Immunocastration of male pigs

Dissertation

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Für Ulrike Weiler

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LIST OF ABBREVIATIONS

except abbreviations used in Chapter 2

®	Registered Trademark
2nd	second
ADG	average daily gain
approx.	approximately
e.g.	exempli gratia
et al.	et alia
GnRH	Gonadotropin-Releasing-Hormone
HPLC	High Performance Liquid Chromatography
i.e.	id est
ID	Idaho
Inc.	Incorporated
LH	Luteinizing Hormone
n	numbers
ng/ml	nanogram/milliliter
pCBG	porcine Corticosteroid Binding Globulin
RIA	Radioimmunoassay
UK	United Kingdom
US	United States of America
USDA	United States Department of Agriculture
µg/g	microgram/gram

1 GENERAL INTRODUCTION

The surgical castration of male piglets has been carried out in the production of pork for centuries [1,2]. Within the European Union, surgical castration of male piglets is allowed up to the seventh day of life without anesthesia or analgesia [3], even if this is painful and violates the physical integrity of piglets [4]. These circumstances are facing increasing societal concerns [1] and thus in 2010 led European stakeholders to voluntarily commit to ending surgical castration by 2018 [5]. Today, however, still about 63% of all male piglets are castrated surgically, most of them without adequate pain relief [6]. Traditionally, the main reasons for castrating male piglets were to avoid a sex-specific off-odor, the so called boar taint, to produce carcasses of higher quality (more fat), but also to ensure a positive impact on animal behavior, as barrows show a less agonistic behavior compared to boars [7]. The sex-specific off-odor can be ascribed to the accumulation of the two compounds androstenone and skatole in adipose tissue along puberty [8]. Pork production with boars was thus limited for decades to those European countries which raised boars with very low slaughter weights before boars entering puberty, such as the UK [7].

In the 1960s, the concept of pork production with boars instead of barrows was discussed within the European pork industry, since consumers demanded leaner carcasses and thus pork production with boars seemed to be an attractive economic advantage [9] based on the more efficient feed conversion and growth performance of boars due to their higher anabolic potential [8,9]. However, this did not lead to an overall change of the production system as androstenone as one key boar taint compound is a metabolite of testosterone and thus, strongly linked to the growth potential of boars [8]. In the 1970s, pork production with boars was discussed in an environmental context, since the more efficient feed conversion resulted in less nitrogen excretion [10] and lower amounts of manure [7]. These further important arguments for pork production with boars did not lead to a change of the production system either, as the boar taint problem still remained unsolved. In the past few decades, however, the most prominent aspect discussed has been animal welfare [1,11], since surgical castration in most cases has been carried out without adequate analgesia or anesthesia and is painful for the young piglets [4,12]. The increasing public awareness and scientific developments have resulted in new animal welfare standards being reflected in industry initiatives and legislative regulations [13]. Within the European Union, there are countries that traditionally produce boars and countries that mainly castrate male piglets. These heterogeneous market conditions lead to various strategies across Europe with countries that rely on intact boars and countries that continue to castrate male piglets with different animal welfare standards [14]. In contrast to the debates in the previous decades, where product quality and welfare aspects were predominant, the current debate about surgical castration of piglets and potential alternatives tries to take into account all dimensions of sustainability (economy, social, environment and animal welfare) [14,15].

For a long time, surgical castration was the most effective way to prevent boar taint, but it also removes the above-mentioned anabolic potential of boars [15]. The idea of using immunological methods (immunocastration) to reduce boar taint while still using the growth potential of boars is quite old. First attempts aimed to induce antibody formation against androstenone, the luteinizing hormone (LH), and finally against the gonadotropin-releasing hormone (GnRH), thus reducing boar taint while maintaining the anabolic advantages of boars [16–22]. An active immunization against GnRH seemed to be the most effective immunological method to reliably prevent boar taint [17]. However, it turned out that a vaccine must be developed that is economical, contributes to a high level of operational safety, and allows the positive advantages of pork production with boars to be used for as long as possible [17]. This led to the concept of developing a vaccine that only effectively suppresses testicular functions after two vaccinations [23], thus suppressing spermatogenesis and testosterone as well as androstenone synthesis in Leydig cells [24]. These requirements were met with the development of Improvac® (Zoetis Inc., Parsipanny, New Jersey, US), the first commercial vaccine for use in pigs to provide an active immunization against GnRH [25]. Improvac® is only effective after two vaccinations. After the first vaccination, the immune system only produces some GnRH antibodies which are not sufficient to suppress testicular functions [26]. After the second vaccination, the so-called booster vaccination, the antibody production against GnRH, increases considerably [26] and suppresses the hypothalamic–pituitary–gonadal axis [27]. **Figure 1** illustrates the endocrine regulation of testicular functions according to Claus et al. [8], and further the effects of Improvac® on this cascade. The release of the hypothalamic factor GnRH binds to GnRH receptors of the pituitary. This induces the secretion of LH, which in turn binds to receptors in the target tissue, the Leydig cells in the testicles. In the Leydig cells, LH stimulates the synthesis of testosterone and estrogen, and the release of the pheromone androstenone. The androgenic effects of testicular steroids, in particular testosterone, affect different target tissues and lead to a pronounced sexual dimorphism in muscle growth [28,29], more efficient feed utilization and thus a lower nitrogen excretion [10], and a tendency towards more aggressive behavior

[8]. Testosterone also acts as a feedback signal on the hypothalamus and controls the further release of GnRH [8]. Immunocastration interrupts the entire androgenic effects by interrupting the endocrine cascade which controls testicular hormone secretion [30]. For this reason, after the second vaccination, immunocastrates are from a physiological point of view like barrows [27].

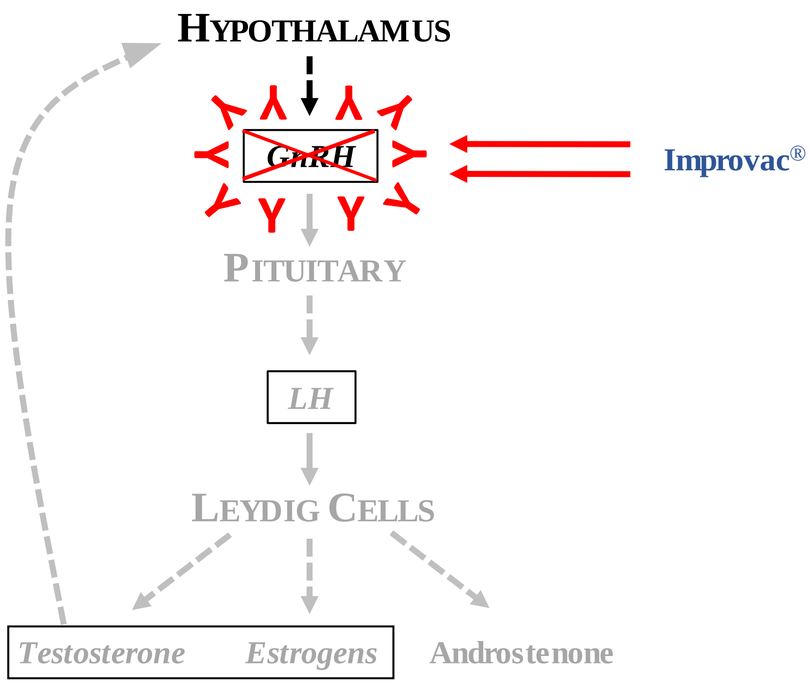

Figure 1. Endocrine regulation of testicular functions in boars and the impact of Improvac® on the endocrine cascade by inhibiting Gonadotropin-releasing hormone (GnRH) and thus testicular functions. Endocrine glands: capital letters; Hormones: italic letters; Pheromone: bold letters.

