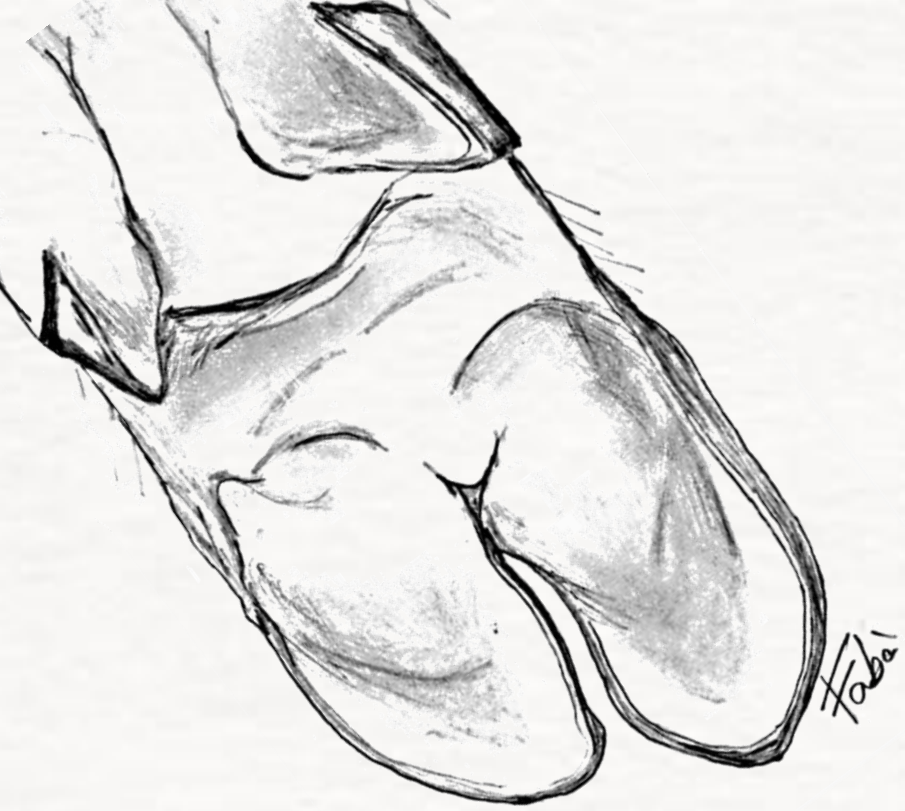
Study of nutritional strategies to improve rearing, quarantine, and adaptation periods of replacement gilts

Lluís Fabà Camats



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Lluís Fabà Camats | 2018

Departament de Ciència Animal i dels Aliments

Universitat Autònoma de Barcelona

FACULTAT DE VETERINÀRIA

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Study of nutritional strategies to improve rearing, quarantine, and adaptation periods of replacement gilts

TESIS DOCTORAL PRESENTADA PER:
Lluís Fabà Camats

SOTA LA DIRECCIÓ DELS DOCTORS:
Josep Gasa Gasó y **David Solà Oriol**

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FACULTAT DE VETERINÀRIA

Il·lustració de la portada de Lluís Fabà. Vista plantar de peu de porc a llapis.

Josep Gasa Gasó, Catedràtic del Departament de Cèencia Animal i dels Aliments de la Facultat de Veterinparia de la Universitat Autònoma de Barcelona, y **David Solà Oriol**, investigador del Servei de Nutrició i Benetar Animal (SNIBA),

Certifiquen:

Que la memòria titulada "**Study of nutritional strategies to improve rearing, quarantine, and adaptation periods of replacement gilts**", presentada per Lluís Fabà Camats amb la finalitat d'optar al Grau de Doctor en Veterinària, ha estat realizada sota la seva direcció i, considerant-la finalizada, autoritzen la seva presentació per a ser jutjada per la comisió corresponent.

Per a que consti en efectes oportuns, firmen la present a Bellaterra, 30 de novembre de 2018.

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Address: Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain
E-mail: Lluis.Faba@uab.cat; lluis.faba@gmail.com

Dedicada a la creixent Big Family

als de casa Mercè, Jaume, Xavier, Toni,
i Laura

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El present projecte presenta unes dimensions considerables i ha implicat treballar amb animals propietat de tercers, en condicions comercials, i de forma prolongada en el temps. Característiques que dificulten l'execussió dels dissenys experiments però també impliquen un aprenentatge més enllà de l'educació científica típicament compresa en el desenvolupament d'una tesis doctoral. Darrera la finalització amb èxit del projecte hi ha la serietat i col·laboració de diferents actors. En primer lloc, vull agrair a Tecnología & Vitamínas S.L. (Farm Faes, S.A.) la voluntat per desenvolupar un ambiciós projecte juntament amb el SNiBA de la facultat de veterinària de la UAB. En el disseny, vull agrair al David, Josep, Mike i a l'Evelia la possibilitat de discussió. Aquest marge per a la conversa durant el disseny dels estudis va ser un procés molt interessant i que es va mantenir viu en el transcurs dels experiments. En l'execució, cal remarcar que sense l'Evelia aquest projecte clarament semblaria un altre. Treballar amb ella ha estat altament eficaç, fàcil, i a més, molt agradable. De forma semblant agrair a l'Antón, Josep, Conchi, Carlos, Fernando, i Ester tot el temps i ajuda. Un altre pilar important ha estat la col·laboració amb l'IRTA de Munells i en especial amb la Maria i l'Albert. També voldria agrair l'ajuda de l'Albert Canturri com a anatomopoatòleg. I la col·laboració del Departament de Sanitat i Anatomia Animal, en especial al valuós temps de l'Enric. També a tota l'ajudà en forma de temps, mans i bon humor aportada per part dels companys i amics becaris del departament i SNiBA; es i serà un dels millors records. També guardo especial caliu per aquells amb qui he compartit despatx, que últimament han "gaudit" de la meva versió "menys" expressiva. Merci per la comprensió! … ara força per vosaltres! i que quedin molts dies i ocasions per creuar-nos!

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A l'ajuda més important. Quan es fa una cosa, en general, no se'n pot fer un altra i això es una decisió. Ironicament, gran part del cost de temps involucrat en la realització de la tesis l'ha assumit la persona amb qui he decidit compartit camí i temps. Sembla contradictori i per això em disculpo i agraeixo la comprensió! En aquest tros de camí que es deia tesis, m'has acompanyat i ajudat més del que et penses. Ara toca feliciplats i confeti!

ABSTRACT

The present PhD thesis hypothesized reductions of lameness, osteochondrosis (OC) and claw lesions due to dietary treatments (nutrients with specific roles in bone and claw development) fed to rearing gilts, and (or) by reducing growth rates. Similarly, additional nutrients (acting over the immune system) could enhance performance under hostile Porcine Reproductive and Respiratory Syndrome virus (PRRSv) environment. In **Chapter III**, 360 young gilts were reared under dietary Con (control); TM (trace minerals as additional 10, 20 and 50 mg/kg of copper, manganese, and zinc, respectively); Met (102% methionine:lysine); and TM plus Met; to evaluate lameness, performance, and claw lesions (to first parity). Gilt lameness (7.8%) reduced (P<0.05) average daily gain (ADG). Gilt lameness was highest (14.8%, P<0.01) for Con compared with TM (2.0%), Met (5.3%), or TM plus Met (6.5%). On the sow farm, overall lameness increased to 21%. Con females showed highest (P < 0.01) prevalence of lameness (20.8%) than TM (6.5%), Met (11.1%), or TM plus Met (7.6%) at weaning. In **Chapter IV**, a subsample of heavy gilts (10/dietary treatment) finishing rearing (Chapter III) were classified for final ADG as low (838 ± 36.3 g) or high (922 ± 31.1 g) and used to study gait, OC, tibia strength, metacarpal mineralization, and using computerized tomography, whole bone density (scaled by density degree). Dietary treatment TM increased (P<0.05) 0.75 mm the tibia length vs. Con and TM plus Met, and the whole bone density vs. Con and Met. Proportion of highly dense bone increased in TM and Met vs. Con (P<0.05). Tibia strength and metacarpal ash increased (P<0.05) for TM compared with Con. Total score of gross OC lesions was lower in TM plus Met compared with Con (P<0.05). However, growth classification showed no general effects. In **Chapter V**, 240 gilts were used under 2×2 factorial arrangement with factors: 1) control or TM plus Met (as Chapter III); and 2) standard (requirement) lysine (Lys) or low Lys (19% lower); to evaluate lameness, performance, and claw lesions (up to first parity). Low Lys did not affect feed intake but reduced 6.35% ADG and 3.80% the final BW compared with standard (P<0.001). Gilt lameness (7.9%) did not affect growth (P>0.05), and was not affected by dietary treatments. Sow farm lameness and claw lesions were unaffected by dietary treatments. In **Chapter VI**, 100 PRRSv naïve gilts were distributed to 4 dietary treatments as Con (control), VitA (vitamin A 18,000 IU/kg), TT (0.34 tryptophan:Lys and 0.80 threonine:Lys), and Ω3 (10 g/kg fish oil); which were exposed to PRRSv at day 11 post-allotment; and a fifth group as Sham (Con in separate facility); to evaluate growth, viremia, and acute immunity. A 100% viremia was observed at day 2 post-infection (dpi). Positive gilts 9 dpi were 7.8 kg lighter than Sham. Gain 9 dpi increased in TT vs. Ω3 (P=0.036) and Con (P=0.054). Gain 9-to-15 dpi increased (P=0.041) in TT vs. Con. Viremia was not modulated through diet, but IFNα and IL8 increased acute for VitA and IL6 for TT, yet, without performance effects. In **conclusion**, TM or Met enhanced bone, combined reduced OC, but lameness resulted controversial. Reducing 19% Lys reduced growth (6.35%) but not lameness, nor combined with TM plus Met. The TM and Met during growth could not overcome sow farm lameness and claw lesions. Feeding TT before and during PRRS marginally improved early weight gain compared with Con and Ω3.

RESUMEN

Las hipótesis de la tesis fueron que la suplementación dietética (con nutrientes importantes para la formación del huesos y pezuñas) y/o la reducción del ritmo de crecimiento durante la recría reduce las cojeras, osteocondrosis (OC) y lesiones de pezuñas. Además, la suplementación con ciertos nutrientes (importantes para el sistema inmune) mejoraría el rendimiento productivo en un ambiente hostil con el virus del síndrome respiratorio y reproductivo porcino (PRRSv). En el **Capítulo III**, se recriaron 360 nulíparas utilizando cuatro dietas, Con (control); TM (10, 20 y 50 mg/kg adicionales de cobre, manganeso y zinc, respectivamente); Met (102% metionina:lisina); o TM+Met; y se estudiaron como en el Capítulo III. Las cojeras durante la recría (7.8%) redujeron (P<0.05) la ganancia media diaria (GMD). Además, se observó mayor (P<0.01) prevalencia para Con (14.8%) que TM (2.0%), Met (5.3%), o TM+Met (6.5%). La prevalencia de cojeras en las reproductoras fue el 21% y, al destete, Con presentó mayor (20.8%, P<0.01) prevalencia que TM (6.5%), Met (11.1%), o TM+ Met (7.6%). En el **Capítulo IV**, una sub-muestra de nulíparas (10/tratamiento) al final de recría (Capítulo III) fueron clasificadas por GMD baja (838 ± 36.3 g) o alta (922 ± 31.1 g) y se evaluaron la locomoción, OC, resistencia de tibia, mineralización metacarpiana y densidad ósea mediante tomografía computarizada (escalada por densidad). El TM aumentó (P<0,05) 0,75 mm la longitud de la tibia comparado con Con y TM+Met, y mayor densidad ósea que Con y Met. También, TM y Met presentaron mayor (P<0.05) proporción de hueso de alta densidad que Con. La resistencia de la tibia y la cantidad de cenizas en metacarpo aumentaron (P<0.05) en TM vs. Con. La valoración global de lesiones de OC fue menor (P<0.05) en TM+Met que en Con. Sin embargo, la clasificación por GMD no mostró efectos. En el **Capítulo V**, se recriaron 240 nulíparas en un diseño factorial 2 × 2 con: 1) control o TM+Met; y 2) lisina (Lys) estándar (requerimientos) o Lys bajo (19% menor) para estudiar cojeras, rendimiento productivo y lesiones de pezuñas (hasta primer destete). La Lys baja no afectó al consumo de pienso, pero redujo 6.35% la GMD y 3.80% el peso final comparando con Lys estándar (P<0.001). Las cojeras en recría (7.9%) no afectaron al crecimiento (P>0,05) y tampoco fueron afectadas por la dieta. En granja de reproductoras, el tratamiento dietético no afectó ni cojeras ni lesiones de pezuña. En el **Capítulo VI**, se recriaron 100 nulíparas con cuatro tratamientos dietéticos Con (control), VitA (vitamina A 18,000 IU/kg), TT (0,34 triptófano:Lys y 0,80 treonina:Lys) y Ω3 (10 g/kg de aceite de pescado) y expuestas al virus del PRRSv el día 11 post-asignación; y un quinto tratamiento Sham (Con separado en instalaciones PRRS libres). Se estudiaron el crecimiento, la viremia y la respuesta inmune. A día 2 post-infección (dpi) se observó 100% de viremia. A 9 dpi, las nulíparas cursando PRRS pesaban 7.8 kg menos que Sham. La ganancia 9 dpi aumentó en TT comparando con Ω3 (P=0.036) y Con (P=0.054). Entre 9 y 15 dpi, la ganancia de TT fue mayor (P=0.041) que Con. La dieta no afectó la viremia, pero IFNα e IL8 incrementaron con VitA, e IL6 con TT, aunque no hubo relación con el rendimiento productivo. En **conclusión**, TM o Met reforzaron el desarrollo óseo; combinados redujeron la valoración de OC, pero el efecto resultó contradictorio para las cojeras. Limitando un 19% la Lys, se redujo 6.35% la GMD pero no las cojeras, que tampoco se afectaron combinado con TM+Met. Suplementar TM y Met durante la recría no mejoró las cojeras y lesiones de pezuña en granja de reproductoras. Suplementar de TT antes y durante PRRS mejoró ligeramente la ganancia de peso en comparación con Con y Ω3.

RESUM

En la present tesi, es varen hipnotitzar reduccions en coixeses, osteocondrosis (OC) i lesions de peülles via proporcionar una suplementació dietètica (de nutrients importants en ossos i peülles) i/o reduir el creixement durant la recria. També, que nutrients (importants per al sistema immune) millorarien el rendiment productiu en un ambient hostil incloent el virus del síndrome respiratori i reproductor porcí (PRRSv). Al **Capítol III**, 360 nul·lípares es recriaren sota els tractament dietètic Con (control); TM (10, 20 i 50 mg/kg addicionals de coure, manganès i zinc, respectivament); Met (102% metionina:lisina); o TM+Met; i s'avaluà coixeses, rendiment productiu i lesions en peülles (fins al primer destete). Les coixeses en recria (7.8%) van reduir (P<0.05) el guany mig diari (GMD). A més, s'observà una major (P<0.01) prevalença en Con (14.8%) que TM (2.0%), Met (5.3%), o TM+Met (6.5%). En reproductores, les coixeses varen presentar 21% de prevalença. Al deslletament, Con presentava major (20.8%, P<0.01) prevalença que TM (6.5%), Met (11.1%), o TM+Met (7.6%). Al **Capítol IV**, una sub-mostra de nul·lípares (10/tractament) finalitzant la recria (Capítol III) varen ser classificades per GMD baix (838 ± 36.3 g) o alt (922 ± 31.1 g) i s'avaluà la locomoció, OC, resistència de tíbia, mineralització metacarpiana i densitat òssia mitjançant tomografia computeritzada (escalada per densitat). El TM va augmentar (P<0,05) 0,75 mm la longitud tibial comparant amb Con i TM+Met, i una major densitat òssia que Con i Met. També, TM i Met varen presentar major (P<0.05) proporció d'os altament dens que Con. La resistència tibial i cendres en metacarp varen augmentar (P<0.05) en TM vs. Con. La valoració global de lesions d'OC va ser menor (P<0.05) en TM+Met que en Con. No obstant, la classificació per GMD no va mostrar efectes. En el **Capítol V**, es recriaren 240 nul·lípares sota un disseny factorial 2×2 amb: 1) control o TM+Met; i 2) lisina (Lys) estàndard (requeriments) o Lys baixa (19% menys) per estudiar coixeses, rendiment productiu i lesions de peülles (fins primer destete). Lys baixa no afectà al consum de pinso, però va reduir 6.35% el GMD i 3.80% el pes final comparant amb Lys estàndard (P<0.001). Les coixeses en recria (7.9%) no afectaren al creixement (P>0,05) i tampoc reduïren via dieta. En granja de reproductores, ni coixeses ni lesions de peülla van ser afectades pels tractaments dietètics. En el **Capítol VI**, es recriaren 100 nul·lípares sota quatre tractament dietètic Con (control), VitA (vitamina A 18,000 IU/kg), TT (0,34 triptòfan:Lys i 0,80 treonina:Lys) i Ω3 (10 g/kg d'oli de peix), exposats a PRRSv a dia 11 post-assignació; i un cinquè tractament Sham (Con separat en instal·lacions lliures de PRRSv). Es va estudiar el creixement, la virèmia i la resposta immune. El segon dia post-infecció (dpi) s'observà 100% de virèmia. A 9 dpi, les nul·lípares cursant PRRS pesaven 7.8 kg menys que Sham. El guany 9 dpi augmentà en TT comparant amb Ω3 (P=0.036) i Con (P=0.054). També, el guany en TT va ser major (P=0.041) que en Con entre 9 i 15 dpi. La dieta no va afectar la virèmia, però IFNα i IL8 van incrementar amb VitA, i IL6 amb TT, malgrat sense presentar una relació amb el rendiment productiu. En **conclusió**, TM o Met reforçaren el desenvolupament d'ossos, combinats reduïren l'OC, però l'efecte en coixeses resultà diferent entre estudis. Limitant el 19% la Lys, es reduí 6.35% el GMD però no les coixeses, tampoc combinant amb TM+Met. Els TM i/o Met durant la recria no milloraren les posteriors coixeses i lesions de peülla en truges. El TT abans i durant PRRS va millorar lleugerament el guany de pes en comparació amb Con i Ω3.

ABBREVIATIONS

ADFI, average daily feed intake
ADG, average daily gain
AECC, articular-epiphyseal cartilage complex
AI, artificial insemination
APL, alkaline phosphatase
Asym, asymmetry between digit claws
AUC, area under the curve
Av, average
BE, boar exposure
BF, backfat
BW, body weight
Ca, calcium
CI, confidence interval
CON, control basal-diet (Chapter III, IV)
Con, control basal-diet (Chapter V, VI)
COX, cyclooxygenase
CP, crude protein
Ct, threshold cycles
Cu, copper
CV, coefficient of variation
Cys, cysteine
d, day
DewB, dewclaw broken or lost
DHA, docosahexaenoic acid
dpi, day post-infection
EFS, electronic feeding system
EO, endochondral ossification
EPA, eicosapentaenoic acid
EU, European Union
Fe, iron
FI, feed intake
FTU, phytase units
G:F, gain:feed
h, hours
HeelF, fissure crack in transition heel/toe
HeelG, excessive growth of the heel tissue
HG, high-growth rate group
HoofL, excessive growth of the hooves
HU, Hounsfield Units
ICS, intercellular cementing substance
IFNα, interferon-α
IL, interleukin
IU, international units
K, potassium
LD, loin depth
L**G**, low-growth rate group
LMD, longissimus dorsi muscle depth
LPS, lipopolysaccharide
Lys, lysine
MET, additional Met dietary treatment (Chapter III, IV)
Met, methionine
Mg, magnesium
MIN, additional trace minerals dietary treatment (Chapter III, IV)
MM, additional Met and trace minerals dietary treatment (Chapter III, IV)
MMPs, metalloproteinases
Mn, manganese
Na, Sodium
NE, net energy
OC, osteochondrosis
P, phosphorus
PBA, piglets born alive
PC, principal component
PCA, principal component analysis
PCR, polymerase chain reaction
Procollagen-II, porcine cross-linked c-terminal telopeptides of type II collagen
PRRSv, porcine reproductive and respiratory syndrome virus
qRT-PCR, real-time quantitative reverse transcription PCR
$\mathbf{R^2}$, coefficient of determination
RD, "real decreto"
S, sulphur
SE, standard error
Si, silicon
SID, standardized ileal digestible
ß.CTx, C-telopeptide of the beta 1-chain of type I collagen of bone and cartilage
Thr, threonine
TM, trace minerals also as dietary treatment (Chapter V)
TNFα, tumor necrosis factor-α
TT, aditional Trp and Thr dietary treatment (Chapter VI)
VitA, additional vitamin A dietary treatment (Chapter VI)
VL, viral load
VR, void ratio

TNFα, tumor necrosis factor-α
TT, aditional Trp and Thr dietary treatment (Chapter VI)
USA, United States of America
VitA, additional vitamin A dietary treatment (Chapter VI)
VL, viral load
VR, void ratio
WBD, whole bone density
WCH wall crack horizontal
WCV, wall crack vertical
wk, week
WL, white line lesion
YBP, years before present
Zn, zinc

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CHAPTER I

Introduction and Literature Review

The present thesis insights into the importance of gilt rearing phase and young female management. Initially, introduction and literature review (Chapter I) is divided into three main subjects: 1) a general introduction to modern swine industry, 2) sow replacement and longevity, and 3) lameness and its main causes, with further attention on nutritional strategies that may have intervention potential. This will lead to following chapters including the objectives of this thesis (Chapter II), and different experimental works (Chapters III, IV, V, VI). These experiments were conducted during the gilt development phase and Chapters III, IV, and V multidisciplinary evaluated dietary treatment effects on lameness and lameness consequences during growth and also as carryover into the sow herd. In addition, Chapter VI is an experiment focused on growth and acute immune response under Porcine Reproductive and Respiratory Syndrome when gilts were provided various nutritional strategies. Finally, these experimental works are followed by a general discussion (Chapter VII) and general conclusions (Chapter VIII).

1.1. General introduction

1.1.1. Swine industry, part of a global challenge

The current world population is estimated to grow from 7.6 to 8.6 billion by 2030, attaining 9.8 billion in 2050, and 11.2 billion in 2100; which roughly means 83 million people every year. Growth is expected to be particularly significant in developing countries. For example, India population (1.3 billions) would surpass that of China (1.4 billions) in about seven years, and Nigeria will become the third largest country before 2050 (United Nations, 2017). This, carries a growing demand for food and water, while natural resources are limited and unequally distributed. Already 3.6 billion inhabitants are in water-scarce areas, which is projected near 5.7 billion by 2050 (WWAP, 2018). Recent estimates also indicate that about 10.9% of the world population (821 million) is already chronically undernourished (FAO, 2018). However, near 30% (and 39% of the adults) were either obese or overweight in 2016 (World Health Organization, 2018). Therefore, strategic decisions in the future crop and meat industries, but also on the education and food share are required when assuming the challenge "more for more with less".

Feed and food production have a footprint on land, water, and greenhouse gas emissions (Windisch et al., 2013; Flachowsky et al., 2018). Livestock production is responsible for using one-third of global land and generating 14% of human-caused greenhouse gas emissions (Gerber et al., 2013). Crop yield and animal production should consider the most convenient locations (i.e. close to the resources) and the species and production systems more efficient accordingly. Footprints are lower for poultry than pork industry, which in turn are lower than in ruminants such as cattle, lambs, and goat (Gerbens-Leenes et al., 2013; Flachowsky et al., 2018). Therefore, poultry and pork have potential to become main edible meat protein sources worldwide. Feed conversion efficiency, feed composition, and feed origin are the main factors driving this trends. Conversely, manufacture of the feed used in these intensive meat industries has greater footprint compared with other feeding systems typically used in ruminants, especially when sourced from deforestation regions (Gerber et al., 2013; Flachowsky et al., 2018). Still, when producing large amounts of meat, the intensive systems result more sustainable (Gerbens-Leenes et al.,

2013; Phalan et al., 2016; Swain et al., 2018). Nonetheless, such trends of current capitalism shaping the meat industries cannot be this simply justified. Advancement in overall optimization and sustainability are greatly required; and first, by reducing losses and food waste (Blanke, 2015). According to FAO (2011), 1.3 billion t, 24% of produced of edible food per year is lost or wasted. It is intriguing if first logic behind large scale food industrialization is to provide food for all, or to make more business. According to previous discussed evidences, it can be suggested that legislation is required to address sustainability. Given the scale of projected growth and food demand, consistent strategies must be directed from combining efforts.

1.1.2. Modern pig production

The first domestication of pigs likely occurred in the Near East about 9,000 years before present (YBP), begun by 3500 YBP in Europe, and was introduced into America in 1539 by Hernado de Soto (Giuffra et al., 2000; Mitchem, 2011). Therefore, throughout the human world history, pigs played a vital role as a source of food, but also in cultural, religious, and social aspects. Omnivorous, highly prolific, and with a high versatility to adapt on a wide range of environmental conditions, are characteristics that placed pigs as the most consumed source of meat. Nowadays, within a dynamic industry that is mostly based in intensive production.

World inventory of swine in 2016 was 981.7 million pigs (FAO, 2018). The swine industry in volume is led by China (46.5%), the European Union (EU, 15.2%), and the United States of America (USA, 7.28%). Looking at the EU (data without Croatia), current inventory is 148.8 million and from 2007 to 2017 has decreased 10.7 million (Eurostat, 2018). Interestingly, with such reduction and having 2.98 million less of sows, the amount of final produced meat has increased 1.84% within the same year range (Eurostat, 2018). To figure the reasons behind the evolution of modern pig industry, this section will focus on Spanish industry (20% of EU swine inventory) as example of such progress.

The swine industry was already evolving to intensive 20 years ago, and three principal factors continue shaping it: a) the shrinking margin per kg of product, consequence of globalized pricing of feedstuff and meat; b) the genetic improvements (i.e. enlarging litter size); and, c) the transitioning from ‘traditional’ raising pigs to ‘expertise’ with an industrialized approach and using scientific knowledge. Nowadays, there are more integrative companies and less small producers, the companies are bigger, and the farms are larger and more specialized than before (BDporc, 2017). In Spain, the sow productivity increased from 23.8 to 29.3 weaned piglets per productive sow/year (23.1%) between 2007 and 2017 (BDporc, 2007, 2017). The industry keeps improving, only during the last 5 years and from weaning to slaughter, growth performance increased 3.10%, whereas gain:feed ratio and mortality reduced 5.65% and 4.16%, respectively (Observatori Porcí, 2011, 2016). Altogether, and similar to other countries such as Germany, Denmark, USA, etc., these changes pushed the swine industry to be more intensive and efficient (Eurostat, 2018). In fact, Spain reduced 7.8% of the breeding sows, but produce 18.4% more fattening pigs and 23.5% more tones of meat in 2017 than 2007 (Eurostat, 2018). On one hand, there are many improvements, but on the other hand, some indexes such as the pre-weaning mortality (near 17%), and sow annual replacement (near 50%) are not improving. In fact, great variability is

observed among farms, and the coefficient of variation from all previous mentioned indexes range between 13% and 38% (BDporc, 2007, 2017).

More efforts should be directed to reduce premature sow elimination and replacement, which would decrease costs and increase the number of piglets produced per sow and lifetime. Near 15% of females fail to achieve production while carry an investment that will be not returned. Furthermore, 15% to 38% of the sows entering the sow farm do not reach a second gestation. Additionally, mortality and such premature removals (i.e. reproductive problems, lameness, lesions, illness, etc.) are often a welfare issue. Improving this, will increase efficiency, sustainability, and also the animal welfare in the industry; key concepts for future generations with raised concerns about ethical aspects of this production. These subjects will be reviewed in the following sections.

1.2. Sow replacement and longevity

Sow **replacement** could be defined as the act of introducing young females into production to substitute aged sows, mortality, and other removals due to problems. **Longevity** definition, requires some consensus in the literature as can be defined in different manners: stayability (productive lifetime in days), parity number at removal, lifetime total born piglets, lifetime productivity (number of weaned pigs), or pigs born alive per day of life (Yazdi et al., 2000; Knauer et al., 2007; Tarrés et al., 2006; Sasaki and Koketsu, 2011). Despite these different possibilities, more time or more cycles are generally associated with more lifetime piglets. Therefore, different definitions seem to yield similar results in terms of predictability (Hoge and Bates, 2011). By definition, replacement success or failure will affect longevity, and both are thought to be influenced by gilt management and gilt conditioning.

The definition of gilt **conditioning** includes any measure directed to qualify the females with a metabolic stage for an optimum and profitable lifetime (Rozeboom, 2015). Indeed, there is a recognized agreement that conditioning influence to some unknown degree the further productivity, risk of culling, lifetime performance, and herd stability (Engblom et al., 2007; Roongsitthichai and Cheuchuchart, 2013; Thingnes et al., 2015; Koketsu et al., 2017; Bergman et al., 2018). Related or not, strong evidences state that rearing, quarantine-acclimation, and entrance into production to first parity are highly sensible to undesired culling. Such effect on longevity is a medium-to-long term outcome, therefore, likely influenced by many other on-farm experiences and factors. Additionally, conditioning targets include confounding i.e. it is difficult to separate effects of age, BW, growth rate, and fat and protein tissue stores at first service (Bortolozzo et al., 2009; Knauer et al., 2010; Díaz et al., 2017). These facts complicate research and the design of effective strategies. Evolving with the swine industry, producers frequently have capability and willingness to implement gilt strategies, but uncertainty may lead to disregard them. Other on-farm priorities usually take more attention, and often, are easier to make decisions on, implement changes, and evaluate responses.

From a practical standpoint, this section will review the importance of sow **replacement** and gilt **conditioning**. The context used entail the 4 principal factors that directly affect sow herd or that are recognized as important for sow **longevity**: 1) ratio and frequency of replacement, which define the parity distribution and herd steadiness; 2) replacement immune status, which together with that of the sow herd define a major risks for

pathogen outbreaks (little addressed in this review); 3) update of genetic potential, which should improve herd's productivity over time (not extended in this review); and, 4) gilt conditioning and strategies to reach specific targets (i.e. puberty attainment and targets to first service). Open questions will address factors (1), (3), and (4) in the 1.2.1. subsection. The factor (4) is further reviewed in depth for specific conditioning targets in the 1.2.2 subsection. Factor (2) is only introduced in 1.2.3 subsection to discuss gilt health and immunity consequences during rearing and quarantines. Finally, subsection 1.2.4 concludes.

1.2.1. Importance of sow replacement

*1.2.1.1. "Is sow **longevity** driven by **replacement** or by culling?"*

Ideally, to remove or maintain females in a sow farm should be a decision limited to low performing sows and aged parities (i.e. >8th parity); while replacement should carry genetic improvement. The balance between removal-maintain and replace is expressed as the replacement annual ratio, which is commonly accepted between 40 and 50% (Lucia et al., 2000; Engblom et al., 2007; BDporc, 2017). Sow replacement aims an ideal distribution of parities to maximize the number of sows on their highest productivity. Highest sow performance occurs between 2nd and 5th parity. In fact, the number of piglets born alive is greater from 3rd to 5th parity, but farrowing rate is usually higher between 2nd and 4th (Iida et al., 2015; Koketsu et al., 2017).

A near-perfect situation would be annually replacing 30% of females after completing 8 parities, and producing near 90 piglets per sow and lifetime (Rozeboom, 2015). However, unplanned removals shape longevity and replacement. Typically, removals were either stated as voluntary (determined broadly by farm manager, i.e. economic reasons) or involuntary (forced reasons beyond the farm manager, i.e. chronic illness; Fetrow et al., 2006). Among voluntary or involuntary, most removals could be classified as undesired, because such females retain investment and potential which imply a loss of efficiency and sustainability. Often, undesired removals highly concentrate in young and aged females, being the first, those that represent the majority (Engblom et al., 2007; Iida et al., 2015). In a recent study, Bergman et al. (2018), described that 14.7% out of 65,313 females entering the sow herd did not farrow for the first time and were removed. Likewise, from those that farrowed, 1 out of 6 sows were removed within 1st and 2nd parity. Conversely, removals above 5th parity may be planned and directed to a specific limit (i.e. 6th, 7th, or 8th parity) which is commonly attributed to genetic improvement needs (Engblom et al., 2007; Abell et al., 2016). According to cycle length, accepting 40-50% replacement ratio, 10 to 15% of removals between parities, and voluntary removal at 7th or 8th parity, near 18-25% of the sow herd should be gilts. To calculate the target of gilt proportion, this equation is representative [Gilt target per batch % = $100/\sum^{n}_{i=0}(1-$ proportion of removals between each parity$)^{n-1}$; where "n" is the voluntary maximum parity number at removal] (Gasa et al., 2015). Such proportion of gilts should be maintained within each batch because sows produce differently among parities. A fluctuating distribution of parities per batch would risk farm steadiness on fertility, farrowing rate, flow of piglets, and even immune stability (Carney-Hinkle et al., 2013; Koketsu et al., 2017).

Sow longevity is determined by many factors that affect culling and hence replacement. For example, sow biology, productivity, parity, number of tits, season, management, housing, planning, and mortality (Tarrés et al., 2006; Engblom et al., 2007;

Engblom et al., 2008a; Iida et al., 2015; Koketsu et al., 2017). However, the daily decision on voluntary removals is made by the farm manager. This decision is likely subjective to sow's parity, previous performance, reproductive status, health, the market price for culled sows, and the needs for the following breeding target. The latter, imply access to replacement gilts (i.e. 21% of the batch) on the right reproductive status. Therefore, a properly planning of replacement can indirectly affect actual criteria for removal. Deficiencies on such planning increase the risk of negative dynamics such as to inseminate non-appropriate females (i.e. aged sows, gilts out of BE the target, etc.), fluctuating parity distribution, or increase replacement per se.

Replacement rate has not improved in parallel to other productive traits. In fact, it is commonly observed 30 to 60%, and averages near 50% (Roongsitthichai et al., 2015; PigCHAMP, 2016; BDporc, 2017). One may attribute this to a more rapid genetic improvement, but data suggest that there are other factors driving this increase of replacement. Higher removals concentrate early on the productive life of sows, and 15 to 38% of the sows entering the sow farm do not reach a second gestation (López-Serrano et al., 2000; Lucia et al., 2000a; Roongsitthichai et al., 2013). In a study from Engblom et al. (2007), information from 14,234 removals were recorded. They reported reproductive disorders (26.9%) as the most common removal reason followed by planned removal (18.7%, old age), udder problems (18.1%), low productivity (9.50%), lameness or foot lesions (8.60%), and traumatic injuries (7.08%). Although great variation is observed, it can be summarized that removals due to reproductive disorders and sub-optimal performance represent near 50% (Lucia et al., 2000a; Engblom et al., 2007; Anil et al., 2009a; Roongsitthichai et al., 2013); whereas age planned removals vary between 19 and 41% (Boyle et al., 1998; Heinonen et al., 1998; Jr et al., 2000).

*1.2.1.2. "What does bring higher return, sow **replacement** or keep sows ageing?"*

Unplanned removals are a direct cost for the replacement, include the cost of previous investment on the female (i.e. feed during development, employee, facility, veterinary costs, vaccinations with other treatments, and others), and an opportunity cost for the missed potential (i.e. non-productive days). Nonetheless, in relative terms to direct costs, replacement has a moderate importance. In Spanish sow herds, the replacement cost per sow/year is low-intermediate (7.40%) compared with facilities and labor (41.6%), or feed (38.3%); while is similar to treatments (9.62%) and higher than insemination (3.25%; Observatori del Porcí, 2017). However, opportunity costs are uncertain and often not taken into account. Undesired removals and increased replacement increase death intervals and non-productive days, which reduce overall piglets produced per year, longevity, and sow lifetime productivity (Koketsu et al., 2017). In addition, high replacement usually increases the proportion of piglets from first farrowing, which compared to multiparous sow piglets have higher mortality and worsen performance throughout both, nursery and grow-finish phases (Carney-Hinkle et al., 2013).

Conversely, high replacement is sometimes seen as none-problematic because may increase herd productivity and could even be more profitable (Faust et al. 1993; Stalder et al., 2003). Otherwise, such statement cannot be competitive with the index of annualized lifetime piglets born alive (or similarly for weaned pigs); a better image of herd productivity, efficiency, and sustainability than the traditional use of weaned piglets per sow and year (Iida

et al., 2015; Koketsu et al., 2017). Abell et al. (2016) calculated the relative economic weights for reproductive traits based on genetic variance. Lifetime born alive had the greatest relative economic importance compared with litter size of born alive and weaned, litter birth weight, litter weaning weight, and wean to estrus interval. Altogether, this suggests that production strategies, management, and genetic selection should apply more efforts to enhance longevity and lifetime productivity.

Genetic lag from nucleus selection herds to commercial herds is at least 1.5 to 3.4 years (Mitchell et al., 1982; Harris and Newman, 1994; Abell et al., 2010). Assuming a 1.5-year lag and improvements of 0.3 piglets/sow, 1.36 kg/litter, and 3 d less to market on the new generation, Abell et al. (2010), reported that the economical balance between maintain or replace a sow would not cover the variable costs associated with gilt development until 7th parity. Various studies reported that 3 to 5 parities per sow are required to be profitable, and after, sows should provide benefits (Lucia et al., 2000b; Stalder et al., 2003). Therefore, genetic lag, average sow productivity, actual genetic improvement, costs of maintaining aged sows, and costs of gilt development for each specific system case need to be considered. Planned culling should not be based on improving genetic potential without previous calculations.

Animal selection has also been proposed to maximize longevity, although, this is not often included in genetic programs or assessments. The extended time required to accumulate lifetime data may be an explanation. Additionally, poor genetic correlation is reported for longevity between purebreds and crossbreds, likely due to differences in removal criterion either for selection or performance (Abell et al., 2016). Performance records from early parities in purebreds and crossbreds highly correlate with lifetime longevity and performance of crossbreds (Abell et al., 2016; Engblom et al., 2016). Indicating that this would be the best way to select for longevity. Similarly, multiple genetic markers and genes associated to sow longevity have been described and should be further investigated (Mote et al., 2009; Kang et al., 2018).

*1.2.1.3. "Is required to select among gilts for **replacement**?"*

There is a general agreement to select among gilts and eliminate those with high risk to reduce performance during development and before replacement. Poorly conformed sows and locomotion difficulties are associated with increased risk of culling and reduced productivity (Tarrés et al., 2006; De Fernàndez Sevilla et al., 2008; Fernández-Ibáñez et al., 2009; Le et al., 2016). Abnormal conformation has been related with joint and feet problems, and increased risk of lameness and culling (Engblom et al., 2007; Einarsson et al., 2008; L. Pluym et al., 2013ab; De Koning et al., 2015). These authors, reported that deficient conformation includes extreme X or O shaped hind legs, straight or bow hind legs, straight and sickled hocks, swaying hind quarters, and steep or weak pasterns of the front legs were associated to joint problems (see Figure 1.1 for drawings). Nonetheless, controversy and lack of consistent correlations over time are often attributed to subjectivity of in vivo observations (Koning et al., 2015). Objective measures in development and used in research (i.e. kinematic assessments of locomotion and force plates with automated analysis), may provide a more reliable tool for gait and lameness assessments (Grégoire et al., 2013; Karriker et al., 2013; Kristjansson et al., 2013; Liesbet M. Pluym et al., 2013bc; Suontama et al., 2013; McNeil et

al., 2018). Furthermore, evidence indicates that poor conformation is partly heritable, and therefore, seedsstock companies should add higher selection pressure on sound legs, which in turn would enhance sow longevity (Aasmundstad et al., 2014; Le et al., 2016).

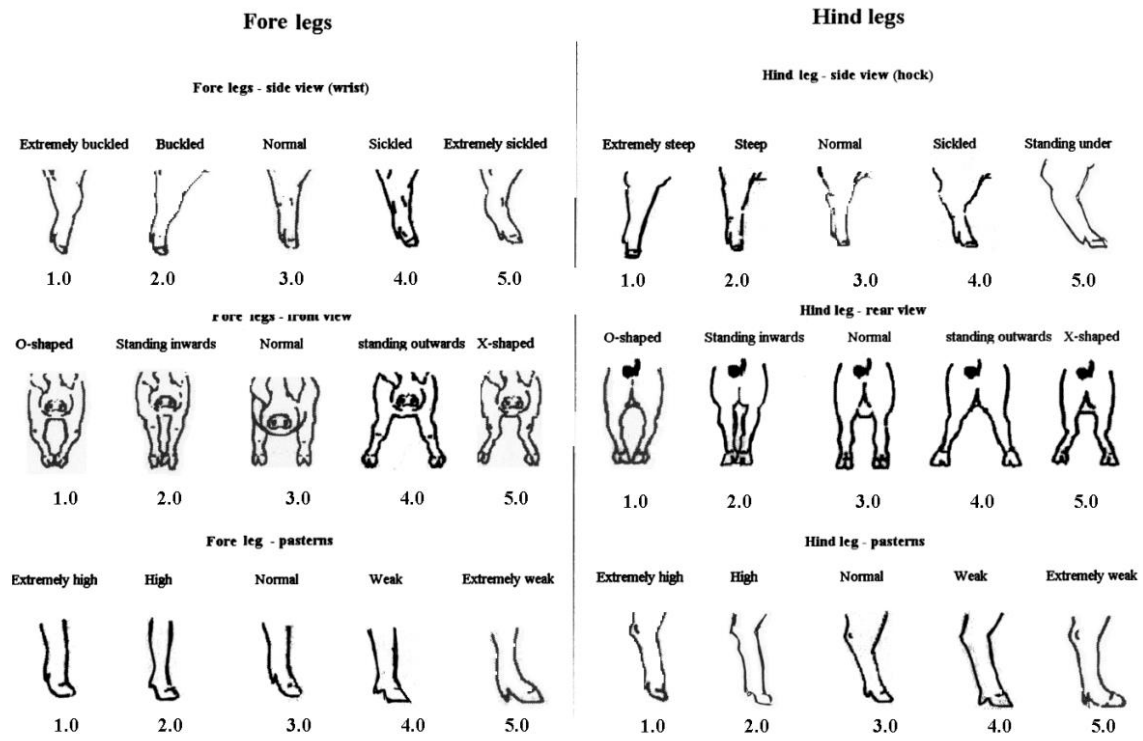

Figure 1.1. Drawing of conformation traits and a system for scoring fore and hind legs. Source: Canadian Centre for Swine Improvement Inc. (CCSI, 2001).

There is no recommended percentage of gilts to be discarded, probably because the swine industry must seek zero. Therefore, this information is not commonly reported and often combine mortality and removals. Across studies, this has shown 3.3% to 28% range (Lucia et al., 2000a; Díaz et al., 2013, 2015, 2017; Thingnes et al., 2015). Causes include mortality, poor body condition, leg problems, and others. Among those, leg problems represented between 18% and 73% of the cases. Furthermore, lameness is also a major cause for culling young females already inside the breeding farm, and has become a great and a permanent problem in the swine industry (see *1.3. Lameness on gilts and sows*). Up to the date, overall efficacy of replacement becomes a worrying issue. Near 15% of gilts are lost from rearing to entering the breeding herd (Díaz, 2013, 2015, 2017), and an additional 14.7% of females do not reach a single farrowing (Bergman et al., 2018).

*1.2.1.4. "What is gilt fitness and **conditioning**?"*

Producers, veterinarians, researchers, and nutritionists typically set targets to first service. Gill (2006) and Rozeboom (2015) defined gilt fitness and conditioning concepts taking a holistic approach towards body condition, and soundness of legs and feet to succeed a productive and profitable lifetime. This, include management, feeding, and nutritional programs aligned to the lean gain potential of modern genotypes.

Remarkable works on research and in-depth reviews noted the effects and interactions of age at puberty attainment, growth, BW, and body composition on reproductive

performance, lifetime productivity, and longevity (Gill, 2006; Bortolozzo et al., 2009; Rozeboom, 2015). The importance of gilt development is acknowledged and the understanding over the last years has increased. Conversely, while targets to first service are suggested, at the same time, there is a general agreement that such targets have confounding and a limited predictive use. For example, 1st parity productivity seems to be more determining than age at first service for lifetime productivity (Figure 1.2). The different experiences that females undergo during their productive lifetime could also explain part of this variability.

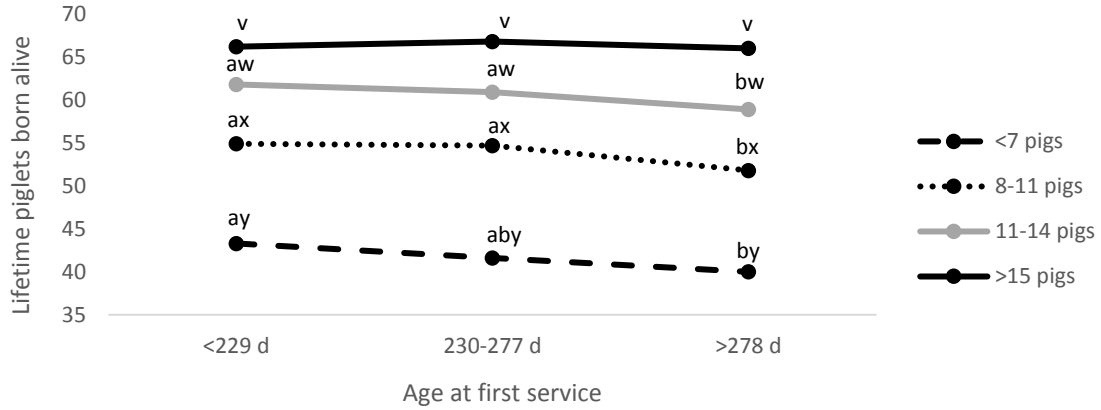

Figure 1.2. Lifetime piglets born alive (PBA) for 4 groups categorized by the basis of the 10th, 50th and 90th percentiles of PBA at 1st parity and classified in 3 categories of age at first service. Modified from Lida et al. (2015). *Their study includes 476,816 parity records of 109,373 sows entered into 125 southern EU herds.
[a-b] Different superscripts within PBA categories differ at $P < 0.05$.
[v-y] Different superscripts within age at first service categories differ at $P < 0.05$.

