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Tesis Doctoral

IMPACT OF IMMUNOCASTRATION AND EVALUATION OF NUTRITIONAL STRATEGIES ON PIGS INTENDED FOR THE PDO TERUEL HAM

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Impact of immunocastration and evaluation of nutritional strategies on pigs intended for the PDO Teruel ham

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INDEX OF CONTENTS

INDEX OF FIGURES

INDEX OF TABLES

COMPENDIUM OF ARTICLES

This doctoral thesis is presented as a compendium of six research papers, four previously published and two under review in journals that are indexed in the Journal Citation Reports™ (JCR) database. The references of these articles are listed below.

1. Pérez-Ciria, L., Carcò, G., Miana-Mena, F. J., Mitjana, O., Falceto, M. V., & Latorre, M. A. (2021). Immunocastration in Gilts: A Preliminary Study of the Effect of the Second Dose Administration Time on Growth, Reproductive Tract Development, and Carcass and Meat Quality. *Animals*, *11*(2), 510. https://doi.org/10.3390/ani11020510.
2. Pérez-Ciria, L., Miana-Mena, F. J., Falceto, M. V., Mitjana, O., & Latorre, M. A. (2021). Effect of Immunocastration and Diet on Growth Performance, Serum Metabolites and Sex Hormones, Reproductive Organ Development and Carcass Quality of Heavy Gilts. *Animals*, *11*(7), 1900. https://doi.org/10.3390/ani11071900.
3. Pérez-Ciria, L., Miana-Mena, F. J., Álvarez-Rodríguez, J., & Latorre, M. A. (2022). Effect of Castration Type and Diet on Growth Performance, Serum Sex Hormones and Metabolites, and Carcass Quality of Heavy Male Pigs. *Animals*, *12*(8), 1004. https://doi.org/10.3390/ani12081004.
4. Pérez-Ciria, L., Miana-Mena, F. J., López-Mendoza, M. C., Álvarez-Rodríguez, J., & Latorre, M. A. (2021). Influence of Immunocastration and Diet on Meat and Fat Quality of Heavy Female and Male Pigs. *Animals*, *11*(12), 3355. https://doi.org/10.3390/ani11123355.
5. Pérez-Ciria, L., Ripoll, G., Sanz, M. A., Blanco, M., Miana-Mena, F. J., & Latorre, M. A. Impact of gilt immunocastration on weight losses and instrumental and chemical characteristics of Teruel dry-cured ham. Manuscript submitted to *Meat Science* on 10/22/2022 (ref.: MEATSCI-D-22-00784). Under review.
6. Pérez-Ciria, L., Ripoll, G., Sanz, M. A., Blanco, M., & Latorre, M. A. Effect of male pig immunocastration on instrumental and chemical characteristics of Teruel dry-cured hams. Manuscript submitted to *Spanish Journal of Agricultural Research* on 11/07/2022 (ref. number: 19967). Under review.

SUMMARY

During years, a considerably proportion of carcasses of pigs destined for the protected designation of origin “Teruel ham” is being declared not suitable for this purpose. The main cause is the lack of fat cover, which avoids an excessive dehydration of the pieces during the dry-curing process. Also, a limited intramuscular fat content has been found in the meat from these animals, parameter positively related to tenderness and juiciness. The focus of genetic selection on getting lean has caused the lack of fat. This problem mainly affects female pigs, because male pigs destined for this protected designation of origin are surgically castrated to avoid boar taint, and castration increases fat accretion. On the other hand, surgical castration is an invasive method and therefore alternatives of that are being sought in the European Union for animal welfare reasons.

In order to increase fat retention in female pigs, the current work evaluated the possibility of immunocastration and also testing nutritional strategies that could increase fatness. In this type of animals, which are slaughtered at approximately 130 kg of body weight, it is accepted that only two doses against gonadotropin-releasing factor are necessary for immunocastration, but the optimal time to inject the second dose has not been defined (the first one just stimulates the immune system and the second one is the really effective). Therefore, another aim of this work was to determine the optimum moment to apply the second dose of immunocastration in this kind of females. To carry out the aforementioned objectives, two experiments were planned. In the Experiment 1, a total of 48 Duroc x (Landrace x Large White) gilts, destined for Teruel dry-cured ham production, were distributed in four experimental groups; entire gilts and immunocastrated for second time at 12, 9 or 7 weeks before slaughter (12 animals in each group). In this trial, average daily gain, serum sex hormones, reproductive organs, and carcass, meat and fat quality were studied. In the Experiment 2, a total of 192 Duroc x (Landrace x Large White) gilts intended for Teruel dry-cured ham elaboration (96 entire and 96 immunocastrated) were used. Three experimental diets (a control diet, a high-energy diet and a low-crude-protein and -amino-acids diet) were offered to both types of females during the grower and the finisher periods (from 76 to 102 kg and from 102 to 134 kg of body weight, respectively). In this second trial, growth performances, serum metabolites and sex hormones, reproductive organs, and carcass, meat, fat and dry-cured ham quality were evaluated.

In order to found an alternative to surgical castration in male pigs, the present work also assessed immunocastration in them. It could reduce fat accretion, in comparison to surgical castration, which could be a negative aspect for Teruel dry-cured ham production, but it is key to check it in this kind of animals. Therefore, the present work with males also aimed to analyze the effects of providing nutritional strategies that could increase fatness. For it, the Experiment 3 was carried out with a total of 144 Duroc x (Landrace x Large White) male pigs destined for Teruel-dry-cured ham production (72 surgically castrated and 72 immunocastrated), being tested in both types of males, the same experimental diets researched in the Experiment 2 during the grower and the finisher periods (from 80 to 110 kg and from 110 to 137 kg of body weight, respectively). In this trial, growth performances, serum sex hormones and metabolites, and carcass, meat, fat and dry-cured ham quality were researched.

The Experiment 1 showed that earlier immunocastrated gilts for second time (at 9 and 12 weeks before slaughter) presented certain (numerically) higher subcutaneous fat thickness in carcass and intramuscular fat content in pork compared to later vaccinated gilts (at 7 weeks before slaughter). In the Experiment 2, immunocastration of female pigs increased growth rate (P=0.0007) and also subcutaneous fat thickness (P=0.011) and intramuscular fat content (P=0.018 for meat and P=0.049 for dry-cured ham). In the case of males (Experiment 3), immunocastration improved (P<0.0005) average daily gain and feed conversion ratio, in comparison to surgical castration, but generated lower subcutaneous fat thickness in carcass (P=0.0004) and intramuscular fat content in meat (P=0.001). Moreover, in the fat of dry-cured hams, male pig immunocastration did not reduce (P<0.005) the concentrations of some compounds responsible for boar taint (skatole and indole) as much as surgical castration, although both concentrations were low.

On the other hand, the nutritional strategies tested (increasing dietary energy by 0.15 Mcal of net energy per kg or decreasing dietary crude protein by 2 percentage points and amino acids *vs*. commercial diets) had no effect on subcutaneous fat thickness and intramuscular fat content. Nevertheless, both in female and male pigs, the high-energy diet improved the efficiency transforming feed into weight gain, and the low-crude-protein and -amino-acids diet did not impair growth performances.

It can be concluded that immunocastration is an interesting technique to use in gilts intended for Teruel dry-cured ham production and the optimum time for the

application of the second dose of immunocastration in them seems to be between 9 and 12 weeks before slaughter. However, in male pigs destined for Teruel dry-cured ham elaboration, surgical castration is preferable than immunocastration. On the other hand, increasing dietary energy by 0.15 Mcal of net energy per kg or decreasing dietary crude protein by 2 percentage points and amino acids in commercial diets, during the grower and the finisher periods (from 80 to 135 kg of body weight), are not suitable strategies for increasing fatness, although these nutritional approaches may still be beneficial to pig farmers by improving or not worsening growth performances.

RESUMEN

Durante años, una proporción considerable de las canales de cerdos destinados a la denominación de origen protegida “Jamón de Teruel” está siendo declarada no apta para este fin. La principal causa es la falta de cobertura grasa, la cual evita una deshidratación excesiva de las piezas durante el proceso de curado. Además, se ha encontrado un contenido limitado de grasa intramuscular en la carne de estos animales, parámetro relacionado positivamente con la ternura y la jugosidad. El enfoque de la selección genética en conseguir magro ha causado la falta de grasa. Este problema afecta principalmente a las hembras, ya que los machos destinados a esta denominación de origen protegida son castrados quirúrgicamente para evitar el olor sexual, y la castración aumenta la acumulación de grasa. Por otro lado, la castración quirúrgica es un método invasivo y por lo tanto se están buscando alternativas a esta práctica en la Unión Europea por razones de bienestar animal.

Para aumentar la retención de grasa en las cerdas, el presente trabajo evaluó la posibilidad de la inmunocastración y también probó estrategias nutricionales que podrían aumentar el engrasamiento. En este tipo de animales, los cuales son sacrificados a aproximadamente 130 kg de peso vivo, se acepta que solo son necesarias dos dosis contra el factor de liberación de la gonadotropina para la inmunocastración, pero no se ha definido el momento óptimo para inyectar la segunda dosis (la primera solo estimula al sistema inmunitario y la segunda es la realmente efectiva). Por lo tanto, otro objetivo de este trabajo fue determinar el momento óptimo para aplicar la segunda dosis de inmunocastración en este tipo de hembras. Para llevar a cabo los objetivos antes mencionados, se planificaron dos experimentos. En el Experimento 1 un total de 48 cerdas Duroc x (Landrace x Large White), destinadas a la producción de jamón curado de Teruel, se distribuyeron en cuatro grupos experimentales; cerdas enteras e inmunocastradas por segunda vez a las 12, 9 o 7 semanas antes del sacrificio (12 animales en cada grupo). En este ensayo, se estudiaron la ganancia media diaria, las hormonas sexuales séricas, los órganos reproductores y la calidad de la canal, la carne y la grasa. En el Experimento 2, se utilizaron un total de 192 cerdas Duroc x (Landrace x Large White) destinadas a la elaboración de jamón curado de Teruel (96 enteras y 96 inmunocastradas). Tres dietas experimentales (una dieta control, una dieta alta en energía y una dieta baja en proteína bruta y en aminoácidos) se ofrecieron a ambos tipos de hembras durante los períodos de crecimiento y finalización (de 76 a 102 kg y de 102 a 134 kg de peso vivo,

respectivamente). En este segundo ensayo, se evaluaron los rendimientos productivos, los metabolitos y las hormonas sexuales en el suero, los órganos reproductores y la calidad de la canal, la carne, la grasa y el jamón curado.

Con el fin de encontrar una alternativa a la castración quirúrgica en los cerdos machos, el presente trabajo también evaluó la inmunocastración en ellos. Esta técnica podría reducir la acumulación de grasa, en comparación con la castración quirúrgica, lo que podría ser un aspecto negativo para la producción de jamón curado de Teruel, pero es clave comprobarlo en este tipo de animales. Por lo tanto, el presente trabajo con machos también tuvo como objetivo analizar los efectos de proporcionar estrategias nutricionales que podrían aumentar el engrasamiento. Para ello se llevó a cabo el Experimento 3 con un total de 144 cerdos machos Duroc x (Landrace x Large White) destinados a la producción de jamón curado de Teruel (72 castrados quirúrgicamente y 72 inmunocastrados), siendo testadas en ambos tipos de machos las mismas dietas experimentales investigadas en el Experimento 2 durante los períodos de crecimiento y finalización (de 80 a 110 kg y de 110 kg a 137 kg de peso vivo, respectivamente). En este ensayo se investigaron los rendimientos productivos, las hormonas sexuales y los metabolitos en el suero, y la calidad de la canal, la carne, la grasa y el jamón curado.

El Experimento 1 mostró que las cerdas inmunocastradas antes por segunda vez (a las 9 y a las 12 semanas antes del sacrificio) presentaron cierto (numéricamente) mayor espesor de la grasa subcutánea en la canal y contenido de grasa intramuscular en la carne en comparación con las cerdas vacunadas más tarde (a las 7 semanas antes del sacrificio). En el Experimento 2 la inmunocastración de las hembras aumentó la tasa de crecimiento (P=0.0007) y también el espesor de la grasa subcutánea (P=0.011) y el contenido de grasa intramuscular (P=0.018 para la carne y P=0.049 para el jamón curado). En el caso de los machos (Experimento 3), la inmunocastración mejoró (P<0.0005) la ganancia media diaria y el índice de conversión, en comparación con la castración quirúrgica, pero generó menor espesor de la grasa subcutánea en la canal (P=0.0004) y contenido de grasa intramuscular en la carne (P=0.001). Además, en la grasa de los jamones curados, la inmunocastración de los machos no redujo (P<0.005) las concentraciones de algunos compuestos responsables del olor sexual (escatol e indol) tanto como la castración quirúrgica, aunque ambas concentraciones fueron bajas.

Por otro lado, las estrategias nutricionales testadas (aumentar la energía dietética en 0,15 Mcal de energía neta por kg o disminuir la proteína bruta dietética en 2 puntos

porcentuales y los aminoácidos *vs.* dietas comerciales) no tuvieron efecto sobre el espesor de la grasa subcutánea y el contenido de grasa intramuscular. Sin embargo, tanto en cerdos hembras como machos, la dieta alta en energía mejoró la eficiencia transformando el alimento en ganancia de peso, y la dieta baja en proteína bruta y en aminoácidos no perjudicó los rendimientos productivos.

Se puede concluir que la inmunocastración es una técnica interesante para usar en cerdas destinadas a la producción de jamón curado de Teruel y el momento óptimo para la aplicación de la segunda dosis de inmunocastración en ellas parece ser entre 9 y 12 semanas antes del sacrificio. Sin embargo, en cerdos machos destinados a la elaboración de jamón curado de Teruel es preferible la castración quirúrgica a la inmunocastración. Por otro lado, aumentar la energía dietética en 0,15 Mcal de energía neta por kg o disminuir la proteína bruta dietética en 2 puntos porcentuales y los aminoácidos en las dietas comerciales, durante los períodos de crecimiento y finalización (de 80 a 135 kg de peso vivo), no son estrategias adecuadas para aumentar el engrasamiento, aunque estos enfoques nutricionales pueden aun así ser beneficiosos para los ganaderos al mejorar o al no empeorar los rendimientos productivos.

LIST OF ABBREVIATIONS

AA	Amino acids
ADFI	Average daily feed intake
ADG	Average daily gain
BW	Body weight
CP	Crude protein
EG	Entire gilts
EM	Entire males
EU	European Union
FCR	Feed conversion ratio
FSH	Follicle-stimulating hormone
G:F	Gain-to-feed ratio
GnRF	Gonadotropin-releasing factor
GnRH	Gonadotropin-releasing hormone
IG	Immunocastrated gilts
IM	Immunocastrated males
IMF	Intramuscular fat
LH	Luteinizing hormone
Lys	Lysine
MUFA	Monounsaturated fatty acids
N	Nitrogen
NE	Net energy
PDO	Protected designation of origin
PGI	Protected geographical indication
PUFA	Polyunsaturated fatty acids
SCF	Subcutaneous fat
SCM	Surgically castrated males
SFA	Saturated fatty acids

1. INTRODUCTION

In recent years, a considerably proportion of pig carcasses destined for the protected designation of origin (PDO) “Teruel ham” is declared not suitable for this purpose (Latorre et al., 2008). The main cause is the lack of fat cover (Latorre et al., 2009), since genetic selection has focused on getting lean. This parameter is especially relevant for dry-cured ham production because it avoids an excessive dehydration of the pieces and improves organoleptic characteristics (Bosi & Russo, 2004). Likewise, a modest content of intramuscular fat (IMF) has been detected in dry-cured hams of these animals (Rodríguez-Sánchez et al., 2014), parameter positively related to juiciness and negatively to hardness (Ruiz-Carrascal et al., 2000). These problems mainly affect females (Latorre et al., 2009), since males are surgically castrated to avoid boar taint, and castration increases fat accretion (Weatherup et al., 1998).

Feeding could resolve in part these problems. Nutrients that more affect fatness are the level of energy and the content of crude protein (CP) and amino acids (AA). The rise of dietary energy level maintaining CP and AA contents or the reduction of dietary CP and AA levels maintaining energy content seem to increase fatness (Suárez-Belloch et al., 2016; Suarez-Belloch et al., 2013), but the results are not consistent. On the other hand, growth performances could be penalized with a high restriction of CP and AA contents and the price of the sources of fat could condition the formulation of diets with high-energy level.

The castration of females could be other solution, since it also increases fat retention (Peinado et al., 2008), but it is prohibited by surgical methods in female pigs reared indoors in the European Union (EU) (Official Journal of the European Union, 2009). Besides, according to the regulation of the PDO Teruel ham (Boletín Oficial de Aragón, 2021), gilts should not present estrus at slaughter. Therefore, immunocastration could resolve these issues. In Spain, this technique has been applied especially in Iberian females rearing outdoors to avoid pregnancies by wild boars. Consequently, the protocol of immunocastration was designed for them. Gilts intended for Teruel dry-cured ham elaboration are younger and lighter at slaughter, and thus, the protocol of immunocastration should be adapted for them.

In the case of male pigs, as agreed in the European Declaration on alternatives to surgical castration of pigs in 2010 (European Declaration on alternatives to surgical castration of pigs, 2010), in the EU, it is possible that surgical castration without pain relief could be banned for welfare reasons. Thus, immunocastration could also be a

solution for that. However, part of the literature (Grela et al., 2020; Poulsen Nautrup et al., 2018) suggests that the level of fat accretion of immunocastrated males (IM) could be lower than that of surgically castrated males (SCM), which could be a negative aspect for Teruel dry-cured ham production. In this context, it would be reasonable to study feeding plans to optimize the quality of dry-cured hams.

On the other hand, immunocastrated gilts (IG) and IM are likely to have different feeding patterns than entire gilts (EG) and SCM, respectively. Therefore, the study of their feeding is of particular importance.

This doctoral thesis will try to address the recently presented issues. Firstly, a literature review focused on the effects of immunocastration, both in female pigs and in male pigs, and of increasing dietary energy level maintaining CP and AA contents or decreasing dietary CP and AA levels maintaining energy content is presented. Secondly, the hypotheses, the objectives and the experimental approach of the present doctoral thesis are described. Thirdly, in the Results section, the academic articles that contain all the information about the experiments carried out are included. Lastly, a general discussion and conclusions of the doctoral thesis are presented.

2. LITERATURE REVIEW

2.1. Pig production

According to Food and Agriculture Organization of the United Nations (2022), in 2020 pork was the second most produced meat in the world, behind chicken meat (109.8 *vs.* 119.5 million t). The top five pork producers were China (41.1 million t), United States of America (12.8 million t), Germany (5.1 million t), Spain (5.0 million t) and Brazil (4.5 million t), providing 62.4% of the pork in the world.

In the EU-27 countries, in 2020, the census of pig heads was 146.2 million. The top five pig producers were Spain (32.8 million heads), Germany (26.1 million heads), France (13.7 million heads), Denmark (13.4 million heads) and Poland (11.7 million heads), providing 66.8% of the heads (Eurostat, 2022).

In Spain, pig production has a great importance in the economy, since in 2020 the final pig production accounted for 42.8% of the final livestock production and 16.4% of the final agricultural production. During that year, 56.5 million pigs were slaughtered and the autonomous community with the highest number of pigs was Aragon, closely followed by Catalonia. On average, in 2020 each Spanish inhabitant consumed 49.6 kg of pork. Besides, from the 5.0 million t of pork produced yearly in our country, around 3.0 million t were exported, principally to China, France, Italy and Portugal (Ministerio de Agricultura, Pesca y Alimentación, 2021b).

De Briyne et al. (2016) reported that, in Spain, from the total pig males, approximately 80% are entire, 15% surgically castrated and 5% immunocastrated. Also, within SCM, still 92% are castrated without analgesia or anesthesia, only 1% are castrated with analgesia and anesthesia and 7% with analgesia. Besides, in 2020, around 2.0 million pigs (6.2%) achieved more than 110 kg of body weight (BW) at slaughter (Ministerio de Agricultura, Pesca y Alimentación, 2021b). These pigs are principally destined for the production of dry-cured pieces, since a heavier carcass weight and a higher fatness is necessary to ensure good quality of the final product.

2.2. Dry-cured products in Spain

Spain has a great tradition in the elaboration and consumption of dry-cured products, especially dry-cured hams. In 2020, a total of 303,000 t of dry-cured hams and shoulders and 224,000 t of cured cold cuts were produced and 61,943 t of dry-cured hams and 66,493 t of cured cold cuts were exported (Asociación Nacional de Industrias de la Carne de España, n.d.). In that year, on average a Spanish inhabitant consumed 2.2 kg of dry-cured hams and shoulders (Ministerio de Agricultura, Pesca y Alimentación, 2021c).

There are seven types of dry-cured hams with labels of differentiated quality linked to a geographic area: five PDOs and two protected geographical indications (PGI) (Figure 1). The PDOs "Guijuelo", "Dehesa de Extremadura", "Jabugo" and "Los Pedroches" use Iberian pigs, which is an autochthonous breed. These animals can be reared outdoors a period of their life, in a special ecosystem called "Dehesa". The other three labels (PDO "Teruel ham", PGI "Serón ham" and PGI "Trevélez ham") use commercial breeds that are reared in confined conditions (indoors) all their life. In 2020, most of these hams are commercialized in Spain (93.7%) and only 4.6% in the EU and 1.7% in third countries. Among these labels, the PDO Teruel ham is the one that commercialized most hams (349,161 hams, representing 50.0%), and which presents the highest economic value in terms of hams (29.7 million euros) (Ministerio de Agricultura, Pesca y Alimentación, 2021a).

Figure 1. Protected designations of origin and protected geographical indications of dry-cured hams in Spain.

2.3. Protected designation of origin “Teruel ham”

The PDO Teruel ham was the first PDO of ham created in Spain and the unique of “white pigs” (not autochthonous). In 1984, the regulation of this PDO was approved by the Department of Agriculture of the Government of Aragon and the following year the Ministry of Agriculture of Spain ratified it (Sanjuán et al., 2004). In 2014, the dry-cured shoulder was included in this PDO (Official Journal of the European Union, 2014).

The production of Teruel hams increased drastically from its beginnings to 2008, when the production was situated in 743,738 pieces (Ministerio de Medio Ambiente y Medio Rural y Marino, n.d.). From that moment to 2014, it suffered a gradual decline until 220,678 pieces due to the economic crisis (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2015). From 2015 to 2019, this production increased, marking 375,932 hams in 2019. Despite the COVID-19 pandemic, the production of Teruel hams in 2020 only reduced 5.42% (355,549 pieces). In 2020, 148 farms, 34 dry-curing facilities, 19 packing rooms, 7 feed mills, 9 processing rooms and 9 slaughterhouses were included in this PDO (Consejo Regulador de la Denominación de Origen Protegida Jamón de Teruel, 2021).

The PDO Teruel ham is regulated (Boletín Oficial de Aragón, 2021) to guarantee the quality of the final product. The main requirements are the following:

- The production area (geographic area of breeding and slaughter-butchering of pigs) has to be in the province of Teruel, in the northeast of Spain.
- Feed mills have to be situated in Teruel province or in its neighboring provinces.
- Dry-curing facilities must be in Teruel province at an altitude equal to or greater than 800 m above sea level.
- The dam line of pigs has to be Landrace (standard) or Large White or the cross of these breeds and the sire line has to be Duroc.
- The diet of pigs has to contain a minimum of 50% of cereals.
- Male pigs have to be castrated before their entrance in the fattening facilities and female pigs do not have to present estrus at slaughter.
- Warm carcass weight has to be greater or equal to 86 kg.
- Fat thickness, measured in the lumbar area at the height of the tip of the ham, has to measure more than 16 mm and less than 45 mm.

- The minimum duration of the dry-curing process of the hams has to be 60 weeks and of the shoulders 36 weeks.
- Dry-cured ham weight has to be greater or equal to 7 kg and dry-cured shoulder weight has to be greater or equal to 4.5 kg.

2.4. Relevant factors in dry-cured products

2.4.1. Fat content and composition

Nowadays, the percentage of fat in carcass of commercial pigs is approximately 20-25% (Font-i-Furnols, Luo, et al., 2020; Martínez-Ramírez et al., 2014; Realini et al., 2010). Adipose tissue is deposited in distinct anatomical positions as subcutaneous, intermuscular (between muscles), internal (visceral) and intramuscular (within muscle) fat. The first three categories represent 65%, 30% and 5%, respectively, of the total separable fat. On the other hand, IMF is not anatomically separable and its percentage varies according to the anatomical localization of muscles (Mourot & Hermier, 2001). During the first part of growth, fat depots develop in order of decreasing intensity: subcutaneous, internal, intermuscular and intramuscular (Henry, 1977).

A sufficient amount of fat, especially fat covering the ham, is important to reduce the weight loss of hams during the dry-curing process, since adipose tissue contains less water than muscular tissue and hinders exchanges between muscle and external environment (Bosi & Russo, 2004). The thicker the subcutaneous fat (SCF) layer, the more difficult it will be to absorb salt, and the dehydration will be slower than in the lean areas. Therefore, fat balances the dry-curing process, prevents sudden and uncontrolled drying of the pieces and controls the curing effects of the salt (Bello-Gutiérrez, 2008).

Especially, IMF content has a remarkable effect on texture and appearance of dry-cured hams and intervenes in the development of the specific aromas of dry-cured products, since is a precursor of a large number of volatile compounds (Bello-Gutiérrez, 2008; Ruiz-Carrascal et al., 2000; Sabio et al., 1998). This parameter is positively related to juiciness, by stimulating the secretion of saliva, and juiciness positively influences dry-cured ham acceptability (Ruiz-Carrascal et al., 2000; Ruiz et al., 2002). Likewise, IMF is closely related to marbling (Ruiz-Carrascal et al., 2000), being considered positive or negative depending on the countries (Hocquette et al., 2010). On the other hand, IMF is negatively related to dryness and fibrousness (Ruiz-Carrascal et al., 2000), parameters that influence negatively on dry-cured ham acceptability and that depend on the degree of dehydration achieved during the dry-curing process (Ruiz et al., 2002). In addition, hardness is also negatively related to IMF (Ruiz-Carrascal et al., 2000), due to IMF disrupts the structure of the endomysium, separating and diluting the collagen fibers of perimysium and disorganizing the structure of intramuscular connective tissue, which

contributes to increasing meat tenderness (Hocquette et al., 2010). However, hardness does not influence dry-cured ham acceptability (Ruiz et al., 2002). It is worth noting that the minimum percentage of IMF to achieve consumer satisfaction seems to be about 2% (Bejerholm & Barton-Gade, 1986).

The fat composition of dry-cured products is relevant from a technological, sensorial and nutritional point of view. If the fat cover is thicker, there is more content of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) (from *de novo* synthesis). On the contrary, the content of polyunsaturated fatty acids (PUFA) (from the diet) is lower (Gandemer, 2002). As SFA content increases, fat cover becomes firmer, more cohesive and less susceptible to oxidation due to the high melting point of SFA, improving storage stability of dry-cured products and avoiding rancidity and off-flavors, although it has poorer health properties (Hugo & Roodt, 2007).

2.4.2. Boar taint

Boar taint is an unpleasant odor and flavor for sensitive consumers that is perceived mainly when heating and eating the meat from some boars, i.e. entire males (EM) (Bonneau & Weiler, 2019; Brunius et al., 2011; Zamaratskaia & Squires, 2009). Two substances are the main contributors for boar taint: androstenone (5α-androst-16-en-3-one) and skatole (3-methylindole). Other compounds, such as indole, might also contribute to boar taint, but to a lesser degree (Squires & Bonneau, 2014).

Androstenone is a steroid, with no anabolic effects, produced almost exclusively by testicular Leydig cells of EM (Čandek-Potokar et al., 2017; Zamaratskaia & Squires, 2009) (Figure 2). In the case of females and castrated males, ovary and adrenal cortex, respectively, could be small sources of this compound (Claus et al., 1971). The synthesis of androstenone in testicles is controlled by the activation of hypothalamic-pituitary-gonadal axis, as in the case of other testicular steroids, being low in young pigs and progressively increases at sexual maturity. Therefore, puberty is a relevant aspect in androstenone synthesis (Zamaratskaia & Squires, 2009). Once produced, it can be metabolized in testicles (Squires et al., 2020). The metabolism of androstenone has two phases; hydroxylation (Phase I) and conjugation (Phase II) (Squires & Bonneau, 2014). Following synthesis in testicles, androstenone is released into the blood stream (Squires et al., 2020). Part of this compound is transported to the submaxillary salivary gland, where it is bind to a specific protein (pheromaxein) and secreted through saliva, acting as

a pheromone to promote sexual behaviors in female pigs (Čandek-Potokar et al., 2017). Owing to its lipophilic character, androstenone also accumulates in adipose tissue (Squires & Bonneau, 2014). This accumulation is a reversible process. Androstenone is also metabolized in the liver and excreted in urine and feces (Rius-Solé, 1999). The conjugates formed in the liver can enter into the bile and be delivered to the small intestine, from which bacteria can produce free androstenone, which can be absorbed by the enterocyte and transport back to the liver by blood, which is known as enterohepatic circulation (Squires & Bonneau, 2014).

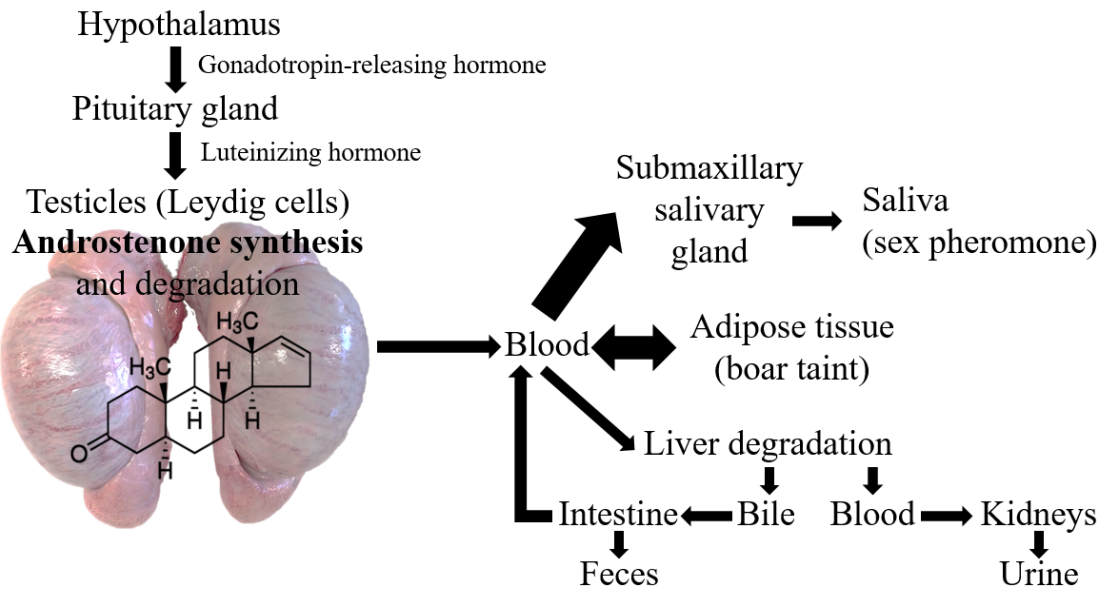

Figure 2. Formation, degradation, accumulation and elimination of androstenone. Own elaboration based on the information obtained from Čandek-Potokar et al. (2017), Zamaratskaia & Squires (2009), Squires et al. (2020), Squires & Bonneau (2014) and Rius Solé (1999).

On the other hand, skatole is a product of bacterial degradation of the tryptophan amino acid in the large intestine (Figure 3), with no known physiological function (Čandek-Potokar et al., 2017). The amount of skatole generated is mainly regulated by the availability of tryptophan, been a major source the turnover of the intestine mucosa cells, and the composition and activity of the intestinal bacteria (Squires & Bonneau, 2014). Once produced, a part of skatole is excreted through feces and the remaining part is absorbed through the intestine wall into the blood (Zamaratskaia & Squires, 2009). The liver is the organ that mainly metabolizes a part of the absorbed skatole and the remaining part is deposited in the peripheral tissues, and due to its lipophilic character, most of it

accumulates in adipose tissue (Jensen, 2006; Rius Solé, 1999; Wesoly, 2015). The accumulation of skatole in this tissue is a reversible process (Rius Solé, 1999). The metabolism of skatole has two phases; Phase I entails the addition of a hydroxyl group that can be used to attach a conjugate in Phase II. The metabolites produced can be excreted in the urine or bile (Zamaratskaia & Squires, 2009). The level of skatole is also related to sexual maturity, since androstenone seems to play an important role inhibiting skatole hepatic metabolism and 17β-estradiol, another steroid, favors skatole formation in the intestine (Čandek-Potokar et al., 2017; Han et al., 2019). Consequently, skatole concentration in fat of EM are higher than in that of barrows, i.e. castrated male pigs, and EG (J. Morales et al., 2010; Stupka et al., 2017).

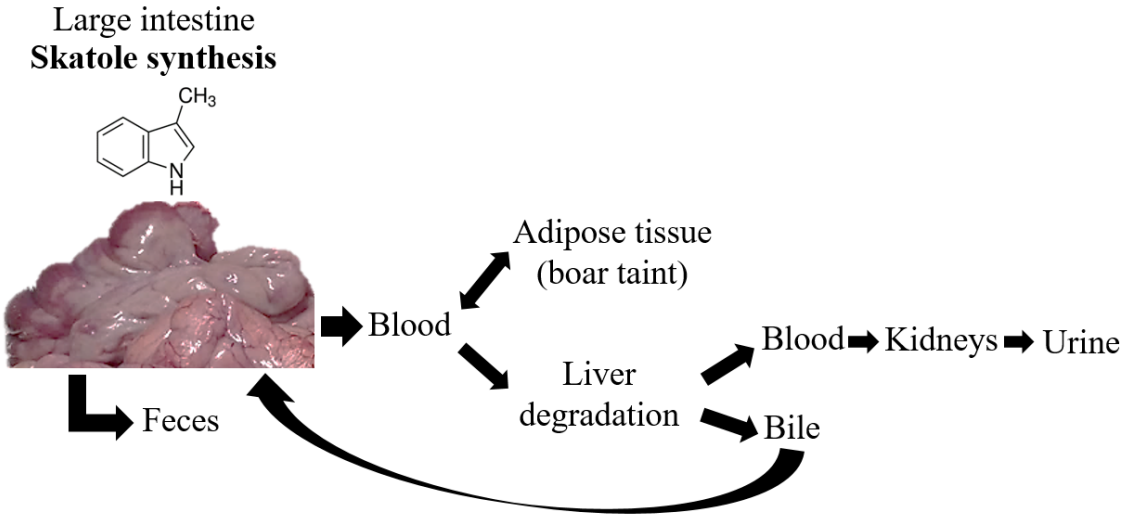

Figure 3. Formation, degradation, accumulation and elimination of skatole. Own elaboration based on the information obtained from Čandek-Potokar et al. (2017), Zamaratskaia & Squires (2009), Jensen (2006), Rius Solé (1999) and Wesoly (2015).

The human sensitivity to androstenone is highly variable, not everyone are able to perceive it (Bonneau & Weiler, 2019; Claus et al., 1994). About one third of people cannot smell it, whereas another third are highly sensitive, especially women, and consider androstenone as very unpleasant urine- or sweat-like odor. The remaining third perceive this compound, but describe it as pleasant (sweet- and floral-like sensation) (Bonneau & Weiler, 2019; Running & Hayes, 2016; Weiler et al., 2000). In the case of skatole, approximately 99% of consumers are sensitive to this substance and perceive it as fecal- or manure-like odor or, to a lesser extent, to naphthalene odor, and a bitter taste (Čandek-Potokar et al., 2017; Squires & Bonneau, 2014; Weiler et al., 2000). The

thresholds values to which sensitive consumers would negatively react seem to be 0.5 or 1.0 µg/g of fat for androstenone and 0.2 or 0.25 µg/g of fat for skatole (Walstra et al., 1999). The level of boar taint in pork can be affected by gender, age at slaughter, genetic, diet, management practices and meat processing methods (Čandek-Potokar et al., 2017; Zamaratskaia & Squires, 2009). Traditionally, male pigs have been surgically castrated to eliminate this off-odor and flavor.

2.5. Surgical castration of male pigs and alternatives

2.5.1. Surgical castration of male pigs

This technique consists of incising the scrotum (normally two vertical incisions are performed), extracting the testicles and removing them by severing the spermatic cords, having the piglet restrained to minimize any movement. Surgical castration of male pigs is allowed in the EU by other means than tearing of tissues and has to be carried out by a veterinarian or a trained person. Only if castration is performed after the seventh day of life, it shall be carried out under anesthesia and prolonged analgesia practiced by a veterinarian (Official Journal of the European Union, 2009).

The main cause to carry out surgical castration of male pigs is to avoid boar taint (Bonneau & Weiler, 2019). Additionally, this technique reduces aggressive and sexual behaviors due to the lack of testicular hormones, being SCM easier to manage and presenting lower carcass lesions than EM (Cronin et al., 2003; Fàbrega et al., 2010). Surgically castrated males have also greater carcass yield than EM, owing to the higher weight of the reproductive tract in the latter, removed during the slaughtering process (J. Morales et al., 2010; Škrlep et al., 2020). Besides, the higher capacity of SCM to deposit fat (Kress et al., 2020; J. Morales et al., 2010; Škrlep et al., 2020) is beneficial in the case of heavy pigs destined for traditional high quality products and improves the eating quality by increasing the IMF content (Bonneau & Weiler, 2019; Pauly et al., 2012). Fat of SCM seems to be firmer and more resistant to oxidation than that of EM, due to the higher content of SFA and the lower of PUFA, being a positive aspect for meat technological processes and for the storage stability and flavor of dry-cured products (Hugo & Roodt, 2007; Pauly et al., 2012).

On the other hand, this practice is painful for piglets because the tissues associated with castration are innervated (European Food Safety Authority, 2004). The performance of this technique and the higher pre-weaning mortality in SCM (J. Morales et al., 2017) supposes a mayor cost for pig farmers. Additionally, it has been seen that SCM eat more feed and convert feed to BW gain less efficiently than EM (European Food Safety Authority, 2004). Owing to the higher feed conversion ratio (FCR) and the lower nitrogen (N) retention rate of SCM, these males present a greater N excretion (European Food Safety Authority, 2004; Elsbernd et al., 2015; Metz et al., 2002). Therefore, surgical castration has an unfavorable impact on the costs for pig farmers and also on the

environment. Besides, due to the fact that consumers demand lean meat (Rault et al., 2011) and SCM present lower lean meat percentage than EM (Pauly et al., 2012), the selling price for their carcasses is lower (Bonneau & Weiler, 2019). Lastly, the lower levels of PUFA (Pauly et al., 2012) and protein content (Grela et al., 2020) in SCM might indicate nutritional disadvantages of this meat compared with that from EM (Squires & Bonneau, 2014).

2.5.2. Alternatives

Owing to economic and ethical concerns, alternatives to surgical castration of male pigs are been sought. Several of the existing alternatives are going to be detailed below, although among them, rearing of EM and immunocastration are currently the most promising.

2.5.2.1. Sperm sexing and rearing of female pigs

This technique consists of separating X (female)- and Y (male)-chromosome-bearing pig-sperm to produce only female pigs. Entire gilts have no problem with boar taint (Font i Furnols et al., 2009; Weiler et al., 2013), and therefore, carrying out their castration to avoid this off-odor is no necessary, increasing animal welfare. This type of females seems to present a FCR and a level of fat deposition intermediate between EM and SCM (EM < EG < SCM) (Gispert et al., 2010; J. Morales et al., 2010; Weiler et al., 2013). The only commercially available method is flow cytometry, based on differences in size between the X- and Y-chromosomes (European Food Safety Authority, 2004). Currently, sperm sexing is unsuitable for commercial use in pigs, owing to the high number of sperm required for optimal fertility, the low sexed-sperm production per unit of sorting time, the high sensitivity to manipulation of the sperm of EM and the high costs (Čandek-Potokar et al., 2017; Škrlep et al., 2014; Vazquez et al., 2009).

2.5.2.2. Rearing of entire male pigs

The advantages and disadvantages of rearing EM are reversed to those of surgical castration described in Section 2.5.1. With the raising of EM, pain, workload and post-operational complications associated with surgical castration are avoided (European Commission, 2017). In addition, the rearing of EM supposes a lower cost for farmers and impact on the environment, a higher muscle content and a healthier fat (more unsaturated) (Bonneau & Weiler, 2019). On the other hand, EM rearing increases likelihood of the

presence of boar taint and problems with their management, reduces welfare associated with aggressiveness and mounting behavior (European Commission, 2017) and worsens carcass yield, meat quality and technological quality of the fat (softer) (European Food Safety Authority, 2004; Škrlep et al., 2020). Besides, this practice is difficult to carry out in heavy pigs destined for high-quality dry-cured products for several reasons (European Commission, 2017):

- increased incidence of boar taint in heavy pigs because they tend to be more sexually mature (old age at slaughter);
- boar taint is more easily perceived by consumers in high fat products;
- high fat content and more saturated fat are required in many of these products;
- castration of male pigs can be officially required;
- minimal age at slaughter can be specified and, in these cases, the quicker growth of EM than SCM could be a problem;
- heavier pigs require longer growing periods, and older EM pose safety concerns for farmers;
- sexually mature males and females must be reared in separate batches.

The reduction of boar taint risk in EM is potentially possible slaughtering pigs before puberty, through selective breeding, dietary manipulations, flooring, housing and pig management, sorting methods that detect tainted carcasses and processing methods to inhibit boar taint perception in meat/ meat products. These strategies will be developed below. It should be noted that information about these practices is included in a report of the European Commission (European Commission, 2021), and therefore, this has been the main source of information used.

i) Slaughter at a young age. It could reduce boar taint because younger pigs may not have reached puberty. This strategy is easy to implement, but farmers may receive a lower price per carcass, since younger pigs tend to be lighter than older ones, and it may not be feasible when pigs have to be slaughtered at high weights to achieve the require quality, as in the case of pigs destined for high-quality dry-cured products. In addition, slaughtering pigs at a young age does not guarantee the total elimination of boar taint (Čandek-Potokar et al., 2017), possibly due to individual and breed differences in sexual development (European Food Safety Authority, 2004).

ii) Selective breeding. There are mainly two techniques; the first one, selecting on breed since certain breeds, such as Pietrain, are considered to be at lower risk for boar taint, and the second one, selecting within a breed low-taint boars. Selective breeding reduces the risk of boar taint from the beginning, but it does not guarantee the total elimination of this off-odor and flavor, the dosages of sperm of low-taint boars are sometimes more expensive than normal dosages and the availability of these products varies per country. Besides, genetic selection against boar taint reduces the levels of anabolic hormones, and thus, it produces negative effects on growth performance of EM (Čandek-Potokar et al., 2017).

iii) Dietary manipulations. Different feeding strategies have been investigated in order to reduce boar taint, especially the content of skatole. Some of them are shown below. Fermentable carbohydrates, such as inulin or raw potato starch, can reduce the production of skatole by the intestine microflora, and therefore, its accumulation in fat. Three hypotheses could explain the effects of fermentable fiber: a) fermentation generates butyrate, minimizing cell apoptosis and the amount of cellular debris that is a source of tryptophan for skatole synthesis; b) fermentable fiber increases microbial activity and incorporation of available tryptophan into bacterial biomass, reducing the quantity of tryptophan available for skatole synthesis; and c) the composition of the microbiota is altered to reduce the amount of bacteria that produce skatole (Squires et al., 2020). Besides, there are two commercial feeds, combining fibers and additives (from Belgium and Netherlands), which reduce skatole levels by giving them to EM the last weeks before slaughter. Also, the addition of non-nutritive sorbent materials (5% activated charcoal or 5% polyoxyethylene sorbitan monostearate) to finishing diets of EM for 28 days followed by either 14 or 28 days of recovery can reduce the levels of fat androstenone (Jen & Squires, 2011). These materials seem to bind androstenone in the intestine, reducing its reuptake, i.e. interrupting the enterohepatic circulation. However, further studies are needed to determine the exact mechanism, evaluate the efficacy of this practice under commercial production conditions and identify other cheaper binding adsorbents (Squires et al., 2020). Dietary manipulations reduce the risk of boar taint, but they do not guarantee the total elimination of this off-odor and flavor. Besides, the cost of specific ingredients is high and it is not normally a solution for production systems with prolonged fattening (Čandek-Potokar et al., 2017).

iv) Flooring, housing and pig management. Many of these practices pursue to improve the welfare of pigs, reducing their stress, which is related to a reduction in boar taint. However, it is unclear to what extent these practices affect the prevalence of this off-odor and flavor. Keeping pigs clean may help to decrease the skatole levels, since this compound can be absorbed from the manure (Squires & Bonneau, 2014). One way to improve hygiene is to use fully or partially slatted floors because they help to eliminate feces and urine. Nevertheless, fully slatted floors could increase the risk of injury. On the other hand, sex-separated rearing can reduce the levels of hormones associated with boar taint in EM because they cannot see or smell female pigs. This practice allows that expensive feeds with taint-reducing additives can be supplied only to EM. In addition, transporting pigs in sex-separated groups to the slaughterhouse can facilitate the logistics of processing EM and detecting boar taint. Nevertheless, sex-separated rearing may lead to increased aggression among EM and require additional infrastructures in the farm. Another practice that reduces stress levels is wean to finish grouping, i.e., pigs are rearing together with their siblings until slaughter. This practice can also help to prevent the spread of diseases, but requires additional logistics to separate pigs if abattoirs request sex-separated groups and the smaller pens may lead to extra cost. Other practices for reducing stress are the following: making sure pigs have enough space, keeping group sizes small, removing ill pigs from the pen, using partly open pen walls, ensuring temperature control, proper ventilation and suitable lighting, keeping pigs supplied with enough fresh water, providing enrichment and reducing competition at feeding. These practices improve pig welfare and reduce negative behaviors, but implementing them may increase costs.

v) Detection of tainted carcasses. Carcasses with high boar taint are not suitable for fresh pork products and their meat are processed in order to reduce boar taint perception. The percentage of tainted carcasses should be low (4-5%), since these have a lower value (Bonneau & Weiler, 2019; Squires et al., 2020). Currently, in some Danish, German, Belgian, Dutch, French and Spanish slaughterhouses boar taint is measured by a sensory quality control (human nose method) and in other Danish slaughterhouses by a colorimetric method (Font-i-Furnols, Martín-Bernal, et al., 2020). Besides, several chemical testing methods are under development, although they are not used in commercial conditions. Thus, the human nose is the most widely used method in the EU for detecting boar taint, delivering solid results when it is implemented correctly. It

consists of trained testers heating and smelling a fat sample of each carcass, since when pig fat is heated, androstenone and skatole are volatilized (Squires & Bonneau, 2014). This method needs enough trained staff and has a cost. The most advisable part of the body to carry out this technique is the neck, since the fat in this location is easy to reach and it can be heated without harming the surrounding meat. Human nose method has two variants: *on line*, when the detection of boar taint occurs in the slaughter line using hot air devices, soldering irons with metal tips or gas-powered soldering irons; and *off line*, when fat samples are taken from carcasses and analyzed later using hot water or microwaves. The most common are *on line* methods.

vi) Processing methods to inhibit boar taint perception. There are several methods to mask boar taint in meat/meat products. The effectiveness of these methods depends on the level of boar taint that meat has before its processing. Some of the most used techniques are the following: dilution/mix of tainted meat with non-tainted, fermentation, smoking, thermal treatment and use of flavor masking substances such as spices. Moreover, if pork products are consumed cold, the detection of boar taint is much less pronounced (Squires & Bonneau, 2014).

2.5.2.3. Surgical castration with anesthesia and/or analgesia

Anesthesia consists of the use of drugs to induce loss of sensation, especially of pain, and it can be general, regional or local. General anesthesia generates unconsciousness and total lack of sensation, while regional and local anesthesia only causes loss of sensation to a specific area of the body. For surgical castration of male pigs, general anesthesia can be injected intramuscularly or inhaled, regional anesthesia is injected in the epidural space and local anesthesia is administrated by subcutaneous, intratesticular or intrafunicular (i.e. into the spermatic cord) injection or applying a topical gel (European Commission, 2017; European Food Safety Authority, 2004). On the other hand, analgesia consists of the use of drugs to lose the ability to feel pain without the loss of consciousness. For surgical male-pig castration, the injections of analgesics should be intramuscularly (European Commission, 2017).

The advantages and disadvantages of different methods of anesthesia and analgesia used or proposed in male-pig castration in the EU are summarized in Table 1. From this table it can be extracted that to effectively avoid pain associated to castration, anesthesia and analgesia should be combined, although this is an expensive procedure,

especially if veterinarians are required (Bonneau & Weiler, 2019). Besides, the number of anesthetics and analgesics authorized for pig castration in the EU is limited and varies greatly between countries (De Briyne et al., 2016). In addition, the withdrawal periods of the drugs used has to be considered if meat of very young pigs is consumed (European Commission, 2017). Therefore, surgical castration with pain relief improves welfare (no or less pain associated to castration) compare to surgical castration without pain relief, but it increases costs (due to the cost of equipment and drugs and the time consuming) and needs for authorization and specially trained personnel (Bonneau & Weiler, 2019; Čandek-Potokar et al., 2017). Likewise, surgery is carried out, and thus, the risk of post-surgical complications persist (J. Morales et al., 2017). In addition, it can be assumed that behavior and growth performance of these pigs and the quality of their products will be similar to that of SCM without pain relief.

Table 1. Advantages and disadvantages of different methods of anesthesia and analgesia used or proposed in male-pig castration in the European Union. Modified from European Commission (2017).

	General anesthesia with CO_2/O_2 gas with or without NSAID	**General anesthesia with isoflurane/sevoflurane/ N_2O gas with or without NSAID**	**General anesthesia with ketamine/azaperone with or without analgesia**	**Local anesthesia with lidocaine with or without analgesia**	**Preemptive analgesia (meloxicam-NSAID/ flunixine-NSAID/metamizol)**	**Post-surgical analgesia (meloxicam-NSAID/ flunixine-NSAID/metamizol)**
Advantages (+)	-Fast and short acting efficient anesthesia -Analgesia for post-operative pain is required -Simple equipment	-Short and fast acting -Efficient only in combination with analgesia	-Deep and effective anesthesia	-Effective only if properly administered in combination with an analgesic drug	-Only effective for post-operative pain depending on half-life -Easy to apply -Relatively low costs for drug and extra labor	-Only effective for the period after injection depending on half-life -Easy to apply -Costs for drug relatively inexpensive
Disadvantages (−)	-Aversive during initiation -Handling stress -Risk of suffocation -Risk of over-/under dosage -Potential hygienic risk if not properly cleaned and disinfected -Moderately high cost for equipment -Moderate costs for gases	-Stress of handling -Risk of over-/under dosage (anesthetic depth decreases with weight and age) -Requires authorization and specific training for farmers, careful handling and substantial hygienic measures -Weight of piglets has to be considered -Potent climatic effect if not properly controlled -Leakages increase risk of user inhalation -Risk for spreading diseases when sharing equipment -Advanced equipment -High costs for equipment -High costs for gas -Costly hygienic measures	-Very long anesthetic sleep with risk of hypothermia, dehydration and deprivation of milk -Little control for dosage (individual variation) -Enormous labor effort (monitoring piglets) -Strictly under control of veterinarian -Risk of ketamine abuse for human consumption (hallucinogenic drug) -Very high labor costs and management efforts: costs for drugs	-Stress of handling -Injection may induce pain if not done properly (slow injection with buffered solution) -Requires authorization and specific training for farmers -Moderate costs for drugs	-Pain and stress during castration not alleviated if not combined with anesthesia -Problem of monitoring the actual use	-Pain and stress during and immediately after castration not alleviated if not combined with preemptive analgesia and anesthesia -Problem of monitoring the actual use
Overall evaluation	− − −	+ −	+ − −	+ + −	+ −	+ − −

NSAID: non-steroidal anti-inflammatory drugs.

2.5.2.4. Local destruction of testicular tissue by chemical compounds

This practice consists of injecting chemical substances into testicles to destruct spermatogenic and hormone-producing cells. In the case of male pigs, a solution of $KMnO_4$ and CH_3COOH (Giri et al., 2002) and other of $ZnC_4H_6O_4$ (Fahim, 1994) were the chemical compounds tested. This technique appears to be easy to perform, safe and not expensive and produces no hemorrhage. However, it is not recommended to carry out, because there is lack of information on the possible pain caused to pigs and on the reduction of boar taint (European Food Safety Authority, 2004).

2.5.2.5. Down-regulation of the hypothalamic-pituitary-gonadal axis by exogenous hormones

The administration of steroid agonists or antagonists or the continuous administration of the gonadotropin-releasing hormone (GnRH) down-regulate the hypothalamic-pituitary-gonadal axis, arresting the testicular function. This practice is effective inhibiting sexual development, but little is known about its effectiveness in reducing boar taint (European Food Safety Authority, 2004). In addition, the use of these hormones in meat-producing animals is not permitted in the EU and consumers would find it unacceptable (Prunier et al., 2006).

2.5.2.6. Immunocastration

Immunocastration is based on the vaccination of animals against hormones that regulate the reproductive function (Čandek-Potokar et al., 2017). This immunization can target either the luteinizing hormone (LH) or the GnRH, also denominated as gonadotropin-releasing factor (GnRF) (European Food Safety Authority, 2004). However, immunization against LH is less effective than that against GnRH (Falvo et al., 1986). In Europe, the unique commercial available vaccine for this purpose (Improvac®, Zoetis) contains a synthetically produced analogue of GnRF, which has no hormonal activity, conjugated with an immunogenic carrier protein (diphtheria toxoid) and combined with an aqueous adjuvant (Diethylaminoethyl-dextran) to increase the level and duration of the effect (Čandek-Potokar et al., 2017; European Medicines Agency, 2022). This analogue stimulates the formation of antibodies against endogenous GnRF secreted from the hypothalamus (Figure 4). The antibodies produced neutralize endogenous GnRF, inhibiting LH and follicle stimulating hormone production by the hypophysis (pituitary gland), whose target organs are the testicles. Consequently, the formation of testicular steroid hormones is prevented, reproductive organs regress and some metabolic changes occur, leading to changes in behavior (reduced aggression and increased appetite and

feed intake) and body composition (higher fatness and faster growth) compared to EM, and to the elimination of androstenone formation, and, therefore, of boar taint (Škrlep et al., 2014).