As already mentioned above, the current debate about alternatives to surgical castration is not only concerned with the boar taint issue and economic performance, but also with the consequences for the environment, animal welfare, consumer acceptance and pork quality [15]. The consequences of immunocastration on all aspects of sustainability are well known and described in more detail in MANUSCRIPT I and here only briefly summarized. Immunocastration improves some animal welfare aspects, such as avoiding painful surgical castration but also reducing the risk for more agonistic behavior of boars [31–34]. In addition, the anabolic effects up to the second vaccination have a positive impact on growth performance [35,36], so that immunocastrates have a more efficient feed conversion with lower nitrogen excretion than barrows [37]. In terms of meat quality, immunocastrates are comparable to barrows and have similar lean meat percentages, tenderness and fatty acid compositions [35]. However, the timing of the second vaccination is also decisive here. A recent study by Čandek-Potokar et al. [38] shows that the meat quality of immunocastrates in the production of traditional dry-cured ham products lies between that of boars and barrows. The period of 4 weeks between second vaccination and slaughter is too short and has a negative effect on the processing characteristics, as the lean meat percentage is too high. Immunocastration also has the potential to be economically profitable, as the better growth performance of immunocastrates and their more efficient feed conversion compared to barrows can compensate the costs for vaccination and thus make immunocastration competitive with the other alternatives [39,40]. Consumer studies show that the current practice of surgically castrating male piglets is not well-known. Consumers are basically open to immunocastration as long as it can be guaranteed that the welfare of the pigs is improved, the product quality is constant, and food safety is guaranteed [41]. Consumer acceptance of immunocastration can be further increased through purposeful and quality-oriented communication with consumers [42,43].

Immunocastration is not only used in pork production but also in other farm animals to avoid surgical castration. This has the advantage that the pain induced during surgical castration itself, the risk of wound infections and potential losses are avoided [24]. The suppression of testicular steroids and of associated agonistic behavior problems also improves the welfare of male animals that have been immunologically castrated [30]. This has resulted in several commercial immunocastration products being offered on the market, e.g. Improvac® and Valora® (Ceva Santé Animale, Libourne, France) for pigs [25], Bopriva® (Zoetis Inc., Parsipanny, New Jersey, US) for cattle [44], Equity® (Zoetis Inc., Parsipanny, New Jersey, US) for horses [45] and GonaCon® (USDA, Pacarello, ID, US) for wildlife and feral horses [46]. In Europe, however, Improvac® is the only approved product (by the European Medicines Agency in 2009) for commercial use in pigs [47]. Although Improvac® has been approved for almost 10 years in the European Union [47], the market share of immunocastrates is only 2.8% of all male pigs [6]. Nonetheless, there are countries that have a considerable share of immunocastrates. In Europe, for example, Belgium produces approx. 15% immunocastrates, while on a global level Brazil and Australia have a market share of immunocastrates of above 50% [41,48,49]. The low market acceptance among various European stakeholders when it comes to immunocastration is mainly related to a lack of practical experience and uncertainties as to whether this technique will be sufficiently accepted by other stakeholders [14]. Immunocastration offers the pork chain a chance to use the positive androgenic effects on growth performance and the lower environmental impact of boars. However, these advantages must be adjusted by the timing of the second vaccination and ensured by a reliability of the vaccination to avoid boar taint [14]. Both androstenone and skatole need 1 to 2.5 weeks to be released and metabolized from the adipose tissue of boars after the formation of testicular steroids has been eliminated [50,51], so the second vaccination must be applied at least 4 weeks before slaughter to ensure low boar taint values [14]. A potential uncertainty results from the fact that carcasses from some immunocastrates reveal boar taint, despite being vaccinated twice. It is reported that up to 3% of immunocastrates are so-called non-responders [48], which means that slaughterhouses and retailers are not convinced that the technique works reliably. The market uncertainty in terms of meat and carcass quality can lead to the result that immunocastrates are priced as boars, thus resulting in an economic disadvantage [39].

1.1 Overview and main research objectives

The main objective of this doctoral thesis was to address some of the key problems mentioned above in regard to the low market acceptance of immunocastration within the European pork chain. First, a comprehensive review article based on scientific publications was composed to analyze the impact of immunocastration on pork production based on the three pillars of sustainability and to compare immunocastration with surgical castration and pork production with boars. Two particular knowledge gaps within the pork chain were identified. As described above, the reliabilty of immunocastration has been questioned, as the phenomenom of non-responders is discussed controversially within the pork chain. The reasons which may lead to an insufficient immune response after Improvac® vaccinations are unclear so far. As social stress can have a negative impact on the immune system, we have experimentally investigated whether social mixing has a negative impact on the reliability of immunocastration. The hypothesis was that more challenging housing conditions may lead to higher incidences of non-responders. Due to the lack of market experience, it is furthermore uncertain how carcasses of immunocastrates will be priced in Germany, as the leading slaughter companies have different carcass pricing systems for barrows/gilts and boars. In addition, it is so far unclear to what extent fines for boar taint will be implemented if market shares of boars and immunocastrates further increase. The aim was therefore to investigate the effects of carcass pricing systems and fines for boar taint on the profitability of German pig production. In this context, the hypothesis was that both a pricing of immunocastrates like boars and the introduction of fines against boar taint would worsen the competitiveness of pork production with boars and immunocastrates.

1.2 Objectives and methodological approaches of included manuscripts

This doctoral thesis is based on an extensive physiological experiment with male pigs (boars, immunocastrates and barrows) that were raised during the fattening period under different housing conditions (enriched, standard and social mixing). Blood was collected from all pigs via puncture of the *vena jugularis externa* at different times to test the effects of sex group (gonadal status) and housing condition on different physiological parameters. The blood samples were analysed for GnRH-antibodies and testosterone concentrations using various laboratory methods, including GnRH-iodination and RIA. Furthermore, adipose tissue was sampled at slaughter for the determination of boar taint compounds by HPLC. Different parts of the genital tract were also collected and weighed at slaughter and performance data recorded during the entire experiment. The data were evaluated on the basis of a linear mixed model. Moreover, the performance data of pigs housed under standard conditions were set in relation to an economic data set and the competitiveness of pork production with immunocastrates and boars was analyzed using different carcass pricing systems and risk scenarios for boar taint and compared to pork production with barrows.

MANUSCRIPT I

Sustainability of Pork Production with Immunocastration in Europe

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Immunocastration is one alternative to surgical castration of male piglets without anesthesia or analgesia. Until the second vaccination, immunocastrates are from a physiological perspective similar to boars and then like barrows. The particular advantages of pork production with boars are a better feed conversion than with barrows and the resulting lower environmental impact. Disadvantages of fattening boars are a higher potential for agonistic behavior, problems with processing meat from boars due to a higher proportion of unsaturated fatty acids, and the risk of boar taint. Surgical castration, on the other hand, is painful when performed without appropiate analgesia or anesthesia. The feed conversion in barrows is more inefficient than in boars, but a high product quality is guaranteed. Agonistic behavior in barrows is less pronounced than in boars. Depending on the timing of the second vaccination, immunocastrates may resemble boars or barrows, with all the respective advantages and disadvantages. The aim of this review article is therefore to assess immunocastration globally with regard to the three pillars of sustainability, to compare the advantages and disadvantages of pork production with boars or barrows, and to describe the corresponding consequences for the whole pork chain.

