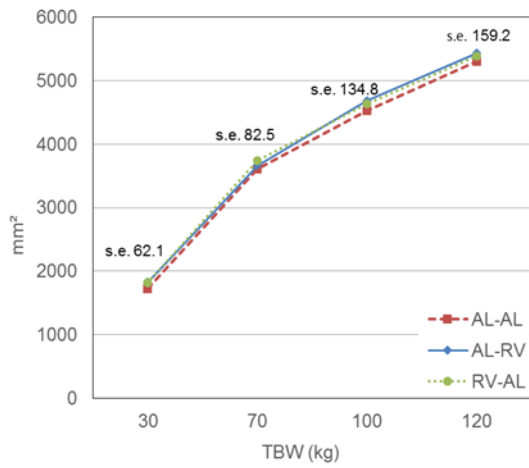
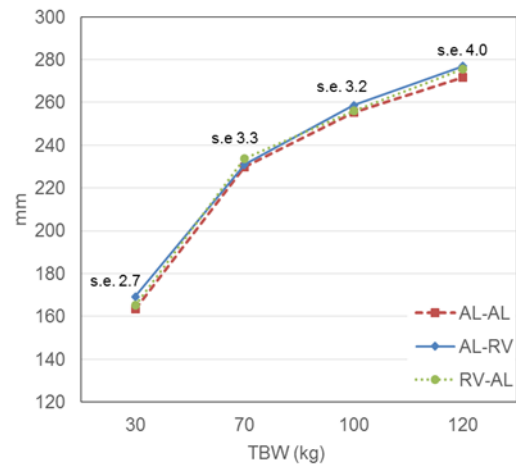


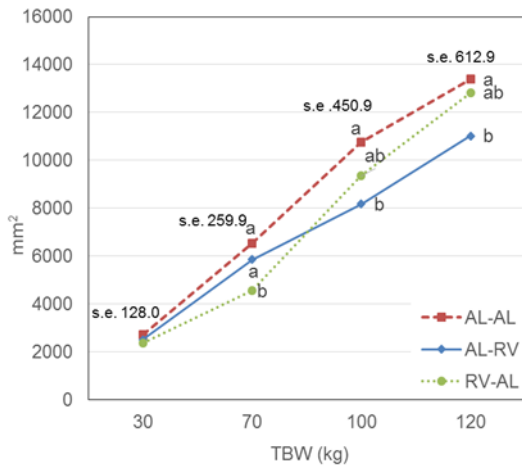
Figure 17 Measures obtained from computed tomography images between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae: (a) loin area, (b) loin perimeter, (c) subcutaneous fat area, and (d) subcutaneous fat thickness. Different lower case or capital letters within TBW indicate significant differences,  $P < 0.05$  or  $P < 0.1$ , respectively.



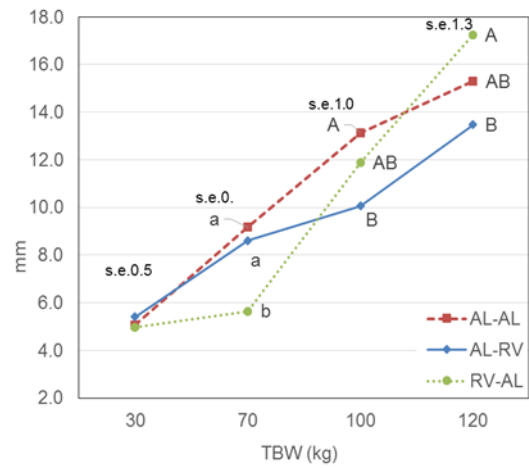
(a)



(b)

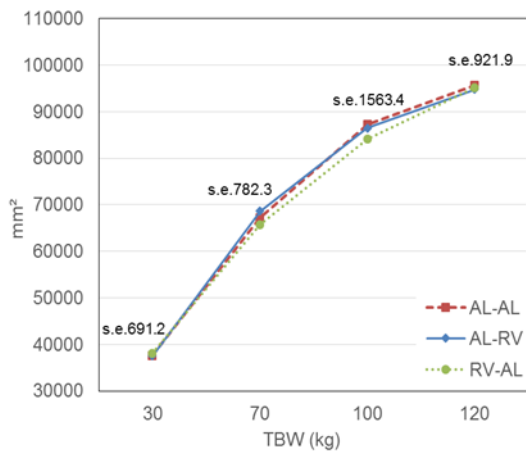


(c)

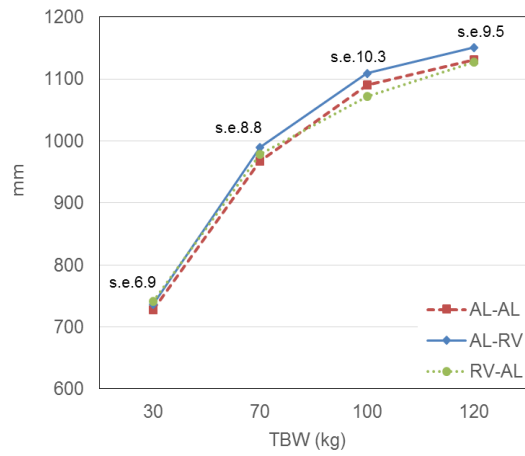


(d)

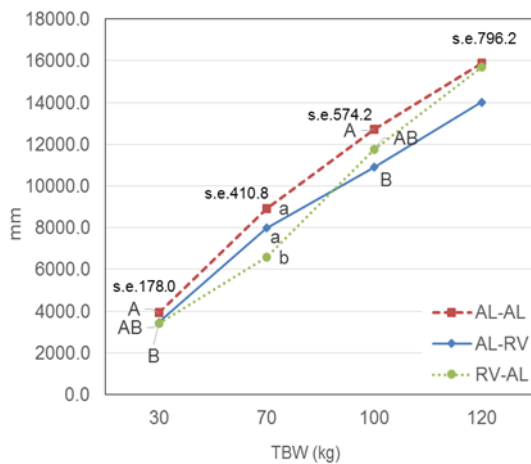
Figure 18. Measures obtained from computed tomography images between the 11<sup>th</sup> and 12<sup>th</sup> ribs: (a) loin area, (b) loin perimeter, (c) subcutaneous fat area, and (d) subcutaneous fat thickness. Different lower case or capital letters within TBW indicate significant differences,  $P < 0.05$  or  $P < 0.1$ , respectively.



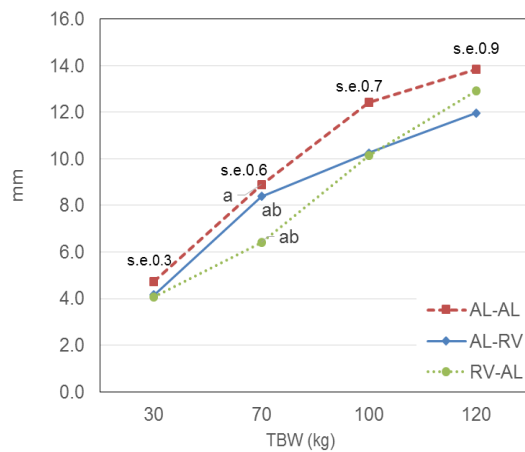
(a)



(b)



(c)



(d)

Figure 19. Measures obtained from computed tomography images in the ham: (a) total area, (b) perimeter, (c) subcutaneous fat area, and (d) subcutaneous fat thickness. Different lower case or capital letters within TBW indicate significant differences,  $P < 0.05$  or  $P < 0.1$ , respectively.

#### 4.1.2.2. Carcass and cut composition

Because of the advantages of CT, which is non-destructive and capable of scanning the same animal during growth, the carcass and cut compositions were measured from the CT images at each TBW in the same animal.