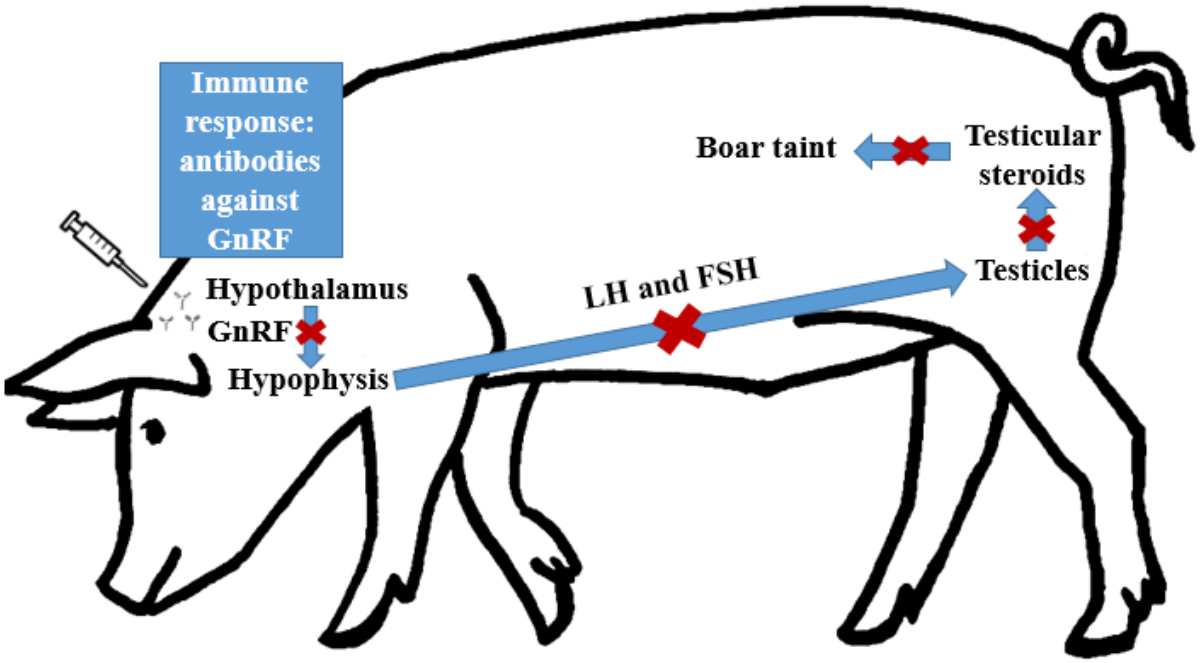

Figure 4. Physiological response of male pigs to immunocastration. The vaccine carries an antigen (gonadotropin releasing factor-GnRF analogue-protein conjugate), triggering the formation of antibodies that neutralize endogenous GnRF. Thus, the hypophysis is not stimulated to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn fails to signal testicles to produce testicular steroids and therefore prevents boar taint development (Čandek-Potokar et al., 2017). Own elaboration based on the Figure 3 of Čandek-Potokar et al. (2017).

The main protocol for immunocastration with Improvac® requires the subcutaneous injection with a safety vaccinator of two doses of 2 mL behind the ear (European Medicines Agency, 2022). The first one should be injected at no earlier than 8 weeks of age and only primes the immune system of the pig (European Commission, 2021; Rault et al., 2011), generating a slight increase of anti-GnRH antibody titers (Zamaratskaia, Andersson, et al., 2008) that is not sufficient to block hypothalamic-pituitary-gonadal axis (European Medicines Agency, 2022; Kress et al., 2019). The second one, the really effective, should be given at least four weeks after the first dose and it is normally given four to six weeks before slaughter, although there is no withdrawal period, to be sure that boar taint compounds are eliminated from the adipose tissue (European Commission, 2021; European Medicines Agency, 2022; Škrlep et al., 2014). In the first week after the second dose, anti-GnRH antibody titers in IM increase markedly, causing immunological suppression of their testicular function and thereby

decreasing the concentration of testicular steroids (Claus et al., 2007; European Medicines Agency, 2022). Consequently, after the second dose, IM change their metabolism from boar-like to barrow-like (Čandek-Potokar et al., 2017). Vaccination schedules can be adjusted depending on the desired results; to maximize the amount of IMF and backfat and to minimize undesirable behaviors the second dose should be apply as early as possible, while to improve FCR and to obtain leaner carcasses this dose should be administer as late as possible (European Commission, 2021). The effects of the vaccination are temporary (European Medicines Agency, 2022). According to the manufacturer of Improvac®, these can last for as long as 10 weeks after the final injection, but according to Zamaratskaia, Rydhmer, et al. (2008) as long as 22 weeks. Moreover, a third dose should be administered if the intended effect is not observed (non-responders), or in early vaccinated pigs when the time between the second dose and the slaughter exceeds 10 weeks or in heavy or older pigs, to ensure that endogenous GnRF is inactivated and that boar taint is eliminated (Čandek-Potokar et al., 2017; European Commission, 2021).

Immunocastration is less painful than surgical castration without pain relief (Zamaratskaia & Rasmussen, 2015), although it can produce minor pain at the injection site (European Food Safety Authority, 2004). Moreover, this immunization avoids post-operational complications related to surgical castration (European Commission, 2017), simplifies handling by consisting only of the injection of vaccines (Di Pasquale et al., 2019) and seems to be effective in cryptorchids (Gutzwiller & Ampuero Kragten, 2013). If pigs respond adequately to the immunocastration doses, this practice seems to prevent effectively boar taint (European Commission, 2017). Immunocastrated males are physiologically EM at least until the second dose, and consequently, IM seems to present better FCR and greater lean meat percentage than SCM (Dunshea et al., 2013; Poulsen-Nautrup et al., 2018), being an economic advantage in standard pigs. In addition, this technique is applicable for production systems that require prolonged fattening (Čandek-Potokar et al., 2017), as in the case of pigs destined for the production of high-quality dry-cured hams.

On the other hand, immunocastration has a cost (vaccine price, workforce for its administration and possible cost for analyzing carcasses) (Di Pasquale et al., 2019; European Food Safety Authority, 2004) and needs trained and certified vaccine administrators (European Commission, 2021). It should be noted that this trainee only lasts half a day or a day and it is normally delivered by the company Zoetis free of charge (European Commission, 2021). In addition, this technique is more difficult to carry out in heavy/older pigs or in free-range pigs

(European Commission, 2017). As previously commented, at least until the second vaccination IM behave like EM, presenting more aggressive behavior and attempted mounts than SCM, although after the second vaccination IM behave similarly to SCM (Cronin et al., 2003). As mentioned earlier, a small percentage of pigs (lower than 12%) could not respond to immunocastration (Font-i-Furnols et al., 2012; Kubale et al., 2013; Zeng, Turkstra, Meloen, et al., 2002). These animals might be missed at vaccination moments in group-housing systems or might not react correctly to immunocastration doses, because immunological response is variable between individuals or they could have health problems, malnutrition or stress that reduce the immune system response (Čandek-Potokar et al., 2017; Jaros et al., 2005; Kress et al., 2019; Škrlep et al., 2012). It could also be due to the application of the second dose outside the recommended period (Font-i-Furnols et al., 2012). Therefore, after the second dose some monitoring should be done to detect non-responders based on their behavior and testicle size (Bonneau & Weiler, 2019), although it is difficult to detect them before slaughter (European Commission, 2017). Besides, as previously commented, in some cases a third dose of immunocastration could be needed. On the other hand, slaughterhouses may be reluctant to kill IM, since more labor and equipment modifications are needed to handle them in comparison to SCM (Squires et al., 2020). In addition, immunocastration seems to reduce carcass yield and fat deposition compared to surgical castration (Poulsen-Nautrup et al., 2018), which might deteriorate fresh meat and dry-cured products quality. Another handicap of immunocastration is that consumers may be unwilling to accept this method since they have fear for possible residues in meat (European Food Safety Authority, 2004), even though Improvac® has not withdrawal period (European Medicines Agency, 2022). Lastly, although the dosing gun used for immunocastration has several safety mechanisms, there is a risk of accidental self-injection by the staff performing immunocastration, and it may produce the same effects in humans as in pigs (European Commission, 2021).

2.6. Consequences of immunocastration in male pigs (*vs.* surgically castration)

2.6.1. Growth performance and blood sex hormones

From the application of the first to the second dose of immunocastration, in the meta-analysis of Batorek, Čandek-Potokar, et al. (2012), it was found that IM grow less than SCM, although in some later studies (Braña et al., 2013; Caldara et al., 2015; Stupka et al., 2017) this effect was appreciated only numerically. This finding may be because, during this period, IM consume less feed than SCM (Batorek, Čandek-Potokar, et al., 2012; Batorek, Škrlep, et al., 2012; J. I. Morales et al., 2013). In addition, IM are more efficient converting feed into weight gain (Batorek, Čandek-Potokar, et al., 2012; Batorek, Škrlep, et al., 2012; Caldara et al., 2015). Until the second vaccination, IM, like EM, present higher blood concentrations of testosterone and estradiol than SCM (Han et al., 2019; Yamsakul et al., 2017; Zamaratskaia, Andersson, et al., 2008). These results confirm that IM are physiologically similar to EM at least until the second dose. Androgens like testosterone and estrogens like estradiol have a negative influence on feed intake (Claus & Weiler, 1994) and lead to more aggressive behavior and attempted mounts that reduce the active time that pigs spend in feeding behavior (Cronin et al., 2003). Furthermore, greater levels of these hormones entail a higher anabolic effect in IM, and thus a better FCR (Batorek, Čandek-Potokar, et al., 2012; Claus et al., 2007). It could be explain because androgens promote protein synthesis and inhibit their degradation, and estrogens, through the stimulation of growth hormone release and additionally insulin-like growth factor 1, contribute to increasing protein synthesis, having the protein gain a lower energy cost than lipid gain (Claus & Weiler, 1994; Patience, 2012).

From the second dose of immunocastration to the slaughter, according to the results shown by some meta-analysis (Batorek, Čandek-Potokar, et al., 2012; Dunshea et al., 2013), IM grow faster, seem to show higher average daily feed intake (ADFI) and continue presenting better FCR than SCM. After the second dose, blood testosterone and estradiol levels in IM decrease drastically to those of SCM, remaining at a low level until the sacrifice (Brunius et al., 2011; Han et al., 2019; Zamaratskaia, Andersson, et al., 2008). This could be explained because approximately one week after the injection of the effective dose of immunocastration, i.e. the second, LH levels decrease strongly limiting testicular steroidogenesis (Claus et al., 2007). The drop of the levels of testicular hormones in IM would contribute to increasing their feed intake. Besides, Batorek, Škrlep, et al. (2012) detected in IM the presence of relatively small amounts

of leptin after the second injection compared to SCM, being a protein that suppress feed intake (Barb et al., 1998). Therefore, these findings could explain the greater feed intake found in IM compared to SCM. On the other hand, after the second vaccine IM seem to present greater levels of insulin-like growth factor 1 than SCM (Batorek, Škrlep, et al., 2012; Han et al., 2019), being this compound essential to mediate the anabolic effects of growth hormone (Metz & Claus, 2003), contributing to improve FCR. Therefore, the higher average daily gain (ADG) found in IM is mainly due to their better FCR and also to their higher ADFI (Batorek, Čandek-Potokar, et al., 2012).

Finally, considering the entire fattening period, there is some unanimity that IM present higher ADG and lower FCR than SCM (Batorek, Čandek-Potokar, et al., 2012; Grela et al., 2020; Poulsen-Nautrup et al., 2018). Regarding ADFI, there is not consensus among authors; some of them (Batorek, Čandek-Potokar, et al., 2012; J. I. Morales et al., 2013; Van den Broeke et al., 2020) observed lower feed intake in IM compared to SCM, and others (Batorek, Škrlep, et al., 2012; Braña et al., 2013; Grela et al., 2020) did not detect any difference.

2.6.2. Carcass, meat and fat quality

There is some unanimity in the literature (Batorek, Čandek-Potokar, et al., 2012; Dunshea et al., 2013; Poulsen-Nautrup et al., 2018) about the lower carcass yield of IM compared to SCM. According to Boler et al. (2014), it could be explained by the weight of the testicles in IM and also by their heavier intestinal tract, liver, kidneys and additional reproductive tract. Likewise, there is some agreement in the literature (Batorek, Čandek-Potokar, et al., 2012; Dunshea et al., 2013; Poulsen-Nautrup et al., 2018) that IM present lower backfat thickness than SCM. It could be explain because IM are physiologically like EM until immunocastration becomes effective, i.e. shortly after the second dose, and EM present higher levels of testosterone (Han et al., 2017; Yamsakul et al., 2017) which seem to increase lean mass and to reduce fat mass (Wittert et al., 2003). Besides, the smaller the time between the second dose of immunocastration and the slaughter, the lower fat deposition is expected (Lealiifano et al., 2011). According to Daza et al. (2016) and Pinna et al. (2015) there are no significant differences between IM and SCM regarding ham size (length and perimeter). Djurkin Kušec et al. (2021) did also not observe effect of immunocastration on ham length. However, these last authors detected that IM Landrace x (Pietrain x Large White) present lower ham perimeter than SCM of the same genetic, whereas IM Landrace x Pietrain and IM Landrace x (Pietrain x Duroc x Large White) show the same ham circumference as SCM of the same

genetics. Regarding main lean cuts as percentage of carcass, there is not consensus in the literature. Several authors (Batorek, Škrlep, et al., 2012; Caldara et al., 2013; J. I. Morales et al., 2011) observed similar ham yield between IM and SCM. However, other authors (Font-i-Furnols et al., 2012; Martinez-Macipe et al., 2016; Pauly et al., 2009) detected that IM present higher ham yield than SCM and Daza et al. (2016) obtained the opposite effect as a tendency. In the case of shoulder yield, Lowe et al. (2014) and Pauly et al. (2009) observed that it is higher in IM than in SCM, but other works (Caldara et al., 2013; Gispert et al., 2010; Martinez-Macipe et al., 2016) did not show any difference. Among other factors, longer or shorter periods between the second dose of immunocastration and the slaughter and the genetics used could explain the differences across studies.

Immunocastration of male pigs may influence also on meat quality. In the case of pork water-holding-capacity (measured as drip loss and/or thawing loss and/or cooking loss), in the meta-analysis of Batorek, Čandek-Potokar, et al. (2012) and in the review of Harsh et al. (2017) similar results were obtained between IM and SCM. However, in other studies (Aluwé et al., 2013; Grela et al., 2020; Muniz et al., 2021) IM had inferior water holding capacity than SCM and in the report of Caldara et al. (2013) the opposite effect was observed only with respect to drip loss. As to color parameters, literature also provides divergent results. Batorek, Čandek-Potokar, et al. (2012) and Harsh et al. (2017) did not observe differences in color measurements between IM and SCM, whereas other authors (Andreo et al., 2018; Caldara et al., 2013; Škrlep et al., 2020) obtained them in some of these parameters. Likewise, results about meat tenderness are again study-dependent. Batorek, Čandek-Potokar, et al. (2012) and Harsh et al. (2017) detected that pork from IM have similar shear force than that from SC, while others authors (Li et al., 2015; Martinez-Macipe et al., 2016) observed that meat from IM are tougher and Grela et al. (2020) obtained the opposite effect. In the case of pork composition, in the review of Harsh et al. (2017) and in the studies of J. I. Morales et al. (2013) and Seiquer et al. (2019) meat from IM present higher moisture percentage than that of SCM. However, other authors (Grela et al., 2020; J. I. Morales et al., 2011; Škrlep et al., 2020) did not observe any effect. The results in terms of protein are controversial. Grela et al. (2020) and Seiquer et al. (2019) obtained that loins of IM have greater protein proportion than those of SCM, whereas J. I. Morales et al. (2013) referred the opposite effect and Caldara et al. (2013), J. I. Morales et al., (2011) and Škrlep et al. (2020) did not observe differences between these groups. In the case of IMF, in the review of Harsh et al. (2017) IM present lower IMF percentage than SCM, whereas in the meta-analyses of Batorek, Čandek-Potokar, et al. (2012) and Poulsen-Nautrup et al. (2018) this

effect is only observed numerically. Therefore, there is not consensus regarding meat composition, which is a relevant variable, and for this reason more research should be done in this regard.

Fat quality might be influenced by the type of castration in male pigs too. In the case of SCF, several authors (Daza et al., 2016; Font-i-Furnols et al., 2012; Mackay et al., 2013) observed that IM have similar total SFA percentage than SCM. However, other authors (Pauly et al., 2009; Škrlep et al., 2020) found that IM present lower total SFA proportion than SCM. A similar effect to the one found with total SFA proportion was also detected in the case of total MUFA percentage (Font-i-Furnols et al., 2012; Mackay et al., 2013; Škrlep et al., 2020). On the other hand, several authors (Costa e Silva et al., 2017; Pauly et al., 2009; Škrlep et al., 2020) observed that IM present greater total PUFA percentage than SCM, whereas others (Daza et al., 2016; Font-i-Furnols et al., 2012) did not find significant differences between these groups. A lower number of reports provides results on PUFA/SFA and n-6/n-3 ratios and on total n-3 and n-6 percentages. Daza et al. (2016) and Font-i-Furnols et al. (2012) did not find differences with the type of castration in these parameters, but Costa e Silva et al. (2017) observed that IM present higher total n-3 and n-6 percentages than SCM. In the case of IMF, a similar pattern as for SCF was observed (Daza et al., 2016; Seiquer et al., 2019; Škrlep et al., 2020). Differences between studies may be due to the time elapsed between the second dose of immunocastration and the slaughter, and the genetics used.

In respect of boar-taint compounds (androstenone, skatole and indole), a great deal of reports in this field (Batorek, Škrlep, et al., 2012; Pauly et al., 2009; Zamaratskaia, Andersson, et al., 2008) observed that immunocastration reduces these concentrations as much as surgical castration. Nevertheless, Weiler et al. (2013) detected that IM present higher androstenone and indole concentrations than SCM, which could be due to a shorter period between the second dose of immunocastration and the slaughter.

2.7. Consequences of immunocastration in female pigs (*vs.* entire gilts)

Immunocastration can also carry out in female pigs. In Spain, until 2022, the only product approved for this purpose was Vacsincel® (Zoetis), which has the same qualitative and quantitative composition and similar mechanism of action as Improvac®. In females, the immunization against GnRF produces a temporary immunological suppression of ovarian function. As previously commented, the protocol of administration of Vacsincel® is designed for outdoor Iberian gilts, which are slaughtered at a heavy BW (approximately 160 kg of BW). It consists of the subcutaneous injection of four doses (2 mL each one) behind the ear. The doses have to be administer at 18, 22, 34 and 46 weeks of age (Agencia Española de Medicamentos y Productos Sanitarios, 2022). However, it was seen that in gilts slaughtered at a lighter BW (approximately 130 kg), as in the case of gilts destined for Teruel dry-cured ham production, only two doses of immunocastration would be necessary (Mitjana et al., 2020; Rodrigues et al., 2019). Nevertheless, during the realization of the present thesis, in this type of gilts, the optimum moment for application of the doses was not defined. According to Allison et al. (2021) the best protocol of immunocastration in market gilts will depend on local conditions and production goals but, with *ad libitum* feeding, in general, a shorter second dose-slaughter period will favor efficient growth, while a longer period will increase fat deposition.

It should be noted that, in 2022, the European Commission approved the use of Improvac® also in female pigs. In this case, the goal is to reduce unwanted pregnancies in gilts intended for slaughter, of special interest when female pigs are raising together with male pigs, and to reduce the associated sexual behavior. Currently the protocol of administration of Improvac® in female pigs is the following: a first dose at no earlier than 14 weeks of age and a second dose approximately 4 weeks later (European Medicines Agency, 2022).

2.7.1. Growth performance, reproductive organs and blood sex hormones

From the first to the second dose of immunocastration, a great deal of authors (Daza et al., 2014; Rodrigues et al., 2019; Van den Broeke et al., 2016) did not observe differences between EG and IG in growth performance, since it seems that, as in the case of males, the first dose only primes the gilt's immune system. However, in this period, Daza et al. (2016) found that IG grow slower and tend to eat less and to be less efficient converting feed into weight gain

than EG. Additionally, Bohrer et al. (2014) also detected that IG have lower ADFI than EG. These last results are unexpected.

From the second dose of immunocastration to the slaughter, several authors (Bohrer et al., 2014; Rodrigues et al., 2019; Van den Broeke et al., 2016) observed that IG grow faster than EG and Zeng, Turkstra, Tsigos, et al. (2002) detected this effect only numerically. Rodrigues et al. (2019) and Van den Broeke et al. (2016) also obtained that IG eat more feed than EG and Bohrer et al. (2014) observed this effect numerically. The increase in ADFI by IG could be explained in part by the decrease of gonadal hormones in these gilts, although other mechanisms may be involved (Van den Broeke et al., 2016). Regarding efficiency, there is no unanimity in the literature. Rodrigues et al. (2019) and Van den Broeke et al. (2016) did not find differences between EG and IG, whereas Bohrer et al. (2014) observed that IG are more efficient than EG and Gómez-Fernández et al. (2013) obtained the opposite effect from 10 days post-second dose to slaughter.

From the first dose of immunocastration to the slaughter, some authors (Bohrer et al., 2014; Daza et al., 2014; Rodrigues et al., 2019) found that IG have higher ADG than EG, while others (Daza et al., 2016; Xue et al., 2019) did not observe significant effect of immunocastration. Likewise, Daza et al. (2014) and Rodrigues et al. (2019) detected that IG present greater ADFI than EG, whereas Bohrer et al. (2014) and Daza et al. (2016) did not find differences between these groups. Finally, regarding efficiency, several authors (Daza et al., 2014; Rodrigues et al., 2019; Xue et al., 2019) obtained similar results between IG and EG. However, Bohrer et al. (2014) observed that IG are more efficient than EG and Gómez-Fernández et al. (2013) obtained the opposite effect from 21 days before the first dose to the slaughter.

Gilts correctly immunized against GnRF present lighter and smaller internal parts of the reproductive tract (ovaries, oviducts, uterine, vagina and vestibule) than EG (Dalmau et al., 2015; Mitjana et al., 2020; Rodrigues et al., 2019). Some authors (Hernández-García et al., 2013, 2015) did not detect in IG macroscopically visible follicles, whereas EG presented immature and/or mature follicles and/or *corpora lutea* and/or *corpora albicantia*. Others (Mitjana et al., 2020; Xue et al., 2019) found that some IG show macroscopically visible follicles, although gilt immunocastration prevent the presence of developed follicles (>6 mm) present in EG. Moreover, Dalmau et al. (2017), Rodrigues et al. (2019) and Xue et al. (2019) did not detect any IG exhibiting estrus. However, Dalmau et al. (2015), Di Martino et al. (2018)

and Zeng, Turkstra, Tsigos, et al. (2002) detected a little percentage of IG (<17%) that shows mature follicles and/or *corpora lutea* and Allison et al. (2021), Bohrer et al. (2014) and Dalmau et al. (2015) observed that some IG (<21%) exhibit estrus. The main reasons that could explain these facts are that the time elapsed between the last dose and the slaughter was too long, that some gilts could not respond properly to immunocastration doses or might be missed at vaccination moments in group-housing systems.

Researches about blood sex hormones (Dalmau et al., 2015; Van den Broeke et al., 2016; Xue et al., 2019) report that gilt immunocastration reduces progesterone levels. This reduction was detected in different moments; Van den Broeke et al. (2016) from just before the administration of the second dose of immunocastration, Hernández-García et al. (2013) from the second dose, Dalmau et al. (2015) from the third dose and Xue et al. (2019) at slaughter. However, Mitjana et al. (2020) did not find a significant reduction in progesterone concentration with gilt immunization against GnRF. In the case of estradiol, Esbenshade & Britt (1985) detected that estradiol levels decline to basal levels in IG after they become acyclic. Nevertheless, Mitjana et al. (2020) and Van den Broeke et al. (2016) did not observe significant differences in estradiol levels between EG and IG. The moment of the estrus cycle in which gilts were when the blood was drawn could contribute to the lack of difference in the estradiol concentration in the report of Van den Broeke et al. (2016). Estradiol levels increase only between 6 to 2.5 days before estrus and decline until basal levels on day 4.5 of the estrus cycle (Esbenshade et al., 1982). In the case of Mitjana et al. (2020), the lack of differences in the concentrations of sex hormones could be explained because both EG and IG were prepubertal at slaughter, and thus, basal concentrations of progesterone and estradiol were expected in both groups.

2.7.2. Carcass, meat and fat quality

A great deal of reports in this field (Daza et al., 2014; Rodrigues et al., 2019; Xue et al., 2019) did not detect differences between EG and IG in carcass dressing percentage, although Gómez-Fernández et al. (2013) found higher carcass yield in IG than in EG and Martinez-Macipe et al. (2016) observed the opposite effect. There is certain unanimity in the literature (Daza et al., 2014, 2016; Van den Broeke et al., 2016) about the higher fat thickness generated by gilt immunocastration. This effect seems to be greater when the period between the second dose and the slaughter is longer (Allison et al., 2021). Nevertheless, some authors (Di Martino et al., 2018; Martinez-Macipe et al., 2016; Xue et al., 2019) did not detect effect of gilt

immunocastration on fat thickness. Di Martino et al. (2018) explains that these discrepancies might be attributed to different production systems, genetics and feeding programs. On the other hand, in the literature (Daza et al., 2014, 2016; Martinez-Macipe et al., 2016) it was not observed that gilt immunocastration influenced on carcass length and ham size. However, there is no unanimity in respect of ham and shoulder yields. Some authors (Daza et al., 2014; Izquierdo et al., 2013; Martinez-Macipe et al., 2016) observed that ham yield of IG is similar to that of EG, while others (Daza et al., 2016; Gómez-Fernández et al., 2013) detected that IG present or tend to present lower ham yield than EG. In the case of shoulder yield, some authors (Daza et al., 2014, 2016; Izquierdo et al., 2013) did not find influence of gilt immunocastration, whereas Gómez-Fernández et al. (2013) observed that IG have lower shoulder yield than EG. Daza et al. (2014) explain that these different results might be related in part to the amount of fat removed in the trimming of the pieces.

Regarding meat quality, several authors (Bohrer et al., 2014; Gamero-Negrón, Sánchez del Pulgar, Ventanas, et al., 2015; Van den Broeke et al., 2016) did not observe influence of gilt immunocastration on water holding capacity. Likewise, gilt immunization against GnRF seems to have scarce effects on color parameters (Bohrer et al., 2014; Daza et al., 2014; Gamero-Negrón, Sánchez del Pulgar, Ventanas, et al., 2015), although Daza et al. (2016) observed that pork from IG is redder and presents more chroma than that from EG. Some authors (Bohrer et al., 2014; Martinez-Macipe et al., 2016; Xue et al., 2019) did also not detect differences in texture, while Van den Broeke et al. (2016) found that pork from IG is more tender than that from EG. Regarding chemical composition, moisture and protein contents appear to be unaffected by gilt immunocastration (Bohrer et al., 2014; Gamero-Negrón, Sánchez del Pulgar, & García, 2015; Gamero-Negrón, Sánchez del Pulgar, Ventanas, et al., 2015). However, in respect of IMF, Gamero-Negrón, Sánchez del Pulgar, & García (2015) found that dry-cured loins from IG have higher IMF content than those from EG, and Daza et al. (2014) and Van den Broeke et al., (2016) detected this effect as a tendency in fresh loins. Others authors (Bohrer et al., 2014; Daza et al., 2016; Gamero-Negrón, Sánchez del Pulgar, Ventanas, et al., 2015) only observed numerically this effect, and conversely, Xue et al. (2019) with traditional Chinese gilts obtained the opposite effect.

Few studies have evaluated the impact of gilt immunocastration on fat quality. In SCF, Daza et al. (2014) found that IG present higher total SFA percentage and lower total MUFA, PUFA, n-3 and n-6 contents and PUFA/SFA ratio than EG. In IMF of fresh loins, these authors also observed that gilt immunocastration increases total SFA proportion, reduces total MUFA

content and PUFA/SFA ratio and tends to reduce total PUFA and n-6 percentages. Likewise, Gamero-Negrón, Sánchez del Pulgar, & García (2015) in IMF of dry-cured loins detected that immunization against GnRF increases total SFA content and decreases total PUFA percentage. However, Daza et al. (2016) and Gamero-Negrón, Sánchez del Pulgar, Ventanas, et al. (2015) did not observe differences between EG and IG in the fatty acid profile of SCF and IMF. That lack of effect in these last studies could be due to the minor differences in backfat depth and similar IMF content between EG and IG. Besides, in the report of Daza et al. (2016) the shorter time between the second dose of immunocastration and the slaughter could contribute to that lack of effect.

2.8. Impact of increasing dietary energy level maintaining similar crude protein and amino acid contents

This section carries out a review literature of studies in which feed was provided *ad libitum*, since pigs destined for Teruel dry-cured ham are fed in this way, and the feeding level can modify growth rate and/or carcass and muscle composition (Lebret, 2008).

2.8.1. Growth performance and blood metabolites

In growing pigs, from 24 to 54 kg of BW, Hossain et al. (2018) observed that increasing dietary energy level improves ADG and gain-to-feed ratio (G:F), without being affected ADFI. These results indicate that, in this stage, pigs are in an energy-dependent phase of growth, i.e. protein deposition increases with the increase of energy intake (De la Llata et al., 2001). Hossain et al. (2018) hypothesized that the improvement of ADG with the high-energy diet could be attributed to increased nutrient intake. From 70 to 100 kg of BW, Moreira et al. (2021) detected that the increase in dietary net energy (NE) levels provides a linear reduction in ADFI. These authors explain that, on the one hand, traditionally this reduction is associated to the fact that pigs consume feed to meet their energy requirements. On the other hand, these authors suggest that ADFI reduction could be due to the increase in diet lipid inclusion, since the higher lipid absorption by small intestine stimulates the synthesis and release of apolipoprotein A-IV by enterocytes, which inhibits feed intake (Moreira et al., 2021; Tso & Liu, 2004). Besides, Moreira et al. (2021) obtained a quadratic effect on ADG and FCR as dietary NE increased. On the other hand, Knowles et al. (1998), applying a smaller increase in dietary energy, did not observe any effect on ADG, ADFI and G:F, both in SCM (74-117 kg of BW) and in EG (74-102 kg of BW), meaning that these diets contained sufficient energy for potential growth.

In finishing pigs, from 94 to 130 kg of BW, Suarez-Belloch et al. (2013) detected that ADG is not modified by increasing dietary NE level, suggesting that during this phase pigs are not in an energy-dependent phase (De la Llata et al., 2001). These authors also observed that ADFI is lower and FCR tends to improve as NE level increases. Fracaroli et al. (2017), from 100 to 130 kg of BW, did also not detect effect of increasing dietary energy level on ADG and they obtained a quadratic response in G:F. On the other hand, these authors did not observe influence of NE levels on ADFI. This finding could be because energy intake could be below the potential for maximum energy intake, and thus, increasing dietary energy content would not reduce feed intake (De la Llata et al., 2001).

When diets are applied for a longer period (approximately from 30 to 115 kg of BW) diverse effects are observed. Hong et al. (2016) and Matthews et al. (2003) did not detect effect of increasing dietary energy density on ADG, ADFI and G:F, whereas Marçal et al. (2019) observed that G:F increases when dietary energy level is increased. On the other hand, Liu et al. (2007) detected a quadratic increase in ADG and a linear or quadratic decrease in ADFI and FCR as the energy density in the diets increased. The discrepancies between studies may be explained by the use of different energy units and levels of energy tested, the composition of diets (content of fiber) and the distinct genetics utilized.

Regarding blood metabolites, there are not many reports that have studied the effect of increasing dietary energy level maintaining similar CP and AA contents on blood concentrations of albumin, urea, cholesterol and triglycerides, especially on the latter three. In any case, there is some unanimity in the literature (Hossain et al., 2018; Kim et al., 2018; Moreira et al., 2021) about the lack of effect of increasing energy content of the diet maintaining similar dietary CP and AA levels on blood concentrations of albumin, urea, cholesterol and triglycerides.

2.8.2. Carcass, meat and fat quality

Several authors (Hong et al., 2016; Liu et al., 2007; Suarez-Belloch et al., 2013) did not observe effect of the rise of energy of the diet on carcass yield. However, Fracaroli et al. (2017) and Marçal et al. (2019) detected higher carcass yield with the increase in the NE level of the diet. These authors suggest that it could be because low-energy diets have higher fiber content than high-energy diets, increasing the weight of gastrointestinal tract and therefore reducing carcass yield. In respect of fat thickness, it seems that it increases with the rise of dietary energy content (Liu et al., 2007; Marçal et al., 2019; Suarez-Belloch et al., 2013), although some authors (Kerr et al., 2003; Knowles et al., 1998; Moreira et al., 2021) reported that dietary energy content has no effect on carcass fatness. The lack of effect could be due to the smaller increase in dietary energy level, the different pig BW when dietary treatments began or the shorter experimental period. According to Serrano et al. (2013), in pigs fed high-energy diets, surplus energy intake is converted to body fat. About the main piece in carcass, Suarez-Belloch et al. (2013) reported that the increase in dietary energy content does not affect ham size and reduces linearly the ham yield, although Moreira et al. (2021) did not observe this last effect.

In respect of meat quality, several authors (Liu et al., 2007; Matthews et al., 2003; Suarez-Belloch et al., 2013) obtained similar water holding capacity indicators as energy level

is increased. However, Moreira et al. (2021), applying a higher energy increase, observed that drip loss decreases linearly. Moreira et al. (2021) and Suarez-Belloch et al. (2013) detected similar meat lightness by increasing dietary energy content, while Matthews et al. (2003) obtained that this parameter tends to be increased in pigs fed high-energy diets. On the other hand, Matthews et al. (2003) and Suarez-Belloch et al. (2013) did not observe influence of dietary energy content on pork redness, whereas Moreira et al. (2021) obtained that redness decreases linearly as dietary NE levels increase. None of the previous works detected effect of dietary energy level on yellowness and Suarez-Belloch et al. (2013) did not detect it on chroma and hue angle parameters either. Some authors (Hong et al., 2016; Suarez-Belloch et al., 2013) did not observe impact of dietary energy level on shear force, while Matthews et al. (2003) obtained that shear force tends to be decreased in pigs fed high-energy diets. Regarding chemical composition, some authors (Hong et al., 2016; Suarez-Belloch et al., 2013) did not detect significant effect of increasing dietary energy level on meat moisture, protein and IMF contents. However, Liu et al. (2007), testing a greater energy increase, observed that IMF content increases linearly with the increase of dietary energy content.

Very few studies have evaluated the impact of increasing energy content of feed maintaining CP and AA contents on fat quality. In SCF, Suarez-Belloch et al. (2013), increasing dietary NE content in 140 kcal/kg, mainly by increasing the inclusion of animal blended fat, did not find any effect on total SFA, MUFA and PUFA percentages. However, these authors did observe a linear increase of C16:0 proportion and reductions of C20:0, C22:0, C20:1 and C20:4 as NE in diet increased. On the other hand, in SCF and in IMF, Alencar et al. (2017), increasing dietary NE content in 500 kcal/kg by adding soybean oil, obtained that total SFA and MUFA percentages decrease and total PUFA proportion increases. These last results are mainly due to the fact that soybean oil has a high concentration of linoleic fatty acids (Zambiazi et al., 2007). Therefore, the nature of the diet is able to modify the fat composition of pigs (Mourot & Hermier, 2001).

2.9. Impact of decreasing dietary crude protein and amino acid levels maintaining energy content

This section carries out a review literature of studies in which feed was provided *ad libitum*, since pigs destined for Teruel dry-cured ham are fed in this way, and the feeding level can modify growth rate and/or carcass and muscle composition (Lebret, 2008).

2.9.1. Growth performance and blood metabolites

In growing pigs, from 24 to 70 kg of BW (D'Souza et al., 2003) and from 37 to 67 kg of BW (Lee et al., 2020), no effect on productive performances when reducing dietary CP and lysine (Lys) contents was found. However, Chiba et al. (2002) and Yang et al. (2008), with pigs from 20 to 50 kg of BW and from 35 to 55 kg of BW, respectively, applying a greater CP and Lys restriction obtained a decrease in ADG and G:F and an increase in ADFI. The reduction in growth rate could be a consequence of limited CP and Lys intake (Lebret, 2008) and the rise in feed intake could be an attempt of pigs to meet their daily requirements (Henry, 1985). This feed overconsumption can carry out an increase in fat deposition and energy cost of gain (Henry, 1985), explaining the lower efficiency converting feed into BW (Wood et al., 2013). Teye et al. (2006) and Bunger et al. (2015), in pigs from 40 to 100 and 115 of BW, respectively, also obtained reduced growth rate and worse feed conversion efficiency by reducing CP and Lys contents.

In finishing pigs, from 70 to 110 kg of BW, Knowles et al. (1998) also observed lower G:F by reducing CP and AA levels, although ADG and ADFI were not significantly affected. From 90 to 130 kg of BW, Suárez-Belloch et al. (2015a) detected that CP and AA restriction reduces ADG and ADFI and increases FCR. This decrease in ADFI could be explained because pigs could have reached a point that they cannot compensate for reduced dietary CP and AA concentrations and thus their ADFI declines (Ferguson & Gous, 1997). Rodríguez-Sánchez et al. (2011), from 100 to 130 kg of BW, applying a lower CP and Lys restriction than Suárez-Belloch et al. (2015a), also detected a reduction in ADG and ADFI, but they did not observe effect on G:F. On the other hand, Tejeda et al. (2020) from 116 to 174 kg of BW did not obtain impact of CP and Lys restriction on ADG.

When dietary CP and Lys reduction was tested during a longer period (approximately from 20 to 120 kg of BW), no significant effects were observed on growth performance (Millet et al., 2006; Monteiro et al., 2017; Nguyen et al., 2018). The discrepancies detected among all

mentioned studies could be due to the different pig BW when dietary treatments start, the growth rate of pig breed and the level of reduction of CP and AA contents.

With regard to blood metabolites, there is unanimity in the literature (Mule et al., 2006; Suárez-Belloch et al., 2015b; Yang et al., 2008) about the decrease of albumin concentration in response to CP and AA reduction from approximately 25 to 55 kg of BW. According to Ruusunen et al. (2007), the rapidly growing muscles need high amount of AA, and thus, the synthesis of exported proteins in the liver, such as albumin, may be attenuated in pigs fed a low-CP and AA diet, since restrictions in AA availability would first appear in the synthesis of exported proteins. Likewise, several reports (Mule et al., 2006; Suárez-Belloch et al., 2015a; Yang et al., 2008) found that urea concentration decreases with CP and AA restriction, which could be due to a lower N intake and/or a more efficiently utilization of N for growth (Fabian et al., 2004). Regarding cholesterol, in some trials [experiment 2 of Mule et al. (2006) and Suárez-Belloch et al. (2015b)], from around 25 to 55 kg of BW, it was observed an increase in cholesterol concentration promoted by CP and AA reduction, which could indicate that dietary CP and AA restriction could have a hypercholesterolemic effect (Mule et al., 2006). Nevertheless, during a similar period, in other studies [experiment 1 of Mule et al. (2006) and Yang et al. (2008)] in which the CP and AA restriction applied was lower, this effect was not observed as significant. On the other hand, some authors (Suárez-Belloch et al., 2015a; Yang et al., 2008) did not detect effect of CP and AA reduction on triglyceride concentration. However, Suárez-Belloch et al. (2015b) testing a higher CP and AA restriction obtained an increase in triglyceride levels.

2.9.2. Carcass, meat and fat quality

Several authors (Monteiro et al., 2017; Rodríguez-Sánchez et al., 2011; Tejeda et al., 2020) did not observe impact of CP and Lys restriction on carcass yield, although Suárez-Belloch et al. (2015a) detected that CP and Lys reduction decreases quadratically carcass yield. Regarding fat thickness, some works (Millet et al., 2006; Rodríguez-Sánchez et al., 2011; Suárez-Belloch et al., 2016) showed that it increases linearly as dietary CP and Lys contents decrease, although others (Suárez-Belloch et al., 2015a; Teye et al., 2006; Wood et al., 2013) reported that fat thickness is unaffected. The greater fat thickness found in some studies could be explained by the excess energy intake in relation to Lys intake (Friesen et al., 1994). Other possible explanations may be a greater amount of dietary energy available for fat accretion due to lower energy expenditure for catabolization of excess dietary CP (Kerr et al., 2003) and lower

maintenance energy needs owing to the lower weight of kidney and liver as dietary CP and Lys contents decrease (Friesen et al., 1994; Wang et al., 2018). In terms of ham size (length and perimeter), Rodríguez-Sánchez et al. (2011) and Suárez-Belloch et al. (2015a) did not obtain influence of CP and Lys restriction. However, no differences were observed in ham yield (Rodríguez-Sánchez et al., 2011; Suárez-Belloch et al., 2015a; Tejeda et al., 2020) when reducing dietary CP and AA levels and, in the case of shoulder yield, Suárez-Belloch et al. (2015a) and Tejeda et al. (2020) did not observe any impact, whereas Rodríguez-Sánchez et al. (2011) detected a linear reduction.

Regarding meat quality, there seems to be unanimity in the literature (Millet et al., 2006; Monteiro et al., 2017; Suárez-Belloch et al., 2015a) about the lack of influence of CP and AA restriction on water holding capacity indicators (drip, thawing and cooking losses). Some authors (Millet et al., 2006; Suárez-Belloch et al., 2015a; Tejeda et al., 2020) detected similar pork color parameters by reducing CP and Lys contents. However, Teye et al. (2006) observed that lightness, redness and yellowness are higher in meat from pigs fed low-CP and -Lys diets and Lee et al. (2020) obtained the opposite effect regarding redness. These last authors explained that dissimilarities between studies could be due to different strains of pigs and methods of analysis. In terms of pork texture, some authors (Millet et al., 2006; Suárez-Belloch et al., 2015a; Teye et al., 2006) did not observe effect of CP and Lys restriction, although Rodríguez-Sánchez et al. (2011) obtained that shear force tends to increase linearly with this restriction. No effect of CP and Lys reduction was detected on moisture percentage (Rodríguez-Sánchez et al., 2011; Suárez-Belloch et al., 2015a; Tejeda et al., 2020). Likewise, Rodríguez-Sánchez et al. (2011) and Tejeda et al. (2020) did also not detect impact on protein proportion, although Suárez-Belloch et al. (2015a) obtained a linear decrease of protein percentage with CP and AA restriction. In the case of IMF, several authors (Suárez-Belloch et al., 2015a; Teye et al., 2006; Wood et al., 2013) detected higher content with CP and Lys reduction, even though others (Millet et al., 2006; Rodríguez-Sánchez et al., 2011) did not obtain this effect as significant. According to Teye et al. (2006), inadequate levels of dietary CP and Lys limit the synthesis of protein and increases the amount of energy available for fat accretion. Dissimilarities between studies could be explain by different pig breed used, periods of testing diets and level of reduction of CP and AA contents.

The impact of reducing CP and AA contents, maintaining the energy level, on fat quality, has been evaluated in few studies. In SCF, Rodríguez-Sánchez et al. (2011) and Tejeda et al. (2020) by reducing CP and Lys contents from 100 to 130 kg of BW and from 116 to 174

kg of BW, respectively, did not observe effect on total SFA, MUFA and PUFA proportions. However, Suárez-Belloch et al. (2015a), carrying out a similar restriction as Tejeda et al. (2020) from 90 to 130 kg of BW, detected that dietary CP and Lys reduction decreases linearly total PUFA percentage. In IMF, Teye et al. (2006), who carried out a CP and Lys restriction from 40 to 100 kg of BW, observed that this reduction increases total SFA and MUFA proportions and decreases total PUFA percentage and PUFA/SFA ratio, being a reflection of the higher IMF content. Likewise, Wood et al. (2013) detected lower PUFA/SFA ratio in pigs fed low-CP and -Lys diets. Nevertheless, when CP and Lys restriction was applied at a heavier BW, Suárez-Belloch et al. (2015a) did not observe effect on total SFA, MUFA and PUFA percentages and Tejeda et al. (2020) only in IMF of *serratus ventralis* muscle detected lower total PUFA proportion with CP and Lys reduction.

3. HYPOTHESES, OBJECTIVES AND EXPERIMENTAL APPROACH

3.1. Hypotheses

The main hypotheses of the present doctoral thesis were the following:

I. In female pigs destined for the PDO Teruel ham, the optimum time for injection the second dose of immunocastration could be in the point in which fat deposition is increased and ovarian follicles are small enough that gilts are not in estrus.

II. Immunocastration of gilts intended for the PDO Teruel ham could increase fat deposition, both SCF and IMF, and therefore it could optimize carcass, meat and dry-cured ham quality. Likewise, a moderate increase in dietary energy level maintaining CP and AA contents, or a drop in dietary CP and AA levels maintaining energy content, might increase fat deposition without penalizing growth performances.

III. Immunocastration of male pigs destined for the PDO Teruel ham could be an alternative to surgical castration, but also immunocastration could lead to a reduction in fatness in comparison to surgical castration. The rise of dietary energy content maintaining CP and AA levels or the drop in dietary CP and AA contents maintaining energy level might reduce/compensate this effect.

3.2. Objectives

The objectives to test and verify these hypotheses were the following:

I. To define the optimum time for the application of the second dose of immunocastration in female pigs destined for the PDO Teruel ham.

II. To study the impact of immunocastration on growth performance, serum metabolites, serum sex hormones, reproductive tract development and carcass, meat, fat and dry-cured ham quality of female pigs intended for the PDO Teruel ham.

III. To evaluate the impact of the type of castration (surgical *vs.* immunological) on growth performance, serum sex hormones, serum metabolites and carcass, meat, fat and dry-cured ham quality of male pigs destined for the PDO Teruel ham.

IV. To assess the effects of increasing dietary energy level maintaining CP and AA contents or decreasing dietary CP and AA contents maintaining energy level on growth performance, serum metabolites and carcass, meat and fat quality of pigs, in entire and immunocastrated females and in surgical castrated and immunocastrated males destined for the PDO Teruel ham.

3.3. Experimental approach

Three experiments were designed to address the objectives.

Experiment 1 (Paper 1)

The first experiment mainly met the first objective. Additionally, this trial contributed to getting the second objective. A total of 48 Duroc x (Landrace x Large White) gilts of 27 kg of BW were housed in a commercial farm (Torrijo del Campo, Teruel) in four pens (12 gilts per pen) with similar average BW each pen. There were four experimental treatments (one pen per treatment) based on the time of injection of the second dose of immunocastration: EG and IG providing the second dose at 12, 9 and 7 weeks before slaughter (with approximately 60, 75 and 90 kg of BW, respectively). The first dose of immunocastration was previously administered to IG groups when they weighed 30 kg of BW and the medicine used was Vacsincel® (Zoetis). The management and feeding program were the same for all the animals. The gilts were weighed individually at the beginning and at the end of the trial, as well as the days of application of the second doses to IG groups to calculate ADG. Besides, blood samples were taken on the days of injection of the second doses and at the end of the trial to evaluate serum sex hormone levels. All gilts were slaughtered the same day with 125 kg of BW. During the evisceration, all reproductive tracts were individually collected to study them in the laboratory. Then, carcass weight was individually recorded to calculate carcass yield. On the left side of each carcass, carcass length, ham length, ham perimeter and fat depth were measured. Carcasses were processed, and ham and shoulder from the left side of each carcass were individually weighed to calculate their yields. In addition, in the quartering, samples of meat of loins and hams and of SCF of hams were taken to assess later meat and fat quality.

Experiment 2 (Papers 2, 4 and 5)

The second experiment accomplished the second objective and part of the fourth. A total of 192 Duroc x (Landrace x Large White) gilts of 40 kg of BW were housed in a commercial farm (Foz-Calanda, Teruel) in 24 pens (eight gilts per pen). They were allotted to blocks of increasing BW and each block contained all the experimental treatments. Half of the gilts were immunocastrated at 58 and 77 kg of BW with Vacsincel® (Zoetis) and the other half remained intact throughout the trial. Three experimental diets were offered *ad libitum* to both IG and EG during the grower (76-102 kg of BW) and the finisher (102-134 kg of BW) periods: a control diet; a diet with a greater energy level than the control, but with similar CP and AA contents; and a diet with lower CP and AA percentages than the control, but with the same energy level.

The change between the grower and finisher feeds was carried out on a fixed day. Thus, there were six experimental treatments: two types of gilts (EG *vs.* IG) × three diets (control *vs.* high energy *vs.* low CP and AA). The gilts were weighed individually at the beginning and at the end of the trial, and also when the immunocastration doses were injected (the second dose coincided with the first day of supply of the experimental grower diets) and the first day of supply of the experimental finisher diets. Likewise, feed consumption per pen was controlled during the suppling of the experimental diets. Therefore, ADG, ADFI and G:F could be calculated. Besides, blood samples were taken on the days in which immunocastration doses were administered, at the end of the grower period and at the end of the trial (coinciding with the end of the finisher period) to study serum sex hormones and metabolites. Animals were slaughtered in three batches when they achieved approximately 134 kg of BW. During the evisceration, some reproductive tracts were individually collected to evaluate them in the laboratory. Then, carcass weight was individually recorded to calculate carcass yield. On the left side of each carcass, fat depth, ham length and ham perimeter were measured. Carcasses were processed, and ham and shoulder from the left side of each carcass were individually weighed to calculate their yields. In addition, in the quartering, samples of meat of loins and hams and of SCF of hams were taken to assess later meat and fat quality. Several hams from both EG and IG that fed the high-energy diet were dry-cured during approximately 19 months. Throughout this process, individual weight of the hams was recorded several times to calculate weight losses. Later, dry-cured ham quality was studied.

Experiment 3 (Papers 3, 4 and 6)

The third experiment was designed to achieve the third objective and part of the fourth. A total of 144 Duroc x (Landrace x Large White) male pigs of 35 kg of BW were housed in a commercial farm (Foz-Calanda, Teruel) in 18 pens (eight pigs per pen). They were allotted to blocks of increasing BW and each block contained all the experimental treatments. Half of the pigs were surgically castrated the first week of life. The other half were immunocastrated at 25, 58 and 79 kg of BW with Improvac® (Zoetis). The same experimental diets as in the Experiment 2 were offered *ad libitum* to both SCM and IM during the grower (80-110 kg of BW) and the finisher (110-137 kg of BW) periods. Likewise, the change between the grower and finisher feeds was carried out on a fixed day. Thus, there were six experimental treatments: two types of castration (SCM *vs.* IM) × three diets (control *vs.* high energy *vs.* low CP and AA). The pigs were weighed individually 22 d after the administration of the first dose of immunocastration, when the second dose was applied, when the third dose was injected (coinciding with the first

day of supply of the experimental grower diets), the first day of supply of the experimental finisher diets and at the end of the trial. Likewise, feed consumption per pen was controlled during the suppling of the experimental diets. Therefore, ADG, ADFI and FCR could be calculated too. Besides, blood samples were taken on the days in which second and third immunocastration doses were administered, at the end of the grower period and at the end of the trial (coinciding with the end of the finisher period) to study serum sex hormones and metabolites. Animals were slaughtered in three batches when they achieved approximately 137 kg of BW. In the slaughterhouse, carcass weight was individually recorded to calculate carcass yield. On the left side of each carcass, fat depth, ham length and ham perimeter were measured. Carcasses were processed, and ham and shoulder from the left side of each carcass were individually weighed to calculate their yields. In addition, in the quartering, samples of meat of loins and hams and of SCF of hams were taken to assess later meat and fat quality. Several hams from both SCM and IM that fed the high-energy diet were dry-cured during approximately 19 months. Throughout this process, individual weight of the hams was recorded several times to calculate weight losses. Lastly, dry-cured ham quality was studied.

4. RESULTS

Paper 1. Effect of the administration time of the second immunocastration dose on growth, serum sex hormones, reproductive tracts and carcass, meat and fat quality of female pigs

animals

MDPI

Article

Immunocastration in Gilts: A Preliminary Study of the Effect of the Second Dose Administration Time on Growth, Reproductive Tract Development, and Carcass and Meat Quality

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Simple Summary: Nowadays, a significant proportion of pig carcasses destined to high-quality dry-cured ham elaboration are declared unsuitable for this purpose. The main reason is the lack of backfat thickness, affecting females in particular because males are castrated. Moreover, the estrus in gilts is undesirable because it carries out productive losses. Immunocastration could resolve these problems. The protocol of immunocastration in white-breed gilts is not well established, especially in terms of the second injection. Therefore, the objective of the current trial was to evaluate the impact of immunocastration and to determine the optimum time for the second dose application in gilts intended for dry-cured ham production. In this trial, we concluded that gilt immunocastration is positive, increasing carcass fatness and decreasing reproductive tract development. Moreover, the optimum time to administer the second dose of immunocastration for this type of gilt seems to be between 9 and 12 weeks before sacrifice.

Abstract: Increasing fatness and avoiding puberty are desirable in gilts intended for high-quality dry-cured ham production. A total of 48 Duroc x (Landrace x Large White) females of 26.5 ± 3.70 kg body weight (BW) were used to evaluate the impact of immunocastration and to find the optimum application time of the second dose for immunocastration on growth; sex hormones; reproductive tract development; and carcass, meat, and fat quality. Gilts were allocated to four experimental treatments (n = 12): control (entire gilts, EG) and immunocastrated gilts (IG), providing the second dose at 12, 9, or 7 weeks before slaughter (with approximately 60, 75, or 90 kg BW, respectively). Mean slaughter BW was 125 kg. Immunocastrated gilts had lighter reproductive tracts and greater fat thickness than EG. Fat from IG was more saturated and less polyunsaturated than that from EG. Numerically, gilts immunocastrated 9 and 12 weeks before slaughter presented higher fatness than those immunocastrated 7 weeks before slaughter. In conclusion, immunocastration is a good strategy to improve the fatness of gilts destined to dry-cured ham elaboration, with the optimum time for the second dose application seemingly between 9 and 12 weeks before slaughter.