MANUSCRIPT II

The Economic Impact of German Pig Carcass Pricing Systems and Risk Scenarios for Boar Taint on the Profitability of Pork Production with Immunocastrates and Boars

Published in *Agriculture* **2019**, 9, 204

Based on MANUSCRIPT I, it turned out that the economic performance of immunocastration is crucial for its implementation and acceptance among pig producers. Due to the currently low market share of immunocastrates in Germany, it is futhermore unclear how carcasses of immunocastrates are priced at the slaughterhouse, since gilts/barrows and boars are priced differently in Germany. Boar taint is currently not fined at German slaughterhouses, although it minimizes the value of affected carcasses and objective methods for determining the two boar taint compounds (androstenone and skatole) at the slaughter line have become available. However, if the market shares of intact boars (boars and immunocastrates) further increase, fines for boar-tainted carcasses can be expected. The aim of MANUSCRIPT II was to investigate the extent to which a switch from pork production with barrows to pork production with boars or barrows impacts on the competitiveness of pig production in different regions in Germany, taking into account different pricing systems and sanctioning mechanisms for boar taint. Performance data of pigs (standard housing conditions) from the trial described in MANUSCRIPT III was used and set in relation to the economic data set of agri benchmark. In addition, the substances responsible for boar taint, androstenone and skatole, were measured and economically evaluated on the basis of different thresholds for boar taint.

MANUSCRIPT III

Influence of Housing Conditions on Reliabilty of Immunocastration and Consequences for Growth Performance of Male Pigs

Published in *Animals* **2020**, 10, 27

Non-responders to immunocastration are reported in some scientific publications, described as pigs that, despite being vaccinated twice with Improvac®, have boar-tainted carcasses. The reasons for an insufficient antibody response to Improvac® vaccination are unknown.

Therefore, the aim of this study was to examine whether stress due to more challenging housing conditions has an impact on the immune response after Improvac® vaccinations. For this purpose, male pigs (boars, immunocastrates and barrows) were housed under different housing conditions (enriched, standard and repeated social mixing), and the antibodies against GnRH were determined at different times (before and after each vaccination and at slaughter). Testosterone concentrations were also analyzed as an indicator for testicular functions, and fat samples were collected at the slaughter line to measure concentrations of boar taint compounds. Furthermore, the performance data of the animals were recorded in order to compare the competitiveness of the different sex groups and housing conditions. The data were evaluated on the basis of a linear mixed model.

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2 MANUSCRIPTS

This chapter contains all manuscripts that were prepared in context with this doctoral thesis. The content and text of the manuscripts are included in this thesis as they were published. The layout was adapted to this doctoral thesis. All three manuscripts have already been published in international peer-review journals.

I Sustainability of Pork Production with Immunocastration in Europe
Published in *Sustainability* **2019**, 11 (12), 3335

II The Economic Impact of German Pig Carcass Pricing Systems and Risk Scenarios for Boar Taint on the Profitability of Pork Production with Immunocastrates and Boars
Published in *Agriculture* **2019**, 9 (9), 204

III Influence of Housing Conditions on Reliability of Immunocastration and Consequences for Growth Performance of Male Pigs
Published in *Animals* **2020**, 10 (1), 27

I

Sustainability of Pork Production with Immunocastration in Europe

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Abstract

Immunocastration, a technique to replace surgical castration of piglets, consists of two consecutive vaccinations to induce antibodies which transiently suppress testicular functions and avoid boar taint. It is a method to ensure both a high product quality and a high level of animal welfare. The impact of immunocastration on the three pillars of sustainability has been studied extensively. While all aspects of sustainability have been studied separately, however, a contemporary global overview of different aspects is missing. In immunocastrates, performance results are better than in barrows, but worse than in boars. The environmental impact of pork production with immunocastrates is lower than with barrows, but higher than with boars. The level of aggression is considerably lower in immunocastrates compared to boars. Societal concerns are mainly related to food safety, and are not supported by scientific evidence. After second vaccination, immunocastrates switch from a boar- to a barrow-like status. Therefore, the timing of second vaccination is a fine-tuning tool to balance advantages of boars with environmental and economic benefits against increased risk of welfare problems and boar taint. Nevertheless, both synergic and conflicting relationships between the pillars of sustainability must be communicated along the value chain to produce tailored pork products.

Keywords: sustainability; immunocastration; carbon footprint; animal welfare; food safety; pork production; boars; surgical castration

1. Introduction: The Castration Dilemma in Pork Production

In Europe, many citizens are concerned about the impact of intensive production conditions of farm animals on animal welfare and the environment [1,2,3]. A critical evaluation of the aspects which cause public disapproval is necessary and sustainable improvements have to be introduced, where negative conditions can be avoided. A main problem is that conflicting aims may occur which must be balanced with different market needs as well as stakeholder requirements [4]. Such a situation currently applies in Europe in the debate about castration of male piglets [5,6,7]. Surgical castration is painful and hurts the animals' integrity, it is therefore a major welfare issue [6]. For centuries, male piglets designated for pork production have been surgically castrated in Europe to improve behavior and product quality [5,8,9,10]. The fattening of boars has advantages such as requiring fewer resources to produce the same amount of meat due to more efficient feed conversion ratio, reduced nitrogen excretion, and a higher protein accretion compared to barrows and gilts. However, disadvantages in pork production with boars remain, including boar taint and welfare problems due to increased aggression and mounting behavior. Those may limit the acceptance of pork production with boars by farmers, the meat industry and consumers [9,11,12,13]. In boars, a sex-specific off-odor of the carcass may develop in some growing boars during puberty, which can be mainly ascribed to two substances, androstenone and skatole. Androstenone is a male pheromone which is formed in the Leydig cells of the testes and has a urine-like smell [12,13]. Skatole is a metabolite of the amino acid tryptophan with a fecal odor and is synthesized in the colon by microbial degradation [14]. Boars may accumulate more skatole than barrows or gilts in adipose tissue because the hepatic degradation of skatole is reduced, due to lower activities of CYP2E1 and CYP2A enzymes if concentrations of androstenone or testosterone are high [15]. A cross-national European study by Walstra and co-authors [16] showed that while 29% of the carcasses reveal high androstenone concentrations, only 11% show elevated skatole concentrations and that slaughter weight and genotype modify this percentage considerably (high androstenone level: range 18–42%, high skatole level: range 5–23%). Androstenone and skatole are perceived differently by consumers depending on individual sensitivity. Whereas most consumers are sensitive to skatole [17], Lunde and co-authors [18] described a specific anosmia for androstenone, which explains the variable percentage of consumers perceiving androstenone. Both compounds share the disadvantage that consumers who are sensitive to the substances rate them as very unpleasant and may therefore reject pork from boars more often [19,20]. In addition, aggressive and sexual behavior of boars may lead to animal welfare problems such as leg weakness or penile injuries [11,21].

Growth is the result of a predominance of anabolic over catabolic metabolic processes. In pigs, it is orchestrated by the activity of sex-independent anabolic hormones such as growth hormone and IGF-I, and of catabolic hormones such as glucocorticoids. Gonadal hormones in boars already interact during the fattening period, with the endocrine regulation of growth by decreasing catabolic processes (e.g., androgens) or increasing anabolic processes (e.g., estrogens via stimulatory action on IGF-I secretion). This leads to more efficient growth, increased nitrogen retention and higher protein accretion rate when compared to barrows [22]. Both androgens and estrogens also decrease the voluntary feed intake and improve the feed conversion ratio, explaining the known differences between barrows, gilts, and boars [12]. Taken together, all those factors lead to higher muscle and lower fat deposition. Thus, boars have a higher lean meat content than barrows [23]. Moreover, boars have a higher concentration of polyunsaturated fatty acids (PUFA) in adipose tissue, which may be healthier for human consumption but is regarded as a problem for processed meat products [24,25]. Therefore, boars are more efficient in the fattening period but create problems in terms of product quality and animal welfare [9,11,21,26,27].