The carcass composition results are shown in Table 6. The lean content was significantly lower in the AL-AL pigs than the RV pigs (RV-AL at 70 kg and AL-RV at 100 kg). At the final weight, significant differences were not observed in the lean content. Only the fat composition (in kg) presented a significant interaction between the feeding effect and TBW. In this case, significant differences were not obtained between feeding treatments at 30 and 120 kg, although at 70 and 100 kg, the fat weight was higher in the AL-AL pigs than the RV pigs in this period (RV-AL at 70 kg and AL-RV at 100 kg). The lack of differences at 120 kg indicates a compensatory effect. When the proportion is considered, a significant interaction can be found ( $P < 0.10$  for ash content and  $P < 0.05$  for fat, moisture and protein content). Differences between feeding treatments were obtained at 70 and 100 kg. The fat proportion follows the same pattern as the fat weight and protein should logically follow an opposite pattern, *i.e.*, when fat is higher, protein is lower. The feeding restriction treatments did not significantly influence the carcass composition (ash, moisture and protein) at the final weight, which is consistent with the results obtained by Mason et al. (2005) in pigs and Keady et al. (2017) in steers. Heyer and Lebret (2007) reported that feed restriction reduced adipose tissue and increased slightly lean deposition at the muscle level from 30 to 70 kg TBW.

The cut composition results are presented in Table 7. The interaction between TBW and feeding treatment was only significant for the fat parameters. The feeding treatments had an effect at 70 and 100 kg, although no effect was observed at the beginning and end of the experiment. When the fat content of the entire carcass was considered (Table 6), the animals in the RV-AL treatment had significantly lower fat contents in all cuts (for entire individuals) than those in the AL-RV and AL-AL treatments at 70 kg. At 100 kg, the animals fed AL-RV had significantly lower fat than those fed AL-AL at 100 kg, and the fat content of the

animals fed RV-AL was in between that of the two other treatments. These results are in accordance with previously presented linear and area fat measurements, thus demonstrating again the compensatory growth for RV-AL pigs. Other works (Bikker et al.1996; Ellis et al.1996; Heyer and Lebret 2007) have reported an increase in the proportion of fat tissue with increasing energy intake.

Table 6. Carcass composition predicted from computed tomography images from the whole live pig by target body weight (TBW) and feeding treatment (FT<sup>1</sup>).

TBW (kg)	30			70			100			120			Error <sup>3</sup>	P-value <sup>4</sup>	
	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL		FT	TBWxFT
Lean %	64.14	65.08	64.96	61.45 <sup>b</sup>	62.37 <sup>ab</sup>	64.32 <sup>a</sup>	59.15 <sup>b</sup>	61.46 <sup>a</sup>	60.61 <sup>ab</sup>	58.42	59.90	58.46	1.44	0.1223	<.0001
Composition (kg) <sup>2</sup>															
Ash	0.78	0.81	0.73	1.86	1.97	1.84	2.66	2.62	2.54	3.14	3.10	3.02	0.55	0.3361	0.9492
Fat	3.41	3.27	3.00	10.00 <sup>a</sup>	9.71 <sup>a</sup>	8.36 <sup>b</sup>	18.62 <sup>a</sup>	15.34 <sup>b</sup>	16.12 <sup>ab</sup>	25.50	22.23	24.40	1.46	0.0093	0.0091
Moisture	17.20	17.46	17.05	34.92	35.59	34.49	49.75	46.71	46.34	58.27	55.00	54.23	2.14	0.2811	0.7648
Protein	4.59	4.66	4.35	10.61	10.92	10.64	15.45	14.59	14.30	18.29	17.20	16.92	1.23	0.2128	0.7608
Composition (%) <sup>2</sup>															
Ash	2.93	2.96	2.91	3.06	3.13	3.13	2.96	3.11	3.02	2.79	2.92	2.77	0.38	0.1386	0.0709
Fat	11.25 <sup>a</sup>	11.04 <sup>ab</sup>	10.63 <sup>b</sup>	15.81 <sup>a</sup>	15.36 <sup>a</sup>	13.81 <sup>b</sup>	21.56 <sup>a</sup>	18.61 <sup>b</sup>	19.57 <sup>b</sup>	24.95	22.26	23.98	1.32	0.0077	0.0007
Moisture	66.25	66.31	67.01	61.09 <sup>b</sup>	61.22 <sup>ab</sup>	62.40 <sup>a</sup>	55.98 <sup>b</sup>	58.28 <sup>a</sup>	57.86 <sup>a</sup>	53.38	55.64	54.58	1.27	0.0106	0.0383
Protein	18.09	18.15	18.06	18.10 <sup>b</sup>	18.28 <sup>ab</sup>	18.47 <sup>a</sup>	17.60 <sup>b</sup>	18.15 <sup>a</sup>	17.84 <sup>ab</sup>	16.97	17.45	16.93	0.63	0.0649	<.0001

<sup>1</sup>AL-AL: Feeding ad libitum (AL) during all period of growth; AL-RV: Feeding AL and then volume limited to 84% until slaughter; RV-AL: Feeding volume limited to 78% of AL and then AL until slaughter.

<sup>2</sup>Predicted using the equations obtained by Zomeño et al. (2016) from live pig images to estimate composition of minced carcasses.

<sup>3</sup>Square root of the covariance parameter estimate, equivalent to the root mean squared error.

<sup>4</sup>P-value for the TBW was significant (P<0.001) for all variables.

Table 7. Cuts composition predicted from computed tomography images from the whole live pig by target body weight (TBW) and feeding treatment (FT<sup>1</sup>).

TBW (kg) FT	30			70			100			120			Error <sup>4</sup>	P-value <sup>5</sup>	
	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL	AL-AL	AL-RV	RV-AL		FT	TBWxFT
Main cuts (kg) <sup>2</sup>															
Lean <sup>3</sup>	6.06	6.24	5.90	13.47	13.93	13.52	20.03	18.85	18.39	23.53	22.13	21.42	1.50	0.273	0.762
Fat <sup>3</sup>	1.37	1.29	1.23	4.30 <sup>a</sup>	4.09 <sup>a</sup>	3.34 <sup>b</sup>	7.62 <sup>a</sup>	6.16 <sup>b</sup>	6.61 <sup>ab</sup>	9.71	8.46	9.21	0.92	0.007	0.001
Bone <sup>3</sup>	0.97	0.97	0.91	1.79	1.81	1.73	2.40	2.31	2.26	2.79	2.65	2.60	0.49	0.198	0.959
<i>Ham</i> <sup>2</sup>															
Weight	3.28	3.30	3.18	7.41	7.47	7.03	11.15	10.15	10.21	13.29	12.34	12.45	0.94	0.035	0.237
Lean	2.47	2.55	2.40	5.40	5.56	5.34	7.80	7.36	7.24	9.05	8.58	8.41	0.87	0.255	0.792
Fat	0.44	0.42	0.40	1.30 <sup>a</sup>	1.24 <sup>a</sup>	1.03 <sup>b</sup>	2.28 <sup>a</sup>	1.86 <sup>b</sup>	2.00 <sup>ab</sup>	2.90	2.54	2.76	0.50	0.007	0.001
Bone	0.32	0.32	0.30	0.58	0.59	0.56	0.77	0.75	0.73	0.90	0.85	0.84	0.28	0.198	0.959
<i>Loin</i> <sup>2</sup>															
Weight	1.81	1.83	1.74	4.79	4.83	4.51	7.48	6.76	6.81	9.03	8.34	8.42	0.80	0.035	0.237
Lean	1.24	1.28	1.19	3.04	3.15	3.05	4.52	4.27	4.17	5.28	5.00	4.85	0.70	0.294	0.800
Fat	0.26	0.24	0.23	1.13 <sup>a</sup>	1.08 <sup>a</sup>	0.86 <sup>b</sup>	2.13 <sup>a</sup>	1.70 <sup>b</sup>	1.84 <sup>ab</sup>	2.77	2.40	2.62	0.50	0.007	0.001
Bone	0.32	0.32	0.29	0.60	0.61	0.58	0.81	0.78	0.76	0.95	0.90	0.88	0.29	0.198	0.959
<i>Shoulder</i> <sup>2</sup>															
Weight	1.86	1.87	1.80	4.24	4.27	4.02	6.12	5.68	5.71	7.09	6.75	6.80	0.92	0.045	0.267
Lean	1.31	1.34	1.27	2.80	2.90	2.81	4.09	3.87	3.78	4.77	4.51	4.37	0.66	0.281	0.776
Fat	0.35	0.33	0.32	1.01 <sup>a</sup>	0.97 <sup>a</sup>	0.81 <sup>b</sup>	1.57 <sup>a</sup>	1.36 <sup>b</sup>	1.44 <sup>ab</sup>	1.82	1.71	1.81	0.33	0.008	<.0001
Bone	0.21	0.21	0.20	0.40	0.41	0.39	0.54	0.52	0.51	0.63	0.60	0.59	0.23	0.198	0.960
<i>Belly</i> <sup>2</sup>															
Weight	1.19	1.20	1.15	2.90	2.92	2.74	4.59	4.12	4.15	5.60	5.12	5.18	0.64	0.032	0.223
Lean	0.77	0.79	0.75	1.68	1.74	1.69	2.59	2.40	2.33	3.11	2.86	2.76	0.58	0.221	0.683
Fat	0.30	0.28	0.27	0.97 <sup>a</sup>	0.93 <sup>a</sup>	0.76 <sup>b</sup>	1.75 <sup>a</sup>	1.42 <sup>b</sup>	1.53 <sup>ab</sup>	2.25	1.96	2.14	0.44	0.007	0.001
Bone	0.12	0.12	0.11	0.21	0.21	0.20	0.28	0.27	0.26	0.32	0.31	0.30	0.16	0.200	0.966
<i>Tenderloin</i> <sup>2</sup>															
Weight	0.20	0.20	0.19	0.45	0.46	0.45	0.67	0.63	0.61	0.79	0.74	0.72	0.28	0.270	0.756