Keywords: immunocastration; gilts; reproductive tract; carcass; meat and fat quality

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1. Introduction

In Spain, the only protected designation of origin (PDO) dry-cured ham from non-autochthonous pigs is "Teruel ham". In recent decades, a lack of fat cover has been detected in this type of ham [1] because genetic selection has focused on getting lean. Likewise, a limited content in intramuscular fat (IMF) has been observed in these pieces by trained panelists [2]. The relevance of fatness in Teruel ham is unquestionable; subcutaneous fat guarantees an adequate dry-curing process and IMF is related to juiciness and tenderness [3,4]. These problems have been found mainly in gilts [5–7] because males are castrated (barrows) and castration increases the retention of fat tissue [8]. Moreover, according to this PDO regulation [9], gilts in estrus phase should be avoided at slaughter, and also some authors [10,11] have indicated that feed intake and growth is reduced during estrus. Immunocastration could be a possibility to resolve these issues. It consists in the application of several vaccines whose active substance is a gonadotrophin-releasing factor (GnRF) analogue protein conjugate, temporarily suppressing the sexual development [12]. Immunocastration has been more researched in male pigs as an alternative to surgical castration, observing that it increases the level of fat in carcass and in pork compared to boars [13]. In gilts, it seems that this practice has a similar effect on fatness, but it has been less studied and is mainly focused on autochthonous breeds reared outdoors, with the goal of avoiding undesirable pregnancies [14]. This immunization should be evaluated deeper in white-breed gilts, and the protocol of vaccination should be adapted for them, considering that they are younger and lighter at slaughter than autochthonous gilts. Therefore, the aim of this trial was to evaluate the impact of immunocastration on growth; sex hormone levels; reproductive tract development; and carcass, meat, and fat quality, as well as determining the optimum time for the application of the second dose of immunocastration in crossbred gilts intended for Teruel dry-cured ham.

2. Material and Methods

2.1. Animal Husbandry and Feeding

A total of 48 Duroc x (Landrace x Large White) gilts of 26.5 ± 3.70 kg of body weight (BW) (74 ± 3 d of age) were selected from 12 litters (4 females per litter assigning each to 1 treatment). All sows were mated to the same boar. On arrival at the facilities (Torrijo del Campo, Teruel, Spain), which was a commercial fattening farm, pigs were individually weighed and allotted to 4 pens (12 animals in each) according to their initial BW (similar average BW per pen). Each pen (1.1 m^2/animal) had 80% slatted floor and an outside park and was equipped with 1 drinking bowl and a hopper-type feeder.

There were 4 experimental treatments to evaluate the optimum time for the second dose administration of immunization against GnRF: entire gilts (EG) (control) or immunocastrated gilts (IG) with 56.6 ± 5.08 kg BW (12 weeks before slaughter; IG-12), 75.2 ± 6.46 kg BW (9 weeks before slaughter; IG-9), or 87.7 ± 6.62 kg BW (7 weeks before slaughter; IG-7). The first dose of immunocastration was previously administered to vaccinated groups with approximately 30 kg BW (1 week after entering the facilities). A trained veterinary carried out the administration of Vacsincel (Zoetis Spain S. L., Alcobendas, Madrid, Spain), the product used to perform the immunocastration, using a safety vaccinator with the animals loose in their corresponding pens.

The feeding program—grower diet from 27 to 70 kg BW and finisher diet from 70 to 125 kg BW—was the same for all animals during the experimental period. It consisted in pelleted commercial diets based on cereal and vegetable protein sources (grower diet: 9.20 MJ/kg of net energy and 16.9% of crude protein and finisher diet: 9.75 MJ/kg of net energy and 14.4% of crude protein). Feed intake was not controlled. Pigs had free access to feed and water throughout the trial and were slaughtered on the same day with 125.2 ± 8.47 kg BW (200 ± 3 d of age).

2.2. Control of Growth

Individual BW was recorded at day 0 (arrival to facilities) and at day 125 (pre-slaughter day). Moreover, the BW was individually taken at days 42, 62, and 77 of the trial (administration times of the second dose to the vaccinated groups IG-12, IG-9, and IG-7, respectively). These data were used to calculate the average daily gain (ADG) for each stage and for the overall experimental period.

2.3. Blood Sampling and Analyses

On the days of the administration of the second doses (days 42, 62, and 77 of the trial) and the day before slaughter (day 125 of the trial), a blood sample of 5 mL was taken from each pig by jugular venipuncture into a sterile tube with no additives (Vacutainer Brand, Becton Dickinson Vacutainer Systems, Plymouth, South West England, United Kingdom). Blood samples were obtained 3 h after the second dose injection and conserved at 4 °C until centrifugation at 1600× *g* for 10 min at 4 °C. After this process, serum was stored at −20 °C. Serum analyses were carried out in an external laboratory (Laboratorios Albéitar, Zaragoza, Spain) with competitive immunoassays using enzyme-labeled chemiluminescent technology (IMMULITE, Siemens Healthineers España, Getafe, Madrid, Spain). For progesterone, total coefficient of variation (CV) ranged between 6.5% (31.4 ng/mL) and 13.2% (1.04 ng/mL), depending on the concentration. In the case of estradiol, intra-assay CV ranged between 6.3% (480 pg/mL) and 15% (46 pg/mL), and inter-assay CV ranged between 6.4% (482 pg/mL) and 16% (56 pg/mL).

2.4. Reproductive Tract Collection, Carcass Measures, and Meat and Fat Sampling

Before slaughter in a commercial abattoir (Jamones y Embutidos Altomijares S.L., Formiche Alto, Teruel, Spain), pigs were fasted for 15 h and electrically stunned. During the evisceration, all reproductive tracts were individually collected in plastic bags and stored at 4 °C until subsequent studies in the laboratory.

Afterwards, warm carcass weight was individually recorded to calculate carcass yield. At 45 min postmortem, carcass length (from the posterior edge of the pubis symphysis to the anterior edge of the first rib), ham length (from the anterior edge of the pubis symphysis to the hock joint), and ham perimeter (at its widest side) were measured on the left side of each carcass. In addition, on the same carcass side, fat depth (skin included) between the third and fourth last ribs and over the gluteus medius muscle (GM) (at its thinnest point) was measured. After refrigeration for 4 h, carcasses were processed, and ham and shoulder from the left side of each carcass were individually weighed to calculate their yields in carcass.

The study of meat and fat quality was carried out with 40 fresh hams and loins (10 per treatment, always the left ones). For this, samples of approximately 150 g of the GM and the longissimus thoracis muscle (LT) were excised. Moreover, from each ham, near the GM, a sample of around 150 g of subcutaneous fat (including skin, fat layers, and lean) was taken. All the samples were vacuum packaged. The samples of the LT were stored at 4 °C, while those of the GM and subcutaneous fat were preserved at −20 °C until subsequent analyses.

2.5. Study of the Reproductive Tracts

The different parts of the reproductive organs from each gilt were dissected and studied separately. Both ovaries were weighed and measured (length, width, and depth). In addition, the follicles of each ovary were counted according to their size (<2 mm: very small, 2–4 mm: small, 4–6 mm: intermediate, and >6 mm: big follicles). Moreover, oviducts, uterine horns, uterine corpus, cervix, and vagina were weighed, and their lengths were taken. Finally, vaginal vestibule and vulva lengths were also measured.

2.6. Meat Quality Traits

Color, cooking losses, and hardness were measured in the fresh LT. The day after slaughter, color was assessed using a spectrophotometer (CM-2002, Konica Minolta Holdings. Inc., Osaka, Kansai, Japan) in CIE$L^*a^*b^*$ space [15], with illuminant D65 and an observer angle of 10°. The mean of three random readings was used to measure lightness (L^*), redness (a^*), and yellowness (b^*). Moreover, chroma ($C^* = \sqrt{a^{*2} + b^{*2}}$) and hue angle ($H^\circ = \tan^{-1}(b^*/a^*) \times 57.29$) were calculated [16]. Afterwards, cooking losses were evaluated by the method described by Honikel [17]. Firstly, samples were weighed, placed in individual plastic bags, and cooked in a water bath at 75 °C to reach the core temperature of 70 °C (Precisterm, J.P. Selecta S.A., Barcelona, Cataluña, Spain). During the cooking, the internal temperature was monitored through a thermocouple type T connected to a data logger (testo 177-T4, Testo GmbH, Lenzkirch, Freiburg, Germany). Then, the cooked samples were cooled, blotted dry, and weighed again. Cooking losses were calculated by dividing the difference of pre- and post-cooked weights by the pre-cooked weight and were expressed as a percentage. Hardness was also determined by the method described by Honikel [17]. The cooked samples were cut in prism-shaped pieces with a 100 mm^2 (10 × 10 mm) cross-section with the fiber direction parallel to a long dimension of at least 30 mm. Eight prisms per sample were sheared perpendicular to the fiber orientation, with a Warner–Bratzler shear blade attached to an Instron Universal testing machine (Model 5543, Instron Ltd., High Wycombe, Buckinghamshire, UK) attached to a computer.

Chemical composition (moisture, protein, and IMF) was analyzed in the GM according to the procedures of Boletín Oficial del Estado [18]. When it was required, the samples were thawed for 24 h at 4 °C and minced. Moisture was determined using an oven (Memmert UFE500, Schwabach, Mittelfranken, Germany), protein with a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Skåne, Sweden), and IMF by an ANKOM^{XT15} Extration System (ANKOM Techonology, Macedon, NY, USA) after the samples were hydrolyzed by an ANKOM^{HCL} Hydrolysis System.

2.7. Fatty Acid Profile of Subcutaneous Fat

Each fat sample was separated into inner layer and outer layer, and each layer was independently analyzed because they may have different metabolic activity [19]. Lipids were extracted following the method of Bligh and Dyer [20]. Fat extracts were methylated in the presence of sulfuric acid and later analyzed using a gas chromatograph (HP-6890, Hewlett Packard Co., Avondale, PA, USA) equipped with a flame ionization detector and a capillary column (HP-Innowax, 30 m length × 0.32 mm id × 0.25 µm cross-linked polyethylene glycol) [21]. The proportions of total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA), and also PUFA/SFA ratio, total ω-3 and ω-6, and ω-6/ω-3 ratio were calculated from the individual fatty acid proportions.

2.8. Statistical Analyses

Data were analyzed with the Statistical Analysis System, Version 9.4 (SAS Institute, Cary, NC, USA). Body weights; ADG; reproductive tracts; and carcass, meat, and fat quality data were assessed using the GLM procedure. Initial or final BW were included as covariates, when significant ($p < 0.05$), for ADG or for carcass quality, respectively.

Serum progesterone concentration was not statistically analyzed because most of values, irrespective of the treatment, were below the detection level of the equipment utilized (0.20 ng/mL). Therefore, a descriptive analysis was carried out with this parameter. Estradiol was analyzed using the MIXED procedure with repeated measures. The model included treatment, sampling time, and their interaction as fixed effects, as well as gilt within treatment as experimental unit. Compound symmetry was the covariance structure chosen because it was the model with the smallest Akaike and Bayesian Information Criteria values. Tukey test was used to assess the differences between the least square means of sampling times.

The number of ovarian follicles and the percentage of gilts with follicles in each category of size were analyzed using the GENMOD procedure. In the first case, negative binomial distribution and log link function were applied, and in the second case, binomial distribution and logit link function were used.

In all the statistical analyses described above, preplanned orthogonal comparison was used to evaluate EG versus IG. Moreover, the tendency response inside immunocastrated groups (lineal or quadratic) was analyzed with orthogonal polynomials.

Normality of the residuals was checked with Shapiro–Wilk test and homoscedasticity with Levene's test. In cases in which normality or homoscedasticity were not achieved, variables were transformed with $\sqrt{x}$, Napierian logarithm, $1/x$ or x^2 before statistical analyses in order to normalize residual distributions. When data transformation was carried out, results were shown in tables as means and standard deviations of the original data, and coefficients of determination and *p*-values obtained with the transformed data.

The experimental unit was the animal and a *p*-value ≤ 0.05 was considered to be a significant difference.

3. Results and Discussion

It has to be noted that this was a preliminary study and the number of replicates per treatment was limited. Moderate values of the coefficient of determination were obtained in many variables studied.

3.1. Weight Gain Pattern

Table 1 shows that there was no significant difference ($p > 0.05$) between EG and IG in terms of growth in the studied period, and therefore the BW at slaughter was similar ($p = 0.785$) for both types of gilts. Our results agree with those of Zeng et al. [22] and Di Martino et al. [23]. On the other hand, Daza et al. [24] detected greater ADG in IG during the next month and a half right after the second vaccination, while other authors [11,25,26] even observed that effect until the slaughter. It is worth noting that all those authors did not find differences on ADG between the first and the second dose, which was expected because the first vaccine only primes the pig immune system [27]. However, the second vaccine is the one that really affects the reproductive system, which could generate greater physiological effects. The discrepancies among experiments may be due to the different genetics used, ages at slaughter, or timing of dose application for immunocastration.

Within IG groups, from day 0 (arrival to facilities) to day 42 (second injection for IG-12) of the trial, ADG was similar ($p > 0.05$). It was logical because the management was the same and the time of application of the first vaccine was also the same for all cases (1 week after arrival). From day 42 to day 62 (second injection for IG-9), no differences ($p > 0.05$) were observed on ADG. However, from day 62 to day 77 (second injection for IG-7), a linear effect was detected; the earlier the second dose was applied, the greater the ADG ($p < 0.0001$). From day 77 to day 125 (end of the trial), the longest period, there was a quadratic response; the gilts immunocastrated for a second time 9 weeks before slaughter presented greater ($p = 0.001$) ADG than the other vaccinated groups. This finding might suggest that 9 weeks before slaughter could be the optimum time for the administration of the second dose of immunocastration, but in the overall trial period (0 to 125 d), all IG groups presented similar ($p > 0.05$) ADG.

Table 1. Body weights (BW) and average daily gains (ADG) (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Item	EG	IG-7	IG-9	IG-12	R^2 [2]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
BW, kg								
Initial	26.5 ± 3.2	25.7 ± 3.5	27.0 ± 4.4	26.9 ± 3.9	0.02	0.963	0.425	0.584
Final	124.6 ± 8.4	122.1 ± 8.2	129.2 ± 6.4	124.8 ± 10.0	0.09	0.785	0.431	0.059
ADG [3], kg/d								
0 to 42 d	0.671 ± 0.057	0.722 ± 0.085	0.685 ± 0.094	0.708 ± 0.098	0.06	0.233	0.673	0.320
42 to 62 d	0.903 ± 0.145	0.952 ± 0.126	0.969 ± 0.087	1.001 ± 0.139	0.08	0.100	0.342	0.871
62 to 77 d	1.002 ± 0.125	0.846 ± 0.159	1.006 ± 0.125	1.085 ± 0.090	0.34	0.579	<0.0001	0.375
77 to 125 d	0.769 ± 0.142	0.717 ± 0.124	0.812 ± 0.082	0.665 ± 0.122	0.37	0.306	0.450	0.001
0 to 125 d	0.785 ± 0.078	0.772 ± 0.072	0.818 ± 0.048	0.784 ± 0.082	0.18	0.784	0.474	0.067

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] R^2: coefficient of determination. [3] The second dose was administered at the following days of the trial: 42 in IG-12, 62 in IG-9, and 77 in IG-7. The first dose was injected at the same time for all of them (with approximately 30 kg of body weight, 1 week after the beginning of the trial).

3.2. Serum Sex Hormones

All gilts presented basal concentrations of progesterone (<1 ng/mL) throughout the trial (Table S1). In Iberian x Duroc gilts, in which at least three doses are required because they are slaughtered at around 160 kg, Dalmau et al. [28] found no differences between EG and IG until the third vaccination (at 238 days of age). However, from that moment onwards, IG showed lower progesterone concentration than EG. Xue et al. [29], with Chinese gilts, observed the same effect the day before slaughter, and Hernández-García et al. [30], with purebred Iberian gilts, found the effect from the second vaccination onwards. The greater effect detected with Iberian and Chinese female pigs could be explained in part because these breeds are autochthonous and reach puberty earlier [31]. Moreover, progesterone concentration varies through the estrus cycle, with the highest point being around 10 days after estrus, and therefore the time to draw blood could be relevant [32].

No significant ($p = 0.183$) interaction treatment x sampling time was found in serum estradiol concentration. Estradiol levels were similar ($p > 0.05$) between EG and IG, in agreement with Van den Broeke et al.'s [26] findings, and irrespective of the administration timing for the second dose (Table S2). Esbenshade and Britt [33] did observe that estradiol concentration declined to basal levels in IG (vaccinated at around 8, 10, and 11 months of age) after they became acyclic. Regarding sampling time (Figure 1), the concentration of estradiol increased ($p < 0.0001$) as gilts grew, irrespective of the treatment.

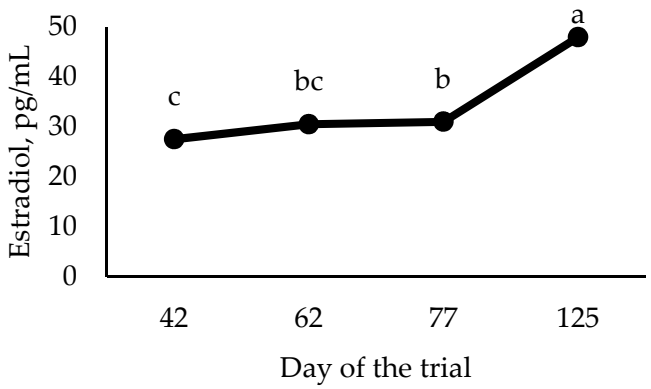

Figure 1. Serum estradiol concentration during the trial. Statistical evaluation was carried out with data after their transformation. Data are presented as back-transformed least square means. Values that differ significantly ($p < 0.05$) are noted with different letters (a, b, or c).

3.3. Reproductive Tracts

Immunocastrated gilts had a lighter ($p < 0.0001$) reproductive tract than EG, which was due to the differences found ($p < 0.01$) in the weight of ovaries, oviducts, uterine horns, uterine corpus, cervix, and vagina (Table 2). Furthermore, ovary size was minor ($p < 0.0001$) and length of uterine horns ($p < 0.0001$) was shorter in IG than in EG. These results agree with others found in the literature [11,28,30], corroborating that immunocastration suppresses the development of reproductive organs.

Table 2. Reproductive organ size and weight (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Item	EG	IG-7	IG-9	IG-12	R^2 [2]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Ovaries								
Weight, g	6.91 ± 1.73	2.68 ± 1.45	1.46 ± 0.53	2.22 ± 2.15	0.68	<0.0001	0.493	0.084
Size, cm	12.00 ± 3.48	3.78 ± 3.13	2.00 ± 1.05	4.08 ± 4.57	0.61	<0.0001	0.833	0.109
Oviducts								
Weight, g	1.79 ± 0.40	1.24 ± 0.29	1.30 ± 0.87	1.43 ± 0.75	0.24	0.001	0.944	0.536
Length, cm	16.9 ± 2.0	15.2 ± 2.1	14.8 ± 2.6	15.8 ± 4.8	0.08	0.112	0.652	0.551
Uterine horns								
Weight, g	72.8 ± 29.1	28.3 ± 12.4	31.9 ± 42.5	32.7 ± 32.0	0.27	0.0004	0.744	0.901
Length, cm	63.4 ± 9.4	47.8 ± 7.3	47.2 ± 11.3	45.8 ± 14.5	0.33	<0.0001	0.424	0.837
Uterine corpus								
Weight, g	2.94 ± 1.32	1.00 ± 0.27	1.08 ± 1.16	1.32 ± 1.34	0.35	<0.0001	0.509	0.844
Length, cm	2.79 ± 0.75	2.32 ± 0.64	2.21 ± 0.89	2.54 ± 1.10	0.07	0.141	0.540	0.477
Cervix								
Weight, g	37.1 ± 13.7	13.2 ± 3.9	14.0 ± 10.7	17.1 ± 16.5	0.41	<0.0001	0.450	0.791
Length, cm	15.0 ± 3.3	13.1 ± 2.1	13.8 ± 2.1	13.7 ± 4.2	0.05	0.147	0.777	0.605
Vagina								
Weight, g	31.1 ± 7.6	15.3 ± 5.6	12.6 ± 7.1	17.5 ± 14.4	0.42	<0.0001	0.579	0.274
Length, cm	10.75 ± 2.32	9.95 ± 1.63	9.82 ± 1.69	9.45 ± 2.58	0.05	0.161	0.583	0.882
Vestibule length, cm	12.3 ± 0.6	11.7 ± 0.8	11.9 ± 1.3	11.1 ± 1.5	0.13	0.067	0.305	0.263
Vulva length, cm	3.14 ± 0.55	3.20 ± 0.51	2.94 ± 0.44	3.03 ± 0.47	0.04	0.633	0.402	0.337
Total genital tract weight, g	153.0 ± 47.1	61.9 ± 21.6	64.0 ± 64.0	64.9 ± 56.9	0.42	<0.0001	0.900	0.975

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] R^2: coefficient of determination.

Within IG groups, no differences ($p > 0.05$) were found in the size and weight of the reproductive organs. Therefore, the three moments of administration of the second dose of immunocastration appeared to be equally effective in avoiding reproductive tract development in gilts of this crossbred and slaughter weight.

Table 3 provides the study on the ovarian follicles. The total number of follicles was lower ($p = 0.011$) in IG than in EG, with IG having less ($p = 0.0001$) small follicles. When the percentage of gilts with follicles in each category of size was studied, we found that IG presented a greater ($p = 0.0003$) proportion of females with very small follicles and a lower ($p < 0.05$) percentage of females with small and intermediate follicles than EG. Consequently, immunocastration prevented the presence of more developed follicles, supporting the results of Xue et al. [29], who observed that IG showed immature follicles (3–4 mm) or did not show visible follicles. This effect was more pronounced in the works of Zeng et al. [22] and Hernández-García et al. [30], which did not find visible follicles in any gilt immunocastrated.

Table 3. Study of the ovarian follicles (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Item	EG	IG-7	IG-9	IG-12	*p*-Value [2]		
					EG vs. IG	IG Linear	IG Quadratic
Number of follicles							
<2 mm	14.8 ± 30.2	55.9 ± 36.9	39.2 ± 34.8	27.7 ± 21.8	0.069	0.260	0.992
2–4 mm	59.5 ± 25.6	13.4 ± 24.5	2.1 ± 6.3	18.5 ± 25.3	0.0001	0.621	0.003
4–6 mm	4.17 ± 5.15	0.73 ± 2.41	0.42 ± 1.44	1.27 ± 4.22	0.090	0.685	0.504
>6 mm	0 ± 0	0 ± 0	0.42 ± 1.44	0 ± 0	-	-	-
Total	78.4 ± 25.3	70.0 ± 32.2	42.1 ± 32.8	47.5 ± 23.8	0.011	0.066	0.082
Gilts with follicles, %							
<2 mm	33.3 ± 49.2	90.9 ± 30.2	91.7 ± 28.9	81.8 ± 40.5	0.0003	0.531	0.680
2–4 mm	100.0 ± 0	54.5 ± 52.2	25.0 ± 45.2	90.9 ± 30.2	0.004	0.048	0.004
4–6 mm	58.33 ± 51.49	9.09 ± 30.15	8.33 ± 28.87	9.09 ± 30.15	0.0007	1.00	0.940
>6 mm	0 ± 0	0 ± 0	8.33 ± 28.87	0 ± 0	-	1.00	0.144

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2]: *p*-Value could not be obtained.

Within IG groups, a quadratic effect was observed both in the number of follicles and in the percentage of gilts with follicles; the gilts immunocastrated 9 weeks before slaughter had a lower ($p = 0.003$) number of small follicles and a lower ($p = 0.004$) percentage of females with small follicles than IG-7 and IG-12. It is worth noting that one gilt belonging to the IG-9 group presented some follicles of 7–8 mm. Zeng et al. [22] and Dalmau et al. [28] also detected two and one gilt, respectively, to which the doses of immunization against GnRF would have been injected, but that presented mature follicles at slaughter. This could be explained by the fact that these animals did not respond to immunocastration or that some of the doses were not injected correctly [22,34].

3.4. Carcass Quality

The effect of gilt immunocastration and the impact of the application time of the second dose on carcass characteristics are presented in Table 4. Carcass weight was similar ($p = 0.775$) for EG and IG. Moreover, no significant differences ($p > 0.05$) between both groups were detected in carcass yield and in size of carcass and ham. These results are consistent with those of Daza et al. [24,35], and was expected because the slaughter weight and age in both trials were similar.

Fat depth measured at both points (between the third and fourth last ribs and at the GM) was thicker ($p < 0.05$) in IG than in EG, being positive in the case of pigs intended for the elaboration of dry-cured ham. There is certain unanimity in the literature about the greater fat cover generated by immunocastration [10,24,26,35]. In the case of immunocastrated male pigs, Dunshea and D'Souza [19] attribute this effect to a greater feed intake and to a gradual reduction in steroid production during the first two weeks post-second vaccination.

The weights and yields of ham and shoulder and total (ham + shoulder) weight were similar ($p > 0.05$) between EG and IG, in agreement with Izquierdo et al. [36], Daza et al. [24], and Rodrigues et al. [11]. Gómez-Fernández et al. [25] also found no differences in ham and shoulder weights between EG and IG, but these authors observed that IG had lower ham and shoulder yields than EG. This effect was obtained in the current study in the case of total (ham + shoulder) yield; immunocastrated gilts presented lower ($p = 0.024$) total yield than EG. The different results found in terms of yields could be explained because the amount of fat removed in the trimming of the pieces may influence in these parameters.

With respect to the administration time of the second dose for immunocastration, it did not affect ($p > 0.05$) any of the carcass characteristics. However, it has to be noted that numerically IG-12 showed fatter carcasses (thicker backfat thickness) than the other vaccinated groups, probably because the period as immunocastrated animals was longer.

Table 4. Carcass characteristics (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Trait	EG	IG-7	IG-9	IG-12	R^2 [2]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Carcass weight, kg	96.5 ± 7.1	95.3 ± 7.8	99.9 ± 5.2	96.9 ± 9.2	0.94	0.775	0.573	0.187
Carcass yield, %	77.4 ± 1.7	77.8 ± 1.5	77.3 ± 0.9	77.6 ± 2.3	0.12	0.811	0.550	0.228
Length [3], cm								
Carcass	86.8 ± 3.0	85.5 ± 3.2	87.8 ± 2.7	86.0 ± 1.9	0.21	0.663	0.862	0.169
Ham	38.7 ± 0.9	38.4 ± 1.0	38.5 ± 1.1	38.3 ± 1.6	0.26	0.323	0.465	0.581
Ham perimeter	78.4 ± 2.5	77.3 ± 3.1	77.9 ± 1.7	77.5 ± 2.3	0.53	0.087	0.695	0.285
Fat thickness [3], mm								
Between third/fourth last ribs	22.9 ± 4.1	24.8 ± 4.5	26.7 ± 2.9	27.1 ± 7.1	0.39	0.033	0.426	0.440
At GM [4]	15.7 ± 4.6	18.4 ± 3.1	20.5 ± 3.1	20.9 ± 8.9	0.40	0.013	0.428	0.466
Trimmed cut weight [3], kg								
Ham	13.6 ± 1.0	13.0 ± 0.9	13.6 ± 0.8	13.4 ± 1.0	0.79	0.260	0.649	0.113
Shoulder	7.93 ± 0.53	7.66 ± 0.48	7.93 ± 0.30	7.75 ± 0.55	0.73	0.374	0.254	0.256
Total [5]	21.6 ± 1.5	20.7 ± 1.4	21.5 ± 1.0	21.1 ± 1.5	0.82	0.220	0.397	0.098
Trimmed cut yield [3], % carcass								
Ham	14.1 ± 0.3	13.9 ± 0.4	13.5 ± 0.5	13.8 ± 0.6	0.31	0.075	0.866	0.390
Shoulder	8.21 ± 0.28	8.23 ± 0.26	7.91 ± 0.27	8.02 ± 0.33	0.60	0.065	0.333	0.589
Total [5]	22.3 ± 0.5	22.2 ± 0.5	21.4 ± 0.6	21.9 ± 0.9	0.52	0.024	0.807	0.344

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] R^2: coefficient of determination. [3] Data recorded from the left side of each carcass. [4] GM: gluteus medius muscle. [5] Ham + shoulder.

3.5. Meat Quality

As shown in Table 5, immunocastration had no effect ($p > 0.05$) on color traits (L^*, a^*, b^*, C^*, and H°), cooking losses, and hardness, confirming the findings of other authors [10,29,37]. However, the effect of immunization against GnRF on chemical composition of meat from gilts is more controversial. In the present trial, there was no influence ($p > 0.05$), corroborating the results of Bohrer et al. [10] and Gamero-Negrón et al. [14]. However, Daza et al. [24] and Van den Broeke et al. [26] observed that meat from IG tended to have greater IMF content than that from EG. In the present experiment, IMF content was 12% greater in IG than in EG, in line with backfat depth findings, which were 14% higher between the third and fourth last ribs and 27% greater at the GM in IG. Nevertheless, the effect on IMF content was only numerical ($p > 0.05$) due to the high variability of data and a small number of animals included in the experiment (10 animals). This parameter is relevant because it has a positive impact on some texture and appearance parameters of hams, such as oiliness, brightness, juiciness, and marbling [3].

Table 5. Meat quality (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Trait	EG	IG-7	IG-9	IG-12	R^2 [2]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Color [3]								
Lightness, *L**	47.2 ± 3.1	47.6 ± 2.0	48.6 ± 2.6	47.6 ± 3.0	0.04	0.529	0.989	0.336
Redness, *a**	3.35 ± 1.40	3.07 ± 0.54	3.13 ± 0.93	3.24 ± 0.80	0.01	0.612	0.693	0.936
Yellowness, *b**	7.27 ± 1.10	6.85 ± 0.75	7.21 ± 1.01	7.01 ± 1.53	0.02	0.628	0.761	0.518
Chroma, *C**	8.06 ± 1.46	7.53 ± 0.64	7.90 ± 1.12	7.76 ± 1.52	0.02	0.547	0.686	0.590
Hue angle, *H*°	66.0 ± 7.3	65.6 ± 5.2	66.7 ± 5.5	64.7 ± 6.7	0.02	0.896	0.733	0.518
Cooking losses [3], %	26.1 ± 1.3	26.5 ± 1.8	25.0 ± 2.3	25.0 ± 3.5	0.07	0.607	0.185	0.457
Hardness [3], kg	1.62 ± 0.22	1.72 ± 0.31	1.86 ± 0.45	1.82 ± 0.42	0.06	0.290	0.555	0.537
Chemical composition [4], %								
Moisture	72.0 ± 1.4	72.3 ± 0.9	71.0 ± 2.0	71.8 ± 2.7	0.06	0.707	0.597	0.202
Protein	22.7 ± 0.5	23.2 ± 0.6	22.7 ± 0.7	22.2 ± 1.0	0.21	0.897	0.005	0.878
Intramuscular fat	3.68 ± 1.85	2.99 ± 0.83	4.88 ± 2.35	4.51 ± 3.09	0.10	0.668	0.147	0.202

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] R^2: coefficient of determination. [3] Laboratorial analyses were carried out with samples of the longissimus thoracis muscle. [4] Laboratorial analyses were carried out with samples of the gluteus medius muscle.

Within IG groups, the effect of the administration time of the second dose was limited because only protein content was influenced; the delay in the application of the second injection generated greater ($p = 0.005$) protein content in meat. Again, it is worth noting that IMF content was numerically ($p > 0.05$) considerably greater in earlier vaccinated gilts (IG-9 and IG-12) than in those immunized later (IG-7). It is because the later the second dose was administered, the longer IG behaved as EG from a reproductive point of view.

3.6. Fat Quality

The impact of immunocastration on fatty acid profile of subcutaneous fat is shown in Table 6 (inner layer) and Table 7 (outer layer). Immunocastrated gilts had greater ($p = 0.010$) proportion of total SFA than EG in the inner layer, due to the greater contents in C16:0 ($p = 0.024$) and C18:00 ($p = 0.033$). In both inner and outer layer, total PUFA percentage was lower ($p = 0.04$) in IG than in EG because of the lower ($p \leq 0.05$) contents in C18:2n-6, C18:3n-3, and C20:4n-6. Daza et al. [24] found similar results in analyzing both layers together. Our findings about fat composition were expected because greater C18:0 proportion and lower C18:2n-6 content have been related to pigs with thicker backfat thickness [38]. According to Madsen et al. [39], the lower total PUFA content (and total ω-6; $p \leq 0.05$) detected in fat from IG could lead to a better storage stability and flavor of the pieces due to their lower susceptibility to oxidation spoilage, being especially desirable in the case of dry-cured hams. In both inner and outer layer, PUFA/SFA ratio was lower ($p < 0.05$) in IG than in EG, which would generate firmer fat, being better for meat technological processes [40]. However, EG had greater ($p = 0.011$) total ω-3 percentage in the outer layer, which would implicate healthier pork because these fatty acids decrease triglyceride levels, favorably affect platelet function, and reduce blood pressure in hypertensive people [41]. In general, our results about fatty acid composition confirm those obtained by Daza et al. [24].

Table 6. Fatty acid profile (mean ± standard deviation) of the inner layer of the subcutaneous fat of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Trait [2], %	EG	IG-7	IG-9	IG-12	R^2 [3]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Individual FA								
C14:0	1.29 ± 0.10	1.26 ± 0.05	1.35 ± 0.11	1.28 ± 0.15	0.10	0.811	0.613	0.075
C16:0	23.8 ± 1.1	24.7 ± 0.5	25.4 ± 1.0	24.5 ± 1.1	0.25	0.024	0.741	0.033
C16:1n-7	1.98 ± 0.18	1.80 ± 0.29	1.98 ± 0.31	1.79 ± 0.38	0.09	0.406	0.922	0.142
C16:1n-9	0.516 ± 0.070	0.424 ± 0.173	0.446 ± 0.125	0.479 ± 0.179	0.06	0.260	0.410	0.965
C17:0	0.345 ± 0.052	0.339 ± 0.060	0.285 ± 0.026	0.342 ± 0.085	0.18	0.295	0.884	0.024
C17:1	0.294 ± 0.050	0.261 ± 0.054	0.246 ± 0.016	0.270 ± 0.066	0.10	0.092	0.663	0.571
C18:0	14.0 ± 1.4	15.8 ± 1.6	15.3 ± 0.9	15.3 ± 1.6	0.15	0.033	0.371	0.611
C18:1n-7	2.45 ± 0.35	2.36 ± 0.60	1.96 ± 0.66	2.34 ± 0.40	0.10	0.410	0.818	0.100
C18:1n-9	39.8 ± 1.3	39.2 ± 1.9	39.9 ± 1.4	39.8 ± 1.8	0.03	0.823	0.477	0.524
C18:2n-6	12.9 ± 2.1	11.3 ± 1.1	10.7 ± 1.2	11.4 ± 2.3	0.15	0.039	0.929	0.385
C18:3n-3	1.015 ± 0.149	0.903 ± 0.088	0.850 ± 0.097	0.895 ± 0.156	0.17	0.027	0.892	0.330
C20:1n-9	0.99 ± 0.08	1.08 ± 0.10	1.03 ± 0.12	1.14 ± 0.17	0.19	0.095	0.375	0.092
C20:3n-3	0.137 ± 0.025	0.130 ± 0.035	0.129 ± 0.020	0.159 ± 0.032	0.20	0.846	0.035	0.158
C20:3n-6	0.097 ± 0.013	0.091 ± 0.024	0.079 ± 0.017	0.097 ± 0.018	0.15	0.348	0.493	0.051
C20:4n-6	0.238 ± 0.039	0.201 ± 0.040	0.196 ± 0.035	0.189 ± 0.054	0.14	0.040	0.563	0.953
C22:5n-3	0.124 ± 0.038	0.132 ± 0.040	0.111 ± 0.026	0.125 ± 0.025	0.07	0.950	0.610	0.156
Groups of FA								
Total SFA	39.5 ± 2.3	42.1 ± 2.0	42.3 ± 1.5	41.4 ± 2.2	0.21	0.010	0.449	0.430
Total MUFA	46.0 ± 1.5	45.2 ± 1.9	45.6 ± 1.5	45.8 ± 1.5	0.03	0.476	0.405	0.871
Total PUFA	14.5 ± 2.3	12.8 ± 1.3	12.1 ± 1.3	12.9 ± 2.5	0.15	0.039	0.932	0.361
PUFA/SFA	0.370 ± 0.073	0.305 ± 0.037	0.287 ± 0.037	0.314 ± 0.076	0.18	0.020	0.821	0.353
Total ω-3	1.28 ± 0.19	1.17 ± 0.12	1.09 ± 0.12	1.18 ± 0.17	0.15	0.063	0.839	0.170
Total ω-6	13.2 ± 2.1	11.6 ± 1.2	11.0 ± 1.2	11.7 ± 2.4	0.15	0.038	0.940	0.384
ω-6/ω-3	10.34 ± 0.44	9.99 ± 0.67	10.13 ± 0.35	9.84 ± 0.79	0.08	0.213	0.605	0.379

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] FA: fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. [3] R^2: coefficient of determination.

Table 7. Fatty acid profile (mean ± standard deviation) of the outer layer of the subcutaneous fat of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively) [1].

Trait [2], %	EG	IG-7	IG-9	IG-12	R^2 [3]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Individual FA								
C14:0	1.36 ± 0.08	1.33 ± 0.05	1.43 ± 0.12	1.34 ± 0.14	0.12	0.907	0.889	0.043
C16:0	23.2 ± 0.5	23.5 ± 0.9	23.9 ± 0.8	23.6 ± 1.0	0.08	0.208	0.793	0.279
C16:1n-7	2.29 ± 0.26	2.18 ± 0.18	2.34 ± 0.30	2.10 ± 0.31	0.13	0.515	0.554	0.073
C16:1n-9	0.591 ± 0.160	0.493 ± 0.086	0.567 ± 0.089	0.550 ± 0.173	0.06	0.375	0.362	0.390
C17:0	0.346 ± 0.051	0.362 ± 0.070	0.318 ± 0.031	0.352 ± 0.092	0.06	0.807	0.529	0.207
C17:1	0.338 ± 0.046	0.332 ± 0.056	0.311 ± 0.020	0.321 ± 0.070	0.04	0.424	0.473	0.692
C18:0	11.8 ± 1.1	13.0 ± 1.1	12.3 ± 1.0	12.8 ± 1.2	0.15	0.076	0.688	0.135
C18:1n-7	3.21 ± 0.37	2.98 ± 0.47	3.18 ± 0.67	3.11 ± 0.62	0.02	0.630	0.620	0.557
C18:1n-9	40.2 ± 0.8	40.4 ± 1.5	40.8 ± 1.4	41.0 ± 1.5	0.05	0.403	0.333	0.902
C18:2n-6	13.9 ± 1.4	12.7 ± 1.1	12.3 ± 1.2	12.1 ± 2.2	0.13	0.052	0.476	0.874
C18:3n-3	1.119 ± 0.115	1.022 ± 0.078	0.984 ± 0.088	0.961 ± 0.154	0.20	0.020	0.260	0.878
C20:1n-9	0.944 ± 0.140	1.008 ± 0.070	0.989 ± 0.055	1.085 ± 0.128	0.22	0.084	0.112	0.162
C20:3n-3	0.165 ± 0.028	0.162 ± 0.017	0.146 ± 0.023	0.172 ± 0.028	0.17	0.652	0.389	0.030
C20:3n-6	0.107 ± 0.008	0.113 ± 0.012	0.099 ± 0.018	0.107 ± 0.029	0.07	0.913	0.525	0.167
C20:4n-6	0.268 ± 0.048	0.216 ± 0.012	0.239 ± 0.034	0.210 ± 0.053	0.23	0.017	0.750	0.111
C22:5n-3	0.207 ± 0.070	0.192 ± 0.049	0.151 ± 0.051	0.135 ± 0.069	0.20	0.087	0.047	0.603

Table 7. *Cont.*

Trait [2], %	EG	IG-7	IG-9	IG-12	R^2 [3]	*p*-Value		
						EG vs. IG	IG Linear	IG Quadratic
Groups of FA								
Total SFA	36.7 ± 1.3	38.2 ± 1.8	37.9 ± 1.7	38.1 ± 1.5	0.11	0.062	0.887	0.684
Total MUFA	47.6 ± 0.8	47.4 ± 1.8	48.2 ± 1.6	48.2 ± 1.8	0.05	0.679	0.290	0.553
Total PUFA	15.7 ± 1.6	14.4 ± 1.2	13.9 ± 1.3	13.7 ± 2.5	0.14	0.043	0.421	0.864
PUFA/SFA	0.431 ± 0.056	0.378 ± 0.040	0.369 ± 0.044	0.363 ± 0.076	0.16	0.026	0.579	0.953
Total ω-3	1.49 ± 0.17	1.38 ± 0.09	1.28 ± 0.11	1.27 ± 0.20	0.25	0.011	0.125	0.489
Total ω-6	14.2 ± 1.4	13.0 ± 1.1	12.7 ± 1.2	12.5 ± 2.3	0.13	0.050	0.474	0.896
ω-6/ω-3	9.57 ± 0.21	9.46 ± 0.55	9.89 ± 0.67	9.82 ± 0.66	0.09	0.555	0.194	0.276

[1] The second dose application time corresponded with 90, 75, and 60 kg of body weight for IG-7, IG-9, and IG-12, respectively. [2] FA: fatty acids; SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids. [3] R^2: coefficient of determination.

Within IG groups, the significant differences were punctual. There were linear effects in two fatty acids—the C20:3n-3 increased ($p = 0.035$) in the inner layer with earlier vaccination and C22:5n-3 increased ($p = 0.047$) in the outer layer with later vaccination. Moreover, some quadratic effects were observed; the gilts immunocastrated for a second time 9 weeks before slaughter had greater C16:0 ($p = 0.033$) and lower C17:0 ($p = 0.024$) and C20:3n-6 ($p = 0.051$) proportions in the inner layer, and greater C14:0 ($p = 0.043$) and lower C20:3n-3 ($p = 0.030$) percentages in the out layer than the other vaccinated groups.

4. Conclusions

Under our experimental conditions, it can be concluded that immunocastration of gilts notably reduces the reproductive tract development without penalizing the animal growth. This technique increases the fat thickness covering the ham, an essential aspect for dry-cured ham production, although it does not have a significant effect on intramuscular fat content of meat. Fat composition is also affected by immunization against GnRF, generating higher proportion of saturated fatty acids and lower of polyunsaturated fatty acids. Despite the fact that no considerable differences were found between the immunocastrated gilts groups, the application of the second dose of immunocastration between 9 and 12 weeks before slaughter seems to be the optimum time in this type of gilt because it numerically increases carcass fatness and intramuscular fat, which are desirable aspects for dry-cured ham production and consumption.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-2615/11/2/510/s1: Table S1: Blood progesterone concentrations (ng/mL) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively), sampled in different moments of trial. Table S2: Serum estradiol concentrations (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9, or 12 weeks before slaughter (IG-7, IG-9, and IG-12, respectively).

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Data Availability Statement: Data available on request due to restrictions of privacy.

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Immunocastration in Gilts: A Preliminary Study of the Effect of the Second Dose Administration Time on Growth, Reproductive Tract Development, and Carcass and Meat Quality

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Table S1. Blood progesterone concentrations (ng/mL) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9 or 12 weeks before slaughter (IG-7, IG-9 and IG-12, respectively) sampled in different moments of the trial [1].

Treatment	Day 42 (2nd dose IG-12)	Day 62 (2nd dose IG-9)	Day 77 (2nd dose IG-7)	Before slaughter
EG	<0.200	<0.200	<0.200	0.279
EG	0.390	0.272	0.299	0.251
EG	<0.200	<0.200	<0.200	<0.200
EG	0.291	0.266	0.461	0.322
EG	<0.200	<0.200	<0.200	<0.200
EG	0.200	0.237	<0.200	0.264
EG	<0.200	0.204	<0.200	<0.200
EG	<0.200	<0.200	<0.200	0.316
EG	<0.200	<0.200	<0.200	<0.200
EG	0.223	<0.200	<0.200	0.334
EG	<0.200	<0.200	<0.200	<0.200
EG	.	.	.	.
IG-7	<0.200	<0.200	<0.200	<0.200
IG-7	<0.200	<0.200	<0.200	<0.200
IG-7	<0.200	<0.200	0.224	<0.200
IG-7	0.251	<0.200	0.670	0.504
IG-7	<0.200	<0.200	<0.200	0.227
IG-7	<0.200	<0.200	0.267	0.305
IG-7	0.442	0.250	0.542	.
IG-7	<0.200	0.423	.	.
IG-7	<0.200	<0.200	.	.

IG-7	<0.200	<0.200	<0.200	<0.200
IG-7	<0.200	<0.200	0.432	<0.200
IG-7	<0.200	<0.200	0.536	<0.200
IG-9	0.281	0.225	0.396	0.252
IG-9	<0.200	0.230	0.811	0.215
IG-9	<0.200	<0.200	<0.200	<0.200
IG-9	<0.200	<0.200	0.242	0.448
IG-9	0.275	<0.200	0.256	0.205
IG-9	<0.200	<0.200	<0.200	0.233
IG-9	<0.200	0.224	<0.200	<0.200
IG-9	<0.200	<0.200	<0.200	<0.200
IG-9	0.380	<0.200	<0.200	<0.200
IG-9	<0.200	<0.200	<0.200	<0.200
IG-9	<0.200	<0.200	<0.200	<0.200
IG-9	.	.	.	.
IG-12	<0.200	<0.200	0.327	0.253
IG-12	<0.200	<0.200	<0.200	0.318
IG-12	0.259	0.282	<0.200	0.314
IG-12	<0.200	<0.200	<0.200	<0.200
IG-12	0.224	0.238	0.352	0.317
IG-12	<0.200	<0.200	<0.200	<0.200
IG-12	<0.200	<0.200	<0.200	0.334
IG-12	<0.200	<0.200	<0.200	<0.200
IG-12	0.207	<0.200	<0.200	<0.200
IG-12	0.331	<0.200	<0.200	<0.200
IG-12	.	.	.	.
IG-12	.	.	.	.

[1] The second dose application time corresponded with 90, 75 and 60 kg of body weight for IG-7, IG-9 and IG-12, respectively.

Table S2. Serum estradiol concentrations (mean ± standard deviation) of entire gilts (EG) and immunocastrated gilts receiving the second dose at 7, 9 or 12 weeks before slaughter (IG-7, IG-9 and IG-12, respectively) [1].

Trait	**EG**	**IG-7**	**IG-9**	**IG-12**	***p*-Value**		
					EG vs IG	**IG linear**	**IG quadratic**
Estradiol, pg/mL	36.7 ± 14.9	37.5 ± 13.0	38.2 ± 15.6	31.8 ± 12.9	0.795	0.134	0.477

[1] The second dose application time corresponded with 90, 75 and 60 kg of body weight for IG-7, IG-9 and IG-12, respectively.

Paper 2. Effect of immunocastration and diet on growth performance, serum metabolites and sex hormones, reproductive tracts and carcass quality of female pigs

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Effect of Immunocastration and Diet on Growth Performance, Serum Metabolites and Sex Hormones, Reproductive Organ Development and Carcass Quality of Heavy Gilts

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Simple Summary: Currently, a considerable proportion of the carcasses intended for the production of Teruel dry-cured ham are declared unsuitable for this purpose, mainly due to their lack of fat. This problem is detected especially in females, because males are castrated to avoid boar taint, and castration increases fat deposition. Immunocastration (immunization against gonadotrophin releasing factor) could resolve this issue, as that collateral effect has been reported in the literature. Increasing energy or reducing protein and amino acids in the diet could also result in greater fatness. Additionally, immunocastrated gilts could have different feeding pattern to entire females, and thus it is interesting to study feeding plans. Therefore, a study was conducted to evaluate the influence of immunocastration and provided diet on growth performance, serum metabolites and sex hormones, reproductive organ development, and carcass quality. In this trial, it was concluded that immunocastration is a positive strategy to apply in gilts intended for Teruel dry-cured ham production, because it increases growth rate and fatness. On the other hand, irrespective of immunocastration, a rise in dietary energy or a drop in dietary crude protein and amino acids in gilts from 76 to 134 kg could also be beneficial strategies for pig farmers.

Abstract: It is desirable to increase fatness in gilts destined for Teruel dry-cured ham production. A total of 192 Duroc × (Landrace × Large White) gilts of 40.3 ± 4.80 kg body weight (BW) were used to assess the impact of immunocastration and feeding on growth performance, serum metabolites and sex hormones, reproductive organ development, and carcass quality. Six treatments were arranged factorially (2 × 3) with two types of gilt (entire gilts (EG) vs. immunocastrated gilts (IG)) and three experimental diets (control vs. high energy vs. low crude protein and amino acids) provided from 76 to 134 kg BW (n = 4 per treatment, being the replicate the pen with eight pigs). Immunocastration was carried out at 58 and 77 kg BW. The IG grew faster and showed lighter reproductive tracts and greater fatness than EG. The experimental feeds had limited effect on carcass quality, but the high-energy diet improved gain-to-feed ratio and the low-protein and -amino-acids diet did not impair growth performance. In conclusion, immunocastration was a better strategy than the tested diets to increase the fatness of gilts intended for Teruel dry-cured ham, although increasing energy or decreasing crude protein and amino acid levels in the diet could be beneficial strategies for pig farmers.

Keywords: immunocastration; dietary energy; dietary protein; growth performance; sex hormones; reproductive organs; carcass quality; gilts

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1. Introduction

"Teruel ham" is a Spanish protected designation of origin (PDO) dry-cured ham with national relevance and certain international recognition. It is based on white-crossbreed pigs (not autochthonous) slaughtered at a heavy body weight (BW) (around 135 kg). To guarantee the quality and homogeneity of the end product, the Consortium of that PDO requires a fat depth over the gluteus medius muscle (GM) > 16 mm [1], which is necessary to optimize the dry-curing process [2]. The Teruel ham industry complains about the difficulties in achieving that fat thickness, with this problem being detected especially in females [3], because males are surgically castrated to avoid boar taint, and castration increases subcutaneous fat content [4]. One strategy to try to resolve this issue could be the castration of gilts, which would be expected to produce a similar effect to that in males. However, surgical castration of female pigs reared indoors is banned in the European Union [5]. Therefore, immunocastration might be an alternative. It consists of the application of several vaccines in which the active substance is a gonadotrophin-releasing factor (GnRF) analogue–protein conjugate. This analogue stimulates the gilt immune system to produce anti-GnRF antibodies that neutralize the pig's GnRF, blocking the production of follicle-stimulating hormone and luteinizing hormone. Therefore, estrus is temporarily suppressed [6]. Additionally, immunocastrated gilts (IG) could have different feeding pattern to entire females (EG); thus, it is reasonable to study feeding plans in this type of animals, which might provide positive or even additional effects to immunocastration on animal fattening. In this sense, the increase of dietary energy level and the reduction of crude protein (CP) and amino acids (AA) contents could be interesting, because these strategies seem to be successful in EG [7,8]. Therefore, the objective of this trial was to evaluate the impact of immunocastration and diet on growth performance, serum metabolites and sex hormones, reproductive organ development, and carcass quality of gilts intended for Teruel dry-cured ham production.

2. Materials and Methods

2.1. Animal Husbandry and Experimental Design

All the experimental procedures used in this experiment followed the ethical committee requirements of the University of Zaragoza (ref. PI29/18). Animals were cared for and managed according to the Spanish Policy for Animal Protection [9]. A total of 192 Duroc × (Landrace × Large White) gilts with 40.3 ± 4.80 kg BW (84 ± 3 days of age) were used. On the arrival at the farm (Foz-Calanda, Teruel, Spain), pigs were individually identified and weighed, and housed in groups of eight in pens of 9 m^2 with 50% slatted floor, equipped with one drinking cup and one grow feeder. Animals were allotted to blocks of increasing BW and each block contained all treatments.

Half of the gilts were immunocastrated by injection of two doses of Vacsincel® (Zoetis Spain S.L., Alcobendas, Madrid, Spain), with the first injection at 58.1 ± 6.39 kg of BW (102 ± 3 days of age) and the second one at 77.0 ± 8.12 kg of BW (122 ± 3 days of age), according to the conclusions obtained in a previous study [10]. The other half of the gilts remained intact throughout the trial. Three experimental diets were offered to both IG and EG during the grower and finisher periods (Table S1 and Table 1): (i) a control diet with a similar nutritional profile to the recommendations of FEDNA [11]; (ii) a diet with a greater energy content than the control diet, but with similar CP and AA percentages; and (iii) a diet with lower CP and AA contents than the control diet, but with the same energy level. In all diets, ideal protein content was maintained [11] and the change between the grower and the finisher feeds was carried out on a fixed day. The grower diets were supplied from 122 to 149 ± 3 days of age (76–102 kg BW) and the finisher diets were given from 150 ± 3 days of age to the day before slaughter (102–134 kg BW). Therefore, there were six experimental treatments; two types of gilt (EG vs. IG) × three diets (control vs. high energy vs. low CP and AA).

Table 1. Estimated and analyzed nutrient composition of the experimental diets (%, as-fed basis).