Surgical castration is effective in preventing those problems, but also removes the anabolic advantage of boars [12]. In most European countries, surgical castration of male piglets without anesthesia or analgesia is still permitted within the first seven days of life [28]. Usually, farmers castrate male piglets without any pain-relieving methods [29,30,31]. Already in October 2010, representatives of major stakeholders committed themselves to a roadmap to voluntarily end surgical castration of male pigs in Europe by 1 January 2018 [32]. Despite this commitment, about 63% of all male piglets in Europe were still surgically castrated in 2017, most of them without any pain relieving methods or anesthesia even though alternatives exist [30]. Today, these figures have not changed considerably in Europe.

Immunocastration is an active immunization against GnRH [33] and could be a sustainable alternative to solve the above-mentioned problems of pork production with boars and surgical castrates, and thus could make European pork production more competitive. In contrast to other parts of the world such as Australia and Brazil [10], immunocastration in Europe is not frequently used, with a low percentage of 2.8% in 2017 [30]. Little practical experience of stakeholders and no targeted communication about the consequences of immunocastration [34] of European pig genotypes for management, feed requirements, and product quality exist in the market. These knowledge gaps may explain why a method that might have economic, ecological, and societal advantages still has no market relevance at the moment. Market acceptance could be increased, if the sustainability of immunocastration is scientifically demonstrated. In order to evaluate immunocastration from a sustainability point of view, this review examines factors which are part of the three pillars of sustainability (society, economy, and environment) and how their interactions can lead to both synergic and conflicting relationships within the three pillars.

2. The Principle of Immunocastration

Immunocastration is an active immunization against GnRH, a key hormone of the endocrine cascade regulating reproductive functions. In consequence, the pig’s immune system starts the production of antibodies against the hypothalamic hormone GnRH and postpones the pubertal development by suppressing the hypothalamic pituitary gonadal axis [33]. The treatment consists of at least two injections of the vaccine Improvac® during the fattening period. The manufacturer recommends vaccinations at an age of about 12 weeks and again at 4–6 weeks before slaughter. Within a time interval of at least 4 weeks between the first and second vaccination [10]. After the first application of Improvac®, some GnRH antibodies are already formed but their concentration is not sufficient to limit gonadal axis activity [35]. Within one week after the second vaccination, the production of GnRH antibodies increases markedly and suppresses testicular steroid synthesis and in consequence spermatogenesis [36,37]. The drop in testosterone and estradiol concentrations occurs within a week, followed by a reduction in IGF-I secretion [36]. Due to the lack of testosterone in the hormonal feedback system, both immunocastration and surgical castration initially increase the release of GnRH by the hypothalamus but lead to a reduced GnRH synthesis in the hypothalamus further on [38]. In Europe, only one product (Improvac®) is available to date for immunocastration of male pigs. The vaccine is manufactured by Zoetis (formerly Pfizer Ltd., formerly CSL Limited, Parkville, Victoria, Australia) and has been approved by the European Commission in May 2009 for use in pigs within the European Union [39]. Due to the strong interlinked regulation of boar taint compounds and testicular hormones, immunocastration always affects their formation in a similar way [40]. Thus, the only way to maintain the anabolic advantage of boars is the appropriate timing of the antibody formation leading to a tailored cessation of the testicular steroid synthesis. This avoids the accumulation of boar taint till slaughter, although the anabolic effects of testicular hormones are still maintained during the main part of the fattening period [10]. At the same time, male aggressive and sexual behavior can also be reduced as described in Section 3.3. Active immunization against GnRH was already discussed in the 1970s as a potential means by which the reproductive system of mammals might be shut down for various practical and clinical reasons [33]. In 1998, a patent (International application number: PCT/AU1998/000532) has been submitted and was published under the international publication number WO 1999/002180 (21 January 1999; Pfizer Inc. New York, NY, USA). As GnRH itself has no immunogenic effect and does not stimulate antibody production, a proprietary strategy must be used to deceive the immune system and recognize GnRH as an antigen [33]. This strategy includes the use of GnRH or a modified GnRH (truncated or repeated, with or without amino acid substitution) as antigenic target linked to a carrier substance [41]. Antigens which are conjugated through its C terminus seem to produce a higher specific antibody response than constructs in which GnRH is conjugated through its N terminus [42]. In case of Improvac®, the antigenic part of the construct is the C-terminal fragment of GnRH (AS 2–10) conjugated to a diphtheria toxoid and adsorbed to DEAE-dextran (Patent US 8741.303 B2; 3 June 2014; McNamara).

3. Potential of Immunocastration for Sustainable Pork Production

3.1. On-Farm Application of Immunocastration

Even if the first vaccination could be applied at 8–9 weeks of age [10], such an early vaccination may not be recommended if piglets are sold and not raised on the same farm where they have been born, as the vaccination cannot be controlled afterwards and a 100% vaccination rate is required to avoid behavioral and quality problems. As a consequence, the first vaccination is usually carried out early in the fattening period at an age of about 12 weeks. The endocrine changes induced by the second vaccination lead to a switch from boar-specific feed intake, metabolism, and behavior to that of barrows with a further delay of about one week as described in Section 3.3, Section 3.6 and Section 3.7 below. The recommended time between the second vaccination and slaughter is about 4 to 5 weeks to allow the release of already accumulated androstenone and skatole from adipose tissue. Even if long-term studies revealed a resumption of testicular function after 10 to 24 weeks [43,44], a third vaccination is only suggested if animals are slaughtered at a higher age [45].

Several studies have been conducted to evaluate the effect of a variation in the timing of the second vaccination. As further described below, the decision has to be balanced between the conflicting aims of desirable boar-like growth efficiency, lean meat content, and the superiority of barrows in behavior, as well as the quality of adipose tissue and meat. Such differences are obvious in the meta-analysis of Nautrup and co-authors [46] who compared immunocastrates vaccinated for the second time more and less than 4.5 weeks before slaughter. The animals vaccinated later were more boar-like in their growth and carcass characteristics than the immunocastrates vaccinated more than 4.5 weeks before slaughter. Whereas, in some studies, the vaccination protocol of first vaccination/second vaccination at 10/14 and 16/20 weeks of age or 11/21 and 11/18 weeks of age did not lead to significant differences in growth performance and carcass composition [47,48], differences in dressing percentage [47], carcass yield [49], and welfare problems [50] were reported. The early vaccination protocol, however, was not recommended with regard to boar taint [50].