Contemporary sow dam-lines are leaner, have increased growth aptitude with enlarged adult size, and produce a more total born and weaned piglets per litter than 20 years ago (1.1.2. Modern pig production). Logic says that gilt preparedness with a minimum BW or body condition (amount of lean and fat tissue) may be more important nowadays, and also, more challenging to maintain over the lifetime. In fact, young female failure, mortality, and replacement are indexes not improved during the last decade (BDporc, 2007, 2017; Bergman et al., 2018). Genotypic differences (i.e. appetite, rate of accretion, mass of fat, lean, etc.) and productive traits are also acknowledged (Gill, 2006; Knauer et al., 2010). Indeed, precise targets are thought necessary to manage different maternal line gilts and most seedstock companies suggest them. However, to review these differences in depth was not purpose herein.

1.2.2. Importance of gilt **conditioning** targets

At the end of this chapter, there are presented two tables that summarize the reviewed literature in this subsection. Tables, include previous works based on gilt conditioning or targets (i.e. growth rate, dietary treatment, and age or BW at first service) and classified in puberty attainment (Table 1.4) and sow productivity (Table 1.5). Additional information is also included to provide interpretation help on possible confounding effects.

1.2.2.1. Puberty attainment

Puberty in gilts is the occurrence of first estrus and the onset of reproductive capability (usually between 200 and 220 days of age, Evans and O'Doherty, 2001). Age was earlier suggested the most determining factor for puberty, however, vast variability (near 80% of the mean) and low predictability compared with other factors (i.e. genetics, nutrition, boar exposure, and season) seem to discard age as the first decisive factor (Rozeboom et al., 1995; Evans and O'Doherty, 2001). Large differences for age at puberty are observed between breeds (i.e. 97 to 235 d of age; Duroc> Hampshire > Large White > Landrace > Meishan; Evans and O'Doherty, 2001). Puberty is a complex biological process that involves the hypothalamus secretion of gonadotropin-releasing hormone (GnRH), adrenal maturation, sexual development, and gametogenesis (Evans and O'Doherty, 2001). The GnRH secretion is the first known step in the cascade to activate pituitary and gonadal function, whilst regulation occurs via inhibitory and/or stimulatory actions. The exact mechanisms behind of gilt maturation are unclear (Zhuo et al., 2014), however, nutritional and hormonal factors seem to provide puberty predictability. High consumption of fat (near 370 g/d vs. 64 g/d) resulted as 12 d early attainment of puberty, which was associated with changes on secreted hormones and gene expression in the hypothalamus - pituitary - gonadal axis (Zhuo et al., 2014).

Research on gilt conditioning has often studied the relationship between puberty and growth rate, which may be an indicator of an enhanced metabolic state and performance (Rozeboom, 2015). Earlier, growth rate was reported to modulate puberty with a quadratic relationship. Puberty would delay with growth being both, extremely low (400 to 530 g/d) or high (>600 g/d), but not when intermediate (Beltranena et al., 1991). Even though, current data agree that growth rate >550 g/d, with ad libitum access to feed, and rapid or even very fast growth rate (near 800 g/d from birth to puberty) do not delay the occurrence of pubertal estrus (Bortolozzo et al., 2009; Kummer et al., 2009; Filhaa et al., 2010; Patterson et al., 2010). Nonetheless, (Díaz et al., 2017) recently reported that puberty can be delayed with limiting growth through lessen dietary Lys and without feed restriction. Ad libitum access (100 to 212 d of age) to low or medium Lys diets reduced growth rate 17.5% or 9.7% compared with a high Lys diet (720 or 788 vs. 873 g/d, respectively). High and medium Lys resulted with 6 and 10 d (respectively) earlier attainment of puberty than the Low fed gilts. Estimated these growth rate alternatively from birth (i.e. birth BW = 1.5 kg), the 3 previous groups (low, medium, and high Lys) grew >600 g/d (Díaz et al., 2017). This growth slightly contrasts with previous threshold of 550 g/d to delay puberty. The difference may be explained via genetic differences. Particularly, if limiting lean accretion impairs reproduction (Knauer et al., 2010) accordingly with dam-line leanness and growth potential. Use of deficient Lys diets seems an interesting approach with a practical value that needs further study in modern lines.

Puberty attainment could interact with growth rate and age at boar exposure, besides, a threshold of age or BW may be limiting any interaction. In two different studies, using an early boar stimulation (near 144 d of age), resulted with an earlier puberty attainment and increased proportion of mature gilts than a later (130-149 d vs. 150-170 of age) exposure (Filha et al., 2009), or only in fast vs. slow growing gilts (658-800 vs. 492-656 g/d, Kummer et al., 2009). However, growth rate did not influence puberty at older ages of boar exposure

(150 to 170 d; Filha et al., 2010). In another study (Magnabosco et al., 2014) gilts were classified retrospectively to boar stimulation age (two levels), and for growth rate (three levels). Authors described no interaction, but first estrus manifestation was greater either with the greatest ADG (630-790 vs. 580-625 and 500-575 g/d) or for the older gilts at boar exposure (156-170 vs. 140-155 d). Again, effect of high growth rate or age at boar exposure on reproductive performance disappeared with a minimum of 130 kg at first service (Magnabosco et al., 2014). Suggesting that minimum BW may be more important than the growth rate and boar exposure effect on puberty attainment.

Earlier age at puberty attainment was associated with higher fertility and productivity (Young et al., 2004, 2008). Young et al. (2008) reported that gilts with greater ADG (>860 g/d) attaining puberty younger (<185 d of age) had greater number of total born and born alive piglets (+2.5 and +2.3, respectively) over first 3 parities than older ones (>185 d of age). Conversely, too early puberty attainment (<110 d of age) decreased the number of piglets born alive over first 3 parities, while an earlier entrance to production may be confounding (Tummaruk et al., 2007). More consistently, an early boar contact decreases age at puberty, but onset it latter require fewer exposition days, enhances manifestation, and increases the synchronization (Filha et al., 2009; Magnabosco et al., 2014). Numerous authors agree that starting a standardized protocol of boar stimulation between 130-150 d of age, can identify up to 75% of cycling gilts within 40 d (Amaral Filha et al., 2009; Paterson et al., 2010). Similarly, Paterson et al. (2010), stated that 50 to 75% of the future gilts with greatest lifetime productivity could be detected within those 40 d of boar stimulation when beginning from 140 d of age. Yet, 7.9% of the females never cycle (Gibson and Jackson, 2012), and reproductive problems (43-65%) are still the main reason for gilt removal (Lucia et al., 2000; Engblom et al., 2008b). Therefore, improvements on gilt reproductive function are needed.

According to the above reviewed literature, ad libitum feed allowance without specific nutrient restriction results with a wide distribution of gilt growth (520-830 g/d), and a little relationship between growth and puberty attainment. Nonetheless, a high growth rate seems positive for puberty attainment with an early boar exposure until approximately 150-190 d of age or up to 130 kg of BW. Above such threshold, puberty precocity may be more limited by innate variability in LH secretion than by growth performance (Beltranena et al., 1993), hence, genetic differences.

1.2.2.2. Body weight, age, and growth

Previous research on gilt conditioning is often based on BW and despite the confounding with age and growth targets, it seems that BW is the most important target. For example, as discussed earlier, the positive association between growth rate and puberty attainment had a threshold near 130 kg BW (Magnabosco et al., 2014). Among the literature, a minimum BW at first service is the most accepted need to achieve an optimal physiological state throughout lifetime; likely, a safer approach as BW includes all body tissue stores (Newton and Mahan, 1993; Williams et al., 2005; Rozeboom, 2015).

Kummer et al. (2006), observed equivalent productivity with similar BW at first service (near 148 kg) for high (>700 g/d) or low (<700 g/d) growth rate with 198 d and 223 d of age, respectively. However, a third group with 164 kg BW, >700 g/d, and 223 d of age at service produced almost 1 piglet more than previous. Williams et al. (2005) reported

maximal number of pigs born on first 3 parities when gilts were inseminated within 135 and 150 kg of BW. Hoving et al. (2010), described that 2nd parity reproductive performance was poorer when gilts were first time inseminated at 124 kg than at 145 kg BW. Roongsitthichai et al. (2013) reported an increase of 0.6 total born piglets at 2nd parity when gilts entered the breeding unit >150 kg BW than between 136 and 140 kg BW. Conversely, no differences were observed with BW <130 kg.

Generally, gilts entering the breeding herd overweight (i.e. >170 kg) and (or) confounding with overaged (i.e. >250 d), are suggested to increase risks of removal via more difficulties on heat detection and increased anestrus (Williams et al., 2005; Young et al., 2008; Filha et al., 2009) and also for being associated with lameness (Pluym et al., 2013a; Thingnes et al., 2015). High BW and growth rate during rearing are accepted to cause joint problems on growing animals, which is suggested to increase leg weakness and lameness (Ewers et al., 2001; Grevenhof et al., 2011; Koning et al., 2014; Quinn et al., 2015). Busch and Wachmann (2011), described that for 100 g/d increase in ADG increases 20% the risk of joint lesions (osteochondrosis). Nonetheless, several interrelated factors such as genotype, diet composition, growth rate, and mechanical stress are thought to be important for lameness occurrence and will be further discussed in *1.3. Lameness on gilts and sows* section.

In practice, rapid growing females are in risk of overweight before first service. This, is due to quarantine-acclimation prolonged periods (i.e. up to 90 d). Indeed, this is anticipated to protect herd's health status, a major priority during replacement. To minimize the derived problems from this, it was proposed to inseminate 25 d earlier the fast growing gilts (i.e >700 g/d), or target <135 kg BW (and before 200 d of age), or otherwise to rear gilts feed restricted (Kummer et al., 2006; Bortolozzo et al., 2009; Roongsitthichai et al., 2013; Quinn et al., 2015). However, these strategies are not always possible in practice. As described earlier, Díaz et al. (2017) limited growth with Lys deficient diets ad libitum, which could be a more feasible approach than feed restriction in practical conditions. Nonetheless, caution must be taken with limiting growth because <550 g/d (from birth to 100 kg BW) may negatively affect litter size and reproductive function from 1st to 5th parity (Tummaruk et al., 2001).

Williams et al. (2005) recommended that at least 180 kg BW at first farrowing were needed to minimize protein loss during lactation. Kim et al. (2016) classified 2,404 farrowing records (over 6 parities) from 585 gilts according to BW at d 109 of gestation (190, 200, 210, 220, 230, and 240 kg). The lowest culling rate and the highest number of lifetime piglets born alive was observed for 210 kg BW gilts before first farrowing. Therefore, to achieve this BW at farrowing, at least 150 to 160 kg BW would be required at breeding, which is higher than the 135 kg BW previous recommendation by Williams et al. (2005). In agreement, Schenkel et al. (2010) observed that even with BW at first service above 135 kg, the litter size of 2nd parity was reduced for those females that lost >10% BW in their first lactation. In fact, a severe negative balance and BW loss during lactation may impair reproductive function and following prolificacy; which is especially important in young sows and consequences include risks for culling (Soede and Kemp, 2015). Therefore, a minimum BW is required. Nonetheless, overweight and overfat at first farrowing is not desired either, because can increase stillborn and reduce lactation feed intake and litter growth (Cools et al., 2014; Goncalves et al., 2015; Kim et al., 2016). Typical increase of feeding level during late gestation (based on fetal, placenta, fluids, and mammary gland requirements, Kim et al.,

2005; Solà-Oriol and Gasa, 2017) arise risk of over-fattening those gilts already heavy. However, recent studies demonstrated that gilts and sows have great resilience to modify piglet birth weight with different levels of dietary energy and/or Lys in late gestation (Goncalves et al., 2015; Thomas et al., 2018).

To look at first service and first farrowing BW together, including gestation and lactation feeding strategies (Theil, 2015) might provide most of the opportunity to minimize gilt problems and culling. For example, Thingnes et al. (2015), reported that increasing gilt BW 3 wk before farrowing generally increased the risk of sow death or culling. Authors, also reported that culling risk was lower with increased energy intake during rearing but only when first service was performed before 250 d of age. When service was after 250 d of age and gilts had higher energy intake during rearing, the risk of culling increased even more. Culling also reduced when gilts were fed high energy throughout rearing and gestation, likely related with being younger at entrance and maintain so in the breeding herd.

Form the above reviewed research and reviews, it can be summarized that between 135 and 160 kg BW is an optimal range of BW to target at first service. This recommendation is already susceptible of great outcome variability as previously discussed, and to narrow it more seems unreliable. Part of this varying outcome is likely related to different genetics and different experiences or management among studies. Hence, more accurate targets may be possible within specific breeds. Extreme BW at first service (high or low), rather than a precise target seem more determining for longevity outcome. Further, replacement success or failure will still greatly depend on feeding management, prolificacy at 1st parity, and BW or body stores loss during first gestation and lactation.

1.2.2.3. Body condition, fat, and protein tissue stores

Body condition scoring is a subjective evaluation with an extended use and utility in practice. It provides an overall assessment of muscularity but represents a poor indicator of subcutaneous fat (Gill, 2006). This scoring does not substitute BW or specific tissue amounts, but results a practical complementary method to look at herd's nutritional status (i.e. adjust feeding allowance in gestation, speculate likelihood on appetite in lactation, risk of return to estrus, risk of lameness, etc). More appropriate are the amounts of fat and protein tissue stores, which are typically estimated or simply associated from measures of backfat (BF) depth and loin (longissimus thoracis muscle) depth or area. These measures are commonly collected using ultrasound technology at P2 position (6 cm from the midline at the level of the last rib).

The BF at first service was assumed key to maximize early reproductive function, but more recently, this has lessened with some controversy. Some authors reported positive associations of BF at first service with subsequent reproductive performance and longevity (Tummaruk et al., 2001, 2007; Tarrés et al., 2006; Kummer et al., 2009). Contrariwise, Young et al. (2004) noted that thin gilts (backfat <5 mm) at first service did not reduce reproductive performance. Gill (2006), limited 50% Lys during rearing and 27% during first pregnancy, which increased BF at service (16.5 vs. 20.7 mm). The author reported a 10 d puberty delay, a lessened growth rate, and reduced BW up to 6th parity with limited Lys. Additionally, higher Lys was associated with reduced longevity, being lameness the major removal reason, whilst also had increased the number of born alive piglets at 1st parity.

Previous data seem contradictory and have supported both, that BF level at first service can affect longevity and not. In practice, the BF level at first service may be more important for longevity as a carryover to farrowing (i.e. increasing the likelihood of overfat and culling risk), than affecting itself the longevity. Among studies, high BF at farrowing (i.e. >0.22 mm) is known to boosts dystocia and increase stillborn (Cools et al., 2014; Langendijk, 2015; Theil, 2015). According to these authors, high BF at farrowing is associated with reduced feed intake in lactation; which increases the catabolism and the typical negative balances described in the lactating sow (i.e. nitrogen, amino acids, and energy). Other consequences can be a lowered litter growth and reduced 2nd parity prolificacy, likely increasing risks for early culling. Additional noise may include variability on perception of fat and thin females by the famr staff, with consequent ratio adjustments (Fitzgerald et al., 2009). These authors, reported that fat sows are worse detected than thin ones, and to increase the feed ratio of an already fat sow would increase above described problems. Moderate BF at first service seems the most adequate to avoid extremes.

Over the last 15 years, interest on body protein stores and their metabolism increased. These, arisen from the evidence that minimum BW was need at first service, and were suggested more determining factors than BF (Clowes et al., 2003a, 2003b; Williams et al., 2005; Gill, 2006; Rozeboom, 2015). According to previous authors, a certain protein amount may be required to achieve optimum reproductive function of gilts. Similarly, Knauer et al. (2011) described positive genetic correlation (r^2 = 0.38) with increasing loin muscle area at puberty (212 d of age and 137 kg BW) to achieve a first farrowing. The minimum amount of lean tissue required at first farrowing may directly related with a minimum amount of lean tissue achieved at first service. Lean tissue at first service is therefore highly important to minimize the unavoidable catabolism for milk production during lactation (Theil, 2015). Clowes et al. (2003), reported that young sows that mobilized 9 to 12% of protein stores during lactation, decreased the milk quality and also the litter growth beyond 20 d of lactation. This, could also suppress ovarian function and reduce uterus weight at weaning increasing the risk for culling. Schenkel et al. (2010) estimated that >10% BW and body protein losses were thresholds during first lactation, beyond that, described a lowered prolificacy at 2nd parity. Conversely, regardless of BW and body protein losses, the heavier at first farrowing produced more piglets on 2nd parity than the lighter ones. Therefore, BW may be more important and a minimum lean growth or lean tissue amount seem best ensured with a minimum BW. Although, this may change with more precise methods to predict body composition.

1.2.3. Gilt health and immunity

The immune status of replacement gilts, together with that of the sow herd define a major part of herd health stability and risk for outbreaks. In practice, health stability is defined as animals having active immunity either from prior vaccination or infection but, without the presence of overt clinical signs or pathogen shedding (Yeske, 2010). Therefore, it is a necessary step to vaccinate and immunize females towards endemic pathogens on the destination farm, as well as, to cool down any possible infectious disease that gilts carry or were acclimated before entering the sow herd (Batista et al., 2002; Yeske, 2010; Roos et al., 2016; Garza-Moreno et al., 2017). The use of gilts from highest health status as possible

reduces the risks of adding new pathogens to the sow herd. For example, Porcine Reproductive and Respiratory Syndrome virus (PRRSv), *Actinobacillus pleuropneumonie, Mycomplama hyopneumoniae*, swine influenza virus, *Escherichia coli*, etc. Conversely, other frequent realities are to decide matching or to end up matching, the health of the multiplier origin with the herd's one. At this point, veterinarian-to-veterinarian ongoing communication between the two farms is even more essential. It is not herein objective to discuss risks, problems, acclimation details, vaccination and medication programs, and the importance of having a controlled flow, but these are key issues to minimize odds of disease and outbreaks. This, would require a broad and complex discussion. Differently, the present subsection will introduce the following debate: gilts are often exposed to variety of immune challenges such as several vaccinations and sometimes pathogen feedback from the sow herd, but, do current diets provide the most adequate status to face these challenges? Herein, some nutritional components with potential to enhance immune status are overviewed. Likely, this could be adapted to nursery and growing-finishing pigs produced under poor healthy environments.

Early in most infections, the innate immune response activates Toll-like receptors that downregulate oxidative phosphorylation in favor of glycolysis (Badaoui et al., 2013; Tannahill et al., 2014), therefore, reducing efficiency. Immune proteins (i.e. cytokines and acute-phase proteins) are synthetized and released to the bloodstream (Lunney et al., 2010). Another frequent consequence of infection is oxidative stress which together with the overabundance of inflammatory cytokines cause tissue damage (Chiou et al., 2000). Therefore, immune system activation, inflammation and following antibody production increase energy and amino acids demand, meanwhile, disease often cause lethargy and anorexia which under increased requirements leads to protein catabolism (Che et al., 2011; Gabler, 2014). In addition, not only metabolism and requirements change, the acute response antagonizes growth factors that ultimately result with suppressed growth (Spurlock et al., 1997; Broussard et al., 2001). Already very low physiological concentrations of tumor necrosis factor alpha (TNFα) and interleukin (IL) 1β reduce both, protein synthesis and muscle cell development by inducing a state of resistance in insulin-like growth factor I receptor (Broussard et al., 2003, 2004). Therefore, nutritionally anticipating to inflammation, and (or) metabolic changes may be a successful strategy to mitigate the negative impact of disease on pig performance (Greiner et al., 2001, 2001b; Liu et al., 2013; Rochell et al., 2015; Moran et al., 2017).

Over the last 10-15 years, feed additives and nutritional strategies caught research attention to mitigate or prevent disease (i.e. as possible alternatives to antimicrobials). Usually, those are aiming to reduce pathogen load, or enhance immunity, gut health, digestive function, and set beneficial microbes to ultimately increase performance, but a wide variety of responses are reported (Adewole et al., 2016; Celi et al., 2017; Pluske et al., 2018). Additionally, research has mostly focused on nursery piglets (Wang et al., 2006; de Lange et al., 2010; Che et al., 2011; Liu et al., 2013; Moran et al., 2017), and lesser on older pigs and other critical situations such as gilt acclimation, multiple-vaccinating, or outbreaks (Gabler, 2014; Speiser et al., 2015; Gabler, 2016; Schweer et al., 2018). Some examples of nutritional strategies earlier tested to improve immune response and performance towards systemic infection will be reviewed here.

Soybean and components from soybean have been frequently studied. For example, isoflavones genistein and daidzein were attributed with both positive and negative immune-modulating effects. In vitro, genistein increase natural killer cell activity (Zhang et al., 1999) and antiviral replication and (or) attachment (Yura et al., 1993); but, reduced phagocytosis rates via lessen tyrosine kinase activity (Steele and Brahmi, 1988) and lower T and B lymphocyte production (Chang et al., 1995). Similarly, feeding daidzein to mice, was shown to increase cytotoxic T cell activity, phagocytosis rate in macrophages, and antibody production (Zhang et al., 1997). Feeding genesin concentrations of 200 to 400 ppm to young pigs resulted an active immune modulator that enhanced systemic serum virus elimination and body growth in PRRSv challenged pigs (Greiner et al., 2001a), whilst the same doses of daidzein only enhanced growth weakly (Greiner et al., 2001b). Increasing dietary soybean meal (SBM) concentration from 21% to 32% in finisher pigs (Johnston et al., 2010) and from 12.5% to 22.5% in nursery pigs (Rocha et al. 2013), improved growth in PRRSv infected pigs. Similarly, Rochell et al. (2015), found that a 17 to 29% increase enhanced growth, immune response, and reduced viral load of pigs under PRRS. Whereas more recently, none of these effects were observed when increasing SBM from 10 to 30% in growing pigs (Schweer et al., 2018). Such controversy could be attributed to genetic variation of PRRSv (Kappes and Faaberg, 2015) and (or) genetic background and age of pigs (Lunney et al., 2010; Islam et al., 2013), which highly influence PRRS outcome.

Plant extracts, are secondary plant metabolites with some degree of antimicrobial (Sökmen et al., 2004), anti-inflammatory (Sosa et al., 2005; Liu et al., 2012), and anti-viral (Sökmen et al., 2004) properties. Those have potential for improving pig health and performance as shown via increasing determinant gene expression for gut mucosal immunity and gut barrier function (Manzanilla et al., 2004; Allan and Bilkei, 2005; Liu et al., 2013). Some examples are capsicum oleoresin, garlic botanical, and turmeric oleoresin (Liu et al., 2013). In a PRRSv infection, plant extracts were shown to delay fever and some cytokine changes over time post-infection, improve immune responses, and growth efficiency (only for turmeric oleoresin extract) but without affecting growth (Liu et al., 2013). This, suggests difficulties to influence performance outcome after infection even when nutritional strategies are capable of modulating immunity.

Vitamin E, a major lipid-soluble antioxidant, has been notably studied due to its essential properties preventing lipid peroxidation of cell membranes and oxidative stress (Toepfer-Berg et al., 2004). Antioxidant effect is highly important in immune cell function such as for leukocytes, where polyunsaturated fatty acids are threaten for oxidative damage (Coquette et al., 1986). Han and Meydani (2000), observed that viral titers of influenza, virus load in lung, as well as IL1β and TNFα secretion were reduced in mice that were dietary supplemented vitamin E (500 mg/kg) compared with a control (30 mg/kg). Nonetheless, vitamin E, did not reduce morbidity nor enhanced growth during acute infection of PRRSv (Toepfer-Berg et al., 2004). Vitamin A, with recognized role for mucosa immune capacity and participating in wide variety of roles of innate and adaptive immune responses may also provide advantage under infection (Kunisawa and Kiyono, 2013). Retinoic acid, metabolite from vitamin A can produce anti-inflammatory effect via inhibiting cyclooxygenase 2 (Villamor and Fawzi, 2005); but its potential has not been studied in pig diseases.

Omega-3 polyunsaturated fatty acids are reported to improve immune cells functionality and reduce cellular inflammation (Liu et al., 2003; Calder, 2008; Li et al., 2014). Further, these polyunsaturated fatty acids modify the fatty acids composition of immune cells and enhance phagocytosis capacity (Calder et al., 2008). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from omega-3 are acknowledged to decrease inflammation; but may also reduce T cell signaling and antigen presentation (Sanderson et al., 1997; Shaikh and Edidin, 2007). A 5% substitution of corn oil for fish oil (rich in omega-3) in weanling piglets, showed a tendency to compensate gain after a lipopolysaccharide (LPS) challenge in pigs (Gaines et al., 2003). In another study, 7% substitution was shown to reduce cellular inflammation and improve performance under LPS challenge (Liu et al., 2003). Similar high levels of omega-3 (10% fish oil) improved ADFI and ADG in a model of vascular graft infection with Staphylococcus aureus (Langerhuus et al., 2012).

Amino acids are also important for immune system. Threonine (Thr) is a major component in g-globulin; and similarly, it is suggested that tryptophan (Trp) demand increases during inflammation (Cuaron et al., 1984; Li et al., 2007; Le Floc'h et al., 2008; Le Floc'h et al., 2009). In combination Thr and Trp were used in a PRRSv live vaccine challenge (Xu et al., 2015), which increasing ratios to 0.26 Trp:Lys and 0.80 Thr:Lys reduced lung damage and tended to have higher growth performance than using NRC (1998) recommendations. In their study, Xu et al. (2015), observed increased gene expression of Toll-like receptor 3 and 7, which are potent inducers of innate immunity against viral infections.

Altogether, data suggest that there is some chance for nutritionally enhance immune status of animals. Nonetheless, to reinforce immune response and cause an earlier elimination of pathogen, reduce pathogen load, and minimize symptoms (such reduce performance loss) seems contradictory in some cases and with a poor response in most published data. The role of nutrition in immunity needs to be further elucidated. Likely, interactions with other factors as genetic variability of pathogens or animal background, age of the pigs, and concomitant pathogens are important. This debate is not sufficiently addressed for replacement gilts and vaccination, acclimation, and challenges often concentrate in a short period of time before entering the sow herd. Improving immune status during vaccination and minimize these disease consequences on performance is therefore interesting.

1.2.4. Summary from sow replacement

The rearing of gilts and replacement of sows become critical aspects for efficiency and sustainability in the swine industry. Although little data is reported, near 10-15% of gilts are eliminated during rearing. Furthermore, intensive protocols of vaccination and pathogen acclimation are applied, and generally, nutritional factors are not taken into account. On the sow farm, culling will include near 50% of undesired removals which often concentrate in young parities. Furthermore, culling aged sows by routine should be based on actual genetic lag and costs (likely $>7^{th}$ parity). Lameness during rearing and reproductive problems, low productivity, and lameness during productive lifetime are permanent culling causes; although, variability demonstrates that indexes can be improved. Overall, proper planning and breeding targets are the first necessary step for both farm steadiness, and to achieve gilt conditioning targets at first service.

To the date, gilt conditioning (or fitness) to first service is acknowledged as poorly predictive of subsequent lifetime, yet, it is important and not fully elucidated either. A minimum BW seems the most important target. Data demonstrate that any target of BW within 135 and 165 kg at first service may be feasible, although, different degrees of complexity and risks may arise (Table 1.1). Likewise, refinement is probably linked to genetics and other targets (puberty attainment, ADG, age, and dietary strategies) combined with management during the first cycle. Variety of 'right' combinations may exist to enhance prolificacy and lifetime but lack of understanding prevents recommendations. Simplifying, it appears clearer that extreme BW and body condition (low or high) at first service and farrowing increase the risk of problems such as lameness (if high), reproductive failure (both), stillborn piglets (if high), further prolificacy (if lactation BW loss is high), and culling (both).

Therefore, generate noticeable improvements in productivity or longevity from more precise targets at first service seems unreliable without further management considerations. Evidences indicate that amount of protein tissue at farrowing and balance during the first lactation are greatly important, and with recent and future research, those may become feasible targets. Simplifying, BW target at first service should be predictive of minimal BW lost during lactation. Being conservative, the use of targets and feeding protocols that minimize risk of too light or heavy gilts at farrowing should be preferred when a controlled gilt management is not feasible.

Table 1.1. Combination of targets to first service with possible strategies and main reported risks.

	ADG, g/d	**Age, d**	**BW, kg**	**Risks or problems**
Range	550-850	210-250	130-170	
Rigorous controlled targets	700 (=)	235 (=)	145(=)	Difficulty to control variability
Concentrate diet	+	=	+	Overweight, lameness, heat detection
Concentrate diet; BE and AI earlier	+	-	-	Small size, low performance, lameness?
Concentrate diet; BE and + AI earlier	+	-	=	Lameness during rearing?
Concentrate diet; older at AI	+	+	+	Overweight, lameness, heat detection, culling?
Limit FI or diet Lys	-	=	-	Risk of low performance
Limit FI or diet Lys; older at AI	-	+	=	Difficulty to control, culling?
Limit FI or diet Lys; + older at AI	-	+	+	Time, overweight, lameness? culling?
Limit FI or diet Lys; BE and AI earlier	-	-	-	Small size, low performance

Concentrate diet = maximize growth; AI = artificial insemination; BE = boar exposure; FI = feed intake; Lys = lysine.

1.3. Lameness on gilts and sows

Lameness is a clinical manifestation caused by variety of locomotion disorders that are frequently encountered in many production animal species. Generally, lameness condition is characterized by alterations in the normal gait and posture, a reduced mobility, leg weakness, pain, discomfort, and a probable decline of performance (Anil et al., 2005; Anil et al., 2009a; Nalon et al., 2013ab). It is a major welfare concern that causes premature culling and economic loses in the swine industry (Welfare Quality, 2009; Heinonen et al., 2013). The objective of the present section is to present existing information about principal causes of lameness in gilts and sows with a special focus on osteochondrosis (OC) and claw health; two different pathologies indicated as major risks for sow lameness and culling associated to lameness.

1.3.1. Lameness consequences

Early studies from Osborne (1950) and Penny et al (1963) were pioneer on lameness assessments in pigs. In 1993, Dewey et al. stated concerns about the variety of locomotion problems that were causing lameness and culling in sows. Since then, the understanding of lameness causes has greatly increased but it kept being an important problem to the date as reviewed by Heinonen et al. (2013). Premature culling due to lameness ranges from 18 to 73% in rearing gilts (Díaz et al., 2015, 2017), and is near 20% in young sows, and near 10% for overall sow removals (Boyle et al., 1998; Lucia et al., 2000a; Engblom et al., 2007, 2008a; Anil et al., 2009a). The costs of lameness are also important, and were recently estimated between €290 and €330 per affected sow (Niemi et al., 2017). In growing-finishing pigs, lameness appears to be less studied although it was reported as one of the main causes for antibiotic treatment (Christensen et al., 1994). In fact, it is suggested to penalize performance as 25-50 g of ADG, and to cost between €11 and €38 per growing pig (Jensen et al., 2012).

The on-farm prevalence of lameness in sows has been mostly reported between 8.8% and 16.9% (Heinonen et al., 2006; KilBride et al., 2009; Boyle et al., 2010; Jensen et al., 2010; Pluym et al., 2013a), nonetheless, some authors report extreme ranges between 4% and 40% (Willgert et al., 2014). Despite that lameness should be a condition easily visible, such large variability may respond to differences on lameness definition, scorings, and methodology. Otherwise, management practices, environmental conditions and facilities (i.e. flooring), genetics, leg conformation, herd health, nutrition, and mechanical stress, are all suggested to play a role in lameness occurrence. Furthermore, among parities and also across productive phases lameness incidence may change. Pluym et al. (2013a), reported that prevalence was highest (8.1%) when moving sows into the gestation unit than when moving them to the farrowing pens (4.1%) or insemination cages (5.5%); which suggests a clear effect of management and facilities.

The greatest consequence of lameness on productivity is through increasing risk of earlier culling (i.e. 1.7 times greater than non-lame sows; Anil et al., 2009a) and consequently shortening the productive lifetime (i.e. 2.6 vs. 4.0 parities at culling; Pluym et al., 2013a). Otherwise, there exists controversy regarding other consequences. Fitzgerald et al. (2009) observed no effect on litter size, piglets born alive, stillborn, or average birth weight. Conversely, Anil et al. (2009a), described a reduction born alive for lame compared with non-lame sows, while Pluym et al. (2013), reported an increase in mummified fetuses.

Additionally, lameness (Anil et al., 2009a) or heel lesions (Pluym et al. 2013) may affect sow mobility in lactation and increase piglet crushing.

The disorders that originate lameness may occur along pig's productive lifetime at different ages (Figure 1.3). Therefore, causes of lameness have been classified in different manners (i.e. Blowey, 1992; Engblom et al., 2008; Jensen and Toft, 2009; Heinonen et al., 2013). Herein, there will be reviewed only the most frequent disorders affecting gilts and sows. Osteochondrosis and claw lesions will be extensively addressed, whilst other important causes as infectious arthritis, bone pathologies, and physical injuries only overviewed. All these disorders do not have the same prevalence nor imply the same odds of progressing to lameness. For example, Heinonen at al. (2006) described that lameness were caused 49.1% by OC, 14.3% infected wounds, 10.5% claw lesions, 8.8% infectious arthritis, 7.0% infected claw lesions, and 7.0% by overgrown claws.

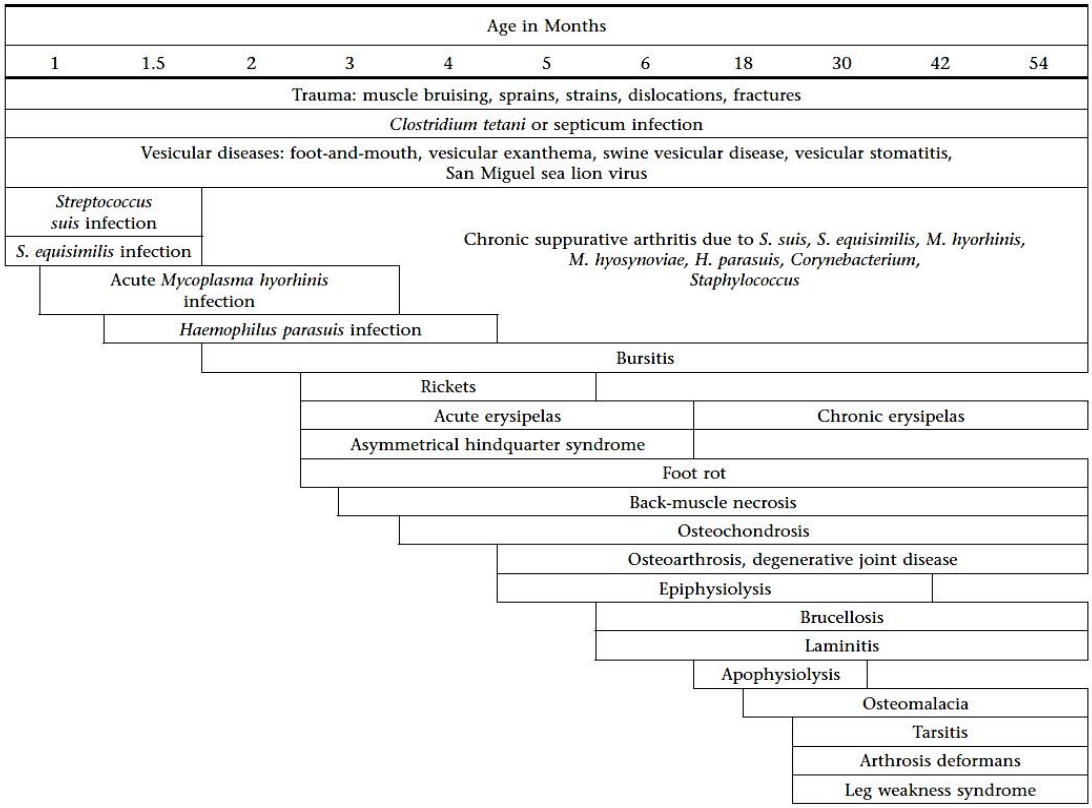

Figure 1.3. Ages of pig's productive life at which diseases and disorders cause of lameness more frequently occur. Source: Straw et al. (2006); Zimmerman et al. (2012).

1.3.2. Osteochondrosis

For over 75 years, OC has been an important focus of researcher's attention in multiple animal species (Thomasen, 1939; Orth, 1999; Ytrehus et al., 2007; McCoy et al., 2013). As described above, OC has been associated as a direct cause of lameness and a frequent finding in culled sows (Heinonen et al., 2006; Ytrehus et al., 2007; Engblom et al., 2008a; Koning, 2015). Prevalence is found high and varies from 41 to 100%, while the prevalence of the more severe cases associated with lameness (OC dissecans, later detailed) is reported from 1 to 21% (Grøndalen & Vangen, 1974; Nakano and Aherne, 1984; Jørgensen,

1995; Jørgensen and Andersen, 2000; Ytrehus et al., 2004; van Grevenhof et al., 2011; Aasmundstad et al., 2013; Olstad et al., 2014; Stavrakakis et al., 2014; de Koning, 2015).

Osteochondrosis is a noninfectious degenerative condition of the physeal and epiphyseal growth cartilage seen in growing animals. Earlier, various nomenclature was used to define similar non-traumatic and non-inflammatory joint lesions. Current consensus for OC consists of a group of syndromes caused by vascular failure that leads to a focal impaired of endochondral ossification (EO); which occurs in the physeal cartilage and the articular-epiphyseal cartilage complex (AECC, Zimmerman et al., 2012; McCoy et al., 2013; Olstad et al., 2015). Additionally, although OC appears as focal lesions, it occurs multifocal at predilection sites for over 50% of the cases (Jorgensen et al., 1995). An overview of EO, pathogenesis, and different factors typically speculated to interact with OC will be presented.

1.3.2.1. Endochondral ossification

Mature bone tissue has an organic and an inorganic extracellular matrix, the first provides flexibility and elasticity, whereas the second does strength and rigidity (Clarke, 2008). During growth, bones include specialized growth cartilage instead of only bone tissue. This cartilage grows and undergo EO. Briefly, EO is a process in which animals replace specialized cartilage for bone. In long bones, this process happens in the metaphyseal growth plate (between the metaphysis and epiphysis) of both bone ends and in the AECC of the epiphyses; which results with bone elongation (Ytrehus et al., 2007; Koning, 2015; Figure 1.4). The ossification progression advances faster than the cartilage production which eventually causes the complete transformation to bone and leaving only the articular cartilage (Ytrehus et al., 2007).

Summarizing as Orth (1999) and Myllyharju (2014), the chondrocytes in the growth plate and AECC are initially recruited from the resting zone to the proliferative zone, then undergo hypertrophy differentiation, and finally apoptosis (Figure 1.4). Within this environment, epiphyseal arteries provide nutrients, oxygen, and growth factors to enhance proliferate the chondrocytes. Those, also produce the extracellular matrix, which is mainly constituted by macromolecules such as collagen Types II, IX, and XI and proteoglycan (i.e. aggrecan). Type II is the major collagen in the extracellular matrix and forms thick (40 nm) and thin (16 nm) fibrils (Kenne et al., 1995, Kadler et al., 2008). Collagen XI is found bind to type II and is thought to be involved with fibril diameter (Kenne et al., 1995, Kadler et al., 2008). Collagen IX is associated with II and XI types by the surface and interact with other components in the matrix. Aggrecan, is the main proteoglycan in the cartilage, has aggregating function, and include chondroitin sulfate with keratan sulfate chains attached. Many aggrecans bind to hyaluronic acid forming high molecular weight complexes that include negative charged high density and retain water osmotically, which provides high biomechanical endurance to the cartilage. There are other components in the extracellular matrix with acknowledged important functions i.e. interact with growth factors and angiogenesis (i.e. Perlecan; Kruegel and Miosge, 2010), or agregant adaptors for the matrix assembly (i.e. martilin types 1 and 3; Klatt et al., 2011). Although, further details are beyond the scope herein.

Advancing the ossification front, chondrocytes differentiate to hypertrophic and interact with subchondral bone. They express several new proteins including cartilage matrix

protein and fibronectin, which role in the EO is not well understood. Also, produce collagen type X, which participates in both, matrix mineralization and degradation, and influences the architecture of trabecular bone near the subchondral bone (see above mentioned authors). Furthermore, hypertrophic chondrocytes express numerous proteins associated with the mineralization expansion (Johansson et al., 1997), such as osteocalcin, osteopontin, bone sialoprotein, *alkaline phosphatase* (*ALP*), and proteolytic enzymes (i.e. Calpain II). Some vitamins and minerals can impact chondrocyte differentiation and will be further discussed (i.e. vitamin A and D, Ca, P, Cu, Zn, etc.). Finally, chondrocytes undergo apoptosis adjacent to the chondro-osseous junction, while bone vascularization and mineralization occurs.

1.3.2.2. Osteochondrosis pathogenesis

Earlier, several etiological factors such as hormonal imbalance, genotype, diet composition, growth rate, and mechanical stress were speculated as potential responsible of OC (Ythreus, et al., 2007). Current evidences and consensus point out vascular failure as the initiating cause. A damage located near the ossification front is followed by ischemic chondronecrosis at intermediate depth of the growth cartilage (reviews by Ytrehus et al., 2007; McCoy et al., 2013; Olstad et al., 2015). The disease is initially subclinical and appears about 12 wk of age although can be seen already the first week of life (Ytrehus et al., 2004; Van Grevenhof et al., 2012; McCoy et al., 2013; Tóth et al., 2016). Such an early occurrence of lesions in pig's life suggests that weight is not likely the initial cause, although, it may contribute to aggravate its progression.

These previous authors state that numerous factors may direct to vascular disruption including bacteremia, fragility of cartilage matrix and collagen structures, and deposits of immature bone. It could be that matrix structures (i.e. collagen type II) near the ossification front fail to provide optimal support (Laverty and Girard, 2013). This would probably affect all cartilage in general but, Hellings et al. (2016) demonstrated that surrounding of some cartilage canal vessels were surrounded by different type of collagen (type I and type II) in horses. These differences around canal vessels may result with different vulnerability to eventually collapse. Recently, Finnøy et al. (2017) tested a novel technique based in non-linear optical microscopy to obtain information about the collagen in cartilage canals and the cause of vascular failure in pigs. This technique, by photon-excited fluorescence and second harmonic generation presents high resolving for morphological changes at microscopic scale (Finnøy et al., 2016). Authors, supported the hypothesis that cartilage canals of young pigs also vary in their presence of collagen fibres at the canal margin and include variation in fibril organization and calcification of the surrounding cartilage.

Insufficient anastomose between blood vessels from growth cartilage and those in subchondral bone would often present compensatory attempts with intense branching for vascularization near necrotic areas, therefore, lesions of ischemic necrosis can eventually heal (Olstad et al., 2015). Otherwise, such lesions would be followed by irregular ossification, and abnormalities in shape and weaken structures could fracture articular cartilage under mechanical stress and load. Yethrus et al. (2004, 2007) defined this progression of OC in 3 stages (Figure 1.4): OC "latens" (vascular lesions on the epiphyseal cartilage visible only by microscopy), "manifesta" (ischemic lesions accompanied with necrosis and delay in endochondral ossification), and "dissecans" (fissured articular cartilage into underlying

bone); with only the last including clinical signs. Remarkably, healing of lesions is highly evidenced as being >50% about 6 months of age, presumably, if lesions were small and on latens and manifesta stages (Busch and Wachmann, 2011; Van Grevenhof et al., 2012; K. Olstad et al., 2014). The "manifesta" OC would heal through an initial phagocytosis via osteoclast and chondroclast, thereafter, by intramembranous ossification. Otherwise, such lesions can also persist as focal necrotic lesions i.e. subchondral pseudocyst (Ytrehus et al., 2007; Olstad et al., 2015). Most frequently and severely affected joints are the elbow, knee, and hock joints (Grevenhof et al., 2011; Hill et al., 1984). Whilst predilection sites for OC "manifesta" and "dissecans" are the medial humeral condyles and the medial aspect of the femoral condyles (Ytrehus et al., 2007; Hill et al., 1984). Why some lesions and OC locations heal while others have more likelihood to develop to OC "dissecans" needs to be understood.

The earlier introduced factors (i.e. hormonal imbalance, genotype, diet composition, growth rate, and mechanical stress) that were presumably associated with OC etiology, may not be the initial cause of OC but still be relevant. It would be more correctly to readdress such factors as either as possible causes of the initial vascular failure or otherwise as capable of modifying the disease outcome (Olstad et al., 2015). Contradictory results were often reported for some of these factors, perhaps, this is indicative of various interactions or lack of understanding.