<sup>1</sup>AL-AL: Feeding ad libitum (AL) during all period of growth; AL-RV: Feeding AL and then volume limited to 84% until slaughter; RV-AL: Feeding volume limited to 78% of AL and then AL until slaughter. <sup>2</sup>Prediction using the equations obtained for live pig images from Pietrain x (Landrace x Large White) by Font-i-Furnols et al. (2014) to estimate the carcass composition from dissection. <sup>3</sup>Lean5: lean content of the ham, shoulder, loin, belly and tenderloin from dissection; Fat4 and Bone4: predicted fat and bone content of the ham, shoulder, loin and belly obtained by dissection. <sup>4</sup>Square root of the covariance parameter estimates, which are equivalent to the root mean squared error

#### 4.1.2.3. Final carcass quality measurements

The results of the carcass quality characteristics after slaughter are shown in Table 8, which indicates that all the studied variables showed non-significant differences among feeding treatments, thus indicating a lack of effect of RV applied at the growing or finishing phases on the final carcass quality. However, considerable numerical differences were observed for fat thickness between the AL-RV pigs (20.9 mm) and the RV-AL (23.3 mm) or AL-AL pigs (22.1 mm). Considerable numerical differences were also observed for the lean meat values between the AL-RV pigs (57.7) and RV-AL pigs (55.6) and for the minimum fat thickness over the muscle GM between the AL-RV (16.0 mm) and RV-AL pigs (20.1 mm). Some of these variables have important economic consequences, such as the % lean meat. Since the objective of the study was to determine whether feed restriction resulted in similar performance and carcass characteristics compared with AL feeding, the type II error must be considered very relevant. The present results do not rule out the possibility that RV-AL results in carcasses with greater fat than the other treatments. Likely, an effect on carcass grade could be found if the RV period or the degree of restriction were greater. In fact, Madsen and Bee (2015) reported a high lean meat content and low fat content in carcasses from pigs subjected to RV feeding in the growing and finishing periods compared with those subjected to AL-AL and RV-AL feeding, whereas Cho et al. (2006) did not find differences in carcass grade and back fat depth when RV (90% of consumed feed for the last two weeks) was applied during different periods before slaughter, including RV during all growing-finishing periods. Thus, under the conditions of the present experiment, a restriction in volume at different moments of the pig growth cycle does not affect the final carcass quality.



Table 8. Carcass and meat quality measurements by feeding strategy<sup>1</sup>

	AL-AL	AL-RV	RV-AL	RMSE	P-value
Live weight (kg)	126.82	127.66	130.62	5.68	0.283
Warm carcass weight (kg)	104.46	105.40	107.02	4.85	0.474
Yield (%)	82.37	82.59	81.90	1.18	0.394
Cold left carcass weight (kg)	53.02	53.68	54.44	2.55	0.439
Fat thickness <sup>2</sup> (mm)	22.13	20.91	23.28	3.50	0.305
Muscle depth <sup>2</sup> (mm)	66.12	63.29	63.17	4.63	0.247
Lean meat <sup>2</sup> (%)	57.10	57.66	55.55	3.46	0.370
F-ZP <sup>3</sup> (mm)	18.21	16.02	20.07	4.69	0.168
M-ZP <sup>4</sup> (mm)	78.80	80.86	80.09	5.68	0.685
Last rib fat thickness (mm)	45.16	47.08	49.46	9.44	0.602
Last rib fat thickness (mm)	28.94	25.87	26.63	4.87	0.298
Moisture (%)	73.64	73.79	74.06	0.53	0.296
Intramuscular fat%	2.06	1.90	1.79	0.41	0.432
pH 45 SM	6.63	6.61	6.62	0.18	0.962
pH 45 LT	6.67	6.53	6.56	0.22	0.378
pHu SM	5.58	5.58	5.57	0.07	0.930
pHu LT	5.62	5.62	5.62	0.09	0.977
ECuSM (mS)	4.19	5.55	4.19	1.96	0.173
EC LT(mS)	3.86	4.38	3.58	1.16	0.415
Marbling NPPC5	1.59	1.47	1.43	0.59	0.838
Drip loss (%)	2.36	3.34	2.55	1.97	0.461
Cooking loss (%)	34.53	33.75	34.43	2.42	0.708
EJC6	2.92	2.55	2.54	0.51	0.230
<i>L</i> *	48.65	49.37	49.18	1.79	0.660
<i>a</i> *	8.60	7.77	8.32	1.15	0.247
<i>b</i> *	1.61	1.24	1.38	0.55	0.315
Shear force (N)	5.42	5.37	5.01	0.73	0.454

<sup>1</sup>AL-AL: Feeding *ad libitum* (AL) during all period of growth; AL-RV: Feeding AL until 70 kg and then volume limited to 84% until slaughter; RV-AL: Feeding volume limited to 78% of AL in growth period and then AL until slaughter. <sup>2</sup>Fat and muscle thickness measured with Fat-O-Meat'er between the 3<sup>rd</sup> and 4<sup>th</sup> last rib at 6 cm from the midline and lean meat % obtained from these two measures according to the Spanish official equation (Commission Implementing Decision 384/UE/2012 of 12 July 2012). <sup>3</sup>F-ZP: minimum fat thickness over the muscle *gluteus medius*. <sup>4</sup>M-ZP: muscle thickness between the medullar canal and the cranial edge of the muscle *gluteus medius*. <sup>5</sup> National Pork Producers Council (NPPC, 1999) scale from 1 (very low) to 10 (very high). <sup>6</sup> Japanese Scale colour (Nakai et al., 1975) from 1 (pale) to 6 (dark colour). pH 45: pH measured at 45 min *post mortem*; pHu: Ultimate pH; ECu: Ultimate electrical conductivity.