3.2. Reliability of Immunocastration

An important criterion for being successful in the market is the reliability and efficacy of the method. Several reviews have described the phenomenon of non-responders [8,10,51]. As with all vaccines, a small percentage of animals will not or will just poorly respond to the vaccine for both disease vaccines and Improvac® vaccination protocols with a two-fold application of the vaccine increase the effectiveness and may provide almost 100% efficacy [52]. It is assumed that on average 0–3% of the Improvac®-vaccinated animals were non-responders [10]. The rate and definition of non-responders, however, varies between studies and depends on the criteria investigated. Thus, non-responders were defined as animals with enlarged testes (similar to the testes size of boars) or with boar taint (above the threshold of 0.5–1 ppm androstenone), despite two assumed vaccinations. Reasons given for non-responding include that these animals might have been accidentally missed during vaccination or might have had a suppressed immune system due to health problems or stress at the time of vaccination. It has to be kept in mind that only healthy animals are suitable for vaccinations. In a study by Sødring and Naadland [53], about 1% of all immunocastrates slaughtered in Norway in 2017 were tested for boar taint as the success of vaccination seemed doubtful due to the size of their testes. 29% of these suspicious animals had androstenone values above 1 ppm and were classified as non-responders. A recent study by Kress and co-authors [35] hypothesized that a stressful unstable social environment could reduce the chance of adequate immunization. Even under intensive housing conditions and additional stress before and after the vaccinations, however, all immunocastrates showed a sufficient immune reaction with high GnRH antibody titers and low testicular steroid production. Similarly, the meta-analyses by Batorek-Lukač and co-authors [23] and Nautrup and co-authors [46] show that immunocastration prevents boar taint effectively and is a reliable method. It seems that if the vaccine is handled and stored correctly, and if the manufacturer's vaccination recommendations are met, almost 100% of the vaccinated animals produce sufficient antibodies and react accordingly [35]. This, however, does not rule out that occasionally insufficient immunizations occur under practical conditions, e.g., if animals are vaccinated only once by accident. As such animals have no higher risk for high boar taint levels than boars, it remains a corporate risk decision of the slaughter house, whether or not to test for boar taint at the slaughter line. With an assumed proportion of 3% non-responders [10] and a tainted carcasses rate of 30% among boars [16], the risk of tainted carcasses in immunocastrates is 0.9%. Assuming a reproducibility of the currently used human nose test at slaughter line to detect boar taint of 23% [54], the risk of marketing tainted carcasses of immunocastrates is far below the currently marketed carcasses of boars with off-odor.

3.3. Consequences of Immunocastration for Animal Welfare, Behavior, and Health in Pork Production

A major benefit of immunocastration is an increase in **animal welfare** by preventing painful surgical castration and the risk of wound infection in piglets. The study by Morales and co-authors [55] shows that the piglet mortality during the first week post-partum is higher in surgically castrated piglets than in intact piglets (6.3% vs. 3.6%). Especially piglets with a low or medium live weight at birth have a significantly higher mortality rate than uncastrated piglets (low:12.2% vs. 6.2%; $p < 0.05$, medium 5.5% vs. 2.7%; $p < 0.05$).

Even with immunocastration, pigs have to be treated by humans. However, immunocastration is carried out later in life during the fattening stage and farmers are given a longer time span to apply the vaccination [56]. Mimicking the injection procedure of Improvac®, McGlone, and co-authors [57] investigated the effects of intramuscular or subcutaneous injection of 1 mL of saline on pain and stress in finishing pigs. In general, no significant changes in activity behavior (such as lying, eating, walking, drinking) and physiology (cortisol concentrations) were noted 1 h after the injections. Thus, injection per se does not affect welfare, although the injection of Improvac® may cause a skin reaction in a small number of cases. While there were no visible site reactions at slaughter, some reactions could be detected by palpation in 6.25% of immunocastrates [58]. Compared to surgical castration, such rather local reactions may trigger minor discomfort in immunocastrates. Moreover, such adverse reactions can be avoided if the vaccine is applied according to the manufacturer’s recommendations (subcutaneous injections at the base of the ear) by trained persons. As with all vaccinations, a severe allergic reaction may happen on extremely rare occasions (1.31 per million vaccine doses) within a few minutes of vaccination [59]. Immunocastration is also effective in cryptorchids and avoids the more sophisticated surgical procedure or the even higher risk of boar taint, if the animals are untreated [60].

If the second vaccination is fully effective, immunocastrates show differences in **social behavior**, e.g., less aggression and mounting than boars, and are very similar to barrows demonstrated that the effect of immunocastration on behavior can prevail for a long time [61,62,63,64,65]. Even 16 or 22 weeks after the second vaccination, significant behavioral differences in social, manipulating, and aggressive behavior exist between immunocastrates and boars. On the other hand, the change in behavior appears relatively soon after second vaccination as described above (see Section 3.1) Thus, pigs that received their second vaccination only 1 week before behavioral observation did not differ from those who received Improvac® injection 3 weeks before observation [66]. It can therefore be concluded that the beneficial effects of immunocastration on behavior cover a relatively long time span from (at least) 1 week after second injection well until slaughter. Guay and co-authors [67] investigated the effect of immunocastration on human-pig interactions and handling during transport. There were only a few differences, e.g., more chewing and rubbing on the test person's pants and boots in immunocastrates compared to barrows. Other measures, such as the total time of approaching people did not differ between the two groups. Most studies on immunocastration have been conducted under experimentally controlled conditions, but some studies were also performed on commercial farms. The results obtained under field conditions resemble the experimental farm findings in showing that fighting and mounting is substantially reduced in immunocastrates compared to boars [62,68].

Such aggressive and sexual behavior is relevant for animal welfare, as it may also lead to **health** problems (e.g., scratches and wounding) in boars. In addition, mounting activity has led to lameness and skeletal problems for mounting and mounted animals in 15% of all boars [27]. In the study of Einarsson [58], scratches and lesions in the head region (assessed at slaughter) were highest in boars, much reduced in immunocastrates, and absent in barrows. Schmidt and co-authors [69] reported higher skin lesion scores in the shoulder region (caused by mounting behavior) in immunocastrates before second vaccination compared to barrows, which disappeared after second vaccination. Recent findings show that penile injuries are a major welfare problem in boars [21]. Before boars enter puberty, the penis frenulum prevents the penis from extruding. As soon as the boars enter puberty, they can completely extrude the penis. If a particular boar shows mounting behavior and extrudes its penis, it can trigger other pen mates to bite its penis. This phenomenon occurs both in domestic and wild boars and causes obvious animal welfare problems [21,26]. Immunocastration can reduce the incidence of penile injuries and the risk of severe injuries but does not completely prevent this problem. The vaccination protocol also affects the percentage of immunocastrates with penile injuries: the percentage is low (16.7%) if animals are vaccinated early, and increases up to 41.7% if animals are vaccinated late [50]. A recent study by Kress and co-authors [35] suggests a reduction in aggressive behavior and in penile injuries in immunocastrates compared to boars regardless of their housing environment (stressful, conventional, outdoor access). Another question which requires a lot more research is the application of immunocastration to mature boars. The findings of Bilskis and co-authors [70] reveal that testosterone and libido (characterized by pre-mating behavior) of mature boars (>2 years) can be reduced by immunocastration.

In a study by Cronin and co-authors [61], **feeding behavior** was also determined. At an age of 21 weeks, boars spent much less time in the feeders than immunocastrates and barrows. Weiler and co-authors [71] investigated this effect in more detail. Feed intake of boars was lower than in barrows and immunocastrates due to a reduction of number and duration of meals consumed per day. Immunocastration affected feed intake behavior with meal size increasing by 25%. Considerable increases in feeding behavior at least one week after second vaccination were also observed by Schmidt and co-authors [69] and Van den Broeke and co-authors [72]. Restrictive feeding of immunocastrates after second vaccination can lead to more aggressive behavior and higher incidences of skin lesions, comparable to the level among boars [73]. It is therefore recommended not to feed immunocastrates restrictively during the late finishing period.