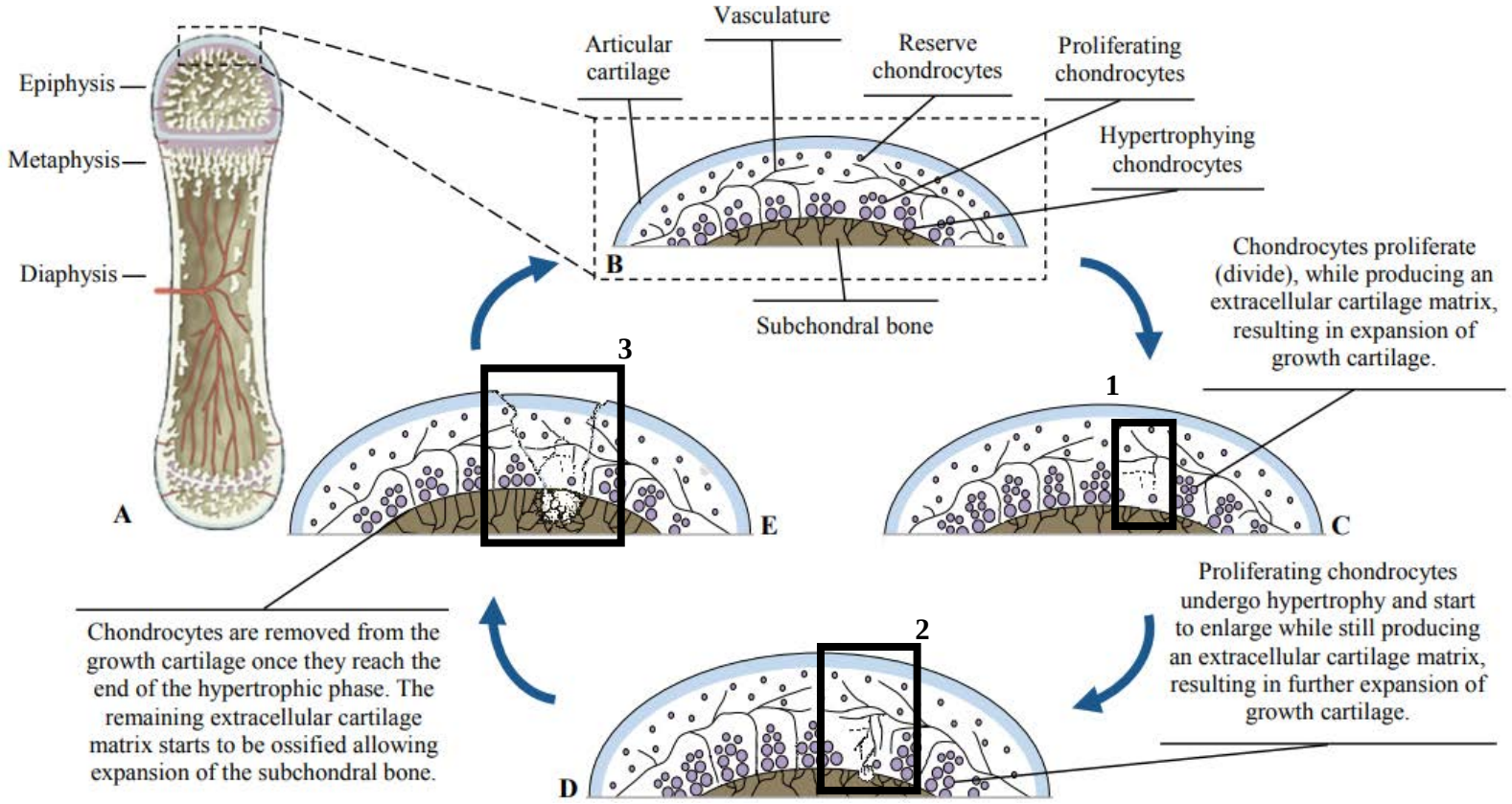

Figure 1.4. Scheme[1] of endochondral ossification and pathogenesis of oestochondrosis progression (not illustrated to scale) in the epiphyseal growth cartilage below the articular surface in long bones (**A**). Epiphyseal growth consists of chondrocytes at different zones (reserve, proliferating, and hypertrophying) nourished by vessels residing in cartilage canals (**B**). Growth is attained by proliferation (**C**) and hypertrophy (**D**) of chondrocytes which produce the extracellular cartilage matrix. Between hypertrophic and subchondral zones, chondrocytes are removed and the remaining extracellular matrix is eventually ossified (**E**). This process repeats itself in young growing animals. Within frames OC pathogenesis is described as Ytrehus et al. (2007). A focal interruption of cartilage vessels may cause ischemic necrosis of the cartilage they supply (osteochondrosis latens; framed **1**). A small defect may resolve, but large defects will resist vascularization and ossification and persist as necrotic cartilage while the ossification front advances around it (osteochondrosis manifesta, framed **2**). Lesions may become totally surrounded by bone tissue and gradually replaced by fibrous tissue, which may later undergo membranous ossification. Yet, the overlying articular cartilage may eventually rupture (osteochondrosis dissecans lesion, framed **3**). [1]Figure adapted from de Koning (2015) which in turn modified **A** from van Weeren (2006).

1.3.2.3. Inhered factors

Pig domestication and modern genetic selection likely diminishing selective pressure for leg robustness while focusing mainly on productive features (Etterlin, 2016). Indeed, articular OC in wild boars is reported inexistent or marginal compared with domestic pigs (i.e. 13% vs. 100% in talus, respectively, Etterlin, 2016; Etterlin et al., 2017). Conversely, increased growth rate capacity (Aasmundstad et al., 2013) and lean characteristics (Jørgensen and Andersen, 2000; Kadarmideen et al., 2004) of modern pigs have shown phenotypic and genetic negative associations with OC. Heritability of OC wide ranges from low to moderate (0.08 to 0.50) among studies (Lundeheim, 1987; Stern et al., 1995; Jørgensen and Andersen, 2000; Yazdi et al., 2000). Most authors reported similar values between dam-lines whilst others found inconsistent heritability (Yazdi et al., 2000) or OC prevalence (Jørgensen and Andersen, 2000; Kadarmideen et al., 2004), which suggests differences among pig populations. Furthermore, specifoc joint OC shown lower heritability values than scoring a sum of joints as a trait, therefore indicating both, heritable predilection and probable interactions with other factors. Altogether, data suggests that is feasible to select pigs against OC while previous selection probably indorsed the contrary. Such evidences are important results that should be considered in genetic selection programs, especially for the dam-lines.

1.3.2.4. Hormones and biomarkers

Most evidences to date (Yakar et al., 2018), recognize the important role of growth hormone and growth factors in the differentiation and maturation of chondrocytes. Works in horses have shown that higher levels of glucose, insulin, and insulin-like growth factor 1 (IGF-1) were related with increased prevalence of OC (Semevolos et al., 2001; Verwilghen et al., 2009). Conversely, lower levels of IGF-1 had also been associated with higher prevalence of OC (Oldruitenborgh-Oosterbaan et al., 1999). These above, although important and hypothetical influencing OC, are factors not contemplated in recent research. Perhaps, because their presence in the blood stream is linked to many functions and variables that complicate the assessment.

Otherwise, being OC affected by genetic variability, there may be chance to detect animals suffering of OC by assessments of biomarker proteins, which result from gene expression. Biomarkers of bone and cartilage turnover have shown prospective potential to detect cases of sever OC (Frantz et al., 2010). These, are protein metabolism products result from such tissues synthesis or degradation that are associated with the loss of homeostasis during the disease (Garnero et al., 2000; Garvican et al., 2010). In 5- to 6-month age gilts, relevant predictive use was found for increasing cartilage synthesis biomarker (i.e. C-propeptide of type II collagen), likely increased as an attempt to promote repair (Frantz et al., 2010). Nonetheless, biomarkers of cartilage turnover appear to depend on stage of the disease and corresponding severity. Variability amongst studies is observed, but in general, OC severity was related with biomarker concentrations of decreased bone formation and increased cartilage synthesis (Billinghurst et al., 2004; Frantz et al., 2010). Development of OC seems highly dynamic with simultaneously developing and resolving lesions, therefore more research is required to test and refine predictability of biomarkers over time.

1.3.2.5. Conformation

Anatomical conformation of the legs and joints is suggested to be crucial to tolerate weight load and mechanical stresses on the joint surface (Ytrehus et al., 2007; Stavrakakis, 2014; De Koning et al., 2015). Although conformation and OC were found to correlate, no consistent associations for specific conformations were found via visual assessed (Koning et al., 2015). Stavrakakis (2014), used biomechanical quantitative measurements to assess joint and stride kinematics, which improve sensibility when compared with visual methods. Previous author reported that conformation and gait abnormalities had differential joint flexion and symmetry (left vs. right flexion) than sound animals; therefore, mainly affecting the swing phase of the gait cycle (strides). Otherwise, pigs with OC lesions had increased angular changes during the stance phase (weight-bearing) and also asymmetry (Khumsap et al., 2010; Stavrakakis, 2014). Ideally, such gait variables could be used to detect pigs with joint lesions that do not display lameness or leg weakness.

Despite biomechanical methods seem more accurate, variability is observed in both type of systems. Often, OC is found without any conformational deficiency or stiffness (i.e. 29%; Stavrakakis, 2014), or lameness (i.e. 38%; Engblom et al., 2008a); while sometimes, severe lesions can be observed without signs of lameness (Crenshaw et al., 2013; Etterlin et al., 2015) or conformation deficiencies (Stavrakakis, 2014). In other words, OC and conformation deficiency may cause locomotion problems independently or together, whereas both may be present but cause no problems. Additionally, complexity and high technical degree are included with objectively measures of gait and conformation, which complicates on farm applicability. Altogether, to determine the precise contribution from unfavorable conformation on OC is therefore difficult. Nonetheless, this approach and methodology may still result feasible in genetic selection programs and research as conformation traits are heritable (Aasmundstad et al., 2014; Le et al., 2016; Stock et al., 2017). Likely, more efforts to elucidate the contribution of conformation deficiencies to OC and to reduce conformation deficiencies in modern genetic-lines should be in collaboration with genetic selection.

1.3.2.6. Mechanical stress

Historically, sufficient evidences support that mechanical stress or trauma influence OC outcome (Orth et al., 1999; Ythreus et al., 2007; McCoy et al., 2013). Ythreus et al. (2007) stated that the trauma required to precipitate OC dissecans from subclinical manifesta lesions was minimal, often including normal physiological forces. Similarly, mechanical stress early in pig's life (29 kg BW) increased OC "dissecans" at slaughter (Nakano and Aherne, 1988), yet, the role of such trauma on initiating OC seems poorly understood. However, the hypothetical effect on initiating the vascular failure seems less plausible yet not discarded (Olstad et al., 2015). Indeed, the contribution of mechanical stress is suggested to vary between OC stages (Olstad et al., 2014). Olstad et al. (2015), stated that for trauma to be the initial responsible of OC, this, had to be capable of causing vascular failure within a single cartilage canal while leaving vessels in immediately adjacent canals intact and no other sign of previous traumatic lesion. Although, this could not be totally discarded because ossification front advances rapidly and may obscure structural changes soon after occurrence of lesions.

In practical terms, environmental factors such as housing system and the type of floor would mean differences in mechanical stress. Some authors found effects of housing on OC (van Grevenhof et al., 2011; Etterlin et al., 2014, 2015) whilst others did not (Jorgensen, 2003; Scott et al., 2006). Presumably, risks for traumatic events to occur and their influence on joint loading and OC, could be higher using a concrete-slatted floor (likely more slippery) than if pigs were penned using a floor with some type of bedding (Koning, 2015). Additionally, this may be age-dependent if vulnerability is different throughout pigs' life, and if high BW load is assumed as increasing risk for OC or to progress to sever OC. Koning et al. (2014), performed a factorial design with growing gilts switching at 10 wk of age conventional concrete-slatted floor vs. wood shavings (as deep bedding) or not. Contrarily, wood shavings floor increased the odds for severe OC in the long term, and age effects were not observed. Authors speculated that results could be related with increased activity. In fact, the type of floor can modify behavior and increase activity of pigs (Scott et al., 2006; Grevenhof et al., 2011). In pigs, free range vs. confined had more OC prevalence, and increased severity but not lameness; which is contrariwise (Etterlin et al., 2015). Authors suggested that strengthening of joint supportive tissue and pain relief were promoted by exercise. To the date, strategies on type of floor and OC are uncertain and contradictory, which may be further obscured by the fact that repeatedly, severe OC lesions were observed without visual impairments on gait indicating great resilience to pain (Etterlin et al., 2014, 2015, Koning et al., 2015).

1.3.2.7. Growth rate

A rapid growth rate would lead to increased weight bearing at the joint level earlier in gilt development than a lower growth rate. Joint loading and trauma were previously associated with increased risk to affect OC outcome. Whether this only relates to increasing risk of occurring cartilage fissures near necrotic lesions (severe OC outcome), or also affect the initial vascular lesion cause of OC is unclear (Nakano and Aherne, 1988; Ytrehus et al., 2004; Olstad et al., 2015). Furthermore, pressure on joints and frequency of impacts can alter chondrocytes function i.e. their survivability or reparative capability (Quinn et al., 1999; Ewers et al., 2001; Davisson et al., 2002; Stokes et al., 2007).

In practical terms, growth rate and weight bearing could be modulated with dietary density and (or) feed intake level. Earlier works, studied lowering of nutrient density in the diet (Nakano et al., 1984; Woodard et al., 1987) and restricted intake (Nakano et al., 1979) which did not affect prevalence and severity of OC at slaughter. More recently, some authors related growth rate with increasing risk of OC (Busch and Wachmann, 2011; Grevenhof et al., 2012; Quinn et al., 2015) but others could not find this relationship (Ytrehus et al., 2004, 2007; Quinn et al., 2015; Tóth et al., 2016). Grevenhof et al. (2012) associated increasing growth rate with OC, but this was reported controversial among joints. Gilts with lesions in elbow had higher BW than the average, meanwhile, those with lesions in femoropatellar joint had lower BW. Such controversial results may imply that other factors are relevant, for example age. Feeding ad libitum between 10 and 26 wk of age was shown to increase risk of OC in gilts when compared with feeding initially ad libitum (4 to 10 wk) followed by restricted allowance (10 to 26 wk), or restricted throughout (Koning et al., 2014). Authors, also reported that ad libitum throughout had higher odds of being affected by OC than gilts

fed initially at libitum (4 to 10 wk) and following by restriction (10 to 26 wk). Adding complexity, age interaction may be important early in OC development but also later when clinical signs may appear and obscure the initial relationship. Indeed, pain and discomfort are known to weaken feeding behavior and reduce ADG (Weary et al., 2009), and even before lameness, OC may lower ADFI by 25% (Munsterhjelm et al., 2015).

As earlier discussed, OC is heritable and there exists a genetic correlation between growth rate and OC. Furthermore, BW load and mechanical stress may increase risk of OC. Nonetheless, none of such statements attribute more importance to one than another, and both factors may be similarly associated by genetics and therefore confounding.

1.3.2.8. Diet composition and bone development

It is acknowledged that some nutrients are essential for bone and cartilage development (Riet et al., 2013). Nonetheless, most to date research agrees that under dietary sufficiency the diet intervention on OC is likely limited (Olstad et al., 2015), hence, diet changes seem not the best approach to reduce OC prevalence. Indeed, as OC has been determined heritable, genetic selection against OC should be the priority for reducing population prevalence. This may appear difficult, at least initially, because growth rate and lean characteristics were also associated with OC (Jørgensen and Andersen, 2000; Kadarmideen et al., 2004; Aasmundstad et al., 2013), while are primary desired traits in genetic selection. Meanwhile, factors that affect OC outcome through healing or disease progression are less understood, and nutrients with important roles on bone and cartilage development may be relevant in this sense.

Bone structure, composition, and content have been documented to vary according to nutrition (Riet et al., 2013), but the degree of nutrition importance on bone pathological processes is more controversial and likely related to deficiencies or toxicity. In general terms, the diet composition could be relevant to some extent; i.e. high content of fiber or protein may interact with calcium (Ca) absorption, or i.e. amino acids are highly important for the synthesis of the extracellular matrix (Heaney and Layman, 2008). Similarly, high levels of saturated vs. polyunsaturated fatty acids may decrease bone quality (Watkins et al., 2003), yet fish oil did not improve OC severity (Frantz et al., 2008). General composition of the diet has not been investigated towards OC and itself seems not the most relevant factor on a typical scenario conditions. Otherwise, diet components with most likely intervention on cartilage and bone development are minerals, micro-minerals, or some functional components (i.e. vitamins). These, including Ca, phosphorus (P), or micro minerals, as well as the use functional additives have a practical implication for bone development in conventional swine diets and their roles are discussed below.

Minerals

By mid-20th century, researchers using purified diets were evidencing the essentiality of minerals, and in 1981, 22 mineral elements were believed to be essential (with proven metabolic roles) for animal life (Underwood and Suttle, 1999). Seven major or macronutrient minerals (Ca, P, potassium, sodium, chlorine, magnesium, and sulphur) and 15 trace or micronutrient mineral elements (iron, iodine, zinc, copper, manganese, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon, nickel, and arsenic). Minerals perform

4 broad types of functions: structural, physiological, catalytic, and regulatory. All can be relevant in bone development and the most important will be discussed.

Calcium and P, are notoriously important for bone development and are homeostatically regulated together. In the diet, they are leveled as Ca:P ratio with a minimum of 1:1 required, which actually is variable according to P level, i.e. 1.25:1 when P is at NRC (2012) requirement level (50 to 80 kg BW; Lagos et al., 2018). Lagos et al. (2018) also determined that needs of Ca were higher to maximize bone mineralization than to maximize growth (still <1.35:1 when P was at requirement level). The deficiency of Ca weakens the growth plate and causes that osteoid (unmineralised organic bone matrix) is not produced normally or becomes replaced by fibrous tissue. If prolonged, total content of bone minerals is reduced and lameness may occur (Underwood and Suttle, 1999). Under P deficiency, pigs reduce growth rate, and in the bone, osteoid formation lacks mineralization (Underwood and Suttle, 1999). Dietary calcium availability is usually high whereas for P varies greatly among sources; which is the main reason for worldwide adding phytase to animal diets while reducing costs and P environmental impact. Altogether, Ca:P imbalances affect bone development but there are no evidences proving interaction with OC (Ythreus et al., 2007).

Sulphur (S), is necessary for binding proteoglycan and hyaluronic acid, and therefore, to provide adequate absorptive capacity to the cartilage (Myllyharju, 2014). Therapeutic effects have been attributed to dietary sulphur via fulfilling extracellular needs (Cordoba and Nimni, 2003; Ouattara et al., 2016). Methionine is the primary dietary source of S and was shown to reduce OC severity when supplemented to gilts (Frantz et al., 2008). Indeed, Huang et al. (2014) reported that lowering the Met intake delayed bone differentiation in rats. Other important minerals are sodium (Na) and potassium (K), which are necessary for electrolyte balance and cell transportation. Such balance is essential for intestinal absorption of minerals, but also for protein transports between tissue compartments (i.e. bone, Underwood and Suttle, 1999). Although, no effects were reported on bone metabolism and mineralization for Na and K supplementation (Budde and Crenshaw, 2003). Conversely, magnesium (Mg) is important to activate vitamin D into form 1,25-(OH)2D3, and therefore, required for Ca absorption. Magnesium also mediates with calcitonin, parathyroid hormone, and when lacking, has shown a reduction on oxidative phosphorylation causing bone resorption (Manicourt et al., 1981). Furthermore, Mg has been suggested to enhance OC healing. Counotte et al. (2014), reported 21.9% OC in growing foals supplementing Mg (4 g/d) up to 12 months of age, whilst the control group had 41.9% OC. In a second experiment in the same study, authors diagnosed OC by radiology image. Using the same daily supplement of Mg reduced 14.3% OC between 5 and 12 months of age whereas the percentage of affected animals did not change for non-supplemented foals.

Trace minerals (TM) have variety of important physiological functions and are recognized as essential dietary components (Underwood and Suttle, 1999). Therefore, recommendations of daily requirements are reported for most of them by the principal entities providing requirement tables and information for feeding pigs worldwide. Conversely, such recommendations may be notorious different (Table 1.2.) between needs for growth and production (NRC, 2012), compared with recommended levels as a compilation of practical experience, requirements, and regulations (FEDNA, 2013). This suggests that even TM importance is clear, various views may coexist. In part, this could be attributed to the fact that

requirements are based on performance research while practical believes may add concerns on interactions amongst feed additives, or other assumptions (i.e. maximize development of bone and horn tissues). Nonetheless, research is lacking and often contradictory. Variety in the proportion and type of feed ingredients typically included in the diets among regions and countries would also explain some differences.

Requirements of TM have been discussed. Data in growing-finishing pigs suggest that ingredient contribution allow to reduce TM supplementation when response variable is growth performance (Creech et al., 2004; Shelton et al., 2004; Gowanlock et al., 2013; Ma et al., 2018). Whilst looking at mineral status variables, data suggest to do not reduce or remove TM supplements (Shelton et al., 2004; Veum et al., 2009; Ma et al., 2018). Some authors suggest that TM requirements for proper development of structural tissues (i.e. bone, joints, and claws) are not clear and may be higher than for growth (i.e. in turkey, Ferket et al., 2009; and pigs, Barneveld and Vandepeer, 2008). Conversely, sows of different parity fed conventional levels of TM during productive life, shown no differences in TM status and OC at culling (Crenshaw et al., 2013). For one reason or another, the levels usually used in commercial conditions are higher than those reported as requirements for growth.

Zinc (Zn) is directly related with the quality of the extracellular matrix and bone development by mediating with several of its components (i.e. osteoblast) or as cofactor for metalloproteinases and growth factors (Matsui and Yamaguchi, 1995; Ovesen et al., 2009). Bone ash and strength linearly increase with increased dietary Zn to NRC (1998) requirements (Veum et al., 2009). Zn is also, known to influence *ALP* which regulates mineral deposition (Clarke, 2008). Revy et al. (2004), observed that additional Zn or phytase increased *ALP*; whilst phytase increased serum and metacarpal Zn, bone strength, and ash content. This, suggests a positive effect on bone development when available Zn is increased, but whether dietary levels above growth requirement provide benefits on OC development is not evidenced. Similarly, manganese (Mn) is involved in bone metabolism and cartilage formation by participating in the synthesis of proteoglycans (Tomlinson et al., 2004). Both, too low and too high dietary Mn are undesired, deficiency shorten bones and may cause lameness whilst toxicity can cause stiffness and stilted gait (McDowell, 2003). Through the Mn-superoxide dismutase, Mn also has a regulatory function with the synthesis-degradation balance of cartilage (Koike et al., 2013). Silicon (Si) is another important mineral required for maximizing the synthesis of hydroxyproline and necessary in the formation of collagen and proteoglycans (Carlise, 1981). Si deficiency reduced collagen formation in rats (Seaborn and Nielsen, 2002), whilst supplemented it in pigs (1,000 mg Si/kg of diet) reduced OC severity score (Frantz et al., 2008).

Table 1.2. Recommended dietary and daily allowances for iron, zinc, copper, and manganese depending on body weight and productive stage of swine.

	Iron			Zinc			Copper			Manganese		
	NRC, 2012		FEDNA,[1] 2013	NRC, 2012		FEDNA, 2013	NRC, 2012		FEDNA, 2013	NRC, 2012		FEDNA, 2013
Body weight range, kg	mg/kg	mg/d	mg/kg	mg/kg	mg/d	mg/kg	mg/kg	mg/d	mg/kg	mg/kg	mg/d	mg/kg
5-7	100	26.6	90 (80-125)	100	26.6	120 (100-130)	6	1.60	120 (100-130)	4	1.1	120 (100-130)
7-11	100	46.8		100	46.8		6	2.80		4	1.9	
11-25	100	90.5	75(70-100)	80	72.4	110(110-120)	5	4.53	110(110-120)	3	2.7	110(110-120)
25-50	60	90.2		60	90.2		4	6.01		2	3.0	
50-75	50	105.9	50(50-90)	50	105.9	80(90-110)	3.5	7.41	80(90-110)	2	4.2	80(90-110)
75-100	40	100.2		50	125.3		5	7.52		2	5.0	
100-135	40	111.5		50	139.4		3	8.36		2	4.6	
Gestating sow	80	168.0	75(60-95)	100	210.0	100(95-120)	10	21.0	11(10-15)	25	52.5	40(40-50)
Lactating sows	80	477.3	100(95-120)	100	596.6	100(95-120)	20	119.3	11(10-15)	25	149.2	40(40-50)
Mature boars	80	190.0	60(60-90)	50	118.8	120(95-140)	20	190.0	10(8-15)	20	47.5	35(25-50)

[1] Recommended (range).

The most discussed TM related with OC is copper (Cu), which catalyzes the Cu-dependent lysyl oxidase and provides cartilage strength through collagen crosslinks (Bridges et al., 1984). Knight et al. (1990) and Hurtig et al. (1993) described a reduced severity of OC in horse foals that had been supplemented with Cu. Furthermore, failure of bone deposition on the cartilage matrix has also been documented in Cu deficiency (McDowell, 2003). Similar data, shown that supplementing Cu (250 mg/kg) and Mn (100 mg/kg) combined improved OC severity scores and cartilage biomechanical properties evaluated on ~130 kg BW gilts (Frantz et al., 2008). Recently, growth cartilage, bone geometry, and bone mineral content worsened in broiler supplemented only 25% of Cu NRC (1994) requirements (Muszyński et al., 2018). Conversely, some studies have shown no positive effects on OC after Cu or other TM dietary supplementation (Ytherus et al., 2007, Tóth et al., 2016). Indeed, Tóth et al. (2016) recently evaluated inorganic sources supplemented with additional organic sources of TM (150 + 50 g/kg Zn, 50 + 20 mg/kg Cu, and 16.5 + 10mg/kg Mn) without apparent effects on OC neither early (12 wk) or later (24 wk) on pigs' life. Whilst reported 100% prevalence at 12 wk, only few cases of sever OC (3 out of 200) were observed at 24 wk of age. They concluded that base-levels used in all their dietary treatments (all above recommendations for growth) produced similar effect on OC development. Therefore, some data supports the importance of Cu whilst other suggest no benefits from supplements above growth requirements. Olstad et al. (2015), whom wrote the most updated review on OC in pigs and horses, questioned:

"Copper deprivation was associated with reduced crosslinking of collagen, but is there any evidence that crosslinking of elastin in blood vessel walls is affected also, resulting in weakening and vascular failure?"

Therefore, having reported conflicting results and lacking some understanding other more practical hypothesis may arise as: a) may the potential of TM supplements depend on other factors as inhered risk of OC or mechanical stress at different life phases (i.e. such as heavy weight or growth rate)?, or otherwise, b) are there in-feed interactions amongst minerals or other dietary components (i.e. antimicrobials, ZnO, phytate, etc.) causing punctual and time-dependent TM deficiencies that affect OC?

Another important fact to note is the presence of interactions among minerals. For example, excessive Zn can displace Cu metallothionein and Cu-dependent enzyme lysyl oxidase (Hill et al., 1983), which may cause abnormal cartilage and bone formation resulting in lameness. Nonetheless, often those interactions need exceptionally high levels i.e. 5000 mg/kg ZnO (Hill et al., 1983). Similarly, iron (Fe) dietary concentration should be maintained at adequate levels according to Zn and Cu. Selenium and Fe, although apparently less relevant in terms of bone development are also important minerals for tissue development.

Other components

Vitamins are organic compounds distinct from amino acids, carbohydrates, and lipids that are required in small concentrations for normal growth and reproduction usually classified as either fat-soluble (A, D, E, and K) or watersoluble (B-vitamins group and vitamin C, NRC, 2012). Some vitamins can be synthesized from other elements in the feed or by microorganisms in the intestinal lumen, therefore, less or not needed in the diet. Vitamins deficiency or toxicity cause variety of negative consequences in pigs and some

influence bone development and metabolism (NRC, 2012). Briefly, vitamins B and more precisely folacin are associated with bone longitudinal growth and deficiency can lead to abnormalities (Riet at al., 2013). Vitamin C, whilst known as necessary for collagen formation and involved in the synthesis of 1,25-(OH)2D3, can be sufficiently synthetized by the pig (NRC, 2012). Most importantly, vitamin D plays an essential role in bone development and metabolism. The primary effect of its deficiency is a reduction on Ca and P availability, followed by disruptions on bone metabolism and development, besides of altering many more physiological functions (Clarke, 2008). Nonetheless, vitamin A, B, C, D, and E supplementation, do not influence OC outcome (Nakano et al., 1987; Ytherus et al., 2007).

Dietary carbohydrates directly influence glucose, insulin, and IGF-1 responses (Brand et al., 1998, 2000) and confounding with increasing BW are suggested to intervene with chondrocytes and OC (Koning et al., 2016). Nonetheless, as earlier discussed, contradictory results and broad spectrum of modulating factors and responses associated with blood levels of these hormones and biomarkers complicate the association with OC. Glucosamine sulphate constitute a part of proteoglycans, and its supplementation or additional glycosaminoglycans (i.e. glucosamine and chondroitin sulfate) have been speculated to enhance proteoglycan formation and therefore enhance cartilage structure and resilience (Neil et al., 2005; Crenshaw, 2006). Additionally, glucosamine and n-3 fatty acids are suggested to reduce cartilage degradation through block matrix metalloproteases, which are known to over-degrade cartilage (Neil et al., 2005; Goggs et al., 2005). However, improving OC effects were not confirmed in gilts (Frantz et al., 2008).

Amino acids such as glycine, lysine (Lys), cysteine, hydroxyproline, and hydroxylysine form part of collagen fibres (Percival, 1997), therefore, if supplemented may maximize its proper production and quality. Frantz et al. (2008) supplemented growing gilts with non-essential amino acids glycine (3:1 to Lys) and proline (2:1 to Lys) and observed a reduced total severity scores compared with a control. They also combined methionine (1.1:1 to Lys) and Thr (1:1 to Lys) which improved total OC score. Another combination tested was a diet with leucine, isoleucine, and valine (at 2:1, 1:1 and 1:1 of Lys, respectively), which itself did not show improvements on OC total severity score. Nonetheless, authors statistically grouped the previous 3 dietary treatments based in amino acids supplementation and applied contrast with grouped control and other dietary treatments. This shown, lessened number of external abnormalities on the articular surface, improved external abnormality severity score, and total OC score. Although the combining effect is confounding, this results suggest a potential effect of amino acids on OC. Authors, attributed the importance to the methionine donator capacity of S and through its role in the proteoglycan formation, as previously discussed.

1.3.2.9. Summary of osteochondrosis

Osteochondoris, a disease reported near 100% prevalence in domestic pigs but minimal in the wild boar. It is a degenerative condition of growth cartilage that disrupts joint development with an important (>50%) healing and a varying outcome. When severe (near 10-15% of cases), causes pain and lameness, although animals can be resilient and hide clinical signs. It is heritable and genetically associated with fast growth rate, meanwhile,

greater BW load during pigs' growth may also affect OC. These two factors confound and the importance for each one should be investigated. Conflicting results and lack of understanding are questioning intervention of nutritional factors on OC, and more research is required to elucidate their role on the vascular disruption as initial cause of OC, the outcome, and severity. Similarly, interactions in commercial practices i.e. amongst feed components should be examined as may alter actual availability of some essential components for bone development and be responsible of inconsistencies. Ultimately, greatest opportunity to reduce OC prevalence seems to be through animal selection, which should enhance growth but also select against OC and leg weakness.

1.3.3. Claw lesions

Pig, are tetradactylous with two central digits responsible for bearing weight, the third digit (medial) and the fourth digit (lateral). Those are relatively larger than the two non-bearing weight accessory digits or dewclaws (second and fifth digits), whilst the first digit is absent. Each digit contains the claw or hoof, which embraces the distal phalanx and the distal parts of the second phalanx within a horn capsule. The capsule consists of four types of horn tissue, soft keratinized tissue for the heel and soft horn in the sole, whilst harder horn tissue makes up the outside wall or hoof (Figure 1.5). The junction between the two latter tissues is the white line and allows flexibility during movement (Nalon et al., 2013a). The level at which the wall encounters the skin is named the coronary band.

Claw health is one important cause of pain, lameness and a reason for culling sows (Heinonen et al., 2013; Pluym et al., 2013). Prevalence of claw lesions in sows is notoriously high, being reported between 59% and 100% (Gjein and Larssen, 1995; Anil et al., 2007; Knauer et al., 2007; KilBride et al., 2009; Pluym et al., 2011, 2013a; Lisgara et al., 2016). Meanwhile, it has been estimated that 5% to 20% of lameness might be due to foot lesions (Dewey et al., 1993; Kirk et al., 2005; Heinonen et al., 2006). There are different types of claw lesion (discussed later), and risk of lameness may be different amongst such types. For instance, lesions in the white line have been documented with higher odds to cause lameness whilst other claw lesions were not significantly associated to lameness (Anil et al., 2007). Conversely, great variability is reported among studies and type of lesion attributing more or less consequences to some claw lesions. From none gait problems (Kroneman et al., 1993; Bradley et al., 2007) or productive consequences (Bradley et al., 2007; Heinonen et al., 2006) for some claw lesions, to some other claw lesions being related with lameness (Gjein and Larssen, 1995; Anil et al., 2007; Pluym et al., 2013ab) or reproductive and productive impairment (Anil et al., 2009a; Bradley et al., 2009; Fitzgerald et al., 2012; Lisgara, 2017). This could be explained through the high incidence of small claw lesions, the degree of severity, the presence of infection, or differences in location (i.e. hind vs. front feet) affecting the likelihood of becoming lame (Gjein and Larssen, 1995; Bonde et al., 2004; KilBride et al., 2009). Furthermore, lameness and claw lesions can be related one with the other inversely. Lame gilts spend more time lying down, and if floor consists of hard surfaces, this would likely increase the risk of wounds and claw lesions (KilBride et al., 2009).

The integrity of the claw depends on the structural strength of its tissues and their junctions to cope with environmental experiences (Anil et al., 2007; Nalon et al., 2013ab).

Occurrence of lesions is suggested to be complex and multifactorial and will be reviewed herein.

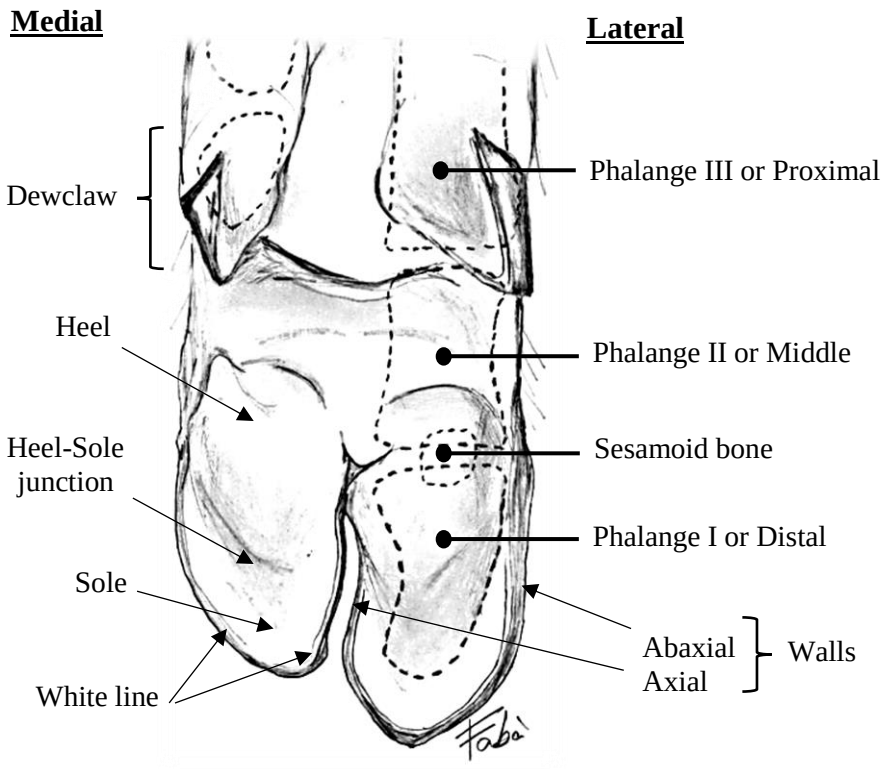

Figure 1.5. Schematic representation of sow foot and claw principal components on a ventral view.

1.3.3.1. Horn formation and pathogenesis

Bone phalange, subcutuaneus, dermis, vascular basement membrane, and epidermis constitute the inside of the claw. The dermis layer, situated underneath the epidermis, contains blood vessels, nerves and the connective tissue to support epidermis (Lisgara, 2017). The distal layers from epidermis, yield the claw from pushing cells to following levels of epidermis proliferation and differentiation. Claw horn formation, described as by Tomlinson et al. (2004) results from proliferation, keratinisation and cornification of keratinocytes in the claw epidermis. Such process includes the synthesis, aggregation, and stabilization of keratin proteins by keratinocytes. These proteins are cross-linked with disulphide bonds and consist of a stable protein complex. During keratinization, the intercellular cementing substance (ICS) is synthetized. The ICS stabilizes cell to cell adhesion with keratin proteins and includes glycoproteins and complex lipids (phospholipids, glycolipids, and acylglycosylceramides). Towards the end of differentiation, basophilic dense keratohyalin granules accumulate in the cells, making up the stratum granulosum (Lisgara, 2017). Following with cornification, death of keratinocyte occurs and keratohyalin granules dissolve to merge with filamentous keratin proteins and cross linkage with ICS (Tomlinson et al., 2004). In time, this process leads to claw growth. Amstel and Doherty (2010), described that growth rates for the inner claws (front and rear) and outer claws was 6.7 and 5.8 mm/month, respectively; which were higher than the overall wear rate of 5.1 mm/month (in gilts 113-150 kg BW in a 3 x 4 m pen including access to concrete walkway.)

Factors such as the biomechanical properties of the different types of horn tissue, the pig weight distribution through the claw, and the characteristics of most commonly used floors lead to high susceptibility for lesions in some anatomical areas of the claw (Anil et al., 2007; Díaz et al., 2013; Nalon et al., 2013a). Most common lesion are overgrowth or erosion (toes, heel), followed by cracks (heel, white line, claw wall), variable proportions of separations and cracks along the heel-sole junction, skin lesions near the claw, excessive (dew) claw length, and dewclaw hoof broken or missing (Anil et al., 2005,2007; Bradley et al., 2007; Pluym et al., 2011). Gjein and Larssen (1995) first describe a scoring and classification system for claw lesion, including six principal lesions and a scoring scale including five degrees of severity. More recently, similar scoring systems were used including more or less lesion types and severity scales of three (Bradley et al., 2007; Foot First Team, 2010) or four levels (Pluym et al., 2011).

Lesions in the heel usually begin as bruising which can be followed by erosion (with hemorrhage underneath) and evolve to ulceration. Similarly, lesions in the sole area frequently start with underneath bruising as areas of hemorrhage dark color, often occurring on sideways of the heel-sole junction. Repetitive trauma can produce hyperkeratinisation, excessive granulation, and necrosis on the heel (Penny et al., 1963). Similarly, erosion and ulceration can leave sole hyperkeratinization as a sequel. Lesions in the withe line usually initiate as wear, break, or separation between hard and soft horn tissues, which may evolve with extensive separation and become severe (Bradley et al, 2007). Such separation easily fills in with dirt material causing infection and inflammation. Cracks on the wall can be vertical (starting at the white line) or horizontal (from the coronary band; Penny et al., 1963). Coronary band may present abscesses and infection of the laminae, and even loss of the horn with extended infection to the dermis.

Claw lesions can be found on young animals (Kilbride et al., 2009). Even though, lesions seem to be more frequently increasing with age and parities (Dewey et al., 1993, Anil et al., 2007; Pluym et al., 2011). Conversely, in another study, 1st and 2nd parity sows had more lesions than superior parity sows (Bradley et al., 2007). Since claw lesions are highly prevalent and represent a reason for culling, the frequency across parities should be taken with caution as culling policies would influence it (Pluym et al., 2011). Claw lesions seem to originate as multifactorial and have been associated with genetics (i.e. small inner claws and abnormal toe angles) related with conformation and location predisposition to lesions (i.e. outer claws of the rear feet), nutritional factors (Riet et al., 2013; Varagka et al., 2016), age (Anil et al., 2007), management and flooring conditions (Ehlorsson et al., 2003; Amstel and Doherty, 2010; Olsson et al., 2016); and will be overviewed herein.

1.3.3.2. Multifactorial occurrence of claw lesion

Nutritional factors

The production of horn is influenced by the supply of nutrients, oxygen, active molecules, and hormones via blood and from the dermis to the avascular epidermis (Mülling et al., 1999; Tomlinson et al., 2004; Riet et al., 2013). If such supply is compromised, the horn could be disturbed and become vulnerable to biomechanical, chemical, or microbial damage (Tomlinson et al., 2004). Nutrient deficiencies may reduce ICS yield or compromise its quality, which would lessen horn production, alter the morphometric characteristics of

horn, and increase the likelihood of cracks and wear (Tomlinson et al., 2004; Lisgara et al., 2016; Varagka et al., 2016). Furthermore, the diffuse supply through the epidermis could be also diminished with tissue compression and overload, especially if damage of the vascular walls has occurred (Muelling, 2009; Vermunt and Greenough, 1995). Other factors associated with diffusion impairment described in cows and horses are metabolic stress, systematic diseases, histamine, lactate, endotoxin, activation of matrix metalloproteinases (MMPs), and vasoactive factors (Muelling, 2009; Pollitt, 2004).

Regarding nutritional factors, the developing keratinocytes may lessen horn production under amino acids shortage i.e. sows in late gestation or lactation because their requirements increase (Tomlinson et al., 2004; NRC, 2012; Riet at al., 2013). Sulphur amino acids are important for horn integrity. Cysteine (Cys) participates with disulphide bonds in the epidermal laminea to form the cellular envelope which offers high cell-wall resistance and rigidity against proteolytic enzymes (Tomlinson et al., 2004). Methionine (Met) and its effects on claws is not studied in sows, whereas contradictory results are described in cows. Vermunt and Greenough, (1995) observed softer horn which attributed to a limiting effect of high Met to Cys concentration. Otherwise, no effects were observed in another study (Galbraith et al., 2006), whereas in vitro, Met shown increasing protein synthesis in epithelial horn-forming cells (Hepburn et al., 2008).

Calcium interacts with horn formation by regulate both keratinisation and cornification via epidermal transglutaminase (TG) activation; which participates on the formation of cross-links between fibers of keratin and the cell wall (Mülling et al., 1999; Tomlinson et al., 2004). One consequence of Ca deficiency is the reduction of TG activity which leads to dyskeratotic horn production (Mülling et al., 1999; Tomlinson et al., 2004). Nonetheless, increasing the dietary intake of both, Ca and P over three parities did not affect the frequency and severity of claw lesion (Arthur et al., 1983).

Trace minerals and vitamins are important in the process of producing horn and preserve keratinized tissues healthy (Riet et al., 2013). Early work in biotin, essential cofactor in a variety of enzyme systems for in mammalian metabolism (Olson and Suttie, 1977), pointed out its importance for claw health (Kornegay, 1986). Some studies shown that biotin supplementation could reduce the prevalence and severity of claw lesions (Penny et al., 1980; Bryant et al., 1985; Greer et al., 1991) and also improve hoof wall hardness and pad resilience (Misir and Blair, 1986; Simmins and Brooks, 1988). Conversely, other authors did not find these benefits (Watkins et al., 1991). Such variability amongst studies can be attributed to variety of factors (i.e. ingredients contribution, supplementing level and duration, bioavailability differences, or other factors affecting claw health), yet to be fully elucidated, biotin is still viewed as important (Riet et al., 2013).

Trace minerals Zn, Cu, and Mn have been acknowledged as necessary in the keratinization process by having catalytic, structural, and regulatory functions in activating enzymes (Mülling et al., 1999; Riet et al., 2013; Lisgara et al., 2016; Varagka et al., 2016). The requirements of TM are based on growth and productivity (Table 1.2), and have been suggested insufficient for proper horn formation (Lisgara et al., 2016; Varagka et al., 2016). The reserves of TM in sows may weaken over several parities (Mahan and Newton, 1995) and this would be aggravated with the increasing level of productivity in modern sows (*1.1.2. Modern pig production*"). Mahan and Newton (1995), described a decreasing TM status in

3rd parity sows of high litter weight at weaning comparing with sows of low litter weight, and both, had lower TM status than none-bred females. More recently, research has focused on the source of TM. Anil et al. (2009b) reported a reduced prevalence of claw lesions and lower severity with a partial substitution of Zn, Cu, and Mn from inorganic to organic (chelated to an amino acid) sources. Similarly, two recent studies from Lisgara et al. (2016) and Vergaka et al. (2016) presented similar results with a partial substitution of inorganic sources with organic, as Zn (45 of 125 mg/kg), Cu (14 of 15 mg/kg), and Mn (25 of 40 mg/kg). Additionally, Vergaka et al. (2016) observed that under organic sources, females were more likely to have none or less histologic changes than those with inorganic. Whilst the most frequently observed histological change regardless of TM source was lamellar hyperplasia in the epidermis of the claws. Further, horn density was determined higher, and vertical and horizontal diameters of the horn tubules were smaller within hoof horn of sows fed organic TM compared with those fed inorganic TM diet; a comparative advantage (Vergaka et al., 2016).

Therefore, there could be a limiting threshold level for supplementing TM and optimizing TM stores in sow diets depending on the TM source. Briefly, current supplements of TM with premix for swine diets include inorganic salts (typically oxides or sulphates). In the stomach and gastrointestinal tract, these salts break down and free the ions. This allows either to continue as soluble or to interact with ligands and bind them to insoluble and non-retainable chelates like with phytate. This would result with less available TM and P while increasing fecal excretion (Peters, 2006; Gerber and Steinfeld, 2008). The TM already chelated with organic ligands such as specific amino acids, are more soluble and stable the intestinal lumen than inorganic sources (Suttle, 2010; Liu et al., 2016). Utilizing intestinal uptake process of amino acids may also increase availability (Liu et al., 2017). Studies comparing organic sources of TM in pigs have documented higher (Matsui and Yamaguchi, 1995; Veum et al., 2004; Liu et al., 2014; Liu et al., 2016) or equal retention (Swinkels et al., 1996; Cheng et al., 1998). Therefore, organic sources may help achieving TM needs minimizing intestinal lumen interactions (i.e. phytate complexes) and in compliance with regulatory limits of inclusion in the feed (Directive 2016/1095/EU and 2018/1039/EU).

Genetic, conformation, and location factors

Conformation and location are recognized as determining for claw lesions. The medial digits are smaller than the lateral ones, which is associated with more exposure and weight bearing on lateral claws (Penny et al., 1963; Anil et al., 2007; Amstel and Doherty, 2010). This divergence increases with time and the lateral claws have increased incidence of lesions. Indeed, lateral claws are responsible for 75% of weight bearing and concentrates near 80% of the lesions (Webb, 1984; Tubbs, 1988). Therefore, weight distribution seems decisive on the occurrence of lesions and likely influence as well severity. Sows including markedly unevenness with smaller medial digit would have more chance to develop a lesion (Kroneman et al., 1993; Enokida et al., 2011). Similarly, severity has been documented greater in hind than in fore limbs (Gjein and Larssen, 1995; Kirk et al., 2005; Anil et al., 2007). In fact, Amstel and Doherty (2010), suggested that this was because fore limbs were more stable than the hind, with the medial hind claw being the least steady claw and the hind lateral the more frequently affected. Furthermore, differences in strength and resilience capacity of horn tissue

have been attributed to some horn areas, i.e. weak junctions between soft and hard horn (Kroneman et al., 1993; Budras et al., 1996; Anil et al., 2007). For example, heel bulb and its junction and abaxial wall bear most of the weight in the lateral claw, but the medial digit bears most of the weight through its tip (Webb, 1984). Unevenness between medial and lateral digits is associated with genetics, and selection may help to control the development of claw lesions (Fan et al., 2009; Pluym et al., 2013b).