The final carcass characteristics (Table 8) are consistent with those reported previously as shown in Figure 19 and Table 6 and Table 7 in live pigs at 120 kg just before slaughter. These findings support the use of CT as a non-destructive method, which can be used to predict the carcass quality of pigs before slaughter. Moreover, this method presents advantages in accuracy and a lack of time restrictions on the evaluation of the growth performance and body composition of pigs.

#### 4.1.3. Meat and Sensory Quality by Feeding strategy

The results of the meat quality characteristics based on the feeding strategy are presented in Table 8, which shows that no significant differences ( $P < 0.05$ ) were observed in any of the meat quality measurements among the three feeding treatments. Previous works have shown a decrease in intramuscular fat under RV (Heyer and Lebret 2007; Affentranger et al. 1996), which is inconsistent with the present results. However, other studies (Kristensen et al. 2002; 2004) are consistent with the present work and did not find a significant effect of feeding treatment (AL-AL compared with several combinations of RV) based on the colour parameters ( $L^*$ ,  $a^*$  and  $b^*$ ), intramuscular fat content and ultimate pH. Nevertheless, the same authors found an effect on shear force, which was higher in pigs fed AL-RV than RV-AL (growing phase from 29 to 90 d and finishing phase from 91 to 165 d, and RV 60% of AL).

Table 9 shows the sensory scores given by trained panellists base on feeding strategy. The results show that significant differences occurred in pork meat odour, which was slightly higher in the AL-AL pigs relative to the AL-RV pigs, and in pig odour, which was slightly higher in the RV-AL pigs relative to the AL-RV pigs. Regarding flavour, meat from pigs fed AL-RV presented higher ( $P = 0.002$ ) acid scores than those from the other feeding treatments. A tendency ( $P < 0.10$ ) can also be seen in the metallic scores, which were higher in meat from animals fed AL-RV compared with AL-AL. Nevertheless, in all cases, the scores are similar and such differences might not be relevant. When the texture attributes were considered, significant differences were found in hardness, which was 0.4

and 0.6 points higher in animals fed AL-AL than AL-RV and RV-AL, respectively. Additionally, tenderness tended to be higher in animals fed AL-RV than AL-AL and RV-AL. Fibrosity was also significantly higher in animals fed AL-AL than AL-RV. No significant differences were found in juiciness and the other evaluated attributes. Kristensen et al. (2004) reported that meat from LT of gilts restricted to 69% volume in the growing period (from 28 d to 80 d of life) and then fed AL until slaughter at day 140 showed higher tenderness scores than that from gilts fed AL during all growing and finishing periods. This effect was not detected in meat from gilts in the *biceps femoris* muscle. Furthermore, the results were different when meat from castrated pigs was considered. Thus, the effect of the restriction in the tenderness might depend on the muscle, the time of restriction and the gender of the pig. In another work, (Heyer and Lebret 2007) did not find significant differences in tenderness; however, they reported differences in juiciness (meat from animals fed AL presented slightly higher juiciness scores than those from animals restricted to 65% in volume during the growing period from 30 to 70 kg), which is inconsistent with the results presented here.

Considering these results, differences between studies may be due to the duration and quantity of the restriction, the gender of the animals used in the experiment and the muscle studied. In our experiment, the restriction period was divided by different TBWs and not the growing days of pig. Additional details of different feeding periods must be evaluated to confirm the effect of feeding treatment on meat tenderness.

Table 9. Sensory characteristics (trained panel and consumer acceptability) of the meat from pigs fed different restriction strategies<sup>1</sup>.

	AL-AL	AL-RV	RV-AL	RMSE	P-value
<b>TRAINED PANEL<sup>2</sup></b>					
<i>Odour attributes</i>					
Pork meat	4.1 <sup>a</sup>	3.8 <sup>b</sup>	4.0 <sup>ab</sup>	0.8	0.042
Pig	1.3 <sup>ab</sup>	1.1 <sup>b</sup>	1.5 <sup>a</sup>	0.8	0.005
Abnormal	0.9	0.9	0.9	0.4	0.524
<i>Flavour attributes</i>					
Pork meat	4.0	3.8	3.9	0.8	0.260
Pig	1.0	1.0	1.2	0.7	0.196
Abnormal	0.8	0.8	0.8	0.4	0.513
Acid	1.7 <sup>b</sup>	2.2 <sup>a</sup>	1.9 <sup>b</sup>	0.9	0.002
Sweet	1.8	1.7	1.9	0.7	0.222
Metallic	1.5 <sup>B</sup>	1.7 <sup>A</sup>	1.7 <sup>AB</sup>	0.7	0.065
<i>Texture attributes</i>					
Hardness	4.8 <sup>a</sup>	4.4 <sup>b</sup>	4.2 <sup>b</sup>	1.0	0.001
Initial juiciness	1.9	1.8	1.9	0.7	0.952
Final juiciness	3.5	3.5	3.6	0.9	0.713
Tenderness	4.0 <sup>B</sup>	4.3 <sup>A</sup>	4.0 <sup>B</sup>	0.9	0.038
Fibrosity	3.5 <sup>a</sup>	3.2 <sup>b</sup>	3.4 <sup>ab</sup>	0.6	0.030
Chewiness	5.0	4.8	4.8	1.0	0.228
<b>CONSUMER TEST<sup>3</sup></b>					
Overall acceptability	5.9	6.0	6.3	1.5	0.182
Tenderness	5.5	5.5	5.9	1.9	0.211
Odour	6.1	6.2	6.3	1.5	0.514
Flavour	6.1	6.2	6.3	1.6	0.681

<sup>1</sup> AL-AL: Feeding ad libitum (AL) during all period of growth; AL-RV: Feeding AL and then volume limited to 84% until slaughter; RV-AL: Feeding volume limited to 78% of AL and then AL until slaughter.

<sup>2</sup> Scores from 1 (low/weak) to 10 (high/strong).

<sup>3</sup> Scores from 1 (I dislike it extremely) to 9 (I like it extremely) without the intermediate level 5 (neither like nor dislike).

All significant differences in the sensory characterization of meat from the different feeding strategies were numerically very low, which may explain why consumers scores were not significantly different in the overall acceptability, tenderness, odour and flavour between the three different feeding strategies (see results in Table 9), which was suggested by Heyer and Lebret (2007). Intramuscular fat and/or marbling is considered to have an influence on some sensory qualities (Bejerholm and Barton-Gade 1986; Font-i-Furnols et al. 2012;

Fernandez et al. 1999a). In the present project, no differences in intramuscular and marbling were detected (Table 8), and this may have had an influence on the lack of sensory differences between meats from different feeding strategies.

Consumers were asked to rank several characteristics of the meat by purchasing preference, and the results of this ranking are presented in Figure 20. The findings show that meat colour, less fat marbling, less superficial fat and rearing organically are the most important purchasing preferences, which are consistent with studies showing that visual characteristics of the meat, such as the colour, marbling and fat, together with the price have the most important influence on the purchasing decision (Font-i-Furnols et al. 2012). In this questionnaire, people preferred to buy meat with less marbling, which is consistent with the work of Brewer et al. (2001), who reported that people show a reduced intention to purchase loin chops with high marbling compared with leaner chops. Moreover, our findings are also consistent with Fernandez et al. (1999b) who reported that increasing the intramuscular level can decrease the purchasing preference of meat. However, Font-i-Furnols et al. (2012) found that approximately half of the consumers prefer meat with more intramuscular fat while half prefer meat with low intramuscular fat content, which is probably related to the fact that a high amount of consumers choose leaner meat because of the lower calories (Cannata et al. 2010). Meat from entire males is the least important characteristic of the meat with regard to purchasing preference by consumers. Nevertheless, different opinions likely occur among consumers depending on their sensitivity to boar taint, a sensory defect that can be found in meat from entire males (Font-i-Furnols et al. 2012).