3.4. Consequences of Immunocastration for Growth Performance, Carcass, and Meat Quality

Immunocastrates change their anabolic potential from that of boars to that of barrows after the second vaccination [10,33,36,74]. Before second vaccination, immunocastrates have a lower average daily gain and a more favorable feed conversion ratio than barrows, up to the second vaccination [23]. As a consequence, the higher boar-specific anabolic potential and the reduced feed intake can be exploited until the second vaccination as reviewed in detail by several authors, e.g., [23,46,75]. After the second vaccination, feed intake of immunocastrates increases significantly [23,71,72] but compared to barrows, they grow still more efficiently [23]. A recent meta-analysis by Nautrup and co-authors [46] including 78 studies showed that, over the entire fattening period, immunocastrates have higher average daily gains than boars and barrows, whereas their feed conversion ratio is intermediate between barrows and boars.

In most of the studies with a fixed duration of fattening, hot carcass weights also differ. Hot carcass weights of immunocastrates are lower than those of barrows, but higher than those of boars [46]. In terms of dressing percentage, boars are inferior to barrows mainly due to higher weights of the genital tract. The dressing percentage of immunocastrates is even poorer due to a higher volume of the gastrointestinal tract [75]. In terms of lean meat content of the carcass, boars are superior to immunocastrates, which have a higher backfat thickness.

Compared to barrows, however, the carcasses of immunocastrates are leaner [23]. Regarding the valuable parts of the carcass, immunocastrates have heavier shoulders and hams than barrows, but lighter bellies. Compared to boars, the carcass traits of immunocastrates are quite similar, but the bellies are heavier [23,46]. In total, the carcass yields of immunocastrates are more favorable than those of boars as well as barrows.

The meat quality of immunocastrates is similar to that of barrows. Both have higher levels of intramuscular fat and lower shear force values than boars [23]. Furthermore, the accumulation of boar taint in adipose tissue is significantly reduced by immunocastration [23,46]. The fatty acids composition of immunocastrates is also comparable to that of barrows and has less PUFAs than boars, which is particularly important in the production of dry-cured products [10]. The meta study of Nautrup co-authors [46] and the review of Čandek-Potokar and co-authors [10] both confirm these findings and suggest that the timing of the second vaccination allows for a product quality tailored to the demands of different pork markets.

3.5. Suitability of Immunocastration for Alternative and High Quality Production Systems

Immunocastration is mainly used in the production of male animals for standard conventional pork products [56]. Nonetheless, the methodology can be used for alternative production systems. In the production of traditional high quality pork products such as dry-cured hams and shoulders, animals are slaughtered at higher live weights [10,45]. In Iberian high quality production systems, boars and gilts are castrated surgically either to prevent boar taint or undesirable performance losses in female animals during estrus [76], or in extensive free-ranging housing systems in order to prevent unwanted pregnancies during fattening [77,78]. However, castration of females jeopardizes animal welfare and increases production costs and infection risks [76,79]. For traditional products, immunocastration is a good alternative to surgical castration or fattening of entire boars or gilts, as neither performance nor product quality are negatively influenced [45,77,80,81,82]. In a study by Pinna and co-authors [45] with heavy pigs (165 kg live weight) produced for Parma ham, three vaccinations were recommended to prevent boar taint reliably.

Immunocastration could also have positive effects on organic pig production: In a study by Grela and co-authors [83] boars, immunocastrates, barrows, and gilts were fattened under organic conditions. Growth performance as well as feed conversion ratio and lean meat content were more efficient in immunocastrates and boars than in barrows or gilts. Immunocastration was evaluated most favorably both from production and meat quality perspectives. Immunocastration can be considered as a suitable method for organic pig farming. As mentioned above, organic production systems should also take into account that, for longer fattening periods, animals should be vaccinated a third time in order to reliably prevent boar taint. Looking at the Council Regulation on organic production and labelling of organic products [84] at the European level it remains unclear how immunocastration is classified. According to the European veterinarian code, Improvac® is classified in a subgroup of hormone-like substances [85] and according to the EU Council Regulation mentioned above, no hormone-like substances may be used in organic pork production (EC no. 834/2007). From a scientific point of view, immunocastration is not a hormone application at all, so the EU leaves the decision of whether immunocastration is permitted on a national level or not to the EU-members.

In a study by Bilskis and co-authors [70] the efficacy of immunocastration was tested in cull boars from artificial insemination (AI) programs. It showed that even in mature boars (>2 years), testosterone levels decreased significantly after the third vaccination to a level found in young immunocastrates. In a further study with AI boars by Oliviero and co-authors [86], it was also shown that a single dose of Improvac® has no negative effect on the fertility of young AI boars. Immunocastration thus allows to use boars for AI services and to prevent boar taint in case of culling by a second vaccination. Such boars can be sold and used for meat products similarly to sows. Immunocastration provides possibilities for alternative production systems to maintain added value by higher animal welfare standards while at the same time delivering high quality products, thus bringing together the two formerly conflicting aims.

3.6. Consequences of Immunocastration for Feeding Requirements

Before the second vaccination, a sufficient amino acid provision is required to support the high protein deposition levels in boars. Thereafter, animals change their metabolism within two weeks [87,88] as described in Section 3.1. In contrast to the increased fat deposition after second vaccination [23,89], the protein deposition seems to remain nearly constant [90]. However, because of the increased feed intake after the second vaccination as described in Section 3.3, the feed intake can be limited or the amount of protein per kg of feed, to limit the increase in nitrogen emission. Quantitative restriction of feed intake has been shown to limit protein deposition in immunocastrates [91] but triggers behavioral problems as described in Section 3.3. Utilization of dietary fibers to dilute protein and energy, on the other hand, does not satisfactorily decrease the intake of protein and amino acid in immunocastrates [92]. Alternatively, protein and amino acid to energy ratios can be decreased rapidly from the second vaccination onwards in order to limit the excess in protein and amino acid intake. Studies have therefore proposed to decrease the dietary lysine to energy ratio by 20–35% [89,93], but this second option largely depends on the level of feed intake [91]. Moreover, the way animals use dietary energy affects the efficiency of energy utilization as described below in Section 3.7. Labussière and co-authors [94] showed that five weeks after the second vaccination, immunocastrates exhibit a lower basal heat production (783 vs. 856 kJ/kg BW $^{0.60/\mathrm{day}}$) than boars but an increased heat increment (25.6 vs. 21.6% of ME) when animals were fed the same diet. The difference in basal metabolism can be directly linked to the level of testosterone and anabolic hormones. The difference in energetic efficiency is indicative of the utilization of dietary protein as an energy source for ATP provision and lipid deposition [90], which is less efficient than the utilization of carbohydrates and lipids for such purposes [95]. Most of the time, feeding recommendations are nevertheless supported by measurements in animals in a steady state, e.g., before the second vaccination, or when the transition phase has finished. It has also been shown that modified feeding behavior following the second vaccination [71] is associated with modified glucose metabolism [96,97], which may affect energy efficiency. Because discrepancies between animals in their transition from boar to barrow status may occur, the kinetics in metabolism changes should be considered carefully because of the large variations in speed of feed intake increase between animals or groups of animals [71].