Management factors

Housing systems and type of floor have been extensively studied in swine, and some have been related with claw problems. Generally, ample evidence has associated group housing with an increased frequency of claw lesions in various studies (Anil et al., 2007; Karlen et al., 2007; Pluym et al., 2011; Cador et al., 2014). Nonetheless, some of those also reported lesser lameness even with higher prevalence of claw lesions (Karlen et al., 2007), or reduced incidence and severity of lesions in some cases with using straw bedding (Ehlorsson et al., 2003; Kilbride et al., 2010). Likely, the more freedom in movements and activity in loose housing is beneficial for most behavior changes (welfare) and locomotion ability (Anil et al., 2003). Whereas such loose systems may increase social conflict and competition (Kroneman et al., 1993; Gjein and Larssen, 1995; Olsson et al., 2016) and challenge the sow claws with increased likelihood of slippery, trauma, scratch, and catching between slats. Among systems of loose housing, differences may also appear. For instance, electronic feeding system (EFS) was suggested to produce greater activity related with aggression when females await cue to entry into the feeder (Anil et al., 2007). Similarly, Cador et al. (2014), confirmed increased leg problems in large groups of sows and ESF systems than in small groups with walk-in, lock-in stalls.

In a recent study, gilts were challenged with repeated regrouping to increase social conflict and agonistic activity (Olsson et al., 2016). They observed increased body lesion scores at the end of the regrouping and higher claw lesion scores over time. Furthermore, factors such as animal flow, group management (i.e. static or dynamic groups), and feeding system also influences the level of lesions (Olsson et al., 2011; Li and Gonyou, 2013). Altogether, this suggests disadvantages in systems frequently regrouping or including high levels of social and agonistic activity. Additionally, prevalence of claw lesions has been documented to increase not only in gestation but in lactation room, which could be associated to more time laying down on the roughness of the metallic floors, and difficulties to stand up in the farrowing cage (Anil et al., 2007; Pluym et al., 2011, 2013a).

Since group housing in pregnant sows is mandatory in the EU, there is need for improving flooring types and management systems to avoid increasing risk of claw problems. Straw or deep bedding were proposed, but those prevent natural wear, which is not desired either, and are not applicable in all climates and manure systems (Kilbride et al., 2009; Pluym et al., 2013b). Slatted floors or high animal density have been documented to cause more claw lesions than solid concrete floors or when some bedding was included (Jorgensen, 2003). Therefore, flooring type and characteristics (i.e. slip-resistance and abrasiveness) and density of animals should be further studied in terms of causality. European union regulate sow density according to group size and parity (Table 1.3), but whether any of these possibilities yield better or worse results in terms of lameness and claw lesions is unknown. Such factors

may determine the odds for a traumatic event to occur but also whether or not those become a claw lesion (McKee and Dumelow, 1995). Current Directive 2001(2001/88/CE) in the EU determines that void ratio (unit of area of holes: unit of area floor) in slat floor must be equal or below 15% (Figure 9.1 in Chapter IX. Appendix 1), with maximum slot width of 2.0 cm and minimum rip with of 80 mm for in group housing pens for both, gilts and sows. Other type of materials as rubber floors have shown with benefits for heel health, heel-sole cracks, white line and claw length in loose housed sows (Bos et al., 2016). Conversely, plastic floors may not improve claw problems (Olsson and Svendsen, 2002).

Table 1.3. Female density, free space, and pen width in group housing during gestation.

Group housing	N of females	Free surfacen m^2	Minimum pen sides, m
Gilts and 1st parity	<6	1.81	2.4
	6-39	1.64	2.8
	≥40	1.48	2.8
Multiparous	<6	2.48	2.4
	6-39	2.25	2.8
	≥40	2.03	2.8
Multiparous sows (80%) gilts (20%)	<6	2.34	2.4
	6-39	2.13	2.8
	≥40	1.92	2.8

Source: Spanish regulatory *"real decreto"* RD 1135/2002, in accordance to European Union 2001(2001/88/CE).

1.3.3.3. Summary of claw lesions

Claw problems include different lesions on frequent locations among claw tissues. Such lesions have a notoriously high prevalence (59% to 100%) and are associated with pain and lameness to some extent. If severe, or infected, or located in some specific locations (i.e. white line) these have been more clearly associated with lameness and culling of sows. It has become controversy whether or not claw lesions induce other consequences in productivity, however, including great variability, consensus of main influence is through longevity.

The integrity of the claw mainly depends on: 1) properties and strength of its tissues and their junctions, 2) claw conformation, and 3) environmental experiences to cope. Tissue properties and strength are influenced by production and quality of horn, which in turn, is interfered by keratinization. This, can be disrupted through insufficient supply of nutrients (vitamins and minerals), oxygen, active molecules, and hormones via blood and from the dermis towards the epidermis. In this sense, TM (with catalytic, structural, and regulatory functions) and more precisely from organic sources (found more available), have shown to reduce prevalence and severity of claw lesions likely via improving horn formation and its properties (higher density, lessen tubules diameter). Further, inflammation or heavy weigh could also disturb blood supply. Unfavorable conformation such as asymmetry of digits seem determinant, as it is recognized that lateral claws, suffering lesions the most, cope with 75% of weight bearing. Ultimately, environmental conditions as health status, facilities, and flooring, as well as management and system characteristics such floor type, animal flow,

remixing, group size, and feeding method are experiences to cope by the claws. These, are the most important causes of lesions and suppose a challenge in modern pig production.

1.3.4. Other important causes of lameness

1.3.4.1. Infectious arthritis

Infectious arthritis is an inflammation of one or more joints caused by microorganisms. It is most commonly found on 2- to 4-month age pigs as acute, while older animals usually presented chronic forms and even subclinical cases. Many of the infectious agents are commensal bacteria which can take advantage of opportunity (i.e. depressed immune status and initial trauma). The agents commonly responsible of arthritis are *Mycoplasma hyosynoviae, M. hyorhinis, Erysipelothrix rhusiopathiae, Haemophilus parasuis, Arcanobacterium pyogenes, Streptococcus suis, Streptococcus equisimilis, and Staphylococcus* (Zimmerman et al., 2012). Joint lesions are characterized by increased synovial fluid which may change color, synovial membranes inflamation, fibrinous periarteritis, external swelling, and eventual abscesses. Clinical signs may include pyrexia, inability to rise or move, lameness, and death. Furthermore, young animals and acute infections are often associated with other lesions (i.e. pneumonia, Zimmerman et al., 2012).

This joint pathology is a frequent finding on removed sows. Among 96 females found dead or euthanized, 44.8% revealed chronic and purulent arthritis, being 2 out of 5 tested culture positive to *Streptococcus equisimilis* and *Arcanobacterium pyogenes* (Engblom et al., 2008a). Authors described that from those diagnosed with arthritis, 77% had clinical signs of lameness, 43% were initially diagnosed as fracture, and 37% had swollen joints. In another study, *Mycoplasma hyosynoviae* was found by culture in 20% (17 of 86) of lame pigs and also in 8.4% (7 of 83) of non-lame pigs (Nielsen, 2001). It can be presumed that associating lameness with arthritis may be difficult, whilst to make a final diagnosis of the pathogen is also conflicting. Kirk et al. (2005), diagnosed 13.5% arthritis (35 of 265) but only isolated bacteria on 42.8% of those (*Arcanobacterium pyogenes* was 10 of 15; *Streptococcus spp*. 4 of 15; and *Staphylococcus aureus* 1 of 15). On the one hand, such data imply that infectious arthritis are highly prevalent (13-45%), though not being the most common diagnosed cause of culling due to lameness (i.e. 8.8%; Heinonen at al., 2006). On the other hand, infectious agents can be isolated as subclinical (i.e. 8.4%), yet may yield chronic lesions without being finally isolated (i.e. 57.2%). The actual proportion of lameness caused by arthritis lesions is not clear, and some bias may occur when looking at culled animals. Additionally, sows may accumulate various potential causes of lameness (i.e. claw lesions, OC, and arthritis) and risk factors (i.e. social conflict and agonistic activity, inappropriate floor, nutritional deficiencies, etc.).

1.3.4.2. Bone pathologies and injuries

Other possible causes of lameness are bone pathologies and on-farm injuries. These could be ample discussed but similarities and causality confound with concepts discussed earlier in nutritional factors affecting OC and bone development. As well as management and flooring affecting competitive behavior, fights and claw health. Briefly, a bone pathology derived from OC is osteoarthritis as extended complication of OC dissecans (Ythreus et al., 2007). Other commonly found bone problems are osteomalacia, osteoporosis, or ‘Downer sow syndrom’ as softening or thinning bones caused by extreme decalcification mostly

caused by inadequate dietary levels of calcium, phosphorus or vitamin D. Often developing during lactation or vitamin D deficiency (Anil et al., 2005; Dewey and Straw, 2006). This changes typically happen fast and before the sow can reconstitute bone integrity during the next gestation period, contributing to an overall loss of bone mass (NRC, 2012). Such loss can lead to hypocalcemia and bone fractures, which typically take place late in gestation or at weaning (i.e. especially axial skeleton bones; Dewey and Straw, 2006).

Trauma and injures can occur in all ages but are more common with animal transportation, on-farm movements (i.e. weaning), and when regrouping (Heinonen et al., 2013). Types of injury are several and generally include wound and mild injuries due to inadequate facilities or management, but sows can even fracture bones when are stuck and struggle to free a leg, fall, fight, or otherwise, likelihood as secondary to weakening bone disease (see above).

1.3.5. Summary of lameness

Lameness is a persistent problematic in gilts and sow and an important welfare concern with great consequences in swine herd's sustainability, mainly via affecting longevity. Perhaps, the fact of being a persistent problem is related with unsolved negative dynamics (i.e. disregard leg soundness, genetic selection, or intensifying the industry), or otherwise, because it is of great complexity. The three main problems to face locomotion problems and lameness are: 1) multicausality, 2) multifactoriality within causes, and 3) limitations to detect, measure, and diagnose locomotion impairment. Altogether difficulty to mitigate or prevent causes through intervening factors.

The greatest opportunity to reduce current lameness may be through addressing main factors that promote different causes of lameness at once. For example, throughout animal selection. Osteochondrosis is heritable, as well as are the deficient conformations that likely relate with OC (i.e. X or O hind legs shape) or with increasing odds of claw lesions (i.e. unevenness). Similarly, for environmental conditions that including health status, facilities, and flooring, as well as management and system characteristics (animal flow, remixing, group size, and feeding method); which influence likely the most the prevalence of locomotion disorders of all type. Conversely, whilst facilities and flooring are to some extent regulated, the systems and management are not so; perhaps indicating lack of knowledge or attention.

Adequate levels of minerals, TM, and vitamins, are essential for claw horn formation and bone development. Actual dietary needs for proper development of these structures is unclear, yet, those may be at some degree above needs for growth. In fact, the industry includes dietary levels above requirements, which within legal levels, may be justified as some minerals were reported with higher requirements for mineralization than for growth (Ca). Importantly, deficiencies must be avoided, but interactions among feed components may be important in this sense. The effects of high growth rate on OC are through genetic association but may also be from weight load on growing joints. Indeed, to modulate growth rate through the diet could be an effective and applicable practice. However, it has shown controversial results, plus age and joint interactions. Limiting growth through the diet can be applied via various strategies. Earlier, 15% to 20% reduction of growth rate from 10 wk of age to the end of rearing was necessary to reduce OC, and the methods earlier used often combined feed restriction with lowering nutrient concentration in the diet. Only reducing

some nutrient (i.e. Lys) concentration seems more applicable but is poorly studied to reduce OC and lameness, whereas earlier use of severe feed restriction is less applicable and includes welfare concerns.

To one's understanding, common sense must apply to reduce or avoid the incidence of OC lesions and claw problems by first, genetic selection and proper use of facilities, management, and health. Then, optimizing nutrition not only for growth but also to ensure development and healthy maintenance of structural tissues.

Table 1.4. Literature associating gilt conditioning with puberty attainment.

Authors	Breed	Study	Categories	n	Puberty age, d	Puberty, %	Other data reported		
								BE to estus, d	BW, kg
Kummer et al., 2009	Camborough 22 (C22®) South Brazil	ADG: birth to ~144 d of age; Puberty rate ~190 d of age	577 g/d	47	164[a]	95[a]		20.0[a]	99.4 ± 13[b]
			724 g/d	56	155[b]	76[b]		10.8[b]	113 ± 10[a]
Magnabosco et al., 2014	Landrace x Large White (DanBred) Santa Catarina State Brazil	ADG: birth to BE (~156 d of age); Puberty rate 30 d after BE	500-575 g/d	223	172[a]	65.5[b]	Puberty	16.4[a]	98.0[c]
			580-625 g/d	216	171[ab]	64.3[b]		14.4[ab]	104.4[b]
			630-798 g/d	226	169[b]	74.3[a]		13.5[b]	112.9[a]
		Age at BE; Puberty rate 30 d after BE	140-155 d	365	168[b]	60.8[b]		15.1	104.7
			146-170 d	300	174[a]	70.0[a]		14.2	105.5
								ADG, g/d	
Tummaruk et al., 2009	Landrace × Yorkshire Thailand	Age at 1st observed estrus	4 month	31			Birth to ~134 kg BW	627[a]	
			5 month	660				604[b]	
			6 month	1619				588[c]	
			7 month	689				516[d]	
			8 month	204				541[e]	
			9 month	89				543[e]	
			10 month	37				546[de]	
								BW, kg	BF, mm
Amaral Filha et al., 2009	Camborough 22 (C22®) Midwest Brazil	Age at BE x ADG: birth to BE (~156 d of age); Puberty rate 40 d BE	130-149 d: 550-649 g/d	170	165[c]	82.3[ac]	Puberty	89[e]	9.8[d]
			130-149 d: 650-725 g/d	400	162[bc]	79.2[c]		98[c]	10.6[c]
			130-149 d: 726-830 g/d	181	160b	85.6[bc]		108[b]	11.3[ab]
			150-170 d: 550-649 g/d	201	172[a]	88.6[ab]		96[d]	10.6[c]
			150-170 d: 650-725 g/d	349	171[a]	89.7[b]		107[b]	11.1[bc]
			150-170 d: 726-830 g/d	185	174[a]	89.2[ab]		120[a]	11.9[a]
Patterson et al., 2010	L42 dams × L19 sire (PIC) Canada	Age at puberty; Puberty 40 d after of BE	<153 d	70	105[c]	100		134[c]	
			154 to 167 d	117	118[b]	100		146[b]	
			168 to <180 d	71	129[a]	100		156[a]	
			no puberty <180 d	91		0			

Table 1.4. (Continue)

Authors	Breed	Study	Categories	n	Puberty age, d	Puberty, %	Other data reported			
									BW, kg	ADG, g/d
Roongsitthichai et al., 2013	Landrace x Yorkshire Thailand	Age 1st AI	<224 d	1039	201		Entering breeding unit		138.9[b]	627
			>224 d	1575	206				139.6[a]	588
		Age 1st farrowing	<343 d	1089	201				139.0[b]	622
			>343 d	1059	205				139.7[a]	581
Calderón Díaz et al., 2017	Large White × Landrace Utha, USA	Diets varying SID lysine (46 kg to 139 kg BW, 220 d of age)	0.68% 42 d; 0.52% 78 d	214	209 ± 2[a]	27.7	Entering gilt pool		130	720[c]
			0.79% 42 d; 0.60% 78 d	214	202 ± 2[b]	31.0			138	788[b]
			0.90% 42 d; 0.68% 78 d	214	198 ± 2[b]	37.7			148	873[a]
Tummaruk et al., 2009	Landrace × Yorkshire Thailand	BW entering the gilt pool	46-70, kg	471	193[d]					
			71-80, kg	232	196[d]					
			81-90, kg	406	207[bc]					
			91-100, kg	600	206[c]					
			101-110, kg	267	206[c]					
			111-120, kg	140	211[ab]					
			121-149, kg	179	213[a]					
									Rate, %	BW, kg	BF, mm
Beltranena et al., 1991	Camborough × Canabrid (PIC) Canada	Feed intake levels 47 kg BW to 2nd heat	Ad libutum	57	170		2nd estrus		75.4	114.3	16.5
			2.0 kg/d	43	185				88.4	107.8	12.6
			2.0 kg/d - 1st estrus - 2.8 kg/d	41	187				82.9	104.4	12.0

Puberty = first observed estrus; ADG = average daily gain; BF = backfat; BW = body weight; BE = boar exposure; birth BW = 1.5; SID = standarized ileal digestible

Table 1.5. Literature associating gilt conditioning with sow production.

Authors	Breed	Study	Categories	n	FR, %	1st litter, n		Other data reported		
									Interval, d	
Tummaruk et al., 2001	11 herds Landrace; 8 herds Yorkshire Sweden	ADG: birth to 100 kg BW; n = 5577	350-450 g/d			Born alive	9.3[b]	Weanin to estrus	6.1[abc]	
			450-500 g/d				9.6[b]		6.6[a]	
			500-550 g/d				9.7[b]		6.3[ab]	
			550-600 g/d				9.9[a]		6.15[c]	
			600-650 g/d				9.9[a]		6.1[c]	
			650-800 g/d				10.0[a]		6.0[c]	
									2nd litter, n	3rd litter, n
Kummer et al., 2006	Camborough 22 (C22®) South Brazil	ADG: birth to average 144 d of age	>700 g/d; AI at <210 d	164	88.4[a]	Total born	11.7[b]	Total born	11.0 ± 4	11.2 ± 4
			>700 g/d; AI at >210 d	165	87.9[a]		12.8[a]		11.5 ± 4	11.4 ± 3
			<700 g/d; AI at >210 d	239	88.7[a]		11.8[b]		10.6 ± 3	11.5 ± 3
Roongsitthichai et al., 2013	Landrace x Yorkshire Thailand	ADG: birth to 139 ± 6 kg BW	<550 g/d	433	78.9[a]		9.9		10.8[ab]	11.3[ab]
			551-600 g/d	2328	82.4[a]		10.2		10.5[b]	11.2[ab]
			601-650 g/d	2348	81.8[a]		10.1		10.8[a]	11.2[a]
			>650 g/d	583	80.5[a]		10.3		10.6[ab]	10.9[b]
									BW, kg	Age, d
Kummer et al., 2009	Camborough 22 (C22®) South Brazil	ADG: birth to average 144 d of age	577g/d	58	90.7	Viable embrios	12.0	1st AI	121 ± 11[b]	193 ± 7
			724g/d	58	94.5		11.1		143 ± 12[a]	193 ± 7
Kummer et al., 2009	Camborough 22 (C22®) Midwest Brazil	ADG: birth to 1st AI	600-700 g/d	345	92.6[a]	Total born	12.0[b]	1st AI	147[c]	219
			701-770 g/d	710	92.7[a]		12.5[a]		160[b]	218
			771-870 g/d	366	93.6[a]		12.9[a]		172[a]	216
Magnabosco et al., 2014	Landrace x Large White (DanBred) Santa Catarina State Brazil	ADG: birth to BE (average 156 d of age)	500-575 g/d	223	94.1		15.8	Puberty	98.0[c]	172[a]
			580-625 g/d	216	92.5		15.4		104[b]	171[ab]
			630-798 g/d	226	92.3		15.4		113[a]	1691[b]
		Age at BE	140-155 d	365	92.2		15.8		105	168[b]
			146-170 d	300	93.7		15.3		106	174[a]

Table 1.5. (Continue)

Authors	Breed	Study	Categories	n	FR, %	1st litter, n		BW, kg	Age, d
								4th litter, n	5th litter, n
Tummaruk et al., 2001	11 herds Landrace; 8 herds Yorkshire Sweden	Age at 1st AI; n = 5577	5-7 month			Born alive	9.2[c]	Born alive 11.5[ab]	11.5[ab]
			8 month				9.5[b]	11.5[a]	11.5[a]
			9 month				9.8[a]	11.3[ab]	11.3[bc]
			10 month				10.0[a]	11.0[b]	11.2[bc]
			11 month				10.1[a]	10.7[ab]	10.7[c]
						1st litter, n		2nd litter, n	3rd litter, n
Amaral Filha et al., 2008	Camborough (C22®) Midwest Brazil	BW and age at 1st AI	130-150 kg; 211 ± 9 d	298	89.9[a]		12.1[b]	9.6 ± 4	11.7 ± 3
			151-170 kg; 219 ± 9 d	1007	90.7[a]		12.4[b]	9.8 ± 3	11.7 ± 3
			170-200 kg; 225 ± 8 d	421	92.9[a]		12.8[a]	9.8 ± 4	12.0 ± 3
Patterson et al., 2010	L42 dams × L19 sire (PIC) Canada	Age at puberty	<153 d	70	84.6		10.7	11.1	12.7
			154 to 167 d	117	85.0		10.7	11.9	12.0
			168 to <180 d	71	81.0		11.6	11.5	12.6
			no puberty <180 d	91	-		10.6	11.3	12.8
Roongsitthichai et al., 2013	Landrace × Yorkshire Thailand	Age at puberty	<180 d	487	76.0[b]	Total born	10.2	Total born 10.8	11.2
			181-200 d	916	83.2[a]		10.2	10.8	11.2
			201-210 d	1074	83.5[a]		10.1	10.5	11.1
			211-220 d	772	82.5[ab]		10.1	10.6	11.2
			>220 d	391	82.2[ab]		10.1	10.8	11.1
		BW entering the breeding unit	<130 kg	295	80.1[a]		10.0	11.0[ab]	11.2[ab]
			131-135 kg	680	81.4[a]		10.1	10.7[ab]	11.3[a]
			136-140 kg	1652	80.9[a]		10.1	10.5[b]	11.1[a]
			141-145 kg	723	83.0[a]		10.2	10.8[ab]	11.4[a]
			146-150 kg	301	81.9[a]		10.3	10.8[ab]	10.7[b]
			>150 kg	147	87.6[a]		10.4	11.1[b]	11.2[ab]

Authors	Breed	Study	Rearing	1st gestation	n			BW, kg	BF, mm		BW, kg	BF, mm
Thingnens et al., 2015	Landrace × Yorkshire Norway	Energy level rearing (> 25 kg BW) + Energy level 1st gestation	13.2–29 MJ NE/d;	27.3 MJ NE/d	114		12.9[a]	Early gestation 181[a]	16.4[ab]	Late gestation	225[a]	17.7[a]
				22.3 MJ NE/d	118		12.6[a]	183[a]	16.8[a]		218[b]	17.3[ab]
			10.6–23 MJ NE/d	27.3 MJ NE/d	105		13.0[a]	179[a]	16.0[b]		222[ab]	17.4[ab]
				22.3 MJ NE/d	121		12.8[a]	183[a]	16.5[ab]		220[ab]	16.8[b]

FR = farrowing rate; ADG = average daily gain; BW = body weight; BE = boar exposure; birth BW = 1.5; BF = backfat; Puberty = first observed estrus.

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CHAPTER II

Hypothesis and Objectives

The literature review has highlighted the importance of sow replacement and gilt development on herd sustainability. Equally important, it has revealed the complexity to define strategies that effectively enhance longevity and lifetime productivity. Nutrition, genetics, and management affect gilt development and risk factors for culling. Lameness, being itself highly complex and multifactorial, is identified as one major problem (8% to 17% prevalence) which negatively affects sow longevity and animal welfare. Principal causes of lameness are OC, a disease including joint defective endochondral ossification, and claw lesions. Those, had been associated with genetic, conformation, growth rate, management, flooring conditions, and nutritional factors. Trace minerals such as Cu, Mn, and Zn, and mineral S are involved in cartilage, bone, and horn development, and their potential to reduce risk factors of lameness is controversial. Furthermore, during the rearing and development of females, hostile conditions and numerous vaccination are often challenging gilts, yet those may be justified to stabilize herd sow health status. Intensive vaccination and acclimation to endemic pathogens may include metabolism and nutritional requirement changes that are poorly regarded in terms of nutrition and can affect gilt performance.

Nowadays swine industry has the willingness to find solutions to above mentioned problems and to contribute improving sustainability. Manifesting such interest, Tecnología & Vitaminas S.L. (today Farm Faes, S.A.) collaborating with researchers from the Animal Nutrition and Welfare Service (SNiBA) from the Department of Animal and Food Science at "Universitat Autònoma de Barcelona" (UAB), obtained a CDTI funding project (Spanish Innovation Agency, Ministry of Industry Tourism and Trade, code IDI-20150749). In this context, project lasted 3 years while other companies such as private farms joined to provide conditions to conduct experimental trails. Such collaborations, especially from the farm owners and employees, is very appreciated and needed to implement applied research and for the industry to progress. Similarly, IRTA (Institut de Recerca i Tecnologia Agroalimentaria) and Department of Animal Health and Anatomy from UAB collaborated in some parts of this project.

In the present project, there was hypothesized that additional organic Cu, Mn, and Zn and the amino acid Met (as S donor) would reduce the incidence of lameness and lameness risk factors (OC, and claw lesions) while enhancing bone properties and joint health (reducing OC gross lesions). In addition, OC and lameness would be reduced in animals of slower growth rate (that carried lower lifetime weight load) or increased in heavy fast growth gilts. Similarly, that lameness would reduce with limited dietary Lys as means to reduce growth rate, or including TM plus Met, while combined both (TM plus Met with reduced Lys) would interact and present lowest incidence of lameness. Finally, it was hypothesized that some nutrients with some immunity roles (vitamin A, Trp and Thr, and omega-3 polyunsaturated fatty acids) could enhance growth performance under hostile environment with PRRSv.

The present PhD thesis has been developed to attain a main objective "to design nutritional strategies for improving gilt development via reducing lameness problem or enhancing performance under hostile health conditions", which are culling risk factors that replacement gilts frequently face in commercial conditions. To achieve this main objective and test the above hypotheses, five specific objectives were formulated:

Hypothesis and Objectives

1. To study the occurrence, prevalence, and impact of lameness on rearing gilts and up to 1st parity. (Results presented in Chapters III, IV, and V)

2. To evaluate the effects of feeding additional organic Zn, Cu, and Mn, or Met, or the combination to growing gilts on lameness, performance, body composition, and the carryover effects on lameness, claws lesions and sow productivity to 1st parity. (Results presented in Chapter III)

3. To evaluate the effects of feeding additional organic Zn, Cu, and Mn, or Met, or the combination on gait score, bone density, and OC in replacement gilts of different growth rate. (Results presented in Chapter IV)

4. To evaluate the effects of feeding additional organic Zn, Cu, and Mn plus Met and (or) to reduce a 19% the dietary Lys from growth requirements, as mean to reduce ADG in growing gilts on lameness, body composition, performance, and the carryover effects on lameness, claws lesions, and early sow productivity to 1st parity. (Results presented in Chapter V)

5. To evaluate the effects of vitamin A, or Trp and Thr, or omega-3 polyunsaturated fatty acids, which have some immunity roles, on growth performance and acute immune response under hostile environment with PRRSv. (Results presented in Chapter VI)

To reach the objectives, three experiments were planned and conduct
conditions.

CHAPTER III

Effects of supplementing organic microminerals and methionine during the rearing phase of replacement gilts on lameness, growth, and body composition

Effects of supplementing organic microminerals and methionine during the rearing phase of replacement gilts on lameness, growth, and body composition

Lluís Fabà,† Josep Gasa,† Mike D. Tokach,‡ Evelia Varella,‖ and David Solà-Oriol†

†Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; ‡Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506-0201; and ‖Tecnología & Vitaminas S.L., Alforja 43365, Spain

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ABSTRACT

Lameness is a primary reason for culling and mortality within a sow herd. This study evaluated the impact of feeding organic trace minerals and methionine (Met) to growing gilts (134 d) on lameness, performance, body composition and claw health (to first parity), productivity (to second parity), and reproductive performance through 2 parities. Young gilts [28.8 ± 8.8 kg of body weight (BW), n = 360] were BW blocked (10 gilts/pen) and randomly allotted to 1 of 4 dietary treatments: control (CON, basal diet); CON plus organic minerals (MIN, at 10, 20 and 50 mg/kg of Cu, Mn and Zn, respectively; Aplomotec Plus, Tecnología & Vitaminas, S.L, Alforja, Spain); additional Met (MET, at 102% Met: Lys); and MET plus MIN (MM). Feed was provided ad libitum. Lameness, BW and body composition were measured 7 times during rearing, at gilt service, d 109 of gestation, and first weaning. Gilts fed the MM diet had lower average daily feed intake (5.1%) and final BW (2.1%) than CON gilts ($P < 0.05$); whereas MIN and MET were intermediate and not different from each other. Similarly, final backfat (BF) was greatest in CON ($P < 0.05$), whilst CON and MIN increased final loin depth compared with MM ($P < 0.05$) with MET not being different. During rearing, 7.7% of all gilts presented lameness, which appeared between 106.8 and 129.7 kg BW confidence interval. Gilts that had been or were lame had reduced BW and average daily gain compared with never lame gilts ($P < 0.05$). Lameness during rearing was highest ($P < 0.01$) in gilts fed CON diet (14.8%), with no differences amongst MIN (2.0%), MET (5.3%), or MM (6.5%). In the sow herd, 21% of sows showed lameness and 24% of those were associated with claw lesions. At weaning, gilts fed CON diet had highest ($P < 0.01$) prevalence of lameness (20.8%) with no differences amongst MIN (6.5%), MET (11.1%), or MM (7.6%). Over the first 2 parities, 27.3% of gilts were culled. On farm lameness was associated with 0.7 more stillborn piglets ($P < 0.10$), 1 mm more BF loss in first lactation ($P < 0.05$), and increased weaning-to-estrus by 3 d ($P < 0.05$). In conclusion, lameness during rearing was decreased by supplementing organic trace minerals, methionine, and their combination; which also reduced lameness during lactation.

Key words: trace minerals; osteochondrosis; claw health; longevity; animal welfare

CHAPTER IV

Effects of additional organic microminerals and methionine on carcass composition, gait score, bone characteristics, and osteochondrosis in replacement gilts of different growth rate

Effects of additional organic microminerals and methionine on carcass composition, gait score, bone characteristics, and osteochondrosis in replacement gilts of different growth rate

Lluís Fabà,† Josep Gasa,† Mike D. Tokach,‡ Maria Font-i-Furnols,‖ Ester Vilarrasa,[$] and David Solà-Oriol†

†Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain. ‡ Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201, United States of America. ‖ IRTA – Product Quality, Finca Camps i Armet, 17121 Monells, Girona, Spain. [$] Tecnología & Vitaminas S.L., Alforja 43365, Spain.

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ABSTRACT

Osteochondrosis (OC) is a multifactorial defective endochondral ossification that causes lameness and early culling in gilts and sows. Previous research suggested that nutrition and growth rate could influence OC development and progression. As part of a broader study [n = 360 gilts; 28.8 ± 8.8 kg body weight (BW)] designed to evaluate the effect of four dietary treatments: 1) basal diet (CON); 2) CON plus organic micro-minerals (MIN, copper, manganese and zinc at 10, 20 and 50 mg/kg respectively; 3) additional methionine (MET, at 102% methionine:lysine); and, 4) the combination (MM), on lameness and performance, a sub-sample of 40 heavy replacement gilts (10 gilts/treatment, 171.5 ± 8.1 kg of BW) was used. Within treatment, gilts were classified for final average daily gain (ADG) as low (LG, 838 ± 36.3 g/d; n = 20) or high (HG, 922 ± 31.1 g/d; n = 20). Dietary treatment and growth classification were the fixed effects to evaluate gait, OC, tibia bending measures, metacarpal mineralization; and using computerized tomography, the carcass composition, bone size, and whole bone density (WBD). The WBD was expressed as volume of Hounsfield values (HU), where higher values indicate increased density. Gilts fed MIN diet had 0.75 mm larger tibia and a 9.5% increase of WBD > 140 HU compared to CON and MET ($P < 0.05$). The volume of high dense bones (> 1000 HU) was also increased in MIN and MET compared with CON ($P < 0.05$). Tibia bending moment and breakage strength were greater ($P < 0.05$) in MIN than in CON, being MET and MM intermediate. Metacarpal ash, calcium, and phosphorus content, but not proportions, were higher in gilts fed MIN than CON ($P < 0.05$). Total score of OC lesions was lower in MM gilts compared to CON ($P < 0.05$). The OC total score increased with ADG from 35.8 to 109.8 kg of BW ($R^2 = 0.10$; $P < 0.10$). However, between 109.8 and 171.5 kg of BW the ADG decreased with higher OC score ($R^2 = 0.14$; $P < 0.05$). In conclusion, supplementing growing gilts with MIN enhanced bone strength and bone density, MET increased the proportion of highly dense bone (>1000 HU), and MM dietary treatment reduced OC lesion score compared with CON.

Key words: trace minerals, osteochondrosis; lameness; bone; pigs; sows.

INTRODUCTION

Osteochondrosis (OC) is focal disturbance in endochondral ossification seen in growing animals (Olstad et al., 2015). The cartilage superficial OC lesions fracture is suggested to be a major cause of lameness in gilts (Koning et al., 2015), while lameness is a primary reason for gilt failure (Engblom et al., 2008). The OC prevalence is variable and can be high (41 to 100%) although by 6 months of age healing is described as being above 50% (Busch and Wachmann, 2011; Olstad et al., 2014; Grevenhof et al., 2012). Osteochondrosis is the result of a blood supply failure to the epiphyseal growth cartilage and ischemic chondronecrosis, which originating cause is controversial and may be influenced by genetics, growth rate, nutrition, conformation, and mechanical stress (Ytrehus et al., 2007; Koning et al., 2014; Olstad et al., 2015; Quinn et al., 2015; Le et al., 2016). For instance, Busch and Wachmann (2011) described that every 100 g increase in wean-to-finish average daily gain (ADG) increased risk of OC by 20%.

Some nutrients are essential for bone and cartilage development and if supplemented may reduce OC enhance healing (Riet et al., 2013). Zinc (Zn) impacts bone mass and is important for matrix quality (i.e. key in IGF-1, osteoblast, parathyroid hormone activations;

Matsui and Yamaguchi, 1995; Veum et al. 2009). Manganese (Mn) and copper (Cu) also participate in bone and cartilage matrix formation by being involved with proteoglycans and lysyl oxidase, respectively (Riet et al., 2013).

The availability of inorganic trace minerals (TM) is often variable or unknown (Reese and Hill, 2010), which may lead to deficiency. A common practice to prevent this is to increase dietary levels above recommendations, which results in extra cost and environmental impact (Creech et al., 2004; Suttle, 2010). Organically bound minerals, may have higher retention compared with inorganic minerals, could minimize those problems and reduce content in the manure (Liu et al., 2016). Additionally, the European Union recently limited the inclusion of Zn to 150 mg/kg in sows and piglets while 120 mg/kg in growing-finishing pigs (Directive 2016/1095/EU). Cu is limited to 150 mg Cu/kg up to 4 weeks post weaning, 100 mg/kg from 4^{th} to 8^{th} week, and 25 g/kg for other phases (Directive 2018/1039/EU). Other nutrients, such as methionine (Met) and threonine (Thr), have shown potential to reduce OC severity in growing pigs (Frantz et al., 2008). Such effects were attributed to Met, which is a source of sulfur known to enhance osteoblast differentiation and increase osteocalcin (Bottiglieri, 2002; Ouattara et al., 2016), and would benefit collagen and bone formation.

We hypothesize that supplementing Cu, Mn, and Zn with additional Met would enhance bone and cartilage development, similarly could be on animals of lower growth rate that carried lower lifetime weight load, or that both may interact. Therefore, the objective of this study was to assess the effect of supplementing the diet with organic TM (Cu, Mn, and Zn), Met or the combination on carcass composition, gait score, bone density, and OC in replacement gilts of different growth rate.

MATERIALS AND METHODS

The animals used were produced and housed in commercial swine facilities. The Ethical Committee on Animal Experimentation (CEEAH) at the Universitat Autònoma de Barcelona reviewed and approved the procedures and protocols for the experiment according to the guidelines of the European Union (Directive 2010/63/EU).

Experimental Design, Housing, and Dietary Treatments

This is a complementary study part of a broader trial designed to evaluate the effect of four dietary treatments on lameness and performance (Fabà et al., 2018). A total of 360 young gilts [28.8 ± 8.8 kg body weight (BW)]; DanAvl Dania Hybrid line, Landrace × Yorkshire, DanBred Internacional, Sant Cugat del Vallés, Spain) were blocked and randomly assigned to 1 of 4 treatments: 1) control (CON, basal diet); 2) CON plus organic minerals (MIN, Cu, Mn, and Zn at 10, 20 and 50 mg/kg respectively; Aplomotec Plus, Tecnología & Vitaminas, S.L, Alforja, Spain); 3) additional Met [MET, at 102% Met:lysine (Lys)]; and 4) MET plus MIN (MM). The experimental diets were formulated for 3 different phases of 14, 75, and 45 d to include 2,550, 2,425, and 2,310 kcal net energy/kg and 1.15, 0.90, and 0.70% standarized ileal digestible (SID) Lys, respectively (Table 4.1). For each experimental period, feeds were formulated to meet or exceed nutrient requirements (FEDNA, 2013) for a proper gilt growth. For more details of the diets, see Fabà et al. (2018). Feed was pelleted and provided *ad libitum* using dry feeders with one space for 134 d. Gilts had free access to fresh water and enrichment items (biting iron chains and solid plastic balls).

Table 4.1. General composition of experimental diets1 (phases I, II and III) offered to growing gilts (as-fed basis), %.

	Phase I (0-14 d)				Phase II (15-91 d)				Phase III (92 -136 d)			
	Treatment[2]											
Analyzed composition	**CON**	**MIN**	**MET**	**MM**	**CON**	**MIN**	**MET**	**MM**	**CON**	**MIN**	**MET**	**MM**
Moisture, %	11.3	11.1	11.6	11.7	11.6	11.4	12	11.8	12.7	12.6	12.4	12.9
Net energy[3], kal/kg	2,550	2,550	2,550	2,550	2,425	2,425	2,425	2,425	2,310	2,310	2,310	2,310
Crude protein, %	18.1	18.0	17.8	17.7	16.5	16.6	16.2	16.3	13.9	14.1	13.7	14
Crude fat, %	6.58	6.42	6.07	6.03	4.15	1.25	3.88	4.00	1.99	2.03	1.96	2.00
Crude fiber, %	3.39	3.34	3.23	3.3	3.59	3.4	3.5	3.58	4.53	4.49	4.52	4.55
Lysine, %	1.25	1.26	1.24	1.25	1.00	0.98	0.97	1.01	0.77	0.75	0.78	0.77
Methionine (Met), %	0.43	0.41	1.30	1.28	0.27	0.25	0.98	1.02	0.26	0.24	0.51	0.54
Hydroxy-analogue Met, %	0.16	0.14	1.03	1.02	0.19	0.23	0.73	0.76	-	-	1.02	0.99
Calcium[3], %	0.75	0.75	0.75	0.75	0.89	0.89	0.89	0.89	0.96	0.96	0.96	0.96
Phosphorus[3], %	0.56	0.56	0.56	0.56	0.59	0.59	0.59	0.59	0.57	0.57	0.57	0.57
Copper, mg/kg	15.6	26.3	15.2	25.1	15.5	25.4	15.9	25.8	16.4	26.1	16	26.5
Manganese, mg/kg	66.4	58.2	62.1	82.7	66.1	86	68.5	88.2	65.3	84.4	67.1	84.7
Zinc, mg/kg	122.5	173.4	121.1	176.4	120.4	172.8	125.1	175.5	123.4	174.1	124.1	173.9

[1]More details of the experimental diets are published in a complementary work (Fabà et al., 2018). Briefly, the approximate inclusion levels of ingredients were: 25% corn; 25% barley; 10% wheat (30% in phase III); 20% soybean meal (10% in phase III); 10% wheat (30% in phase III); 2% to 10% bakery byproduct or wheat middlings; 4.0%, 2.7%, or 0.3% fat (phases I, II and II, respectively); 0.4% salt; and synthetic amino acids, cacluim carbonate, and di-calcium phosphate were added to sufficient growth requirements (FEDNA, 2013).

[2]Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control; MIN, which provided the diet with 10, 20 and 50 mg/kg of chelated copper, manganese, and zinc, respectively (0.1%; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, added methionine (Met); or MM, combination of MIN and MET.

[3]Calculated values.

Forty of the 360 gilts were used in the study described herein. Initially, gilts were distributed into 36 pens and 3 blocks of BW (as 10 animals/pen and 0.90 m^2/gilt with 60% slatted and 40% solid floor in each pen). At the end of rearing, gilts were selected (171.5 ± 8.12 kg of BW and 221 ± 7.68 d of age) as 10 gilts per treatment among the 120 heaviest gilts in the study. For each treatment, gilts were originally provided from 4 pens with 4 gilts from one pen and the other 6 gilts from 3 different pens. Within each dietary treatment, the 10 gilts were proportionally divided according to growth rate as low growth (LG, 838 ± 36.3 g/d of ADG) or high growth (HG, 922 ± 31.1 g/d of ADG). The criteria to choose heavy gilts and ADG classification was that heavy animals with HG gilts were a population with higher risk of OC (Busch and Wachmann 2011). The 40 gilts were slaughtered in a commercial slaughterhouse and their left half carcass studied on a similar BW to gilts at first service.

Growth, Carcass Traits and Whole Bone Density by Computerized Tomography

Measures of BW collected were on d 0, 41, 67, and 134 of the experimental period. The backfat (BF) and loin muscle depth were determined at 6 cm from the midline and at the level of the last rib using an ultrasound scanner (AV-3000V Digital Handheld Electronic B Ultrasound Scanner, AMBISEA Technology Corp., Ltd; Hong Kong, China). The 40 gilts were fasted for 12 h and transported to a local slaughterhouse where exsanguination occurred post CO_2 stunning. Within 15 min after slaughter, hot carcass weight was recorded to calculate dressing percentage (= 100 × hot carcass weight / live weight), and then placed in a chilling room (4°C).

Left half carcasses were transported to IRTA-Monells research institute (Institut de Recerca i Tecnologia Agroalimentaria, Monells, Spain). Carcasses were prepared following the European Reference Method (Walstra and Merkus, 1995) and were scanned with the General Electric HiSpeed Zx / I computed tomography (CT) device (GE Healthcare, Madrid, Spain). Acquisition parameters were 140 kV, 145 mA, 10 mm-thick, helical, 512 × 512 matrix, displayed field of view 500 mm and reconstruction algorithm STD+ (Font-i-Furnols et al., 2009). From the images of the whole half carcasses, it was obtained the distribution of volume by Hounsfield (HU) values with Matlab [Version 7.5.0.342 (R2007b), The MathWorks, Inc., MA]. Using the distribution of HU values, it was calculated the lean tissue percentage of the carcass using prediction equations previously developed by Font-i-Furnols et al. (2009).

The HU distribution was also used to determine the whole bone density (WBD)., which was calculated in two manners: 1) estimated by the formula developed by Picouet et al. (2010), and 2) considering bone density at HU >140 as previously described (Font-i-Furnols et al., 2015). For 2) method, whole half carcass HU values higher than 140, 500, 1000, and 1500 were obtained, thus marrow is excluded. The use of these cut-off ranges were defined accordingly to Gaudré et al. (2014), and therefore, used to compare proportions of different density threshold. The higher the HU value, the higher the density of the bone (Batawil and Sabiq, 2016). Hence, volume and/or proportion of bone from different ranges of HU values is associated with higher or lower bone density. The density for specific bones was not measured and WBD refers to entire half left carcass.

From the collected images and using Horos software (v1.1.7, The Horos Project, GNU Lesser General Public License, Version LGPL 3.0), femur and tibia length were

measured. Furthermore, a transversal image from the middle of the femur and tibia bones provided the cortical bone area (total bone area minus marrow area).

Tibia Bending Test

Bending test of fresh tibia was determined using an MTS Material Testing Apparatus (Model 810, MTS Systems Corporation, Minneapolis, USA) in Escola Tècnica Superior d'Enginyeria Industrial de Barcelona (Departament de Resistència de Materials i Estructures; Universitat Politècnica de Catalunya, Spain) according to methodology described by Veum and Ellersieck (2008). Tibia was held on two supports spaced 120 mm apart and force was applied to the midpoint with crosshead speed constant at 6 mm/min. The bending maximum force as breakage strength (kN), bending depth (mm), total energy (area under the curve, kN), and bending moment (kN – mm) were reported. Bending moment, defined as applied force adjusted for the distance over which it is applied (Crenshaw et al., 1981), was calculated using the formula: bending moment = (F × L)/4, where F is a measure of the maximum load (kN) and L is the distance between the bottom two fulcra (mm).

Analysis of Minerals in Third Metacarpal and Serum

The 3rd metacarpal of the left half carcass were collected, systematically boiled and cleaned of adherent tissue, diagonally cut in two pieces, weighed, dried (109ºC 12h), chemically cleaned (in Acetone for 24h), dried (109ºC 12h), weighed again, and burned in a muffle-oven overnight (550ºC). The ash percentage was calculated and the metacarpal content of calcium (Ca), phosphorus (P), Zn, Mn, and Cu were analyzed using atomic absorption spectrophotometry at proper wavelength ICP-MS (Perkin-Elmer, model Optima 4300DV; MA). Blood samples (10 ml) were collected at exsanguination post stunning (using siliconized blood-collecting tubes) and levels of Zn and Cu were analyzed in serum using ICP-OES spectrometry (Perkin-Elmer, model Optima 4300DV; MA).