Item	Grower Diet (76 to 102 kg Body Weight)			Finisher Diet (102 to 134 kg Body Weight)		
	Control	High Energy	Low CP and AA	Control	High Energy	Low CP and AA
Estimated nutrient composition						
Net energy, Mcal/kg	2.33	2.48	2.33	2.33	2.48	2.33
Dry matter	88.3	88.6	88.3	88.4	88.7	88.4
Ash	4.07	4.05	3.89	3.88	3.83	3.68
CP	16.0	16.0	14.0	14.5	14.5	12.5
Ether extract	3.08	6.10	3.00	3.02	5.81	2.81
Neutral detergent fiber	12.3	11.8	13.0	12.9	12.1	13.5
Starch	47.4	45.2	49.9	49.2	47.5	52.0
Digestible AA						
Lysine	0.77	0.77	0.67	0.63	0.63	0.54
Methionine	0.24	0.24	0.21	0.21	0.20	0.18
Methionine + cysteine	0.49	0.49	0.44	0.44	0.43	0.39
Threonine	0.50	0.50	0.43	0.43	0.43	0.36
Tryptophan	0.16	0.16	0.14	0.15	0.15	0.13
Analyzed nutrient composition						
Gross energy, Mcal/kg	3.99	4.12	3.92	3.91	4.12	3.95
Dry matter	88.7	88.2	88.0	88.0	89.4	88.1
Ash	4.18	4.19	4.17	3.85	3.98	3.65
CP	16.2	15.9	14.4	14.5	15.1	12.7
Ether extract	3.55	5.88	3.44	3.00	5.65	3.73
Neutral detergent fiber	10.9	10.2	10.5	10.5	8.96	10.2
Starch	42.1	40.3	44.0	44.5	47.8	49.0
Total AA						
Lysine	0.98	0.98	0.79	0.76	0.77	0.71
Methionine	0.28	0.27	0.25	0.24	0.25	0.23
Threonine	0.62	0.60	0.59	0.56	0.58	0.51

CP: crude protein; AA: amino acids.

2.2. Feed Sypply and Analyses

Feed in pellet form and water were provided ad libitum throughout the trial. Gross energy of the experimental diets was analyzed using an adiabatic bomb calorimeter (Model 1356, Parr Instrument Company, Moline, IL, USA). Dry matter, ash, and CP were determined following methods 934.01, 942.05, and 2001.11, respectively, of the Association of Official Analytical Collaboration (AOAC) International [12]. Ether extract was analyzed using the Am 5-04 procedure of the American Oil Chemists' Society [13]. Neutral detergent fiber was determined with an ANKOM^{220} Fiber Analyzer (Ankom Technology, Macedon, NY, USA) as described by Mertens [14]. Starch content was analyzed enzymatically using a commercial kit (Total Starch Assay Kit K-TSTA 07/11, Megazyme, Bray, Wicklow, Ireland). The AA profile was determined in an external laboratory (Ofice S.L., Castellgalí, Barcelona, Spain) using high-performance liquid chromatography–fluorescence.

2.3. Growth Performance Measurements

Individual BW was recorded several times: at the beginning of the study, when the first dose of Vacsincel was applied, when the second dose was injected (coinciding with the first day pigs were given the experimental grower diets), the first day of the experimental finisher diets, and the day before slaughter. The records were used to calculate average daily gain (ADG) per pen for each phase and for the overall trial period (from 84 $\pm$ 3 days of age to the day before slaughter). Feed consumption per pen was controlled during the grower and the finisher periods to calculate average daily feed intake (ADFI) per pen. Finally, ADG and ADFI per pen were used to calculate gain-to-feed ratio (G:F).

2.4. Blood Sampling and Analyses

A blood sample of 10 mL from one pig per pen, chosen at random, was taken at several points: the days on which immunocastration doses were administered (the second one coinciding with the beginning of the grower period), at the end of the grower period, and at the end of the finisher period (coinciding with the day before slaughter). The

sampled animals were always the same, and each blood sample was obtained by jugular venipuncture and introduced into a sterile tube with no additives (Vacutainer Brand, Becton Dickinson Vacutainer Systems, Plymouth, Devon, UK). It was conserved at 4 °C until its centrifugation (1600× *g*, 10 min, 4 °C) and serum was then stored at −20 °C. Serum analyses were carried out by an external company (Laboratorios Albéitar, Zaragoza, Spain).

In the case of serum metabolites, the concentrations of albumin, urea, cholesterol, and triglycerides were evaluated at the end of the both the grower and the finisher diets using GernonStar equipment (RAL S.A., Barcelona, Spain). Albumin was analyzed via a colorimetric method (reagent GN 86125); intra-assay coefficient of variation (CV) was 0.50% and inter-assay CV ranged between 0.70 and 0.80% (5.70 and 3.35 g/dL, respectively), depending on the concentration. Urea was determined via a kinetic method (reagent GN 70125); intra-assay CV ranged between 0.77 and 2.79% (120 and 37 mg/dL) and inter-assay CV between 1.65 and 2.65% (126 and 40 mg/dL). Cholesterol and triglycerides were analyzed with an enzymatic–colorimetric method (reagent GN 21100 for cholesterol and GN 90125 for triglycerides). Cholesterol intra-assay CV ranged between 0.76 and 1.22% (185.3 and 99.8 mg/dL) and inter-assay CV between 4.36 and 6.91% (96.3 and 185 mg/dL). Triglycerides intra-assay CV ranged between 0.99 and 1.57% (196 and 128 mg/dL) and inter-assay CV between 3.15 and 7.70% (126 and 201 mg/dL).

In the case of sex hormones, the concentrations of progesterone and estradiol were evaluated on the days of application of the immunization doses and on the day before slaughter using competitive immunoassays with enzyme-labeled chemiluminescent technology (IMMULITE, Siemens Healthineers, Madrid, Spain). Progesterone total CV was 6.5–13.2% over the calibration range of 31.4–1.04 ng/mL, respectively. Estradiol intra-assay CV was 6.3–15% at 480–46 pg/mL, respectively, and inter-assay CV was 6.4–16% over the calibration range of 482–56 pg/mL, respectively.

2.5. Slaughtering, Reproductive Organ Collection, and Carcass Measures

The slaughter was planned at a fixed BW (close to 135 kg), and thus pigs were slaughtered at 178, 185, and 199 ± 3 days of age. On the day before slaughter, feed was withdrawn for 5 h and pigs were transported 130 km to a commercial abattoir (Cartesa, Teruel, Spain), where they were kept in lairage for 10 h with full access to water but not to feed. At the slaughterhouse, animals were stunned in CO_2 atmosphere, exsanguinated, scalded, dehaired, singed, and eviscerated. The genital tracts of a total of 27 gilts (12 EG and 15 IG, chosen at random, which had eaten the same feeding plan (high-energy diet)) were collected in individual plastic bags and conserved at 4 °C until their subsequent evaluation in the laboratory.

For the study of carcass quality, a total of 132 gilts were chosen at random, being 22 from each experimental treatment (type of gilt × diet). After carcasses were split lengthwise, hot carcass weight was individually recorded to calculate carcass yield. Fat depth (skin included) over the GM (at its thinnest point), ham length (from the anterior edge of the pubis symphysis to the hock joint), and ham perimeter (at its widest side) were measured on the left side of each carcass. After refrigeration at 2 °C (approximately 1 m/s air speed and 90% relative humidity) for 5 h, carcasses were processed, and, to fit commercial requirements (round shape), hams and shoulders were trimmed of external fat. The ham and the shoulder from the left side of each carcass were then individually weighed to calculate their yields in the carcass.

2.6. Study of Reproductive Organs

The collected genital tracts were dissected and each part was studied separately. Uterine horns, uterine corpus, cervix, and vagina were weighed and their lengths were measured. Vaginal vestibule and vulva were also measured. Each ovary was weighed and its length, width, and depth were measured. The follicles of each ovary were counted according to their size (<2 mm: very small, 2–4 mm: small, 4–6 mm: intermediate, and >6 mm: big follicles) [15].

2.7. Statistical Analyses

The Statistical Analysis System, Version 9.4 (SAS Institute Inc., Cary, NC, USA), was used. In the case of growth performance, data were analyzed as a randomized complete block design with a 2 × 3 factorial arrangement of treatments using the GLM procedure, with the pen as the experimental unit (n = 4 per treatment). The model included type of gilt (EG or IG) and diet (control, high energy, or low CP and AA) as main effects and the initial BW as the blocking criterion. Interaction (type of gilt × diet) was removed from the final models because it was nonsignificant ($p > 0.05$).

Serum metabolites were analyzed by repeated-measures analysis using the MIXED procedure. The model included type of gilt (EG or IG), diet (control, high energy, or low CP and AA), sampling time (at the end of the grower period or at the end of the finisher period), and their interactions as fixed effects, with the gilt as the experimental unit (n = 4 per treatment at each sampling time). Unstructured, compound symmetry, unstructured, and variance components were the covariance structures chosen for albumin, urea, cholesterol, and triglycerides, respectively, since these were some of the models with the smallest Akaike and Bayesian information criteria values.

Progesterone was not statistically analyzed because many values in both types of gilt were below the detection level of the equipment used (0.20 ng/mL); consequently, a descriptive analysis was carried out. Estradiol was analyzed using the MIXED procedure with repeated measures. The model included type of gilt (EG or IG), sampling time (at first dose of immunocastration, at second dose, or the day before slaughter), and their interaction as fixed effects, and gilt within type of gilt as the experimental unit (n = 12 per type of gilt at each sampling time). The effect of the diet on sex hormones was not analyzed. Compound symmetry was the covariance structure chosen, since it was the model with the smallest Bayesian information criterion value.

Reproductive organs were analyzed using the GLM procedure. The model included type of gilt (EG or IG) as main effect and the gilt as the experimental unit (n = 12 for EG and n = 15 for IG). The number of ovarian follicles and the percentage of gilts with follicles in each size category were analyzed using the GENMOD procedure. In the first case, a negative binomial distribution was applied, and in the second one, a binomial distribution was considered. Least square means and 95% confidence intervals were transformed from the log and logit scales, respectively.

Carcass quality was analyzed as a factorial design (2 × 3) using the GLM procedure with the gilt as the experimental unit (n = 22 per treatment). The model included type of gilt (EG or IG) and diet (control, high energy, or low CP and AA) as main effects. Interaction (type of gilt × diet) was removed from the final models because it was nonsignificant ($p > 0.05$). Additionally, slaughter weight was included as a covariate in parameters for which it was significant ($p < 0.05$).

Tukey test was used to analyze the differences between least square means for all parameters studied.

Normality of the residuals was checked with Shapiro–Wilk test using the UNIVARIATE procedure. In cases in which normality was not achieved, variables were transformed with $\sqrt{x}$ or Napierian logarithm or x^3 prior to statistical analysis. In these cases, the results are presented as back-transformed least square means with 95% confidence intervals within parentheses. A p-value < 0.05 was considered a significant difference and a p-value between 0.05 and 0.10 a tendency.

3. Results and Discussion

Except for serum triglyceride concentration, the rest of the results are presented as main effects, since no significant interactions were detected.

3.1. Growth Performance

Table 2 shows the effect of immunocastration and diet on growth performance of heavy gilts. From the first to the second dose of immunization against GnRF, no effect

(p = 0.222) was observed in ADG, confirming the findings of a great number of works in this field [6,16,17]. This was expected because the first dose only primes the immune system of the pig [18]. From the second dose of immunocastration to the time of slaughter (coinciding with the overall experimental diet period), IG ate more feed (p = 0.006) and grew faster (p = 0.002) than EG, with no difference in G:F (p = 0.292). This is in agreement with the results of other authors [6,19]. These effects of immunocastration were greater in the finisher phase (from approximately 100 kg BW to the slaughter) by 8% in ADFI (p = 0.0005) and by 11% in ADG (p = 0.001). In the grower period, the differences were in the same direction but lower; in ADFI it was only a trend (p = 0.098) and in ADG it was only numerical (p = 0.175). Daza et al. [17] detected higher ADG in IG just after the second injection, and the reason could be that the second dose was applied earlier in their trial. The higher voluntary appetite detected in IG in the current experiment could be explained by a quieter behavior, although this was not evaluated. In male pigs, it has been seen that immunocastration reduces aggressive and sexual behaviors after the second dose [20], which could increase visits to the feeder in group-housed pigs, leading to an increase in feed intake [21]. The lack of effect found in the current trial on feed efficiency was not reported by some other authors. From the second dose to time of slaughter, Bohrer et al. [16] detected that immunocastration improved G:F, and Gómez-Fernández et al. [22] observed the opposite effect. The different responses reported in literature about the impact of immunocastration on growth performance might be attributed to the different genetic used, age, and weight of gilts when the immunization doses were applied and time elapsed between the second dose and the slaughter. For the overall trial period (from 40 kg BW to slaughter), IG showed greater (p = 0.0007) ADG than EG, and as a consequence, IG needed 7.4 days less (p = 0.005) on the farm to achieve the slaughter weight target, representing a great advantage for pig farmers.

Table 2. Effect of immunocastration and diet on growth performance (least square means) of heavy gilts.

Item [1]	Type of Gilt [2]		SEM [3] (n = 12)	Diet [4]			SEM [3] (n = 8)	p-Value [5]	
	EG	IG		Control	High Energy	Low CP and AA		Gilt	Diet
Body weight, kg									
Initial	40.1	40.5	0.09	40.5	40.2	40.1	0.11	0.004	0.066
First dose	57.0	58.0	0.34	57.7	58.1	56.7	0.41	0.044	0.067
Second dose [6]	75.3	77.1	0.43	76.2	76.4	75.9	0.53	0.011	0.762
Start finisher period	100.8	103.6	0.74	102.8	102.2	101.7	0.91	0.016	0.680
Day before slaughter	134.0	133.6	1.03	131.9	135.1	134.4	1.26	0.786	0.206
ADG Initial–1st dose, kg/day	0.940	0.974	0.0180	0.954	0.997	0.919	0.0220	0.193	0.068
ADG 1st–2nd dose [7], kg/d	0.924 (0.878–0.966)	0.960 (0.917–0.999)	-	0.929 (0.872–0.979)	0.927 (0.870–0.978)	0.970 (0.918–1.016)	-	0.222	0.392
Grower period [8]									
ADG [7], kg/d	0.911 (0.871–0.952)	0.950 (0.909–0.991)	-	0.950 (0.900–1.001)	0.919 (0.870–0.970)	0.921 (0.872–0.972)	-	0.175	0.599
ADFI, kg/d	2.80	2.92	0.051	2.90	2.82	2.86	0.062	0.098	0.635
G:F	0.328	0.324	0.0057	0.328	0.328	0.323	0.0070	0.686	0.846
Finisher period [9]									
ADG [7], kg/d	0.785 (0.754–0.817)	0.872 (0.839–0.906)	-	0.764 [b] (0.726–0.802)	0.860 [a] (0.820–0.902)	0.861 [a] (0.821–0.903)	-	0.001	0.002
ADFI, kg/d	2.90	3.13	0.037	2.99 [ab]	2.94 [b]	3.12 [a]	0.046	0.0005	0.035
G:F	0.271	0.278	0.0059	0.255 [b]	0.294 [a]	0.275 [ab]	0.0072	0.378	0.005
Overall diet period [10]									
ADG, kg/d	0.837	0.906	0.0138	0.843	0.885	0.886	0.0169	0.002	0.153
ADFI, kg/d	2.86	3.04	0.039	2.95	2.88	3.02	0.047	0.006	0.153
G:F	0.293	0.300	0.0043	0.286 [b]	0.308 [a]	0.296 [ab]	0.0053	0.292	0.037
Overall trial period [11]									
ADG, kg/d	0.869	0.927	0.0100	0.879	0.910	0.905	0.0123	0.0007	0.185
Length, d	108.6	101.2	1.63	104.6	105.0	105.0	2.00	0.005	0.988

[1] ADG: average daily gain; ADFI: average daily feed intake; G:F: gain-to-feed ratio. [2] EG: entire gilt; IG: immunocastrated gilt. [3] SEM: standard error of the mean. [4] Grower period: control (2.33 Mcal net energy (NE)/kg, 16% crude protein (CP) and 0.77% standardized ileal digestible (SID) lysine (Lys)); high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys); and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [5] No significant interactions (type of gilt × diet) were found ($p > 0.05$). [6] Start of the grower period. [7] Statistical evaluation was carried out with data after their transformation. Data are presented as back-transformed least square means with 95% confidence intervals within parentheses. [8] From the second dose to approximately 100 kg. [9] From approximately 100 kg to the day before slaughter. [10] From the second dose to slaughter or when the experimental diets were tested. [11] From approximately 40 kg to the day before slaughter. Within a row, means without a common superscript ([a,b]) differ ($p < 0.05$).

Regarding feeding, no significant differences ($p > 0.05$) between diets were detected in any parameter during the grower period (from approximately 75 to 100 kg BW). The lack of effect of increasing dietary energy while maintaining CP and AA contents agrees with the work of Knowles et al. [23] in similar pigs. Additionally, the lack of influence of decreasing dietary CP and AA levels confirms the results of Pires et al. [24], but disagrees with other authors who detected higher ADFI and worse G:F with restricted diets [23,25] in that range of BWs. These discrepancies could be mainly explained by the different intensities in the decrease in the CP and AA levels tested, being less pronounced in our case. During the finisher period (from approximately 100 to 135 kg BW), gilts fed the diet with high energy level and that with low CP and AA contents grew faster ($p = 0.002$) than those fed the control diet. Pigs that ate the high-energy diet showed lower ($p = 0.035$) ADFI than those that ate the low-CP and -AA diet, with those fed the control diet being in an intermediate position. Thus, gilts fed the high-energy diet presented greater ($p = 0.005$) G:F than those fed the control diet, with those that ate the low-CP and -AA diet being in an intermediate position. With respect to increasing dietary energy level, the G:F result agrees with Suarez-Belloch et al. [7], but the reasons are different. In the case of the latter report [7], the result was due to a lower feed intake with similar daily BW gains, justifying the idea that growing pigs adjust their feed consumption to maintain their voluntary energy intake constant under a wide range of dietary energy concentrations [26]. In the current trial, it was because of a higher ADG with similar ADFI. Our hypothesis is that in pigs above 100 kg BW with great capacity of the digestive tract, and especially under commercial conditions, energy intake is probably below the potential for maximum energy intake and, therefore, an increase in the energy of the diet would not decrease feed intake, thus increasing growth rate, according to the work of De la Llata et al. [27]. Regarding the decrease of dietary protein and AA level during the finisher period, the results obtained were not expected. There is a considerable unanimity in the literature about the lower ADFI when CP and/or AA are restricted, accompanied with worse ADG and feed efficiency [28,29]. However, in the current experiment, the response was a higher ADG, similar to that presented by pigs fed the high-energy diet, and similar ADFI and G:F to control pigs. We do not have an explanation for these results; maybe the restriction in AA levels was very limited.

Finally, evaluating the overall period during which experimental feeds were provided (approximately from 75 to 135 kg BW), only G:F was significantly ($p = 0.037$) different among dietary groups; gilts fed the high-energy diet had greater G:F than those fed the control diet, with animals fed the low-CP and -AA diet in an intermediate position. Therefore, the effects of diet detected in the finisher period were mitigated in the global period because of the lack of impact during the grower phase. These results imply that increasing the dietary energy in gilts destined for Teruel ham from 76 to 134 kg BW could compensate pig farmers, depending on the price of fat sources. Additionally, in this period and in these types of gilts, the application of a diet with low CP and AA contents would reduce feeding cost, because this diet is cheaper, without penalizing growth performance, as well as contributing to reduced nitrogen losses to the environment [30]. When the period of administration of the experimental diets was broader (around 30–115 kg BW), several authors [31–33] found no effect of increase dietary energy on ADG and ADFI. Regarding feed efficiency, Marçal et al. [32] obtained similar results as ours, but other authors [31,33] did not observe an impact of increased energy level. In relation to the dietary low CP and AA, when the diet supplementation period was broader (around 20–120 kg BW), several studies [34–36] have reported no effect of restricted diets in growth performance, as in the current trial. However, it has to be considered that worse feed conversion ratio and slower growth has been shown by others [37,38]. On the other hand, Schiavon et al. [39] found that pigs fed at low CP and AA levels from 86 to 145 kg BW grew faster and ate more feed than those fed at high CP and AA levels, because many of the pigs restrictively fed the low-CP and -AA diet were forced to consume more feed. These authors [39] suggested that an animal will eat sufficiently to satisfy its genetic requirements for nutrients, even though

some factors (diet, climate, disease, or housing) may cause it to either increase or decrease feed intake from its potential.

3.2. Serum Metabolites

As can be seen in Table 3, immunocastration had no effect ($p > 0.05$) on concentration of serum metabolites (data of triglycerides not shown). Van den Broeke et al. [19], injecting the second GnRF vaccination at a heavier BW (at 105 kg BW), did observe at slaughter time that gilt immunocastration increased serum urea, and their justification was the higher daily protein intake. In the current trial, IG also showed higher urea content than EG, but the difference was only numerical (nonsignificant).

Table 3. Impact of immunocastration and diet on serum metabolites (least square means) of gilts.

Item	Albumin, g/dL	Urea, mg/dL	Cholesterol, mg/dL
Type of gilt			
Entire	3.34	26.3	73.8
Immunocastrated	3.19	28.7	65.9
SEM [1] (n = 24)	0.107	1.49	3.04
Diet [2]			
Control	3.09	30.3	68.6
High energy	3.41	26.0	69.8
Low CP and AA	3.28	26.2	71.1
SEM [1] (n = 16)	0.131	1.82	3.72
Sampling time			
At the end of the grower period	2.89	25.7	63.2
At the end of the finisher period	3.63	29.3	76.4
SEM [1] (n = 24)	0.100	1.22	2.87
p-value [3]			
Type of gilt	0.339	0.274	0.087
Diet	0.265	0.187	0.877
Sampling time	0.0001	0.011	0.012

[1] SEM: standard error of the mean. [2] Grower period: control (2.33 Mcal net energy (NE)/kg, 16% crude protein (CP) and 0.77% standardized ileal digestible (SID) lysine (Lys)); high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys); and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [3] No significant interactions (type of gilt × diet, type of gilt × sampling time, diet × sampling time, type of gilt × diet × sampling time) were found ($p > 0.05$).

No influence ($p > 0.10$) of experimental diets was observed on serum albumin, urea, and cholesterol contents. These results are in agreement with previous reports [23,33,40] in which diets with different energy and similar CP and AA contents were compared. On the other hand, when a greater and earlier restriction of CP and AA was practiced, Mule et al. [41], Ruusunen et al. [42], and Suárez-Belloch et al. [43] detected lower albumin level, and Chiba et al. [44], Fabian et al. [45], and Kerr et al. [46] observed lower serum urea concentration. The authors explained these results; the effect on albumin was because the limitations in the availability of AA first appear in the synthesis of exported proteins [42], and the effect on urea was due to a lower nitrogen intake [39], implying that pigs fed low levels of CP and AA use nitrogen more efficiently for growth [47]. Therefore, the results of the current trial about serum albumin and urea confirm the previous idea of a limited CP and AA restriction. Suárez-Belloch et al. [43] found an increase in cholesterol concentration associated with limited CP and AA in diets, indicating a possible hypercholesterolemic effect in those animals [41]. Additionally, it is worth noting that as pigs grew older, they showed greater concentrations of albumin, urea, and cholesterol ($p = 0.0001$, $p = 0.011$, and $p = 0.012$, respectively).

A significant interaction ($p = 0.031$) between diet and sampling time on serum triglyceride concentration is shown in Figure 1. At the end of the grower period, gilts fed the high-energy diet showed higher triglyceride levels than those in the other two groups. However, at the end of the finisher period, that effect was mitigated and no difference was

found among the three experimental diets, confirming the lack of significance detected on carcass fatness. Kim et al. [40], in lighter pigs, did not observe differences between diets with different energy contents. As in the current trial, Suárez-Belloch et al. [29] also found no influence of CP and AA restriction on triglyceride levels. However, in other report, Suárez-Belloch et al. [43] detected that the reduction of CP and AA contents promoted a linear increase of triglycerides at the end of the grower phase.

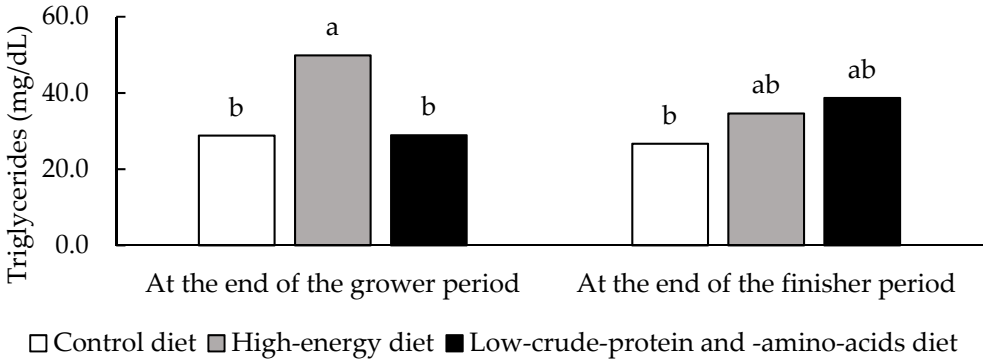

Figure 1. Significant interaction ($p = 0.031$) between diet and sampling time on serum triglyceride concentration of heavy gilts. The average body weight of gilts at the end of the grower period was 102 kg and at the end of the finisher period was 134 kg. Statistical evaluation was carried out with data after their transformation. Data are presented as back-transformed least square means. Different letters (a,b) denote significant differences between treatments ($n = 8$) ($p < 0.05$).

3.3. Serum Sex Hormones

All gilts, both EG and IG, presented basal serum progesterone concentrations (<0.400 ng/mL) when the doses of immunocastration were applied. The day before slaughter, 17% of EG had reached puberty, because these EG showed high levels of progesterone (32.2 and 31.4 ng/mL), while all IG continued to present low levels (<0.600 ng/mL) (data not shown). With the same type of white-breed gilts, Mitjana et al. [48] did not detect differences in this parameter at slaughter between EG and IG, but it has to be noted that the gilts in that study were younger (approximately 125 kg BW). However, with Chinese and Iberian gilts, other authors did find significantly higher concentrations of progesterone in EG than in IG on the preslaughter day [49] or even some months before slaughter [50,51]. The greater effect observed with Chinese and Iberian gilts could be because these breeds reach puberty earlier [52]. Van den Broeke et al. [19] also reported differences in progesterone level in white-breed gilts from just before the second vaccination, but the immunocastration doses were applied later (at 70 and 105 kg BW) than in our trial and in Mitjana's [48] experiment. Therefore, the gilts of Van den Broeke et al. [19] were more sexually developed when they were immunized against GnRF, and thus the difference between EG and IG was expected to be greater.

Data about serum estradiol concentration are shown in Table 4. No differences ($p = 0.795$) between EG and IG were detected in estradiol levels, corroborating the findings of Van den Broeke et al. [19], Mitjana et al. [48], and Pérez-Ciria et al. [10], and no significant ($p = 0.787$) interaction type of gilt × sampling time was found. Additionally, estradiol concentration increased in serum as gilts grew older ($p = 0.0005$), in agreement with Pérez-Ciria et al. [10].

Table 4. Effect of immunocastration on serum estradiol concentration (least square means) of heavy gilts.

Item	Estradiol, pg/mL
Type of gilt	
Entire	27.6
Immunocastrated	26.7
SEM [1] (n = 36)	2.39
Sampling time	
At first dose of immunocastration	22.0 [b]
At second dose of immunocastration	27.4 [ab]
Day before slaughter	32.1 [a]
SEM [1] (n = 24)	2.17
p-value	
Type of gilt	0.795
Sampling time	0.0005
Type of gilt × sampling time	0.787

[1] SEM: standard error of the mean. Within a column, means without a common superscript ([a,b]) differ ($p < 0.05$).

3.4. Reproductive Organs

The IG presented lighter (p = 0.015) reproductive tracts than EG, since most organs were lighter (p = 0.004 for uterine horns, p = 0.010 for uterine corpus, p = 0.0001 for cervix, and p = 0.024 for vagina) (Table 5). Additionally, ovaries tended to be smaller (p = 0.065) and uterine horns (p = 0.004), uterine corpus (p = 0.022), cervix (p = 0.005), and vulva (p = 0.021) were shorter in IG than in EG. All of these results indicate that immunization against GnRF inhibited the development of reproductive organs, confirming the results of Hernández-García et al. [50], Dalmau et al. [51], and Mitjana et al. [48].

Table 5. Impact of immunocastration on reproductive organs (least square means) of heavy gilts.

Trait	Type of Gilt		SEM [1] (n = 12)	p-Value
	Entire	Immunocastrated		
Ovaries				
Weight, g	5.85	4.60	0.922	0.354
Size, cm^3	10.76	7.34	1.213	0.065
Uterine horns [2]				
Weight, g	106.6 (77.6–140.2)	49.2 (31.1–71.4)	-	0.004
Length, cm	65.6 (57.2–74.7)	47.8 (41.0–55.1)	-	0.004
Uterine corpus [2]				
Weight, g	5.30 (3.46–7.89)	2.24 (1.29–3.57)	-	0.010
Length, cm	4.10 (3.21–5.22)	2.72 (2.14–3.47)	-	0.022
Cervix				
Weight, g	62.3	29.5	5.03	0.0001
Length, cm	16.0	13.5	0.55	0.005
Vagina				
Weight, g	31.0	21.2	2.87	0.024
Length, cm	10.44	9.38	0.589	0.214
Vestibule length, cm	13.1	12.5	0.31	0.166
Vulva length, cm	3.93	3.15	0.217	0.021
Total reproductive tract weight, g	186.8	117.1	17.80	0.015

[1] SEM: standard error of the mean. [2] Statistical evaluation was carried out with data after their transformation. Data are presented as back-transformed least square means with 95% confidence intervals within parentheses.

As can be seen in Table 6, no difference was detected (p = 0.324) between EG and IG in the total number of ovarian follicles; however, ovaries of IG showed lower (p = 0.034) numbers of big follicles (>6 mm) than those of EG. Additionally, the IG group presented a lower proportion of gilts with intermediate and big follicles than the EG group (p = 0.01 and p = 0.037, respectively). These results indicate that ovarian activity was less intense in IG, confirming the previous results of Pérez-Ciria et al. [10]. Hernández-García et al. [50] detected greater differences between EG and IG in terms of follicular development, because they did not find any visible follicle in the case of IG, and EG presented 3–11 mm follicles

and corpora lutea. On the other hand, Dalmau et al. [51], Xue et al. [49], and Mitjana et al. [48] did find that some IG showed follicles as in the present experiment, although the differences in follicular size between EG and IG were more marked.

Table 6. Effect of immunocastration on ovarian follicles of heavy gilts [1,2].

Trait	Type of Gilt		*p*-Value
	Entire	Immunocastrated	
Number of follicles			
<2 mm	5.73 (1.25–26.32)	11.60 (2.37–56.82)	0.535
2–4 mm	24.4 (11.9–49.9)	35.8 (16.9–75.6)	0.469
4–6 mm	10.91 (4.68–25.44)	7.50 (3.06–18.39)	0.556
>6 mm	2.00 (0.33–11.94)	0 (0–0)	0.034
Total	43.0 (30.8–60.0)	54.9 (38.8–77.6)	0.324
Gilts with follicles, %			
<2 mm	45.5 (20.3–73.2)	40.0 (15.8–70.3)	0.801
2–4 mm	90.9 (56.1–98.7)	80.0 (45.9–95.0)	0.473
4–6 mm	90.9 (56.1–98.7)	40.0 (15.8–70.3)	0.010
>6 mm	27.3 (9.0–58.6)	0 (0–0)	0.037

[1] Data are presented as back-transformed least square means with 95% confidence intervals within parentheses. [2] $n = 12$.

Overall, immunocastration was an effective method of preventing puberty, although the effects were more evident in the anatomical development of the reproductive tract than in the sex hormone levels. However, it should be noted that three gilts belonging to the IG group were in the phases of estrus or diestrus at slaughter; these animals were removed from the data analysis and description of serum sex hormones, reproductive organs, and ovarian follicles. The reasons for this are unknown, but it has been also observed in other works. It could be due to the reproductive organs having been taken around 11 weeks after the second dose of immunocastration. Claus et al. [53] observed in male pigs that 10 weeks after the second injection, antibody titers to GnRF were almost as low as before the second dose; this may indicate that some IG could have reverted. As in the current trial, Bohrer et al. [16], who administered the second injection of immunocastration 10 weeks before slaughter, found that some IG reached puberty, while Rodrigues et al. [6], who applied the second dose 6 weeks preslaughter, did not observe that any IG showed signs of estrus. It also has to be considered that since gilts were loose in the pen when the doses of immunocastration were applied, some doses might not have been correctly injected. However, the most plausible explanation is that those gilts did not react to immunocastration doses, and, as Zeng et al. [54] observed, might develop lower GnRF antibody titers than the other IG. The reason for the lower antibody production is not clear.

3.5. Carcass Quality

Table 7 shows that no effect ($p = 0.998$) of immunocastration on carcass weight was observed, because the slaughter was at a target BW (close to 135 kg). Carcass yield was also not influenced ($p = 0.851$) by immunization against GnRF, in agreement with previous reports [6,17,19]. However, fat thickness at the GM was greater ($p = 0.011$) in IG, corroborating the findings of a great number of studies in this field [10,17,55]. This result may lead to a decrease in rejected carcasses intended for Teruel dry-cured ham production and optimize the dry-curing process of the pieces [56]. Hams of IG were similar in length ($p = 0.144$) but narrower ($p = 0.019$ for ham perimeter) than those of EG. Immunocastration resulted in a reduction ($p = 0.034$) in the weight of the main pieces, especially the shoulder ($p = 0.012$), but this was not reflected when they were expressed as percentage of carcass ($p > 0.05$). This result agrees with the results of Daza et al. [57], who did not detect differences in these variables. However, Pérez-Ciria et al. [10] observed that IG had lower total yield (ham + shoulder) than EG. The discrepancies in the weights or

yields of trimmed cuts between studies may be due to the slaughter criterion (fixed BW or age).

Table 7. Impact of immunocastration and diet on carcass quality (least square means) of heavy gilts.

Trait	Type of Gilt [1]		SEM [2] (n = 66)	Diet [3]			SEM [2] (n = 44)	p-Value [4]	
	EG	IG		Control	High Energy	Low CP and AA		Gilt	Diet
Slaughter weight, kg	134.3	133.7	1.22	131.9	135.9	134.2	1.49	0.711	0.161
Carcass weight, kg	104.6	104.6	0.93	105.4	103.6	104.9	1.13	0.998	0.509
Carcass yield, %	77.7	77.9	0.69	79.0	76.7	77.8	0.84	0.851	0.137
Fatness at the GM [5,6], mm	21.2	23.7	0.66	21.9	22.8	22.6	0.81	0.011	0.698
Ham length [6], cm	40.1	39.8	0.14	39.9	40.1	39.9	0.18	0.144	0.531
Ham perimeter [6], cm	78.2	77.5	0.22	78.1	77.9	77.5	0.27	0.019	0.299
Trimmed cut weight [6], kg									
Ham	13.5	13.2	0.08	13.4	13.5	13.2	0.10	0.087	0.119
Shoulder	8.79	8.63	0.043	8.80	8.71	8.62	0.053	0.012	0.060
Total [7]	22.2	21.9	0.12	22.2	22.2	21.8	0.14	0.034	0.062
Trimmed cut yield [6], % carcass									
Ham	12.9	12.7	0.14	12.8	13.0	12.6	0.17	0.354	0.211
Shoulder	8.48	8.27	0.083	8.38	8.49	8.26	0.102	0.087	0.278
Total [7]	21.4	21.0	0.22	21.1	21.5	20.8	0.27	0.172	0.187

[1] EG: entire gilt: IG: immunocastrated gilt. [2] SEM: standard error of the mean. [3] Grower period: control (2.33 Mcal net energy (NE)/kg, 16% crude protein (CP) and 0.77% standardized ileal digestible (SID) lysine (Lys)); high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys); and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [4] No significant interactions (type of gilt × diet) were found ($p > 0.05$). [5] GM: gluteus medius muscle. [6] From the left side. [7] Ham + shoulder.

Regarding experimental diets, limited effects on carcass quality were found. The weight and yield of carcasses were not affected ($p > 0.05$) by feeding, probably because of the similar slaughter weight, which confirmed other reports comparing different energy contents or CP and AA levels [7,35,58]. Similarly, diets did not influence ($p > 0.05$) ham size. In terms of carcass fatness, although a thicker fat depth at the GM was expected in animals fed the tested diets, this was not observed ($p = 0.698$). It is worth noting that those animals fed the high-energy diet or the low-CP and -AA diet had 1 mm thicker fat thickness than those fed the control diet, but this result was nonsignificant. Other authors did find differences (Suarez-Belloch et al. [7] increasing in 140 kcal above 2280 kcal/kg; and Sirtori et al. [59] restricting CP and AA contents). The hypothesis being that those nutritional strategies generate an excess of energy/Lys ingested which is then transformed into fat [60,61]. The lack of effect in the present study might have been due to the high variability of data, the shorter experimental time, or the fact that the nutrient levels tested were more prudent. Feeding also had limited effect on the weight of main pieces; only shoulder tended to be lighter ($p = 0.060$) with the diet low in CP and AA than with the other diets. Ruiz-Ascacibar et al. [62] found a similar effect and Suárez-Belloch et al. [29] only observed this effect numerically. No impact ($p > 0.10$) of dietary treatments on ham and shoulder yields was detected.

4. Conclusions

Under our experimental conditions, it can be concluded that immunization against GnRF is an interesting strategy to apply in gilts intended for the PDO Teruel ham, because it improves animal growth rate, decreases the number of fattening days, and increases fat thickness at the GM, which is a very desirable aspect for the dry-curing process. On the other hand, in gilts from 76 to 134 kg BW, a rise in dietary energy by 0.15 Mcal of net energy/kg or a drop in dietary CP by 2 percentage points and in AA do not improve carcass fatness, but could be beneficial for pig farmers whether the gilts are entire or immunocastrated; the first dietary strategy improves feed efficiency and the second one does not impair growth performance.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11071900/s1, Table S1: Ingredients of the experimental diets (%, as fed-basis).

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Institutional Review Board Statement: The study was conducted according to the Spanish Policy for Animal Protection [9] and approved by the Ethical Committee of the University of Zaragoza (reference PI29/18; approval date: 27 June 2018).

Data Availability Statement: Data available on request due to restrictions of privacy.

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Effect of Immunocastration and Diet on Growth Performance, Serum Metabolites and Sex Hormones, Reproductive Organs Development and Carcass Quality of Heavy Gilts

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Table S1. Ingredients of the experimental diets [1] (%, as-fed basis).

	Grower diet (76 to 102 kg of body weight)			Finisher diet (102 to 134 kg of body weight)		
Ingredient	**Control**	**High energy**	**Low CP and AA**	**Control**	**High energy**	**Low CP and AA**
Corn	35.0	33.9	35.0	35.0	32.5	35.0
Wheat	18.0	18.0	18.4	17.0	18.0	18.1
Barley	17.6	15.0	21.0	21.1	21.8	25.0
Oat	9.00	8.72	11.0	11.0	8.00	12.0
Soybean meal 47% CP	17.8	18.7	11.9	13.6	14.4	7.69
Palm oil	0.53	3.65	0.34	0.36	3.36	0.08
Calcium carbonate	0.79	0.78	0.80	0.85	0.85	0.86
Sodium chloride	0.45	0.45	0.45	0.45	0.45	0.45
Monocalcium phospate	0.26	0.27	0.31	0.13	0.13	0.18
L-Lysine 50%	0.23	0.21	0.30	0.14	0.12	0.23
L-Threonine	0.02	0.02	0.02	-	-	0.01
DL-Methionine	0.02	0.02	0.01	-	-	-
L-Tryptophan	-	-	-	0.01	0.01	0.01
Premix [2]	0.40	0.40	0.40	0.40	0.40	0.40

[1] Grower diet: control (2.33 Mcal net energy (NE)/kg, 16% crude protein (CP) and 0.77% standardized ileal digestible (SID) lysine (Lys)); high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys); and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher diet: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys); and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [2] Provided the following per kilogram of complete diet: 6.5 IU Vitamin A; 1.5 IU Vitamin D3; 15 mg α-tocopherol; 3 mg Vitamin B2; 1 mg Vitamin B6; 0.02 mg Vitamin B12; 15 mg nicotinic acid; 8 mg pantothenic acid; 100 mg choline chloride; 100 mg Zn (ZnO); 50 mg Mn (MnO); 250 mg Fe ($FeCO_3$); 10 mg Cu ($CuSO_4 \cdot 5H_2O$); 0.2 mg Se (Na_2O_3Se); 2 mg BHT; 1 mg I (KI); 500 FYT 6-phytase.

Paper 3. Effect of castration type and diet on growth performance, serum sex hormones and metabolites, and carcass quality of male pigs

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Effect of Castration Type and Diet on Growth Performance, Serum Sex Hormones and Metabolites, and Carcass Quality of Heavy Male Pigs

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Simple Summary: Castration is indispensable in male pigs intended for the production of high-quality dry-cured ham; however, for welfare reasons, alternatives are being sought and, among them, immunocastration stands out. The literature shows that immunocastration might generate lower fat deposition than surgical castration, which is undesirable for the dry-curing process. Therefore, it would be interesting to confirm the impact of immunocastration in male pigs and to test feeding plans that benefit fatness. The aim of this trial was to assess the effect of type of castration (surgical vs. immunological) and diet (control vs. high energy vs. low crude protein and amino acids) on growth performance, serum sex hormones and metabolites, and carcass quality of male pigs destined for the Protected Designation of Origin Teruel ham. Although immunocastration improved productive performance, it reduced carcass fatness. Also, alternative diets to control improved the feed conversion ratio but did not influence carcass quality traits. In conclusion, under a quality point of view, surgical castration would be preferable over immunocastration for dry-cured ham production, and the use of moderately high-energy or low-crude-protein and -amino-acids diets from 80 to 137 kg of body weight would not provide improvements.

Abstract: A trial was carried out to study the effect of type of castration and diet on pigs destined for Teruel ham production, which is a Spanish protected designation of origin for dry-cured ham. A total of 144 Duroc × (Landrace × Large White) male pigs were used. Half of them were surgically castrated and the other half were immunocastrated with three doses at approximately 25, 58 and 79 kg of body weight. Furthermore, three diets (control vs. high energy vs. low crude protein-CP- and amino acids-AA) were tested from 80 to 137 kg of body weight. Growth performance, serum sex hormones and metabolites, and carcass quality were evaluated. Immunocastrated males grew faster and had better feed conversion ratio than surgically castrated males, but presented lower carcass fatness. Pigs fed the high-energy diet and the low-CP and -AA diet were more efficient at transforming feed into gain than those fed the control diet, but no effect was detected on carcass quality. In conclusion, surgically castrated males are preferable than immunocastrated males for Teruel dry-cured ham elaboration. Besides, a high-energy diet or a low-CP and -AA diet might improve productive performances, but does not provide any benefit in terms of carcass quality.

Keywords: male pigs; immunocastration; dietary energy; dietary protein; growth performance; sex hormones; serum metabolites; carcass quality

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1. Introduction

The protected designation of origin (PDO) "Teruel ham" is a Spanish PDO for the production of high quality dry-cured hams from crossbred pigs (not autochthonous). To guarantee their quality, the Consortium requires, among other aspects [1], pigs slaughtered with heavy body weight (BW) (at least 86 kg of carcass weight) and fatty (fat depth measured over the gluteus medius muscle (GM) greater than 16 mm). Then, animals reach sexual maturity and, in the case of males, the castration becomes essential to avoid boar taint in the pork and, in fact, male castration is other requirement of this PDO. Traditionally, it is performed by surgical methods but, for welfare reasons, alternatives are sought, among which is immunocastration. This practice usually consists in the application of two doses of a gonadotrophin releasing factor (GnRF) analogue protein conjugate. The first dose only primes the pig's immune system, and the second one stimulates the production of a large quantity of antibodies against GnRF, neutralizing the pig's endogenous GnRF and reducing testicular function [2]. The consequences of immunocastration in males on body fat retention are not clear, but some studies [3–5] show that it might generate leaner carcasses than surgical castration. It would be a handicap for the elaboration of Teruel ham, taking into account that a considerable fat thickness is required to avoid an excessive curing of the pieces [6]. However, no sufficiently robust experiment to assess immunocastration in males destined to that type of dry-cured ham has been developed. For this reason, it would be interesting to study the impact of immunocastration of these males and the feeding plans for them that could benefit body fat retention. In this regard, the increase of the energy content [7,8] and the decrease of the crude protein (CP) and amino acids (AA) levels [9,10] in the diet have been related to increases of body fat accretion. Therefore, the aim of the current trial was to evaluate the effect of the type of castration (surgical vs. immunological) and the feeding provided (control diet vs. high-energy diet vs. low-CP and -AA diet) on growth performance, serum sex hormones and metabolites, and carcass quality of male pigs destined for Teruel dry-cured ham elaboration.

2. Materials and Methods

2.1. Animals Husbandry and Experimental Design

A total of 144 Duroc × (Landrace × Large White) male pigs were used. Half of them were surgically castrated during the first week of life. The other half received the first dose for immunocastration (Improvac®, Zoetis Belgium SA, Louvain-la-Neuve, Belgium) at the end of the post-weaning period, with 56 ± 3 days of age (around 25 kg BW), upon request of the PDO Teruel ham Consortium. At the age of 78 ± 3 days, pigs were moved to the fattening farm (Teruel, Spain), individually identified with ear tags, weighed (35.3 ± 4.10 kg BW) and group-penned (eight pigs/pen; space allowance 1.1 m^2/animal). Each pen had a 50% slatted floor, a grow feeder, and a drinking cup. Animals were allotted to blocks of increasing BW and each block contained all treatments. A second dose of Improvac® at 57.7 ± 5.60 kg BW (101 ± 3 days of age) and a third dose at 79.2 ± 7.20 kg BW (122 ± 3 days of age) to ensure the effect of this product were injected to the pigs previously vaccinated.

Three experimental diets were provided to both surgically castrated males (SCM) and immunocastrated males (IM) during the grower and finisher periods. The grower phase lasted from 122 to 149 ± 3 days of age (around 80–110 kg BW) and the finisher phase lasted from 150 ± 3 days of age to the day before slaughter (around 110–137 kg BW). The tested diets were the same as those used in a previous study about immunocastration in gilts [11]; (i) a control diet with a nutritional profile similar to the recommendations of FEDNA [12]; (ii) a diet with a higher energy content than the control diet, with similar CP and AA percentages; and (iii) a diet with lower CP and AA contents than the control diet, with the same energy level. In all experimental feeds, the ideal protein concept was maintained [12] and the change between the grower and the finisher feeds was carried out on a fixed day. Table 1 shows the analyzed nutrient composition of the diets. More information about them (ingredients, estimated nutritional value and a more complete description of the analyzed nutrients) as well as the methods used to analyze the chemical

composition are reported in Pérez-Ciria et al. [11]. Feeding in pellet form and water were provided ad libitum throughout the trial. Therefore, there were six experimental treatments: two types of castration (surgical vs. immunological) × three diets (control vs. high energy vs. low CP and AA).

Table 1. Analyzed nutrient composition of the experimental diets (%, as-fed basis).

Item	Grower Diet (80 to 110 kg BW)			Finisher Diet (110 to 137 kg BW)		
	Control	High Energy	Low CP-AA [1]	Control	High Energy	Low CP-AA [1]
Gross energy, Mcal/kg	3.99	4.12	3.92	3.91	4.12	3.95
Crude protein	16.2	15.9	14.4	14.5	15.1	12.7
Ether extract	3.55	5.88	3.44	3.00	5.65	3.73
Neutral detergent fiber	10.9	10.2	10.5	10.5	8.96	10.2
Total lysine	0.98	0.98	0.79	0.76	0.77	0.71
Total methionine	0.28	0.27	0.25	0.24	0.25	0.23
Total threonine	0.62	0.60	0.59	0.56	0.58	0.51

BW: body weight. [1] low in crude protein and amino acids.

2.2. Performance Measurements

Individual BW was measured at several times: on the arrival at the fattening farm (22 days after the first dose of Improvac®), when the second dose was administered, when the third dose was applied (coinciding with the day in which pigs received the experimental grower diets), the first supply day of the experimental finisher diets, and the day before slaughter. Data of BW were used to calculate average daily gain (ADG) per pen. During the grower and finisher periods, feed consumption per pen was recorded in order to calculate average daily feed intake (ADFI). Average daily gain and ADFI were used to estimate the feed conversion ratio (FCR).

2.3. Blood Sampling and Analyses

Ten milliliters of blood from one representative pig per pen, always the same and close to the average BW, were taken around 10–12 a.m. at several times: when the second and third immunocastration doses were injected, at the end of the grower period and at the end of the finisher period (coinciding with the previous day of slaughter). More information about blood sampling and processing until its analysis is described in Pérez-Ciria et al. [11].

In the case of sex hormones, the concentrations of testosterone and estradiol were determined in the samples taken in the days of application of the second and third immunization doses and also the day before slaughter using competitive immunoassays with enzyme-labeled chemiluminescent technology (IMMULITE, Siemens Healthineers, Madrid, Spain).

The concentrations of albumin and urea, as protein biomarkers, and triglycerides and cholesterol, as lipid biomarkers, were determined in the samples taken at the end of both the grower and finisher periods. For it, the same methods reported in Pérez-Ciria et al. [11] were followed using the same equipment (GernonStar equipment, RAL S.A., Barcelona, Spain).

2.4. Slaughtering and Carcass Measures

The slaughter BW target was 135–140 kg, and thus loads were programmed in three times; with 178, 185, and 199 ± 3 days of age, achieving 137 kg BW on average. Pigs were slaughtered after a fasting period of 13 h in a commercial abattoir (Teruel, Spain) located 130 km from the farm. In the current trial, a total of 102 animals were chosen at random for the study of carcass quality, 17 from each experimental treatment (type of castration × diet). The following measures were recorded of each pig: hot carcass weight, fat depth over the GM, ham length, ham perimeter and ham and shoulder weights. Later, carcass yield, total (ham + shoulder) weight and ham, shoulder and total yields were calculated. Details about slaughter and measurements taken on the carcass are described in Pérez-Ciria et al. [11].

2.5. Statistical Analyses

Data were analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Growth performance parameters were assessed as a randomized complete block design with treatments arranged factorially (2 × 3) using the GLM procedure. Serum sex hormones and metabolites were analyzed by repeated-measures analysis using the MIXED procedure. The covariance structures chosen were: variance components for testosterone, albumin, urea and cholesterol; heterogeneous autoregressive for estradiol; and compound symmetry for triglycerides. Carcass quality was analyzed as a factorial design (2 × 3) using the GLM procedure. The pig was the experimental unit, except in the case of growth performance parameters, which was the pen. Tukey test was utilized for pairwise comparison of the least square means. More complete information about statistical analyses is described in Pérez-Ciria et al. [11], where the same procedures were used. A p-value < 0.05 was considered as significant and a p-value between 0.05 and 0.10 as a tendency.

3. Results and Discussion

Except for serum testosterone and cholesterol concentrations, the remaining results are presented as main effects, as no significant interactions were found.

3.1. Growth Performance

From the beginning of the trial (22 days post-1st dose) to the second dose of Improvac® (a total of 23 days), the IM tended (p = 0.056) to grow slower than SCM (Table 2). Batorek et al. [13] already reported a similar response in a meta-analysis. In this period, IM are physiologically similar to entire males, since the first dose of immunocastration only primes the pig's immune system [2]. The lower ADG in IM than in SCM might be due to a lower feed intake; it was not recorded in the present trial but it has been observed by other authors [14,15]. This effect has also been found when entire males and SCM were compared [16–18], which could be related to higher levels of testicular hormones in entire males than in SCM [19]. Cronin et al. [20] have reported more aggressive and sexual behaviors in entire males, which would reduce their eating times. Between the second and the third dose of immunization against GnRF (a total of 21 days), the differences in ADG between IM and SCM disappeared (p = 0.828). According to the manufacturer of Improvac®, within one week post-2nd dose, an induction of anti-GnRF antibodies can be expected, and that leads to a reduction of aggressive and sexual behavior around one to two weeks post-2nd vaccination. However, from the third dose to the day before slaughter (a total of 56, 63 or 77 days, depending on the day of slaughter), IM grew faster (p = 0.0004), tended (p = 0.063) to eat more feed, and had lower (p < 0.0001) FCR than SCM. This improvement in growth rate observed in IM in comparison to SCM has been supported by a great deal of reports [13,21,22]. Batorek et al. [23] and Brunius et al. [19] observed that IM exhibited greater serum insulin-like growth factor-1 concentration than SCM at three weeks post-2nd dose and one day prior to slaughter, respectively. Therefore, IM would have a higher anabolic potential than SCM [19], contributing to improved feed efficiency and causing faster growth [23]. In addition, Morales et al. [15] suggested that the extra growth detected in IM after the second vaccination might result from a compensatory response to the reduced growth after the first injection. Regarding the increase of feed intake in IM, it should be noted that the levels of serum testosterone begin to drop after the second dose, which might increase the appetite in these animals. Also, Batorek et al. [23] found that IM presented relatively low amounts of serum leptin compared with SCM at 24 days post-2nd vaccination, and it is known that leptin reduces appetite [24]. For the overall trial (from 22 days post-1st dose to the day before slaughter), IM grew faster (p = 0.007) than SCM, although it did not have any influence (p = 0.337) on the time the pigs stayed on the farm.

Table 2. Impact of type of castration and diet on growth performance (least square means) of heavy male pigs.