3.7. Consequences of Immunocastration for the Environmental Burden

While pork is of high nutritive value, the pig’s omnivorous nature and the way it is fed nowadays negatively affects the perception of pork because of environmental concerns. Major points that influence this perception include the consumption of edible proteins for humans, the global warming potential of meat production, and the excretion of nutrients (most important nitrogen and phosphorus) leading to water eutrophication and soil acidification [98]. Diet composition is an important factor here. Today, pigs are most often fed cereals, legumes, and by-products from the cereal and oil food industry [99]. With the selection towards higher efficiencies, the environmental burden per kg of pork has diminished [100]. Key driver is feed conversion ratio. A study by Reckmann and Krieter [101] showed that feed conversion ratio was the performance parameter in finishing that had the largest impact on global warming, eutrophication, and acidification potential. In the same study, increased lean meat percentage was also linked with decreased environmental impact, although partially because of the link with improved feed conversion ratio. The functional unit of expressing the environmental impact—per pig place, per kg of pig, per kg of carcass, or per kg of meat and the time period included (life cycle assessment or fattening period)—may also affect the interpretation [98,100,102]. In boars, with no pharmaceutical products used for castration, while the feed conversion ratio is low, lean meat percentage is high and carcass yield better than in immunocastrates [23,75,103]. Expressed per kg of pig, carcass or meat, this type of male pig raising is therefore expected to be most environmentally friendly. Hence, boars are compared to different scenarios for pork production the most environmental friendly one [99]. Still, the estimated impact may differ between farms and management strategies and the assumptions made. In a study by Bandekar and co-authors [102], it was concluded that boars had a slightly higher global warming potential than the baseline scenario with barrows. However, they compared slaughtering male pigs at a low slaughter weight (91 kg) with keeping barrows until 125 kg and using ractopamine in barrows. So their model assumed only small differences in feed efficiency despite the lower slaughter weight in boars. This result may not be valid in Europe, where ractopamine is not used and where boars and barrows are slaughtered at a similar weight, but with a larger difference in feed efficiency than assumed by Bandekar and co-authors [102].

An improved environmental impact may be expected with immunocastrates versus barrows, at least per kg of pig due to differences in performance [75]. Indeed, the carbon footprint of a pigs' feed intake was significantly higher in barrows compared to boars and immunocastrates, with intermediate results for gilts [104]. Nitrogen efficiency was also higher in immunocastrates than in barrows and slightly lower than in boars [105]. Immunocastration is therefore considered to lessen the environmental impact of pork compared to barrow production [102,106,107]. Comparing barrows receiving ractopamine throughout finishing with immunocastrates receiving ractopamine after second vaccination, Bandekar and co-authors [102] estimated a reduction of 2.39%, 2.57%, and 2.96% in global warming potential, energy use and water use per kg pig, respectively. As the dressing percentage in immunocastrates is somewhat lower than in boars and barrows [23,75,103], the difference between barrows and immunocastrates in environmental impact per kg of carcass may be less pronounced. On the other hand, the lean meat percentage is higher in boars and immunocastrates than in barrows. Thus, also per kg of meat, immunocastrates can be expected to have a lower impact than barrows. This was confirmed by the study of De Moraes and co-authors [107], who calculated a 3.7% improvement of global warming potential per kg live weight and of 5.0% per kg of meat in immunocastrates versus barrows.

Apart from the differences in performance and hence the amount of feed consumed, the environmental cost of the product for vaccinating pigs also needs to be taken into account. In a study carried out by the manufacturer of Improvac® and an independent consulting firm [107], the calculated contribution of the product manufacturing accounted for 0.01% of the global warming potential, compared to 36% assigned to the feed consumed and 30% to the slurry management. In barrows, there is increasing pressure for using analgesics and anesthetics during castration. Isoflurane, currently used in some European countries for castration under anesthesia, is a potent greenhouse gas. Environmental burden by the use of isoflurane or others anesthetic drugs has to be considered in the assessment of environmental sustainably [108].

One shortcoming in most studies comparing different types of male pig production is that they often do not correct for altered nutrient requirements and thus for possibly different diets in these different production systems. Compared to boars, barrows have lower amino acid requirements. Hence, barrow diets may contain less soybean meal than boar diets. While their feed conversion ratio is worse, the environmental impact per kg of feed consumed by barrows may be lower than in boars. A classic reductionist approach may overestimate the difference in environmental impact. Similarly, the finishing diet of immunocastrates may be optimized to minimize their environmental impact. It needs to be taken into account that male pigs are only half of the pigs born on a farm. On individual farm level, the effect may depend on whether male and female pigs are raised and fed together or separate as described above (Section 3.6). While ceasing the castration of piglets may improve the environmental sustainability of pork production, this will only happen with optimal management and especially feeding. Key principles such as precision feeding, the use of enzymes such as phytase to increase nutrient digestibility, and the application of free amino acids to reduce total crude protein content may have a more pronounced effect than that achieved by just the castration decision. Further insights in the sustainable feeding of pigs, in particular immunocastrates, are therefore crucial.

3.8. Consequences of Immunocastration for Economy

Immunocastration is highly controversial in international pork markets and globally rarely used in practice. There are however some international differences, while some countries reject the method completely [10,30], in other countries such as Brazil and Australia, immunocastration is already widely used with more than 50% of all male pigs vaccinated [10,34,109]. Based on a press release by Zoetis in June 2018, more than 2.5 million doses of the vaccine Improvac® are sold each month worldwide [110]. This means that with an average of two vaccinations per animal, about 15 million immunocastrates are produced annually. In 2018, 1.27 billion pigs were slaughtered globally [111]. Assuming that half of the slaughtered pigs are male, 634.6 million of male pigs were slaughtered in 2018. The global proportion of immunocastrates is then about 2.36%. Within three years, both the absolute number and the proportion of immunocastrates have doubled worldwide between 2015 and 2018 [8,110,111].

The cost per dose of Improvac® range between 1.4 € and 1.5 € [112]. With two vaccinations plus labor costs (45–50 s for both vaccinations per pig), the additional expenses amount to 3–4 € per pig [113,114]. Decreasing costs are likely, as depending on the size of the farm-larger purchasing volumes may lead to discounts which create economies of scale. Additionally, it is possible that generic pharmaceuticals or other vaccines may increase the cost competition of suppliers, further reducing vaccination costs per animal. At present, only one product for pigs is available on the European market (Improvac®), and the manufacturer has a monopoly in this segment. A review of Vondeling and co-authors [115] shows that pharmaceutical prices fall by 6.6–66% after patents expire. A recent study by Verhaagh and Deblitz [114] even estimated price reduction at 55%, based on historical discounts after patent expiration. This could further increase the economic profitability of immunocastration. Along the pork supply chain, additional costs may arise from factors such as specialization of production systems, special sorting of immunocastrated animals or carcasses, removing the testes, boar taint detection, or rejected carcasses due to boar taint. As described above (Section 3.2), the risk of immunocastrates for displaying boar taint is very low. This explains why many economic studies do not consider the cost of boar taint detection at slaughter line in their research [113,116]. In addition, the economic efficiency of pork production systems with immunocastrates is influenced by the pricing scheme that is applied. An analysis by Niemi and co-authors [116] shows that additional costs of 1 € per metric ton of pork arise if immunocastrates are sold according to the boar pricing scheme. If the production systems adapt and immunocastrates are sold similar to barrows, a value added of € 21 per metric ton pork arises. Irrespective of these aspects, the studies by de Roest and co-authors [113] and Verhaagh and Deblitz [114] show increased production costs of immunocastrates on farm level due to vaccination costs, labor costs, and feed costs. On average, these additional production costs are compensated by higher revenues due to higher performance resulting in more pigs produced per place and year. As a consequence, immunocastrates represent a viable alternative for European pig producers.