Macroscopically Assessment of Joint Lesions

The elbow, carpal, femoral-iliac, knee, and tarsal joints of the left half carcass were opened and examined. All articular faces were photographed and gross evaluated for external abnormalities (defects in cartilage surface) as OC lesions on the articular cartilage. The joints were macroscopically evaluated by a board-certified veterinary pathologist, based on the methodology described by Kirk et al. (2008), and without knowledge of the dietary treatment assignment on the individual samples as blind assessment. Different locations were examined, and the presence of erosions, ulcerations, repair reactions, marginal osteophytes, and infolding of the joint were accounted as OC lesions (see the aforementioned study for the description and figures of lesion characteristics). Joint faces were scored for presence of lesions and their severity. The proportion of gilts with moderate or severe lesions was calculated; furthermore, the number of faces with lesions and total score of OC were collected for each gilt. The presence of lesions was scored as presence (1) or absence (0) of lesion on each articular face. The severity on each face was as scored as: none (0), small (1) when the lesion involved less than 5% of the cartilage surface, moderate (2) when the lesion was 5 to 10%, and severe (3) when fragmented cartilage was visible and lesion exceeded 10% of the cartilage surface (Figure 4.1). Proportion of moderate or severe lesions was the number of

gilts with at least 1 moderate or severe lesion, respectively. The number of faces with lesion was the sum of faces with lesions for each gilt. Finally, total OC score was calculated as the sum of severities accumulated throughout all evaluated joint faces (0-9). Because none of the left carcasses evaluated showed more than 3 articular faces with lesions, the classification was defined as: articular face of tibia, femur, and "other joints" (gilts with elbow, carpal, or tarsal OC lesions).

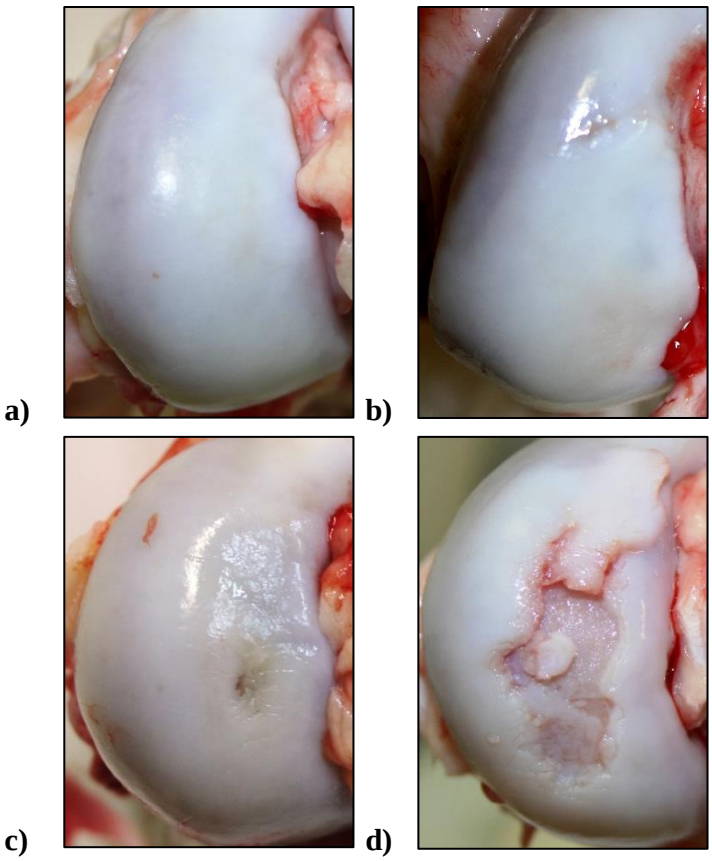

Figure 4.1. Illustration of femur lateral condyle to macroscopically scoring the severity of osteochondrosis gross lesions: a) none (0); b) small (1), when the lesion involved less than 5% of the epiphysis cartilage surface; c) moderate (2), when the lesion was 5 to 10%, and d) severe (3) fragmented cartilage and lesion exceeded 10% of the cartilage surface.

Locomotor capacity

Even though gilts were selected to be non-lame (no limp), gait was evaluated and scored using an adapted methodology from Mustonen et al. (2011) as: 0, no-difficulty; 1, slight-difficulty and slower exercise; 2, moderate-difficulty with shortened stride and some problems to exercise; and 3, severe-difficulty with evident limp lameness and exercising problems (none was selected at this level).

Statistical Analysis

The procedures were performed using SAS v9.3 (SAS Inst. Inc., Cary, NC). Gilt was the experimental unit. The normality and homoscedasticity of variables was evaluated using the Shapiro-Wilk test and examining the normal plot. Data with a normal distribution (BW, BF, loin depth, bone characteristics and WBD assessed with computerized tomography, ash

and minerals in third metacarpal, and serum minerals) were analyzed using the general linear model (PROC GLM). The OC lesions scorings were evaluated using Fisher's Exact test for presence or absence of lesions (PROC FREQ). The gait score, OC severity, number of lesions, and total lesion score were tested using Binomial or Poisson models (PROC GENMOD). Also, the t-test was used to compare severity among different joints (PROC TTEST). The main fixed effects of the model were dietary treatment and growth rate groups. In the general model, there was no evidence of dietary treatment × growth rate group interactions and was removed from the model. In all parametric analysis, Tukey–Kramer adjustments were used to determine significant ($P < 0.05$) differences. Linear regression was used to study the relationship between ADG for different phases (d 0 to 41, d 0 to 67, d 41 to 67, d 41 to 134, d 67 to 134 and d 0 to 134) and total OC lesion score using PROC REG.

RESULTS

The levels of Cu, Mn, and Zn were formulated to 10, 40 and 110 mg/kg, respectively, in the basal diet with an additional 10, 20, and 50 mg/kg added, respectively, to dietary treatments MIN and MM. The analyzed levels, which included the feed ingredients contribution, resulted between 10% and 50% higher levels than the formulated values (Table 4.1).

Growth, Carcass Traits and Whole Bone Density by Computerized Tomography

Performance, carcass characteristics, bone measures, and WBD results are presented in Table 4.2. There was no evidence of ADG differences across dietary treatments. Because ADG was the variable used for growth classification, HG had higher slaughter BW and carcass weight than LG (P <0.001). Also, LG tended to have 2% greater dressing percentage than HG (SE = 1.02; P = 0.066). Carcass weight (132.7 ± 6.10 kg) and dressing percentage (77.4 ± 3.29 kg) were not different among dietary treatments. Nevertheless, some carcass measurements indicated differences. Gilts from CON group had 3.4 mm greater BF depth compared to MIN, while MET and MM did not differ and had intermediate values (SE = 0.85; P = 0.041). Similarly, through computerized tomography, it was observed that MIN and MET gilts had 4.3% leaner carcass percentage than CON; and MM was intermediate and not different (SE = 1.07; P = 0.013).

Table 4.2. Effect of dietary treatment provided to rearing gilts and growth rate group on growth performance, carcass and bone characteristics.

	Treatment[1]				Growth[2]			*P*-value	
	CON	**MIN**	**MET**	**MM**	**LG**	**HG**	RMSE	Diet[1]	Growth[2]
n	10	10	10	10	20	20			
Final performance									
Age, d	219	222	222	222	222	221	7.41	0.782	0.607
Body weight, kg	171	174	170	172	167	177	5.3	0.175	<0.001
Average daily gain, g/d	874	899	877	873	838	922	27.9	0.189	<0.001
Carcass									
Hot weight, kg	134	132	131	134	131	136	5.18	0.840	0.002
Dressing, %	78.5	76.0	77.1	77.8	78.4	76.4	3.01	0.360	0.066
Backfat depth, mm	19.1^{a}	15.7^{b}	17.2^{ab}	16.5^{ab}	17.2	17.1	2.47	0.041	0.861
Loin depth, mm	70.1	69.7	70.0	70.2	68.1	71.9	6.09	0.998	0.439
Lean tissue, %	47.3^{b}	51.6^{a}	51.6^{a}	51.3^{ab}	49.9	51.0	3.18	0.013	0.307
Bone measures[3]									
Femur length, cm	20.3	20.9	20.6	20.6	20.4	20.7	0.51	0.124	0.096
Femur area, cm^2	9.46	9.25	9.09	9.31	9.01	9.50	0.88	0.979	0.119
Tibia length, cm	17.8^{b}	18.6^{a}	18.1^{ab}	17.9^{b}	17.8	18.4	0.64	0.007	0.003
Tibia area, cm^2	5.91	6.01	5.88	5.90	5.83	6.02	0.47	0.874	0.243
Tibia bending test[4]									
Maximum force, kN	5.59^{b}	6.38^{a}	5.95^{ab}	5.83^{ab}	5.87	6.01	0.51	0.022	0.404
Bending moment, kN - mm	168^{b}	191^{a}	179^{ab}	175^{ab}	176	180	15.4	0.022	0.409
Bending depth, mm	2.77	2.75	2.75	2.52	2.52	2.87	0.53	0.726	0.058
Total energy, kN	17.9^{b}	22.6^{a}	17. 2^{b}	19.4^{ab}	19.4	19.1	3.80	0.026	0.848

Table 4.2. (Continue)

Bone density[5]									
Density, g/dm^3	1.53	1.55	1.55	1.53	1.55	1.54	0.029	0.141	0.144
Volume HU > 140, dm^3	3.52^{b}	3.98^{a}	3.62^{b}	3.69^{ab}	3.56	3.85	0.27	0.008	0.004
Volume HU ≥ 500, dm^3	0.73^{b}	0.89^{a}	0.84^{ab}	0.75^{ab}	0.81	0.80	0.12	0.029	0.940
Volume HU ≥ 1000, cm^3	0.59^{b}	0.71^{a}	0.71^{a}	0.60^{ab}	0.66	0.64	0.11	0.048	0.698
Bone HU ≥ 500, %	20.8	22.4	23.3	20.4	22.6	20.9	2.83	0.142	0.086
Bone HU ≥ 1000, %	1.66^{y}	1.80^{xy}	1.97^{x}	1.63^{y}	1.85	1.68	0.29	0.092	0.140
Bone HU ≥ 1500, %	0.014^{xy}	0.014^{xy}	0.028^{x}	0.006^{y}	0.021	0.009	0.016	0.05	0.046

[1]Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control; MIN, which provided the diet with 10, 20 and 50 mg/kg of chelated copper, manganese and zinc, respectively (0.1%; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, added methionine (Met); or MM, combination of MIN and Met.

[2]Growth = classification of the 40 gilts into two groups within the 4 dietary treatments according to ADG: low growth (LG, 838 ± 36.3 g/d ADG) or high growth (HG, 922 ± 31.1 g/d ADG).

[3]Measurements performed using computerized tomography.

[4]Maximum force (kN) was the breakage strength and the highest load of force; Bending moment (kN – mm) was calculated using Crenshaw et al. (1981) formula as: bending moment = (F × L)/4, where F is the maximum load (kN) and L is the distance between two supports (mm). Total energy was calculated as area under the curve: force (kN) x distance (mm).

[5]Bone density was calculated as the distribution of volume according to their Hounsfield (HU) values. Density value was estimated applying the formula developed by Picouet et al. (2010) considering HU >140 (Font-i-Furnols et al., 2015). Bone density was also expressed as the proportion of HU >500, HU >1000, and HU >1500 with respect to the volume with bone density HU >140.

[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.

[x-y]Values within a row with different superscripts trend at $P < 0.10$.

Estimated WBD using Picouet et al. (2010) equation showed no evidence of differences across dietary treatments, but the volume (proportion) of bone above different levels of WBD (with HU values) presented some patterns. Computed tomography commonly expresses density by means of HU values as a standardized and convenient form; higher HU values indicate increased density. The carcass WBD or proportion of bone as ≥140 HU, was higher for MIN than for CON and MET ($P < 0.01$) dietary treatments; with MM being not different and intermediate. Also, gilts fed MIN diet had increased volume of WBD above 500 HU than CON ($P = 0.029$); whilst MET and MM did not differ. Similarly, MIN and MET had 12.4 mm^3 (17.4%) denser bone (≥1000 HU) than CON (SE = 4.01; $P < 0.05$), and MM was intermediate and not different. Looking at percentages of bone relative to WBD ≥ 140 HU, MET showed a tendency ($P < 0.10$) for higher proportion of highly dense bone (WBD ≥ 1000 HU) than CON and MM; whilst MIN was intermediate. Similarly, MET had marginally higher ($P = 0.051$) proportion of the highest density bone (WBD ≥ 1500 HU) than MM; whilst MIN and CON were intermediate. Comparing growth groups, the WBD volume was higher for HG than LG ($P = 0.004$). The HG had marginally higher proportion of bone with WBD ≥ 500 HU than LG ($P = 0.086$), whereas LG had higher percentage of bone ≥ 1500 HU ($P = 0.046$). Bone measurements indicated that length and area of femur were not different across dietary treatments; however, MIN gilts had 0.7 cm larger tibia than CON and MM (SE = 0.20, $P = 0.007$); whilst Met was intermediate.

Tibia Bending Test

Results from tibia bone bending test indicated that breakage strength and bending moment were higher ($P < 0.05$) in tibia bones from MIN dietary treatment than CON; while MET and MM were not different. Total energy was greater ($P = 0.026$) in MIN than CON and MET dietary treatments, with MM intermediate. Contrarily, bending depth was similar across treatments, although, between growth groups, the HG gilts tended to have greater bending depth than LG ($P = 0.058$). Growth classification did not result in other bone bending differences.

Analysis of Minerals in Third Metacarpal and Serum

Metacarpal length was not different amongst dietary treatments nor growth group (Table 4.3). Conversely, the de-fatted dry content of MIN was greater ($P = 0.020$) than that of CON, with MET and MM being intermediate and not different. Additionally, ash, Ca, and P contents in the metacarpal were greater in MIN than in CON ($P = 0.006$, $P = 0.007$, and $P = 0.010$; respectively); being not different than MET and MM. Nevertheless, the ash proportion in dry matter basis and the Ca and P proportion in ash basis were not different amongst dietary treatments. Comparing the growth groups, it was observed that HG had increased metacarpal de-fatted dry weight ($P = 0.029$), ash content ($P = 0.040$), and tended to have increased content of Ca ($P = 0.099$) and P ($P = 0.065$) than LG gilts. There was no evidence of differences for Zn, Cu, and Mn content and proportion in metacarpal bone amongst dietary treatments or growth groups. Similarly, no differences were observed for serum levels of Cu and Zn among dietary treatments or growth groups.

Table 4.3. Effect of dietary treatment provided to rearing gilts and growth rate group on ash percentage and mineral content in the third metacarpal and serum.

	Treatment[1]				Growth[2]			*P*-value	
	CON	**MIN**	**MET**	**MM**	**LG**	**HG**	RMSE	Diet[1]	Growth[2]
n	10	10	10	10	20	20			
Metacarpal									
Length, mm	7.90	8.07	7.95	7.97	7.95	7.99	0.180	0.243	0.442
De-fatted dry content, g	18.8^{b}	20.7^{a}	19.3^{ab}	19.3^{ab}	19.1	20.0	1.11	0.020	0.029
Ash content, g	11.8^{b}	13.1^{a}	12.3^{ab}	12.4^{ab}	12.2	12.7	0.698	0.006	0.040
Ash[3], g/kg	639	637	638	645	642	637	15.2	0.752	0.367
Calcium content, g	4.32^{b}	4.84^{a}	4.50^{ab}	4.58^{ab}	4.48	4.64	0.232	0.007	0.099
Calcium[4], g/kg	366	368	366	368	369	366	5.12	0.685	0.124
Phosphorous content, g	2.09^{b}	2.29^{a}	2.14^{ab}	2.16^{ab}	2.13	2.21	0.113	0.010	0.065
Phosphorous[4], g/kg	174	174	174	174	174	174	1.60	0.907	0.322
Zinc content, mg	2.59	2.82	2.62	2.81	41.3	43.6	0.262	0.245	0.222
Zinc[4], mg/kg	215	214	214	226	218	218	17.8	0.502	0.810
Copper content, µg	6.00	7.26	6.15	8.30	6.23	7.23	2.710	0.292	0.133
Copper[4], mg/kg	5.23	5.63	5.15	6.61	5.24	6.06	2.172	0.459	0.239
Manganese content, µg	9.83	10.2	8.12	10.3	10.1	9.14	2.287	0.231	0.265
Manganese[4], mg/kg	8.08	7.92	6.71	8.25	8.14	7.34	1.732	0.174	0.101
Serum									
Copper, mg/L	2.77	2.77	2.75	2.58	2.69	2.75	0.276	0.436	0.517
Zinc, mg/L	1.56	1.44	1.83	1.52	1.58	1.60	0.422	0.287	0.907

[1]Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control; MIN, which provided the diet with 10, 20 and 50 mg/kg of chelated copper, manganese and zinc, respectively (0.1%; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, added methionine (Met); or MM, combination of MIN and Met.

[2]Growth = classification of the 40 gilts into two groups among the 4 dietary treatments according to ADG: low growth (LG, 838 ± 36.3 g/d ADG) or high growth (HG, 922 ± 31.1 g/d ADG).

[3]Bone dry matter basis.

[4]Bone ash basis.

[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.

Macroscopically Assessment of Joint Lesions

Results from the joint evaluation and OC lesions detected in the joints are presented in Table 4.4. Articular lesions of OC were detected in 80% of gilts. Twenty percent of gilts had only small lesions, while 45% of gilts had moderate lesions, and 15% had severe lesions. Lesions were highly detected in the knee joint (68.8%) compared to elbow (21.8%), tarsal (6.3%), carpal (3.1%), and femoral-iliac joints (0%). As explained in the materials and methods section, incidence of elbow, tarsal and carpal incidence of lesions were grouped as "other joints" for analysis. In the knee joint, 21.8% of lesions were on the femur face and

87.5% on the tibia face. On the other hand, the severity was higher in the femur (0.45) face than on the tibia (0.18) face (CI = 0.335, 0.715; $P < 0.001$). Similarly, severity was higher in "other joints" (0.55; for elbow, carpal, and tarsal joints together) than on tibia (0.18, CI = 0.232, 0.726; $P = 0.037$).

Occurrence of OC lesions and their severity were not statistically different across dietary treatments for the faces of tibia and femur. However, OC lesion severity in grouped other joints was highest ($P = 0.020$) for gilts fed CON diet; and not different amongst MIN, MET, and MM. The MM dietary treatment did not have any OC lesions in the tibia. At the animal level, the CON group tended to have higher moderate to severe lesions than MM ($P = 0.082$), and had a higher total OC lesion score than MM ($P = 0.030$); whilst MIN and MET were intermediate and not different. Comparing growth rate groups (LG and HG), no evidence of differences was detected in OC lesions.

Table 4.4. Effect of dietary treatment provided to rearing gilts and growth rate group on gait score, osteochondrosis (OC), prevalence and severity scores on the cartilage faces of the knee (tibia and femur), elbow, carpal and femoro-iliac joints from the left half carcass.

	Treatment[1]				Growth[2]			*P*-value	
	CON	**MIN**	**MET**	**MM**	**LG**	**HG**	RMSE	Diet[1]	Growth[2]
n	10	10	10	10	20	20			
Gait Score	0.8	0.7	0.3	0.4	0.45	0.65	0.63	0.550	0.504
OC prevalence[3], 0-1									
Tibia	0.1	0.2	0.4	0.0	0.2	0.15	0.70	0.162	0.407
Femur	0.6	0.9	0.8	0.5	0.65	0.75	0.74	0.165	0.462
Other joints[4]	0.4	0.2	0.2	0.2	0.25	0.25	0.74	0.682	0.998
OC severity[5], 0-3									
Tibia	0.1	0.2	0.4	0.0	0.2	0.15	0.70	0.110	0.705
Femur	1.3	1.3	1.1	0.5	0.9	1.2	0.70	0.194	0.354
Other joints[4]	1.0^{a}	0.4^{b}	0.4^{b}	0.4^{b}	0.6	0.6	0.60	0.020	0.672
Articular faces with lesion, n	1.4	1.5	1.5	0.7	1.3	1.3	0.69	0.103	0.606
Gilts with moderate lesion, 0-1	0.7^{x}	0.5^{xy}	0.6^{xy}	0.1^{y}	0.9	1.0	0.70	0.082	0.999
Gilts with sever lesion, 0-1	0.2	0.1	0.2	0.1	0.1	0.2	1.06	0.998	0.999
Total Score[6], 0-9	2.4^{a}	1.9^{ab}	1.9^{ab}	0.9^{b}	1.7	1.85	0.50	0.030	0.596

[1]Treatment provided from 35.8 ± 5.9 to 171.5 ± 8.0 kg of body weight = CON, control; MIN, which provided the diet with 10, 20 and 50 mg/kg of chelated copper, manganese and zinc, respectively (0.1%; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain); MET, added methionine (Met); or MM, combination of MIN and Met.

[2]Growth = classification of the 40 gilts into two groups within the 4 dietary treatments according to ADG: low growth (LG, 838 ± 36.3 g/d ADG) or high growth (HG, 922 ± 31.1 g/d ADG).

[3]Prevalence at each articular face as absence (0) or presence of lesion (1).

[4]Other joints = gilts with elbow, or carpal, or tarsal OC lesions were put together due to lower incidence.
[5]Severity on each articular face macroscopically evaluated: none (0), small (1) when the lesion involved less than 5% of the cartilage surface, moderate (2) when the lesion was 5 to 10%, and severe (3) when the lesion exceeded 10%;
[6]Total Score as sum of severities accumulated throughout all evaluated joint faces. (Figure 4.1).
[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[x-y]Values within a row with different superscripts trend at $P < 0.10$.

Linear regressions between total score of OC and ADG within experimental periods (d 0 to 67, 67 to 134, and 0 to 134) were established. The regression results indicated that ADG from d 0 to 67 (35.8 ± 5.92 to 109.8 ± 6.12 kg of BW) had a positive relationship with lesions. As growth rate increased, the total OC score increased (total OC score = 0.006 × ADG, g/d – 4.661; $R^2 = 0.10$; $P = 0.059$). Thereafter, ADG from d 67 to 134 (up to 171.5 ± 8.05 kg of BW) showed the inverse relationship and ADG decreased with increasing total OC score (total OC score = -0.0047 × ADG, g/d + 5.943; $R^2 = 0.14$; $P = 0.018$). When assessing the whole growing period (d 0 to 134), no relationship between total OC score and growth rate was observed (total OC score = -0.0044 × ADG, g/d + 5.660; $R^2 = 0.02$; $P = 0.301$).

Locomotor capacity

The gait score shown no evidence of differences across dietary treatments or growth rate groups. Without signs of lameness, this score was maximum at 2 for a 3-point scale (10%) and was positively related to the total OC score (OC total score = 1.2095 × gait score + 1.2598; $R^2 = 0.27$; $P = 0.001$).

DISUSSION

As it was previously described, due to the lack of interaction between dietary treatment and growth rate, this was removed from the models and final data was analyzed as main factors.

Trace Minerals

Dietary TM can influence bone and joint quality, but these effects have been mainly reported when TM were fed below requirements (Owen et al., 1973; Ott and Asquith, 1989; Veum et al., 2009; Muszyński et al., 2018). Above requirements, performance maintains, excretion increases, and effects on bone development is null or controversial (Orth, 1999; Creech et al., 2004; Gowanlock et al., 2013; Olstad et al., 2015; Liu et al., 2016). Compared with CON, the dietary treatment MIN increased bending moment, bending maximum force, and WBD. Dietary Zn is known to positively correlate with bone mass (Ovesen et al., 2009). Similarly, increasing dietary Zn to 100% of NRC (1998) and Cu to 160% of NRC (1998) linearly improved bone ash and bone strength in a 28-d study (Veum et al., 2009). Yet, the base-line of TM used herein (10, 40 and 110 mg/kg of Cu, Mn, and Zn) were remarkably higher than NRC (2012) recommendations of 3.0 to 5.0 mg/kg for Cu, 2.0 to 3.0 mg/kg for Mn, and 50 to 80 mg/kg for Zn (as BW ranges in this study). Additionally, MIN and MM

levels were considerably higher and provided for a longer time (134-d trial) than previous mentioned research, which make direct comparisons difficult.

The dietary treatment MIN had greater ash, Ca, and P metacarpal content compared with CON, whilst MET and MM were intermediate. Without proportion differences, such increased contents in MIN would correspond to more ash and heavier metacarpal. Length was not different, therefore, MIN may have increased bone diameter or bone volume and trabecular thickness to influence ash and metacarpal weight; however, this was not measured in the present study. Muszyński et al. (2018) did improve bone geometry, yield, and ultimate strengths, but, supplementing Cu or added phytase under dietary deficiency in broiler. Conversely, deficiency is not contemplated herein. Metacarpal Cu and Mn were not expected to be greatly affected by treatment as bone is not the primary reservoir pool. Organic TM sources increased Cu in muscle and Mn in heart, liver, and kidney but not in bone ash (Ma et al., 2018). However, Zn was expected to increase in MIN and MM herein. Already high dietary base-line levels may have plateau in Zn bone content. Otherwise, interactions may be occurring with high levels of Mn and Cu (Shelton et al., 2004). Comparisons are difficult because TM bone content is not so often measured, and previous studies in grown pigs compare high vs. low or deletion, and present levels were all high with similar proportions.

Similarly, for the metacarpal weight and ash content, gilts fed the MIN diet had 0.75 cm larger tibia than CON, with MET and MM having intermediate values. Obviously, initial length of the tibia was unknown but all groups were homogeneous in BW and age. Weremko et al. (2013) reported that there is a positive relation between bone size and mineralization after feed, protein, or minerals restriction. However, the present difference in tibia length is small and neither dietary nor nutrient restriction was applied. Although, the higher WBD for MIN may not be strictly related to long bones length but may be associated to bone growth and quality. In fact, HG had 0.60 mm larger tibia than LG, but without WBD differences; while the MIN dietary treatment maintained higher WBD than CON above 500 and 1000 HU. Recently, Lagos et al. (2018) shown that Ca and P requirements are higher for mineralization than for growth and a similarly conclusion was suggested earlier for TM requirements in broilers and turkey (Oviedo-Rondón et al., 2006; Ferket et al., 2009). Nonetheless, this should be further investigated in a dose response approach.

According to Orth et al. (1999) and Ythreus et al. (2007), complexity of long bone growth is suggested to be influenced by a number of dietary factors, growth factors, and other cellular signals involved in chondrocyte differentiation. Under such level of interactions, minimum potential to improve bone development is attributed to diet supplements (Olstad et al., 2015; Tóth et al., 2016). Although limited, the present results suggest some improvements and contrast with abovementioned research. It could be that supplementing TM only present some benefits under challenging prevalence of lameness, as for CON gilts which had 14.8% of lameness (Fabà et al., 2018). Furthermore, other authors reported some effects on bone metabolism when increasing TM availability or supplementation. Revy et al. (2004), observed that supplements of Zn or phytase increased *alkaline phosphatase (ALP);* and added phytase increased Zn in metacarpal and serum, bone strength, and ash content. They reported that Zn from organic source and without phytase increased P percentage in metacarpal. This, suggests a positive effect on bone development when Zn is increased. Liu et al. (2016) described that TM from organic sources increased the activity of *Cu,Zn-superoxid dismutase*

(Cu/Zn-SOD), ALP, and *glutathione peroxidase* enzymes in finishing pigs. This would likely enhance bone development and reduce bone loss from reactive oxygen species (Altindag et al., 2008; Clarke, 2008; Smietana et al., 2010). However, the present study did not compare TM sources and whether any benefit comes from the type of source is unknown. Inconsistency across studies suggests that present results may have occurred purely by chance, or otherwise, potential of supplementing TM is limited and interact with other factors (i.e. actual risk of lameness). Results from MIN dietary treatment indicate that improvements may be occurring in bone development with TM supplements compared to CON.

Methionine

In agreement with the hypothesis that Met could influence bone properties, gilts fed MET diet were not different from MIN, and both increased dense bone (WBD >1000 HU) compared with CON. However, this may not directly translate to bone strength as MET had lower total bending energy than MIN. Huang et al. (2014), reported that lowered Met intake delay bone differentiation and results in smaller and thinner bones. Therefore, greater effects should be expected under Met deficiency than Met excess. The role of Met in bone development may be related to its sulfur donator capacity (Frantz et al., 2008; Huang et al., 2014). It is suggested that therapeutic effects of dietary sulfur on bone and cartilage remodeling may result from over-coming deficiency of extracellular sulfur (Cordoba and Nimni, 2003; Ouattara et al., 2016). This would be related to insufficient dietary sulfur amino acids, not for growth but for cartilage development (Cordoba and Nimni, 2003). Other sulfur sources (i.e. coenzyme A, S-adenosyl methionine, glutathione, sulfate etc.) could also supply dietary sulfur and be used with the same objective. However, in this trial, Met was selected as the primary source of dietary sulfur because of previous evidences (Frantz et al., 2008).

Trace Minerals and Methionine

The dietary treatments MIN and MET improved WBD compared to CON, however, the combination MM was intermediate. Similarly, MM also had intermediate values for bone strength which suggest some inconsistency. The reason for this is unknown but would be related with an outbreak of Porcine Reproductive and Respiratory Syndrome (PRRS) that occurred during this trial (Fabà et al., 2018). For 3 wk during the outbreak, MM gilts reduced feed intake by 25% compared with other dietary treatments. This resulted in lower overall feed intake and performance for MM than CON, and would had lowered total mineral intake. The reasons behind lower feed intake and growth rate in MM gilts were unknown, but decreases in feed intake can reduce mineralization (Weremko et al., 2013; Riet et al., 2013) and because of this, MM gilts would show intermediate WBD and bone strength.

Osteochondrosis

The prevalence of OC (80%) is in agreement with previous reports (Grevenhof et al., 2012; Busch and Wachmann, 2011; Koning et al., 2014). Progression of OC can undertake 3 stages: OC latens, manifesta, and dissecans; and only the last includes clinical signs (Ytrehus et al., 2007). Absence of lameness was criteria for gilt selection, therefore, 100% of OC was subclinical, still, gait scoring indicated that mild difficulties in gait moderately correlated with increased severity of OC. Furthermore 2 gilts had macroscopically severe lesions of OC

dissecans without evident lameness (i.e. Figure 4.1.d). Other authors also observed low correlation between OC and clinical lameness (Crenshaw et al., 2013; Etterlin et al., 2015). In this trial, most of the lesions were classified as moderate or small (81.3%). Likewise, Ytrehus et al. (2004) observed 10 times more prevalence of OC manifesta than dissecans in heavy pigs.

Total OC score reduced when combining the organic TM and Met (MM); whilst MIN and MET remained intermediate. Gilts fed the CON diet had highest OC severity for grouped other joints than MIN, MET, or MM. However, no evidence of differences was detected for CON, MIN and MET, in femur and tibia lesions. In contrast, Frantz et al. (2008) observed a reduction of total OC score when supplementing Met, a combination of Cu and Mn, or all combined. In humans, providing dietary S-Adenosyl Met metabolite to osteoarthritis patients improved joint health and functionality (Najm et al., 2004). The use of heavy, grown gilts (221 ± 7.68 d of age) may have reduced the odds to find sever OC because as gilts become older, the possibility of OC healing increases (Aasmundstad et al., 2013; Olstad et al., 2014). Contrarily, MET and MIN strategies might be additive or synergistic. Variability was high and more sample size is required to validate these findings.

Increasing ADG was positively correlated to total OC score between 35.8 ± 5.92 and 109.8 ± 6.12 kg of BW, and subsequently, negatively correlated between 109.8 ± 6.12 and 171.5 ± 8.05 kg of BW. This relationship was weak, although it explains why our growth classification (overall ADG) missed any relationship of ADG to OC. Some studies reported that higher ADG increases risk of OC (Busch and Wachmann, 2011). Other authors could not find this relationship or observed interactions with the diet (Ytrehus et al., 2004, 2007; Quinn et al., 2015; Tóth et al., 2016). Present association is weak but in agreement with Grevenhof et al. (2012), as ADG positively influenced OC up to a BW thershold (90 to 109 kg). In the broader experiment carried out by Fabà et al. (2018), lameness (7.7%) was detected between 106.8 to 129.7 kg of BW, increased with BW, and was related to lowered growth after gilts became clinical lame. Pain and disconfort are known to weaken feeding behavior and reduce ADG (Weary et al., 2009), and even before lameness, OC may lower ADFI by 25% (Munsterhjelm et al., 2015).

The origin of OC is vascular failure, and its first cause is thought to be related with problems when incorporating blood vessels into the advancing ossification, therefore, little support is currently given to other previously suspected factors such as trauma and nutrition (Olstad et al., 2015). Yet intervening causes on OC progression, healing, or heritable factors are still controversial. Present results, together with the previous discussed suggest a role of TM and Met on OC findings in grown gilts.

In conclusion, the dietary treatment did not interact with growth rate groups, but supplementing alone organic Cu, Mn, and Zn for 134 d may increase bone growth, and increased bone density and tibia strength of developing gilts compared with the CON diet. Supplementing high Met alone also increased bone density and had intermediate values of bone bending and breakage strength. The combination MM, presented intermediate bone mineralization and density compared with MIN, but reduced OC total score compared to CON. Finally, the present study supports that ADG can influence OC but explains only 10% of the OC total score variance and does not negate that other factors may be more important.

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CHAPTER V

Effects of supplementing organic micro-minerals and methionine with or without limiting growth during the rearing phase of replacement gilts on lameness, growth, and body composition

Effects of supplementing organic microminerals and methionine with or without limiting growth during the rearing phase of replacement gilts on lameness, growth, and body composition

L. Fabà,† J. Gasa,† M. Tokach,† E. Varella,‡ and D. Solà-Oriol†

†Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra (08193), Spain; †Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201; and ‡Tecnología & Vitaminas S.L., Alforja (43365), Spain.

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ABSTRACT

Previous research suggested that lameness in growing pigs could be reduced with feeding strategies, such as limiting growth rate and supplementing trace minerals (TM) and (or) methionine (Met). The present study evaluated effects of 1) TM and Met, and 2) limiting total lysine (Lys), during the rearing phase (90 d) of gilts (as a means to limit growth rate), on lameness, performance, and sow claw health and productivity (to first parity). Gilts (n = 240; 58.0 ± 11.1 kg body weight) were blocked, distributed into pens of 10 gilts, and pens allocated to a 2 × 2 factorial arrangement. Factors were: 1) **control** or **TM plus methionine** (Met), that provided additional 10, 20, and 50 mg/kg of chelated copper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and a 1.01 Met:Lys ratio and 2) **standard Lys** were formulated to meet growth requirement or **low Lys** to 19% below growth requirements. Feeding was provided through two phases, first between 119 and 163 d of age (phase I), and second between 163 d and 209 d of age (phase II). Diets had 2.43 and 2.31 Mcal net energy/kg, for phase I and II, respectively, and were offered ad libitum. Low Lys did not affect feed intake but reduced 6.35% ADG and 3.80% the final BW compared with standard Lys ($P < 0.001$). Low Lys reduced ADG ($P < 0.001$) and gain:feed ($P = 0.012$) during phase I, but not during phase II. Lameness prevalence was 7.92% during rearing and increased with time ($P < 0.001$). Final BW (151 kg) and ADG (989 g/d) were similar ($P > 0.05$) whether gilts displayed lameness or not. Lameness was low in severity and not affected by dietary factors. However, TM plus Met fed gilts were 19.2 kg heavier ($P = 0.016$) than control at lameness detection. On the sow farm, there was no evidence for differences on lameness and claw lesions among previous dietary treatments. In conclusion, lameness prevalence during the rearing phase was similar independently of TM plus Met supplement, low Lys, or the interaction. Insufficient reduction of ADG and low severity in lameness may have limited the potential of dietary treatments. Moreover, a greater deficiency of Lys would be needed to achieve the degree of growth reduction previously reported to lessen lameness through feed restriction.

Key words: pigs; lameness; growth; minerals, lysine.

INTRODUCTION

Lameness is a persistent problem and a welfare concern in the swine industry with a prevalence reported between 8.8% and 16.9% in sows (Kilbride et al., 2009; Pluym et al. 2013). It is the primary reasons for gilt failure (Engblom et al., 2008; Jensen et al., 2010), and disorders such as osteochondrosis (OC, a defective endochondral ossification) and claw lesions are important causes of lameness in growing pigs and sows (Yazdi et al. 2000; Koning et al., 2015). Additionally, the cost per lame sow is high i.e. estimated to $180 in the US (Deen et al., 2008), and between €290 and €330 in Finland (Niemi et al., 2017).

In a previous study, reduced lameness was observed when supplementing organic trace minerals (TM) copper (Cu), manganese (Mn), and zinc (Zn) in combination or not with high methionine (Met) as 1.02 Met:lysine (Lys) ratio (Fabà et al., 2018). In a subsample study, the additional TM also increased tibia breakage force, bone density, and metacarpal weight and ash content (Fabà et al., 2018). Similarly, Frantz et al. (2008), reported potential effect of TM and Met to reduce OC scores. Furthermore, TM are important for claw health. They are acknowledged as necessary in the keratinization and horn production process by having

catalytic, structural, and regulatory functions in activating enzymes (Riet et al., 2013; Varagka et al., 2016). Nonetheless, extended research support that other factors such as genotype, feed composition, average daily gain (ADG), housing conditions, and mechanical stress interact and greatly influence OC, claw health, and therefore lameness (Orth et al., 1999; Nakano and Aherne 1988; Heinonen et al., 2013; Pluym et al., 2013; Koning et al. 2014a, 2014b; Quinn et al. 2015; Le et al. 2016).

High growth rate has become controversial as a factor increasing risk of OC and lameness in pigs (Busch and Wachmann 2011; Quinn et al., 2015; Koning et al., 2014b) or not (Ytrehus et al., 2004a, 2004b, 2007; Tóth et al., 2016). Therefore, strategies to intentionally limit ADG may have a positive effect on lameness. Previous studies mainly used feed restriction (Quinn et al., 2015; Koning et al., 2014b), however, growth rate could be modulated by altering amino acid density (Rozeboom and Johnston, 2007; Díaz et al., 2017), which would be an easier approach in practical conditions. It was hypothesized that TM and Met dietary supplements during the rearing period of gilts would lessen lameness prevalence, with the benefit further enhanced by limiting ADG through lysine restriction.

The objective of the present study was to evaluate replacement gilt diets on lameness, body composition, performance, and carryover effects on lameness and claws of gilts entering into production using 2 different strategies in a factorial approach: 1) supplementing organic TM (Cu, Mn, and Zn) and Met; and, 2) reducing ADG by lowering dietary Lys by 19%.

MATERIAL AND METHODS

Animal Care and Use

The animals used were produced and housed in north Spain (Lleida) commercial swine facilities. A rearing facility providing gilts to 2 sow sites with similar performance and management conditions. The Ethical Committee on Animal Experimentation (CEEAH) at the Universitat Autònoma de Barcelona reviewed and approved the procedures and protocols for this experiment according to the guidelines of the European Union (Directive 2010/63/EU).

Experimental Design, Housing, and Dietary Treatments

Maternal line gilts (n = 240; DanAvl Dania Hybrid line, Landrace x Yorkshire; DanBred International, Sant Cugat del Vallés, Spain) acquired from a breeding production company were used in a 90 d experiment. Gilts of 15 to 17 wk of age and 58.0 ± 11.1 kg of body weight (BW) were individually identified with ear tags, weighed and blocked into pens of 10. Pens (0.90 m^2/gilt; 60% slatted and 40% solid floor) were randomly allocated to 1 of 4 dietary treatments in a 2 × 2 factorial arrangement being the main factors: 1) TM and Met levels and, 2) Lys concentration. The TM and Met levels were **control** as 10, 40, and 110 mg/kg Cu, Mn, and Zn, respectively, and of inorganic sources and 0.31 to 0.39 Met:Lys ratio or **TM plus Met** that provided the diet with additional 10, 20, and 50 mg/kg of chelated Cu, Mn, and Zn, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and 1.01 Met:Lys ratio. Lysine levels were **standard Lys**, which provided 165 g crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age (phase I), and 140 g CP/kg with 8.0 g Lys/kg between 163 d and 209 d of age (phase II); or **low Lys**, 155 g CP/kg CP with 8.1 g Lys/kg in phase I, and 140 g CP/kg with 6.5 g Lys/kg in phase II. A total of 6 pen replicates per treatment were used. Feed was provided ad libitum and in pelleted form

using a single space dry feeder in each pen. Also, gilts had free access to fresh water provided through drinking nipples. The entire rearing was divided in 2 feed phases of 44 d and 46 d, were diets containing 2,425 and 2,310 kcal net energy (NE)/kg, respectively (Table 5.1). For each experimental period, feeds were formulated to meet or exceed nutrient requirements (FEDNA, 2013) and according to the genetics recommendations (Tybirk, 2015) except for lysine.

Overall rearing phase, 6 gilts died and 4 had non-viable leg conformation. Therefore, the remaining 230 gilts across dietary treatments and blocks were equally divided to 2 similar destination farms. Details of farms management and characteristics were previously described by Fabà et al. (2018). Briefly, the sow farms had similar inventory (1700-1800 sow) and were in the same region (20 km) and integrative company, and fed by the same feed-mill. Equivalent feed and routines were provided. Gilts were penned (5-8 gilts/pen) for 30 d in a quarantine-adaptation barn (4 × 3 m; 40% solid floor and 60% concrete slatted flor). Then, gilts were placed in crates for a minimum of 2 wk before service, which was at least on the second detected heat. After confirm gestation (28 d), gilts and sows were moved to group housing pens of 70 females (as 1.5 m^2/female; 30% solid floor and 70% concrete slatted flor) with electronic feeding system (EFS). The concrete slatted floor areas had 80 mm width beams and 20 mm opening between beams.

The first diet in the sow farm was the phase II control (standard Lys), which was provided for 30 days to all gilts and offered ad libitum. Subsequently, gestation feed formulated to 2,200 kcal NE/kg, and 5.7 g/kg standardized ileal digestible (SID) Lys was provided. From service to 28 d of gestation, feed was individually adjusted according to body composition score (2.2 to 3.2 kg/d). After confirmed gestation, the feeding level was 2.2 kg/d for gilts and a range between 2.3 to 2.5 in multiparous sows depending on body composition score. In the lactation room, sows received 2 kg/d of lactation feed (2,340 kcal NE/kg and 9.3 g/kg SID Lys) until farrowing day, and from the day after onwards feed was progressively increased to ad libitum.

Performance and Body Composition Measurements

Measures of BW, backfat (BF), and loin depth were individually collected every 3 wk (d 119, 141, 164, 184, and 209 of age). The BF and loin depth were collected always by the same trained person at P2 point (6 cm from the midline and the last rib level) using an ultrasound scanner (AV-3000V Digital Handheld Electronic B Ultrasound Scanner, AMBISEA Technology Corp., Ltd; Hong Kong, China). Feed intake was measured by pen on a weekly basis. The ADG, average daily feed intake (ADFI), and gain:feed ratio (G:F) were calculated by pen for each feeding phase and overall. Similarly, SID Lys intake per kg BW gain was also calculated by phases using formulated feed composition.

On the sow farm, BW was measured at first service and BF was collected at service, before farrowing, and at weaning. Productive data was also recorded for the first parity. The reproductive performance included the number of total born, born alive, stillborn, and weaned piglets. The breeding performance recorded were returning to service and weaning-to-estrus interval. Also, removal reasons and the date were recorded. Removals classified as reproductive disorder included not displaying a first estrus, abortion, or consecutive repeating estrus.

Table 5.1. Composition of the experimental diets (phases I and II) offered to growing gilts (as-fed basis), %.

	Phase I (119 to 163 d of age)				**Phase II (**164 d to 209**)**			
	Control		**Trace minerals plus Met**		**Control**		**Trace minerals plus Met**	
Ingredient, kg	**Standard**	**Low Lys**	**Standard**	**Low Lys**	**Standard**	**Low Lys**	**Standard**	**Low Lys**
Corn	20.4	23.8	19.8	22.6	16.5	16.6	15.0	15.6
Wheat	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Barley	25.0	24.8	24.8	25.0	25.0	25.0	25.0	25.0
Wheat middlings	-	-	-	-	6.00	6.00	5.95	6.00
Soybean meal	15.6	13.8	15.8	13.6	7.00	7.55	7.05	7.70
Sunflower meal	6.00	5.65	6.00	6.00	7.00	7.00	7.00	7.00
Bakery byproduct	-	-	-	-	9.15	8.90	10.0	9.25
Fat	2.90	2.45	2.85	2.50	0.30	0.30	0.30	0.30
Calcium carbonate	1.08	1.10	1.06	1.03	1.60	1.60	1.54	1.55
Di-calcium phosphate	1.35	1.38	1.35	1.38	1.01	0.98	1.00	0.99
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Methionine hydroxy-analogue	0.13	-	0.84	0.63	-	-	0.63	0.45
L-lysine HCl	0.50	0.23	0.50	0.24	0.48	0.18	0.48	0.17
L-threonine	0.09	-	0.09	-	0.06	-	0.06	-
L-tryptophan	0.03	-	0.03	-	-	-	-	-
Premix[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Premix trace minerals[2]	-	-	0.10	0.10	-	-	0.10	0.10
Calculated composition, %								

Table 5.1. (Continue)

Net Energy, kcal kg^{-1}	2,425	2,425	2,425	2,425	2,310	2,310	2,310	2,310
Crude protein	16.5	15.5	16.5	15.5	14.0	14.0	14.0	14.0
Total lysine	1.00	0.81	1.00	0.81	0.80	0.65	0.80	0.65
Total methionine	0.39	0.28	1.02	0.82	0.25	0.25	0.80	0.64
Methionine:lysine	0.39	0.35	1.02	1.01	0.31	0.38	1.01	0.98
SID[3] lysine	0.90	0.72	0.90	0.72	0.70	0.56	0.70	0.56
SID methionine	0.36	0.24	0.99	0.79	0.22	0.23	0.77	0.61
SID methionine + cysteine	0.65	0.52	1.28	1.07	0.49	0.49	1.03	0.88
SID tryptophan	0.21	0.17	0.21	0.17	0.15	0.15	0.15	0.15
SID threonine	0.58	0.48	0.58	0.47	0.46	0.41	0.46	0.41
Calcium, %	0.90	0.91	0.91	0.90	1.02	1.01	1.01	1.02
Phosphorus, %	0.59	0.59	0.59	0.59	0.57	0.57	0.57	0.57

[1]Vitamin-minerals premix provided per kg of feed: vitamin B2, 3.5 mg; vitamin B12, 0.035 mg; nicotinamide, 20 mg; folic acid, 1.25 mg; vitamin D3, 2,000 IU; vitamin A, 10,000 IU; vitamin E, 30 mg; vitamin K3, 1 mg; vitamin B1, 1 mg; vitamin B6, 2.4 mg; D-calcium pantothenate, 14 mg; biotin, 0.125 mg; choline chloride, 400 mg; Fe (from FeSO4.H2O), 120 mg; I (from Ca(IO3)2), 0.5 mg; Cu (from CuSO4·5H2O), 10 mg; Mn (from MnO2), 40 mg; Zn (from ZnO2), 110 mg; Se (from Na2SeO3), 0.4 mg; phytase EC 3.1.3.26, 1,500 FTU; and butylhydroxytoluene, 25 mg.