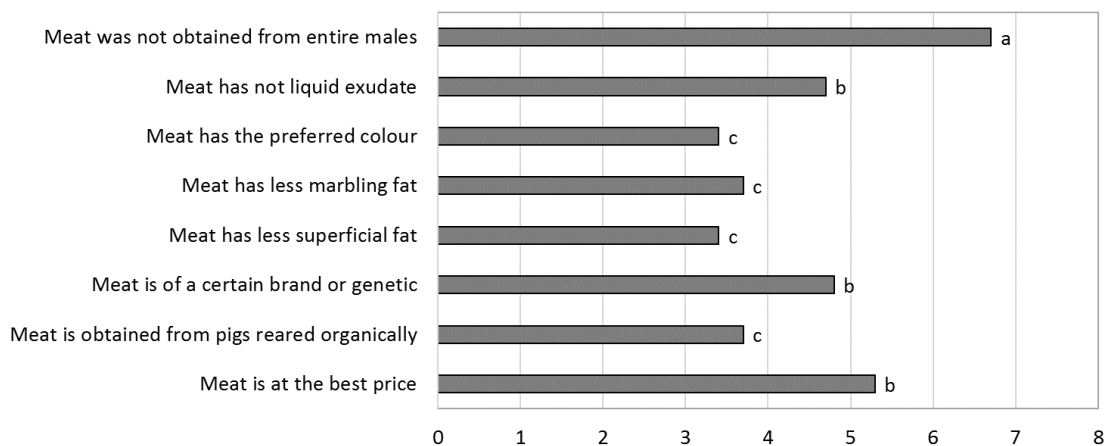


Figure 20. Consumer scores for purchasing preference from 1 (those that the consumer would buy first) to 8 (those that the consumer would buy last).

#### 4.1.4. Overall Evaluation of the Effect of Feeding Strategies on Quality

The relationship between the sensory attributes (trained panel), consumer preferences, meat quality parameters and fat thickness and lean meat content has been determined via a principal component analysis, and it is presented in Figure 21. The figure shows that principal components 1 and 2 explained 22% and 12% of the variation, respectively. The positive side of PC1 is related to high values of certain meat quality variables, such as moisture, drip, CEu, and shear force, and certain sensory attributes, such as pig odour and flavour, meat odour, fibrosity and hardness. The negative side of PC1 is related with the high values of other meat quality attributes, such as pH 45, intramuscular fat and marbling, and certain sensory attributes, such as tenderness, juiciness, acid flavour. Consequently, high marbling is related to high intramuscular fat content, higher tenderness and juiciness scores and lower shear force, which is consistent with the results presented by (Cannata et al. 2010). When the animals were placed in the plot and the consensus by feeding strategy was illustrated, the animals following AL-AL were placed on the positive side of PC1, i.e., meat with higher hardness, shear force and drip losses and lower intramuscular fat, marbling, acid flavour, juiciness and tenderness than those following the AL-RV and RV-AL feeding strategies. These results are consistent with previously reported results.

Regarding PC2, the positive part is related to a high lean meat percentage and higher consumer scores of overall acceptability, tenderness, taste and odour, whereas the negative part is related to high fat thickness and colour. Thus, PC2 could be defined mainly via carcass quality characteristics and consumer acceptability. However, PC2 is not appropriate for differentiating between animals from different feeding strategies because the consensus is close to PC1 in all the feeding strategies. This result makes sense because although important differences were observed in fat content between animals based on the feeding strategy during growth, at the end of the trial, the differences were not significant. The consumer scores were uncorrelated with juiciness and intramuscular fat content, which is consistent with certain previous works (Font-i-Furnols et al. 2009b) but inconsistent with others (Font-i-Furnols et al. 2012; Cannata et al. 2010; Fortin et al. 2005). Furthermore, consumer scores were negatively correlated with instrumental shear force, *i.e.*, consumers gave higher overall and tenderness acceptability scores when the shear force values were lower.

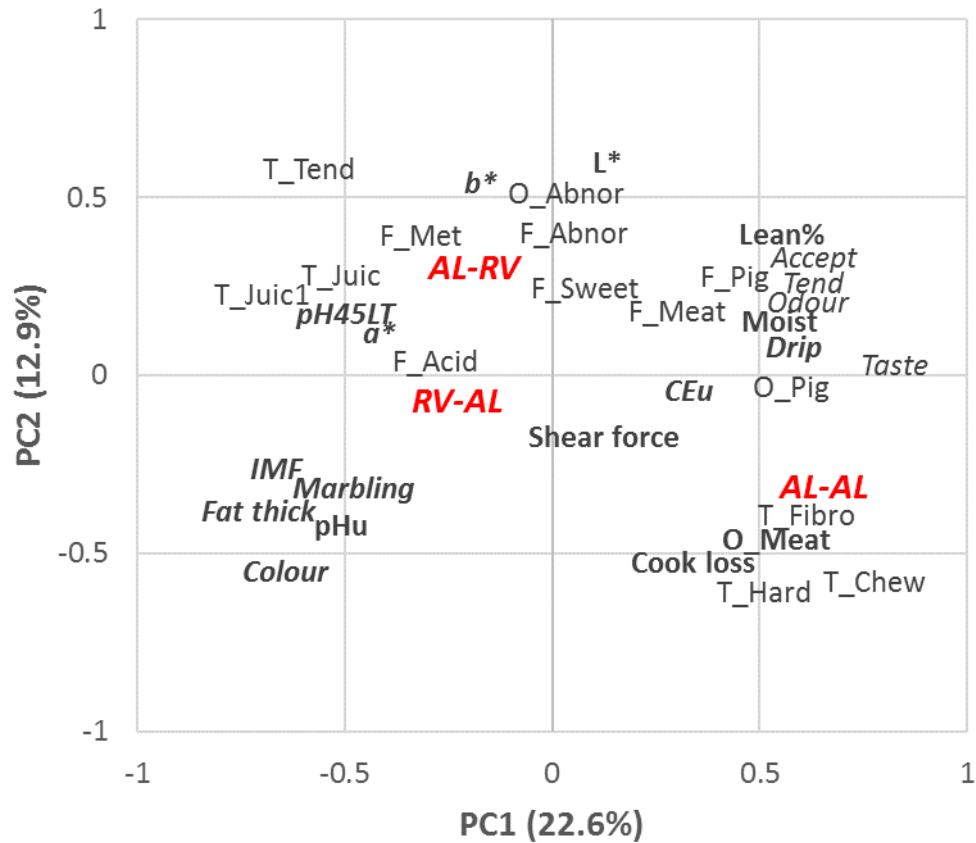


Figure 21. Representation of the consensus of the animals by feeding strategy (AL-AL: fed ad libitum (AL) during all period of growth; AL-RV: fed AL in growing phase and volume limited to 84% until slaughter; RE-AL: Energy restriction (-10% of AL) in growth period and followed by AL until slaughter; RV-AL: Feeding volume limited to 78% of AL and AL until slaughter) and correlations of the quality variables (in italics: consumer scores; in blue, italics and bold: carcass variables; in black and bold: meat quality variables; and in black: sensory attributes –see Table 2-) with the first two principal components (PC1 and PC2).



## 4.2. Experiment 2

### 4.2.1. Productivity results and lameness

The results of animal productivity and body composition during growth are presented in Table 10. The results show an increase in average daily gain, daily feed intake, feed consumption ratio, fat and lean as the pigs grow older and heavier. Significant differences between treatments were found for none of the studied parameters, indicating that, in the conditions of the present experiment, the decrease in dietary P and the inclusion of phytases do not modify the growth performance of the animals. These results are similar to those previously reported showing that a reduction to one-third, or less, of dietary inorganic P during the late finishing phase is sufficient to maintain growth performance and carcass quality and to decrease P excretion (Shaw et al. 2002; O'Quinn et al. 1997).