Item [1]	Type of Castration		SEM [2] (n = 9)	Diet			SEM [2] (n = 6)	p-Value [4]	
	Surgical	Immunological		Control	High Energy	Low CP-AA [3]		Type of Castration	Diet
Body weight, kg									
Initial [5]	36.0	34.7	0.29	35.1	35.3	35.7	0.35	0.011	0.448
Second dose	59.7	57.6	0.37	58.2	58.9	58.9	0.46	0.002	0.528
Start grower period (third dose)	81.5	79.2	0.83	79.7	80.9	80.5	1.01	0.074	0.697
Start finisher period	109.4	109.6	0.86	110.0	108.7	109.8	1.05	0.886	0.662
Day before slaughter [6]	136.1 (133.1–139.1)	138.4 (135.4–141.5)	-	135.7 (132.1–139.5)	138.1 (134.4–141.9)	137.9 (134.2–141.6)	-	0.262	0.565
ADG Initial-2nd dose, kg/day	1.032	0.994	0.0130	1.007	1.025	1.008	0.0159	0.056	0.679
ADG 2nd-3rd dose, kg/day	1.038	1.030	0.0279	1.023	1.050	1.029	0.0342	0.828	0.838
Grower period [7]									
ADG, kg/day	0.995	1.084	0.0282	1.081	0.991	1.047	0.0346	0.046	0.224
ADFI, kg/day	3.30	3.35	0.061	3.47 [a]	3.17 [b]	3.33 [ab]	0.074	0.578	0.040
FCR	3.32	3.10	0.059	3.22	3.20	3.21	0.072	0.020	0.984
Finisher period [8]									
ADG, kg/day	0.860	1.001	0.0220	0.888	0.950	0.954	0.0269	0.0007	0.194
ADFI, kg/day	3.24	3.53	0.077	3.43	3.27	3.47	0.094	0.020	0.319
FCR	3.78	3.53	0.071	3.87 [a]	3.47 [b]	3.64 [ab]	0.087	0.030	0.022
Overall diets period [9]									
ADG, kg/day	0.927	1.040	0.0164	0.981	0.966	1.003	0.0200	0.0004	0.439
ADFI, kg/day	3.27	3.44	0.058	3.44	3.22	3.40	0.072	0.063	0.100
FCR	3.53	3.31	0.027	3.52 [a]	3.34 [b]	3.39 [b]	0.033	<0.0001	0.007
Overall trial period [10]									
ADG, kg/day	0.974	1.026	0.0115	0.996	0.997	1.008	0.0141	0.007	0.795
Length, day	184.2	182.7	1.10	182.7	185.0	182.7	1.35	0.337	0.397

[1] ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio. [2] SEM: standard error of the mean. [3] low in crude protein and amino acids. [4] No significant interactions (type of castration × diet) were found ($p > 0.05$). [5] Twenty two days post-first dose. [6] After the data had been transformed, statistical analysis was performed on it. Data are presented as back-transformed least square means and 95% confidence intervals within parentheses. [7] From the third dose to around 110 kg BW. [8] From around 110 kg BW to the day before slaughter. [9] From the third dose to the slaughter or when the experimental diets were tested. [10] From the entrance to the fattening farm (22 days post-1st dose) to the day before slaughter. Least square means within a row with different superscript ([a,b]) differ ($p < 0.05$).

For the overall period in which the diets were tested (from around 80 to 137 kg BW), ADG was similar for all groups ($p = 0.439$). There is some unanimity in the literature [8,25,26] about the lack of effect of increasing dietary energy concentration on ADG in heavy pigs. This finding could be explained because the growth rate of pigs fed the high-energy level could be limited by the less lysine intake [27]. However, it is worth noting that, although just numerically, pigs given the high energy diet ate less feed ($p = 0.10$) than the other groups, this being especially observed during the grower period (from around 80 to 110 kg BW; $p = 0.04$). The negative relationship between dietary energy level and feed consumption in pigs has also been found by other authors [8,28], and it is justified because pigs adjust their voluntary feed intake to maintain a constant daily energy intake [29]. Regardless the lack of effect of low-CP and -AA diet on ADG and ADFI in comparison to the control diet, it corroborates the results of Knowles et al. [25], although Suárez-Belloch et al. [30] reported a lower ADG and ADFI when a greater reduction of dietary CP and AA was applied (from 17.2 to 10.6% CP and from 0.77 to 0.42% lysine). Furthermore, the high-energy diet and also the low-CP and -AA diet generated lower ($p = 0.007$) FCR than the control diet, which was more pronounced in the finisher period (from around 110 to 137 kg BW; $p = 0.022$). It confirms the results of a previous trial performed by our team using the same diets in gilts [11]. The better results in feed efficiency obtained with a high energy diet in comparison to a standard (control) diet agree with Suarez-Belloch et al. [8], but disagree with Knowles et al. [25], and it could be due to the different nutrient levels tested, feed ingredients, genetic types, target slaughter BW and/or experimental conditions. The better FCR in animals fed the low-CP and -AA diet vs. the control diet was not expected. Applying a greater CP and AA restriction, Suárez-Belloch et al. [30] obtained the opposite effect. This could indicate that, in the present study, the low-CP and -AA diet did not limit growth, and therefore, the CP and AA contents of the control diet could be overestimated. The results obtained with the experimental diets would imply that the increase in 0.15 Mcal of net energy/kg in the diet, from 80 to 137 kg BW, in barrows intended for Teruel dry-cured ham elaboration, could be beneficial for pig farmers, depending on the price of fat sources. In addition, in this period and in this type of animals, a reduction in 2% of CP and 0.10% of standardized ileal digestible lysine would decrease feeding costs, increase the profits of pig farmers and reduce nitrogen losses to the environment [31].

3.2. Serum Sex Hormones

Testosterone concentration in serum was higher in IM than in SCM when the second dose of Improvac® was administered ($p < 0.001$) (Figure 1). These differences were also detected, but numerically much lower, when the third dose was applied ($p = 0.0006$). It was expected because only 21 days had passed. The day before slaughter, no impact of the type of castration was detected on testosterone levels ($p = 0.903$). Han et al. [32] and Yamsakul et al. [33] also observed that IM showed higher serum testosterone concentration than SCM when the second dose was injected, and Zamaratskaia et al. [34] found it in plasma just before the second dose injection. Testosterone concentration began to drop abruptly, in the present study, after the second dose, resulting in no significant difference between male groups the day before slaughter, which is in line with a great deal of reports [19,32,34]. The reason is that luteinizing hormone levels decrease quickly after the second dose [35], and this hormone stimulates testosterone production in Leidig cells [36]. According to Claus et al. [35], the subsequent gradual decrease of testosterone levels in IM seems to be due to the clearance of testosterone stored in adipose tissues, becoming measurable in the blood.

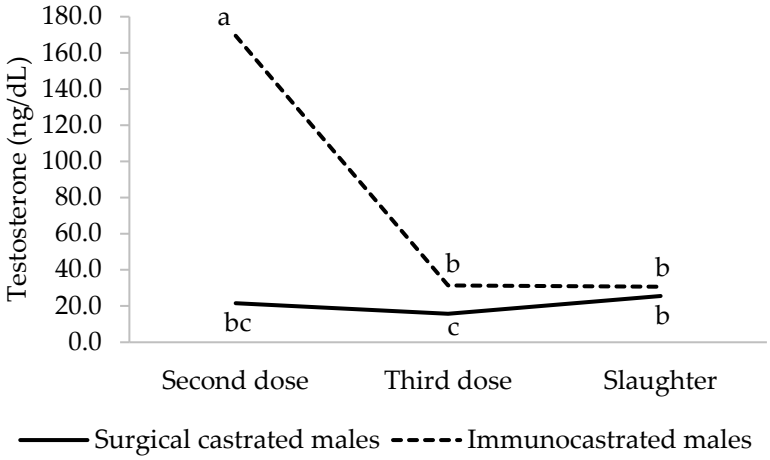

Figure 1. Interaction type of castration × sampling time ($p < 0.0001$) on serum testosterone concentration ($n = 9$ per type of castration at each sampling time). After the data had been transformed, statistical analysis was performed on it. Data are presented as back-transformed least square means. Different letters (a, b or c) are used to indicate values that differ significantly ($p < 0.05$).

Regarding estradiol levels in serum (Table 3), it was greater ($p = 0.034$) in IM than in SCM (31.3 vs. 24.3 pg/mL, respectively), irrespective of the sampling time ($p = 0.801$ for the interaction type of castration × sampling time). Despite this, immunocastration seemed to reduce estradiol secretion, taking into account that Han et al. [37] observed that entire male pigs presented around 70 pg of estradiol/mL of serum between the second dose and the slaughter. Also, in the current study, the estradiol concentration was maintained from the second to the third application and increased from that moment to the slaughter ($p = 0.0002$). The results found about this parameter in the literature are quite variable. Han et al. [37] detected higher estradiol concentrations in IM than in SCM when the second dose was injected but no difference at slaughter, and Van den Broeke et al. [38] did not find differences between IM and SCM at any time point.

Table 3. Impact of type of castration and sampling time on the serum estradiol concentration (least square means) of heavy male pigs.

Item	Estradiol, pg/mL
Type of castration	
Surgical	24.3
Immunological	31.3
SEM [1] ($n = 27$)	2.22
Sampling time	
At second dose of immunocastration	24.6 [b]
At third dose of immunocastration	24.2 [b]
Day before slaughter	34.6 [a]
SEM [1] ($n = 18$)	1.93
p-value [2]	
Type of castration	0.034
Sampling time	0.0002

[1] SEM: standard error of the mean. [2] No significant interaction (type of castration × sampling time) was found ($p = 0.801$). Least square means within a column with different superscript ([a,b]) differ ($p < 0.05$).

3.3. Serum Metabolites

The effect of pig male immunocastration on serum metabolites has been scarcely investigated. In the current trial, no impact of it was detected ($p > 0.10$) on albumin, urea or triglycerides levels (Table 4). The present results would suggest that gonadal steroids suppression did not have carryover effects on nutritional status, as the main blood

protein (albumin), dietary protein breakdown marker (urea), and lipid anabolism marker (tryglicerides), were kept similar.

Table 4. Impact of type of castration, diet and sampling time on serum metabolites (least square means) of heavy male pigs.

Item	Albumin [1], g/dL	Urea, mg/dL	Triglycerides, mg/dL
Type of castration			
Surgical	4.06 (3.89–4.24)	33.9	48.1
Immunological	4.00 (3.81–4.18)	31.9	46.4
SEM [2] (n = 18)	-	1.59	2.91
Diet			
Control	4.05 (3.82–4.29)	37.7 [a]	45.1 [ab]
High energy	4.01 (3.80–4.23)	32.7 [ab]	55.6 [a]
Low crude protein and amino acids	4.02 (3.81–4.24)	28.4 [b]	41.2 [b]
SEM [2] (n = 12)	-	1.95	3.55
Sampling time			
At the end of the grower period	3.35 (3.18–3.51)	30.0	52.1
At the end of the finisher period	4.77 (4.58–4.97)	35.8	42.5
SEM [2] (n = 18)	-	1.59	4.27
p-value [3]			
Type of castration	0.611	0.378	0.686
Diet	0.964	0.008	0.039
Sampling time	<0.0001	0.014	0.217

[1] After the data had been transformed, statistical analysis was performed on it. Data are presented as back-transformed least square means and 95% confidence intervals within parentheses. [2] SEM: standard error of the mean. [3] No significant interactions (type of castration × diet, type of castration × sampling time, diet × sampling time, type of castration × diet × sampling time) were found ($p > 0.05$). Least square means within a column with different superscript ([a,b]) differ ($p < 0.05$).

The type of diet did not affect either albumin concentration, which is in agreement with Pérez-Ciria et al. [11] who evaluated similar feeds in heavy gilts. Kim et al. [39] also obtained the same results when comparing diets with different energy level. Nevertheless, Mule et al. [40] and Suárez-Belloch et al. [41] found that, applying a higher and earlier CP and AA restriction, albumin levels decreased. It could be explained because, with low-CP and -AA diets, the limitations in the availability of AA would firstly appear in the synthesis of exported proteins, such as albumin [42].

However, diet did influence urea concentration; pigs fed the low-CP and -AA diet presented lower (p = 0.008) urea levels than those fed the control diet, with pigs fed the high-energy diet being in an intermediate position. The similar urea content from control and high-energy diets is consistent with the findings of other authors [11,39,43]. Also, the lower urea content from low-CP and -AA diet agrees with several reports [30,40,44] who compared diets with different CP and AA contents. It would indicate that the utilization of low-CP and -AA diets reduces the degradation of protein and AA [45], improving the AA utilization for growth [46], which would reduce the urinary nitrogen excretion, having important implications to reduce manure load.

Furthermore, animals eating the high-energy diet presented greater (p = 0.039) triglyceride levels than those eating the low-CP and -AA diet, with the animals eating the control diet in an intermediate place. These results confirm those of Kim et al. [39] and Suárez-Belloch et al. [30], although in another study, Suárez-Belloch et al. [41] observed that an earlier (at 26 kg BW) and greater dietary CP and AA restriction (from 24 to 14.8% CP and from 1.1 to 0.52% lysine) promoted a linear increase of serum triglycerides. It is worth noting that the higher triglyceride concentration obtained with the high-energy diet suggests that the administration of a high-energy diet could be a good strategy to increase lipid biosynthesis in pigs.

In the present trial, a significant interaction between type of castration and diet was detected for serum cholesterol concentration (p = 0.032, Figure 2); whereas the cholesterol

content was similar for IM and SCM providing the high-energy diet or the low-CP and -AA diet, IM showed higher cholesterol levels than SCM when the control diet was given. Therefore, when providing the high-energy diet or the low-CP and -AA diet, the differences between IM and SCM would be minimized regarding cholesterol levels. The higher serum cholesterol concentration observed in IM fed the control diet could be a negative aspect if the same results were obtained in their tissues, but Harris et al. [47] found that the amount of cholesterol accretion in tissues was not generally influenced by the serum cholesterol concentration of the animal. Cholesterol can be obtained from the diet if it contains animal fat sources. However, in this study, the dietary fat was palm oil, which implies that the outcomes reflect only endogenous synthesis. The lower serum cholesterol in SCM compared to IM fed the control diet would suggest that its hepatic synthesis was downregulated.

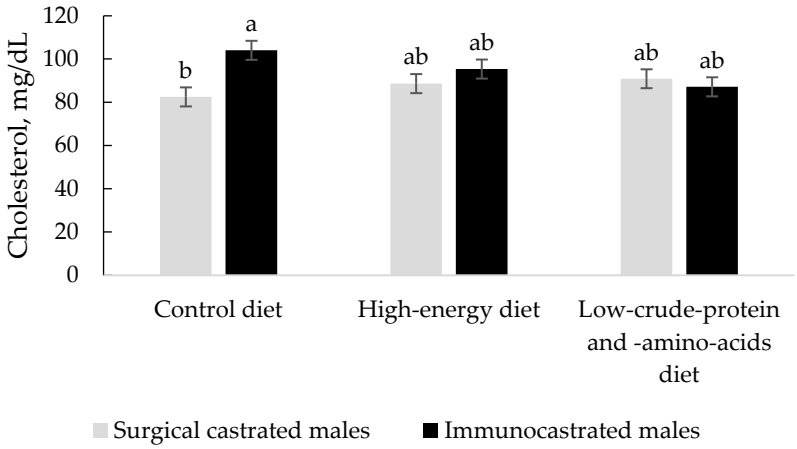

Figure 2. Interaction type of castration × diet ($p = 0.032$) on serum cholesterol concentration ($n = 6$). Least square means that differ significantly ($p < 0.05$) are noted with different letters (a, b).

With regard to the sampling time, albumin, urea and cholesterol levels were higher ($p < 0.0001$, $p = 0.014$ and $p < 0.0001$, respectively) in the finisher period than in the grower period, which corroborates the results reported previously by Pérez-Ciria et al. [11] with the same diets in gilts. It means that these metabolites increase as pigs are older or heavier irrespective of feeding and sex.

3.4. Carcass Quality

The influence of the type of castration and diet on carcass quality can be found in Table 5. The slaughter weight had been pre-fixed and then the BW at slaughter was similar ($p = 0.158$) in animals from both types of castration. However, IM presented lower carcass weight ($p = 0.003$) and carcass yield ($p = 0.006$) than SCM. The lower carcass yield observed in IM agrees with other studies [15,48,49], and it could be mainly due to the weight of the testicles and to the higher weight of the full intestinal tract, liver, kidneys and additional male reproductive tract reported in IM in comparison to SCM [50]. Also, fat thickness over the GM was thinner ($p = 0.0004$) in IM than in SCM, which is consistent with the results of Gispert et al. [51], Morales et al. [14] and Škrlep et al. [3] and not positive for dry-cured ham elaboration. Other authors [48,49] did not find it significant, but numerically observed the same effect. The lower carcass fatness detected in IM, in the current study, was also observed in the intramuscular fat content of the pork of these animals [52]. This is because IM are similar to entire males until immunocastration is effective, shortly after the second dose, and later they behave as castrated males [53], while SCM are barrows from the first week of life. Therefore, the shorter the time interval between the second dose of immunocastration and slaughter, the less fatness could the IM be expected to present [54]. No impact ($p > 0.10$) of type of castration was detected on ham size, in agreement with Pinna et al. [55] and Daza et al. [56]. However, hams from IM were lighter ($p = 0.0009$) than those from SCM, which would be related to the lighter weight of IM carcasses. On

the other side, no influence of type of castration was observed on ham yield in carcass ($p = 0.222$), corroborating the findings of Morales et al. [14,15], although other authors [48,49] observed higher ham yield in IM. No differences between IM and SCM were found in shoulder weight ($p = 0.313$), but shoulder yield was greater ($p = 0.0007$) in IM than in SCM, which agrees with the reports of Lowe et al. [57] and Pauly et al. [58]. The discrepancies between studies in the yield of main lean cuts may be due to the different immunocastration protocols (period between the last dose and slaughter), breed or practices of trimming (more or less amount of fat removed in the pieces).

Despite having pre-fixed the target slaughter weight, there was a slight difference of around 5 kg between the BW mean of pigs fed the high-energy diet and of pigs fed the control diet, resulting in a significant difference between them ($p = 0.009$). However, carcass weight and also carcass yield were similar for all dietary treatments ($p > 0.10$). Several authors reported similar carcass weights by increasing dietary energy [7,8,27] or decreasing CP and lysine of the diet [59–61], although Wood et al. [62] did observe that pigs fed low-CP and -AA levels had lighter carcasses than those fed high levels. Regarding carcass yield, some reports [26,27] show a linear increase as dietary energy content increased, which could be explained because low-energy diets have more fiber content, and an increase in fiber intake increases gastrointestinal tract weight, reducing carcass yield [63]. In the present study, the difference in fiber content between the control and the high-energy diet was not enough to carry out a significant increase of the gastrointestinal tract weight. Also, no influence of diet was found on fat depth over the GM ($p = 0.191$). Suarez-Belloch et al. [8] did observe that fat depth measured at that point was greater with the increase of the dietary energy (in base on fat from animal origin), and Sirtori et al. [64] detected this effect with the lowest CP and AA diet, which could be attributed to the fact that excess of energy intake in relation to lysine intake is transformed into body fat [65,66]. Weight and yield of ham and shoulder were not affected by the feedstuffs ($p > 0.10$). In the current work, the lack of effect on carcass fatness and also perhaps to proportions of the main lean cuts could be due in part to the high variability of data, but probably more to the mild differences in nutrient levels of the tested diets.

Table 5. Impact of type of castration and diet on carcass quality (least square means) of heavy male pigs.

Trait	Type of Castration (C)		SEM [1] (n = 51)	Diet (D)			SEM [1] (n = 34)	p-Value [3]	
	Surgical	Immunological		Control	High Energy	Low CP-AA [2]		C	D
Slaughter weight, kg	136.1	138.6	1.26	134.1 [b]	140.8 [a]	137.2 [ab]	1.53	0.158	0.009
Carcass									
Weight, kg	106.1	103.6	0.56	104.7	105.5	104.3	0.69	0.003	0.491
Yield, %	77.1	75.6	0.38	76.1	76.8	76.2	0.47	0.006	0.559
Fatness over the GM [4,5], mm	23.9	20.6	0.64	21.4	23.4	21.9	0.79	0.0004	0.191
Ham [5]									
Length, cm	39.8	39.7	0.16	39.7	39.8	39.8	0.20	0.515	0.882
Perimeter, cm	79.0	78.4	0.26	78.9	78.9	78.4	0.32	0.109	0.400
Main cut weight [5], kg									
Ham [6]	13.7 (13.5–13.9)	13.3 (13.1–13.4)	-	13.5 (13.3–13.7)	13.5 (13.3–13.8)	13.3 (13.1–13.6)	-	0.0009	0.380
Shoulder	9.04	9.12	0.060	9.19	9.04	9.01	0.074	0.313	0.195
Total [7]	22.7	22.3	0.12	22.6	22.5	22.3	0.15	0.066	0.319
Main cut yield [5], % carcass									
Ham [6]	12.9 (12.7–13.1)	12.7 (12.5–12.9)	-	12.8 (12.6–13.0)	12.8 (12.5–13.0)	12.8 (12.5–13.0)	-	0.222	0.892
Shoulder	8.52	8.82	0.061	8.78	8.59	8.63	0.074	0.0007	0.186
Total [6,7]	21.4 (21.1–21.6)	21.5 (21.3–21.8)	-	21.6 (21.2–21.9)	21.4 (21.1–21.7)	21.4 (21.1–2.7)	-	0.352	0.671

[1] SEM: standard error of the mean. [2] low in crude protein and amino acids. [3] No significant interactions (type of castration × diet) were found ($p > 0.05$). [4] GM: gluteus medius muscle. [5] From the left side of the carcass. [6] After the data had been transformed, a statistical analysis was performed on it. Data are presented as back-transformed least square means and 95% confidence intervals within parentheses. [7] Ham and shoulder. Least square means within a row with different superscript ([a,b]) differ ($p < 0.05$).

4. Conclusions

It can be concluded that SCM would be preferable to IM for Teruel dry-cured ham production, because surgical castration increases carcass fatness, although the slower growth and worse feed conversion ratio in these animals should be considered. On the other hand, increasing dietary energy by 0.15 Mcal of net energy/kg or decreasing dietary CP by 2% and AA, against a standard diet, in male pigs from 80 to 137 kg BW and regardless of the type of castration, seems to be interesting, since they improve feed efficiency but, from a carcass quality perspective, no benefit is noted.

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Institutional Review Board Statement: The study was conducted according to the Policy for Animal Protection of Spain [67] and approved by the Ethics Committee of Zaragoza University (protocol code: PI29/18; date of approval: 27 June 2018).

Data Availability Statement: Data can be available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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Paper 4. Influence of immunocastration and diet on meat and fat quality of female and male pigs

animals

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Influence of Immunocastration and Diet on Meat and Fat Quality of Heavy Female and Male Pigs

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Simple Summary: Sufficient fat cover is necessary for an optimum dry-curing process of Teruel dry-cured hams. However, in recent years, gilts intended for this type of hams are characterized by lack of fat deposition, since male pigs are surgically castrated, to miss boar taint, and castration increases fatness. Thus, immunocastration or the increase of energy in the diet or the decrease of dietary crude protein and amino acids could solve this problem. On the other hand, the surgical castration of male pigs could be banned in the near future in the European Union. Hence, immunocastration could be a solution, as well. However, immunocastrated males seems to present lower fatness than surgically castrated males. Thus, it would be interesting to study feeding plans that increase fatness. Therefore, two experiments were conducted, one with females and another with males, to evaluate the effect of immunocastration and diet on meat and fat quality. In conclusion, immunocastration is a good practice in gilts, as it improves meat quality and technological fat quality. However, in the case of males, this strategy deteriorates meat quality when compared with surgical castration, and it should be accompanied with a high-energy diet or a low-crude-protein diet to guarantee an adequate fat consistency.

Abstract: Two experiments were carried out; one with female pigs and the other with male pigs destined for Teruel dry-cured ham production, to evaluate the effect of immunocastration (entire gilts-EG vs. immunocastrated gilts-IG and surgically castrated males vs. immunocastrated males-IM) and diet (control vs. high energy vs. low crude protein and amino acids) on meat quality and fat composition. Fifteen meat samples and eight fat samples of each treatment were analyzed in both experiments. In the case of males, six fat samples per treatment were analyzed to determine boar taint. Immunocastration is a good strategy in gilts intended for dry-cured ham production because improves meat composition; however, in males, immunocastration impairs the results of pork chemical composition compared with surgical castration. The IG presented a lower polyunsaturated/saturated fatty acids ratio than EG, improving fat technological quality. Diets had little effect on pork or fat quality in gilts, but a high-energy level using oilseeds and a low-crude-protein and -amino-acids diet from 80 to 137 kg of body weight could be interesting in IM to maintain or increase fat consistency, respectively. Moreover, in general, immunocastration is effective in avoiding boar taint in males.

Keywords: immunocastration; high energy; low protein; meat quality; fat quality; pig

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1. Introduction

Currently, a significant percentage of pig carcasses intended for the Protected Designation of Origin (PDO) Teruel dry-cured ham are declared unfit. The main cause is the lack of fat covering the ham [1], which favors the salting process and prevents the excessive drying of the cured pieces [2]. Likewise, trained panelists have detected modest intramuscular fat (IMF) content in the meat of these animals [3], a parameter which is positively related to juiciness and negatively to hardness [4]. These problems appear mainly in females [1,5], since males destined for this PDO have to be castrated, to avoid boar taint, and castration increases the accretion of fat tissue [6]. The usual castration carried out in male piglets in Spain is surgical. The higher carcass fatness generated by this type of castration has been also observed in females [7]. Feeding strategies could resolve, in part, these problems. The rise of dietary energy level [8] or the reductions in crude protein (CP) and amino acids (AA) content [9] seem to increase fat cover at the *gluteus medius* muscle (GM), although the results on the IMF content are not so conclusive. The immunocastration (immunization against gonadotrophin releasing factor-GnRF) of females could be another option for increasing their fatness, because the surgical castration of female pigs reared under intensive conditions is banned in the European Union [10]. On the other hand, in male pigs, for welfare reasons, immunocastration is also emerging as a possible alternative to surgical castration. However, immunocastrated males (IM) seem to present lower fat deposition than surgically castrated males (SCM) [11], which would be undesirable for dry-cured ham production. In this context, it would also be reasonable to study the feeding strategies of these animals in order to optimize their fatness content and composition. Lastly, it should be noted that immunocastrated gilts (IG) and IM could be expected to have different feeding patterns than entire gilts (EG) and SCM, respectively, and thus it would be important to evaluate appropriate feeding plans for them that optimize the quality of the final product. Therefore, two trials were carried out, one with female pigs and the other with male pigs, all of them destined for the PDO Teruel ham, with the aim of assessing the effect of immunocastration and diet on meat and fat quality.

2. Materials and Methods

Two trials were carried out and they are described as follows. Pigs were raised in compliance with the Spanish Policy for Animal Protection [12]. All the experimental procedures used followed the requisites of the Ethical Committee of the University of Zaragoza (ref. PI29/18).

2.1. Pig Husbandry and Experimental Design

In trial 1, a total of 192 Duroc × (Landrace × Large White) gilts of 40.3 ± 4.80 kg body weight (BW) (84 ± 3 days of age) were used. Half of them were immunocastrated using two doses of Vacsincel® (Zoetis Spain S.L., Alcobendas, Madrid, Spain): the first dose at 58.1 ± 6.39 kg of BW (102 ± 3 days of age) and the second dose at 77.0 ± 8.12 kg of BW (122 ± 3 days of age). The other half were EG throughout the trial.

In trial 2, a total of 144 Duroc × (Landrace × Large White) male pigs of 35.3 ± 4.10 kg BW (78 ± 3 days of age) were used. Half of them were surgically castrated during the first week of life and the other half were immunocastrated using three doses of Improvac® (Zoetis Belgium SA, Louvain-la-Neuve, Belgium): the first dose at the end of post-weaning period, at approximately 25 kg of BW (56 ± 3 days of age), as required by the Teruel ham *Consortium*. Immunocastrated pigs were immunized against GnRF for the second time at 57.7 ± 5.60 kg of BW (101 ± 3 days of age) and for the third time at 79.2 ± 7.20 kg of BW (122 ± 3 days of age), to ensure the effect of the immunization.

In both trials, upon arrival at the farm (Foz-Calanda, Teruel, Spain), animals were housed in groups of eight in pens of 9 m^2. Three experimental diets were offered to all of them during the grower and the finisher periods: (i) a control diet, with a nutritional profile similar to the recommendations of FEDNA [13] for this type of animal; (ii) a diet with a greater energy content than the control diet, but maintaining similar CP and AA

percentages; and (iii) a diet with lower CP and AA contents than the control diet, but of similar energy level. In all diets, the ideal protein content was maintained [13] and the change between the grower and the finisher feeds was carried out on a fixed day. The grower diets were supplied from 122 to 149 ± 3 days of age (approximately 78–106 kg of BW) and the finisher diets from 150 ± 3 days of age to the day before slaughter (approximately 106–136 kg of BW). The ingredients, estimated nutrient composition and analyzed nutrient composition of the experimental diets are shown in Tables 1–3, respectively. Feed, in pellet form, and water were provided *ad libitum*.

Table 1. Ingredients of the tested diets (%, as-fed basis).

Ingredient	Grower Diet (78 to 106 kg Body Weight)			Finisher Diet (106 to 136 kg Body Weight)		
	Control	High Energy	Low CP and AA	Control	High Energy	Low CP and AA
Corn	35.0	33.9	35.0	35.0	32.5	35.0
Wheat	18.0	18.0	18.4	17.0	18.0	18.1
Barley	17.6	15.0	21.0	21.1	21.8	25.0
Oat	9.00	8.72	11.0	11.0	8.00	12.0
Soybean meal 47% CP	17.8	18.7	11.9	13.6	14.4	7.69
Palm oil	0.53	3.65	0.34	0.36	3.36	0.08
Calcium carbonate	0.79	0.78	0.80	0.85	0.85	0.86
Sodium chloride	0.45	0.45	0.45	0.45	0.45	0.45
Monocalcium phospate	0.26	0.27	0.31	0.13	0.13	0.18
L-Lysine 50%	0.23	0.21	0.30	0.14	0.12	0.23
L-Threonine	0.02	0.02	0.02	-	-	0.01
DL-Methionine	0.02	0.02	0.01	-	-	-
L-Tryptophan	-	-	-	0.01	0.01	0.01
Premix [1]	0.40	0.40	0.40	0.40	0.40	0.40

CP: crude protein; AA: amino acids. [1] The following were provided per kilogram of complete diet: 6.5 IU vitamin A; 1.5 IU vitamin D3; 15 mg α-tocopherol; 3 mg vitamin B2; 1 mg vitamin B6; 0.02 mg vitamin B12; 15 mg nicotinic acid; 8 mg pantothenic acid; 100 mg choline chloride; 100 mg Zn (ZnO); 50 mg Mn (MnO); 250 mg Fe ($FeCO_3$); 10 mg Cu ($CuSO_4 \cdot 5H_2O$); 0.2 mg Se (Na_2O_3Se); 2 mg BHT; 1 mg I (KI); 500 FYT 6-phytase.

Table 2. Estimated nutrient composition of the tested diets (%, as-fed basis).

Nutrient	Grower Diet (78 to 106 kg Body Weight)			Finisher Diet (106 to 136 kg Body Weight)		
	Control	High Energy	Low CP and AA	Control	High Energy	Low CP and AA
Net energy, Mcal/kg	2.33	2.48	2.33	2.33	2.48	2.33
CP	16.0	16.0	14.0	14.5	14.5	12.5
Digestible AA						
Lysine	0.77	0.77	0.67	0.63	0.63	0.54
Methionine	0.24	0.24	0.21	0.21	0.20	0.18
Methionine + Cysteine	0.49	0.49	0.44	0.44	0.43	0.39
Threonine	0.50	0.50	0.43	0.43	0.43	0.36
Tryptophan	0.16	0.16	0.14	0.15	0.15	0.13

CP: crude protein; AA: amino acids.

Table 3. Analyzed nutrient content of the tested diets (%, as-fed basis).

Nutrient	Grower Diet (78 to 106 kg Body Weight)			Finisher Diet (106 to 136 kg Body Weight)		
	Control	High Energy	Low CP and AA	Control	High Energy	Low CP and AA
Gross energy, Mcal/kg	3.99	4.12	3.92	3.91	4.12	3.95
Dry matter	88.7	88.2	88.0	88.0	89.4	88.1
Ash	4.18	4.19	4.17	3.85	3.98	3.65
Starch	42.1	40.3	44.0	44.5	47.8	49.0
Ether extract	3.55	5.88	3.44	3.00	5.65	3.73
Neutral detergent fiber	10.9	10.2	10.5	10.5	8.96	10.2
CP	16.2	15.9	14.4	14.5	15.1	12.7
Total AA						
Lysine	0.98	0.98	0.79	0.76	0.77	0.71
Methionine	0.28	0.27	0.25	0.24	0.25	0.23
Threonine	0.62	0.60	0.59	0.56	0.58	0.51
FA, % of total FA						
C12:0	3.45	1.66	3.35	3.26	1.72	2.50
C14:0	0.58	0.65	0.41	0.34	0.84	0.83
C16:0	23.3	30.2	25.5	25.0	34.6	27.9
C16:1n-7	0.18	0.19	0.20	0.18	0.17	0.20
C16:1n-9	0.05	0.05	0.06	0.06	0.04	0.06
C18:0	3.20	3.61	3.18	3.18	4.05	3.49
C18:1n-7	0.70	0.61	0.73	0.71	0.62	0.65
C18:1n-9	30.9	33.6	31.1	31.6	34.2	32.9
C18:2n-6	34.9	27.4	32.8	32.6	21.6	28.8
C18:3n-3	1.89	1.46	1.66	1.62	1.05	1.29
C18:4n-3	0.24	0.12	0.28	0.43	0.32	0.44
C20:0	0.16	0.07	0.18	0.38	0.34	0.40
C20:1n-9	0.46	0.32	0.50	0.61	0.40	0.57

CP: crude protein; AA: amino acids; FA: fatty acids.

Therefore, in both trials there were six experimental treatments; two types of gilts (EG vs. IG) or two types of males (SCM vs. IM) × three diets (control vs. high-energy vs. low-CP and -AA).

2.2. Feed Analyses

The determinations of gross energy, dry matter, ash, starch, ether extract, neutral detergent fiber, CP and total AA of the diets are detailed in Pérez-Ciria et al. [14]. Fatty acids were extracted and quantified following the one-step procedure described by Sukhija and Palmquist [15] with minor modifications. Each sample (250 mg) in the presence of toluene (1 mL containing 10 mg/mL of the internal standard–C15:0 and another milliliter of toluene) and acetyl chloride in methanol (3 mL at 1/10) was shaken 30 s at a low speed and later heated for 2 h at 70 °C in a shaking water bath. Then, each sample was cooled to room temperature and 5 mL of potassium carbonate were added. Subsequently, each sample was vortexed for 30 s at a high speed and centrifuged for 5 min at 3500 rpm, and the upper phase was taken and 1 g of anhydrous sodium sulfate was added. Each sample was vortexed and centrifuged again. Finally, the upper phase was collected to identify and quantify fatty acid methyl esters as described in López-Bote et al. [16] using a gas chromatograph (HP 6890 Series GC System) with a flame ionization detector and a capillary column (HP-Innowax: 30 m × 0.32 mm × 0.25 µm cross-linked polyethylene glycol).

2.3. Slaughtering and Meat and Fat Sampling

In both trials, slaughter took place when animals achieved 134 and 137 kg of BW on average, for females and males, respectively (between 178 and 199 days old). The day before slaughter, pigs were not fed for 5 h and were moved to a commercial slaughterhouse (Teruel,

Spain), where they were kept in lairage for 10 h without feed but with *ad libitum* access to water. Animals were stunned in a CO_2 atmosphere, exsanguinated, scalded, dehaired, singed, eviscerated and split lengthwise. After refrigeration at 2 °C (1 m/s air speed; 90% relative humidity) for 5 h, the carcasses were processed according to commercial standards.

In each trial, a total of 90 carcasses (15 per treatment) were chosen at random in order to study their meat and fat quality. From each one, a piece of 100 ± 10 g of the left *longissimus thoracis* muscle (LT) and other similar piece of the left GM were taken. Samples of the LT were used to analyze thawing and cooking losses, color parameters and maximum stress and those of the GM were utilized to determine chemical composition. Additionally, from 48 left-side hams (eight per treatment) randomly chosen, a piece of 100 ± 10 g of the subcutaneous fat (including skin, fat layers and lean) was sampled to analyze the fatty acid profile. In addition, in the trial of males, 36 subcutaneous fat samples (six per treatment) were intended for determining the compounds responsible for boar taint. The subcutaneous fat samples of IM were taken from pigs with a testicular width (both testicles) shorter than 11 cm. That criterion was implemented in Brazil, a country where the use of immunocastration is widespread, for acceptance at the slaughterhouses of IM, based on the reduced risk of boar taint [17]. Additionally, five subcutaneous fat samples of IM that showed a testicular width greater than 11 cm were also intended to be analyzed for boar taint (to be used as positive control samples). All samples (meats and fats) were vacuum-packaged in individual plastic bags and preserved at −20 °C for subsequent analyses.

2.4. Meat Quality Traits

Firstly, the LT samples were thawed for 24 h at 4 °C, removed from plastic bags, blotted dry for 15 min and weighed. Thawing losses were calculated considering the fresh and thawed weight. Afterwards, color was measured with a spectrophotometer (CM-2600d, Konica Minolta Holdings, Inc., Osaka, Japan) in CIELAB space [18], with an Illuminant D65 and an observer angle of 10°, previously calibrated according to manufacturer recommendations. The mean of two random measures in each sample was used to obtain lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*_{ab}) and hue-angle (h_{ab}) values. Later, in those samples, cooking losses were determined by the method described by Honikel [19]. The samples were weighed, placed in individual plastic bags and cooked in a water bath (Precisterm, J.P. Selecta S.A., Barcelona, Spain) at 75 °C to reach a core temperature of 70 °C. During the cooking, the internal temperature was monitored through a thermocouple type T connected to a data logger (testo 177-T4, Testo GmbH, Lenzkirch, Germany). Then, the cooked samples were cooled, blotted dry and weighed again. For the cooking-losses calculation, pre- and post-cooking weights were considered. Then, maximum stress was measured, following the procedure for the Warner–Bratzler shear test described by Honikel [19]. The cooked samples were cut in prism-shaped pieces with a 100 mm^2 (10 × 10 mm) cross-section with the fiber direction parallel to a long dimension of at least 30 mm. Five prisms per sample were sheared at right angles to the fiber axis using a Warner–Bratzler device, with a cross-head speed of 2.5 mm/s, attached to an Instron universal testing machine (Model 5543, Instron Ltd., Buckinghamshire, UK), itself attached to a computer. Maximum stress was defined as the load at maximum peak shear force per unit of cross-section [20].

Chemical composition (moisture, protein and IMF) was determined according to the procedures of Boletín Oficial del Estado [21]. Firstly, the GM samples were thawed for 24 h at 4 °C and minced. Moisture was analyzed using an oven (Memmert UFE500, Schwabach, Germany) over 48 h at 102 °C, protein with a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden) and IMF by an ANKOM^{XT15} Extration System (ANKOM Techonology, Macedon, NY, USA) after the samples had been hydrolyzed by an ANKOM^{HCL} Hydrolysis System.

2.5. Fatty Acid Profile of the Subcutaneous Fat

Firstly, the fat samples spent, individually, 1 min in a microwave at 350 W. Afterward, from each sample, 30 µL of melted fat were taken and 1 mL of hexane and 1 mL of methylated mixture (69% methanol, 29% toluene and 2% sulfuric acid by volume) were added. Samples were placed in the oven at 70–80 °C for 2 h and shaken manually every 15 min. Later, they were cooled and fatty acid methyl esters were recovered from the upper phase, separated and quantified by gas chromatography, as described for the feeds. The percentages of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), and also the PUFA/SFA ratio, total n-3 and n-6 percentages and the n-6/n-3 ratio were calculated from individual fatty acid proportions.

2.6. Boar Taint Compounds

Androstenone, skatole and indole concentrations in the subcutaneous fat samples were analyzed according to the procedures of Hansen-Møller [22], Pauly et al. [23] and Batorek et al. [24], with little modification. Firstly, samples were left at room temperature for 30 min. Then, 10 to 20 g of each, without lean and skin, were cut in cubes and liquefied in a microwave for 2 × 2 min at 350 W. The liquefied fat was removed and centrifuged for 20 min at 10,800× *g* at 40 °C and kept at 50 °C. Then, 0.5 ± 0.01 g of supernatant was placed in 2.5-mL Eppendorf tubes and internal standards were added (1 mL of methanol containing 0.5 mg/L of androstanone and 0.05 mg/L of 2-methylindole). After stirring for 30 s, each tube was incubated for 5 min at 30 °C in an ultrasonic water bath (FB 15061, Fisher Scientific, Illkirch Cedez, France), kept on ice for 20 min and then centrifuged for 20 min at 10,800× *g* at 4 °C. Finally, supernatant was transferred with a syringe of 1 mL into a high-performance liquid chromatography (HPLC) vial for androstenone, skatole and indole analysis with an HPLC system from Agilent Technologies (1200 series) as described by Batorek et al. [24]. Concentrations were expressed as µg/g of liquid fat. The detection limits were 0.20 µg/g for androstenone and 0.03 µg/g for skatole and indole. For data analysis, when the concentrations of skatole or indole were below the limit of detection, they were defined as half of the limit of detection.

2.7. Statistical Analyses

In both trials, data were analyzed as a randomized factorial design (2 × 3) using the GLM procedure of the Statistical Analysis System (SAS 9.4 software, SAS Institute Inc., Cary, NC, USA). In trial 1, the model included the type of gilt (EG and IG) and diet (control, high-energy and low-CP and -AA) as main effects. In trial 2, the model included the type of castration (surgical and immunological) and diet (control, high-energy and low-CP and -AA) as main effects. In both trials, interactions (type of gilt or type of castration × diet) were included in the models for parameters that were significant ($p < 0.05$) and excluded from the final models when they were not significant. Least square means were separated using the PDIFF option.

Residuals' normality was verified with Shapiro–Wilk test using the UNIVARIATE procedure. When normality was not achieved, variables were transformed with $\sqrt{x}$ or $1/x$ or x^2, if it was possible. In cases in which normality could not be found with data transformation, variables were analyzed using non-parametric methods: Mann–Whitney U test was carried out when types of gilt or types of castration were compared and Kruskall–Wallis test when different diets were compared.

The pig was the experimental unit (per treatment: $n = 15$ for meat quality, $n = 8$ for fatty acid profile and $n = 6$ for boar taint compounds). A p-value < 0.05 was considered as a significant difference and between 0.05 and 0.10 as a tendency.

3. Results and Discussion

3.1. Trial 1: Meat Quality and Fat Composition in Female Pigs

The only significant interactions type of gilt × diet were observed for thawing and cooking losses (Figure 1). Whereas, with the control diet, IG had greater thawing losses

than EG, with the high-energy diet there were no differences, and with the low-CP and -AA diet IG presented lower thawing losses than EG ($p = 0.011$). In addition, whereas with the control diet IG had greater cooking losses than EG, with the high-energy diet the opposite effect was observed, and with the low-CP and -AA diet there was no difference ($p = 0.0005$). Therefore, when immunocastration is practiced in gilts, the increase of energy or decreased CP and AA in the feed could generate a reduction in cooking or thawing losses. Some authors [25–27] observed similar cooking losses when EG and IG were fed a standard diet, but it is known that factors relative to the freezing and cooking conditions have great influence in the water losses of meat.

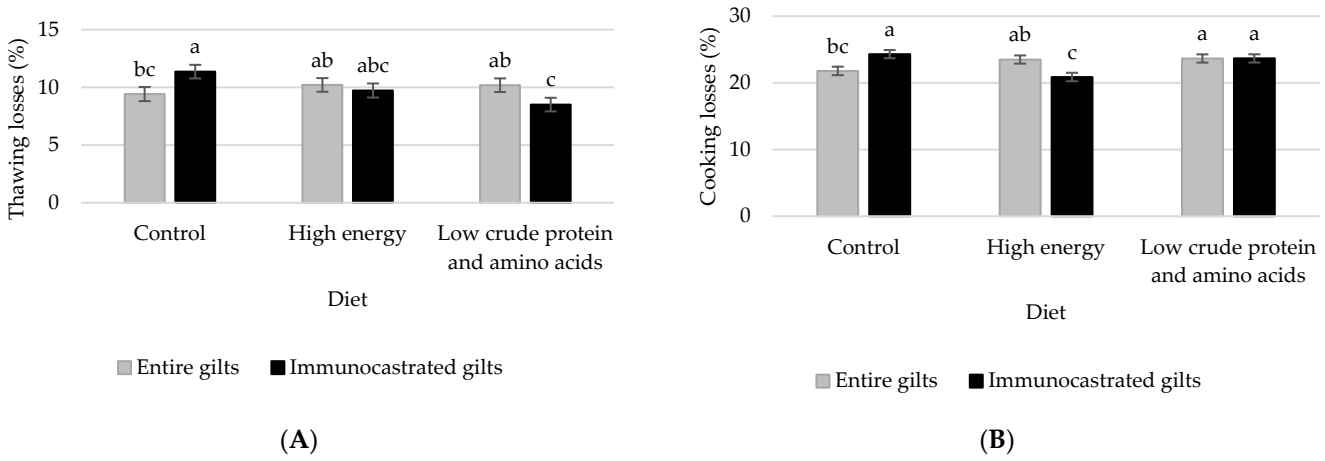

Figure 1. Significant interactions ($p < 0.05$) between type of gilt and diet on water holding capacity indicators (**A**): thawing losses and (**B**): cooking losses of the *longissimus thoracis* muscle. Diets during the grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Diets during the finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). Different letters (a, b, c) denote significant differences between least square means ($p < 0.05$).

The effect of gilt immunocastration and diet on meat quality is shown in Table 4. There was no influence of immunocastration of gilts ($p > 0.10$) on any meat-color trait, in agreement with a great deal of reports [25,28,29]. Maximum stress was also similar ($p = 0.887$) in the loins of both types of gilts. Bohrer et al. [25], Martinez-Macipe et al. [30] and Xue et al. [31] also found no differences between EG and IG in other texture parameters, i.e., shear force. Meat from IG had similar protein proportion ($p = 0.204$) but lower ($p = 0.034$) moisture percentage than that from EG. Bohrer et al. [25] and Pérez-Ciria et al. [27] detected this effect on moisture only numerically. Also, meat from IG presented greater ($p = 0.018$) IMF proportion than that from EG, which could have a positive effect on texture and appearance of dry-cured hams and could reduce ham's weight losses during the dry-curing process [2,4]. There is some unanimity that gilt immunocastration seems to increase IMF percentage [26,28], although some authors [27,29] have detected this effect only numerically (not significantly), which may be due to the use of a small number of animals or to the fact that the breed used was very fatty, mitigating the effect. In fact, in these last studies [27,29], fat thickness at GM muscle was significantly higher in IM than in EG.

Table 4. Effect of immunocastration and diet on the meat quality of gilts.

Trait	Type of Gilt		SEM [1] (n = 45)	Diet [2]			SEM [1] (n = 30)	p-Value [3]	
	Entire	Immunocastrated		Control	High Energy	Low CP and AA		Gilt	Diet
Color traits [4]									
Lightness, L^*	35.0	35.0	0.69	35.2	34.8	35.0	0.85	0.982	0.936
Redness, a^*	2.58	3.13	0.279	2.83	2.70	3.02	0.342	0.168	0.793
Yellowness, b^*	14.8	15.1	0.27	15.1	14.8	15.0	0.32	0.389	0.784
Chroma, C^*_{ab}	15.1	15.5	0.27	15.4	15.1	15.4	0.33	0.281	0.722
Hue angle, h_{ab}	80.2	78.4	1.06	79.5	79.7	78.7	1.30	0.216	0.860
Maximum stress [4], N/cm^2	40.9	41.2	1.61	40.4	42.8	40.0	1.97	0.887	0.566
Chemical composition [5], %									
Moisture	72.2	71.6	0.19	72.0	72.0	71.8	0.24	0.034	0.798
Protein	23.6	23.4	0.11	23.3	23.5	23.7	0.14	0.204	0.145
Intramuscular fat [6]	2.70	3.57	0.239	3.29	3.18	2.93	0.293	0.018	0.494

[1] SEM: standard error of the mean. [2] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [3] No significant interactions (type of gilt × diet) were found ($p > 0.05$). [4] Color and texture analyses were carried out with samples of the *longissimus thoracis* muscle. [5] Proximate composition analyses were carried out with samples of the *gluteus medius* muscle. [6] Least square means and SEM of the original data and p-values obtained with the transformed data.

Dietary treatments had no significant effect ($p > 0.10$) on the color or texture of the meat, corroborating the findings of several works [8,32,33]. In addition, pork chemical composition (moisture, protein and IMF) was not affected ($p > 0.10$) by feeding strategies, in agreement with the results of Suarez-Belloch et al. [8] by increasing energy levels from 2280 to 2420 kcal NE/kg and Rodríguez-Sánchez et al. [33] by reducing CP and lysine (Lys) contents from 14.5% CP and 0.71% total Lys to 14% CP and 0.59% total Lys. However, this could depend on the type of diet evaluated and on the length of the period tested. Suárez-Belloch et al. [32], applying a greater dietary CP and Lys restriction (from 17.2% CP and 0.77% Lys to 10.6% CP and 0.42% Lys) from 90 to 130 kg of BW, observed that meat protein content decreased. In addition, this last study [32] next to the work of Teye et al. [34], in which CP and Lys contents were reduced from 21% and 1.0% to 18% and 0.7%, respectively, from 40 to 100 kg of BW, detected that CP and Lys reduction increased IMF percentage.

The effect of gilt immunocastration and diet on subcutaneous fat composition is provided in Table 5.

Total SFA proportion was greater ($p = 0.003$) in IG than in EG, owing to the higher C16:0 ($p = 0.051$) and C18:0 ($p = 0.001$) contents. The percentage of total MUFA was not affected ($p = 0.771$) by female immunocastration, mainly because the major MUFA (C18:1n-9) was similar ($p = 0.968$) between EG and IG. Total PUFA proportion was lower ($p = 0.006$) in IG, due to the lower C18:2n-6 ($p = 0.007$), C18:3n-3 ($p = 0.017$), C18:3n-6 ($p = 0.015$), C18:4n-3 ($p = 0.024$), C20:3n-6 ($p = 0.054$) and C20:4n-6 ($p = 0.010$) contents. Therefore, PUFA/SFA ratio ($p = 0.004$) and total n-3 ($p = 0.007$) and n-6 ($p = 0.006$) percentages were lower in IG than in EG. It is worth noting that the value of PUFA/SFA ratio obtained in both groups was nutritionally acceptable (the target is $\geq$0.4) [35]. Some authors [27,28] have obtained results similar to ours. However, others [29,36] did not detect significant influence of female immunocastration on fat quality, which could be due to the use of a different crossbreed [29] or the smaller interval between the second dose of immunocastration and the slaughter [36]. As was mentioned previously, there is certain unanimity in the literature about the greater fatness in IG than in EG [25,26,28]. In general, the larger the fat deposits, the higher the proportion of fatty acids from de novo synthesis (SFA and MUFA) and the

lower the percentage of PUFA (provided only by dietary lipids) stored in adipose tissue, since PUFA are diluted [37,38]. The higher proportion of SFA and the lower of PUFA found in IG would imply that these gilts would present more firm and cohesive fat, being better for meat technological processes, but less healthy [39]. In addition, the pork pieces of IG would have better storage stability and flavor, due to a lower susceptibility to oxidative spoilage [39].

Table 5. Impact of immunocastration and diet on subcutaneous fat composition (fatty acids expressed as % of total fatty acids, except in ratios) of gilts.

Trait	Type of Gilt		SEM [1] (n = 24)	Diet [2]			SEM [1] (n = 16)	p-Value [3]	
	Entire	Immunocastrated		Control	High Energy	Low CP and AA		Gilt	Diet
C14:0	1.13	1.16	0.023	1.17	1.10	1.16	0.028	0.327	0.154
C14:1 [4]	0.013	0.012	0.0007	0.012	0.012	0.013	0.0009	0.434	0.738
C15:0	0.054	0.048	0.0023	0.050	0.055	0.048	0.0028	0.062	0.240
C15:1 [4]	0.012	0.010	0.0008	0.011	0.013	0.010	0.0010	0.039	0.223
C16:0	22.0	22.6	0.22	22.2	22.2	22.6	0.27	0.051	0.478
C16:1n-7	1.57	1.55	0.042	1.53 [ab]	1.47 [b]	1.67 [a]	0.051	0.654	0.026
C16:1n-9	0.337	0.294	0.0113	0.299	0.342	0.306	0.0138	0.011	0.067
C17:0	0.307	0.284	0.0142	0.301	0.295	0.290	0.0174	0.259	0.911
C17:1	0.271	0.243	0.0118	0.270	0.244	0.255	0.0145	0.105	0.449
C18:0	11.8	12.7	0.19	12.3	11.9	12.5	0.23	0.001	0.160
C18:1n-7	1.73	1.65	0.030	1.75 [a]	1.60 [b]	1.73 [a]	0.036	0.063	0.013
C18:1n-9	42.8	42.8	0.25	42.8	43.3	42.3	0.31	0.968	0.092
C18:2n-6	15.6	14.3	0.34	14.9	15.2	14.8	0.41	0.007	0.777
C18:3n-3	0.728	0.670	0.0165	0.714	0.701	0.683	0.0202	0.017	0.556
C18:3n-6 [5]	0.040 ± 0.017	0.031 ± 0.008	-	0.032 ± 0.006	0.040 ± 0.022	0.036 ± 0.009	-	0.015	0.236
C18:4n-3	0.058	0.049	0.0029	0.053	0.053	0.055	0.0036	0.024	0.873
C20:0 [5]	0.248 ± 0.038	0.261 ± 0.031	-	0.267 ± 0.025	0.246 ± 0.047	0.251 ± 0.026	-	0.138	0.086
C20:1n-9	0.857	0.913	0.0202	0.956 [a]	0.852 [b]	0.848 [b]	0.0247	0.057	0.005
C20:3n-6 [4]	0.113	0.099	0.0049	0.110	0.107	0.101	0.0060	0.054	0.566
C20:4n-6	0.271	0.238	0.0086	0.258	0.253	0.252	0.0105	0.010	0.899
C20:5n-3 [4]	0.033	0.031	0.0033	0.027	0.034	0.035	0.0041	0.549	0.273
Total SFA	35.5	37.1	0.36	36.3	35.8	36.8	0.44	0.003	0.233
Total MUFA	47.6	47.5	0.25	47.6	47.8	47.2	0.31	0.771	0.303
Total PUFA	16.9	15.4	0.36	16.1	16.4	16.0	0.44	0.006	0.807
PUFA/SFA	0.477	0.418	0.0136	0.445	0.462	0.437	0.0166	0.004	0.533
Total n-3	0.820	0.749	0.0173	0.793	0.787	0.773	0.0212	0.007	0.784
Total n-6	16.1	14.7	0.35	15.3	15.6	15.2	0.43	0.006	0.804
n-6/n-3	19.6	19.6	0.20	19.3	19.8	19.8	0.24	0.905	0.275

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. [1] SEM: standard error of the mean. [2] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [3] No significant interactions (type of gilt × diet) were found ($p > 0.05$). [4] Least square means and SEM of the original data and p-values obtained with the transformed data. [5] This variable has been analyzed using non-parametric methods and its data are presented as mean ± standard deviation. Within a row, least square means without a common letter ([a], [b]) differ ($p < 0.05$).