[2]Vitamin-minerals premix provided per kg of feed: 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain)

[3]Standard ileal digestible; calculated using SID coefficients for the various ingredients obtained from the NRC (2012).

Lameness and gait score

During rearing, lameness and gait score were evaluated by studying walking pattern on a hard, solid floor for a distance of 6 m at average age of 119, 141, 164, 184, and 209 d by the same observer. The gait scoring was adapted from Welfare Quality (2009) and Mustonen et al. (2011) methodologies as: not lame (0); moderate difficulties with gilts having reluctance to move and reduced walking speed, or some difficulties with exercise including slight shortened stride to obvious limp (1); and, severe difficulties with gilts being minimum weight-bearing to incapable of standing (2). Also, gilts were classified as lame (obvious limping) or not lame (no limping), and within lame category whether or not were severe (barely bearing weight on the affected limb) for binomial analysis.

On the sow farm, gait score and lameness were evaluated by the same observer in 3 different times: 1) before first service; 2) at d 109 of gestation; and, 3) at weaning. Lameness evaluation was performed during 2 min observation by walking (6 to 10 m) on a solid flor corridor (moving females to the insemination line at first service and at weaning); or 2 min observation inside the group-housing pens in gestation.

Claw lesions

In the farrowing room, females were evaluated for claw lesions following the protocol previously reported by Fabà et al. (2018a). Briefly, the lesions evaluated were: toe dewclaws; heel sole over-growth and cracks; white line; hoof wall cracks; long hoofs; and asymmetry between digit claws. The score grade was: 0 = none, 1 = mild-moderate (without affecting the integrity of the claw), 2 = severe (affecting the integrity of the claw, including gait difficulties or indicating clinical signs of pain). The assessment included all claws and both, outer and inner digits. Overall, only few (n = 5) lesions were classified as severe and final analysis was for complete prevalence independently of severity. The toe dewclaw, white line, and hoof wall crack are lesions associated with the horn properties and influenced after supplementing organic TM (Varagka et al., 2016). Those lesions were also grouped as hoof lesions, including all females that had at least one of the 3 lesions.

Statistical Analysis

The statistical procedures were performed using SAS v9.4 (SAS Inst. Inc., Cary, NC). The experimental unit was gilt for lameness, claw lesions, and number of piglets, whereas pen (10 gilts) was the unit for BW, BF, LD, ADG, ADFI, Lys intake per kg of BW gain, and G:F ratio. The different variables were evaluated for normality and homoscedasticity by using the Shapiro-Wilk and Levene's test and examining the normal plot (PROC UNIVARIATE). For all parametric analysis, the Tukey–Kramer adjustment was used to determine significant ($P < 0.05$) and marginal ($P < 0.10$) differences among dietary treatments.

The BW, LD, BF, and lameness occurrence over experimental time were analyzed using repeated measures models, including dietary treatment and time (days of experimental study) as fixed effects and block as a random effect, using mixed model methods (PROC GLIMMIX). Additionally, the ADG was analyzed using repeated measures with lameness and time as fixed effects and block and pen as the random effects. In this case, it was analyzed separately for the dietary supplements strategy and the limited Lys strategy due to the low

incidence of lameness (PROC GLIMMIX). Furthermore, phase and overall ADG, ADFI, Lys intake per BW gain, and G:F were analyzed using general linear model (PROC GLM).

Sow farm measurements of BW, BF, and sow productivity were analyzed using general linear model (PROC GLM). Claw lesions, grouped hoof lesions and lameness occurrence at different times were analyzed with Binomial model (PROC GENMOD). The Fisher's Exact test was used for testing removal reasons (PROC FREQ). The main fixed effects included dietary treatment and sow farm. Reproductive performance of females classified as lame (eventually displayed lameness) or not lame (never displayed lameness) on the sow farm were analyzed using nonparametric Binomial model (PROC GENMOD).

RESULTS

Limiting the dietary Lys reduced growth, however, neither such reduction, or the dietary supplementation of TM and Met, or combination of the slower growth and dietary supplements were capable of reducing lameness prevalence compared with the control. Although, age and BW at lameness detection were higher on gilts supplemented with TM and Met than on the control gilts. Results are presented first for performance and lameness during rearing, and second for the measurements in the sow farm.

Rearing

The overall performance shown no interactions between limited Lys and dietary supplements. Feeding low Lys did not affect ADFI but reduced overall ADG ($P < 0.001$) by 6.35% and final BW ($P < 0.001$) by 3.80% compared with standard Lys (Table 5.2). According to the different feed phases, gilts fed low Lys had lowered ADG ($P < 0.001$) and G:F ratio ($P = 0.012$) between 119 and 163 d, but not from 163 d to 209 d. There was no evidence of main effects due TM plus Met supplements on gilt ADG, ADFI, or G:F. Throughout the growing phase, gilts fed low Lys had lower BW than standard Lys ($P < 0.05$). However, an interaction at d 184, showed that gilts fed TM plus Met and low Lys were 2.8 kg heavier (SE = 1.796; $P = 0.034$) than control gilts fed low Lys, not different than control gilts fed the standard Lys, but lighter ($P = 0.034$) than gilts fed TM plus Met and standard Lys.

The BF was not different amongst dietary treatments until the end of rearing. Then, an interaction occurred where gilts fed control and low Lys had reduced ($P = 0.049$) BF compared with the gilts fed TM plus Met with low Lys and the control with standard Lys; whilst TM plus Met with standard Lys being intermediate. Interactions for loin depth were observed with some inconsistency, but seemed to align with those of BW at 184 d. Gilts fed low Lys without supplements (control) had lower loin depth than standard Lys ($P < 0.02$), whilst low Lys supplemented was intermediate at 163 and 184 d of age (Table 5.2). Differently, at the end of rearing (d 209 of age), gilts fed TM plus Met with low Lys had lower loin depth than standard Lys in control, with others being not different. More clearly, at 163 and 184 d of age, gilts fed low Lys diets had reduced loin depth compared with gilts fed standard Lys ($P = 0.05$).

Table 5.2. Effects of organic trace minerals plus methionine (Met) and lowering lysine (Lys) dietary treatments provided (90d) to rearing gilts on performance and body composition.

	Control[1]		**Trace minerals plus Met**[1]			*P*-value		
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]	SEM	MinMet	Lys	MinMet × Lys
Body weight, kg								
119 d	58.3	58.1	58.2	57.8	1.796	0.813	0.889	0.940
141 d	87.2^{x}	84.1^{y}	87.2^{x}	85.2^{xy}		0.685	0.047	0.091
163 d	112.1	106.2	112.5	108.0		0.543	< 0.001	0.491
184 d	132.7^{ab}	126.1^{c}	134.5^{a}	129.9^{b}		0.030	< 0.001	0.034
209 d	153.9	146.9	154.3	149.6		0.189	< 0.001	0.141
Backfat depth, mm								
119 d	7.19	7.33	7.04	7.36	0.353	0.805	0.352	0.695
141 d	9.04	9.32	9.04	9.58		0.640	0.145	0.173
163 d	11.2	10.8	11.1	11.5		0.429	0.953	0.348
184 d	12.9	12.7	13.0	13.3		0.270	0.945	0.407
209 d	15.0^{a}	14.2^{b}	14.3^{ab}	14.9^{a}		0.998	0.603	0.049
Loin depth, mm								
119 d	30.1	30.4	29.4	29.9	0.742	0.605	0.628	0.242
141 d	39.9	39.5	39.9	39.6		0.452	0.271	0.729
163 d	49.5^{a}	48.9^{b}	50.2^{ab}	49.2^{ab}		0.939	0.036	0.019
184 d	62.8^{a}	59.7^{b}	64.1^{a}	61.1^{ab}		0.074	< 0.001	< 0.01
209 d	67.7^{a}	66.3^{ab}	66.4^{ab}	65.3^{b}		0.129	0.106	0.030

Table 5.2. (Continue)

Average daily gain, g								
119 to 163 d	1220	1090	1225	1133	17.5	0.172	< 0.001	0.275
163 to 209 d	907	875	908	900	41.5	0.751	0.635	0.781
Overall	1018	944	1028	972	23.4	0.439	0.011	0.705
Average daily feed intake, g								
119 to 163 d	3055	2953	3040	3001	51.2	0.761	0.183	0.554
163 to 209 d	3403	3263	3393	3245	103	0.892	0.179	0.968
Overall	3238	3204	3215	3220	98.8	0.887	0.971	0.885
Gain:feed ratio								
119 to 163 d	0.40	0.37	0.40	0.38	0.010	0.609	0.012	0.807
163 to 209 d	0.27	0.27	0.27	0.28	0.011	0.834	0.727	0.727
Overall	0.32	0.30	0.32	0.30	0.013	0.173	0.905	0.999

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.

[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.

[a-b]Values within a row with different superscripts differ significantly at $P < 0.05$.

[x-y]Values within a row with different superscripts trend at $P < 0.10$.

Results from the lameness assessments are presented in Table 5.3. During the rearing period, lameness prevalence was 7.92% and appeared in a CI between 119.6 and 139.5 kg of BW. The proportion of lameness at a given moment increased with time and BW ($P < 0.001$). The final BW and overall ADG were not different ($P > 0.05$) comparing gilts that eventually undergo lameness (988 g/d and 150.1 kg, respectively) and those that never had signs of lameness (990 g/d and 151.2 kg, respectively).

Lameness prevalence was not different among dietary treatments. However, lame gilts fed the TM plus Met diets were 19.2 kg heavier (SE = 5.04, $P = 0.016$) and 17.9 d older (SE = 5.17, $P = 0.034$) at lameness detection compared with control fed lame gilts. Figure 5.1 presents ADG by phases and for gilts fed TM plus Met that were classified as lame (gilt that eventually undergo lameness) or never-lame (gilt that never had signs of lameness). Between 163 and 184 d of age, ADG was increased ($P < 0.01$) for mineral plus Met strategy (lame or never lame) compared with control strategy never lame gilts, while control lame gilts were intermediate. Late in growth (184 to 209 d of age), lame gilts had reduced ($P = 0.015$) ADG within the control fed gilts, but gilts from TM plus Met were intermediate for both, lame and never-lame gilts.

The same assessment of ADG and lameness but for Lys dietary strategy is presented in Figure 5.2. Between 119 and 141 d of age, lame gilts fed the low Lys and never-lame gilts fed standard Lys had greater ADG than never-lame females fed low Lys ($P = 0.001$). From 141 to 163 d of age, low Lys lowered ($P < 0.001$) ADG compared with standard Lys for never-lame gilts, however, lame gilts were intermediate for both standard and low Lys. Finishing the rearing phase (184 to 209 d of age), lame gilts fed standard Lys tended to have reduced ($P = 0.097$) ADG compared with never-lame gilts on both, standard Lys and low Lys diets; however, lame gilts on low Lys were intermediate.

Sow Farm

Lameness on the sow farm increased to 17.4% at first service, then decreased to 11.3% at the end of gestation, and increased to 15.2% at weaning. There was no evidence of differences for lameness prevalence and severity across dietary treatments, productive phases, or overall (Table 5.3).

The results from first parity performance are presented in Table 5.4, and show no major carryover effects from dietary treatments provided during growth. Yet, gilts fed TM plus Met with low Lys tended to be 16 d younger (SE = 3.63; $P = 0.009$) at first service than TM plus Met with standard Lys. Additionally, the number of born alive piglets tended to show the same interaction with gilts fed TM plus Met and low Lys having lower number of piglets born alive ($P = 0.091$) than control with low Lys and mineral plus Met with standard Lys. A 17.5% lameness at early gestation tended to increase the odds (1.12; CI = 1.251, 1.116; $P = 0.060$) for reduced total born piglets (16.5 vs. 17.7) and increased the odds (1.11; CI = 1.074, 1.00; $P = 0.044$) for reduced piglets born alive (14.8 vs. 16.0). Similarly, the 11.4% lameness observed at late gestation tended to increase the odds (1.12; CI = 1.274, 0.981; $P = 0.094$) for less total born (16.4 vs. 17.6), and had greater odds (1.15; CI = 1.282, 1.02; $P = 0.017$) for reducing the number of piglets born alive (14.2 vs. 16.0) compared with non-lame females. Furthermore, 11.4% lameness from late gestation and 15.3% at weaning, weakly increased the odds (1.04; CI = 1.008, 1.077; $P = 0.015$; and 1.03; CI = 1.001, 1.067; $P =$

0.040, respectively) for greater weaning to estrus interval days than non-lame females (13.1 vs. 6.17 and 11.1 vs. 6.21, respectively).

Table 5.3. Effects of trace minerals plus methionine (Met) and lowering lysine (Lys) dietary treatments provided (90d) to rearing gilts [from 58.0 ± 11.1 to 151.15 ± 14.1 kg of body weight (BW)] on lameness during rearing and sow productive phases.

	Control[1]		**Trace minerals plus Met**[1]			*P*-value		
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]	SEM	MinMet	Lys	MinMet × Lys
Gilts, n	59	58	56	57				
BW at lameness, kg	120.2	119.8	134.2	144.4	7.75	0.016	0.515	0.456
Age at lameness, d	173.8	175.8	185.3	198.2	6.92	0.034	0.346	0.462
Lameness, %								
During rearing[3]	6.67	8.33	10.0	6.67	19.530	0.775	0.834	0.456
Early gestation[4]	15.5	22.6	14.1	17.6	14.98	0.587	0.309	0.746
Late gestation[5]	15.6	12.1	7.10	10.6	17.76	0.246	0.859	0.421
Lactation[6]	17.1	19.1	8.88	15.8	16.04	0.211	0.304	0.503
Overall	20.5	32.8	19.6	24.0	13.49	0.447	0.532	0.625
Gait score, 0-2								
During rearing	0.15	0.13	0.15	0.15	0.052	0.854	0.789	0.152
Early gestation	0.22	0.29	0.19	0.18	0.124	0.272	0.763	0.486
Late gestation	0.19	0.17	0.09	0.12	0.057	0.140	0.742	0.587
Lactation	0.24	0.26	0.11	0.21	0.096	0.108	0.222	0.344
Overall	0.19	0.23	0.14	0.15	0.046	0.284	0.681	0.871
Severity, %								
During rearing	-	-	-	-	-	-	-	-
Early gestation	39.1	31.2	21.5	7.76	16.40	0.185	0.439	0.792
Late gestation	24.4	54.3	25.0	16.7	21.26	0.364	0.956	0.630
Lactation	47.0	67.7	42.9	32.8	22.90	0.410	0.607	0.793
Overall	66.7	53.8	46.1	38.8	17.01	0.360	0.716	0.534
All severe cases, %	13.7	17.3	8.88	10.6	21.80	0.726	0.449	0.449

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%; Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.

[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.

[3]During the rearing phase, lameness was evaluated for 60 gilts per dietary treatment; for other measurements, it is indicated in the table.

[4]Evaluated at service and at 28 d of gestation.

[5]Evaluated at 109 d of gestation.
[6]Evaluated at weaning.

Table 5.4. Effects of organic trace minerals plus methionine (Met) and lowering lysine (Lys) dietary treatments provided (90d) to rearing gilts (from 58.0 ± 11.1 to 151.15 ± 14.1 kg of body weight) on first parity performance.

	Control[1]		**Trace minerals plus Met**[1]			*P*-value		
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]	SEM	MinMet	Lys	MinMet × Lys
Gilts, n	59	58	56	57				
Service								
BW[3], kg	181.3	180.3	184.4	179.1	2.66	0.721	0.228	0.430
BF[4], mm	15.6	15.8	15.9	15.0	0.47	0.700	0.537	0.188
Farrowing								
BF, mm	14.4	13.8	14.4	14.9	0.46	0.206	0.936	0.195
Rate, %	91.4	82.6	87.8	89.5	4.39	0.373	0.669	0.212
Total born, n	17.3	17.4	17.4	16.4	0.59	0.403	0.351	0.332
Born alive, n	15.6^{xy}	16.3^{x}	16.2^{x}	15.2^{y}	0.58	0.627	0.740	0.091
Stillborn, %	9.3	7.03	5.18	7.68	1.62	0.264	0.933	0.125
Weaning								
BF, mm	12.5	11.7	12.1	12.8	0.42	0.370	0.984	0.091
Weaned, n	14.2	14.4	13.3	14.0	0.55	0.208	0.371	0.672
Days to estrus	6.60	5.92	6.84	8.75	1.54	0.660	0.294	0.401

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.
[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.
[3]BW = body weight.
[4]BF = backfat depth.

The percentage of removals and removal reasons during the first parity are presented Table 5.5. Thirty-one out of 230 females (13.5%) did not reach a second gestation. Of these, 68.1% were sudden deaths, whereas 19.4% were removed due to reproductive problems, 6.9% for lameness, and 5.6% for other reasons. Over the productive phases, 16.7% of those were in gestation, 59.7% during lactation (all sudden deaths), and 23.6% at weaning. Culling and mortality shown no evidence for differences across dietary treatments. Although, removals due to reproductive reason tended to be less frequent amongst gilts fed low Lys strategy (6.25%) than control Lys (33.3%; $P = 0.083$). Additionally, an interaction occurred

with gilts fed TM plus Met and low Lys tending ($P < 0.10$) to have highest incidence of sudden death removals and total removals in lactation.

Table 5.5. Effects of organic trace minerals plus methionine (Met) and lowering lysine (Lys) dietary treatments provided (90d) to rearing gilts (from 58.0 ± 11.1 to 151.15 ± 14.1 kg of body weight) on removal reasons during the first parity.

	Control[1]		**Trace minerals plus Met**[1]			*P*-value		
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]	SEM	Min Met	Lys	MinMet × Lys
Gilts culled, n	6	9	9	7				
Removals, %	10.2	15.5	16.1	12.3	4.59	0.847	0.999	0.758
Sudden death, %	50.0	77.8	44.4	100	18.60	0.999	0.736	0.069
Reproductive, %	33.3	11.1	33.3	0.0	10.60	0.685	0.083	0.365
Lameness, %	16.7	11.1	0.0	0.0	10.62	0.226	0.990	0.554
Others, %	0.0	22.2	22.2	0.0	9.90	0.484	0.226	0.232
Phases								
Gestation, %	33.3	11.1	22.2	0.0	15.73	0.654	0.172	0.488
Lactation, %	50.0	44.4	44.4	100	19.26	0.285	0.285	0.074
Post-weaning, %	16.7	44.4	33.3	0.0	17.60	0.433	0.999	0.219

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.
[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.

Claw Health

Incidence of claw lesions among dietary treatments was evaluated for 87.8% of the females and results are presented in Table 5.6. Of all sows evaluated, 52.4% had at least 1 claw lesion whereas 24.8% of them had 2 lesions or more. Heel lesions were the most common claw lesions (27.0%, together overgrowth and heel cracks). This was followed by lesions of asymmetry (16.1%), hoof wall cracks (10.9%), white line (7.83%), dewclaws broken or missing (4.78%), and long hoofs (3.49%). The grouped hoof lesions (wall cracks, white line and dewclaw) had 23.3% prevalence. When compared across dietary treatments, no differences were observed for any type of lesion.

Table 5.6. Effects of organic trace minerals plus methionine (Met) and lowering lysine (Lys) dietary treatments provided (90d) to rearing gilts (from 58.0 ± 11.1 to 151.15 ± 14.1 kg of body weight) on claw lesions evaluated in lactation room.

	Control[1]		**Trace minerals plus Met**[1]					
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]	SEM	Min Met	Lys	MinMet × Lys
Gilts, n	54	51	46	51	-	-	-	-
Claw lesions[3]	48.1	45.1	54.3	49.0	12.01	0.573	0.673	0.842
Heel growth	31.5	27.5	30.4	33.3	12.97	0.883	0.999	0.875
Asymmetry	18.5	7.8	28.3	19.6	16.33	0.106	0.150	0.097
Wall cracks	11.1	13.7	15.2	9.80	17.60	0.999	0.914	0.921
White line	9.26	9.80	6.52	9.80	20.01	0.816	0.808	0.904
Dewclaw	5.56	7.84	6.52	1.96	26.06	0.539	0.989	0.650
Long hooves	5.56	0.00	4.35	5.88	90.01	0.494	0.723	0.338
Grouped hoof	20.4	25.5	28.3	19.6	12.23	0.990	0.868	0.717

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.

[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.

[3]Claw lesions: heel growth = lesions of excessive growth and cracks on of the heel-sole tissue; asymmetry = unevenness between digit claw size; wall cracks = vertical and horizontal cracks or fissures on the hoof wall; white line = hemorrhagic lesions at the level the junction of wall and sole horn; dewclaw = dewclaw broken or lost; Long hoofs: enlarged size of the hoofs; grouped hoof lesions = include wall cracks, white line and dewclaw lesions.

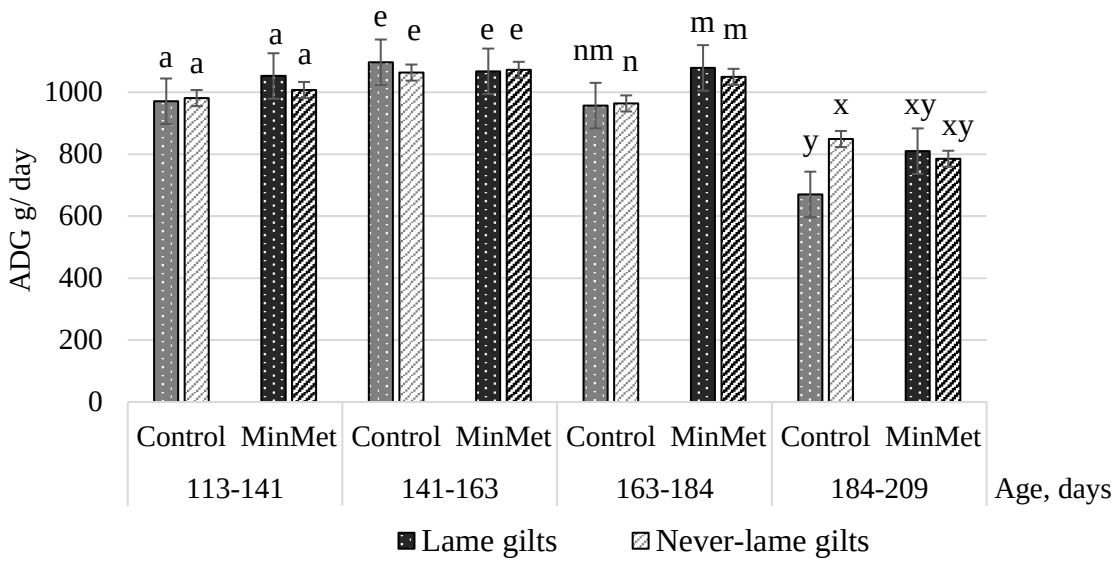

Figure 5.1. Average daily gain (ADG) according to different age phases for control (grey) or dietary supplemented[1] (black) gilts comparing between females that never showed lameness (n = 230) and gilts that became lame (n = 19) during the rearing period.
[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.
[a-b]Different superscripts differ significantly at $P < 0.05$; within 119-141 d ADG.
[e-f]Different superscripts differ significantly at $P < 0.05$; within 141-163 d ADG.
[m-n]Different superscripts differ significantly at $P < 0.05$; within 163-184 d ADG.
[x-z]Different superscripts differ significantly at $P < 0.05$; within 184-209 d ADG.

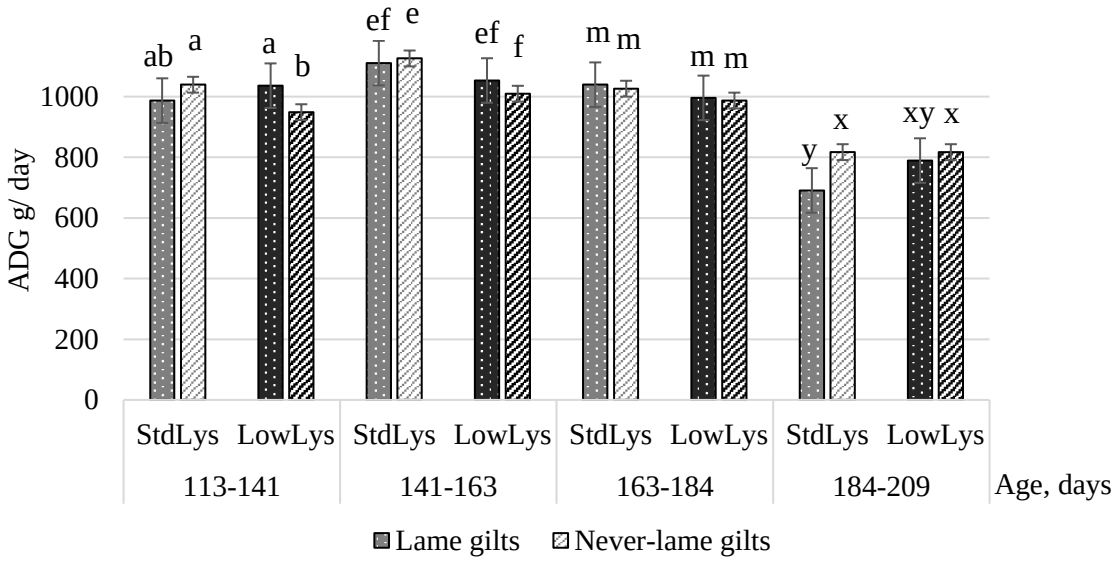

Figure 5.2. Average daily gain (ADG) according to the different age phases for gilts fed standard (grey) or low lysine[1] (black) comparing between females that never showed lameness (n = 230) and gilts that became lame (n = 19) during the rearing period.

[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.
[a-b]Different superscripts differ significantly at $P < 0.05$; within 119-141 d ADG.
[e-f]Different superscripts differ significantly at $P < 0.05$; within 141-163 d ADG.
[m-n]Different superscripts differ significantly at $P < 0.05$; within 163-184 d ADG.
[x-z]Different superscripts differ marginally at $P < 0.10$; within 184-209 d ADG.

DISCUSSION

In agreement with our expectations, the diets with lower Lys reduced ADG. Conversely, neither this, nor the supplement of TM plus Met, or both strategies together affected lameness prevalence. The only effect aligned with our hypotesis might be a delay in lameness occurrence for the gilts supplemented with TM plus Met.

The lack of ADG effect on lameness was, to some extent, not surpirzing since a positive relationship between ADG and lameness was not detected in our previous study (Fabà et al., 2018), and because the relationship between ADG and OC is somewhat inconsistent (Orth et al., 1999; Ytrehus et al., 2007; Olstad et al., 2015). Some research support restrictive growth via dietary strategies (i.e. low energy, low Lys, and/or feed restriction) with a positive effect on reducing OC (Reinald, 1975; Goedegebuure et al., 1980; Koning et al., 2014b) and lameness (Quinn et al., 2015). Whereas other studies do not support such effects (Grøndalen, 1974a; Nakano et al., 1984, Woodard et al., 1987, Carlson et al.,1988). Busch and Wachmann (2011) associated fast ADG with increasing risk of OC whilst others did not (Ytrehus et al., 2004a, 2004b; Tóth et al., 2016); suggesting that other factors (i.e. genetics, age, or even location) are likely more important. For example, Grevenhof et al. (2012) observed inconsistent association between ADG and OC amongst joints. Heavier gilts had more lesions in the elbow, but lighter females had more on femoropatellar joint.

Inherited traits associate OC with fast growth (Grøndalen et al., 1974b; Jørgensen and Andersen, 2000; Kadarmideen et al., 2004; Aasmundstad et al., 2013). However, if the association between fast growth with OC is related to mechanical stress and overload on the joints of growing animals or simply a genetic correlation for the pathology is less clear. Indeed, it was earlier noted that physiological biomechanical forces would be sufficient to affect OC outcome (Ytrehus et al., 2007; Etterlin et al., 2014; Olstad et al., 2015); whilst the initial vascular disruption cause of OC still needs to be elucidated (Olstad et al., 2015). Therefore, a great degree of reduction may be required to lessen OC via weight load reduction, and perhaps, the present lowering was insufficient as low Lys only reduced final BW by 3.8%. Quinn et al. (2015), via feed restriction, found that reducing final BW by 7.30% was associated with less lameness and OC. Koning et al. (2014b), found that gilts fed ad libitum between 4 and 26 wk of age (final BW 132 kg) had higher odds of OC than those fed ad libitum from 4 to 10 wk and then restricted from 10 to 26 wk (reduced 16% BW). Authors, also reported that initially restricting (4 to 10 wk) followed (10 to 26 wk) by ad libitum feeding (reduced 3.0% BW) increased odds of OC compared with initially (4 to 10 wk) ad libitum and followed (10 to 26 wk) by restriction (reduced 16% BW), or restricted throughout (reduced 19% BW). Therefore, assuming that slower ADG can minimize lameness, the

reduction in ADG in the present study is notable less than reduction in studies where benefits were reported.

In agreement with previous works, lowered Lys (Cia et al., 1998; Díaz et al., 2017), or Lys and CP (Li et al., 2018) in the diet reduced growth and loin area and (or) depth. Nonetheless, these authors reported greater BW reductions (7 to 12%) than herein (3.80%). Díaz et al. (2017), used differing total Lys concentrations (low, medium, or high) as 0.81, 0.90, and 0.99%, respectively, from 100 to 142 d of age and 0.65, 0.70, 0.76%, respectively, from 143 to 212 d of age. Hence, our treatments provided similar Lys to their low Lys and high Lys levels (Table 5.1). However, present diets (with wheat, barley, corn, soybean meal, sunflower meal, and wheat byproducts) contained 2.3 to 2.4 Mcal/kg NE, and were lower than the 2.5 to 2.9 Mcal/kg NE in high Lys diets (corn-soybean based) from Díaz et al. (2017). The higher Lys:energy and fiber content herein might result in a relatively high feed and Lys intake, as lower energy density is the first intake determining factor (NRC, 2012; Li and Patience, 2017). As calculated, gilts in standard Lys consumed 25 g SID Lys/kg gain whereas low Lys consumed 19 g SID Lys/kg gain up to 163 d of age. According to Shelton et al. (2011), this would be near or slightly below sufficiency. Altogether, high intake even with low Lys density in the diet could explain the moderate but not severe reduction of BW and the lack of ADG differences in the second feed phase. A greater degree of limiting dietary Lys or ratio to energy would be needed to properly test herein hypothesis.

Interactions for BW at d 184 and loin depth at d 163 and 184 occurred where values were greater for gilts fed control Lys and TM plus Met than for gilts fed control and low Lys. We give little importance to these interactions as differences were small. Although the reason behind the interactions is unknown, high dietary Met could support catabolism for some amino acids if control with low Lys was deficient, however, this is not expected with the present diets. Otherwise, supportive properties to immune system attributed to Met, or antioxidant effect, may have enhanced gilt growth under Lys deficiency (Brosnan et al., 2006; Li et al., 2007). In fact, despite extensive research, additivity, and environmental conditions impacts on amino acid requirements remain poorly defined in practical diets (NRC, 2012).

Providing extra trace minerals plus Met did not reduce lameness prevalence whilst its occurrence seemed to be delayed. Furthermore, lame gilts in control diets tended to have lower ADG than never-lame gilts by the end of rearing, whereas lame gilts fed TM plus Met were intermediate; suggesting little or null intervention. These results contrast with our previous study where identical supplementation of TM and Met, in combination or separately, reduced lameness. Likewise, in the subsample study, the TM alone enhanced bone strength and density, and combined with Met improved OC lesions score (Chapter IV).

Over the years, bone and joint development were studied to enhance bone health and reduce lameness, however, controversy is commonly reported among intervening factors. Deficiencies in TM such as Zn, Cu, and Mn (Ott and Asquith, 1989; Shaw et al., 2006; Veum et al, 2009) and amino acid Met (Huang et al., 2014) used herein, would negatively influence bone development. Conversely, use of TM above requirements is noted as non-effective (Orth, 1999; Creech et al., 2004; Gowanlock et al., 2013; Tóth et al. 2016), or with limited benefits on bone metabolism (Liu et al., 2016), and bones and joint lesions (Frantz et al., 2008; Quinn et al., 2015; Chapter IV). Similarly, benefits had been documented with Met (Frantz et al., 2008; Ouattara et al., 2016). The present results and published data maintain

controversy and do not support a clear benefit from TM supplement on growing pigs' lameness. Using similar TM and levels to this study (all above NRC, 2012), Tóth et al. (2016) evaluated inorganic sources with additional organic ones (150 + 50 g/kg Zn, 50 + 20 mg/kg Cu, and 16.5 + 10 mg/kg Mn). Results showed no improvements in OC either early (12 wk) or later (24 wk) in the pigs' life. Further, only few severe cases of OC (3 out of 200) were detected at 24 wk of age. To discuss such results and the difference between present and our former study, lameness severity may be key. In fact, whereas in the previous study the control gilts had a lameness prevalence of 14.8%, in the present, lameness prevalence in control gilts was 6.67%.

Lameness during the rearing period (7.92%) was similar to that in our previous study (7.75%, Fabà et al., 2018), although, herein was not associated with performance loss. Lame gilts in our previous study had reduced ADG (90 g/d) and final BW (7.0 kg) compared with never-lame gilts. Such effects on performance would indicate high severity (Weary et al., 2009; Munsterhjelm et al., 2015), while absence of performance differences between lame and never-lame in the present study suggests low severity. These differences were unexpected because facilities, genetics, and observer were the same as in our previous study. Different breeder multiplier may partly explain differences because OC is heritable, and more concentrated within populations (Aasmundstad et al., 2013; Le et al., 2016). Although it cannot be confirmed, a lower severity of lameness in the present study may explain the lack of more consistent effects.

When entering into the sow herd, lameness prevalence doubled in our studies. This highlights the high susceptibility of young females (Pluym et al., 2013). The challenge of entering into the sow farm increased lameness similarly for all dietary treatments. Therefore, our previous (Fabà et al., 2018) and present data suggest that dietary supplements do not act as a carryover measure to reduce lameness and claw problems. Different facilities, wider slat void, floor type, social conflicts from management (i.e. movements, animal flow, density, remixing etc.) or training for the electronic feeding system; were all new factors also known to increase lameness and claw lesions (Pluym et al., 2011; Li and Gonyou, 2013; Olsson et al., 2016). Still, if lameness prevalence was to be reduced through TM during rearing, this would enhance latter productivity (Fabà et al., 2018). Data does not negate that TM supplements in sows may reduce lameness via improving claw horn properties as previously reported (Varagka et al., 2016), likely, a continued supplement may provide a better approach. Otherwise, the change may be too severe challenge to find a response. These data indicate that lameness, should be first addressed through management and environment, especially for young sows.

In conclusion, during the rearing phase and under the present conditions, the organic TM (Cu, Mn, and Zn) and methionine supplements, or the standard and low Lys, or combined treatments did not affect prevalence of lameness. Absence of performance loss in lame gilts and low gait scorings suggests low lameness severity during rearing, which may have limited dietary treatments potential. Nonetheless, sow herd lameness increased odds for reduced born alive piglets and increased weaning to estrus interval. The dietary treatments during rearing did not improve sow lameness and claw health into first parity but prevalence increased dramatically, likely due to management and environmental factors. The present low Lys, reduced growth to a lesser extent compared with previous studies that documented reduced

OC and lameness via feed restriction. A greater deficiency of Lys than herein would be needed to further test the potential of the present hypothesis.

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CHAPTER VI

Effects of dietary vitamin A, tryptophan and threonine, or fish oil on performance, viral-load, and acute immune response in growing gilts under Porcine Reproductive and Respiratory Syndrome virus

Effects of dietary of vitamin A, tryptophan and threonine, or fish oil on performance, viral-load, and acute immune response in growing gilts under Porcine Reproductive and Respiratory Syndrome virus

Lluís Fabà,† Enric Mateu,‡ Josep Gasa,† Mike D. Tokach,|| and David Solà-Oriol†

†Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; ‡Centre de Recerca en Sanitat Animal CReSA, Department of Animal Health and Anatomy, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain; and, ||Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201.

ABSTRACT

One hundred gilts [46.6 ± 3.57 kg of body weight (BW)] naïve of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) were BW blocked, distributed as 10 gilts/pen and allotted to 4 dietary treatments: 1) Con, control, 2) VitA, high vitamin A (18,000 IU/kg), 3) TT, increased tryptophan (0.34) and threonine (0.80) ratios to lysine, and 4) Ω3, omega-3 (10 g/kg fish oil); PRRSv infected 11 d post-allotment. The 5) SH, as observational acute-sham was fed the Con diet in a PRRSv negative separate facility. Feed was offered ad libitum for 89 d. Weight and serum samples were collected repeatedly. Using Real-time PCR, serum viral load and the area under the curve were calculated. Serum concentration of interferon-α (IFNα), tumor necrosis factor-α (TNFα), interleukin (IL) 6, IL8, and IL1β were evaluated at d post-infection (dpi) 1, 2, 4, and 7 for a subsample of 11 gilts/treatment. A 100% viremia was observed at 2 dpi. On average, infected gilts were 7.8 kg lighter than SH gilts at 9 dpi, and 12.6 kg at 29 dpi. Gain 9 dpi of gilts fed TT was greater than Ω3 ($P = 0.036$), and tended to be greater than Con ($P = 0.054$); with VitA being not different to Con, TT, or Ω3. Gain 9 to 15 dpi was greater ($P = 0.041$) for TT than the Con, with VitA and Ω3 being intermediate. Also, TT tended to have greater gain compared with Ω3 until 59 dpi ($P < 0.10$); without differences to other treatments. Viral load was not different across dietary treatments. Gilts fed VitA had highest ($P < 0.05$) IFNα at 4 dpi, and higher ($P < 0.05$) IL8 at 7 dpi than Con and Ω3. Similarly, IL6 at 7 dpi was highest for TT ($P < 0.01$). Individual cytokine kinetics did not explain performance differences. Although, using multivariate regression with diets, viral load, and cytokines; gain 9 dpi increased when IFNα at 2 dpi was above the median and decreased with higher viral load. In conclusion, feeding TT before, during, and after a PRRSv infection marginally improved 9 dpi gain compared with Con and Ω3; which may persist versus Ω3 treatment to 59 dpi. Acute-inflammatory response amongst dietary treatments did not translate to growth performance, and the wide range of response should be further investigated.

Key words: Inflammation; infection; PRRS; growing pigs; performance.

INTRODUCTION

Pig production systems strive to minimize disease and control herd's health. Still, in commercial sites pigs face a variety of immune challenges, which often are not taken into account nutritionally. In fact, rearing and quarantine phases often expose replacement gilts to stress factors including long transports, multiple vaccinations, new environments, and a broad spectrum of pathogens (Batista et al., 2002: Garza-Moreno et al., 2017). A major example is the Porcine Reproductive and Respiratory Syndrome (PRRS), causing reproductive impairment in sows and respiratory distress in all pig ages (Neumann et al., 2005) with an estimated cost over $600 million annually to the US industry (Holtkamp et al., 2013).

Traditionally, strategies to control diseases have been biosecurity, vaccine prophylaxis, and antimicrobials. Over the last 10-15 years, the feed additives caught research attention as alternatives to antimicrobials. Such strategies shown wide variety of response and usually aim to reduce pathogen load, enhance immunity, and generally improve gut health, digestive function, and set beneficial microbes (Adewole et al., 2016; Celi et al., 2017; Pluske et al., 2018). Additionally, research has mostly focused on nursery piglets (Escobar et al., 2006; Wang et al., 2006; Lange et al., 2010; Che et al., 2011; Liu et a., 2013; Moran et

al., 2017), and lesser on older pigs and critical situations such as gilt acclimation, multiple-vaccinating, or outbreaks (Gabler, 2014, 2016; Speiser at al., 2015; Schwee et al., 2018).

Early in viral infections (0-14 d), as for PRRS virus (PRRSv), the innate immune response activates Toll-like receptors that downregulate oxidative phosphorylation in favor of glycolysis (Tannahill et al., 2013; Badaoui at al., 2013). Immune proteins (i.e. cytokines and acute-phase proteins) are synthetized and released to the bloodstream (Lunney et al., 2010). Inflammation also increases energy and amino acids demand, which are followed by protein catabolism and antibody production (Che et al., 2011: Gabler, 2014). Altogether, this acute phase antagonizes anabolic growth factors which ultimately suppresses growth and increases morbidity (Spurlock, 1997; Broussard et al., 2003).

Anticipating to inflammation and metabolic changes may be a successful strategy to mitigate the negative impact of disease on pig performance (Greiner at al., 2001; Liu et al., 2013; Rochell et al., 2015; Moran et al., 2016). Vitamin A metabolites, are key for the immune capacity and participate in wide variety of roles of innate and adaptive immune responses (Kim et al., 2004; Villamor and Fawzi, 2005; Kunisawa et al., 2013). Amino acid threonine (Thr) is a major component in g-globulin; and similarly, it is suggested that tryptophan (Trp) demand increases during inflammation (Cuaron et al. 1984; Li et al. 2007; Le Floc'h et al. 2008, 2009). Similarly, the omega-3 polyunsaturated fatty acids are reported to improve immune cells functionality and reduce cellular inflammation (Liu et al., 2003; Calder et al., 2008; Li et al., 2014).

We hypothesize that supplementing growing gilts with vitamin A, the amino acids Trp and Thr, and fish oil rich as source of omega-3 may improve BW gain during PRRSv infection.

MATERIAL AND METHODS

The Ethical Committee on Animal and Human Experimentation (CEEAH) at the Universitat Autònoma de Barcelona reviewed and approved the procedures and protocols for this experiment according to the guidelines of the European Union (Directive 2010/63/EU).

Animals and Experimental Design

A batch of young gilts (Landrace × Yorkshire, DanBred Internacional, Sant Cugat del Vallés, Spain) were acquired from PRRSv positive origin, which strain was endemic in the destination sow farm. One hundred [98.1 ± 6.43 d of age and 46.6 ± 3.57 kg body weight (BW) selected to be naïve to the virus, were individually identified, weighed and blocked as 10 gilts/pen. Pens were randomly allocated to 1 of 4 dietary treatments (exposed to PRRSv) and a fifth sham group (not exposed observational): 1) Con, a negative control; 2) VitA, high vitamin A (formulated to 40,000 IU/kg); 3) TT, increased ratios of Trp and Thr to lysine (Lys) as 0.34 and 0.80, respectively; and 4) Ω3, additional omega-3 (10 g/kg fish oil); and, 5) SH, acute-phase sham that was provided the Con diet in a separate facility PRRSv negative.

For the allotment, gilts were confirmed negative to antibodies and virus in serum using commercially available kits: ELISA (HerdChek PRRS 2XR; Idexx Laboratories) and Real-Time Quantitative Reverse Transcription PCR (qRT-PCR, LSI VetMAX™ PRRSv EU/NA Kit, Life Technologies, Carlsbad, CA). At experimental d 11, Con, Vita, TT, and Ω3 were exposed to PRRSv serum (10^5 to 10^6 genomic copies of PRRSv ml $^{-1}$). From here

onwards, days indicate to day post-infection (dpi). At –7 and –1 dpi, gilts were negative to PRRSv. Therefore, gilts in challenging environment with PRRSv consumed the experimental diets at least 10 d before viremia. Additionally, virus was isolated in porcine alveolar macrophages and classified as European genotype by sequencing of ORFs 2–7.

Because all gilts were required to be PRRSv immunized in the destination sow farm, the SH gilts were PRRSv vaccinated (AMERVAC PRRSV, Laboratorios Hipra SA, Amer, Spain) at experimental d 40. Comparing SH with Con, VitA, TT, and Ω3 was only observational because the design was not complete. Feed was offered ad libitum and in pelleted form using a single space dry feeder in each pen. Also, free access to fresh water was provided through drinking nipple. Pen density was 0.90 m^2/gilt with 60% slatted and 40% solid floor. Feeding was in two different phases from –11 to 9 dpi and from 10 to 79 dpi (Table 6.1) and formulated to meet or exceed nutrient requirements (FEDNA, 2013) for gilts according to the genetic suppliers' recommendations (Tybirk, 2015).

Table 6.1. Composition of the experimental diets (phases I and II) offered to growing gilts (as-fed basis), %.