No significant differences between treatments for weight gain, feed intake, or feed efficiency may indicate that, similar to other reports, the decrease in P and the inclusion of phytases in the diet did not affect the acceptability of the diet to pigs (McGlone 2000; Peter et al. 2001; Shaw et al. 2002). The present work suggests that the levels of inorganic P can be decreased without negative effects on animal performance. Compared with previous studies of growing-finishing pigs (Hastad et al. 2004; Peter et al. 2001; Varley et al. 2010), our study showed that the daily feed intake and average daily gain were much higher, which could explain some of the results.

Table 10. Least square means of the production variables and body composition of gilts by dietary treatment.

	<i>Treatments</i> <sup>a</sup>				<i>Statistics</i>		
	C0	P0	P1	P2	SEM	P-value	
<b>Growing 50-75 kg</b>	Initial weight, kg	54.25	54.58	54.00	53.58	1.38	0.963
	Average daily gain, kg/d	0.98	0.95	0.91	0.99	0.04	0.384
	Daily feed intake, kg	2.58	2.58	2.52	2.60	0.06	0.786
	Feed consumption ratio, kg/kg	2.66	2.73	2.80	2.64	0.08	0.444
	Fat 50 kg <sup>b</sup> , mm	7.02	7.18	6.85	7.16	0.27	0.807
	Lean 50 kg <sup>b</sup> , mm	38.40	38.34	39.02	38.83	0.74	0.913
<b>Finishing 75-120 kg</b>	Initial weight, kg	77.42	77.79	77.38	78.18	0.65	0.790
	Average daily gain, kg/d	1.05	1.04	1.01	1.09	0.02	0.204
	Daily feed intake, kg	3.20	3.16	3.08	3.17	0.09	0.815
	Feed consumption ratio, kg/kg	3.05	3.05	3.05	2.92	0.08	0.590
	Fat 75 kg <sup>b</sup> , mm	9.80	9.73	9.98	9.58	0.40	0.912
	Lean 75 kg <sup>b</sup> , mm	44.71	46.23	44.30	45.01	0.87	0.397
<b>120 kg- Slaughter</b>	Initial weight, kg	119.88	122.38	120.71	121.32	1.00	0.316
	Average daily gain, kg/d	1.13	0.98	0.88	1.07	0.11	0.369
	Daily feed intake, kg	3.56	3.36	3.10	3.46	0.18	0.240
	Feed consumption ratio, kg/kg	3.57	3.71	3.57	3.59	0.46	0.996
	Fat 120 kg <sup>b</sup> , mm	13.98	14.19	14.34	14.63	0.57	0.869
	Lean 120 kg <sup>b</sup> , mm	52.57	53.36	55.39	52.30	1.20	0.243
<b>Global</b>	Days	75.67	77.67	80.50	75.45	2.93	0.567
	Final weight, kg	131.87	132.90	131.15	133.72	1.95	0.776
	Average daily gain, kg/d	1.03	1.00	0.97	1.05	0.03	0.192
	Total consumption, kg	229.36	237.96	229.19	229.53	6.55	0.701
	Feed consumption ratio, kg/kg	2.96	3.04	2.98	2.90	0.07	0.496

<sup>a</sup> C0- positive control diet; P0- negative-control diet; P1- negative-control diet supplemented with 0.01% Phyzime XP 5000; P2 negative-control diet supplemented with 0.02% Phyzime XP 5000.

<sup>b</sup> Fat and loin depths were measured at the last rib, 4 cm from the midline using Piglog 105 ultrasound.

Regarding lameness, the results were in conclusive. No significant differences were found between treatments at the beginning of the experiment (50 kg LW),

showing a lack of initial differences. At 75 kg BW, there were differences ( $P=0.033$ ) between P1 and P2; animals with lameness 2 were more frequent in P2 than in P1 (6 vs 1, respectively). However, none of the pigs presented important lameness scores, since there were no animals with a score of 3 or higher. In the final evaluation (120 kg LW), significant differences were found between CO and P0. These results were opposite to those expected since they resulted from the number of pigs with lameness 3 (three pigs) or 2 (six pigs) in CO compared with lameness 3 (one pig) and lameness 2 (two pigs) in P0. The number of pigs per treatment with lameness scores of 3 (severe) was between 1 and 3, which represents between 8% and 25% of the pigs. No bone fractures were recorded in any animal. Since the control is the treatment with higher incidence of lameness, results suggest that pigs fed the lower P-level diet were able to maintain adequate bone strength.

#### 4.2.2. Carcass and meat quality at slaughter

Measures of carcass and meat quality were taken at the slaughterhouse, and the results are presented in Table 11. No significant differences were found for most of the studied parameters. Only the muscle thickness measured at 6 mm from the midline between the 3<sup>rd</sup> and 4<sup>th</sup> ribs with FOM was significantly higher in P1 than in P0, and electrical conductivity in LT muscle was significantly higher in P0 than in P1. Nevertheless, in both cases, these differences, although significant, were not relevant.

These results are supported by previous studies, where no effects on carcass characteristics or meat quality (O'Quinn et al. 1997; Mavromichalis et al. 1999; Peter et al. 2001) were found, suggesting that a decrease in P levels in finishing diets does not compromise carcass and meat quality.

Table 11. Carcass and meat quality characteristics of gilts by dietary treatment <sup>1</sup>.

	Treatments				Statistics	
	C0	P0	P1	P2	RMSE	P-value
Carcass quality						
Carcass, kg	101.53	102.86	101.26	103.03	4.46	0.714
F34FOM <sup>2</sup> , mm	21.82	22.00	21.44	22.16	2.86	0.943
M34FOM <sup>2</sup> , mm	60.67 <sup>ab</sup>	57.11 <sup>b</sup>	62.46 <sup>a</sup>	60.87 <sup>ab</sup>	4.35	0.042
% lean FOM <sup>2</sup>	56.40	55.59	57.05	56.14	2.87	0.685
Ham fat thickness <sup>3</sup> , mm	22.80	20.35	21.96	19.59	4.82	0.441
Muscle fat thickness <sup>4</sup> , mm	76.35	75.52	76.64	76.25	5.83	0.973
Carcass length, mm	91.17	90.20	90.02	90.75	2.03	0.510
Loin length, mm	93.69	92.43	88.59	93.14	8.09	0.426
Meat quality						
pH 24h SM	5.62	5.56	5.58	5.55	0.08	0.219
pH 24h LT	5.58	5.54	5.58	5.53	0.11	0.602
CE 24h SM (µS)	4.25	3.93	3.88	4.02	0.65	0.547
CE 24h LT (µS)	3.38 <sup>ab</sup>	3.76 <sup>a</sup>	3.34 <sup>b</sup>	3.65 <sup>ab</sup>	0.35	0.034
L*	48.11	48.83	49.23	50.22	2.44	0.264
a*	7.88	7.92	7.60	7.96	1.10	0.863
b*	1.65	1.94	1.85	2.33	0.84	0.313
Colour (Japanese scale)	2.89	2.76	2.76	2.68	0.65	0.896
Drip losses (%)	4.77	4.67	5.13	5.10	1.78	0.953

<sup>1</sup> Different superscripts indicate significant differences ( $P < 0.05$ ) between treatments or diets; C0- positive control; P0- negative-control; P1- negative-control supplemented with 0.01% Phyzime XP 5000; P2 negative-control supplemented with 0.02% Phyzime XP 5000 SM: Muscle *semimembranosus*; LT: Muscle *longissimus thoracis-lumborum*; CE: electrical conductivity.