Dietary treatments only had significant influence on some MUFA. Fat from gilts fed the low-CP and -AA diet presented higher ($p = 0.026$) percentage of C16:1n-7 than that from gilts fed the high-energy diet, placing those gilts fed the control diet in an intermediate position. Animals that ate the low-CP and -AA diet or the control diet had greater ($p = 0.013$) C18:1n-7 proportion than those fed the high-energy diet, and gilts fed the alternative diets to control showed lower ($p = 0.005$) percentage of C20:1n-9 than those fed the control diet. Hence, the increase in dietary energy or the decrease in CP and AA of the diet had no effect ($p > 0.10$) on total SFA, MUFA and PUFA contents, PUFA/SFA ratio, total n-3 and n-6 proportions and n-6/n-3 ratio. Suarez-Belloch et al. [8] and Rodríguez-Sánchez et al. [33] found similar results to those reported in the current manuscript after increasing dietary energy or decreasing CP and Lys in the diet, respectively. Although in the current study there were no differences in IMF content between feeding strategies,

the differences in minor MUFA may be related with dietary-induced modifications in the Stearoyl–CoA desaturase (SCD) gene-family expression, which in turn may contribute to porcine adipocyte differentiation and adipogenesis [40].

3.2. Trial 2: Meat Quality, Fat Composition and Boar Taint Compounds in Male Pigs

The effect of the type of castration and diet on meat quality of male pigs is shown in Table 6. The type of male castration had no impact ($p > 0.10$) on thawing or cooking losses, which agrees with the findings of Pauly et al. [41] and Seiquer et al. [42]. However, other authors [24,43,44] detected that pork from IM had higher cooking losses than that from SCM, justifying this with the increase in protein oxidation in the case of IM, reducing the ability of the muscle to bind water. Meat from IM presented lower ($p = 0.027$) L^* and tended to show lower ($p = 0.084$) h_{ab} than that from SCM, which are parameters strictly linked with human perception of pork color [45], whereas a^*, b^* and C^*_{ab} were similar in both groups ($p > 0.10$). In the literature, the effect of the type of castration on color traits is not unanimous. Some reports [41,46,47] did not find differences between SCM and IM in any color trait but others [48–50] showed, as in the present work, that meat from IM presented lower L^* value. Daza et al. [36], Andreo et al. [50] and Seiquer et al. [42] observed that IM presented lower a^* and C^*_{ab} values, and Škrlep et al. [44] observed the opposite effect. Different genetic types, pre-slaughter handling and conditions of data collection could contribute to pork color [51], generating differences unrelated to animal husbandry.

Table 6. Effect of type of castration and diet on meat quality of male pigs.

Trait	Type of Castration		SEM [1] ($n = 45$)	Diet [2]			SEM [1] ($n = 30$)	*p*-Value [3]	
	Surgical	Immunological		Control	High Energy	Low CP and AA		Castration	Diet
Thawing losses [4,5], %	10.09	9.47	0.389	9.30	9.90	10.14	0.476	0.187	0.459
Cooking losses [4,5], %	23.7	24.0	0.39	23.2 [b]	23.2 [b]	25.2 [a]	0.48	0.491	0.002
Color traits [5]									
Lightness, L^*	34.8	32.2	0.82	33.6	33.4	33.5	1.01	0.027	0.990
Redness, a^*	3.83	4.37	0.299	3.89	3.97	4.44	0.367	0.207	0.505
Yellowness, b^*	14.8	14.2	0.33	14.5	14.3	14.6	0.40	0.182	0.817
Chroma, C^*_{ab}	15.4	14.9	0.31	15.1	15.0	15.4	0.38	0.302	0.775
Hue angle [4], h_{ab}	75.5	72.5	1.22	74.9	73.9	73.2	1.49	0.084	0.695
Maximum stress [4,5], N/cm^2	40.4	37.6	1.91	38.0	41.3	37.7	2.33	0.233	0.332
Chemical composition [6], %									
Moisture	71.4	72.2	0.17	71.7	71.6	72.1	0.21	0.004	0.246
Protein	23.0	23.1	0.08	23.0	23.0	23.1	0.10	0.856	0.876
Intramuscular fat	4.44	3.40	0.220	4.02	4.15	3.58	0.269	0.001	0.293

[1] SEM: standard error of the mean. [2] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [3] No significant interactions (type of castration × diet) were found ($p > 0.05$). [4] Least square means and SEM of the original data and *p*-values obtained with the transformed data. [5] Water losses, color and texture analyses were carried out with samples of the *longissimus thoracis* muscle. [6] Proximate composition analyses were carried out with samples of the *gluteus medius* muscle. Within a row, least square means without a common letter ([a], [b]) differ ($p < 0.05$).

The type of castration had no influence ($p = 0.233$) on meat texture, evaluated as maximum stress, which confirms the results of other works [24,26,44]. It is worth noting that, when trained panelists have evaluated pork from SCM vs. pork from IM, they have

established that IM presents similar tenderness to SCM [30,47]. Regarding the chemical composition of muscle, there was no influence of the type of castration on protein content (p = 0.856), but meat from IM presented greater (p = 0.004) moisture percentage and lower (p = 0.001) IMF percentage than that from SCM. The results observed about IMF proportion between groups are consistent with works of several authors [30,42,48]. The low IMF percentage generated by immunocastration in male pigs would be not desirable because it could have a negative influence on some texture and appearance attributes of dry-cured hams [4]. The reason for such difference between groups would be associated to IM pigs behaving as entire males (EM) until the second dose of immunocastration, and EM (and also IM until the second dose injection) presenting higher levels of testosterone than SCM [52], castrated from the first week of life, which would decrease fat mass [53]. Nevertheless, some authors [24,44,54] did not detect this difference as significant and this might be due to the different genetics used, the age at which immunocastration doses were administered and the time elapsed between the second vaccination and slaughter. Thereby, shortening the time from the second vaccination to slaughter may allow the expression of more differences in IMF accretion between IM and SCM.

The tested diets had no influence (p = 0.459) on thawing losses, but they had an impact (p = 0.002) on cooking losses. Meat from males fed the low-CP and -AA diet presented greater cooking losses than that from males fed the control or the high-energy diet, without observing differences between these last two diets. The higher weight loss during cooking with the CP and AA restriction could impair juiciness [55], being a negative aspect for pork quality. Matthews et al. [56] also found no effect of increasing dietary energy on thawing and cooking losses, and Sirtori et al. [57] observed that animals restrictively fed the lowest CP and AA diet had higher cooking losses as well. The experimental feeds had no significant impact ($p > 0.05$) on any color trait, in agreement with Matthews et al. [56] by increasing dietary energy level, and with Suárez-Belloch et al. [32] and Tejeda et al. [58] by reducing CP and Lys contents. Likewise, meat tenderness was not affected (p = 0.332) by dietary treatments. Reducing CP and AA contents, Millet et al. [59] and Suárez-Belloch et al. [32] did not observe an influence on shear force, either. Therefore, the potential mild negative effects of reduced CP and AA diets on cooking losses may be counterbalanced by similar meat tenderness and a potential reduction in feeding costs. As in the case of gilts (Trial 1, Table 4), and although a higher IMF content in meat was expected by decreasing CP and AA and/or by increasing energy in diets, finally meat chemical composition was similar ($p > 0.10$), irrespective of feeding strategy. Some authors [33,59] also observed no effect when reducing dietary protein or Lys content, but others [32,57] did, mainly in IMF percentage, and it was probably because the restriction of those nutrients was more severe. In the case of dietary energy level, Matthews et al. [56] also detected no influence when increasing energy level in diets, but Liu et al. [60] achieved a higher IMF proportion, probably due to the difference in energy between diets was more pronounced.

Regarding fat composition, some significant interactions between type of castration and diet were detected (Table 7).

The most notable interactions were the following. The high-energy diet affected similarly on total PUFA and total n-6 percentages and PUFA/SFA ratio, irrespective of the type of castration, but IM had higher values thereof with the control diet and lower with the low-CP and -AA diet than SCM ($p < 0.05$). Also, the fat from both types of males had similar content in total SFA percentage when control or high-energy diets were given, but IM presented higher content thereof than SCM with the low-CP and -AA diet ($p < 0.04$). In addition, SCM and IM fed the high-energy diet or the low-CP and -AA diet showed similar total n-3 proportion, whereas IM fed the control diet had higher percentage of total n-3 than SCM fed this diet (p = 0.004). Therefore, in the case of IM, the use of a standard diet would lead to a decrease in the consistency of their fat, whereas a low-CP and -AA diet would improve it, although their fat could be less healthy, and with a high-energy diet the fat consistency would be maintained similarly to that of SCM.

Table 7. Interactions between type of castration and diet with regard to subcutaneous fat composition (fatty acids expressed as % of total fatty acids, except in ratios) of male pigs.

Diet [1]	**Control**		**High Energy**		**Low CP and AA**		**SEM** [2] **(*n* = 8)**	***p*-Value**
Type of Castration	**Surgical**	**Immunological**	**Surgical**	**Immunological**	**Surgical**	**Immunological**		
C16:0	23.3 ^{abc}	22.6 ^{c}	22.8 ^{bc}	23.8 ^{a}	22.7 ^{bc}	23.5 ^{ab}	0.31	0.019
C16:1n-9	0.259 ^{b}	0.318 ^{a}	0.290 ^{ab}	0.277 ^{ab}	0.296 ^{ab}	0.268 ^{b}	0.0150	0.012
C18:2n-6	13.8 ^{bc}	15.7 ^{a}	14.1 ^{bc}	13.8 ^{bc}	15.1 ^{ab}	13.4 ^{c}	0.47	0.002
C18:3n-3	0.660 ^{bc}	0.754 ^{a}	0.651 ^{bc}	0.632 ^{bc}	0.687 ^{b}	0.617 ^{c}	0.0232	0.003
C18:3n-6	0.025 ^{c}	0.033 ^{a}	0.032 ^{ab}	0.030 ^{abc}	0.028 ^{abc}	0.026 ^{bc}	0.0022	0.046
C18:4n-3	0.040 ^{bc}	0.045 ^{ab}	0.052 ^{a}	0.034 ^{c}	0.039 ^{bc}	0.037 ^{bc}	0.0039	0.010
C20:1n-9	0.967 ^{a}	0.881 ^{bc}	0.927 ^{abc}	0.875 ^{c}	0.874 ^{c}	0.962 ^{ab}	0.0351	0.042
C20:4n-6	0.211 ^{b}	0.263 ^{a}	0.230 ^{ab}	0.226 ^{b}	0.233 ^{ab}	0.218 ^{b}	0.0123	0.021
Total SFA	38.0 ^{ab}	37.0 ^{b}	37.1 ^{b}	38.6 ^{ab}	37.2 ^{b}	39.0 ^{a}	0.60	0.039
Total PUFA	14.9 ^{bc}	16.9 ^{a}	15.2 ^{bc}	14.8 ^{bc}	16.2 ^{ab}	14.4 ^{c}	0.50	0.002
PUFA/SFA	0.392 ^{bc}	0.459 ^{a}	0.411 ^{abc}	0.384 ^{c}	0.436 ^{ab}	0.371 ^{c}	0.0181	0.002
Total n-3	0.724 ^{b}	0.829 ^{a}	0.728 ^{b}	0.691 ^{b}	0.751 ^{b}	0.682 ^{b}	0.0259	0.004
Total n-6	14.1 ^{bc}	16.1 ^{a}	14.5 ^{bc}	14.1 ^{bc}	15.4 ^{ab}	13.7 ^{c}	0.48	0.001

SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids. [1] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [2] SEM: standard error of the mean. Within a row, least square means without a common letter ($^{a–c}$) differ ($p < 0.05$).

The effect of the type of castration and diet, as main effects, on the remaining results of subcutaneous fat composition of male pigs is presented in Table 8. The fat from IM presented lower ($p = 0.041$) total MUFA percentage than that from SCM, owing to the lower ($p = 0.028$) C18:1n-9 content. The reason for it would be in line with the preceding discussion of Trial 1; backfat thickness is generally thinner in IM [11], and the less developed the backfat, the less is the proportion of MUFA stored in the adipose tissue arising from de novo synthesis [37]. Mackay et al. [61] and Asmus et al. [62] also found lower total MUFA proportion in the fat of IM, whereas others authors [41,63] failed to detect differences between IM and SCM in this parameter. The lower MUFA content found in IM would be positive from a technological point of view, since MUFA have a negative influence on firmness and cohesiveness of fat tissue [64]. On the other hand, this finding would be less desirable from a health point of view, because MUFA have a beneficial effect on coronary heart disease risk [65]. Besides, IM had similar ($p = 0.920$) n-6/n-3 ratio than SCM, in agreement with the findings of Font-i-Furnols et al. [54] and Daza et al. [36].

In respect of experimental diets, fat from males fed the low-CP and -AA diet had lower ($p = 0.0005$) C15:0 percentage than that from males fed the control or the high-energy diets. Pigs that received the alternative diets to control presented lower percentages of C17:0 ($p = 0.002$) and C17:1 ($p < 0.0001$). Additionally, males fed the high-energy diet presented lower ($p = 0.009$) C18:1n-7 content and tended to show greater ($p = 0.066$) C18:1n-9 percentage than those fed the control or the low-CP and -AA diet. This tendency may be promoted by the higher content of C18:1n-9 in high-energy feed, which was supplemented with greater levels of palm oil. Pigs that received the alternative diets presented a higher ($p = 0.024$) n-6/n-3 ratio, which is not desirable from a health-focused point of view [66]. Madeira et al. [67] also found that CP and Lys reduction increased n-6/n-3 ratio. On the other hand, no influence ($p = 0.403$) of feeding strategy was observed on total MUFA content, which agrees with the works of Suárez-Belloch et al. [32] and Tejeda et al. [58].

Table 8. Impact of type of castration and diet on subcutaneous fat composition (fatty acids expressed as % of total fatty acids, except in ratios) of male pigs.

	Type of Castration		SEM [1] (n = 24)	Diet [2]			SEM [1] (n = 16)	p-Value [3]	
	Surgical	Immunological		Control	High Energy	Low CP and AA		Castration	Diet
C14:0	1.22	1.20	0.022	1.22	1.18	1.23	0.027	0.664	0.488
C14:1	0.012	0.012	0.0006	0.012	0.012	0.011	0.0007	0.786	0.546
C15:0 [4]	0.047	0.051	0.0021	0.055 [a]	0.050 [a]	0.041 [b]	0.0025	0.195	0.0005
C15:1 [4]	0.008	0.008	0.0003	0.009	0.008	0.007	0.0004	0.284	0.105
C16:1n-7 [4]	1.59	1.61	0.045	1.66	1.54	1.60	0.055	0.648	0.266
C17:0	0.282	0.313	0.0118	0.342 [a]	0.284 [b]	0.267 [b]	0.0145	0.064	0.002
C17:1	0.250	0.264	0.0097	0.308 [a]	0.233 [b]	0.231 [b]	0.0118	0.329	<0.0001
C18:0	12.7	13.1	0.24	12.7	12.8	13.2	0.29	0.235	0.418
C18:1n-7 [4]	1.70	1.67	0.034	1.76 [a]	1.58 [b]	1.73 [a]	0.042	0.478	0.009
C18:1n-9	42.4	41.7	0.24	41.6	42.6	41.9	0.29	0.028	0.066
C20:0	0.263	0.252	0.0087	0.254	0.263	0.256	0.0106	0.407	0.809
C20:3n-6	0.096	0.097	0.0031	0.102	0.093	0.095	0.0039	0.768	0.235
C20:5n-3	0.025	0.027	0.0014	0.027	0.025	0.027	0.0018	0.219	0.688
Total MUFA	47.2	46.4	0.26	46.6	47.2	46.7	0.32	0.041	0.403
n-6/n-3	20.0	20.0	0.17	19.5 [b]	20.2 [a]	20.3 [a]	0.21	0.920	0.024

MUFA: monounsaturated fatty acids. [1] SEM: standard error of the mean. [2] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [3] No significant interactions (type of castration × diet) were found ($p > 0.05$). [4] Least square means and SEM of the original data and p-values obtained with the transformed data. Within a row, least square means without a common letter ([a], [b]) differ ($p < 0.05$).

Table 9 shows the influence of type of castration in male pigs and diet on the boar-taint compounds analyzed in the samples of subcutaneous fat from SCM and IM whose testicular width was less than 11 cm. No significant interactions ($p > 0.05$) between type of castration and diet were found, and therefore, the results are shown as main effects. The concentration of androstenone was below the detection limit in all cases (0.20 μg/g). Likewise, skatole and indole concentrations were not influenced ($p > 0.10$) by the type of castration and the contents of both were low (<0.09 μg/g). Thus, SCM and IM had androstenone and skatole levels below the thresholds values for sensory acceptance (0.5–1.0 μg/g and 0.20–0.25 μg/g, respectively) [68]. These findings agree with those published by other authors [24,41,69]. However, Weiler et al. [70], injecting the second dose of immunocastration closer to slaughter, reported that IM had higher levels of androstenone and indole than SCM, although their mean values were equally low (<0.11 μg/g). It has been seen that immunization against GnRF hinders the formation of testicular steroids, including androstenone, by blocking hypothalamic-pituitary-gonadal axis, and therefore prevents the accumulation of this compound in fat tissue [71,72]. Besides, deprivation of testicular steroids, especially 17β-estradiol and androstenone, seems to reduce skatole formation in the intestine and to accelerate its degradation in the liver, and consequently would lead to decrease skatole accumulation in fat [72]. In addition, a similar effect could occur in the case of indole accumulation [69].

Table 9. Effect of type of castration and diet on boar-taint compounds (μg/g) in subcutaneous fat of male pigs.

	Type of Castration		Diet [1]			*p*-Value [2]	
	Surgical	**Immunological**	**Control**	**High Energy**	**Low CP and AA**	**Castration**	**Diet**
n	18	18	12	12	12		
Androstenone [3]	bd	bd	bd	bd	bd	-	-
Skatole [4]	0.028 ± 0.014	0.031 ± 0.018	0.037 ± 0.014	0.027 ± 0.019	0.025 ± 0.012	0.593	0.059
Indole [4]	0.021 ± 0.011	0.027 ± 0.018	0.031 ± 0.019	0.018 ± 0.008	0.022 ± 0.013	0.267	0.149

[1] Grower period: control (2.33 Mcal net energy-NE/kg, 16% crude protein-CP and 0.77% standardized ileal digestible lysine-SID Lys), high energy (2.48 Mcal NE/kg, 16% CP and 0.77% SID Lys) and low CP and amino acids (AA) (2.33 Mcal NE/kg, 14% CP and 0.67% SID Lys). Finisher period: control (2.33 Mcal NE/kg, 14.5% CP and 0.63% SID Lys), high energy (2.48 Mcal NE/kg, 14.5% CP and 0.63% SID Lys) and low CP and AA (2.33 Mcal NE/kg, 12.5% CP and 0.54% SID Lys). [2] No significant interactions (type of castration × diet) were found ($p > 0.05$). In the case of androstenone, *p*-values could not be obtained, since all values were under the detection limit of the equipment (0.20 μg/g). [3] bd: below the detection limit (0.20 μg/g). [4] Data were analyzed with non-parametric methods and are presented as mean ± standard deviation.

Regarding the fat samples of IM that showed a testicular width greater than 11 cm (data not shown), one of them had an androstenone concentration lower than the detection limit (0.20 μg/g) and the remaining presented values between 0.49 and 1.73 μg/g. The values of skatole ranged from 0.08 to 0.42 μg/g and those of indole from 0.06 to 0.23 μg/g. In the current trial, the proportion of IM that exceeded the target testicular width was 11%. Therefore, not all of these IM seem to have high values in all boar-taint compounds, but some of them did. Several reports [54,73,74] have also found a little proportion (between 0.74 and 11.8%) of IM designated as "non-responders". The reasons could be that those pigs are missed at vaccination moments in group-housing systems, or respond poorly to the immunization against GnRF, or have health problems, malnutrition or stress [48,71,75]. Therefore, disregarding the non-responders, immunocastration was effective in the prevention of boar taint.

No significant influence ($p > 0.05$) of the diet was observed on boar-taint compounds, although it should be noted that male pigs fed alternative diets to the control, especially those that received the low-CP and -AA diet, tended ($p = 0.059$) to present lower levels of skatole. This compound is a product of bacterial degradation of the amino acid tryptophan in the large intestine [71]. With the alternative diets, especially with the low-CP and -AA diet, pigs ingested a lower percentage of tryptophan, and therefore it could be expected to obtain a lower skatole concentration. However, it should be noted that tryptophan is mainly absorbed in the small intestine, and consequently is only available to a limited degree for microbial degradation in the large intestine, as the gut mucosa cell debris are a major source of tryptophan [76–78]. In the studies of Westergaard and Mortensen (cited by Malmfors et al. [79]) and Nold et al. [80], feed-protein content had no significant impact on skatole concentration. In the literature, the effect of increasing energy in the diet on skatole concentration is controversial. Lundström et al. [77] observed that providing a diet with high-nutrient density (high energy), skatole concentration decreased, while Neupert et al. [81] and Westergaard and Mortensen (cited by Malmfors et al. [79]) found the opposite effect. Lundström et al. [77] explained that the high-nutrient-density diet had a lesser content of fibre, which will decrease the fermentative process in the large intestine, reducing the number of bacteria and thus the microbial protein that could act as an extra source of tryptophan. However, Claus and Raab [82] described that a rise in energy supply leads to an increase in insulin-like growth factor-1 (IGF-1), rising the degree of mitosis in the intestine, and thus a parallel increase of the apoptotic cells provides the substrate for skatole formation. In the case of androstenone, nutritional influences are attributed to energy or to specific compounds in the ration, although the effect of nutrition, here, is less important than for skatole [83]. Claus et al. [76] found higher androstenone levels with a greater increase in energy content than that of the current trial, maybe because the high-energy diet increases IGF-1, which would stimulate Leydig cell steroidogenic responsiveness, and thus, androstenone formation [84]. Nevertheless, Zeng et al. [85], with a smaller difference of energy between the high- and low-energy diets, did not observe

any effect of feed energy content on androstenone concentration, as in the current study. Further research should be carried out to better understand the effect of dietary energy or AA levels on boar taint compounds.

4. Conclusions

Gilt immunocastration increases IMF content and generates a more adequate fatty acid profile for the curing process, although it also could result in less healthy products. In female pigs, irrespective of whether they are entire or immunocastrated, a high-energy diet or a low-CP and -AA diet at 76 to 134 kg of BW has little influence on meat quality and fat composition. Immunocastration in male pigs deteriorates meat chemical composition compared with surgical castration, because IMF is decreased, but it provides a similar or a better fatty acid profile, in terms of fat consistency, when a high-energy diet or a low-CP and -AA diet, respectively, is given at 80 to 137 kg of BW. However, a low-CP and -AA diet in IM could produce less healthy fat. In general, male pig immunocastration reduces boar-taint compounds as much as surgical castration, although it has to be noted that some animals are "non-responders", implying higher levels of those compounds in their fat.

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Data Availability Statement: Data available on request due to restrictions of privacy.

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Paper 5. Impact of immunocastration on dry-cured ham quality of female pigs

Impact of gilt immunocastration on weight losses and instrumental and chemical characteristics of Teruel dry-cured ham

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ABSTRACT: A total of 32 fresh hams intended for the Spanish Protected Designation of Origin "Teruel ham" were used to evaluate the impact of gilt immunocastration (*vs.* entire gilts) on weight losses during the dry-curing process. After processing, 20 dry-cured hams (10 of each group) were chosen at random to assess instrumental and chemical characteristics. Hams from immunocastrated gilts tended ($P = 0.057$) to present lower weight losses, they were fattier ($P < 0.05$) at both subcutaneous and intramuscular levels and had lower ($P < 0.05$) water activity and volatile compounds that provide unpleasant odors than those from entire gilts. However, immunocastration increased ($P < 0.05$) slightly sodium chloride and sodium nitrite contents, being levels normal. Fatty acid profile was not significantly affected ($P > 0.05$). It can be concluded that, in general, immunocastration could be a good strategy in gilts to improve the quality of Teruel dry-cured ham.

Keywords: gilt, immunocastration, weight loss, instrumental characteristics, chemical composition, Teruel dry-cured ham.

1. Introduction

In recent years, strategies have been sought to reduce the number of unsuitable carcasses for the Spanish Protected Designation of Origin (PDO) "Teruel ham" due to limited fat covering in the ham (Latorre et al., 2008). Fat thickness >16 mm is required for a correct dry-curing process because it prevents an excessive drying of the pieces and improves organoleptic characteristics (Bosi & Russo, 2004). In addition, hams destined for this PDO have moderate marbling (Rodríguez-Sánchez et al., 2014), parameter positively related to juiciness and tenderness (Ruiz-Carrascal et al., 2000). This problem of general lack of fat deposition (at subcutaneous and intramuscular levels) has been found principally in females (Latorre et al., 2008), because male pigs are surgically castrated to avoid boar taint, and castration increases the deposition of fat tissue (Weatherup et al., 1998). Surgical castration of females reared indoors is banned in the European Union (Official Journal of the European Union, 2009) but they could be immunocastrated. In industrial gilts, immunocastration consists of the application of one vaccine, whose active substance is a gonadotrophin releasing factor (GnRF) analogue-protein conjugate, in two doses. Antibodies against GnRF are generated, inhibiting follicle-stimulating and luteinizing hormones, and thus, suppressing temporarily the ovarian function. The effects of gilt immunocastration has been studied on fresh pork characteristics (Daza et al., 2014; Pérez-Ciria, Carcò, et al., 2021) but not on the quality of the dry-cured ham. Therefore, the goal of the current study was to evaluate the effect of gilt immunocastration on weight losses and on instrumental and chemical characteristics of Teruel dry-cured hams.

2. Material and methods

The raising and slaughter of the animals as well as the dry-curing process of the hams followed the regulations established by the *Consortium* of the PDO Teruel ham (Boletín Oficial de Aragón, 2017).

2.1. Experimental samples

A total of 32 fresh hams from Duroc x (Landrace x Large White) gilts were used in the trial. These pieces came from the experimental animals utilized in the work of Pérez-Ciria, Miana Mena, et al. (2021) in which the influence of gilt immunocastration and different diets was evaluated on productive performances and carcass quality. All the experimental procedures used in that trial followed the ethical committee requirements of the University of Zaragoza (ref. PI29/18). Concretely, the hams were chosen at random from gilts that received

a high-energy diet; 16 belonged to entire gilts (EG) (intact throughout the study) and 16 belonged to immunocastrated gilts (IG). The immunization of that group against GnRF was carried out with Vacsincel® (Zoetis Spain S.L., Alcobendas, Madrid, Spain). The priming dose and the booster were applied at 102 and 122 ± 3 days of age, respectively (with 58 and 77 kg of average body weight). The general management and feeding at the farm were the same for all of them. All pigs were slaughtered in the abattoir (Teruel, Spain) at the same day, with 133 kg of average body weight (199 days of age). There, the left ham from each carcass was taken, trimmed and individually weighed.

2.2. Dry-curing process and sampling

All hams were processed as one group. Upon arrival at the ham-curing facilities, hams were classified according to the weight. Then, the residual blood was removed by a bleeding-massaging machine that presses the femoral artery. The six phases of the dry-curing process were the following: i) salting; each ham was introduced in a salting tumbler and 2.5 g of nitrifying salt (a mixture of sodium chloride, maltodextrin, sodium ascorbate and potassium nitrate) per kg of meat mass were applied. Then hams were placed in stackable bins, coated with common salt and kept at 0-2°C and 75-90% of relative humidity (RH) for 0.8 days per kg of meat mass. ii) washing with water and molded. iii) resting; hams were hung in racks with hangers and stored from 3.5 to 5°C and from 80-82 to 72-77% of RH for 90 days. iv) drying; the temperature was gradually increased from 8 to 21°C and the RH reduced from 70-75 to 68-73% for 136 days. Finally, lard was applied manually to the muscular part of the hams to prevent the entry of microorganisms and to avoid over-drying. v) maturing; the temperature continued increasing from 25 to 28°C and the RH was maintained at 70-75% for 79 days. vi) aging; hams stayed in a natural dryer until reaching 32°C for 256 days. The individual weight of all pieces was recorded after salting, resting, drying, maturing and aging.

Once the dry-curing process ended (19 months later), a total of 20 hams (10 from EG and 10 from IG), chosen at random, were manually boned, sectioned in three parts and individually vacuum packaged. The proximal part of each ham (the opposite part to the hoof) was chosen to carry out the laboratorial analyses and was stored at 4°C until then. One month later, one slice of the sectioned surface of each piece was removed with a slicer (Sammic S.L., Azkoitia, Gipuzkoa, Spain) to determine the color parameters in the piece and another slice was cut to carry out the image analyses. After muscle dissection, the *biceps femoris* muscle (170 ± 20 g) was destined to measure texture. Finally, this muscle was minced with a chopper

(Moulinette chopper dpa1, Moulinex®, Groupe SEB Iberica S.A., Barcelona, Spain) to analyze the chemical composition, fatty acid (FA) profile of intramuscular fat (IMF) and volatile compounds.

2.3. Color parameters

Color was evaluated on subcutaneous fat and on the muscles *quadriceps femoris* and *biceps femoris* using a spectrophotometer (CM-2600d, Konica Minolta Holdings, Inc., Osaka, Japan), previously calibrated, with illuminant D65 and observer angle of 10º, in CIELAB color space (CIE, 1986). The average of three random readings of each section was used to obtain lightness (L*), redness (a*), yellowness (b*), chroma (C^*_{ab}) and hue angle (h_{ab}).

2.4. Subcutaneous fat thickness and marbling by image analysis

One photography of each slice was taken following the procedure carried out by Ripoll, Alcalde, Argüello, et al. (2019). All images were transferred to a computer and no image editing was applied other than the cropping of the image. Subcutaneous fat thickness was measured at three points (Fig. 1): at the midpoint of the *quadriceps femoris* muscle, between the *quadriceps femoris* muscle and the *biceps femoris* muscle, and at the right side of the *biceps femoris* muscle. Marbling was estimated in the *biceps femoris* muscle following the methodology described in the work of Mendizabal et al. (2005). The program ImageJ v1.48 (National Institutes of Health, USA) was used to determine subcutaneous fat thickness and marbling.

2.5. Texture

The measure of maximum stress was performed following the procedure described by Honikel (1998). Each sample was cut in prism-shaped pieces with a 100 mm^2 (10 x 10 mm) cross-section with the fiber direction parallel to a long dimension of at least 30 mm. A total of 8-10 prisms per sample were sheared perpendicular to the fiber orientation using a Warner-Bratzler device, with a cross-head speed of 2.5 mm/s, attached to an Instron universal testing machine (Model 5543, Instron Ltd, Buckinghamshire, United Kingdom) attached to a computer. Maximum stress was the load at maximum peak shear force per unit of cross-section (Ripoll, Alcalde, Córdoba, et al., 2019).

2.6. Chemical composition

Moisture, ash, protein and IMF were analyzed following the procedures of Boletín Oficial del Estado (1979). Moisture was determined using an oven (Memmert UFE500, Schwabach, Germany) at 102°C during 48 h and ash by a muffle (Model 10-PR/400, Forns Hobersal S.L., Caldes de Montbui, Barcelona, Spain) at 550°C during 7 h. Protein was analyzed utilizing a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden) and IMF by an ANKOM^{XT15} Extraction System (ANKOM Techonology, Macedon, New York, USA) after being hydrolyzed the samples (ANKOM^{HCL} Hydrolysis System).

Sodium chloride was determined following the procedure described by Matissek et al. (1998). For that, a total of 3 g of each sample were weighted and 50 mL of milli-Q water were added. Samples were agitated in a shaker-incubator (Rotabit, J.P. Selecta S.A., Abrera, Barcelona, Spain) at 190 rpm during 30 min using a magnet and 2 mL of nitric acid were added. Finally, samples were analyzed in a titrator (SM Titrino 702, Metrohm Hispania, Madrid, Spain).

Potassium nitrate and sodium nitrite were also analyzed following the official methods of analysis of meat products described in Boletín Oficial del Estado (1979, 1981, 1982). In the case of potassium nitrate, 4 g of each sample were weighted in an Erlenmeyer flask of 250 mL and 150 mL of ethyl alcohol were added. Samples were agitated in a thermostatic bath (Bunsen BTG, Bunsen, Humanes de Madrid, Madrid, Spain) during 1 h. Once cooled, 5 mL of each of the Carrez reagents I and II, prepared with zinc acetate dihydrate and potassium hexacyanoferrate (II) trihydrate, respectively, were added, and milli-Q water were also added to level the flask at 250 mL. The content of this flask was filtered in a flask of 100 mL until its level. The filtrated was discarded and the remaining part was put in another flask of 250 mL, which was placed in a heating plate (Combiplac, J.P. Selecta S.A., Abrera, Barcelona, Spain) to evaporate ethyl alcohol, until achieving a volume of 50 mL. Then, this volume was transferred to the flask of 100 mL and milli-Q water was added to level it and it was flipped. Later, a total of 10 mL were transferred to a 50 mL flask and 1 mL of brucine-sulfanilic acid and 10 mL of sulphuric acid were added (color reaction) and it was left to rest 10 min in the dark. This flask was made up to 40 mL with milli-Q water and left to rest 15 min in the dark. Later, it was cooled and levelled. Lastly, a spectrophotometer (Shimadzu UV-1700 Pharmaspec, Kyoto, Japan) was used to determine potassium nitrate content at 410 nm. The procedure to determine sodium nitrite was similar, except for the reagent used for the color

reaction, which was prepared mixing equal parts of two solutions. The first solution contained 1.50 g of sulfanilic acid, 50 mL of acetic acid and approximately 200 mL of milli-Q water to make up to 250 mL. The second solution contained 0.075 g of 1-naphthylamine, 50 mL of acetic acid and approximately 200 mL of milli-Q water to make up to 250 mL.

Collagen and water activity (a_w) were determined by near-infrared spectroscopy (measuring range: 850-1100 nm). Each sample was put in a circular small cup of 8.8 mm of depth and 134 mm of diameter that was introduced in the FoodScan™2 equipment (FOSS Iberia S.A., Barcelona, Spain).

The contents of α-tocopherol, γ-tocopherol, δ-tocopherol, retinol and cholesterol were determined following the methods described in the paper of Bertolín et al. (2018) using ultra-high performance liquid chromatography. The equipment used was an ACQUITY UPLC H-Class liquid chromatograph (Waters, Milford, Massachusetts, USA) equipped with a silica-based bonded phase column (Acquity UPLC HSS T3, 1.8 μm × 2.1 mm × 150 mm column; Waters), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ Detector; Waters) and a fluorescence detector (2475 Multi λ Fluorescence Detector; Waters). To determine lipid oxidation, the content of malondialdehyde (MDA) was analyzed, following the methodology of Bertolín et al. (2019) using ultra high performance liquid chromatography coupled to a fluorescence detector.

2.7. Fatty acid profile of IMF

Firstly, all samples were lyophilized. Then the FA extraction and methylation was carried out following the methodology of Lee et al. (2012). A Bruker Scion 436-GC gas chromatograph (Bruker, Billerica, Massachusetts, USA) equipped with SP-2560 capillary column (100 m x 0,25 mm ID x 0,20 μm film thickness; Supelco, Saint Louis, Missouri, USA) was used for FA determination. The identification of the FAs was done using certified reference materials (GLC-401, GLC-463, GLC-532, GLC-538, GLC-642 and GLC-643, Nu-Chek Prep Inc., Elysian, Minnesota, USA). The FAs were quantified based on the guidelines described in ISO 12966-4 (2015) as mg /100 g of muscle (wet matter). The contents of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 and n-6 and the ratios PUFA/SFA and n-6/n-3 were calculated from individual FA concentrations.

2.8. Volatile compounds

Static headspace technique by using a Turbomatrix HS16 sampler (PerkinElmer, Massachusetts, USA) was used to analyze the volatile profile. A total of 4 g of each homogenized sample were placed in vials of 20 mL that were hermetically closed. The samples were thermostatized at 130ºC for 20 min and 1 min of pressurization time. The injection was carried out over 12 s at 25 psi and an inlet temperature of 220ºC. A Clarus 500 gas chromatograph coupled with a mass spectrometer (PerkinElmer, Massachusetts, USA) equipped with a DB-Wax capillary column (60 m x 0.25 mm ID x 0.25 µm film thickness; Agilent Technologies, California, USA) was used to separate and identify the extracted compounds. A flow of 1 mL/min of helium was used as carrier gas. The oven temperature was 45ºC held for 2 min, 45-200ºC at a rate 4ºC/min, and finally to 225ºC at 10ºC/min, and held for 5 min. The mass spectrometer used the electron impact mode with an ionization potential of 70 eV and an ion source temperature of 200ºC. The interface temperature was 220ºC. The mass spectrometer scanned in full scan mode (35-300 m/z). A TurboMass version 5.4.2 Workstation was used for the gas chromatograph-mass spectrometer system. Tentative identification of the volatile components was achieved by comparison of the mass spectra with mass spectral data from the Nist MS Search Program 2.0 library and by comparison of previously reported Retention Index with those calculated using a n-alkane (C7-C25) series under the same analysis conditions according to the equation of Van den Dool & Kratz (1963). The relative percentage was expressed as a mass fraction of the total peaks area and fluorobenzene was used as internal standard. The percentages of total aldehydes, ketones, acids, hydrocarbons, alcohols, furans and sulfur compounds were calculated from individual volatile compound percentages.

2.9. Statistical analysis

All statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Data were analyzed using the GLM procedure. The model included the type of gilt (EG or IG) as fixed effect. Fresh ham weight and final dry-cured ham weight were included as covariates, when significant ($P < 0.05$), to analyze ham weight losses and the rest of the variables studied, respectively. Least square means were compared using the PDIFF option.

Normality of the residuals was checked with Shapiro-Wilk's test using the UNIVARIATE procedure. In cases in which normality was not achieved, variables were

transformed with $\sqrt{x}$ or $\sqrt[4]{x}$ or Napierian logarithm or x^2 or $1/(x+1)$ before statistical analysis if it was possible. When normality could not be found with data transformation, Mann-Whitney U-test was carried out to analyze these variables. Homogeneity of variances was checked with Levene's test. When homoscedasticity was not achieved, Welch's test was applied.

The gilt was the experimental unit (n=16 for weight losses and n=10 for the rest of the variables). A P-value < 0.05 was considered as a significant difference and a P-value between 0.05 and 0.10 as a tendency.

3. Results and discussion

To the best of our knowledge, this is the first paper evaluating the effect of immunocastration on dry-cured hams from gilts. Therefore, in some parameters, studies on immunocastration in male pigs (entire *vs.* immunocastrated males) have been used for the discussion.

3.1. Ham weight losses during processing

As shown in Table 1, even though fresh ham weight was similar (P = 0.720) in EG and in IG, hams from IG tended (P = 0.057) to be heavier after the dry-curing process than those from EG. The reason is that pieces from IG tended (P < 0.10) to present lower weight losses after resting, drying, maturing and aging phases, giving 2.2%-point difference at the end. Therefore, the production of dry-cured hams from IG could be economically positive for ham cellars. Škrlep et al. (2016) did find significant that hams from immunocastrated males had lower processing losses than those from entire males. These results would be expected since immunocastrated pigs, both females and males, usually showed greater fat thickness covering the ham than entire pigs (Daza et al., 2014; Škrlep et al., 2020), and adipose tissue contains less water than muscular tissue and hinders exchanges between muscular part and external environment (Bosi & Russo, 2004).

3.2. Instrumental characteristics

Gilt immunocastration had no effect (P > 0.10) on color parameters of ham subcutaneous fat (Table 2). However, hams from IG tended (P = 0.057) to show lower C^*_{ab} value in the *quadriceps femoris* muscle and this effect was significant (P = 0.017) in the *biceps femoris* muscle. Besides, IG tended (P = 0.055) to have lower b^* value than EG only in the *biceps femoris* muscle. It has to be noted that, according to Zanardi et al. (1999), L^* and h_{ab}

parameters are those strictly linked with human perception of pork color, and none of them were affected ($P > 0.10$) by gilt immunocastration. Therefore, as for color of fat and muscle, hams from IG would result considerably similar to those from EG for consumers.

On the other hand, hams from IG had thicker ($P < 0.05$) subcutaneous fat depth than those from EG at *quadriceps femoris* and *biceps femoris* muscles (Table 3). It was expected because castration increases fat deposition (Peinado et al., 2008), and it would corroborate the differences observed in the weight losses of the pieces. However, this effect was not significant ($P = 0.685$) detected in the marbling, although numerically IG also presented a higher value than EG (5.72 vs 5.29%). A similar effect was observed by Gamero-Negrón et al. (2018) in dry-cured loins from female pigs and by Čandek-Potokar et al. (2020) in dry-cured hams from male pigs when pig immunocastration was researched.

Likewise, there was no difference ($P = 0.433$) on maximum stress between EG and IG (60.5 *vs.* 56.2 N/cm^2, respectively) (data not shown in tables). Škrlep et al. (2016) and Čandek-Potokar et al. (2020) also not found differences on texture profile of the *biceps femoris* muscle between entire males and immunocastrated males.

3.3. Chemical composition

As shown in Table 4, hams from IG showed lower ($P = 0.001$) moisture percentage and higher ($P = 0.049$) IMF content and tended ($P = 0.091$) to present greater ash proportion than those from EG. Gamero-Negrón et al. (2015) also found higher IMF content in dry-cured loins from IG than in those from EG. Likewise, Škrlep et al. (2016) observed that immunocastrated males had greater IMF proportion in the *biceps femoris* muscle than entire males. Our results about IMF supports the differences detected in subcutaneous fat thickness and the numerical effect found on marbling, corroborating that gilt immunocastration increases body fat deposition. Besides, the higher IMF content in hams from IG could improve the development of typical aromatic and textural properties (Kaltnekar et al., 2016).

The pieces from IG presented higher ($P < 0.05$) sodium chloride and sodium nitrite contents than those from EG, which exerts a greater preservative effect. The values of both compounds were within the normal range for this kind of product (Rodríguez-Sánchez et al., 2014). It has to be noted that experts from the World Health Organization and from the Food and Agriculture Organization of the United Nations recommend avoiding daily consumption above 5 g of sodium chloride to prevent diet-related chronic diseases (WHO/FAO, 2003). Potassium nitrate followed the same line, but the effect was not significant ($P = 0.132$). These

differences could be due to salt and nitrites are more concentrated in the muscle of those animals with lower moisture content (IG). Also, some factors during salting could have differed between hams, which might have influenced the formation of the surface brine and the diffusion of sodium, chloride and nitrite ions. Arnau (2007) indicated that these factors are: the way to place hams and the ham situation in the stackable bins during salting, the lean surface shape, the size and pH of the hams, the water holding capacity of the muscles and the content of water in the surface of the hams. Therefore, further studies are needed to understand better the effect of female immunocastration on salt contents of dry-cured ham.

Hams from IG had similar (P = 0.346) collagen proportion than those from EG, confirming the lack of effect found on texture analysis. The lower moisture content and the higher sodium chloride concentration detected in hams from IG led to lower (P = 0.015) a_w than that observed in EG, which would suggest lower microbial growth and thus a greater shelf life (Blesa et al., 2008). In male pigs, results are contradictory; Čandek-Potokar et al. (2020) did not observe differences in a_w of the *biceps femoris* muscle between entire and immunocastrated males and Škrlep et al. (2016) found that immunocastrated males tended to present higher a_w than entire males.

No significant differences (P > 0.05) were found in α-tocopherol, γ-tocopherol, δ-tocopherol and retinol concentrations. It is worth noting that hams from IG tended (P = 0.074) to show greater cholesterol content than those from EG, being this compound a major factor in the pathogenesis of atherosclerosis (Connor & Connor, 2002). However, this cholesterol content (0.89 mg/g of ham) is not worrisome, because the maximum daily consumption recommended by healthy reasons is 300 mg of cholesterol (WHO/FAO, 2003). Both types of hams presented similar (P = 0.905) content of MDA, confirming the results of Gamero-Negrón et al. (2015) with dry-cured loins and shoulders from EG and IG, and those of Čandek-Potokar et al. (2020) by comparing dry-cured hams from entire and immunocastrated males. This result is in accordance with the lack of effect found on tocopherol concentrations, because these compounds have antioxidant function (Di Mascio et al., 1991).

3.4. Fatty acid profile of IMF

Table 5 shows the impact of gilt immunocastration on FA profile of the *biceps femoris* muscle of dry-cured hams. That type of castration had no effect (P > 0.10) on total SFA and MUFA concentrations and either on PUFA/SFA and n-6/n-3 ratios. However, hams from IG tended to present lower total PUFA (P = 0.057), n-3 (P = 0.098) and n-6 (P = 0.062) contents

than those from EG, although, as seen previously, lipid oxidation (the content of MDA) was not affected. These findings were due to the lower values of C18:2n-6 (P = 0.078), C20:4n-6 (P = 0.087), C20:5n-3 (P = 0.037), C22:4n-6 (P = 0.049) and C22:5n-3 (P = 0.018) in IG. Gamero-Negrón et al. (2015), in dry-cured shoulders of Iberian x Duroc females, did not find differences on total SFA, MUFA and PUFA contents between EG and IG, but in dry-cured loins, those authors did observe that IG had greater total SFA percentage and lower total PUFA content. Therefore, further studies are needed to understand better the effect of gilt immunocastration on the fatty acid profile of cured products.

3.5. Volatile compounds

A total of 40 volatile compounds, generated mainly by proteolysis and lipolysis (Toldrá, 1998), were identified in the *biceps femoris* muscle of the experimental dry-cured hams (Table 6), including the following groups: aldehydes (9), ketones (7), acids (3), hydrocarbons (7), alcohols (11), furans (2) and sulfur compounds (1).

Despite the lower percentage of octanal (P = 0.006), (E)-hept-2-enal (P = 0.023), nonanal (P = 0.024), (E)-2-octenal (a trend, P = 0.059) and (E)-2-nonenal (P = 0.040) found in hams from IG, immunocastration had not significant influence (P = 0.603) on the proportion of total aldehydes, being this group the most abundant (around 70% of the total). Škrlep et al. (2016) also not found differences on total aldehydes with male pig immunocastration. These compounds play an important role in ham aroma due to their low perception thresholds and their distinctive characteristic odors (Pugliese et al., 2010). According to Flores et al. (1997), these compounds generate green, oily, fatty and tallowy flavors.

Hams from IG presented similar percentage (P = 0.738) of total ketones than EG, in agreement with the results of Škrlep et al. (2016) comparing immunocastrated and entire male pigs. Within this group of compounds, IG tended (P = 0.080) to present a higher proportion of 2,3-butanedione, which is responsible for buttery notes (Flores et al., 1997), and lower proportions of 2-heptanone (P = 0.011), 3-octanone (P = 0.002) and 2-octanone (P = 0.002) than EG. The 2-heptanone and 2-octanone are methyl ketones having a very strong odor and being neat contributors to ham aroma (García et al., 2013; Sabio et al., 1998). The sensory attributes of 2-heptanone are blue cheese and spicy and a great intensity of these perceptions has been described as a symptom of bad quality, because this compound can be formed by microorganisms (Berdagué et al., 1991; Creuly et al., 1992; Luna et al, 2006; Sabio et al., 1998). In contrast, 2-octanone has been associated to the olfactory characteristics green and

herbaceous (Berdagué et al., 1991). Also, 3-octanone is a remarkable ketone since its very low odor threshold makes it to contribute to ham aroma with spicy, mushroom and dirty notes (García-González et al., 2013).

Gilt immunocastration had no significant influence ($P > 0.05$) on any individual acid or hydrocarbon, nor on the total percentage of acids or hydrocarbons. However, hams from IG presented lower ($P = 0.044$) proportion of total alcohols than those from EG, owing to the lower percentages of 1-pentanol ($P = 0.005$), hexanol ($P = 0.009$), 1-octen-3-ol ($P = 0.002$) and 1-octanol ($P = 0.043$). Conversely, Škrlep et al. (2016) found in hams from male pigs that immunocastration tended to increase the relative abundance of total acids and alcohols. We have no explanation for it but we will study it in more detail in the future. The influence of alcohols in the overall aroma seems to be low, since they have higher flavor thresholds compared to carbonyl compounds (Drumm & Spanier, 1991). Nevertheless, in general, the straight chain primary alcohols, such as 1-pentanol, hexanol and 1-octanol, did affect the overall flavor (Garcia et al., 1991). According to García-González et al. (2008), the aroma notes of 1-pentanol are pungent, strong and balsamic, of hexanol fruity and green and of 1-octanol fatty and sharp. In addition, unsaturated alcohols, such as 1-octen-3-ol, may play an important role in the odor because they have a lower threshold value (Sabio et al., 1998). This compound has a mushroom-like, earthy and dust aroma notes (García-González et al., 2008). Therefore, the lower presence of 1-octen-3-ol in hams from IG could indicate a positive effect on their sensory characteristics.

Hams from IG showed lower ($P = 0.017$) percentage of total furans, owing to the lower 2-ethylfuran ($P = 0.031$) and 2-pentylfuran ($P = 0.002$) proportions. Škrlep et al. (2016) also observed lower normalized areas of 2-ethylfuran and 2-pentylfuran in hams from immunocastrated males than in those from entire males. According to Elmore et al. (1999), it is unlikely that furans contribute significantly to the flavor characteristics because their odor threshold values are relatively high. Nevertheless, 2-pentylfuran has a quite low odor threshold and green fruity notes, and thus, it could play an important role in the overall aroma (García-González et al., 2008).

Finally, hams from IG tended to present lower ($P = 0.069$) percentage of the only sulfur compound detected (dimethyl disulfide) than those from EG. This compound has a low odor threshold (García-González et al., 2008), and thus, it could be an important contributor to ham flavor. In the literature (Flores et al., 1997; García-González et al., 2008) it has been reported

that dimethyl disulfide presents an unpleasant aroma defined as dirty socks or cauliflowers. Therefore, hams from IG could have a better flavor than those from EG. Lastly, it is worth noting that the higher amount of fat found in hams from IG than in those from EG could be an important reason of the differences in the proportions of volatile compounds, although further research has to be carried out in this field.

4. Conclusions

Under our experimental conditions, it can be concluded that gilt immunocastration increases the fat deposition of dry-cured hams, both subcutaneous and intramuscular, tending to reduce weight losses during the dry-curing process, which is positive for Teruel ham elaboration. Immunization against GnRF of gilts does not affect (or very slightly) some quality variables of hams as color, texture, oxidation or fatty acid profile, but it seems to increase the contents of sodium chloride and sodium nitrite, although the concentrations detected were normal ranged for this type of product. Additionally, immunocastration could prolong the shelf life of dry-cured hams by reducing their water activity and favor their aroma by reducing some unpleasant odors.

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Table 1. Impact of gilt immunocastration on weight losses (least square means) of dry-cured hams.

	Type of gilt		SEM[a]	*P*-value
	Entire	Immunocastrated	(*n* =16)	
Ham weight, kg				
Fresh	13.1	13.3	0.355	0.720
Dry-cured	8.63	8.91	0.100	0.057
Weight losses[b], %				
After salting	5.88	5.32	0.290	0.180
After resting	20.4	19.1	0.478	0.070
After drying	27.0	25.4	0.599	0.071
After maturing	32.1	30.1	0.702	0.057
After aging	34.8	32.6	0.771	0.057

[a] SEM: standard error of the mean.

[b] Relative to the fresh-ham weight.

Table 2. Effect of gilt immunocastration on color parameters (least square means) of dry-cured hams.

	Type of gilt		SEM[a]	*P*-value
	Entire	Immunocastrated	(*n* =10)	
Subcutaneous fat				
Lightness, L^*	71.8	74.0	1.054	0.171
Redness, a^*	3.36	3.28	0.277	0.850
Yellowness, b^*	9.90	9.69	0.336	0.670
Chroma, C^*_{ab}	10.5	10.3	0.330	0.632
Hue angle, h_{ab}	71.4	71.1	1.558	0.894
Quadriceps femoris muscle				
Lightness, L^*	37.8	38.1	0.515	0.730
Redness, a^*	11.6	10.9	0.457	0.302
Yellowness, b^*	6.58	5.09	0.623	0.108
Chroma, C^*_{ab}	13.4	12.1	0.464	0.057
Hue angle, h_{ab}	29.2	24.7	2.722	0.259
Biceps femoris muscle				
Lightness, L^*	43.7	43.8	0.762	0.961
Redness, a^*	12.6	11.8	0.313	0.120
Yellowness, b^*	8.80	7.45	0.468	0.055
Chroma, C^*_{ab}	15.4	14.0	0.377	0.017
Hue angle, h_{ab}	34.9	32.0	1.591	0.210

[a] SEM: standard error of the mean.