	Treatment							
	Phase I (0-20 d)				Phase II (21-91 d)			
Ingredient	**Con, SH**	**VitA**	**TT**	**Ω3**	**Con, SH**	**VitA**	**TT**	**Ω3**
Corn	25.6	25.6	25.0	25.4	25.7	25.7	23.1	25.0
Wheat	25.0	25.0	25.0	25.0	22.0	22.0	25.0	14.4
Barley	20.5	20.5	21.4	20.0	25.5	25.5	25.5	33.0
Soybean meal	17.7	17.7	17.0	17.9	16.3	16.3	15.6	16.5
Canola meal	3.0	3.0	3.0	3.0	6.0	6.0	6.0	6.0
Fat	2.9	2.9	2.9	2.4	0.5	0.5	0.5	0.2
Calcium Carbonate	1.0	1.0	1.0	0.9	1.1	1.1	1.0	1.0
Di-calcium phosphate	1.1	1.1	1.1	1.2	1.5	1.5	1.5	1.4
Sal	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Fish oil	-	-	-	1.0	-	-	-	1.0
Methionine	0.13	0.13	0.14	0.13	-	-	-	-
L-lysine HCl	0.57	0.57	0.59	0.56	0.27	0.27	0.29	0.26
L-threonine	0.23	0.23	0.43	0.23	0.06	0.06	0.23	0.06
L-tryptophan	0.03	0.03	0.20	0.03	-	-	0.14	-
Premix[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Analyzed composition								
Moisture, %	10.6	10.4	10.4	10.2	10.8	10.6	10.6	9.89
Net Energy*, kal/kg	2550	2550	2550	2550	2425	2425	2425	2425
Crude protein, %	17.2	17.5	17.2	17.4	15.7	15.6	15.6	15.9
Crude fiber, %	3.44	3.32	3.17	3.37	3.40	3.61	3.53	3.43
Crude fat, %	3.85	3.60	3.74	3.65	2.98	2.85	2.80	3.00
Lysine, %	1.12	1.15	1.10	1.13	0.97	0.96	0.90	0.99
Threonine %	0.78	0.74	0.94	0.75	0.65	0.69	0.75	0.68

Table 6.1. (Continue)

Tryptophan, %	0.22	0.23	0.36	0.20	0.19	0.19	0.30	0.21
Calcium*, %*	0.76	0.76	0.76	0.75	0.92	0.92	0.91	0.90
Phosphorus*, %	0.52	0.52	0.51	0.53	0.60	0.60	0.59	0.59
Vitamin A, IU	6000	19000	5000	5000	5000	18000	5000	7000
Omega-3, %	0.074	0.079	0.078	0.172	0.079	0.078	0.073	0.159

[1] Vitamin-minerals premix provided per kg of feed = vitamin B2, 3.5 mg; vitamin B12, 0.035 mg; nicotinamide, 20 mg; folic acid, 1.25 mg; vitamin D3, 2,000 IU; vitamin A, 10, 000 IU; vitamin E, 30 mg; vitamin K3, 1 mg; vitamin B1, 1 mg; vitamin B6, 2.4 mg; D-calcium pantothenate, 14 mg; biotin, 0.125 mg; choline chloride, 400 mg; Fe (from FeSO4.H2O), 120 mg; I (from Ca(IO3)2), 5 mg; Cu (from CuSO4·5H2O), 10 mg; Mn (from MnO2), 40 mg; Zn (from ZnO2), 110 mg; Se (from Na2SeO3), 0.4 mg; phytase EC 3.1.3.26, 1,500 FTU; and butylhydroxytoluene, 25 mg.
*Calculated values.

Measurements and blood samples

Figure 6.1 schemes the design and data collection. Individual BW was collected on experimental dpi –11, 0, 9, 15, 22, 29, 37, 43, 49, 59, 71, 78. The feed intake was weekly registered by pen and the data used to calculate ADFI and G:F was only after 0 dpi. Blood samples were collected for all gilts via venipuncture from the jugular vein using siliconized blood-collecting tubes (5 ml), at dpi –7, –1, 1, 2, 4, 7, 10, 14, 21, 28, 34 and 56. All serum samples were used to measure viremia and calculate viral load by PRRSv qRT-PCR (LSI VetMAX™ PRRSv EU/NA, Life Technologies, Carlsbad, CA). Because directly threshold cycles (C_t) values from qRT-PCR are contra-intuitive, viral load was expressed as the difference between total C_t in the qRT-PCR test minus actual result C_t (40 – sample C_t). The area under the curve (AUC) was calculated to represent viral load. From this, greater the value greater the viral copies.

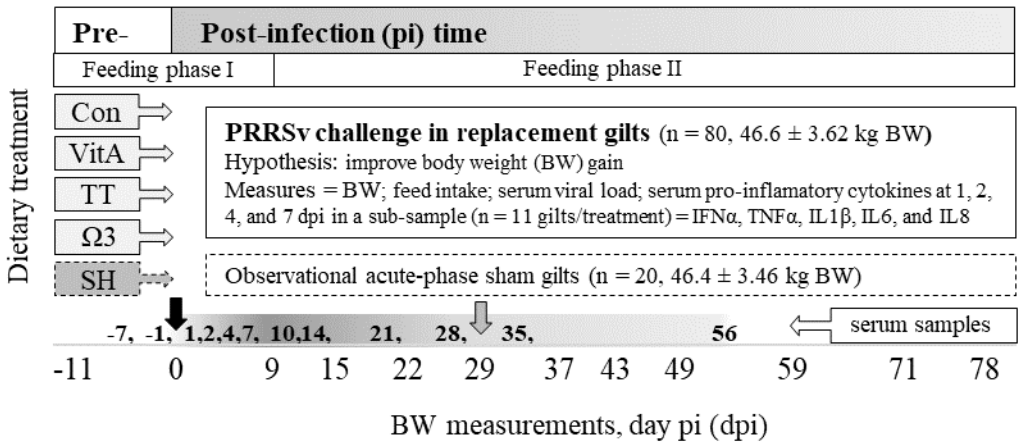


Figure 6.1. Timeline design to study 4 dietary treatments[1] and a sham[2] group of gilts under Porcine Reproductive and Respiratory Syndrome virus (PRRSv)[3].
[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3,

adding omega-3 (10 g/kg fish oil). Gilts were PRRSv infected at 58.8 ± 5.32 kg BW (black arrow).
[2]Sham = SH, acute-phase sham in separate facilities, vaccinated (AMERVAC PRRSV, Laboratorios Hipra SA, Amer, Spain) at 138 ± 6.43 d of age (grey arrow).
[3]Origin and destination farms were PRRSv positive for the same PRRSv1 strain.

A randomly selected subsample of 11 gilts per treatment was used to evaluate the immune response at 1, 2, 4, and 7 dpi of Con, VitA, TT, and Ω3 dietary treatments. The biomarkers used to evaluate acute phase immune response were interferon alpha (IFN-α), tumor necrosis factor alpha (TNFα), interleukin (IL) 1β, IL6, and IL8 levels (Luminex B.V, Het Zuiderkruis 15215 MV's-Hertogenbosch, The Netherlands).

At the end of the experiment, all gilts were confirmed without viremia. Then, gilts were transported to quarantine facilities for 30 d and after to a commercial sow farm were first parity performance discarded any carry over effect.

Statistical Analysis

Analysis of performance and acute immune response

At dpi –11 and 0, BW were equal amongst dietary treatments and SH group, but comparisons with SH were only observational due to lack of replicates and use of different facilities. Gilt was the experimental unit for BW, ADG, viral load, and immune response biomarkers; whereas it was pen for ADFI and gain:feed ratio (G:F). The BW, ADG, ADFI, G:F, AUC, and immune response biomarkers were analyzed as repeated measures using mixed model equation methods (PROC GLIMMIX) from SAS v9.3 (SAS Inst. Inc., Cary, NC). The fixed effects were the dietary treatment, time (dpi), and the dietary treatment × time interaction, whereas block was the random effect. Also, the ADG between –11 to 78 dpi, and ADG, ADFI, and G: F between periods 0 to 59 dpi and 0 to 78 dpi were analyzed with dietary treatment and block as main effects using general linear model (PROC GLM) from SAS v9.3 (SAS Inst. Inc., Cary, NC). Data was reported as least squares means ± SEM, with Bonferroni adjustment for comparisons and considered significant if $P < 0.05$ and a tendency if $P < 0.10$.

Exploratory analysis of post-infection interactions

The following statistical analysis were performed using 3.4.1, R Software (R Core Team, 2017).

Linear and quadratic regressions were used to associate BW gain 9 dpi with peak of viral load (average 1, 2, 4, and 7 dpi) for all 80 gilts. Also, linear and quadratic regressions were used to study the associations gain 9 dpi, ADG 0 to 29 dpi, and ADG 0 to 59 dpi, with immune response biomarkers (IFNα, TNFα, IL1β, IL6, and IL8). Similarly, peak of viral load (1, 2, 4, and 7 dpi average) was linear and quadratic regressed with immune response biomarkers (IFNα, TNFα, IL1β, IL6, and IL8), one to one and as averages (1, 2, 4, and 7 dpi).

Furthermore, the association between gain 9 dpi and serum viral load, dietary treatment, and immune response was analyzed using backwards n techniques in multivariate multiple regression using "car" package (Fox and Weisberg, 2011). First, one to one relationships were studied for BW gain 9 dpi with all variables. The continuous variables through linear and quadratic regression (BW at 0 dpi, viral load, IFNα, TNFα, IL1β, IL6, and IL8 at each dpi 1, 2, 4, and 7, and also the averages). The categorical variables, through

ANOVA (dietary treatment, BW block, classifications as above or below the median: for average 2 and 4 dpi IFNα, and averages 1, 2, 4, and 7 dpi for TNFα, IL1β, IL6, and IL8). Variables with $P > 0.30$ were discarded, and when two variables correlated as $R^2 > 0.30$, only the one with lowest *P*- value was kept. Then, multivariate multiple regression models were performed and selected for lowest Bayesian information criterion (BIC) and eliminating variables with $P > 0.10$ (Schwarz, 1978). The final model with lowest BIC included the dietary treatment, the classification for IFNα at 2 dpi, and average of viral load peak. Having higher BIC, the same model was also presented without dietary treatment effect.

Principal component analysis (PCA) was performed to assess variability and relationships amongst immune response and BW gain 9 dpi using "ChemometricsWithR" package (Wehrens, 2011). A first exploratory PCA included BW at 0 dpi, gain 9 dpi, and viral load, IFNα, TNFα, IL1β, IL6, and IL8 for each 1, 2, 4 and, 7dpi, and also the averages. Secondly, correlated variables ($R^2 > 0.30$) with the same weight direction in the PCA loading matrix were removed. The final PCA analysis model presented include the BW at 0 dpi, gain 9dpi, 2 dpi IFNα, 4 dpi IFNα, and IFNα the 1, 2, 4, and 7 dpi average for TNFα, IL1β, IL6, and IL8.

RESULTS AND DISCUSSION

PRRS Infection and Dietary Treatments

Gilts reduced feed intake for 3 wk once viremia was detected and had negative gain 9 dpi ($P < 0.01$; Figure 6.2 and 6.3); which is similar to previous PRRS infections (Escobar et al., 2004; Scheweer et al., 2017, 2018). On average, the BW from infected gilts (Con, VitA, TT, and Ω3) was 7.8 kg lower than that observed for non-infected SH gilts at 9 dpi. This was maximum at 29 dpi (12.6 kg as 16.2 %) and similar to 18.7% difference observed by Rochell et al. (2015). Growth variability was also increased with the infection. The ADG between –11 and 0 dpi had 22.8% CV, whereas between 9 to 15 dpi, 15 to 22 dpi, and 22 to 29 dpi shown 38.6%, 42.2%, and 30.0% CV respectively. Only reducing to initial levels (23.3% CV) by 43 to 49 dpi (Figure 6.3). Such high performance variability during infection has been attributed to individual differences in immune response (Lunney et al., 2011; Islam et al., 2013; Gabler, 2014).

There were no differences ($P > 0.10$) among dietary treatments in ADG before PRRSv challenge (Table 6.2 and Figure 6.2). Conversely, the ADG from 0 to 9 dpi of gilts fed TT was greater than Ω3 ($P = 0.036$), and tended to be greater than Con ($P = 0.054$); with VitA being not different to Con, TT, or Ω3. Between 9 and 15 dpi, the ADG was greater ($P = 0.041$) on females fed TT than those fed Con, with VitA and Ω3 being intermediate. After, other differences and tendencies were observed (Figure 6.3), but looking at a larger time-frame (0 to 59 dpi), only the dietary treatment TT tended to have increased ADG ($P = 0.081$) than Ω3. In fact, Ω3 shown greater ($P < 0.05$) ADG than TT for some of the intervals (i.e. for 22 to 29 dpi and 43 to 49 dpi periods); which could be explained as compensatory growth (Levesque et al., 2012). However, ADG from –11 dpi to the end of rearing, or ADG, ADFI, and G:F from 0 dpi to the end of rearing shown no evidence of differences across dietary treatments (Table 6.2).

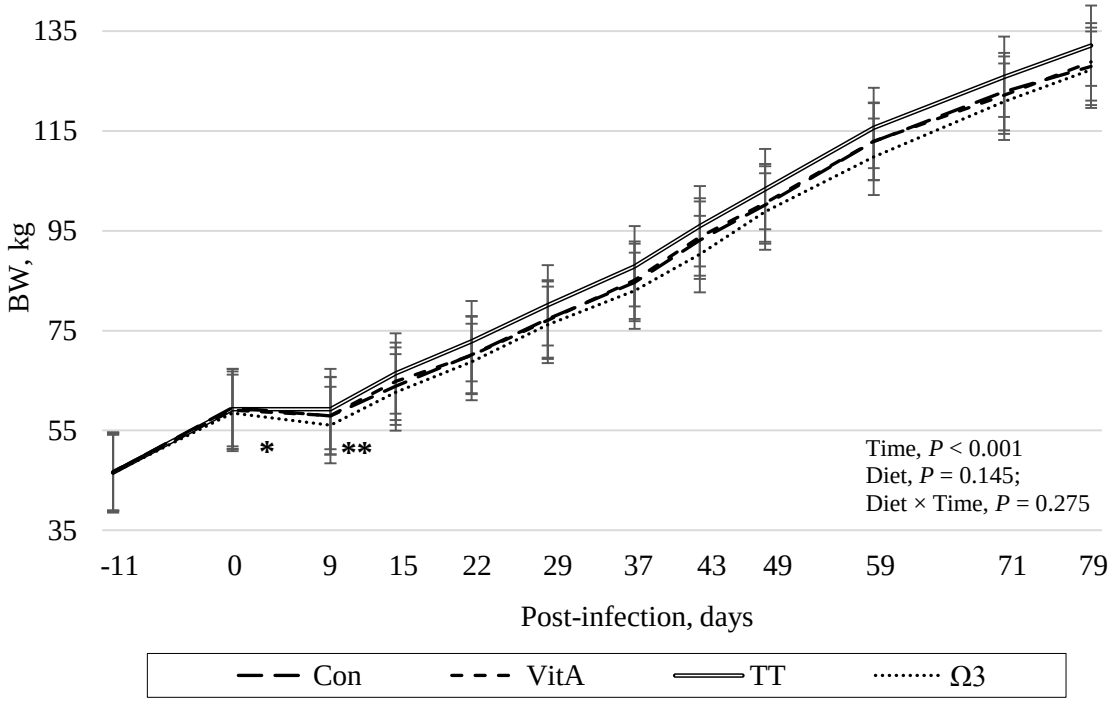


Figure 6.2. Effect of dietary treatment[1] on body weight (BW) evolution in gilts (n = 80) under Porcine Reproductive and Respiratory Syndrome virus[2].

[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega-3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.

[2]Gilts were infected at 58.8 ± 5.32 kg BW.

[*]The BW measures were different over time (SE = 0.536; $P < 0.001$) except between d 0 and d 9 post-infection (SE = 0.536; $P = 0.603$).

[**]The TT 3.23 kg BW > Ω3; SE = 1.99, $P = 0.059$.

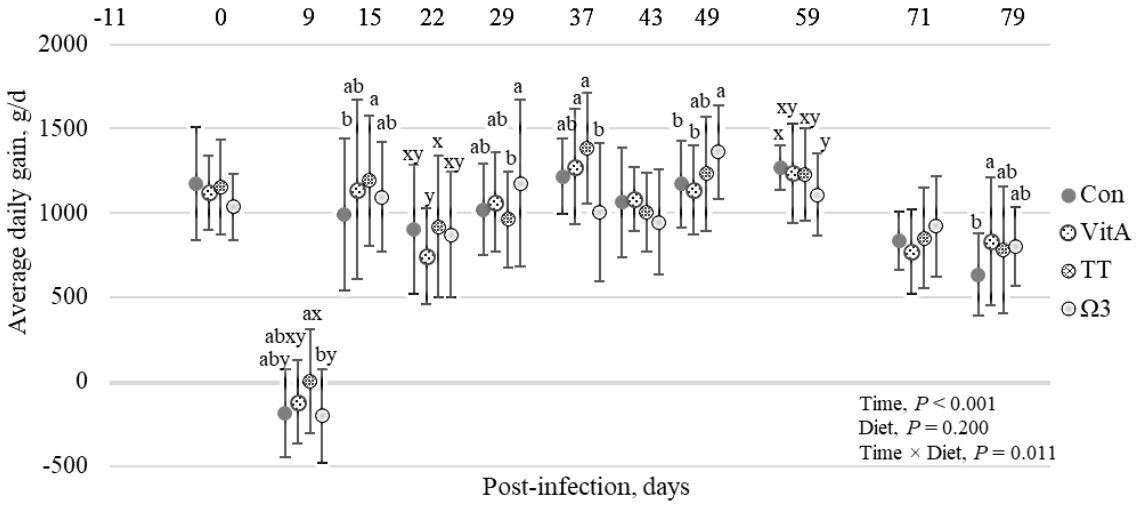


Figure 6.3. Effect of dietary treatment[1] on average daily gain between body weight (BW) measurements in gilts (n = 80, from 46.6 ± 3.62 to 131.2 ± 9.40 kg BW) under Porcine Reproductive and Respiratory Syndrome virus[2].

[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega-3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.
[2]Gilts were infected at 58.8 ± 5.32 kg BW.
[a-b]Values within time measurement and different superscripts differ $P < 0.05$.
[x-y]Values within time measurement and different superscripts tend to differ at $P < 0.10$.

Results suggest that TT may enhance performance compared with Ω3 and Con early post-infection. This could be partly explained by Trp supplementation since, the catabolism of Trp is known to increase under inflammation and poor sanitary conditions (Le Floc'h et al. 2008, 2009). Increased demands of Trp could be related to higher needs for acute phase proteins synthesis; which proportionally require greater amounts of Trp than muscle protein (Reeds et al., 1994). Otherwise, catabolism of Trp into kynurenine through the enzyme indoleamine 2,3 dioxygenase is required for oxidant defenses (Le Floc'h et al. 2009). Similarly, the Trp indole ring breakdown pathway produces molecules involved in inflammation that may be upregulated during immune response (Palego et al., 2016). The consequence could be an inadequate Trp supply for the increased Trp utilization during inflammation. Hence, for one or multiple reasons, Trp requirements may be higher during a PRRSv infection, and together with depressed feed intake, could reduce Trp availability for protein accretion and growth. In addition, despite feed intake shown no differences and sample size (n = 2) was too poor for comparisons, Trp may have enhanced feed intake through appetite stimulating hormones, such as serotonin and ghrelin (Zhang et al., 2007; Li and Patience, 2017).

Table 6.2. Effect of dietary treatment[1] on performance in growing gilts (n = 80) from 46.6 ± 3.57 to 131.2 ± 9.40 kg of body weight (BW) under Porcine Reproductive and Respiratory Syndrome virus (PRRSv) infection[2].

	Treatment[1]					
Item	**Con**	**VitA**	**TT**	**Ω3**	SEM	*P*-value
Dpi 0 to 59						
ADG, g/d	904[xy]	914[xy]	955[x]	880[y]	21.1	0.092
ADFI, kg/d	2.28	2.26	2.32	2.24	0.054	0.816
Gain: Feed	0.40	0.40	0.41	0.39	0.011	0.628
Dpi 0 to 78						
ADG, g/d	865	883	922	878	18.2	0.150
ADFI, kg/d	2.82	2.89	2.83	2.78	0.065	0.786
Gain: Feed	0.31	0.31	0.33	0.32	0.006	0.199
Dpi –11 to 78						
ADG, g/d	903	912	950	897	26.7	0.198

[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega 3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.
[2]Infection at 58.8 ± 5.32 kg BW.

[x-y] Values within a row with different superscripts tend at $P < 0.10$.

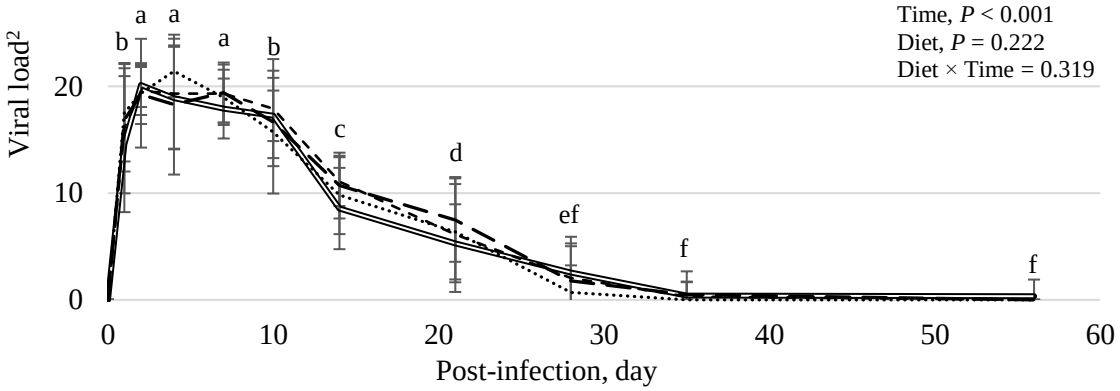
Threonine, the first limiting amino acid for g-globulin production (Cuaron et al., 1984), may also be involved with increased gain on TT dietary treatment at 9 and 15 dpi. Wang et al. (2006) described that requirements of true ileal digestible Thr were 12% higher to optimize g-globulin than to maximize (5.9 g/d) gain and feed efficiency. Similar to the present study, Xu et al. (2015) used 0.26 Trp:Lys and 0.80 Thr:Lys with PRRS live vaccine challenge. Comparing with NRC (1998 and 2012), they observed a tendency for increased growth. Again, this suggests that requirements for Trp and Thr may increase during infection. The present levels in control (0.20 Trp:Lys and 0.67 Thr:Lys) were commercial and slightly above NRC (1998 and 2012). Hence, supplementing 0.34 Trp:Lys and 0.80 Thr:Lys (TT) during a PRRSv infection still suggests a benefit above commercial levels. Nonetheless, because these amino acids were supplemented together, it is not possible to determine a clear response for one or other AA. While TT effect was observed acute (already 9 dpi), the Thr role on g-globulin would likely reinforce humoral response above 14 dpi (Mateu and Diaz, 2008). Suggesting that TT effect may be more explained by the role of Trp than Thr, although this cannot be confirmed with the present data.

The females under dietary treatment TT shown a higher gain than under Ω3 early post-infection and also with a tendency to 59 dpi. One may suggest that Ω3 worsened disease outcome, and indeed, dietary *omega*-3 has shown controversy on immune challenged pigs. Levels near 5.0 g ∑n-3/100 g fatty acids, similar to those in Ω3 diet, did not clearly improve performance (Li et al., 2014; Gaines et al., 2003). Conversely, 10 g ∑n-3/100 g reduced cellular inflammation and improved performance on lipopolysaccharide (Liu et al., 2003) and *Staphylococcus aureus* (Langerhuus et al., 2012) challenged pigs. Dietary *omega*-3 fatty acids would increase eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on immune cell membrane at expenses of arachidonic acid (ARA) places (Calder et al., 2001). This could rise cyclooxygenase and lipoxygenase pathways, decrease (40-70%) eicosanoid production from ARA, and also reduce prostaglandins, thromboxanes, and leukotrienes (Gaines et al., 2003; Calder et al., 2008). Altogether, should induce an anti-inflammatory effect. Nevertheless, dietary fish oil shown a reduction of T cell signaling and antigen presentation in rats (Sanderson et al., 1997; Shaikh et al., 2007), which could reduce effectiveness of response and increase disease severity. Therefore, the hypothesis of supplementing omega-3 to alleviate inflammation and its negative effects still requires mechanisms to be elucidated. Besides, consequences may be different depending on the immune challenge.

Analyzed level of vitamin A in Con, TT and Ω3 diets resulted lower (5,000-7,000 IU/kg) than calculated (10,000 IU/kg). Similarly, VitA diet had 18,000 IU/kg instead of 40,000 IU/kg. Although, these levels were all highly above NRC (2012) recommendations. Increasing vitamin A during a PRRSv infection was novel to the authors' knowledge but provided no evidence for improving performance. Only, for 71 to 79 dpi, VitA had greater ADG than Con, with TT and Ω3 being not different. This, could not be associated to any cause, although, all gilts were vaccinated with a live attenuated gE deleted Aujeszky's disease vaccine at dpi 60.

Viral load

All gilts were PCR positive to PRRSv by 2 dpi, and viremia lasted 23.1 ± 6.33 d as other authors previously reported (Diaz et al., 2005; Zimmerman et al., 2012; Schweer et al., 2018). There were no differences across dietary treatments for viral load (AUC), nor overall viremia length or the peak of the viremia (1 to 7 and 1 to 29 dpi; Figure 6.4).



	Con	VitA	TT	Ω3	SEM	*P* value
Total AUC[2] 40-C_t	342.8	333.2	339.3	322.0	13.69	0.684
21-dpi AUC 40-C_t	332.1	327.4	304.5	320.8	10.75	0.297
7-dpi AUC 40-C_t	180.0	180.5	176.4	181.1	4.99	0.231

Figure 6.4. Effect of dietary treatment[1] on **s**erum viral load[2] in gilts (n = 80) under Porcine Reproductive and Respiratory Syndrome virus[3].

[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega 3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.

[2]Viral Load = expressed as 40 minus Ct (threshold cycles) values from Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). AUC: Area under the curve from the qRT-PCR results as 40 minus actual C_t qRT-PCR.

[3]Gilts were infected at 58.8 ± 5.32 kg BW.

To modulate viral load through dietary changes (Rochell et al., 2015; Scheweer et al., 2018) or by feed additives (Che et al., 2011; Gabler, 2016) has become controversy. Besides, interactions over infection progress and differences due to pig age indicate complexity (Greiner et al., 2001). Furthermore, virus variability or species (PRRSv1 vs. PRRSv2) can also cause differences in viremia and severity amongst studies (Kappes et al., 2015). In fact, in the present trial 9 dpi gain was on average negative, whereas Greiner et al. (2001) described a reduced but positive BW gain 8 dpi. Diet changes have not provided consistent evidence to influence viremia, but genetic background amongst individuals has proved to influence viremia length, pattern, and load (Lunney et al., 2011; Islam et al., 2013). Present variability was 18.0% CV for AUC viral load and 27.8% CV for viremia length. In agreement with previous reports, peak of viremia was within the first 7 dpi (Johnson et al., 2004; Islam et al., 2013; Schweer et al., 2018). Besides, with increasing viral load peak, the BW gain 9 dpi decreased (Figure 6.5); although viremia did not explain most of pigs' performance variance

($R^2 = 0.10$). Even a wide range (26%) of gain differences as highest or lowest performing pigs post PRRSv challenge, was shown without differences in viral load (Gabler, 2014). Viremia results, together with the variability on performance above described, suggest that other factors play important roles in performance response.

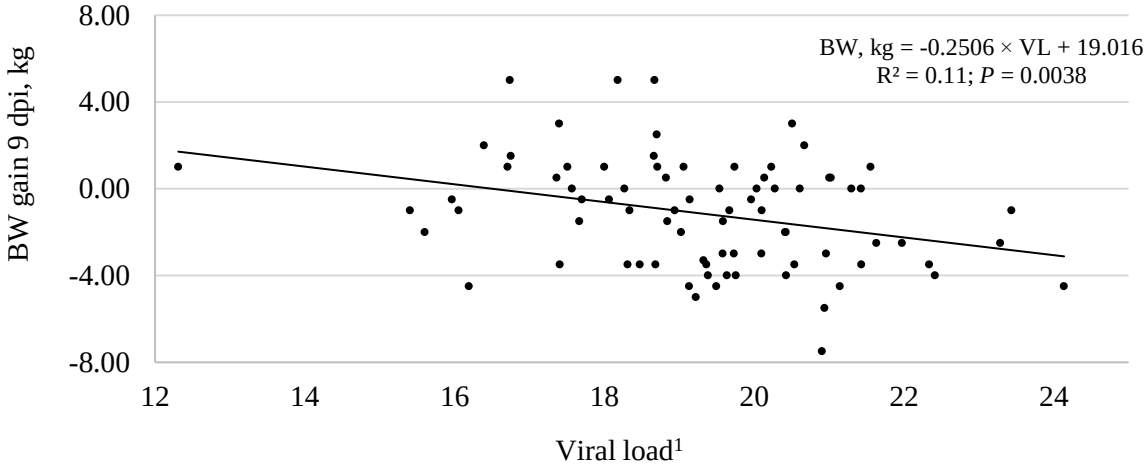


Figure 6.5. Linear regressions for peak of viral load (average 1, 2, 4, and 7 dpi) and body weight (BW) gain 9 d post-infection (dpi) of Porcine Reproductive and Respiratory Syndrome[2] in gilts (n = 80).
[1]Viral Load = expressed as 40 minus Ct (threshold cycles) values from Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) average of the first week post PRRS virus infection.
[2]Gilts infected at 58.8 ± 5.32 kg BW.

Cytokines

In Figure 6.6 are presented the cytokines and cytokine kinetics amongst dietary treatments. Over time, serum concentration of IFNα increased at 2 and 4 dpi compared with 1 and 7 dpi ($P < 0.05$). This IFNα kinetic and the 100% viremia at 2 dpi confirm that infection was happening synchronized. Other time differences for TNFα, IL1β, IL6, and IL8 were not observed. The target for cytokine assessment was acute (1 to 7 dpi) because dietary treatment response on performance was observed already at 9 dpi. However, other studies report cytokine changes latter (i.e. 7 to 14, 21, 28, and 42 dpi) in infection (Diaz et al., 2005; Lunney et al., 2010).

The IFNα at 4 dpi was higher in VitA than in Con and Ω3 gilts ($P = 0.014$), with TT being intermediate and not different. Also, IL8 was higher in gilts fed VitA than in those fed Con and Ω3 at 7 dpi ($P = 0.045$). This effect may be related to vitamin A metabolites. Retinoic acid, can inhibit cyclooxygenase 2 (COX-2) and therefore, produce anti-inflammatory effect (Kim et al., 2004; Villamor and Fawzi, 2005). In fact, inhibited COX-2 has shown increased IFNα in mice (Kirkby et al., 2013). Conversely, increased IL8 is a pro-inflammatory response, which could be explained through the action of vitamin A metabolites on the mucosa level. Indeed, retinol has been associated with IL8 production and skin irritation (Kim et al., 2003).

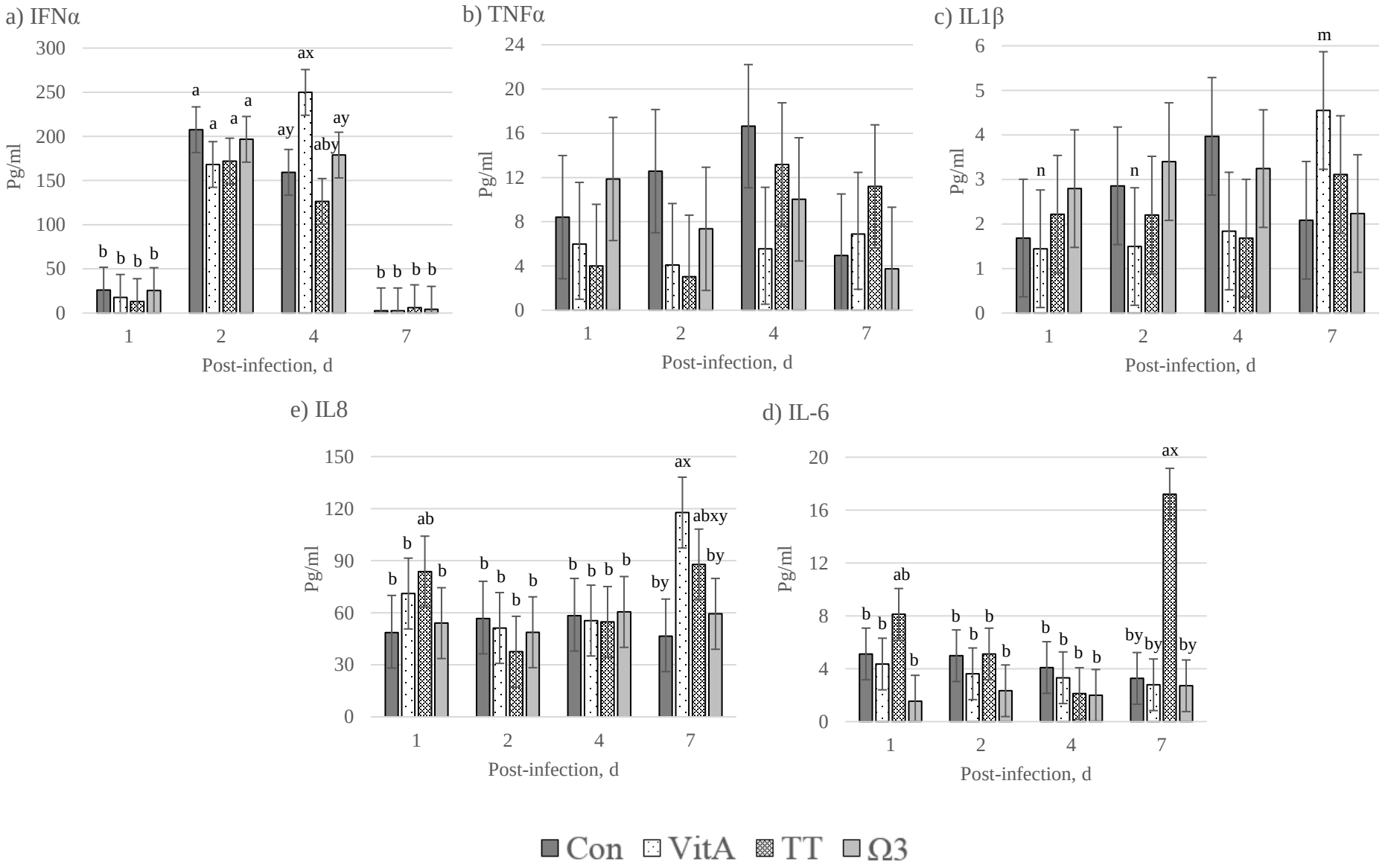

a) IFNα
Pg/ml
300
250
200
150
100
50
0
b b b b
a a a a
ay ax aby ay
b b b b
1 2 4 7
Post-infection, d
b) TNFα
Pg/ml
24
20
16
12
8
4
0
1 2 4 7
Post-infection, d
c) IL1β
Pg/ml
6
5
4
3
2
1
0
n n m
1 2 4 7
Post-infection, d
e) IL8
Pg/ml
150
120
90
60
30
0
b b ab b
b b b b
b b b b
by ax abxy by
1 2 4 7
Post-infection, d
d) IL-6
Pg/ml
20
16
12
8
4
0
b b ab b
b b b b
b b b b
by by ax by
1 2 4 7
Post-infection, d
Con
VitA
TT
Ω3

Figure 6.6. Effects of dietary treatment[1] in gilts[2] (n = 44) under Porcine Reproductive and Respiratory Syndrome infection[3] on serum levels of a) Interferon-α (IFNα); b) Tumor necrosis factor-α (TNFα); c) Interleukin-1β (IL1β); d); Interleukin-6 (IL6); e) Interleukin-8 (IL8); at 1, 2, 4, and 7 d post-infection.

[1]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega 3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.

[2]Subsample of 11 gilts/treatment randomly selected.

[3]Gilts infected at 58.8 ± 5.32 kg BW.

[a-b]Different superscripts differ significantly among dietary treatment × time at $P < 0.05$.

[m-n]Different superscripts differ marginally among dietary treatment × time at $P < 0.10$.

[x-y]Different superscripts differ significantly within time $P < 0.05$.

The IL6 at 7 dpi was increased ($P < 0.001$) on gilts fed TT compared with all other dietary treatments and dpi measures except same TT at 1 dpi. Production of IL6 in TT could be enhanced through Trp and Aryl hydrocarbon receptor (Ahr). Among other roles, Ahr increases IL6 transcription (Tanaka et al., 2014), and requires kynurenine ligand which is catalyzed from Trp (Jux et al., 2009). As a pleiotropic and regulatory cytokine, IL6 has poor interpretation alone (Hunter and Jones, 2015). During acute response, IL6 participates in homeostasis, promotes cell differentiation, and enhance glucose metabolism (Tanaka et al., 2014; Hunter and Jones, 2015). This cytokine did not correlate with post-infection performance, but increasing average (1, 2, 4, and 7 dpi) of IL6 ($R^2 = 0.10$; $P = 0.033$) and increasing IL6 at 7 dpi ($R^2 = 0.14$; $P = 0.013$) correlated negatively and weakly with viral load peak. Finally, and with high variability, there was no evidence for TNFα and IL1β differences amongst diets.

Post-infection Interactions

Severity of inflammation might correlate with post-infection performance (Spurlock, 1997; Broussard et al., 2003). Although, regressions between post-infection performance and immune response did not provide evidence of one to one relationships (data not shown). High variability was earlier documented with poor potential of interpretation to individual cytokine changes (Lunney et al., 2010). Hence, further analyses to study immune response and acute post-infection performance were performed.

The PCA (Figure 6.7), explained that viral load peak and IFNα at 4 dpi were separated from TNFα, IL1β, and IL8 averages (25.1% variance). Alike the regression results (Figure 6.5), viral load negatively correlated with gain 9 dpi, and also, with IFNα at 2 dpi, IL6, and BW (18.2% variance). Interestingly, IFNα 4 dpi was apart from all other variables, while IFNα at 2 dpi was aligned with gain 9 dpi. From multivariate regression (Table 6.3), gain 9 dpi was positively associated with dietary treatment TT and by being above the median for IFNα at 2 dpi; but negatively, with increasing peak of viral load and with dietary treatment Con. The diet Ω3 did not reduce gain 9 dpi in this model, which contrasts with data from all gilts. Likely, because this cytokine analysis included only arbitrary subsampling (n = 11), which Ω3 gilts, increased gain 9 dpi (-1.71 ± 2.46 kg) compared with the assessment complete (n = 20; -1.83 ± 2.50 kg). Repeated the model without the dietary treatment effect, explained 16% of the variance and shown similar effects for IFNα at 2 dpi and the peak of viral load.

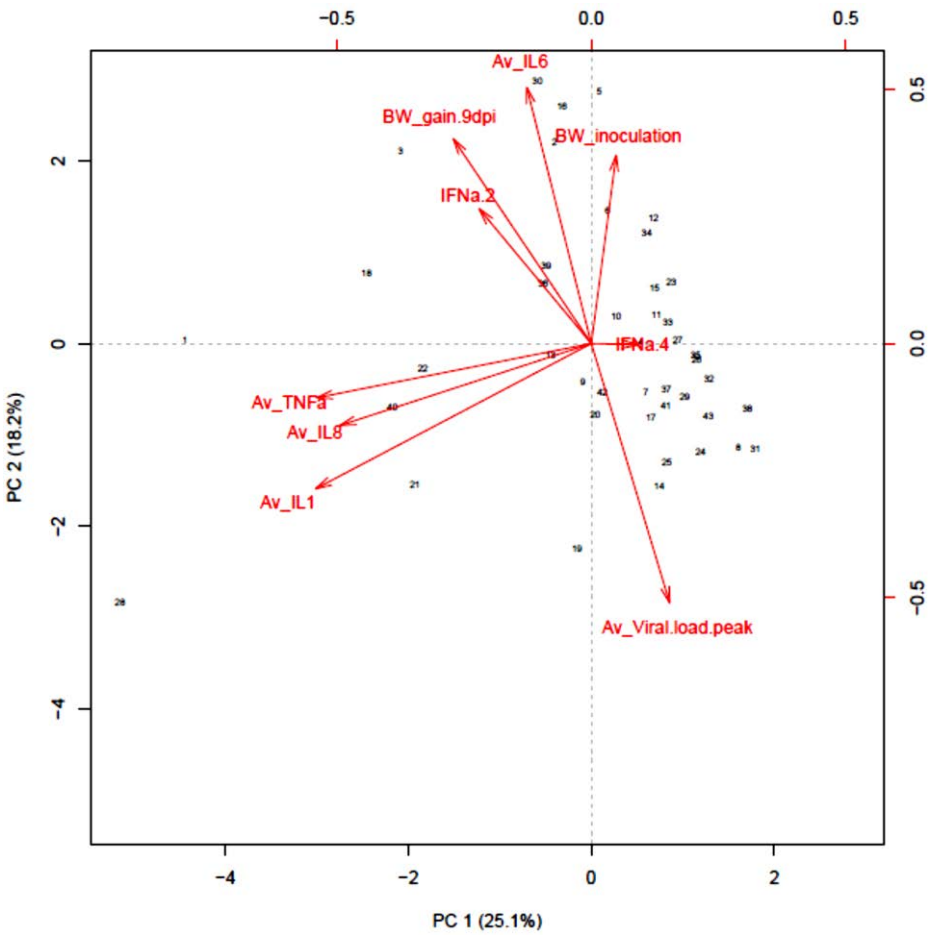


Figure 6.7. Acute phase variables from gilts[1] (n = 44) under a Porcine Reproductive and Respiratory Syndrome virus infection[2] analyzed through principal component analysis (PCA). The left side of the biplot explains the component scores matrix as first and second principal components (PC) variance (%). The right side of the biplot presents the loadings matrix (arrows directing the weight of the variables). Data points are 1 to 44 gilts and variables include: peak of viral load, interleukin (IL) β1, IL6, IL8, interferon-α (IFNa) and tumor necrosis factor-α (TNFa) as averages (Av.) of 1, 2, 4, and 7 d post infection (dpi), and body weight (BW) at infection, BW gain 9 dpi, and total viremia length.

[1]Subsample of 11 gilts/treatment randomly selected.

[2]Gilts infected at 58.8 ± 5.32 kg BW.

Table 6.3. Multivariate regression models of body weight (BW) gain 9 d post-infection[1] (dpi) with Porcine Reproductive and Respiratory Syndrome virus including factor dietary treatment[2], viral load (average 1, 2, 4, and 7 dpi), and interferon alpha (IFNα) classification from 2 and 4 dpi average in gilts (n = 44; 46.6 ± 3.57 BW).

		Pig gain 9 dpi, kg		
	Unit	b	SE	P value
With dietary treatment, adj. R^2 = 0.27				
Intercept		7.944	4.318	0.074
Con		-1.185	0.560	0.041
TT		1.361	0.590	0.026
Ω3		-0.664	0.563	0.245
Viral load	40-$C_{t\ values}$	-0.463	0.222	0.044
IFNα 2 dpi, if below median[3]		-0.700	0.329	0.040
Without dietary treatment, adj. R^2 = 0.16				
Intercept		10.61	4.37	0.019
Viral load	40-$C_{t\ values}$	-0.601	0.225	0.011
IFNα 2 dpi, if below median[3]		-0.679	0.347	0.057

b = coefficient for each factor.

[1] Infection at 58.8 ± 5.32 kg BW.

[2]Treatments = 1) Con, control, 2) VitA, high dose of vitamin A (18,000 IU/kg), 3) TT, high ratio tryptophan and threonine (0.34 and 0.80 ratios to lysine, respectively), and, 4) Ω3, adding omega 3 (10 g/kg fish oil); provided already 10 d before PRRSv infection.

[3]Gilts were classified as below or above 2 dpi IFNα median (165 Pg/ml).

The IFNα response already 2 dpi suggests that this cytokine was probably involved with the acute immune response effectiveness. The PRRSv is known to inhibit production of IFNα compared with other respiratory diseases, however, the virus is also susceptible to it (Buddaert et al., 1998; Baum and Garcia-Sastre, 2010; Loving et al. 2015). Buddaert et al. (1998) shown that IFNα reduced viral yield and antigen expression in vitro, whereas in vivo, decreased virus in lungs. For this trial, viremia did not show such relationship but PCA indicated that gain 9 dpi and IFNα 2 dpi aligned variance against viral load.

In conclusion, treatments had limited effects on gilt performance under PRRS infection. Acute post-infection, results suggest that TT enhanced BW gain compared with Con and Ω3. After, only TT tended to improve ADG from 0 to 59 dpi compared with Ω3. Viremia was not affected through the diet but VitA and TT could modulate acute cytokine production. However, such cytokine changes were not associated with performance, and the mode of action from TT improvement should be further investigated. The 27% of 9 dpi gain variance was explained by the diet, viral load, and IFNα. This gain reduced with increasing peak of viral load and increased when gilts were classified above the median for IFNα at 2 dpi, with other cytokine levels not associated with performance. This trial highlights great variability on both, inflammation and performance response to PRRSv and a possible but marginal intervention through Trp and Thr amino acids.

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CHAPTER VII

General Discussion

The rearing period is highly important for gilt development and specific conditioning. Both, are acknowledged to affect sow longevity and lifetime productivity (Lucia, 2000, Koketsu et al., 2017). Indeed, as reviewed in Chapter I, the growth rate, puberty attainment, and BF, age, and BW at first service could all influence to some unknown degree performance, gilt failure, and culling decisions towards young sows (Rozeboom, 2015). More directly, culling is also driven towards poor condition or low performing gilts as well as to lame animals (Engblom et al., 2007; Díaz et al., 2015, 2017). Culling reasons are multifactorial and include various possible causes (further discussed). In this thesis, the used approach was to study culling risk factors of lameness and disease outcome in commercial conditions.

The present objectives were to study occurrence, prevalence, and impact of lameness during gilt rearing period up to 1st parity (1). To evaluate the effects of additional Zn, Cu, and Mn, or amino acid Met, or all combined on lameness and claw lesion (2). From the same design and using a subsample, to evaluate treatment effect on bone properties and OC gross lesions in gilts of different growth rate (3). Similarly, to study the effects of additional Zn, Cu, and Mn plus Met combined or not with limited dietary Lys (with means to reduce growth) on lameness and claw lesions (4). And finally, to study the effects of additional dietary vitamin A, or Trp and Thr, or fish oil on gilt growth and acute immune response under a challenging environment with PRRS infection (5). A broader goal was to answer these objectives in commercial conditions in order to yield straight applicable conclusions. Most of the results produced in the present thesis are addressed in depth within each of the previous chapters, and are concluded in Chapter VIII. Otherwise, in the present general discussion, the perspective accumulated throughout the previous chapters is used to discuss about three main topics: the challenging rearing period and gilt failure (1), the conflicting results from dietary treatments and lameness (2), and the limitations and considerations for assessing lameness (3).