<sup>2</sup> F34FOM and M34FOM are, respectively, fat and muscle thickness measured with Fat-O-Meat'er (FOM). % Lean FOM =  $66.51 - 0.895 * F34FOM + 0.144 * M34FOM$

<sup>3</sup> Minimum fat thickness over the muscle *gluteus medius* using a ruler.

<sup>4</sup> Minimum distances from the medullar channel to the cranial edge of the muscle *gluteus medius*.

### 4.2.3. Bone characteristics

#### 4.2.3.1. Bone characteristics during growth

All the animals were scanned at different BWs, and, from the images, the absolute and relative volumes associated with several ranges of HU values and the bone densities were calculated. The results are presented in Table 12, independently for treatment and target BW because the interaction was not significant.

It is possible to see that there were only one variable, the volume associated with HU values between +501 and +1000, presented significant ( $P < 0.05$ ) differences between treatments. So, although the total bone volume (HU between 141 and 1400) was not significantly different between treatments, the volumes between HU 401 and 1000 were higher in P2 than in P0, indicating that the volume of denser tissue was higher in P2 than in P0 and was intermediate in the other treatments. In addition, in the proportion of volume associated with these HU values, a tendency can be seen ( $P < 0.10$ ) in the same direction, as well as with the total density of the carcass ( $P < 0.10$ ). These results make sense and can indicate an effect of the phytase at high dose (P2) on the density of the bones in comparison with animals with low P levels and no inclusion of phytase. However, since the pigs fed C0 and P1 were in between, and not significantly different than those fed P0 and P2. It is possible to see that despite the levels of dietary P or the inclusion of phytases, all those pigs have skeletons with similar densities. According to previous experiments (Cromwell et al. 1995; Cromwell et al. 1993; Harper et al. 1997), it was expected that the animals of the P0 group, which lack P in their diets, had lower bone strength and consequently lower bone density than control group animals. Likewise, it was expected that the inclusion of phytases in the P0 diet increase the bone strength in comparison with the negative control for values similar to those of the positive control. Furthermore, Ryan et al. (2011b) reported a decrease of approximately 25%, in the whole body bone mineral density of growing-finishing pigs due to the low levels of P in their diets (1.6 g/kg dig P) when compared to the group fed 2.8 g/kg digestible P. In the present work, differences between P2 and P0 were observed but differences

between C0 and P0, which according to Ryan et al. (2011b) should be evident, were not observed in the present experiment. The absence of significant differences may be because pigs in this experiment had a higher feed consumption compared with those of other works compensating for the low P level in their diets.

Regarding target BW, differences were significant ( $P < 0.0001$ ) for all the variables studied. Ryan et al. (2011a, 2011b) saw an increasing of bone density with time. In this study, the increase in density with time was observed to be in agreement with Ryan et al. (2011b), which translated into a decrease in the proportion of bone with low density (HU141 to HU500) and an increase in the proportion of bone with higher density (HU501 to HU1000 and HU1001 to HU1400).

Table 12. Absolute and relative volume of bones (least squared mean) by range of density (different range of Hounsfield-HU values) evaluated with computed tomography in live pigs from different treatments at different target body weights (TBW).

	Treatment <sup>1</sup>					Target body weight			SEM <sup>5</sup>	P-value <sup>6</sup>
	C0	P0	P1	P2	SEM <sup>5</sup>	50 kg	75 kg	120 kg		
Volume (dm <sup>3</sup> )										
HU141 to HU1400	4.83	4.83	4.83	4.93	0.059	3.34 <sup>c</sup>	4.70 <sup>b</sup>	6.52 <sup>a</sup>	0.080	0.470
HU141 to HU500	4.13	4.17	4.14	4.17	0.058	3.02 <sup>c</sup>	4.05 <sup>b</sup>	5.39 <sup>a</sup>	0.078	0.926
HU501 to HU1000	0.66 <sup>ab</sup>	0.62 <sup>b</sup>	0.66 <sup>ab</sup>	0.73 <sup>a</sup>	0.023	0.31 <sup>c</sup>	0.61 <sup>b</sup>	1.08 <sup>a</sup>	0.031	0.014
HU1001 to HU400	0.03	0.03	0.03	0.03	0.002	0.02 <sup>c</sup>	0.03 <sup>b</sup>	0.05 <sup>a</sup>	0.002	0.714
Proportion (%) <sup>2</sup>										
HU141 to HU500	86.40	87.08	86.47	85.66	0.426	90.25 <sup>a</sup>	86.27 <sup>b</sup>	82.69 <sup>c</sup>	0.575	0.106
HU501 to HU1000	12.93	12.28	12.85	13.69	0.404	9.30 <sup>c</sup>	12.99 <sup>b</sup>	16.54 <sup>a</sup>	0.545	0.081
HU1001 to HU1400	0.67	0.64	0.68	0.65	0.031	0.45 <sup>b</sup>	0.75 <sup>a</sup>	0.78 <sup>a</sup>	0.042	0.741
Density <sup>3</sup>										
	1.46	1.46	1.46	1.47	0.004	1.43 <sup>c</sup>	1.46 <sup>b</sup>	1.49 <sup>a</sup>	0.003	0.076

<sup>1</sup> C0- positive control diet; P0- negative-control diet; P1- negative-control diet supplemented with 0.01% Phyzime XP 5000; P2 negative-control diet supplemented with 0.02% Phyzime XP 5000.

<sup>2</sup> Proportion of the volume associated with each HU value with respect to the total volume of bones (HU141 to HU 1400).

<sup>3</sup> Density calculated according to the equation  $Density = 0.997649 + HU \times 0.001413$  from Picouet *et al.* 2010.

<sup>4</sup> Maximum standard error of the treatment means.

<sup>5</sup> Maximum standard error of the TBW means.

<sup>6</sup> P-value for dietary treatment effect, for TBW effect p-value was < 0.0001 for all the parameters.

#### 4.2.3.2. Bone characteristics at slaughter

After slaughter, carcasses were CT scanned, and the absolute and relative volumes of bones and the whole carcass density were determined. The results are presented in Table 13. Significant differences were found only in the total volume associated with bones (HU values between 141 and 1400), which was higher in P2 than in P0, and was intermediate in C0 and P1. This is probably the reason why the volume associated with HU values between 141 and 500, which is the highest volume of the range of HU values studied, was significantly higher in P2 than in P0. In fact, if the total volume of bones was included as a covariate, the significance disappeared. When the proportion of volume of each range of HU values was studied, no significant differences were found between carcasses from animals of different feeding treatments. Consequently, when the bones of the whole carcass were studied, there was a lack of effect of the feeding treatment in its global bones characteristics. It is difficult to explain why the differences previously observed in live pigs cannot be observed in carcasses. A possible explanation may be the fact that the differences were more important at intermediate weights than at final weights (although no interaction between weight and treatment was found) because of the high daily feed intake that might compensate the restriction in P.



Table 13. Absolute and relative volume of bones (least squared means) by range of density (different range of Hounsfield-HU values) evaluated with computed tomography of pig carcasses from different treatments.

	Treatments <sup>a</sup>				RMSE	P-value
	C0	P0	P1	P2		
All carcass						
Volume (dm <sup>3</sup> )						
HU141 to HU1400	0.21 <sup>ab</sup>	0.20 <sup>b</sup>	0.21 <sup>ab</sup>	0.24 <sup>a</sup>	0.01	0.029
HU141 to HU500	0.17 <sup>ab</sup>	0.16 <sup>b</sup>	0.17 <sup>b</sup>	0.20 <sup>a</sup>	0.03	0.015
HU501 to HU1000	0.04	0.04	0.04	0.05	0.01	0.534
HU1001 to HU400	0.001	0.001	0.001	0.001	0.0007	0.593
Proportion (%) <sup>b</sup>						
HU141 to HU500	79.03	79.18	79.13	80.60	2.17	0.307
HU501 to HU1000	20.46	20.15	20.38	18.85	2.00	0.237
HU1001 to HU1400	0.52	0.67	0.49	0.54	0.33	0.585
Carcass density <sup>c</sup>	1.46	1.46	1.46	1.45	0.02	0.238

<sup>a</sup> C0- positive control diet; P0- negative-control diet; P1- negative-control diet supplemented with 0.01% Phyzime XP 5000; P2 negative-control diet supplemented with 0.02% Phyzime XP 5000.