Table 3. Impact of gilt immunocastration on subcutaneous fat thickness and marbling (least square means) of dry-cured hams.

	Type of gilt		SEM[a]	*P*-value
	Entire	Immunocastrated	(*n* =10)	
Subcutaneous fat thickness, mm				
At the *quadriceps femoris*	12.6	16.3	1.201	0.040
Between *quadriceps* and *biceps femoris*[b]	14.0	16.5	1.434	0.130
At the *biceps femoris*	12.7	17.0	1.144	0.017
Marbling of the *biceps femoris*, %	5.29	5.72	0.728	0.685

[a] SEM: standard error of the mean.

[b] Least square means and SEM of the original data and *P*-value obtained with the transformed data.

Table 4. Effect of gilt immunocastration on chemical composition (least square means) of the *biceps femoris* muscle of dry-cured hams[a].

	Type of gilt		SEM[b]	*P*-value
	Entire	Immunocastrated	(*n*=10)	
Moisture, %	57.1	55.3	0.320	0.001
Ash, %	6.58	7.02	0.175	0.091
Protein, %	31.3	31.8	0.262	0.243
Intramuscular fat, %	4.39	5.54	0.387	0.049
Sodium chloride, g/100g	4.68	5.24	0.176	0.037
Potassium nitrate, mg/kg	98.8	117.1	8.217	0.132
Sodium nitrite, mg/kg	0.42	0.66	0.061	0.014
Collagen[c], %	1.23	1.36	0.097	0.346
Water activity, a_w	0.91	0.90	0.002	0.015
α-Tocopherol, µg/g	4.02	3.97	0.189	0.869
γ-Tocopherol, µg/g	0.28	0.26	0.021	0.639
δ-Tocopherol, µg/g	0.02	0.02	0.001	0.252
Retinol, ng/g	17.4	20.6	1.406	0.126
Cholesterol, mg/g	0.85	0.89	0.017	0.074
Malondialdehyde, mg/kg	0.50	0.49	0.044	0.905

[a] Results expressed on wet matter basis.

[b] SEM: standard error of the mean.

[c] Least square means and SEM of the original data and *P*-value obtained with the transformed data.

Table 5. Impact of gilt immunocastration on fatty acid profile (mg / 100 g of muscle, on wet matter basis) (least square means) of the *biceps femoris* muscle of dry-cured hams.

	Type of gilt		SEM[a]	*P*-value
	Entire	Immunocastrated	(*n*=10)	
C8:0	0.66	0.59	0.081	0.569
C9:0	0.30	0.34	0.037	0.428
C10:0	1.91	1.89	0.162	0.926
C11:0	0.60	0.63	0.070	0.807
C12:0	1.18	1.21	0.107	0.829
C12:1-9c[b]	1.34	0.81	0.489	0.676
C13:0	0.06	0.05	0.016	0.629
C14:0	15.2	15.5	1.259	0.837
C14:1[b]	0.26	0.30	0.050	0.498
C15:0	0.66	0.63	0.054	0.730
C16:0	325	320	18.75	0.831
C16:1-7c	5.26	4.74	0.194	0.079
C16:1-9c	31.1	29.3	2.688	0.654
C16:1-11c	2.73	2.49	0.149	0.278
C17:0	2.72	2.73	0.201	0.974
C17:1-9c	1.62	1.42	0.128	0.271
C18:0	177	174	9.006	0.840
C18:1-9c	456	464	32.53	0.863
C18:1-11c	46.8	45.0	3.032	0.688
C18:2n-6[b]	287	264	9.033	0.078
C18:3n-3	7.60	7.20	0.417	0.504
C18:3n-6	5.14	5.38	0.414	0.688
C19:0	0.39	0.34	0.056	0.493
C19:2n-6[b]	0.13	0.22	0.043	0.149
C20:0	1.50	1.41	0.101	0.560
C20:1	7.28	7.71	0.646	0.644
C20:2n-6	6.72	6.65	0.275	0.869
C20:3n-6[b]	9.72	8.86	0.511	0.221

C20:4n-6[b]	79.5	70.9	3.614	0.087
C20:5n-3	2.24	1.81	0.134	0.037
C21:0[b]	0.08	0.09	0.025	0.949
C22:0	0.31	0.32	0.040	0.794
C22:3n-3[b]	3.60	4.14	0.412	0.445
C22:4n-6	11.6	10.4	0.387	0.049
C22:5n-3	6.15	5.23	0.247	0.018
C22:5n-6	5.96	5.08	0.417	0.150
C22:6n-3	2.09	1.97	0.142	0.566
C24:0	0.29	0.34	0.063	0.576
Total SFA[c]	528	520	30.44	0.860
Total MUFA[d]	552	555	39.03	0.949
Total PUFA[b,e]	428	392	13.53	0.057
PUFA/SFA	0.83	0.76	0.038	0.245
Total n-3	21.7	20.4	0.541	0.098
Total n-6[b]	406	372	13.33	0.062
n-6/n-3	18.9	18.3	0.638	0.534

[a] SEM: standard error of the mean.

[b] Least square means and SEM of the original data and *P*-value obtained with the transformed data.

[c] SFA: saturated fatty acids.

[d] MUFA: monounsaturated fatty acids.

[e] PUFA: polyunsaturated fatty acids.

Table 6. Effect of gilt immunocastration on volatile compounds (chemical compound area/ internal standard- fluorobenzene- area expressed as %) (mean ± standard error) of the *biceps femoris* muscle of dry-cured hams.

	Type of gilt		*P*-value
	Entire	Immunocastrated	
Aldehydes			
3-Methylbutanal	67.70 ± 1.095	69.30 ± 2.622	0.395
Hexanal	1.126 ± 0.326	1.322 ± 0.349	0.794
Heptanal	0.172 ± 0.042	0.082 ± 0.020	0.120
Octanal	0.062 ± 0.014	0.016 ± 0.004	0.006
(E)-Hept-2-enal	0.080 ± 0.025	0.010 ± 0.006	0.023
Nonanal	0.144 ± 0.029	0.049 ± 0.009	0.024
(E)-2-Octenal	0.039 ± 0.009	0.015 ± 0.004	0.059
Benzaldehyde	0.306 ± 0.127	0.252 ± 0.137	0.940
(E)-2-Nonenal	0.019 ± 0.006	0.006 ± 0.001	0.040
Total aldehydes	69.65 ± 0.896	71.07 ± 2.483	0.603
Ketones			
2-Propanone	17.44 ± 0.757	17.50 ± 2.395	0.738
2-Butanone	2.718 ± 0.274	4.519 ± 0.954	0.105
2,3-Butanedione	1.031 ± 0.183	1.428 ± 0.165	0.080
2-Pentanone	0.006 ± 0.001	0.017 ± 0.009	1.000
2-Heptanone	0.635 ± 0.176	0.195 ± 0.073	0.011
3-Octanone	0.053 ± 0.015	0.007 ± 0.002	0.002
2-Octanone	0.054 ± 0.014	0.009 ± 0.002	0.002
Total ketones	21.94 ± 0.902	23.68 ± 2.497	0.738
Acids			
2-Methylpropanoic acid	0.188 ± 0.129	0.021 ± 0.004	0.079
Butanoic acid	0.920 ± 0.194	0.596 ± 0.066	0.320
Hexanoic acid	1.415 ± 0.262	1.368 ± 0.223	0.970
Total acids	2.523 ± 0.342	1.985 ± 0.182	0.256
Hydrocarbons			
Hexane	0.126 ± 0.033	0.119 ± 0.070	0.208
Heptane	0.189 ± 0.063	0.232 ± 0.050	0.527

Octane	1.814 ± 0.369	1.259 ± 0.288	0.157
α-Pinene	0.010 ± 0.004	0.012 ± 0.004	0.519
Methylbenzene	0.290 ± 0.071	0.444 ± 0.074	0.120
Ethylbenzene	0.005 ± 0.003	0.002 ± 0.002	0.732
Limonene [a]	0.007 ± 0.006	-	0.094
Total hydrocarbons	2.440 ± 0.356	2.067 ± 0.297	0.396
Alcohols			
Ethanol	0.474 ± 0.250	0.137 ± 0.032	0.970
2-Propanol	0.094 ± 0.026	0.051 ± 0.022	0.265
2-Butanol	0.004 ± 0.001	0.004 ± 0.001	1.000
2-Methyl-1-propanol	0.067 ± 0.013	0.128 ± 0.041	0.320
Butanol	0.060 ± 0.036	0.001 ± 0.001	0.100
3-Methyl-1-butanol	0.552 ± 0.324	0.293 ± 0.102	0.852
1-Pentanol	0.225 ± 0.030	0.102 ± 0.016	0.005
2-Heptanol	0.023 ± 0.007	0.009 ± 0.003	0.119
Hexanol	0.397 ± 0.082	0.103 ± 0.024	0.009
1-Octen-3-ol	0.132 ± 0.011	0.050 ± 0.009	0.002
1-Octanol	0.011 ± 0.002	0.005 ± 0.002	0.043
Total alcohols	2.038 ± 0.428	0.884 ± 0.170	0.044
Furans			
2-Ethylfuran	0.420 ± 0.113	0.171 ± 0.109	0.031
2-Pentylfuran	0.526 ± 0.289	0.001 ± 0.001	0.002
Total furans	0.946 ± 0.353	0.171 ± 0.109	0.017
Sulfur compounds			
Dimethyl disulfide	0.468 ± 0.125	0.159 ± 0.050	0.069
Total sulfur compounds	0.468 ± 0.125	0.159 ± 0.050	0.069

[a] Undetected value.

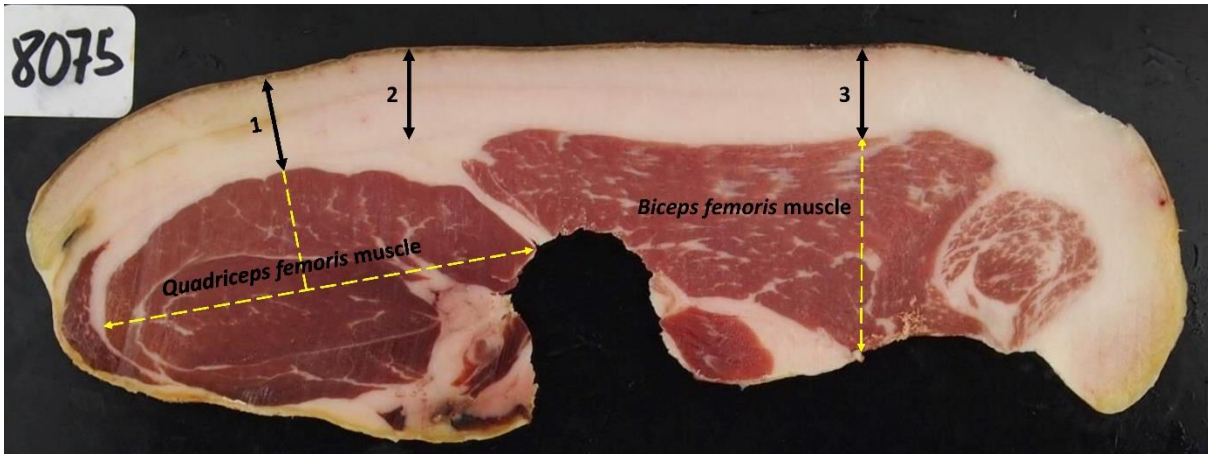

Fig. 1. Subcutaneous fat thickness measured at three points: at the midpoint of the *quadriceps femoris* muscle (1), between the *quadriceps femoris* muscle and the *biceps femoris* muscle (2), and at the right side of the *biceps femoris* muscle (3).

Paper 6. Effect of immunocastration on dry-cured ham quality of male pigs

Effect of male pig immunocastration on instrumental and chemical characteristics of Teruel dry-cured hams

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Topic: Animal production.

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Abstract

Aim of study: To evaluate the effect of the type of male castration (surgical *vs.* immunological) on the characteristics of Teruel dry-cured hams.

Area of study: Teruel and Zaragoza (Spain).

Material and methods: A total of 14 dry-cured hams from Duroc x (Landrace x Large White) male pigs intended for the Spanish Protected Designation of Origin "Teruel ham" were used. Half of them belonged to surgical castrated males (SCM) and the other half to immunocastrated males (IM). Ham weight losses during processing, instrumental and chemical characteristics were analyzed.

Main results: There were no differences ($P > 0.05$) due to the type of castration in ham weight losses throughout processing, thickness of subcutaneous fat, marbling, maximum stress and intramuscular fatty acid profile. However, hams from IM presented lower ($P < 0.05$) chroma than those from SCM. The type of castration had limited influence on chemical composition; only potassium nitrate and retinol contents were affected ($P < 0.05$), being lower in IM than in SCM. For volatile compounds, IM showed lower ($P < 0.05$) percentages of total alcohols and sulfur compounds and higher ($P = 0.012$) proportion of total acids than SCM. All the hams had negligible androstenone content but IM presented higher ($P < 0.05$) skatole and indole concentrations than SCM, being these levels low.

Research highlights: The type of castration in male pigs seems to have scarce influence on the quality of Teruel dry-cured hams, and therefore, immunocastration could be considered as a possible alternative to surgical castration.

Additional key words: barrow; immunological castration; processing losses; instrumental measurements; chemical composition; dry-cured ham.

Abbreviations used: a^* (redness); a_w (water activity); b^* (yellowness); C^*_{ab} (chroma); FA (fatty acid); GnRF (gonadotrophin releasing factor); h_{ab} (hue angle); IM (immunocastrated males); IMF (intramuscular fat); L^* (lightness); MUFA (monounsaturated fatty acids); PDO (Protected Designation of Origin); PUFA (polyunsaturated fatty acids); RH (relative humidity); SCM (surgically castrated males); SFA (saturated fatty acids).

Introduction

Pigs destined for the Protected Designation of Origin (PDO) "Teruel ham" have to be slaughtered at heavy weights (>130 kg of body weight) to meet the quality requirements established by the *Consortium* of this PDO (BOA, 2017). Consequently, male pigs have to be castrated to avoid boar taint, which is an unpleasant odor mainly originated by two compounds present in the adipose tissue: androstenone, a testicular steroid, and skatole, a product of bacterial degradation of tryptophan in the large intestine (Brunius *et al.*, 2011; Škrlep *et al.*, 2014). Besides, another compound, indole, could contribute to the formation of this odor (Brunius *et al.*, 2011). Traditionally, male pigs have been castrated by surgical methods without anesthesia or analgesia, because it is allowed if it is practiced during the first days of animal life (BOE, 2002). However, it generates pain to piglets (Bonneau & Weiler, 2019), and therefore alternatives are being sought in the European Union to this type of castration (European Declaration on alternatives to surgical castration of pigs, 2010). Among them, producing entire males and immunocastration are the two most practical, short-term solutions, likely to thrive (Škrlep *et al.*, 2014). Rearing entire animals is being successfully practiced in several European countries, but it implies a reduction in the slaughter weight, which is not feasible in the PDO Teruel ham. However, immunocastration could be viable in this PDO. It consists of the injection of several vaccines whose active substance is an inactive analogue of gonadotrophin releasing factor (GnRF) conjugated to an immunogenic carrier protein, triggering the formation of antibodies against endogenous GnRF, neutralizing it (Čandek-Potokar *et al.*, 2017; Škrlep *et al.*, 2014). It blocks the stimulation of the hypothalamic-pituitary-gonadal axis, preventing the formation of gonadal steroid hormones and causing regression of reproductive organs and some metabolic changes, which finally leads to the reduction of aggression, the increment of appetite and the elimination of androstenone formation (Škrlep *et al.*, 2014). Nevertheless, according to the meta-analysis of Poulsen Nautrup *et al.* (2018), immunocastrated males (IM) seem to present lower fatness than surgically castrated males (SCM). It could penalize the end product quality, because an insufficient amount of subcutaneous fat covering the piece causes an increase of the seasoning losses and a decrease of the organoleptic characteristics of the dry-cured hams (Bosi & Russo, 2004). However, the effect of immunocastration could differ among production systems. To our knowledge, no scientific paper has been published about the influence of male pig immunocastration on the quality of Teruel dry-cured hams. To check the feasibility of this strategy to produce hams under this PDO, a trial was conducted to assess the effect of the type of castration (surgical *vs.*

immunological) of male pigs on processing weight losses and on instrumental and chemical characteristics of Teruel dry-cured hams.

Material and methods

The raising and slaughter of the animals as well as the dry-curing process of the hams followed the regulations established by the *Consortium* of the PDO Teruel ham (BOA, 2017).

Experimental samples

A total of 14 fresh hams from Duroc x (Landrace x Large White) male pigs were utilized. The pieces came from the experimental animals used in the research of Pérez-Ciria *et al.* (2022), in which the impact of castration type and different feeds was evaluated on productive performances and carcass traits. All the experimental procedures used in that research followed the ethical committee requirements of the University of Zaragoza (ref. PI29/18). Concretely, the hams were chosen at random from pigs receiving the high-energy diet; seven belonged to SCM and seven belonged to IM. The surgical castration was practiced in the first group the first week of life and the immunization against GnRF in the other group was carried out with Improvac® (Zoetis Belgium S.A., Louvain-la-Neuve, Belgium) using three doses; with approximately 25, 58 and 79 kg of body weight (56, 101 and 122 ± 3 d of age, respectively). The general management and feeding at the farm were the same for all of them. All pigs were slaughtered in the abattoir (Teruel, Spain) at the same day, with 142 ± 11.8 kg of body weight (199 ± 3 d of age). There, the left ham from each carcass was taken, trimmed and individually weighed.

Dry-curing process and sampling

Upon arrival at the ham-curing facilities, hams were classified according to the weight. Then, the residual blood was removed by a bleeding-massaging machine that presses the femoral artery. The six phases of the dry-curing process were the following: i) salting; each ham was introduced in a salting tumbler and 2.5 g of nitrifying salt (a mixture of sodium chloride, maltodextrin, sodium ascorbate and potassium nitrate) per kg of meat mass were applied. Then hams were placed in stackable bins, coated with common salt and kept at 0-2°C and 75-90% of relative humidity (RH) for 0.8 days per kg of meat mass. ii) washing with water and molded. iii) resting; hams were hung in racks with hangers and stored from 3.5 to 5°C and from 80-82 to 72-77% of RH for 90 days. iv) drying; the temperature was gradually increased from 8 to 21°C and the RH reduced from 70-75 to 68-73% for 136 days. Finally, lard was

applied manually to the muscular part of the hams to prevent the entry of microorganisms and to avoid over-drying. v) maturing; the temperature continued increasing from 25 to 28°C and the RH was maintained at 70-75% for 79 days. vi) aging; hams stayed in a natural dryer until reaching 32°C for 256 days. The individual weight of all pieces was recorded after salting, resting, drying, maturing and aging.

Once the dry-curing process ended (19 months later), hams were manually boned, sectioned in three parts and individually vacuum packaged. The proximal part of each ham (the opposite part to the hoof) was chosen to carry out the laboratorial analyses and was stored at 4°C until then. One month later, one slice of the sectioned surface of each piece was removed with a slicer (Sammic S.L., Azkoitia, Gipuzkoa, Spain) to determine the color measurements in the piece and another slice was cut to carry out the image analyses. After muscle dissection, the *biceps femoris* muscle (170 ± 20 g) was destined to measure texture. This muscle was minced with a chopper (Moulinette chopper dpa1, Moulinex®, Groupe SEB Iberica S.A., Barcelona, Spain) to analyze the chemical composition, fatty acid (FA) profile of intramuscular fat (IMF) and volatile compounds. Finally, samples of subcutaneous fat (approximately 100 ± 20 g) were taken to determinate boar taint compounds.

Color traits

Color was evaluated on subcutaneous fat and on the muscles *quadriceps femoris* and *biceps femoris* using a spectrophotometer (CM-2600d, Konica Minolta Holdings, Inc., Osaka, Japan), previously calibrated, with illuminant D65 and observer angle of 10º, in CIELAB color space (CIE, 1986). The mean of three random readings of each section was used to obtain lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*_{ab}) and hue angle (h_{ab}).

Subcutaneous fat thickness and marbling by image analysis

One photograph of each slice was taken following the procedure carried out by Ripoll *et al.* (2019a). All images were transferred to a computer and no image editing was applied other than the cropping of the image. Subcutaneous fat thickness was measured at three points (Figure 1): at the midpoint of the *quadriceps femoris* muscle, between the *quadriceps femoris* muscle and the *biceps femoris* muscle, and at the right side of the *biceps femoris* muscle. Marbling was estimated in the *biceps femoris* muscle following the methodology described in the work of Mendizabal *et al.* (2005). The program ImageJ v1.48 (National Institutes of Health, USA) was used to determine subcutaneous fat thickness and marbling.

Texture

The measure of maximum stress was performed following the procedure described by Honikel (1998). Each sample was cut in prism-shaped pieces with a 100 mm^2 (10 x 10 mm) cross-section with the fiber direction parallel to a long dimension of at least 30 mm. A total of 8-10 prisms per sample were sheared perpendicular to the fiber orientation using a Warner-Bratzler device, with a cross-head speed of 2.5 mm/s, attached to an Instron universal testing machine (Model 5543, Instron Ltd, Buckinghamshire, United Kingdom) attached to a computer. Maximum stress was the load at maximum peak shear force per unit of cross-section (Ripoll *et al.*, 2019b).

Chemical composition

Moisture, ash, protein and IMF were analyzed following the procedures of BOE (1979). Moisture was determined using an oven (Memmert UFE500, Schwabach, Germany) at 102°C during 48 h and ash by a muffle (Model 10-PR/400, Forns Hobersal S.L., Caldes de Montbui, Barcelona, Spain) at 550°C during 7 h. Protein was analyzed utilizing a 2300 Kjeltec Analyzer Unit (Foss Tecator, Höganäs, Sweden) and IMF by an ANKOM^{XT15} Extraction System (ANKOM Techonology, Macedon, New York, USA) after being hydrolyzed the samples (ANKOM^{HCL} Hydrolysis System).

Sodium chloride was determined following the procedure described by Matissek *et al.* (1998). For that, a total of 3 g of each sample were weighted and 50 mL of milli-Q water were added. Samples were agitated in a shaker-incubator (Rotabit, J.P. Selecta S.A., Abrera, Barcelona, Spain) at 190 rpm during 30 min using a magnet and 2 mL of nitric acid were added. Finally, samples were analyzed in a titrator (SM Titrino 702, Metrohm Hispania, Madrid, Spain).

Potassium nitrate and sodium nitrite were also analyzed following the official methods of analysis of meat products described in BOE (1979, 1981, 1982). In the case of potassium nitrate, 4 g of each sample were weighted in an Erlenmeyer flask of 250 mL and 150 mL of ethyl alcohol were added. Samples were agitated in a thermostatic bath (Bunsen BTG, Bunsen, Humanes de Madrid, Madrid, Spain) during 1 h. Once cooled, 5 mL of each of the Carrez reagents I and II, prepared with zinc acetate dihydrate and potassium hexacyanoferrate (II) trihydrate, respectively, were added, and milli-Q water were also added to level the flask at 250 mL. The content of this flask was filtered in a flask of 100 mL until its level. The filtrated was discarded and the remaining part was put in another flask of 250 mL, which was placed in a

heating plate (Combiplac, J.P. Selecta S.A., Abrera, Barcelona, Spain) to evaporate ethyl alcohol, until achieving a volume of 50 mL. Then, this volume was transferred to the flask of 100 mL and milli-Q water was added to level it and it was flipped. Later, a total of 10 mL were transferred to a 50 mL flask and 1 mL of brucine-sulfanilic acid and 10 mL of sulphuric acid were added (color reaction) and it was left to rest 10 min in the dark. This flask was made up to 40 mL with milli-Q water and left to rest 15 min in the dark. Later, it was cooled and levelled. Lastly, a spectrophotometer (Shimadzu UV-1700 Pharmaspec, Kyoto, Japan) was used to determine potassium nitrate content at 410 nm. The procedure to determine sodium nitrite was similar, except for the reagent used for the color reaction, which was prepared mixing equal parts of two solutions. The first solution contained 1.50 g of sulfanilic acid, 50 mL of acetic acid and approximately 200 mL of milli-Q water to make up to 250 mL. The second solution contained 0.075 g of 1-naphthylamine, 50 mL of acetic acid and approximately 200 mL of milli-Q water to make up to 250 mL.

Collagen and water activity (a_w) were determined by near-infrared spectroscopy (measuring range: 850-1100 nm). Each sample was put in a circular small cup of 8.8 mm of depth and 134 mm of diameter that was introduced in the FoodScan™2 equipment (FOSS Iberia S.A., Barcelona, Spain).

The contents of α-tocopherol, γ-tocopherol, δ-tocopherol, retinol and cholesterol were determined following the methods described in the paper of Bertolín *et al.* (2018) using ultra-high performance liquid chromatography. The equipment used was an ACQUITY UPLC H-Class liquid chromatograph (Waters, Milford, Massachusetts, USA) equipped with a silica-based bonded phase column (Acquity UPLC HSS T3, 1.8 µm × 2.1 mm × 150 mm column; Waters), an absorbance detector (Acquity UPLC Photodiode Array PDA eλ Detector; Waters) and a fluorescence detector (2475 Multi λ Fluorescence Detector; Waters). To determine lipid oxidation, the content of malondialdehyde was analyzed, following the methodology of Bertolín *et al.* (2019) using ultra-high performance liquid chromatography coupled to a fluorescence detector.

Fatty acid profile of IMF

Firstly, all samples were lyophilized. Then the FA extraction and methylation was carried out following the methodology of Lee *et al.* (2012). A Bruker Scion 436-GC gas chromatograph (Bruker, Billerica, Massachusetts, USA) equipped with SP-2560 capillary column (100 m x 0,25 mm ID x 0,20 µm film thickness; Supelco, Saint Louis, Missouri, USA) was used for FA

determination. The identification of the FAs was done using certified reference materials (GLC-401, GLC-463, GLC-532, GLC-538, GLC-642 and GLC-643, Nu-Chek Prep Inc., Elysian, Minnesota, USA). The FAs were quantified based on the guidelines described in ISO 12966-4 (2015) as mg of FA/100 mg of total FAs (% of total FAs). The percentages of total saturated FAs (SFA), monounsaturated FAs (MUFA), polyunsaturated FAs (PUFA), n-3 and n-6 and the ratios PUFA/SFA and n-6/n-3 were calculated from individual FA percentages.

Volatile compounds

Static headspace technique by using a Turbomatrix HS16 sampler (PerkinElmer, Massachusetts, USA) was used to analyze the volatile profile. A total of 4 g of each homogenized sample were placed in vials of 20 mL that were hermetically closed. The samples were thermostatized at 130ºC for 20 min and 1 min of pressurization time. The injection was carried out over 12 s at 25 psi and an inlet temperature of 220ºC. A Clarus 500 gas chromatograph coupled with a mass spectrometer (PerkinElmer, Massachusetts, USA) equipped with a DB-Wax capillary column (60 m x 0.25 mm ID x 0.25 µm film thickness; Agilent Technologies, California, USA) was used to separate and identify the extracted compounds. A flow of 1 mL/min of helium was used as carrier gas. The oven temperature was 45ºC held for 2 min, 45-200ºC at a rate 4ºC/min, and finally to 225ºC at 10ºC/min, and held for 5 min. The mass spectrometer used the electron impact mode with an ionization potential of 70 eV and an ion source temperature of 200ºC. The interface temperature was 220ºC. The mass spectrometer scanned in full scan mode (35-300 m/z). A TurboMass version 5.4.2 Workstation was used for the gas chromatograph-mass spectrometer system. Tentative identification of the volatile components was achieved by comparison of the mass spectra with mass spectral data from the Nist MS Search Program 2.0 library and by comparison of previously reported Retention Index with those calculated using a n-alkane (C7-C25) series under the same analysis conditions according to the equation of Van Den Dool & Kratz (1963). The relative percentage was expressed as a mass fraction of the total peaks area and fluorobenzene was used as internal standard. The percentages of total aldehydes, ketones, hydrocarbons, alcohols, sulfur compounds, furans and acids were calculated from individual volatile compound percentages.

Boar taint compounds

Androstenone, skatole and indole concentrations in the subcutaneous fat samples were measured by high-performance liquid chromatography as described Pérez-Ciria *et al.* (2021),

using the same laboratorial equipment as in that trial. The concentrations were expressed as µg/g of liquid fat.

Statistical analysis

All statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Data were analyzed using the GLM procedure. The model included the type of castration (surgical *vs.* immunological) as fixed effect. Fresh ham weight and final dry-cured ham weight were included as covariates, when significant ($P < 0.05$), to analyze ham weight losses and the rest of the variables studied, respectively. Androstenone concentration was not statistically analyzed since all values in both types of male pigs were below the detection level of the equipment used (0.20 µg/g of fat); consequently, a descriptive analysis was carried out with this variable.

Normality of the residuals was checked with Shapiro-Wilk's test using the UNIVARIATE procedure. In cases in which normality was not achieved, variables were transformed with $\sqrt{x}$ or $\sqrt[4]{x}$ or Napierian logarithm or x^2 or x^3 before statistical analysis if it was possible. When normality could not be found with data transformation, Mann-Whitney U-test was carried out to analyze these variables. Homogeneity of variances was checked with Levene's test. When homoscedasticity was not achieved, Welch's test was applied.

The pig was the experimental unit. Data are presented in tables as original mean ± standard deviation. A *P*-value < 0.05 was considered as a significant difference.

Results

Ham weight losses during processing

As shown in Table 1, throughout dry-curing process (composed by the phases of salting, resting, drying, maturing and aging), the type of castration did not influence ($P > 0.05$) on ham weight losses. Thus, the final weight of the end product (dry-cured ham) from IM was similar ($P = 0.328$) to that from SCM.

Instrumental characteristics

There were no differences ($P > 0.05$) on color traits of subcutaneous fat between SCM and IM (Table 2). However, hams from IM presented lower ($P < 0.05$) C^*_{ab} value in both muscles studied than those from SCM. Besides, IM also showed lower values of a^* in the *quadriceps femoris* ($P = 0.044$) and of b^* in the *biceps femoris* ($P = 0.045$) than SCM.

As can be seen from Table 3, hams from IM had similar ($P > 0.05$) subcutaneous fat thickness and marbling than those from SCM. Likewise, the type of castration had no effect ($P = 0.283$) on maximum stress (67.57 ± 12.62 *vs.* 60.07 ± 12.38 in SCM and IM, respectively; data not shown in Table 3).

Chemical composition

The moisture, ash, protein and IMF of hams were not affected ($P > 0.05$) by the type of castration (Table 4). Regarding salt contents, sodium chloride and sodium nitrite concentrations were also similar ($P > 0.05$), but IM presented lower ($P = 0.042$) potassium nitrate content than SCM. No influence ($P > 0.05$) of the type of castration was observed on collagen content, a_w, and the contents of tocopherols, cholesterol and malondialdehyde. However, hams from IM presented lower ($P = 0.039$) retinol content than those from SCM.

Fatty acid profile of IMF

Table 5 shows the impact of the type of castration (surgical *vs.* immunological) of male pigs on the FA profile of the *biceps femoris* muscle of dry-cured hams. No significant differences ($P > 0.05$) were detected between SCM and IM in total SFA, MUFA, PUFA, n-3 and n-6 proportions and neither in PUFA/SFA and n-6/n-3 ratios.

Volatile compounds

A total of 39 volatile compounds were identified in the *biceps femoris* muscle of dry-cured hams (Table 6), including the following groups: aldehydes (9), ketones (7), hydrocarbons (6), alcohols (11), sulfur compounds (1), furans (2) and acids (3).

The type of castration had no influence ($P = 0.618$) on total aldehydes percentage. However, within this group, hams from IM presented higher ($P = 0.006$) proportion of heptanal, nonanal and (E)-2-octenal than those from SCM. No impact ($P = 0.708$) of the type of castration was observed on the percentage of total ketones. It is worth noting that inside this group, hams from IM showed lower ($P = 0.009$) proportion of 2,3-butanedione and higher ($P = 0.006$) of 2-octanone. The percentage of total hydrocarbons was also similar ($P = 0.183$) in both experimental groups regardless of the type of castration. Within this group, hams from IM had lower percentage of α-pinene ($P = 0.027$) and limonene ($P = 0.012$) and greater of methylbenzene ($P = 0.024$) than those from SCM. Immunocastration of male pigs reduced ($P = 0.019$) the percentage of total alcohols in comparison to surgical castration, mainly due to the lower proportion of 1-octen-3-ol ($P = 0.009$) and 1-octanol ($P = 0.008$), although hams from

IM presented higher (P = 0.006) 2-methyl-1-propanol percentage than those from SCM. Likewise, hams from IM showed lower (P = 0.039) percentage of total sulfur compounds than those from SCM owing to the lower (P = 0.039) proportion of dimethyl disulfide. The type of castration had no effect (P = 0.183) on the percentage of total furans, although IM presented lower (P = 0.015) proportion of 2-ethylfuran than SCM. Finally, hams from IM did show greater (P = 0.012) percentage of total acids than those from SCM, because of the higher proportions of 2-methylpropanoic (P = 0.006), butanoic (P = 0.009) and hexanoic (P = 0.024) acids.

Boar taint compounds

Table 7 shows the impact of the type of castration of male pigs on the detected boar taint compounds. All hams, both from SCM and from IM, presented low androstenone concentrations, below the detection level of the equipment used (0.20 µg/g) (data not shown). However, hams from IM showed higher skatole (P = 0.004) and indole (P = 0.029) concentrations than those from SCM.

Discussion

Ham subcutaneous fat thickness is the main factor affecting dehydration losses during the dry-curing process, presenting lower losses those hams with thicker subcutaneous fat (Čandek-Potokar & Škrlep, 2011). In a meta-analysis, Poulsen Nautrup *et al.* (2018) reported that IM had less backfat thickness in the carcass than SCM, and thus, it was expected to find this effect in dry-cured hams too. However, in the present study, the type of castration had no significant effect on this parameter and neither on ham weight losses throughout the dry-curing process, which agrees with Pinna *et al.* (2015). Čandek-Potokar *et al.* (2020) did find that IM presented greater ham weight losses, since the fresh hams from these pigs had lower fat thickness than those from SCM. This disagreement among authors could be due, at least in part, to the different period elapsed between the application of the second dose of immunocastration and the slaughter, because this dose is the one that really stimulates the protective immune response (Font-i-Furnols *et al.*, 2012). In the current trial and in the study of Pinna *et al.* (2015), it was injected much earlier than in the work of Čandek-Potokar *et al.* (2020) (approximately 14 *vs.* 4-5 weeks before slaughter), and therefore the metabolic and hormonal status of IM was similar to that of SCM before, which produces similar results between IM and SCM.

Regarding color traits, in contrast to the current trial, Pinna *et al.* (2015) did not find that the muscle tissue of dry-cured hams from IM showed lower a^*, b^* and C^*_{ab} values than that from SCM. According to Zanardi *et al.* (1999), human perception of pork color is strongly

influenced by L^* and h_{ab} values, which were not affected by the type of castration in the subcutaneous fat or in the muscle tissue. Consequently, in terms of color, consumers would find hams from IM to be similar to those from SCM.

In the current trial, the absence of effect of the type of castration on moisture, protein, IMF and sodium chloride contents agrees with the study of Pinna *et al.* (2015). Nevertheless, Čandek-Potokar *et al.* (2020) found that hams from IM presented lower moisture and greater protein and sodium chloride contents than those from SCM, which was also related to the lower subcutaneous fat thickness found in IM that increased the dehydration of their hams. In this last study, IM was in an intermediate position between entire males and SCM according to IMF content and showed lower visually marbling than SCM. In the current study, the high variability found in IMF content and marbling could have contributed partially to the lack of significant differences in these variables. On the other hand, the lower potassium nitrate concentration in hams from IM was not a relevant finding, since this compound has to be converted into nitrite to exert preservative effects (Toldrá *et al*., 2009), and the concentration of sodium nitrite was not affected by the type of castration. The similar collagen content detected in SCM and IM, together with the similar moisture and IMF percentages and FA profile of IMF, led to the type of castration had not influence on maximum stress.

Although Čandek-Potokar *et al.* (2020) detected that IM presented lower a_w than SCM, which could indicate a better shelf life of their hams, in the current trial this variable was similar, probably because both treatments showed similar moisture and sodium chloride contents, in agreement with the results of Pinna *et al.* (2015). However, despite the fact that hams from IM had lower content of retinol, an antioxidant substance (Boulet *et al.*, 2020), it did not translate into differences in malondialdehyde concentration, in agreement with the results of Čandek-Potokar *et al.* (2020). It is worth noting that numerical differences in this variable could suggest a certain tendency towards less oxidation in hams from IM, which would imply that these hams would last longer without going rancid. Additionally, the similar FA profile found between treatments could have contributed to the lack of effect on lipid oxidation. Font-i-Furnols *et al.* (2012), in subcutaneous fat of dry-cured hams, did not find either difference between SCM and IM in total SFA, PUFA, n-3 and n-6 percentages and in PUFA/SFA and n-6/n-3 ratios. From the results of the current trial, it could be deduced that immunocastration does not harm the FA profile of dry-cured hams.

Regarding volatile compounds, generated mainly by proteolysis and lipolysis (Toldrá, 1998), aldehydes play a relevant role in the overall flavor of dry-cured hams due to their low odor thresholds and high concentrations (García-González *et al.*, 2013). Hams from IM by having a greater percentage of heptanal, nonanal and (E)-2-octenal could present more fatty, greasy, ham-like, rancid, leaves, pungent and fruity notes (García-González *et al.*, 2008) than those from SCM. Additionally, the higher percentage of the methyl-ketone 2-octanone in hams from IM could contribute to a greater fruity, floral and green herbaceous scents (García-González *et al.*, 2008; Luna *et al.*, 2006). The hydrocarbons α-pinene, methylbenzene and limonene had relatively low odor thresholds, especially α-pinene (García-González *et al.*, 2008). Therefore, ham consumers could appreciate that hams from IM present lower sharp, pine, citric, fresh and wood olfactory notes and higher strong, plastic and glue notes (García-González *et al.*, 2008; Luna *et al.*, 2006). On the other hand, in general, the flavor of alcohols is considered irrelevant because of their higher odor threshold compared to other carbonyl compounds (Drumm & Spanier, 1991). Therefore, the lower percentage of total alcohols in hams from IM than in those from SCM would not be relevant. However, the alcohols 1-octen3-ol and 1-octanol had lower odor thresholds (García-González *et al.*, 2008), and consequently, these compounds could play an important role in the aroma of dry-cured hams. Thus, hams from IM could show a lower mushroom-like, earthy, dust, fatty and sharp scents than those from SCM (García-González *et al.*, 2008). Sulfur compounds have been associated to undesirable aromas (García-González *et al.*, 2013), and therefore, hams from IM could have a more pleasant aroma than those from SCM, due to the lower percentage of the unique sulfur compound detected, dimethyl disulfide. The olfactory notes of this compound are dirty socks or cauliflowers (Flores *et al.*, 1997; García-González *et al.*, 2008). In spite of the fact that male immunocastration reduced 2-ethylfuran percentage and increased organic acids proportion, the contributions of these compounds to dry-cured ham aroma appear to be low (García-González *et al.*, 2013; Pugliese *et al.*, 2015). It could be interesting to carry out a sensory evaluation to try to see if really consumers appreciated different olfactory notes between hams from SCM and those from IM intended for the PDO Teruel ham.

In respect of boar taint compounds, immunocastration was an efficient strategy decreasing androstenone concentration; in fact, hams from both groups (SCM and IM) showed values under the detection level of the analysis equipment. However, in the case of skatole and indole concentrations, immunization against GnRF failed to reduce their concentrations as much as with surgical castration. It is worth noting that with both surgical and immunological

castration, androstenone and skatole concentrations were under the threshold values for sensory acceptance (0.5 to 1.0 µg/g and 0.20 to 0.25 µg/g, respectively) (Walstra *et al.*, 1999). Therefore, in general, immunocastration would also avoid boar taint in Teruel dry-cured hams.

In conclusion, the type of castration (surgical *vs*. immunological) of male pigs intended for the PDO Teruel ham has limited effect on weight losses throughout the processing, instrumental characteristics, chemical composition and FA profile of IMF of the end product. On the other hand, immunocastration affects some volatile compounds, which could have positive influence on ham flavor, and provides hams with low levels of boar taint contributors. However, previously published results on this topic are not consistent and need further confirmation.

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Tables and figures

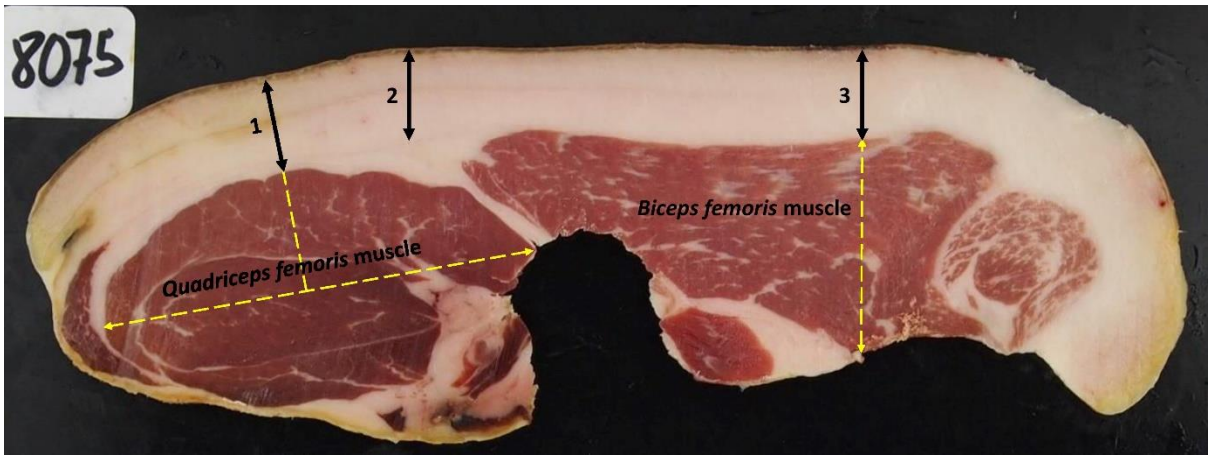


Figure 1. Subcutaneous fat thickness measured at three points; at the midpoint of the *quadriceps femoris* muscle (1), between the *quadriceps femoris* muscle and the *biceps femoris* muscle (2), and at the right side of the *biceps femoris* muscle (3).

Table 1. Impact of the type of castration of male pigs on weight losses (mean ± standard deviation) of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
Ham weight, kg			
Fresh	13.74 ± 1.30	13.79 ± 1.01	0.928
Dry-cured	9.36 ± 1.26	9.17 ± 0.94	0.328
Weight losses[1], %			
After salting	5.52 ± 1.11	6.30 ± 1.00	0.154
After resting	18.90 ± 2.72	20.04 ± 1.76	0.369
After drying	24.99 ± 3.47	26.41 ± 2.33	0.384
After maturing	29.71 ± 3.97	31.09 ± 2.56	0.457
After aging	32.03 ± 4.07	33.63 ± 3.15	0.427

[1]Relative to the fresh-ham weight.

Table 2. Effect of the type of castration of male pigs on color traits (mean ± standard deviation) of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
Subcutaneous fat			
Lightness, L^*	73.24 ± 1.77	73.45 ± 2.03	0.839
Redness, a^*	2.94 ± 0.94	3.49 ± 0.88	0.216
Yellowness, b^*	9.95 ± 1.39	10.11 ± 1.48	0.844
Chroma, C^*_{ab}	10.41 ± 1.43	10.72 ± 1.51	0.700
Hue angle, h_{ab}	73.53 ± 4.68	70.93 ± 4.29	0.300
Quadriceps femoris muscle			
Lightness, L^*	37.49 ± 1.47	37.59 ± 1.55	0.910
Redness, a^*	12.10 ± 1.35	10.73 ± 0.88	0.044
Yellowness, b^*	7.65 ± 2.79	5.29 ± 2.00	0.094
Chroma, C^*_{ab}	14.54 ± 1.43	12.07 ± 1.28	0.005
Hue angle, h_{ab}	31.79 ± 10.84	25.74 ± 8.47	0.267
Biceps femoris muscle			
Lightness, L^*	44.78 ± 2.25	43.73 ± 3.43	0.536
Redness, a^*	13.17 ± 1.22	11.90 ± 1.43	0.116
Yellowness, b^*	8.52 ± 1.01	7.08 ± 1.25	0.045
Chroma, C^*_{ab}	15.72 ± 1.06	13.92 ± 1.19	0.015
Hue angle, h_{ab}	32.93 ± 4.34	30.87 ± 6.00	0.499

Table 3. Impact of the type of castration of male pigs on subcutaneous fat thickness and marbling (mean ± standard deviation) of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
Subcutaneous fat thickness, mm			
At the *quadriceps femoris*	13.59 ± 5.73	14.52 ± 4.93	0.473
Between *quadriceps* and *biceps femoris*	14.14 ± 4.47	15.29 ± 3.38	0.321
At the *biceps femoris*	15.34 ± 3.80	14.17 ± 3.43	0.628
Marbling of the *biceps femoris*, %	6.24 ± 2.96	5.70 ± 3.20	0.750

Table 4. Effect of the type of castration of male pigs on chemical composition (mean ± standard deviation) of the *biceps femoris* muscle of dry-cured hams[1].

	Type of castration		*P*-value
	Surgical	Immunological	
Moisture, %	55.66 ± 1.18	55.80 ± 2.12	0.881
Ash, %	6.77 ± 0.66	6.85 ± 0.62	0.995
Protein, %	31.06 ± 2.08	31.71 ± 1.32	0.524
Intramuscular fat, %	6.06 ± 2.76	5.25 ± 2.74	0.502
Sodium chloride, g/100g	5.17 ± 0.58	5.14 ± 0.63	0.719
Potassium nitrate, mg/kg	120.29 ± 27.34	93.59 ± 14.85	0.042
Sodium nitrite, mg/kg	0.81 ± 0.21	0.62 ± 0.21	0.123
Collagen, %	1.40 ± 0.43	1.36 ± 0.43	0.856
Water activity	0.91 ± 0.01	0.90 ± 0.01	0.857
α-Tocopherol, µg/g	4.29 ± 0.89	3.37 ± 0.95	0.086
γ -Tocopherol, µg/g	0.25 ± 0.07	0.26 ± 0.05	0.752
δ -Tocopherol, µg/g	0.02 ± 0.00	0.02 ± 0.00	0.763
Retinol, ng/g	21.16 ± 6.78	15.50 ± 2.77	0.039
Cholesterol, mg/g	0.88 ± 0.03	0.88 ± 0.03	0.769
Malondialdehyde, mg/kg	0.54 ± 0.23	0.47 ± 0.20	0.554

[1]Results expressed on wet matter basis.

Table 5. Impact of the type of castration of male pigs on fatty acid profile (% of total fatty acids) (mean ± standard deviation) of the *biceps femoris* muscle of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
C8:0	0.045 ± 0.015	0.040 ± 0.015	0.491
C9:0	0.022 ± 0.009	0.018 ± 0.009	0.480
C10:0	0.129 ± 0.026	0.130 ± 0.026	0.944
C11:0	0.044 ± 0.012	0.048 ± 0.014	0.525
C12:0	0.085 ± 0.015	0.090 ± 0.012	0.293
C12:1-9c	0.057 ± 0.041	0.032 ± 0.021	0.201
C13:0	0.007 ± 0.004	0.006 ± 0.005	0.723
C14:0	1.081 ± 0.220	1.038 ± 0.105	0.746
C14:1	0.016 ± 0.010	0.022 ± 0.010	0.250
C15:0	0.043 ± 0.010	0.050 ± 0.013	0.267
C16:0	21.729 ± 0.582	21.686 ± 0.157	0.854
C16:1-7c	0.322 ± 0.025	0.350 ± 0.039	0.134
C16:1-9c	2.203 ± 0.552	2.013 ± 0.469	0.501
C16:1-11c	0.177 ± 0.035	0.201 ± 0.043	0.271
C17:0	0.157 ± 0.034	0.188 ± 0.027	0.065
C17:1-9c	0.109 ± 0.035	0.118 ± 0.021	0.578
C18:0	11.400 ± 0.837	11.886 ± 0.649	0.214
C18:1-9c	30.800 ± 3.910	31.186 ± 2.711	0.834
C18:1-11c	3.314 ± 0.352	2.953 ± 0.364	0.083
C18:2-n6	18.529 ± 2.694	18.529 ± 1.874	1.00
C18:3-n3	0.479 ± 0.061	0.513 ± 0.067	0.342
C18:3-n6	0.443 ± 0.154	0.411 ± 0.143	0.698
C19:0	0.029 ± 0.022	0.031 ± 0.015	0.969
C19:2-n6	0.012 ± 0.012	0.022 ± 0.009	0.094
C20:0	0.093 ± 0.019	0.095 ± 0.015	0.868
C20:1	0.537 ± 0.128	0.539 ± 0.065	0.971
C20:2-n6	0.462 ± 0.055	0.465 ± 0.047	0.927
C20:3-n6	0.677 ± 0.153	0.608 ± 0.073	0.314
C20:4-n6	4.933 ± 1.233	4.674 ± 0.793	0.465

C20:5-n3	0.139 ± 0.021	0.130 ± 0.012	0.347
C21:0	0.007 ± 0.004	0.006 ± 0.005	0.870
C22:0	0.024 ± 0.010	0.026 ± 0.011	0.675
C22:3-n3	0.285 ± 0.080	0.286 ± 0.016	0.325
C22:4-n6	0.771 ± 0.147	0.757 ± 0.114	0.659
C22:5-n3	0.399 ± 0.130	0.376 ± 0.071	0.689
C22:5-n6	0.328 ± 0.058	0.343 ± 0.046	0.598
C22:6-n3	0.118 ± 0.063	0.101 ± 0.034	0.544
C24:0	0.020 ± 0.012	0.019 ± 0.014	0.903
Total SFA[1]	34.886 ± 0.609	35.343 ± 0.516	0.156
Total MUFA[2]	37.529 ± 4.818	37.429 ± 3.294	0.965
Total PUFA[3]	27.571 ± 4.593	27.214 ± 2.955	0.866
PUFA/SFA	0.790 ± 0.127	0.769 ± 0.076	0.723
Total n-3	1.419 ± 0.204	1.407 ± 0.138	0.723
Total n-6	26.143 ± 4.360	25.829 ± 2.805	0.875
n-6/n-3	18.400 ± 1.245	18.386 ± 1.040	0.982

[1]SFA: saturated fatty acids.

[2]MUFA: monounsaturated fatty acids.

[3]PUFA: polyunsaturated fatty acids.

Table 6. Effect of the type of castration of male pigs on volatile compounds (chemical compound area/ internal standard-fluorobenzene-area expressed as %) (mean ± standard deviation) of the *biceps femoris* muscle of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
Aldehydes			
3-Methylbutanal	68.900 ± 6.340	68.643 ± 3.285	0.851
Hexanal	0.455 ± 0.956	1.406 ± 0.824	0.121
Heptanal[1]	-	0.110 ± 0.051	0.006
Octanal	0.015 ± 0.016	0.027 ± 0.011	0.120
(E)-Hept-2-enal	0.005 ± 0.008	0.046 ± 0.054	0.096
Nonanal[1]	-	0.085 ± 0.024	0.006
(E)-2-Octenal[1]	-	0.028 ± 0.019	0.006
Benzaldehyde	0.006 ± 0.005	0.262 ± 0.332	0.572
(E)-2-Nonenal	0.006 ± 0.007	0.008 ± 0.008	0.949
Total aldehydes	69.386 ± 6.581	70.600 ± 3.247	0.618
Ketones			
2-Propanone	14.100 ± 5.907	17.351 ± 1.540	0.534
2-Butanone	2.830 ± 0.745	3.307 ± 0.612	0.325
2,3-Butanedione	4.205 ± 1.624	1.358 ± 0.330	0.009
2-Pentanone	0.050 ± 0.054	0.006 ± 0.002	0.221
2-Heptanone	0.130 ± 0.135	0.177 ± 0.098	0.149
3-Octanone	0.036 ± 0.051	0.009 ± 0.004	0.202
2-Octanone[1]	-	0.009 ± 0.004	0.006
Total ketones	21.350 ± 7.760	22.221 ± 1.549	0.708
Hydrocarbons			
Hexane	0.170 ± 0.150	0.716 ± 0.768	0.097
Heptane	0.413 ± 0.423	0.858 ± 1.646	1.00
Octane	0.900 ± 0.663	1.697 ± 1.295	0.271
α-Pinene	0.247 ± 0.178	0.009 ± 0.008	0.027
Methylbenzene	0.006 ± 0.006	0.297 ± 0.199	0.024
Limonene[1]	0.081 ± 0.109	-	0.012
Total hydrocarbons	1.818 ± 1.204	3.576 ± 3.207	0.183

Alcohols			
Ethanol	0.151 ± 0.120	0.163 ± 0.136	0.754
2-Propanol	2.281 ± 4.297	0.033 ± 0.052	0.053
2-Butanol	0.008 ± 0.011	0.003 ± 0.003	0.789
2-Methyl-1-propanol[1]	-	0.116 ± 0.134	0.006
Butanol	0.003 ± 0.007	0.003 ± 0.004	0.715
3-Methyl-1-butanol	0.503 ± 0.893	0.527 ± 0.526	0.174
1-Pentanol	0.071 ± 0.053	0.108 ± 0.040	0.108
2-Heptanol	0.006 ± 0.004	0.004 ± 0.003	0.179
Hexanol	0.087 ± 0.101	0.096 ± 0.065	0.707
1-Octen-3-ol	1.079 ± 0.756	0.053 ± 0.020	0.009
1-Octanol	0.030 ± 0.029	0.002 ± 0.003	0.008
Total alcohols	4.218 ± 3.860	1.108 ± 0.777	0.019
Sulfur compounds			
Dimethyl disulfide	1.902 ± 1.560	0.242 ± 0.115	0.039
Total sulfur compounds	1.902 ± 1.560	0.242 ± 0.115	0.039
Furans			
2-Ethylfuran	1.160 ± 0.389	0.402 ± 0.235	0.015
2-Pentylfuran	0.016 ± 0.017	0.383 ± 0.370	0.706
Total furans	1.176 ± 0.394	0.785 ± 0.431	0.183
Acids			
2-Methylpropanoic acid[1]	-	0.031 ± 0.018	0.006
Butanoic acid	0.004 ± 0.003	0.503 ± 0.222	0.009
Hexanoic acid	0.151 ± 0.246	0.927 ± 0.580	0.024
Total acids	0.154 ± 0.249	1.461 ± 0.580	0.012

[1]Undetected value.