7.1. Challenging rearing period and gilt failure

It can be stated that rearing and adaptation to the sow farm periods include various experiences that may be viewed as challenges affecting lameness and performance. For example, transportations, numerous vaccinations, pathogen outbreaks, and changes in management and flooring conditions. All those experiences were reported in the present thesis and likely influenced performance, lameness, mortality, and culling. Rearing gilts were several times vaccinated and also suffered PRRS linked to a PRRSv positive multiplier origin and destination sow farm (Chapter III and VI). The vaccinations and the PRRS, while provide immunity to the animals, have a negative impact on performance. As shown in Figure 7.1, vaccinations and the PRRS outbreak matched with feed intake drops, which was observed in both, III and VI Chapters. Those effects were not surprising since modified live vaccines, adjuvant, and the PRRSv are all known to generate an immune response (Lunney et al., 2010; Che et al., 2011; Xu et al., 2015). However, the consequences included in consecutive vaccinations and disease together were uncertain and remain intriguing. For example, although mortality during rearing was not very high (3.80%) it can be lower (i.e. near 1%) in high health conditions, while the present may be indicative of herein challenges or other pathogen processes. Together, the Figure 7.1 and Chapter VI results, clearly indicate that

commercial intensive vaccination and outbreaks imply negative effects on gilt performance. Effects from such stresses are not commonly addressed, and the present thesis contributed to describe consequences and intervention in replacement gilts under hostile conditions. Often, these conditions are justified and necessary to achieve sow herd health status stability. However, intervention is required to minimize performance loss and to enhance animal health. Meanwhile, current feed composition may not be the most adequate to maximize health and performance under multiple vaccinations and hostile commercial conditions.

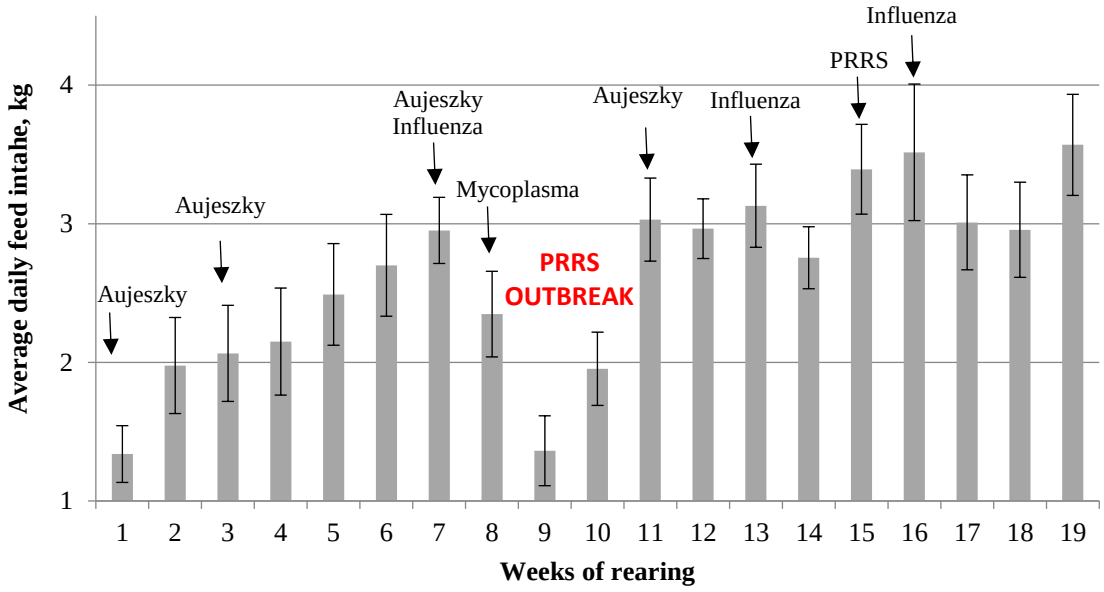

Figure 7.1. Average daily feed intake response during the rearing phase of gilts from Chapter III between 28.8 ± 8.78 kg to 155 ±16 kg body weight.

In Chapter VI, Trp and Thr additional dietary levels were shown to enhance early performance under PRRSv infection. This supplementation (TT) numerically increased 4.2 kg the final BW compared with control (Con), although without significant differences (P = 0.240; SEM = 1.199). A result to highlight was the high variability on PRRS outcome performance, which is explained through reported individual variation (Lunney et al., 2011; Islam et al., 2013). Therefore, a greater sample size than 20 animals and 2 pens should further explore Trp and Thr potential under hostile commercial conditions and PRRSv. From an economical point of view, and again without significant differences (P = 0.218; SEM = 0.010), the costs of feed (€, feed ingredients and additives February 2016) per gain (kg) may help determine whether or not the strategies provide return (Con = 0.563; VitA = 0.565; TT = 0.570; Ω3 = 0.604). Such feed cost for BW gain indicated that Con and TT may show little or null differences; while if counting other costs such as facility occupancy, this could greater benefit the TT return. The cost of PRRS versus the sham gilts observed in Chapter VI, indicated that PRRS had a cost of 9.5 €/pig (on a performance basis). Hence, to minimize consequences from hostile rearing conditions seems interesting. Moreover, the potential of different nutrients or strategies would likely depend on the challenges to what gilts might be exposed. For example, omega-3 polyunsaturated fatty acids or vitamin A did not show positively affect PRRS and vaccinations outcome (at the levels tested in Chapter VI),

however, those may have potential on digestive and (or) bacteria challenges (Liu et al., 2003; Langerhuus et al., 2012; Chen et al., 2016).

Therefore, rearing period is recognized as important to achieve specific targets (i.e. immune status, BW, body condition, etc.) but less addressed for the challenges directly implying performance loss and culling risk factors. The present results indicate that combined high frequency of vaccination and disease could increase time to achieve a certain BW and increase growth performance variability (Chapter VI). These effects may add difficulties to properly plan targets to first service as seen for slaughter weights and variability in pigs (López-Vergé et al., 2018). Furthermore, altered immunity could cause commensal microorganisms such as *Mycoplasma hyosynoviae* to develop non-purulent arthritis and lameness (Nielsen et al., 2001), or reduce the response effectiveness to other vaccination (Rose and Andraud, 2017). More research should investigate the rearing period of replacement gilts to address animal health with nutritional intervention in commercial conditions.

The present thesis so far did not include longevity and lifetime data, which will be further studied thereafter and separately. Although, herein data include early culling in the sow farm. In Chapter III 15.9% of sows did not reach a 2nd gestation and in Chapter V were 13.5%, which resulted similar to earlier reported 1st parity culling (average 16% and range from 6.0% to 30%, Engblom et al., 2007). However, what is remarkable is the high proportion of sudden death sows. Although this is not related with the objective of the present thesis, sudden death is briefly addressed as was an alarming cause for sow failure including 27% in Chapter III and 68% in Chapter V. Those are often related with traumas, circulation failure, heat shock, lesions in the reproductive system, stomach ulcers, and intestinal torsions (Kirk et al., 2005; Vestergaard et al., 2006). Above 60% of cases occurred in the farrowing rooms. However, little is addressed in the literature regarding sow mortality. Using the same genetic line as the present, Jensen et al. (2010) reported 0% to 11% prevalence range of sudden deaths among 33 sow herds, whereas Knage-Rasmussen et al. (2015) reported 5% to 34% over-time ranging in the same farm. High mortality close to farrowing (both before and after) is intriguing and should not be accepted. If mortality is increasing, should be investigated and high productive genetics (>18 piglets/farrowing), as the present, may require update in nutrition and management areas to produce both profit-making and sustainable herds.

Lameness was the other culling risk factor studied, and the principal challenge increasing gilt lameness was the period when females entered the sow farm. This challenge seemed stronger than the dietary treatment effects observed in Chapters III-IV or any inherent difference due to genetic background between Chapters III-IV and V (discussed later). Apparently, the first contact and routines gilts undergo when entered the sow farm were highly important and associated to over 100% increase of lameness incidence (from 4% at late rearing to >10% at early gestation). Generally, more females suffered lameness in the sow farm (about 20%) than during rearing (about 8%) for a similar period of time. Including the present data, lameness prevalence is mostly reported between 7.7% and 17.5% (Boyle et al., 2010; Pluym et al., 2011, 2013ab; Fabà et al., 2018) and is the second or the third reason for culling gilts and sows (6.9% to 20%, Lucia et al., 2000; Anil et al., 2005; Anil et al., 2009a). In average (Chapter III and V) and comparing with the reported range above, lameness prevalence was low-intermediate (7.7%) when looking at the rearing period, but

higher once females were on the sow farm (14% early gestation, 9.9% late gestation, and 13% after lactation). Conversely, culling associated with lameness was reported near the average in Chapter III (12.5%) and lower in Chapter V (6.95%). Although, whereas lameness was a measure registered by the present author (L. Fabà), culling reason criteria was recorded from sow farm data collection. Therefore, culling reasons responded to farm management criteria and were sensible to variation according to other needs (i.e. fulfill breeding batch). This is especially important taking into account culling sample size. Even though culling was in some occasions reported as percentage, removals were n = 81 (1^{st} and 2^{nd} parity, Chapter III) and n = 31 (1^{st} parity, Chapter V); which would not be enough to sample across dietary treatments and culling reasons.

Both, OC and claw lesions are primary causes of lameness mostly associated with genetics (Fan et al., 2009; Enokida et al., 2011; Aasmundstad et al., 2013), and also, experiences such as traumatic events derived from management and flooring conditions (Nakano and Aherne, 1988; Pluym et al., 2011; Etterlin et al., 2014, 2015; Olsson et al., 2016). Dynamic training for electronic feedings systems and new group mixing in adaptation pens before place gilts into insemination area (crates), could be related with early gestation increase of lameness observed herein and by other authors (Olsson et al., 2016; Li and Gonyou, 2013). Differently, the increase of lameness from late gestation to weaning could be associated to metal slats under the sow farrowing crate and claw lesions (Bonde et al., 2004). Altogether, data suggest that most important factors causing lameness in the present works were related with sow farm experiences. Nonetheless, because the present experiments were not designed to study these challenges, this statement cannot be further elucidated.

7.2. Conflicting results from dietary treatments and lameness

The different multiplier origins for gilts in Chapter III and V were likely varying in genetic background as the first gilts were from Denmark and the second were produced in Spain. Differences that may have yield different OC and lameness susceptibility related to genetic background (Jørgensen and Andersen, 2000; Aasmundstad et al., 2013), as well as for leg conformation (Aasmundstad et al., 2014; Le et al., 2015) to explain differences in lameness severity (in terms of performance loss) between Chapter III and V. Unfortunately, OC was only evaluated in gilts from the first experiment (Chapter III and IV) but not in Chapter V. Hence, it cannot be concluded whether or not OC was a difference between genetics and multiplier origins. Conversely, conformation might be slightly different. Gilt conformation was assessed for the 2 experiments although not reported in previous chapters and manuscripts. Conformation, was evaluated in 4 different occasions during rearing for Chapter III and only once at the end of rearing for Chapter V. Results showed no differences across dietary treatments (see Table 9.2 and 9.3, Appendix 2) and no correlation with lameness. Evaluated traits included 3 conformation traits for both front and hind legs and 2 locomotion traits; as modified from Koning et al. (2015). See Appendix 2 for methods and Figure 1.1 (Chapter I) for conformation representations. From repeated measuring in Chapter III, it was observed that gait score and weakening of pasterns (front and hind) increased with time ($P < 0.05$) and therefore, it was decided to measure conformation only at the end of

rearing in Chapter V (Table 9.2). The below Table 7.1 descriptively presents the average of each conformation trait at the end of rearing for gilts from experimental data in Chapter III and Chapter V, and for both, lame gilts and never-lame gilts.

From Table 7.1, it can be summarized that conformation does not seem greatly different between the two studies and neither between lame or never-lame females. Although, Chapter III gilts may have weaker front pastern than in Chapter V, while gilts from Chapter V seem to include weaker pastern, increased X shape, and more buckled hind legs than those from Chapter III. Otherwise, swaying hindquarters and gait score were both found greater in Chapter III than V gilts. Comparing lame with never lame gilts, slightly greater values were found in lame females in both experiments. Hence it seems that due to unknown reasons Chapter III females showed more gait difficulties, which enhances the hypothesis of greater lameness susceptibility and severity. From present data, conformation was not explaining lameness and therefore, gait, lameness and severity during rearing may be a joint related problem (i.e. OC); which could not be answered.

Table 7.1. Average locomotion[1] and conformation[1] characteristics measured at the end of rearing period.

	Chapter III			Chapter V		
	Average	Lame	Never-lame	Average	Lame	Never-lame
n	360	27	319	240	19	212
Average final body weight	156	149	156	151	151	151
Locomotion						
Swaying hindquarters (0-3)	1.23	1.25	1.23	0.39	0.39	0.39
Gait score (0-3)	1.04	1.09	1.03	0.15	0.16	0.14
Conformation						
Front legs						
O shape or X shape (1-2-3*-4-5)	3.49	3.47	3.49	3.44	3.42	3.44
Sickled or buckled (1-2-3-4-5)	3.0	3.0	3.0	3.0	3.0	3.0
Steep or weak pasterns (1-2-3-4-5)	3.88	3.65	3.89	3.71	3.68	3.71
Hind legs						
O shape or X shape (1-2-3-4-5)	3.04	3.06	3.04	3.39	3.52	3.44
Sickled or buckled (1-2-3-4-5)	3.20	3.12	3.21	3.52	3.53	3.51
Steep or weak pasterns (1-2-3-4-5)	3.18	3.12	3.19	3.52	3.53	3.50

[1]Methodology in Table 9.1. Modified from modified from Koning et al. (2015), Steenbergen (1989) and Jørgensen and Vestergaard (1990). See (Figure 1.1, Chapter I).
*Score 3 represents the neutral conformation.

The management and 280 km transportation of gilts produced in Spain (Chapter V) compared with the 2,400 km from Denmark (Chapter V) confounds with the different genetic background. Similarly, other differences such as year (2015-2016 or 2016) or time of the year (October to February in Chapter III vs. October to December in Chapter V) could also

intervene and confound with present results. Gilt age should not be a difference because although data is presented as starting between 15 and 17 wk of age and 58.0 ± 11.1 kg BW in Chapter V, gilts entered the rearing facility at 8 to 12 wk of age and 20.7 ± 5.54 kg of BW (August to December 2016), similarly to Chapter III (9 to 13 wk of age and 28.8 ± 8.78 kg BW). The relative importance of management or genetic differences were unknown but transport length and conditions are recognized to be an important stress (Goumon et al., 2013). In addition, the long transport in Chapter III may have affected lameness or joint health via mechanical stress early in gilts' life, which is documented to affect OC outcome and lameness (Nakano and Aherne, 1988). Similarly, another possibility is that earlier differences in feeding and management during the nursery period of gilts had interacted with the present lameness incidence and severity. The use of TM plus Met was shown to reduce OC in Chapter IV and Frantz et al. (2008) work, therefore, if earlier use of TM was different between Denmark and Spain multiplier origins, this may have resulted in OC differences. To further address this, a broader discussion is required regarding the dietary treatment conflicting results between Chapter III and V.

Trace minerals effect on lameness has been extensively discussed as showing absent, or controversial and inconsistent results. Some data suggest that TM supplementation could be reduced up to 50% without affecting performance (Creech et al., 2004; Shelton et al., 2004; Gowanlock et al., 2013; Ma et al., 2018), while other authors indicated TM supplements as necessary to maintain bone mineral and other tissues (i.e. liver) status (Shelton et al., 2004; Veum et al., 2009; Ma et al., 2018). In the present data (Chapter IV), mineral status in serum or bone was unaffected by TM, but TM increased bone density, strength, and mineral amount (Ca and P). Some authors (i.e. Ferket et al., 2009 in turkey and Barneveld and Vandepeer, 2008 in pigs) speculated that dietary TM should be above the growth requirements due to higher needs for structural tissues development (i.e. bone, joints, and claws) and reduce OC (Knight et al., 1990; Hurting et al., 1993; Frantz et al., 2008). Conversely other authors did not find such effects (Ytrehus et al., 2007; Tóth et al., 2016). The present thesis is also an example of controversy. On the one hand, TM and (or) Met reduced lameness in Chapter III; and in Chapter IV TM brought about some improvements on bone and TM plus Met as reduced gross articular lesions of OC. On the other hand, lameness was not reduced in Chapter V when using the same combined TM plus Met.

Since bone properties or OC were not measured in the Chapter V study, further discussion other than actual differences in genetic background (i.e. OC as discussed above) and earlier experiences from different multipliers (discussed below), or simply linked to severity differences (higher in Chapter III than V) is complicated. It could be that the role of TM and S from Met on cartilage and bone development may be equally or even more important during a healing process. In fact, in humans, providing dietary S-Adenosyl Met metabolite to osteoarthritis patients improved joint health and functionality (Najm et al., 2004). However, whether or not mineral requirements increase under challenging lameness and during healing is unknown. Higher requirements with increasing performance or aging were suggested to cause deficiencies (Mahan and Newton, 1995), but more recently, mineral status (defined as concentration in storage tissues, primarily bone and liver) was unaffected by parity (Crenshaw et al., 2013). The conflicting TM concern is somewhat supported by the use of higher levels in commercial diets (FEDNA, 2013) than the requirements for growth

(NRC, 2012; Table 1.2). Such higher levels in commercial diets likely aim to avoid any deficiency during development and represents the discussed uncertainty while recognize the known roles of TM in bone and horn development (see Chapter I). To validate TM status, a measure of TM content in liver (i.e. Cu) might have been useful in Chapter IV rather than serum, which is usually less sensible to changes.

The presence of interactions among minerals such as Zn, Cu, Ca, and iron (Fe) or with other feed components may also yield conflicting results (Lönnerdal, 2000; Blaabjerg and Poulsen, 2017). These interactions do not seem possible during experimental periods herein, but if feeding practices during nursery phase were different between Denmark and Spain multiplier origins, it may be important. Mineral interactions need to be considered when studying TM supplements in early development of OC (i.e. 4 to 12 week of age). Particularly, since nursery pigs might be fed high doses of $CuSO_4$ (i.e. 250 ppm) and ZnO (i.e. 3,000 ppm) to enhance growth via antimicrobial properties (Jensen et al., 2016). These high levels as growth promoters are currently banned in the EU. In complete feeds, Zn is limited to 150 mg/kg in piglets and sows and 120 mg/kg in growing finishing pigs (Directive 2016/1095/EU) and Cu recently limited to 150 mg Cu/kg diet up to 4 wk post weaning, 100 mg/kg from 4^{th} to 8^{th} week post weaning, whilst after that and for other phases is 25 g/kg (Directive 2018/1039/EU). However, levels near 2,400 ppm of Zn are frequently used under veterinary prescription for post-weaning diarrhea treatment (i.e. for 2 wk). The effects on bone and joint development during the age range when encounter both, OC development (Grevenhof et al., 2012) and veterinary treatments including high levels of ZnO has not been studied. Excessive Zn can displace Cu i.e. Cu-dependent enzyme lysyl oxidase and produce bone growth abnormalities (Hill et al., 1983). Similarly, dietary Fe should be maintained at adequate levels according to Zn and Cu because high levels of Cu (likely near 250 ppm) reduce Fe absorption (Gipp et al., 1974; Dove and Haydon, 1991). Whether $CuSO_4$ or ZnO were used at high doses or if levels used were above or below requirements during nursery in any of the multipliers is unknown. If interaction was to cause increased risk of OC and severity, this points out gilts from Chapter III and would associate to Danish multiplier. However, none of this is certain and to study these interactions earlier in pigs' life than in the present study seems interesting.

In the gastrointestinal lumen, dietary TM can interact with feed components such as phytate, and form complexes that make them unavailable for absorption (Andrieu, 2008; Liu et al., 2016). Opportunity to modulate availability and absorption can be achieved via different sources of TM. For example, metal proteonates as amino acid chelate, also named organic sources, increase availability and reduce excretion in feces compared with inorganic sources (Creech et al., 2004; Hernández et al., 2009; Liu et al., 2014; Liu et al., 2016). Hence, the supplementation or not of microbial phytase, and the TM source should also be taken into account when studying TM supplements. Important differences in growth rate were not observed among dietary treatments, therefore, all TM levels used and including phytase in the present studies seemed not to interact and resulted with sufficient P, Ca and TM availability for growth physiological needs (Creech et al., 2004; Shelton et al., 2004; Garvican et al., 2010; NRC, 2012; Gowanlock et al., 2013). For example, dietary Cu near 6 g/kg in diets for growing-finishing pigs fulfill growth requirement, and above that, liver is the main storage pool for Cu (Hernández et al., 2008, 2009; Gowanlock et al., 2013; Ma et al., 2018).

Limitations in the present study were having not diagnosed the causes of lameness in Chapters III and V or to further study the articular lesions via anatomopathology in Chapter IV. Nonetheless, lameness including severe wounds and abscesses were discarded from the analysis (incidence of 2 cases in Chapter III and 1 case in Chapter V), in order to only target lameness from OC-like leg weakness and claw problems. Furthermore, anatomopathology was not performed since previous authors effectively used gross macroscopic evaluation similarly (Frantz et al., 2008; Grevenhof et al., 2012), besides, we were already targeting other bone development response variables. These type of studies under commercial conditions may require a higher control than herein, such as a directed challenge. To one's understanding and including the data reviewed (see Chapter I), basic hypotheses with a different approach need to be tested before further statements of TM potential in commercial conditions. Olsson et al. (2016), proposed a repeatedly grouping of gilts (at 8, 9, and 10 months of age) as a challenging model to assess flooring and management effects on claw lesions. Similarly, Koning et al. (2014) used a challenging approach for OC via restricting feed intake or not above and (or) below 10 wk of age between 4 and 24 wk of age. Those models could actually be used to assess TM potential on claw lesions and OC. Indeed, a comparable approach to that used by Koning et al. (2014) was tried with reducing dietary Lys in Chapter V. The principal lameness risk factors in the present thesis were experiences during adaptation to the sow farm and group housing; which indeed increased lameness but was not directed. This, included limitations compared with above mentioned studies as were uncontrolled challenges and applied for all gilts. In addition, dietary treatments were not provided once females entered the sow farm. It would be interesting to evaluate the continuing supply of TM plus Met during the sow productive phases. Indeed, other authors reported benefits on claw health from TM (Anil et al., 2009b; Lisgara et al., 2016; Varagka et al., 2016), but in the present experiments was not feasible due to logistic reasons. Either approach, to study TM supplement during entrance to the sow farm up to 1st parity or using a directed challenge during rearing are interesting.

As earlier discussed, most opportunity to reduce lameness may come from animal selection against OC, and also, from research improving facilities and management systems. Differences between Chaper III-IV and Chaper V on TM and Met effects and also regarding lameness severity, point out that lameness severity may interact with such dietary treatment potential. Therefore, challenging pigs with commercial risk factors to force lameness (i.e. claw health, and OC) such as management, genetics lines with different risk of OC or claw lesions (Aasmundstad et al., 2013, 2014), and flooring conditions in a controlled manner could be useful to study TM supplements. Increasing levels of TM or Met inclusion, may answer the actual requirements of TM in current commercial conditions. Furthermore, as reviewed in Chapter I and discussed in Chapter V, BW load and growth rate may intervene with bone and joint development and should be further investigated. Especially, since Lys reduction in Chapter V (19% of requirements) seemed insufficient to reduce BW in herein conditions and reduce lameness. Reduction of final BW was only 3.8% vs. the 7.3% to 19% (via feed restriction) previously reported as effective on reducing OC and lameness (Koning et al., 2014, Quinn et al., 2015). For ad libitum allowance as herein, not only the Lys reduction but the ratio to net energy and the fiber content in the diet should be considered to slow growth and minimize compensatory feed intake response (Li and Patience, 2017).

7.3. Limitations and considerations for assessing lameness

Experimental designs with a commercial approach may complicate the assessment and the results interpretation, while may help to answer actual problems. Treated with caution and including the context to understand possible noise, the present results are highly valuable describing joint health, bone properties, claw health, and lameness in replacement gilts. As above discussed in the present discussion, the context becomes especially important to study intervention of dietary treatments. Especially, if the response variables are already multidimensional, such as lameness, OC and claw lesions are. Hence, some limitations for assessing lameness are discussed below. There are several response variables typically used to assess causes of lameness. For example, gross scoring and counting of OC lesions, histologic changes in specialized tissues (growth cartilage, bone, and horn), bone density, bone strength, bone mineral content, claw lesions, physical properties of tissues, gait scores, lameness scores, biomechanical quantitative measurements, kinematics, etc. (Bradley, 2010; Heinonen et al., 2013; Riet et al., 2013; Olstad et al., 2015; Varagka et al., 2016). Nonetheless, which are the best variables to measure and improve lameness risk factors and lameness outcome? How lameness may be reduced in the coming years? Answers, seem complex and unclear.

Factors and causes of lameness are numerous, but ultimately they have to cause clinical signs such as alteration of gait, posture, and reduce mobility (Anil et al., 2005; Anil et al., 2009a; Nalon et al., 2013a; Nalon et al., 2013b). However, the degree at what these alterations become important is uncertain. There is a lack of data measuring the relationship between the response variables commonly used to study causes of lameness (listed above), with the actual lameness and its consequences (Nalon et al., 2013b). For example, the effects of enhancing bone and joint properties (i.e. density, trabecular structure, strength, OC) on lameness is unknown and difficult to measure. Horn hardness negatively correlated with lameness in cattle (Borderas et al., 2004) while horn tubule structure and density are considered key for providing horn mechanical reinforcement in horses (Lancaster et al., 2013). In pigs, tubule structure and density was suggested as an advantage for laminitis (Varagka et al., 2016). Denser and more resistant horn tubules may reduce claw lesions, but the relative importance of those changes in current prevalence of lameness is unclear for pigs (KilBride et al., 2009). Generally, lameness decreases while claw lesion score increases with age or parity (Lucia et al., 2000; Pluym et al., 2011; Heinonen et al., 2013). Therefore, caution must be taken when associating sow locomotion problems and possible causes. Hypothesizing, if most of OC and claw lesions were associated with genetics, management, and flooring conditions, to reach the most resistant bone and the better horn properties might not make the greatest difference on lameness.

To measure lameness properly is complicated. Females with apparently painful and sever locomotion problems (i.e. OC dissecans, see Chapter I) may be resilient to show symptoms (Main et al., 2000; Crenshaw et al., 2013; Etterlin et al., 2015). Meanwhile, Stavrakakis (2014), described that frequency and magnitude of irregular steps was increased in pigs with subclinical OC. Similarly, some gilts (15%, Chapter IV) in the present study included severe lesions of OC without apparent lameness, whereas gait score positively

correlated with OC total score (Chapter IV). The presence of moderate locomotion changes under OC suggest that not only lameness is important, but also some mild degree of gait abnormalities which are difficult to detect. Clearer, most consensus points out that lame animals reduced the weight bearing on the affected limb (Sun et al., 2011; Pluym et al., 2013c; McNeil et al., 2018). Likely, uncertainty has led to variety of definitions, scoring systems, and methods (objective and subjective) to measure lameness (Nalon et al., 2013a). Visual scoring is the most commonly used method to assess lameness, but usually presents high variability and the observer needs previous training (Welfare Quality, 2009). Although, the method chosen herein was visual scoring for being the most commonly used, inexpensive, and easier implemented. There are different automated and objective systems already reported for lameness evaluation in pigs. The different systems are mainly based on: 1) gait movement or footprint, kinematics, and accelerometers (Thorup et al., 2007; von Wachenfelt et al., 2010; Grégoire et al., 2013; Mohling et al., 2014), 2) pressure and sensitivity to heat (Pairis-Garcia et al., 2014), stance angles (Stock et al., 2017), and 3) force plates (Sun et al., 2011; Pluym et al., 2013c; McNeil et al., 2018). In the future, some automated systems could be integrated in the farm technology i.e. electronic feeding stations, and detect slight changes on feet weight bearing within the feeding time (1 to 10 minutes, McNeil et al., 2018). Even though automated detection is not actual prevention, an early detection of locomotion problems could improve treatment and outcome.

To date, evidences indicate that importance of small changes in gait is variable. The use of objective methods to measure them and study the consequences seem the most adequate for understanding and improving lameness in the near future. However, to study locomotion disorders and causes of lameness, both, subjective and (or) objective methods might still be required (Heinonen et al., 2013). In additioon, some simplification is needed to implement objective systems in practice (Nalon et al., 2013; Stavrakakis, 2014; McNeil et al., 2018).

To overcome the challenging uncertainty for lameness and gait disorders definitions discussed above, and their consequences a clear consensus will be required. One could speculate that any locomotion abnormality becomes important when symptoms can be associated with leg weakness and pain, or when productivity is reduced. KilBride et al. (2009), reported that 14.4% of gilts and 16.9% of sows showed gait abnormalities during gestation, but only 1.0 and 1.8%, respectively, reduced weight bearing on an affected limb. Hence, suggesting a little proportion of lame animals including pain and reduced mobility. Similarly, comparing deficient with sound conformation, deficient were related with abnormalities in gait and joint flexion values or limb flexion symmetry during motion (Stavrakakis et al., 2014). Nevertheless, such abnormalities do not necessary mean lameness, or pain, or performance loss.

In the present thesis, as reported in Chapter III, a performance reduction (7 kg in final BW and 80 g/d in ADG) was directly related with lame sows, whereas no negative impact of lameness on ADG was observed in Chapter V. The prevalence of lameness was similar in both studies but because lameness reduced performance in Chapter III, this seems more important in Chapter III than in V. Gilts from Chapter III were shown a greater gait score than in Chapter V ones (Table 7.1), additionally, they were shown 80% OC prevalence (Chapter IV). Retrospectively, lameness from rearing period reduced onwards reproductive

and productive performance on the sow farm only in Chapter III. Nonetheless, lameness cases observed on the sow farm showed lower productive and reproductive performance for both studies (Chapter III and V). Therefore, again data suggests that lameness in the rearing phase was different between studies by affecting sow performance only in Chapter III. However, lameness on the sow farms may be similar between studies as both reduced sow productivity.

Recovery from a lameness process is a poorly studied concept that may help to clarify lameness severity. Lameness severity may determine the no return point for recovery (cause of culling). Probably, a punctual lameness is less important than a persisting lameness, while performance loss would be determinant for culling. Likelihood of events potentially causing gait changes and punctual lameness seems high in the conditions at what pigs are currently confined and produced. A proof, may be the low correlation between causes of lameness and lameness earlier discussed. Evidences have associated lameness with social conflict and agonistic behavior (Li and Gonyou, 2013; Olsson et al., 2016), floor conditions (KilBride et al., 2009; Bos et al., 2016), animal density (Jorgensen, 2003), and growth rate (Koning et al., 2014). To assess recovery, various evaluations are needed and are included in only few studies have performed them (Pluym et al., 2013abc; Stavrakakis et al. 2013, 2014), sometimes using experimentally induced lameness (McNeil et al., 2018), and usually with very few animals or changing conditions. There are no longitudinal studies available in the literature which, in replacement gilts and commercial conditions up to 1st parity, include repeatedly assessment of lameness (7 times during rearing and 3 times during 1st parity). Taking into account 600 gilts (360 and 240 gilts from Chapters III and V, respectively) a summary of ADG is reported in Table 7.2 including gilts that were able to overcome lameness process or were not. In general terms, data suggests that recovery of lameness may result with better performance than if gilts do not overcome lameness. However, results again seem controversial between studies. Lame gilts reduced performance regardless of recovery in Chapter III, but no effect either of lameness or recovery on ADG was observed in Chapter V.

Table 7.2. Average daily gain (ADG) among never lame gilts and lame gilts eventually recovering or not.

ADG, g/d (n)	Chapter III, n = 360		Chapter V, n = 240		Total, n = 600	
Non-lame	953^{a}	(319)	869	(212)	920^{ax}	(531)
Lame non-recovery	877^{b}	(12)	843	(11)	860^{by}	(23)
Lame recovery	870^{b}	(15)	901	(8)	882^{bz}	(23)
Deaths	-	(14)	-	(8)	-	(22)
P-value lameness	<0.01	SEM = 25.6	0.27	SEM = 27.9	<0.01	SEM = 19.4

$^{a-b}$ Values within a column with different superscripts differ significantly at $P < 0.05$.
$^{x-z}$ Values within a column with different superscripts differ significantly at $P \leq 0.10$.

Including both studies, 46 out to 600 females (7.7%) eventually became lame during the rearing phase and 50% recovered. This is so far the first reference of lameness recovery in replacement gilts under commercial conditions. Logic says that non-recovered lameness should be more important than punctual lameness, however, this may be true only to some

extent. Females that do not overcome lameness seem to have lowered ADG than those overcoming lameness, as numerically in Chapter V and as a tendency when combined both studies data (P = 0.100). More precise and objective methods of lameness evaluation would be required to combine data from the two different studies and elucidate evidences. Nonetheless, as a preliminary idea, the present data indicates that recovery is an interesting concept to further investigate. The reasons behind recovery were unknown. Once detected, the protocol for lame animals was to treat them with antibiotic. Also, lame gilts were isolate in a separate pen when lameness was severe, but this was not possible in most cases and only applied if lameness was highly severe (1 case in Chapter III). Likely, reduce competitive interactions and agonistic experiences with pen-mates should allow easier recovery, but little is known about the healing process of lameness in pigs and would highly depend on the initiating cause. To relate recovery with dietary treatment was found not feasible as divide the already small sample size.

Another difficulty to analyze lameness is sample size, as this becomes critical for observations that include low to moderate prevalence within a population (i.e. 5 to 20% lameness). Indeed, one problem with Chapter V design was that sample size was reduced to 60 females, which may have also contributed to the lack of more clear results. Based in our studies, when we were able to detect changes on lameness prevalence among dietary treatments, the minimum difference was 8.5% (14.8% vs. 6.5%; Chapter III), and the minimal sample required was 85 animals. However, if prevalence is below 8%, or the power effect of treatment is expected less, or capability to detect lameness is reduced, the minimum sample size increases over 100 animals. In the present studies, via 7 repeated observations (every 3 week), 1 or 3 more explorations for gilt conformation, plus a daily rapid observation were believed sufficient to detect most locomotion problems. The uncertainty of lameness and low prevalence in sample size may justify a challenging model with lameness risk factors as earlier discussed.

Altogether, and with the experience herein, future gait and lameness assessments should include a numerous sample size, and when possible, the causes of lameness should be diagnosed. In addition, to state the importance of lameness in each particularly study it would be needed to measure 1) the degree of improvement provided by the treatment strategies on response variables (i.e. horn structure, claw lesions, OC, etc.), and 2) the degree of improvement in the consequences of that particular lameness (i.e. pain, performance, culling due to claw lesion, etc.).

7.4. Literature cited

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CHAPTER VIII

General Conclusions

From the presented results and their interpretation, the following conclusions were obtained:

1- Lameness prevalence is around 8% for the rearing period, occurrence increases with age, and first appearance is between 107 and 140 kg of BW. Lameness may or may not be associated with severe performance losses. In Chapter III, losses include 80 g average daily gain and 7 kg of final BW, while no losses are observed in Chapter V. At the end of the rearing phase (171.5 ± 8.1 kg BW), prevalence of gross osteochondrosis articular lesions is 80%, while prevalence of severe lesions is 15%. The osteochondrosis total score weakly and positively increases (R^2 = 0.27; P = 0.001) with gait score, although, severe osteochondrosis can still be subclinical for lameness.

2- On the sow farm, lameness increases from 4% in late rearing to 10-17% in early gestation, and reduces towards the end of lactation (8.5% to 11%). Similarly, claw lesions (absent during rearing), highly increase to 53% prevalence at first farrowing. Prevalence during first gestation (11 % to 17%) and first lactation (8.5% to 11%) reduces born alive piglets (Chapter V), reduces the weaned piglets (Chapter III), and increases backfat loss in lactation (Chapter III, V) and weaning-to-estrus interval (Chapter III, V).

3- The additional trace minerals as organic Zn, Cu, and Mn slightly enhance bone size, mineral content, and bone strength compared to control (both above growth requirements). Similarly, these additional trace minerals or methionine increase whole body bone density, whereas combined, reduce osteochondrosis total score (Chapter IV). Nonetheless, those dietary treatments affect lameness in Chapter III but not in Chapter V. Additionally, the physiological average daily gain variability (high or low) does not affect gross osteochondrosis (10% of osteochondrosis total score).

4- For the rearing period, a 19% reduction of total lysine over dietary requirements, reduces growth rate (6.4%) and final BW (3.8%) independently of trace minerals plus methionine additional levels. However, lameness prevalence is not affected.

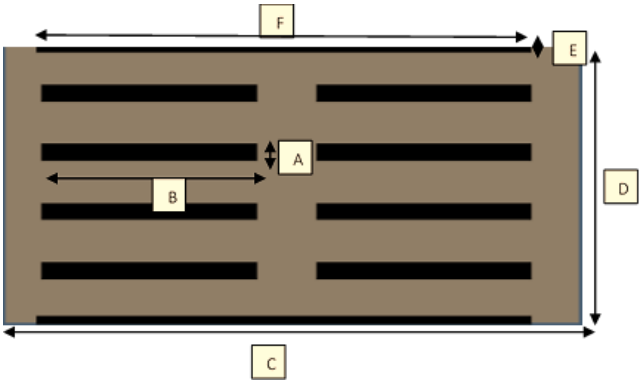
5- The dietary treatment trace minerals, or methionine, or the combination, or low lysine, do not provide any carryover effect into first parity lameness, claw lesions, or productive performance.

6- Under hostile PRRSv environment and infection, dietary treatment vitamin A and *omega*-3 have no positive effects while tryptophan and threonine combined has a limited positive effect on gilt performance. Additional tryptophan and threonine increases BW gain early post infection compared with control and *omega*-3. The PRRSv viremia is not modulated through dietary treatment, however, acute cytokine production is increased by vitamin A (interferon-α and interleukin-8) and TT (interleukin-6) without significant effects on performance.

CHAPTER IX

Appendixes

Appendix 1. European Union regulation on void space and slot width in slatted floors.



Void Surface (VS):
S1 = (A) × (B) × (n of void lines)
S2 = (F) × E) × 2
HS = S1+S2
Concrete surface (CS):
CS = (C) × (D)
% Void ratio = (VS/CS) × 100

Figure 9.1. Illustration of slat assessment for void ration (VR). Always calculated when flooring is full slat, then VR apply as accepted as = or < 15%. If flooring is part slat, when the solid included 1.3 m^2 per sow, VR is not measured. The maximum slot width is 20 mm and minimum rip width is of 80 mm for both gilts and sows in housed during gestation (in accordance with European Union (2001/88/CE, 2008/120/EC).

Appendix 2. Assessment of gilt conformation

Structural conformation of gilts was evaluated 4 occasions (79, 127, and 213 d of age in average during rearing and at service) in gilts from Chapter III and only at the end of rearing (209 d of age) in gilts from Chapter V. Evaluations were performed within the pen (0.90 m2/gilt; 60% slatted and 40% solid floor) and on a 1.5 m wide corridor (solid concrete) during 2 minute observations per animal. The methodology (Table 9.1.) was adapted from methods described by Koning et al. (2015) and modified for 2 locomotive characteristics (score of 0-3) and 6 conformation characteristics (score of 1-5). The locomotive: 1) swaying of hindquarters, as normal state (0) or indicating greater degree of swaying (1-3); 2) gait score, as of fast-smooth (0) to slow with slight difficulty (1), mild difficult (2), and limping or unwilling weight bearing or standing (3). For all structural conformation characteristics, normality was defined in score 3 and severity above or below 3 indicated a certain deviation. These were: "O" shape or "X" shape, sickled or buckled, and steep or weak pasterns (see Figure 1.1 for representations, Chapter I).

Table 9.1. Locomotion and conformation traits.

	Scoring		
Locomotion	0	1-2	3
Swaying hindquarters	No Swaying	Slight - mild	Severe swaying
Gait score	Smoth and fast	Slight - mild	Limping or unwilling weight bearing
Conformation	1	3	5
Front legs			
O shape or X shape	O shape	Neutral	X shape
Sickled or buckled	Sickled	Neutral	buckled
Steep or weak pasterns	Steep	Neutral	weak pasterns
Hind legs			
O shape or X shape	O shape	Neutral	X shape
Sickled or buckled	Sickled	Neutral	buckled
Steep or weak pasterns	Steep	Neutral	weak pasterns

Modified from Koning et al. (2015). Cited in Chapter VII (General discussion literature).

Table 9.2. Structural conformation evaluated 3 times during rearing period (28.8 ± 8.8 to 155.8 ± 14.2 kg of body weight) and at service in replacement gilts which performance results are presented in Chapter III.

		Treatment					
	Age, d	**CON**	**MIN**	**METH**	**MM**	SEM	*P*-value
Swaying hindquarters (0-3)	*79 ± 11*	0.63	0.42	0.36	0.39	0.64	0.27
	127 ± 11	1.73	1.42	1.32	1.30	0.63	0.06
	213 ± 11	1.09	1.03	1.04	1.17	0.60	0.33
	Service	0.59	0.71	0.74	0.56	1.08	0.62
Gait score (0-3)	*79 ± 11*	0.06	0.02	0.04	0.01	0.20	0.80
	127 ± 11	1.04	1.00	1.03	1.07	0.21	0.76
	213 ± 11	1.09	1.03	1.04	1.01	0.21	0.72
	Service	1.26	1.57	1.42	1.13	1.04	0.25
Front legs							
O shape or X shape	*79 ± 11*	3.42	3.36	3.37	3.33	0.27	0.80
(1-2-3-4-5)	*127 ± 11*	3.41	3.37	3.34	3.35	0.27	0.80
	213 ± 11	3.51	3.51	3.38	3.55	0.29	0.88
	Service	3.43	2.90	3.23	3.60	0.83	0.40
Sickled or buckled	*79 ± 11*	2.74	2.96	2.93	4.58	0.36	0.50
(1-2-3-4-5)	*127 ± 11*	2.91	2.91	2.92	2.77	0.35	0.80
	213 ± 11	3.01	3.00	3.02	3.00	0.05	0.96
	Service	3.00	2.97	2.96	3.08	0.15	0.85
Steep or weak pasterns	*79 ± 11*	3.44	3.50	3.37	3.31	0.36	0.84
	127 ± 11	3.32	3.39	3.43	5.16	0.63	0.81
(1-2-3-4-5)	*213 ± 11*	3.93	3.99	3.74	3.84	0.36	0.85
	Service	3.87	3.71	3.63	3.74	0.43	0.67
Hind legs							
O shape or X shape	*79 ± 11*	2.97	2.98	3.00	3.00	0.17	0.90
	127 ± 11	3.01	2.96	8.22	2.96	0.17	0.85
(1-2-3-4-5)	*213 ± 11*	3.09	3.03	3.02	3.02	0.17	0.80
	Service	3.40	3.25	3.23	3.13	0.34	0.63
Sickled or buckled	*79 ± 11*	3.31	3.20	3.25	3.31	0.86	0.80
	127 ± 11	3.23	3.37	3.24	3.25	0.25	0.62
(1-2-3-4-5)	*213 ± 11*	3.22	3.01	3.21	3.19	0.26	0.90
	Service	3.28	3.30	3.33	3.40	0.29	0.89
Steep or weak pasterns	*79 ± 11*	2.98	2.90	3.01	2.93	0.29	0.79
	127 ± 11	2.92	3.02	2.96	2.97	0.26	0.75
(1-2-3-4-5)	*213 ± 11*	3.16	3.26	3.18	3.15	0.22	0.80
	Service	3.17	3.19	3.13	3.19	0.32	0.92

*Score 3 represents the neutral conformation.

Table 9.3. Structural conformation evaluated at late rearing (151.15 ± 14.1 kg of body weight) of replacement gilts which performance results are presented in Chapter V.

	Control[1]		**Trace minerals plus Met**[1]		SEM	P-value		
	Standard Lys[2]	**Low Lys**[2]	**Standard Lys**[2]	**Low Lys**[2]		Min Met	Lys	MinMet × Lys
Swaying hindquarters (0-3)	0.34	0.47	0.38	0.35	1.32	0.225	0.787	0.645
Gait score (0-3)	0.09	0.14	0.14	0.07	0.052	0.854	0.789	0.152
Front legs								
O shape or X shape (1-2-3*-4-5)	3.41	3.38	3.53	3.44	0.072	0.212	0.124	0.654
Sickled or buckled (1-2-3-4-5)	3.05	2.98	3.04	2.96	0.033	0.635	0.035	0.971
Steep or weak pasterns (1-2-3-4-5)	3.56	3.69	3.85	3.75	0.104	0.081	0.878	0.259
Hind legs								
O shape or X shape (1-2-3-4-5)	3.46	3.40	3.10	3.59	0.107	0.822	0.281	0.106
Sickled or buckled (1-2-3-4-5)	3.53	3.55	3.47	3.51	0.089	0.611	0.749	0.927
Steep or weak pasterns (1-2-3-4-5)	3.53	3.45	3.64	3.47	0.091	0.470	0.195	0.610

[1]**Control**, basal diet; and, **trace minerals plus Met (MinMet)**, with additional 10, 20 and 50 mg/kg of chelated cooper, manganese and zinc, respectively (0.1%, Aplomotec Plus, Tecnología & Vitaminas, S.L., Alforja, Spain), and increased Met: Lys ratio as 1.01.

[2]**Standard Lys** provided 165 crude protein (CP)/kg with 10.0 g Lys/kg between 119 and 163 d of age, and 140 g CP/kg with 8.0 g Lys/kg between 164 d to 209 d of age; or **low Lys**, set first to 155 g CP/kg with 8.1 g Lys/kg, and second phase to 140 g CP/kg with 6.5 g Lys/kg.