<sup>b</sup> Proportion of the volume associated with each Hounsfield (HU) value with respect to the total volume of bones (HU141 to HU 1400).

<sup>c</sup> Density of the carcass calculated as  $0.997649 + HU \times 0.001413$  (Picouet *et al.* 2010).

#### 4.2.3.3. Isolated bones analysis

After slaughter, femur and tibia were removed from the carcass and CT scanned. From an axial image of the center of the bones, total, marrow and cortical areas and average of HU values and density were determined. Also the length of the bone was measured. The results are shown in Table 14 and it is possible to see that no significant differences were found between dietary treatments in any of the variables. The lack of differences may be due partly to a high variability within treatments, to the fact that conditions of the different treatments were not different enough to produce these differences in the central part of the bones or to the high feed intake that could compensate for the deficiency in P.

The metacarpus III and IV were also collected after slaughter and scanned. The average HU value was obtained. After a texture analysis the bending strength was calculated and finally, bones were chemically analysed and dry matter and dry ash were determined. The results are presented in Table 14; similar to the femur and tibia, no significant differences were found in any of the parameters studied except the bending strength of the third metacarpus.

The obtained results are surprising since the metacarpus from treatment P0, which is the low P diet, had a higher bending strength, while supposedly, it should have the lowest strength. Since all the other variables studied in bones showed no significant differences between dietary treatments, this result is not relevant and, possibly could be explained by a high variability in bone characteristics within treatment, a high variability of the method of measurement by itself (Crenshaw et al. 1981; Combs et al. 1991; Sedlin and Hirsch 1966).

Bone density, either for the entire body, half of the carcass or isolated bones, was not significantly different for any treatments. This is not in agreement with previous similar studies where the authors found a decrease in bone density when the animals had a lack of P in their diets (Partanen et al. 2010; Ryan et al. 2011a, 2011b; Varley et al. 2011).

The lack of differences in bone density and strength between dietary treatments can be explained by the high feed intake of the pigs compared with other works (Ryan et al. 2011a; Hastad et al. 2004; Apgar et al. 2003; Harper et al. 1997a). This could have compensated for the low P content of the diet. Ryan et al. (2011b) concluded that young pigs are highly sensitive to modifications in dietary P levels compared with finisher pigs. Thus, this statement can be an explanation to the absence of differences in the bones characteristics between treatments found in this study once the experiment started at 50 kg of BW.

Table 14. Characteristics of individual gilt bones evaluated chemically, with texturometer and by means of computed tomography images by dietary treatment.

	Treatments <sup>1</sup>				Statistics	
	C0	P0	P1	P2	RMSE	P-value
Tibia						
Total bone area (mm <sup>2</sup> ) <sup>3</sup>	397	394	406	406	32	0.765
Marrow area (mm <sup>2</sup> ) <sup>3</sup>	64	75	73	73	20	0.570
Cortical bone area (mm <sup>2</sup> ) <sup>3</sup>	333	319	333	333	21	0.322
Length (mm)	183	184	184	184	7	0.967
Average HU <sup>4</sup>	1476	1508	1491	1498	41	0.312
Density <sup>5</sup>	1.88	1.89	1.89	1.91	0.04	0.364
Femur						
Total bone area (mm <sup>2</sup> ) <sup>3</sup>	591	591	581	597	54	0.918
Marrow area (mm <sup>2</sup> ) <sup>3</sup>	183	195	182	189	38	0.822
Cortical bone area (mm <sup>2</sup> ) <sup>3</sup>	408	396	400	408	29	0.706
Length (mm)	197	195	199	195	8	0.649
Average HU <sup>4</sup>	1453	1479	1498	1478	45	0.117
Density <sup>5</sup>	1.76	1.76	1.77	1.79	0.03	0.165
Metacarpus III						
Cortical bone area (mm <sup>2</sup> ) <sup>3</sup>	110	121	116	124	16	0.251
Average HU <sup>4</sup>	597	551	557	559	63	0.358
Bending strength (Kgf)	205.7	254.7	201.5	219.7	47.9	0.048
Dry matter (%)	72.15	70.66	72.14	72.40	2.57	0.379
Metacarpus IV						
Dry ash (%)	39.67	40.27	40.07	40.21	2.07	0.902
Cortical bone area (mm <sup>2</sup> )	107	116	109	121	16	0.180
Average HU <sup>4</sup>	592.8	543.1	530.9	568.5	107.4	0.554
Bending strength (Kgf)	178.1	196.8	193.2	190.2	36.9	0.639
Dry matter (%)	70.64	71.01	70.22	70.75	1.58	0.686

<sup>1</sup> C0- positive control diet; P0- negative-control diet; P1- negative-control diet supplemented with 0.01% Phyzime XP 5000; P2 negative-control diet supplemented with 0.02% Phyzime XP 5000.

<sup>3</sup> Measured in an axial image of the central part of the bone

<sup>4</sup> Average of Hounsfield (HU) values from cortical area of the central part of the bone.

<sup>5</sup> Density of the central part of the bone calculated as  $0.997649 + HU \times 0.001413$  (Picouet *et al.* 2010).

## **CONCLUSIONS**



## 5. Conclusions

In the conditions and with the limitations of the present work, it can be concluded that:

1. Computed tomography technology has been demonstrated to be a very suitable and accurate technology for determining not only body tissue composition but also the density of bones at different moments of pigs' growth, avoiding serial slaughtering. The CT images obtained from live pigs can provide useful information for breeding, health management and nutritional studies in swine industry.

2. There are clear differences in the growth rate and fat composition of the pigs among three different feeding treatment (AL, feeding restricted in volume in growing period and restricted in volume in finishing period) during growth, although these effects are not found in the final product due to compensatory effects. Thus, the work demonstrated that it is possible to obtain the same (or similar) final product with different feeding strategies and the farmers must choose the most suitable to their necessities and possibilities.

3. Feeding restrictions produce certain effects on production parameters that cannot be disregarded, such as the total days at the farm necessary to reach the same final weight, which can increase production costs. Such information may be valuable for the farmers to identify the most economical feeding strategy considering the quality of the productive parameters together with the quality characteristics of the final product.

4. The carcass and meat quality of the final product are not highly affected by the feeding restriction in volume in different growing period, although from the sensorial point of view, meat from animals with some restriction during growth may be slightly less tough than those from animals fed AL during all the growing periods; however, this difference does not appear to have consequences in the consumers' acceptability of the meat. Consequently, feeding restriction strategies

do not impact at meat quality and consumers' acceptability and they can be applied according to farmer's necessities.

5. Feeding restrictions in P at the levels and conditions applied in the present work, was able to ensure the correct bone mineralization and growth performance. Thus, a decrease in dietary P after 50 kg of BW does not negatively affect the growth performance, the meat and carcass quality or the quality of the bones of growing pigs. This indicates that low P-level diet may bring the economic benefits for famers and less harm for the environment.

6. The absence of a negative impact from the low P diet on bone quality may be explained by the age of the animals in the experiment or by the high values of daily feed intake that may have allowed the pigs to compensate for the deficiency of P by increasing the ingestion of food. Another explanation may be that the pigs in this study were fed adequate levels of P before being fed their respective experimental diets and that this can lead to different responses than in pigs fed P deficient diets since weaning. Consequently controlling all these factors it is possible to reduce the dietary P.

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