Table 7. Impact of the type of castration of male pigs on skatole and indole concentrations (μg/g of liquid fat) (mean ± standard deviation) of subcutaneous fat of dry-cured hams.

	Type of castration		*P*-value
	Surgical	Immunological	
Skatole	0.053 ± 0.005	0.066 ± 0.008	0.004
Indole	0.052 ± 0.003	0.059 ± 0.006	0.029

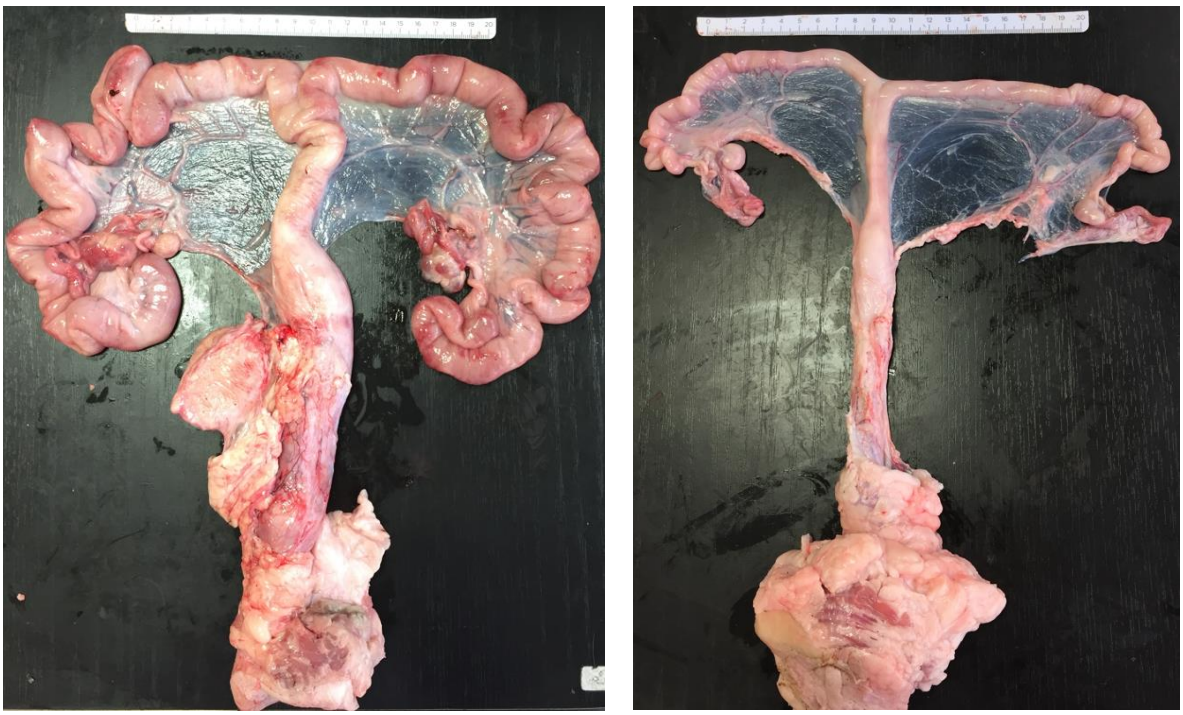
5. GENERAL DISCUSSION

Effects of immunocastration on female pigs intended for the PDO Teruel ham

The second experiment (paper 2) evidenced that, from the first to the second dose of immunocastration, EG and IG presented similar ADG, in agreement with a great deal of authors (Daza et al., 2014; Rodrigues et al., 2019; Van den Broeke et al., 2016). The reason seems to be that the first dose only primes the gilt's immune system. However, from the second dose to the slaughter, IG grew faster and ate more feed than EG, without being significantly affected G:F. In the literature (Gómez-Fernández et al., 2013; Rodrigues et al., 2019; Van den Broeke et al., 2016), similar results according to ADG and ADFI have been found, although those referring to efficiency are inconsistent. Likewise, the higher ADG and ADFI detected in IG did not have any effect on the concentration of serum metabolites (albumin, urea, cholesterol and triglycerides). For the overall experimental period, in the Experiment 2 (paper 2), IG showed higher ADG than EG, whereas in the Experiment 1 (paper 1), no significant differences between both groups were found. The reason shuffled by us was the limited number of animals used for variables related to growth performances in the Experiment 1 (paper 1). However, in the literature, there is also no unanimity; some authors (Bohrer et al., 2014; Daza et al., 2014; Rodrigues et al., 2019) found that IG grew faster than EG, while others (Daza et al., 2016; Xue et al., 2019) did not observe differences.

In the Experiments 1 (paper 1) and 2 (paper 2), IG presented low progesterone concentration (<1 ng/mL). All EG also showed low progesterone levels in the Experiment 1 (paper 1), whereas in the Experiment 2 (paper 2), at slaughter, 17% of EG presented high levels (around 30 ng/mL). In both experiments, no differences between EG and IG were detected in estradiol concentration. Therefore, the serum sex hormones levels may not be the best parameter to assess the efficacy of immunocastration. In our case, the study of the development of the reproductive tract (Figure 5) was more successful; in both experiments, IG presented lighter reproductive tracts and smaller length of the uterine horns, the longest part of the reproductive system, supporting previous research (Dalmau et al., 2015; Hernández-García et al., 2013; Mitjana et al., 2020). Moreover, especially in the Experiment 2 (paper 2), the IG group had a lower proportion of females with more developed ovarian follicles than the EG group. These results indicate that immunocastration reduces ovarian activity, confirming those of the literature (Hernández-García et al., 2013; Mitjana et al., 2020; Xue et al., 2019). It is worth noting that, in the current trials, a small percentage of IG did not appear to respond to immunocastration (<21%) by presenting developed follicles and/or *corpora lutea*. This finding

was observed by other authors (Dalmau et al., 2015; Di Martino et al., 2018; Zeng, Turkstra, Tsigos, et al., 2002) and it could be related to the fact that some gilts fail to generate enough anti-GnRF antibodies or are missed at vaccination moments or too much time elapsing between the last dose of immunocastration and the slaughter.



(a) **(b)**

Figure 5. Effect of gilt immunocastration on reproductive tract development: (**a**) entire gilt; (**b**) immunocastrated gilt.

Female immunocastration had no impact on carcass weight and yield in both experiments (1 and 2-papers 1 and 2), corroborating the findings of some authors (Daza et al., 2016; Izquierdo et al., 2013; Xue et al., 2019). However, gilt immunocastration increased SCF thickness measured in the carcass at *gluteus medius* muscle (Figure 6), supporting previous research (Daza et al., 2014, 2016). This effect was also obtained when SCF thickness was measured at *quadriceps femoris* and *biceps femoris* muscles in dry-cured hams. It could be related to the higher ADFI found in IG from the injection of the second dose of immunocastration. Moreover, it is worth noting that in the Experiment 1 (paper 1), SCF thickness increased numerically when the period from the second dose of immunocastration to the slaughter was increased, which was also detected by Allison et al. (2021). The high variability of data and the smaller number of animals used in the Experiment 1 (paper 1) could

influence the lack of significance. The greater ham fat coverage obtained with gilt immunocastration contributed to the fact that ham weight losses during the dry-curing process tended to be lower. Carcass and ham length were not affected by immunization against GnRF, in agreement with previous reports (Daza et al., 2014, 2016; Martinez-Macipe et al., 2016). Nevertheless, hams from IG seemed to be narrower than those from EG. In the Experiment 2 (paper 2), immunocastration reduced total (ham + shoulder) weight, but this was not reflected when it was expressed as percentage of carcass. On the other hand, in the Experiment 1 (paper 1), total (ham + shoulder) weight was similar between EG and IG, but IG had lower total (ham + shoulder) yield. These discrepancies could be due to the slaughter criterion (fixed age or BW) or the amount of fat removed in the trimming of the pieces.

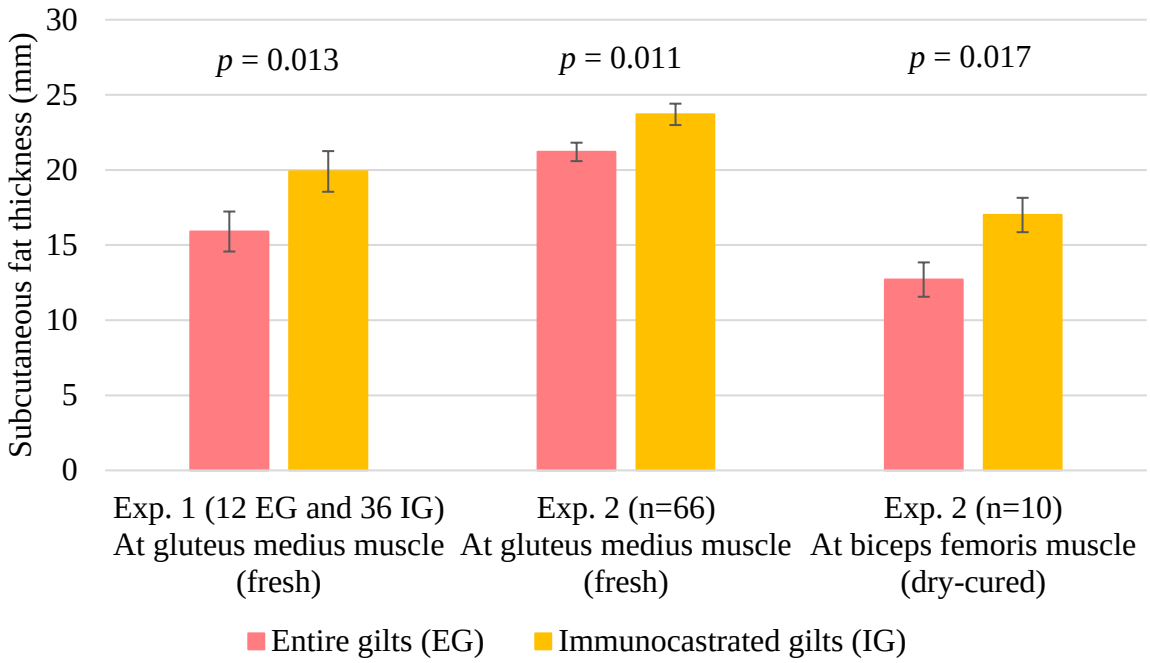


Figure 6. Impact of gilt immunocastration on subcutaneous fat thickness.

In the Experiment 1 (paper 1), gilt immunocastration had no effect on meat cooking losses, whereas in the Experiment 2 (paper 4), also with a standard diet, pork from IG presented higher thawing and cooking losses than that from EG. The differences between both experiments could be because treatments prior to slaughter, such as stunning, and post-slaughter treatments, such as chilling and ageing, may affect meat water holding capacity (Cheng & Sun, 2008). On the other hand, gilt immunocastration had scarce impact on color parameters and texture of meat and dry-cured hams, being consistent with the findings of several authors (Bohrer et al., 2014; Martinez-Macipe et al., 2016; Xue et al., 2019). In both experiments,

protein content was similar between EG and IG although, in the Experiment 1 (paper 1), the sooner the second dose was given, the lower the protein content. In the Experiment 2 (papers 4 and 5), meat and dry-cured hams from IG presented lower moisture percentage and greater IMF content than those from EG (Figure 7). However, in the Experiment 1 (paper 1), this effect was only observed numerically, owing to the higher variability of data and the smaller number of animals studied. Again, it is worth noting that in the Experiment 1 (paper 1) earlier vaccinated gilts for second time (at 9 and 12 weeks before slaughter) presented numerically a considerably greater IMF content in the meat than those vaccinated later (7 weeks before slaughter). The higher IMF in the pork found in the experiment carried out with a greater number of animals (Experiment 2-paper 4) confirms the tendency detected by Daza et al. (2014) and Van den Broeke et al. (2016) using a lower number of gilts. Unexpectedly, although dry-cured hams from IG presented greater SCF, these hams had higher salt and sodium nitrite concentrations than those from EG, although both concentrations were normal for this kind of ham according to results of Rodríguez-Sánchez et al. (2014). Consequently, dry-cured hams from IG showed lower water activity than those from EG, which could improve their shelf life. Besides, gilt immunocastration reduced the percentages of total alcohols and furans and tended to reduce the proportion of total sulfur compounds of dry-cured hams, which could affect positively their aroma. In the future, a sensory evaluation should be carried out to assess if there are really differences in the aroma of the dry-cured hams between EG and IG.

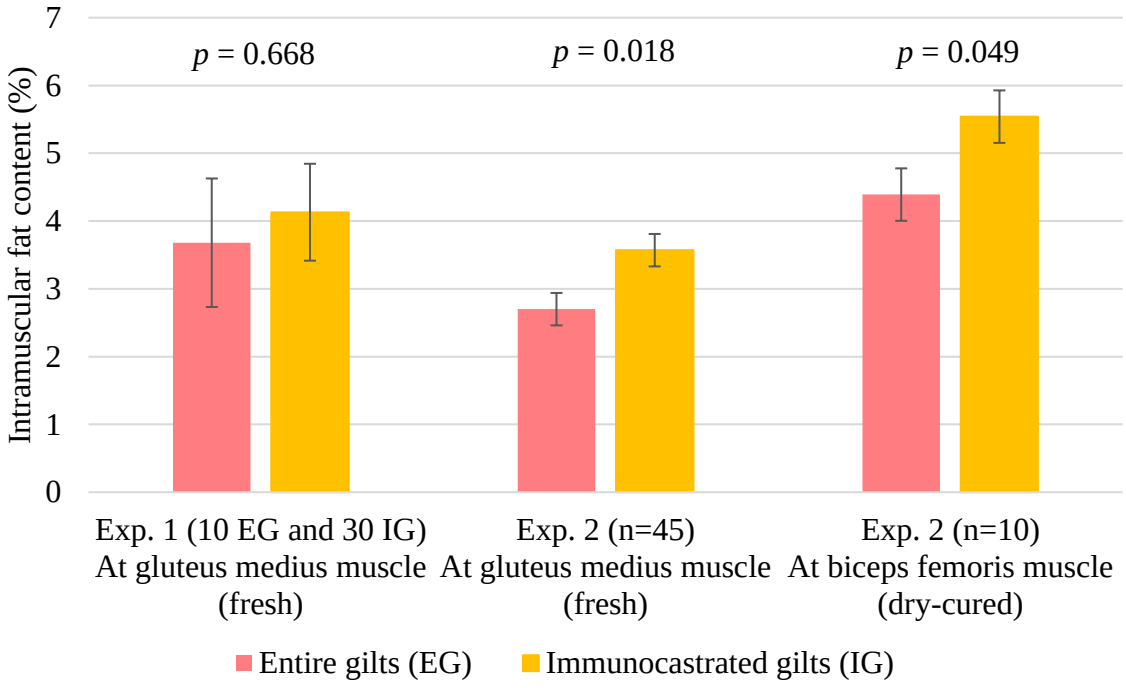


Figure 7. Effect of gilt immunocastration on intramuscular fat content.

In SCF, in the Experiments 1 and 2 (papers 1 and 4), gilt immunocastration increased total SFA percentage and reduced total PUFA, n-3 and n-6 proportions and PUFA/SFA ratio, supporting the results of Daza et al. (2014). These findings could be because a greater SCF thickness was found in IG than in EG, since when more developed is the backfat, higher is the amount of fatty acids arising from *de novo* synthesis, especially SFA, and lower is the percentage of PUFA provided by diet (Gamero-Negrón, Sánchez del Pulgar, & García, 2015; Gandemer, 2002). These results would imply that fat from IG would be firmer and more cohesive, and thus better for technological processes, but less healthy. Moreover, pieces from IG intended to be cured would be less susceptibility to oxidative spoilage than those from EG (Hugo & Roodt, 2007). Nevertheless, regarding the fatty acid profile of IMF, dry-cured hams from IG only tended to present lower contents of total PUFA, n-3 and n-6 than those from EG.

Effects of immunocastration on male pigs intended for the PDO Teruel ham

The Experiment 3 (paper 3) evidenced that, until the second dose of immunocastration, IM tended to have lower ADG than SCM. After the second dose, there was a transient period (21 days) in which no differences between SCM and IM were detected. However, from 21 days after the second dose to the slaughter, IM presented greater ADG, tended to have higher ADFI and showed lower FCR than SCM. Also, for the overall trial period, IM grew faster than SCM. These findings are consistent with those of the meta-analyses of Batorek, Čandek-Potokar, et al. (2012) and Dunshea et al. (2013). The different growth performance between SCM and IM could be explain because IM are physiologically as EM, at least until the second immunization against GnRF, and shortly after, their testicular function is suppressed and their hormonal and metabolic status adjusts to resemble that of SCM (Dunshea et al., 2013). In the Experiment 3 (paper 3), the change in the hormonal status was appreciated when studying the concentration of serum testosterone; after the second dose of immunocastration, it decreased until levels as low as those in SCM at slaughter, supporting previous research (Brunius et al., 2011; Zamaratskaia, Andersson, et al., 2008). Also, from 48 days post-second dose to the slaughter, IM presented similar serum albumin, urea and triglycerides levels than SCM. However, serum cholesterol concentration was higher in IM than in SCM when they ate a standard diet, although this difference was minimized providing a high-energy diet or a low-CP and -AA diet.

Regarding carcass quality, IM presented lower carcass weight and yield than SCM, corroborating the findings of the meta-analysis of Poulsen-Nautrup et al. (2018). Probably the

weight of the testicles in IM and also their heavier intestinal tract, liver, kidneys and additional reproductive tract compared to SCM contributed to reducing their carcass yield (Boler et al., 2014). Effectively, immunocastration reduced SCF thickness compared to surgical castration (Figure 8), measured in the carcass at *gluteus medius* muscle, since IM for a long period of their life are physiologically EM. However, when the thickness of SCF was measured in dry-cured hams from SCM and IM, in fewer replicates per treatment, no differences were observed. Consequently, weight losses of these hams during the dry-curing process were similar. Perhaps using a greater number of hams per group, significant differences could have been detected between both types of pigs. Ham size was similar between SCM and IM. Nevertheless, hams from IM were lighter than those from SCM, which would be related to the lower weight of the carcasses of IM, although no influence of immunocastration was observed on ham yield. On the other hand, no differences between SCM and IM were found in shoulder weight, but shoulder yield was higher in IM than in SCM.

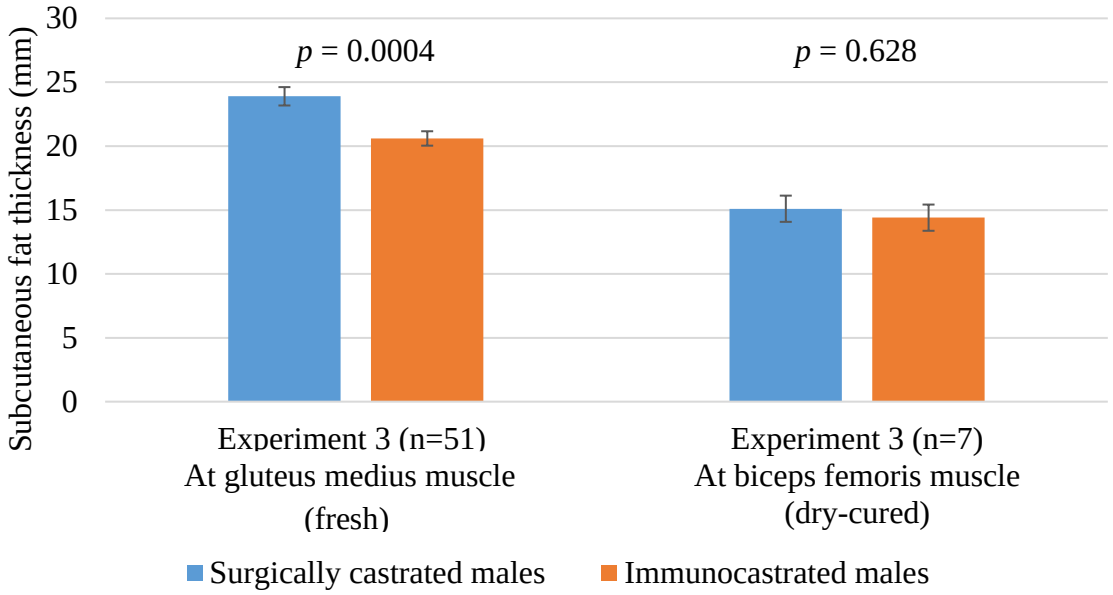


Figure 8. Impact of the type of castration on subcutaneous fat thickness of male pigs.

Meat from IM had similar water holding capacity indicators (thawing and cooking losses) than that from SCM. Likewise, pork redness, yellowness and chroma were similar in both groups. However, meat from IM presented lower lightness and tended to show lower hue angle, parameters strictly linked with human perception of pork color (Zanardi et al., 1999) although, when color was evaluated in dry-cured hams, these last effects were not observed. On the other hand, the type of castration did not affect the texture of the fresh meat or that of

the dry-cured ham. Pork from IM presented higher moisture percentage and lower IMF content than that from SCM (Figure 9), without being affected protein content. These findings are consistent with those of the review of Harsh et al. (2017). However, these differences were not significant in dry-cured hams, which could be due to the high variability of data, especially in the case of IMF, and to the small number of dry-cured hams used. Besides, dry-cured hams from IM showed lower percentage of total alcohols and sulfur compounds and greater of total acids, which could have positive influence on ham flavor. It would be interesting in the future to carry out a sensory evaluation by consumers to see if they appreciate different olfactory notes between hams from IM and SCM destined for the PDO Teruel ham.

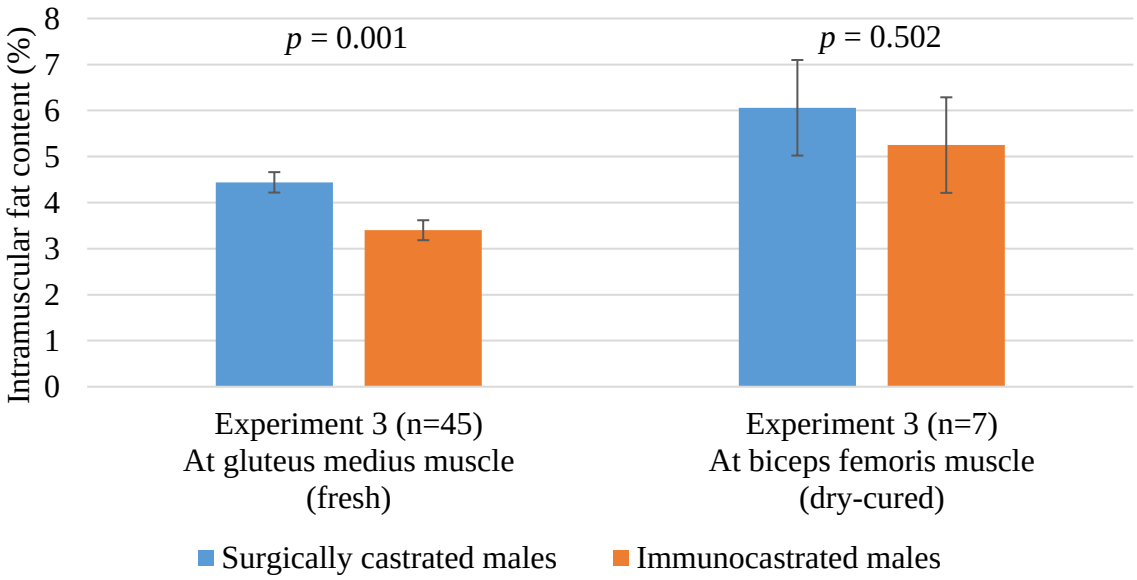


Figure 9. Effect of the type of castration on intramuscular fat content of male pigs.

Regarding the fatty acid profile of SCF, the type of diet used had a relevant role in this parameter. When male pigs were fed a standard diet, immunocastration had no significant effect on total SFA proportion, but it increased total PUFA, n-3 and n-6 proportions and PUFA/SFA ratio in comparison to surgical castration. Using a high-energy diet, these differences disappeared. However, providing a low-CP and -AA diet, IM had greater total SFA proportion and lower total PUFA and n-6 percentages and PUFA/SFA ratio than SCM. Thus, the use of a standard diet in IM would reduce their fat consistency and the storage stability of their pieces, but increase their value for human nutrition in comparison to SCM, whereas a low-CP and -AA diet would provide the opposite effect, and a high-energy diet would keep their SCF characteristics similar to those of SCM (Hugo & Roodt, 2007). In fact, when the fatty acid

profile of IMF of dry-cured hams from SCM and IM that ate a high-energy diet was studied, no differences were observed.

In SCF, both from the carcass (fresh) and from the dry-cured ham (after de dry-curing process), IM and SCM presented an androstenone concentration below the detection limit of the equipment used (0.20 µg/g). In the case of skatole and indole compounds, in the fresh SCF, both type of males had similar concentrations, which agrees with the results of a great deal of reports (Brunius et al., 2011; Pauly et al., 2009; Zamaratskaia, Andersson, et al., 2008). However, in the SCF of dry-cured hams, IM presented higher skatole and indole concentrations than SCM, although these concentrations were also low (<0.08 µg/g). In both kind of SCF samples, immunocastration reduced androstenone and skatole concentrations under the threshold values for sensory acceptance (0.5 to 1.0 µg/g and 0.20 to 0.25 µg/g, respectively) according to the work of Walstra et al. (1999). It is worth noting that, at slaughter line, a small proportion (11%) of IM presented testicles greater than 11 cm and within this proportion, 80% had a high value of androstenone (0.5 to 1.7 µg/g), although the number of pigs sampled was modest. In these pigs, immunocastration was not successful. This finding was also reported by several authors (Font-i-Furnols et al., 2012; Jaros et al., 2005; Zeng, Turkstra, Meloen, et al., 2002). As in the case of females, it could be related to the fact that some pigs cannot react correctly to immunocastration doses or are missed at vaccination moments or too much time elapsing between the last dose of immunocastration and the slaughter.

Effects of increasing dietary energy level maintaining similar crude protein and amino acid contents on pigs intended for the PDO Teruel ham

The Experiments 2 and 3 (papers 2 and 3) showed that increasing dietary NE in 0.15 Mcal/kg, both in female and male pigs, from approximately 80 to 135 kg of BW, improved their efficiency transforming feed into weight gain, which was more pronounced in the finisher period (from approximately 105 to 135 kg of BW). This finding supports a previous research (Suarez-Belloch et al., 2013) carried out also with gilts destined for Teruel ham production. In the case of female pigs (Experiment 2-paper 2), the improvement in feed efficiency with the high-energy diet seemed to be mainly due to the higher ADG in the finisher period, and in the case of male pigs (Experiment 3-paper 3) to the lower ADFI in the grower period (from around 80 to 105 kg of BW). The lower ADFI found in male pigs fed the high-energy diet might be explained because they would have adjusted their feed intake to achieve similar digestible

energy intake as with the control diet (Cole et al., 1967). On the other hand, the greater ADG observed in females could be explained because their energy intake could be below their potential for maximum energy intake (De la Llata et al., 2001), and therefore, by feeding them a diet with a high-energy content, they would not reduce ADFI, increasing their nutrient intake (Hossain et al., 2018). Therefore, depending on the price of fat sources, since in the Experiments 2 and 3 (papers 2 and 3) the high-energy diet was around 17.7 €/Tm more expensive than the control diet (November 2017 prices), and on the price of the kg of pig carcass, increasing dietary NE in 0.15 Mcal/kg from 80 to 135 kg of BW could compensate pig farmers.

In both genders (Experiments 2 and 3-papers 2 and 3), the increase in dietary energy content had no effect on blood albumin and urea concentrations, which is in agreement with several authors (Hong et al., 2016; Kim et al., 2018; Moreira et al., 2021). In female pigs (Experiment 2-paper 2), the high-energy diet also had no impact on cholesterol concentration. Nevertheless, in male pigs (Experiment 3-paper 3), this diet allowed reducing the differences in cholesterol concentration between IM and SCM fed a control diet and serum triglycerides concentration was similar between the control diet and the high-energy diet. However, in the case of females (Experiment 2-paper 2), at the end of the grower period, the high-energy diet increased the concentration of triglycerides, although this effect disappeared at the slaughter. Consequently, increasing NE of the diet in 0.15 Mcal/kg seems not to worse the metabolic status of pigs.

The increase of dietary energy content had no significant effect on the carcass parameters measured in female and male pigs (Experiments 2 and 3-papers 2 and 3). It was expected to find a greater SCF thickness with the high-energy diet, as some authors reported, both using vegetable and animal fat (Liu et al., 2007; Suarez-Belloch et al., 2013). Numerically, females fed the high-energy diet had a SCF thickness 0.9 mm greater than those fed the control diet, and in the case of males, this difference was 2 mm. The lack of statistical significance in this parameter could be due to the high variability of data, the different period in which diets were tested or the more prudent increase in dietary NE.

Likewise, both in female pigs and in male pigs (Experiments 2 and 3-paper 4), increasing dietary energy levels had scarce effects on meat quality, supporting previous research with this similar pigs (Suarez-Belloch et al., 2013). Only in females (Experiment 2-paper 4), the high-energy diet reduced the differences in thawing losses between EG and IG in comparison to a control diet and also cooking losses in IG compared to EG. As in the case of

carcass fatness, the increase in the diet of 0.15 Mcal of NE/kg was not enough to increase the IMF content in pork.

Regarding SCF composition, in female pigs (Experiment 2-paper 4), the increase in dietary energy had no influence on total SFA, MUFA, PUFA, n-3 and n-6 percentages and PUFA/SFA and n-6/n-3 ratios, corroborating the findings of Suarez-Belloch et al. (2013) also with gilts destined for Teruel dry-cured ham production. In males (Experiment 3-paper 4), the high-energy diet reduced the differences in the fatty acid profile between SCM and IM in comparison to a control diet. It could be due, at least in part, to the lower difference in SCF thickness between SCM and IM fed the high-energy diet and those fed the control diet (2.7 mm *vs.* 4.5 mm). Consequently, if IM are used, feeding them with a high-energy diet would allow them to have a fatty acid profile similar to that of SCM. On the other hand, the high-energy diet in both types of male pigs (SCM and IM) increased n-6/n-3 ratio of SCF, which would be detrimental for human health (Russo, 2009).

Effects of decreasing dietary crude protein and amino acid levels maintaining energy content on pigs intended for the PDO Teruel ham

In both female and male pigs (Experiments 2 and 3-papers 2 and 3), a decrease in CP by approximately 2 percentage points and in standardized ileal digestible Lys by around 0.10 percentage points from approximately 80 to 135 kg of BW had no effect on ADG and ADFI. In females (Experiment 2-paper 2), that restriction also had no impact on G:F, whereas in males (Experiment 3-paper 3) improved FCR. These results were not expected because, in finisher pigs also destined for Teruel dry-cured ham production, Rodríguez-Sánchez et al. (2011) and Suárez-Belloch et al. (2015a) found that CP and AA restriction reduced ADG and ADFI. Furthermore, Suárez-Belloch et al. (2015a) detected that reducing dietary CP and AA contents increased FCR. In the Experiments 2 and 3 (papers 2 and 3), perhaps the restriction in AA levels was very limited for the pigs used. Moreover, the contents of CP and AA of the control diet for these pigs could be overestimated, especially in the case of males, since needs for essential AA are lower in IM or SCM than in EG (Fundación Española para el Desarrollo de la Nutrición Animal, 2013). Consequently, the non-deterioration of growth performance with the low-CP and -AA diet would imply a reduction in feeding cost for pig farmers, since this diet was cheaper than the control diet (around 10 €/Tm; November 2017 prices). In addition, in males, the improvement in FCR would increase the profits.

In relation with blood metabolites, in female pigs (Experiment 2-paper 2), no influence of decreasing CP and AA contents was observed on serum albumin, urea, cholesterol and triglycerides concentrations, which corroborates the idea that the CP and AA restriction applied was limited. Likewise, in male pigs (Experiment 3-paper 3), serum albumin and triglycerides were also not affected by the low-CP and -AA diet. However, in these pigs, the low-CP and -AA diet reduced urea concentration in serum, indicator for excess AA catabolism (Dunshea et al., 2013), which agrees with other articles in which low-CP and -AA levels were tested (Mule et al., 2006; Suárez-Belloch et al., 2015a; Yang et al., 2008). It could indicate that males fed the low-CP and -AA diet presented a lower N intake and/or a more efficiently utilization of N (Fabian et al., 2004). This finding could implicate that this diet could reduce N excretion in males, which would have relevant implications to reduce manure load, although further research should be carried out to confirm this hypothesis. On the other hand, the restriction in dietary CP and AA contents allowed reducing cholesterol concentration in IM as much as in SCM.

The possible greater SCF thickness with the restriction of dietary CP and Lys levels (Millet et al., 2006; Rodríguez-Sánchez et al., 2011; Suárez-Belloch et al., 2016) was not observed in any of the experiments carried out. It should be noted that numerically gilts fed the low-CP and -AA diet had 0.7 mm thicker SCF thickness than those fed the control diet and this difference was 0.5 mm in the case of males. The lack of effect could be due to the high variability of data, the different period in which diets were tested or the more prudent restriction in CP and Lys levels. Likewise, the other carcass traits studied were not significantly affected by the reduction tested in dietary CP and AA contents.

Regarding meat quality, in female pigs (Experiment 2-paper 4), the low-CP and –AA diet IG reduced the differences in cooking losses between EG and IG in comparison to a control diet and also thawing losses in IG compared to EG. On the other hand, in male pigs (Experiment 3-paper 4), this diet increased cooking losses. However, in previous research (Rodríguez-Sánchez et al., 2011; Suárez-Belloch et al., 2015a) carried out with similar pigs, no influence of CP and Lys restriction was observed on water holding capacity indicators. In both experiments (2 and 3-paper 4), the low-CP and -AA diet had no impact on color parameters, texture or chemical composition. Therefore, as in carcass fatness, the reduction in dietary CP and Lys in 2 and 0.10 percentage points, respectively, was not enough to increase the IMF content.

With regard to SCF composition, in females (Experiment 2-paper 4), reducing dietary CP and AA levels did not alter total SFA, MUFA, PUFA, n-3 and n-6 percentages and PUFA/SFA and n-6/n-3 ratios, which is in agreement with Rodríguez-Sánchez et al. (2011). In the case of males (Experiment 3-paper 4), the use of a low-CP and -AA diet increased total SFA percentage and reduced total PUFA and n-6 proportions and PUFA/SFA ratio in IM compared to SCM. However, with a control diet, opposite effects were observed. Therefore, in IM, the use of a low-CP and -AA diet would increase their fat firmness and cohesiveness and improve the storage stability of their pieces compared to SCM, although their fat would be less healthy (Hugo & Roodt, 2007). On the other hand, the low-CP and -AA diet, regardless of the type of castration (surgical or immunological) increased n-6/n-3 ratio, effect also observed by Wood et al. (2013) in IMF of *Longissimus* muscle, which would be detrimental for human health (Russo, 2009).

6. CONCLUSIONS

It can be concluded, in pigs intended for the PDO Teruel ham, that:

I. The optimum time for the application of the second dose of immunocastration in gilts seems to be between 9 and 12 weeks before slaughter, considering mainly the evolution of fatness and also the development of reproductive tracts.

II. Immunization against GnRF is an interesting technique to apply in females because offers several advantages, in comparison to the use of entire gilts. Firstly, it improves growth rate. Secondly, this practice reduces considerably the reproductive tract development. Thirdly, it increases subcutaneous fat thickness in carcass and intramuscular fat content in meat, being both key aspects in animals destined for dry-cured ham elaboration. Also, it generates a more adequate fatty acid profile for the curing process, by increasing total SFA proportion and reducing total PUFA percentage in subcutaneous fat. Immunocastration in gilts does not affect the concentration of serum metabolites and has scarce influence on the concentration of serum sex hormones.

III. In males, compared to surgical castration, immunocastration improves body weight gain and feed conversion ratio with scarce effects on serum metabolites. However, it reduces fat accretion, both subcutaneous and intramuscular, and also carcass yield, and does not minimize in the dry-cured hams the concentrations of some compounds responsible for boar taint (skatole and indole) as much as surgical castration, although both levels are low. Currently, taking into account the pros and cons of immunological castration, for the production of dry-cured hams, the use of surgical castrated males would be preferable.

IV. A small percentage of female and male pigs may not be correctly vaccinated or not respond to immunization against GnRF.

V. Increasing energy of commercial diets by 0.15 Mcal of net energy per kg, maintaining crude protein an amino acid contents, both in female and male pigs, from approximately 80 to 135 kg of body weight, is not an effective strategy to increase fatness, neither in carcass nor in meat. However, this rise of energy of the diet increases the efficiency converting feed into weight gain and has little influence on the concentration of serum metabolites. Therefore, depending on the price of fat sources and of the kg of pig carcass, this nutritional strategy could be beneficial for pig farmers. On the other hand, in female pigs, irrespective of whether they are entire or immunocastrated, this increase of energy density of the diet has no effect on the fatty acid profile. Nevertheless, it could be used in immunocastrated males to achieve a similar fatty acid profile to that of

surgically castrated males, since it reduces the differences in fat composition between these types of males in comparison to a standard diet.

VI. Decreasing crude protein of standard diets by 2 percentage points and amino acids, maintaining the energy level, both in female and male pigs, from approximately 80 to 135 kg of body weight, is not successful increasing fatness, neither subcutaneous nor intramuscular. Nevertheless, this nutritional plan decreases serum urea concentration in males but does not penalize feed efficiency in any case, which would be economically beneficial for pig farmers. On the other hand, in female pigs, regardless of whether they are entire or immunocastrated, this restriction in dietary crude protein and amino acid contents has no influence on fat composition. However, it could be used in immunocastrated males to improve technological properties of their fat in comparison to surgically castrated males, although it would be less healthy.

7. CONCLUSIONES

Se puede concluir, en cerdos destinados a la DOP Jamón de Teruel, que:

I. El momento óptimo para la aplicación de la segunda dosis de inmunocastración en cerdas parece ser entre 9 y 12 semanas antes del sacrificio, considerando principalmente la evolución del engrasamiento y también el desarrollo de los aparatos reproductores.

II. La inmunización contra el GnRF es una técnica interesante para aplicar en hembras, ya que ofrece varias ventajas en comparación con el uso de cerdas enteras. En primer lugar, mejora la tasa de crecimiento. En segundo lugar, reduce considerablemente el desarrollo del aparato reproductor. En tercer lugar, aumenta el espesor de la grasa subcutánea en la canal y el contenido de grasa intramuscular en la carne, siendo ambos aspectos claves en animales destinados a la elaboración de jamón curado. Además, esta práctica genera un perfil de ácidos grasos más adecuado para el proceso de curado, al incrementar la proporción de ácidos grasos saturados y al reducir el porcentaje de ácidos grasos poliinsaturados en la grasa subcutánea. La inmunocastración en las cerdas no afecta a la concentración de los metabolitos séricos y tiene una escasa influencia en la concentración de las hormonas sexuales en el suero.

III. En los machos, en comparación con la castración quirúrgica, la inmunocastración mejora la ganancia de peso corporal y el índice de conversión, con escasos efectos en los metabolitos séricos. Sin embargo, reduce la acumulación de grasa, tanto subcutánea como intramuscular, y también el rendimiento de la canal, y no minimiza en los jamones curados las concentraciones de algunos compuestos responsables del olor sexual (escatol e indol) tanto como la castración quirúrgica, aunque ambos niveles son bajos. Actualmente, teniendo en cuenta los pros y los contras de la castración inmunológica, para la producción de jamones curados el uso de machos castrados quirúrgicamente sería preferible.

IV. Un pequeño porcentaje de cerdos hembras y machos puede no estar correctamente vacunado o no responder a la inmunización contra el GnRF.

V. El aumento de la energía de las dietas comerciales en 0,15 Mcal de energía neta por kg, manteniendo los contenidos en proteína bruta y aminoácidos, tanto en cerdos hembras como machos, de aproximadamente 80 a 135 kg de peso vivo, no es una estrategia eficaz para aumentar el engrasamiento, ni en la canal ni en la carne. Sin embargo, este incremento de la energía de la dieta aumenta la eficiencia convirtiendo el pienso en ganancia de peso y tiene poca influencia en la concentración de los metabolitos séricos. Por tanto, dependiendo del precio de las fuentes de grasa y del kg de la canal porcina,

esta estrategia nutricional podría ser beneficiosa para los ganaderos. Por otro lado, en las hembras, independientemente de si son enteras o inmunocastradas, este aumento de la densidad energética de la dieta no tiene efecto sobre el perfil de ácidos grasos. Sin embargo, podría utilizarse en machos inmunocastrados para conseguir un perfil de ácidos grasos similar al de los machos castrados quirúrgicamente, ya que reduce las diferencias en la composición de la grasa entre estos tipos de machos en comparación con una dieta estándar.

VI. Reducir la proteína bruta de dietas estándar en 2 puntos porcentuales y los aminoácidos, manteniendo el nivel de energía, tanto en cerdos hembras como machos, de aproximadamente 80 a 135 kg de peso vivo, no es una estrategia eficaz para aumentar el engrasamiento, ni subcutáneo ni intramuscular. Sin embargo, este plan nutricional disminuye la concentración de urea sérica en machos, pero no empeora la eficiencia alimenticia en ningún caso, lo que sería económicamente beneficioso para los ganaderos. Por otro lado, en las hembras, independientemente de si son enteras o inmunocastradas, esta restricción en los contenidos de proteína bruta y aminoácidos no tiene efecto en la composición de la grasa. Sin embargo, podría usarse en los machos inmunocastrados para mejorar las propiedades tecnológicas de su grasa en comparación con los machos castrados quirúrgicamente, aunque sería menos saludable.

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9. APPENDIX

Impact factor and thematic areas

The impact factor of the journals and the thematic areas corresponding to the articles included in this thesis are presented in square brackets below. This information has been obtained from the Journal Citation Reports™ (JCR) database.

1. Pérez-Ciria, L., Carcò, G., Miana-Mena, F. J., Mitjana, O., Falceto, M. V., & Latorre, M. A. (2021). Immunocastration in Gilts: A Preliminary Study of the Effect of the Second Dose Administration Time on Growth, Reproductive Tract Development, and Carcass and Meat Quality. *Animals, 11*(2), 510. https://doi.org/10.3390/ani11020510 **[Journal Impact Factor (2021): 3.231; Thematic areas: Agriculture, Dairy & Animal Science (Q1); Veterinary Sciences (Q1)].**
2. Pérez-Ciria, L., Miana-Mena, F. J., Falceto, M. V., Mitjana, O., & Latorre, M. A. (2021). Effect of Immunocastration and Diet on Growth Performance, Serum Metabolites and Sex Hormones, Reproductive Organ Development and Carcass Quality of Heavy Gilts. *Animals, 11*(7), 1900. https://doi.org/10.3390/ani11071900 **[Journal Impact Factor (2021): 3.231; Thematic areas: Agriculture, Dairy & Animal Science (Q1); Veterinary Sciences (Q1)].**
3. Pérez-Ciria, L., Miana-Mena, F. J., Álvarez-Rodríguez, J., & Latorre, M. A. (2022). Effect of Castration Type and Diet on Growth Performance, Serum Sex Hormones and Metabolites, and Carcass Quality of Heavy Male Pigs. *Animals, 12*(8), 1004. https://doi.org/10.3390/ani12081004 **[Journal Impact Factor (2021): 3.231; Thematic areas: Agriculture, Dairy & Animal Science (Q1); Veterinary Sciences (Q1)].**
4. Pérez-Ciria, L., Miana-Mena, F. J., López-Mendoza, M. C., Álvarez-Rodríguez, J., & Latorre, M. A. (2021). Influence of Immunocastration and Diet on Meat and Fat Quality of Heavy Female and Male Pigs. *Animals, 11*(12), 3355. https://doi.org/10.3390/ani11123355 **[Journal Impact Factor (2021): 3.231; Thematic areas: Agriculture, Dairy & Animal Science (Q1); Veterinary Sciences (Q1)].**
5. Pérez-Ciria, L., Ripoll, G., Sanz, M. A., Blanco, M., Miana-Mena, F. J., & Latorre, M. A. Impact of gilt immunocastration on weight losses and instrumental and chemical characteristics of Teruel dry-cured ham. Manuscript submitted to *Meat Science* on 10/22/2022 (ref.: MEATSCI-D-22-00784). Under review **[Journal Impact Factor (2021): 7.077; Thematic area: Food Science & Technology (Q1)].**

6. Pérez-Ciria, L., Ripoll, G., Sanz, M. A., Blanco, M., & Latorre, M. A. Effect of male pig immunocastration on instrumental and chemical characteristics of Teruel dry-cured hams. Manuscript submitted to *Spanish Journal of Agricultural Research* on 11/07/2022 (ref. number: 19967). Under review **[Journal Impact Factor (2021): 1.233; Thematic area: Agriculture, Multidisciplinary (Q3)].**

Contribution of the doctoral student

The first author of the articles presented in the previous section has conducted with assistance of her supervisor and other researchers the experimental development and the laboratory analyses. In addition, she has carried out the statistical analyses and the drafting, reviewing and editing of the articles listed above with assistance of her supervisor.

Conference papers derived from the present thesis

Author: L. Pérez-Ciria.
Title: La inmunización contra GnRH porcina como estrategia de bienestar animal y de mejora de la calidad del Jamón DOP Teruel.
Type: oral.
Scientific conference: Jornada Técnica de Presentación de los Resultados de los Proyectos FITE 2019.
Place and date: Teruel (Spain); 03/31/2022

Author: L. Pérez-Ciria.
Title: Objetivos e implicaciones de la inmunocastración en hembras porcinas.
Type: oral.
Scientific conference: Jornada del Aula Porcina de la Facultad de Veterinaria de la Universidad de Zaragoza en la Feria Internacional para la Producción Animal (FIGAN) 2021.
Place and date: Zaragoza (Spain); 09/21/2021.

Authors: L. Pérez-Ciria, M. A. Sanz, M. Blanco, G. Ripoll, J. Álvarez-Rodríguez, F. J. Miana-Mena and M. A. Latorre.
Title: Effect of swine immunocastration on salts and volatile compounds of Teruel dry-cured hams.
Type: poster.
Congress: 72nd Annual Meeting of the European Federation of Animal Science.
Publication: Book of abstracts, p.342.
Place and date: Online; 08/30/2021-09/03/2021.

Authors: L. Pérez-Ciria, G. Ripoll, M. Blanco, J. Álvarez-Rodríguez, F. J. Miana-Mena and M. A. Latorre.
Title: Impact of swine immunocastration on fat quality of Teruel dry-cured hams.

Type: poster.
Congress: 72nd Annual Meeting of the European Federation of Animal Science.
Publication: Book of abstracts, p.346.
Place and date: Online; 08/30/2021-09/03/2021.

Authors: L. Pérez-Ciria, J. Álvarez-Rodríguez, F. J. Miana-Mena, L. Gallo, S. Schiavon, G. Ripoll, M. Blanco, M. A. Sanz and M. A. Latorre.
Title: Actitud de los consumidores españoles e italianos frente a jamones curados de cerdos inmunocastrados.
Type: oral.
Congress: XIX Jornadas sobre Producción Animal de la Asociación Interprofesional para el Desarrollo Agrario (AIDA).
Publication: Book of abstracts, p. 242.
Place and date: Online; 06/01/2021-06/02/2021.

Authors: L. Pérez-Ciria, G. Ripoll, D. Allueva, F. J. Miana-Mena, M. Blanco and M. A. Latorre.
Title: Impact of immunocastration of gilts on instrumental and chemical traits of Teruel dry-cured hams.
Type: oral.
Congress: 71st Annual Meeting of the European Federation of Animal Science
Publication: Book of abstracts, p. 513.
Place and date: Online; 12/01/2020-12/04/2020.

Authors: L. Pérez-Ciria, G. Ripoll, D. Allueva, M. Blanco, F. J. Miana-Mena and M. A. Latorre.
Title: Effect of immunocastration of male pigs on instrumental and chemical traits of Teruel dry-cured hams.
Type: poster.
Congress: 71st Annual Meeting of the European Federation of Animal Science
Publication: Book of Abstracts, p. 519.
Place and date: Online; 12/01/2020-12/04/2020.

Authors: L. Pérez-Ciria, A. Suárez, M. V. Falceto, O. Mitjana, F. J. Miana-Mena and M. A. Latorre.
Title: Efecto de la inmunocastración en cerdos macho destinados a Jamón DOP Teruel.
Type: poster.
Congress: X Congreso Mundial del Jamón.
Place and date: Madrid (Spain); 09/17/2019-09/19/2019.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, G. Ripoll and M. A. Latorre.
Title: Meat and fat quality of pigs intended for Spanish cured ham: effect of male castration and feeding.
Type: oral.
Congress: 70th Annual Meeting of the European Federation of Animal Science.
Publication: Book of abstracts, p.414.
Place and date: Ghent (Belgium); 08/26/2019-08/30/2019.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, G. Ripoll and M. A. Latorre.
Title: Meat and fat quality of gilts intended for Spanish dry-cured ham: effect of immunocastration and feeding.

Type: poster.
Congress: ASAS-CSAS Annual Meeting and Trade Show.
Publication: Journal of Animal Science Vol.97, Suppl. S3, pp. 471-472.
Place and date: Austin (United States of America); 07/08/2019-07/11/2019.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, Z. Amanzougarene, S. Yuste and M. A. Latorre.
Title: Efecto de la inmunocastración y la dieta sobre los rendimientos productivos, los metabolitos séricos y la calidad de la canal de cerdos destinados a Jamón DOP Teruel.
Type: oral.
Congress: XVIII Jornadas sobre Producción Animal de la Asociación Interprofesional para el Desarrollo Agrario (AIDA).
Publication: Book of abstracts, pp. 266-268.
Place and date: Zaragoza (Spain); 05/07/2019-05/08/2019.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, J. I. Abadías, Z. Amanzougarene, S. Yuste, F. D. Sánchez-Fernández and M. A. Latorre.
Title: Efecto de la inmunocastración y del tipo de dieta sobre los rendimientos productivos y metabolitos séricos en cerdas pesadas.
Type: oral.
Congress: VI Congreso de la Asociación Nacional de Veterinarios de Porcino.
Place and date: Zaragoza (Spain); 11/28/2018-11/29/2018.

Authors: L. Pérez-Ciria, F. J. Miana, M. C. López-Mendoza, L. Najes and M. A. Latorre.
Title: Efecto de la inmunocastración y del tipo de dieta sobre las características de la canal de cerdas destinadas a jamón DOP Teruel.
Type: poster.
Congress: VI Congreso de la Asociación Nacional de Veterinarios de Porcino.
Place and date: Zaragoza (Spain); 11/28/2018-11/29/2018.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, Z. Amanzougarene, S. Yuste, M. C. López-Mendoza and M. A. Latorre.
Title: Impacto de la inmunocastración sobre los rendimientos productivos, la calidad de la canal y las hormonas sexuales de cerdas destinadas a la producción de jamón DOP Teruel.
Type: oral.
Congress: II Encuentro de Grupos de Investigación IA2.
Place and date: Zaragoza (Spain); 11/26/2018.

Authors: L. Pérez-Ciria, G. Carcò, F. J. Miana-Mena, O. Mitjana, M. V. Falceto and M. A. Latorre.
Title: Impacto de la inmunocastración sobre el engrasamiento y el desarrollo del aparato reproductor de cerdas destinadas a la producción de Jamón DOP Teruel.
Type: poster.
Congress: II Encuentro de Grupos de Investigación IA2.
Place and date: Zaragoza (Spain); 11/26/2018.

Authors: L. Pérez-Ciria, F. J. Miana-Mena, R. Gutiérrez, L. E. Cobos, M. C. López-Mendoza and M. A. Latorre.
Title: Impact of immunocastration on growth performances and carcass quality of heavy gilts.
Type: oral.
Congress: 69th Annual Meeting of the European Federation of Animal Science.

Publication: Book of abstracts, p.647.
Place and date: Dubrovnik (Croatia); 08/27/2018-08/31/2018.

Authors: L. Pérez-Ciria, G. Carcò, F. J. Miana-Mena, G. Ripoll, J. I. Abadías and M. A. Latorre.
Title: The protocol of immunocastration can affect carcass fatness, meat quality and fat composition of heavy gilts.
Type: poster.
Congress: 64th International Congress of Meat Science and Technology.
Publication: Book of abstracts.
Place and date: Melbourne (Australia); 08/12/2018-08/17/2018.

Authors: L. Pérez-Ciria, O. Mitjana, M. V. Falceto, A. Suárez, F. J. Miana-Mena, M. Fondevila and M. A. Latorre.
Title: Influence of immunocastration on genital organs and sex hormones of heavy gilts.
Type: poster.
Congress: ASAS-CSAS Annual Meeting & Trade Show.
Publication: Journal of Animal Science Vol.96, Suppl. S3, pp. 485.
Place and date: Vancouver (Canada); 07/08/2018-07/12/2018.

Authors: L. Pérez Ciria, G. Carcò, F. J. Miana Mena, O. Mitjana, M. V. Falceto, J. I. Abadías and M. A. Latorre Górriz.
Title: Impact of oestrus suppression in carcass quality intended for Teruel dry-cured ham production.
Type: oral.
Congress: 10th European Symposium of Porcine Health Management.
Publication: Book of abstracts, p.110.
Place and date: Barcelona (Spain), 05/09/2018-05/11/2018.