Even though immunocastration could be very beneficial in terms of animal welfare, product quality, environment, and production efficiency, market shares of immunocastrated male pigs are very low in Europe as shown in **Table 1**. Despite the fact that European stakeholders aimed to end surgical castration by 2018 [32], there is still no common political strategy recognized within the pork chain. European pork markets are too diverse [30] and in addition to pork production with boars (see **Table 1**), each nation develops more or less efficient implementation strategies (see **Table 2**).

Table 1. Population shares of male pigs raised as boars, immunocastrates or barrows in Europe (2017), ranked according to size of pig population [30].

Country	Boars (%)	Immunocastrates (%)	Barrows (%)	Pig Population (x1000)
Germany	20	<1	80	28,046
Spain	80	5	15	25,495
Denmark	<2	0	>97	12,402
Netherlands	65	0	35	12,013
France	22	<0.1	78	11,835
Italy	2	5	93	8561
Belgium	8	15	80	6351
Romania	0	5	95	5180
UK	98	<1	2	4383
Hungary	1	0	99	2935
Austria	5	0	95	2846
Portugal	85	2.5	12.5	2014
Norway	<1	6	94	1644
Switzerland	5	2.5	92.5	1573
Czech	5	5	90	1548
Ireland	100	0	0	1468
Sweden	1	9	90	1354
Finland	4	0	96	1258
Slovakia	0	10	90	637
Latvia	0	0	100	368
Estonia	0	0	100	359
Slovenia	1	0	99	288
Macedonia	0	0	100	200
Luxembourg	1	0	99	90
Iceland	0	0	99	36
Total	**34**	**2.8**	**63**	**132,884**

Table 2. National strategies to substitute surgical castration without pain reliving methods, according to the year of implementation [10,29,30,117].

Country	Year	Alternatives Implemented
Norway	2002	Local anesthesia (lidocaine) with analgesia (meloxicam)
Netherlands	2009	Surgical castration under anesthesia (CO_2) for export market
Germany	2009	End of surgical castration without anesthesia postponed until 2020. Meanwhile analgesia (meloxicam); anesthesia (esp. isoflurane) required only in some organic programs; immunocastration in some high-quality meat programs
Switzerland	2010	Anesthesia (isoflurane)
Denmark	2011/2019	Analgesia (meloxicam); from 2019 on, plus local anesthesia (procaine)
Belgium	2011	Surgical castration with analgesia (meloxicam) for export market; immunocastration domestic retail market
France	2013	Analgesia (meloxicam)
Sweden	2016	Local anesthesia (lidocaine) with analgesia (meloxicam); one smaller retailer prefers immunocastration
Austria	2017	Analgesia (meloxicam)

3.9. Societal Concerns and Immunocastration

Stakeholders' acceptance is crucial for a sustainable use of immunocastration in European pork markets. The perception of the procedure is very heterogeneous and varies between countries. Nations with a high proportion of boars in pork production do not discuss alternatives intensively. Countries where pork production is traditionally based on barrows and gilts have more difficulties abandoning surgical castration without anesthesia or analgesia, as pork production with boars is rejected and immunocastration hardly accepted [34]. Despite intensive animal welfare debates on surgical piglet castration, the study by Tuyttens and co-authors [118] show that pig producers prefer to continue surgical castration, as this seems to be the most efficient and reliable way to produce a high product quality. The studies by Tuyttens and co-authors [118] and Schübeler and Koch [119] point out that farmers are rather neutral about immunocastration and that their knowledge about the method is even lower than about other alternatives to piglet castration.

In a study by Aluwé and co-authors [120], farmers were asked for their attitude towards immunocastration before and after practical experience with this technique. It turned out that experience with immunocastration even had a negative effect on the attitude of farmers. Their main concerns were consumer acceptance, reliable prevention of boar taint, economic efficiency, and the risk of accidental self-injection. However, it cannot be excluded that the farmers gave a lower score because of high expectations prior to the trial. Similar results were obtained in the studies by Tuyttens and co-authors [118] and Schübeler and Koch [119]. A review by Mancini and co-authors [34] showed that the majority of consumers are unaware that male piglets are castrated surgically without anesthesia or analgesia. Furthermore, only a few consumers knew of immunocastration. Among consumers, attributes such as animal welfare, price, product quality and food safety are recognized. When making purchase decisions at the counter, these attributes are weighed and result in the acceptance or rejection of pork from immunocastrates. In terms of animal welfare and product quality, consumers rate immunocastration more positively than pork production with boars or barrows, but are more skeptical about food safety and prices. Thus, the results of consumer studies are unequivocal. A recent study by Di Pasquale and co-authors [121] shows that Italian consumers rate meat from immunocastrates more positively than meat from surgical castrates or entire males, with a low risk perception of immunocastration. This leads to a higher willingness to pay for products from immunocastrated male pigs. The provision of more extensive information on immunocastration had no effect on the decisions of consumers.

Immunocastration can also be an alternative for different production systems as described above. Consumers accept immunocastration for the production of Parma ham if animal welfare, product quality and consumer safety are guaranteed [122]. On the other hand, Heid and Hamm [123] show that German consumers are skeptical about immunocastration in organic pork production because they are worried about residues in meat. Fredriksen and co-authors [124] show that consumer concerns can be minimized by information programs from public authorities. Furthermore, some studies show that information material for target groups increases consumer acceptance of immunocastration—especially if audio-visual techniques are employed [118]. A study by Mörlein and Schübeler [125] investigated which wording should be used by staff at the meat counter to communicate with consumers about immunocastration. It turned out that quality-oriented facts were more important than technical information. For consumers who were more critical and very interested, however, further information material covering technical aspects should also be provided. Moreover, a variety of sensory studies show that pork from immunocastrates is preferred to pork from boars and was rated as similar or even better than pork from barrows [34,46]. A recent study by Čandek-Potokar and co-authors [126] shows that Slovenian consumers prefer pancetta from boars with low boar taint levels to pancetta from immunocastrates or barrows. If boar taint concentrations were high, pancetta from immunocastrates and barrows was considered better.

As mentioned above, one major concern of consumers are possible residues in pork of immunocastrates. As part of the European Medicines Agency [127] approval process for

Improvac®, food safety was evaluated and several studies tested hormonal and oral efficacy of the synthetic antigen used in the vaccine. In sheep, the hormonal efficacy was first tested by intravenous application of the compounds used in Improvac®, then of the complete antigen, in order to measure the LH release. The GnRH fragment itself only had a potency of 0.2% on LH-release when compared to injections of the natural GnRH [128], as the first amino acid, which is involved in receptor binding, is missing [129]. The diphtheria toxoid has also been used for other vaccines and has neither toxic nor hormonal activity [127]. Similarly, the injection of the whole antigen revealed no hormonal activity.

The oral effects of the vaccine were tested in pigs and rats. In pigs, the normal dose of 2 mL Improvac® was administered twice, at the age of 13 and 17 weeks. Neither GnRH antibodies were detected in serum nor decreasing testosterone levels. Even a 70-fold dose of Improvac® applied orally to rats did not change the GnRH antibody concentrations [128]. It was therefore concluded that the vaccine is not orally effective [128] and the withdrawal time was set at 0 days before slaughter [39]. The main risk for the operator is a potential self-injection of the vaccine. In the scientific report of the European Medicines Agency [127], the risk of self-injections is estimated at 0.00004%. However, in order to minimize the risk of self-injections, the manufacturer of Improvac® provides a safety device for vaccination [112]. Nevertheless, the consequences of a potential self-injection have to be estimated. As with all mammals, the hormone GnRH is crucial for reproduction and no species differences in GnRH amino acid sequence exist between pigs and humans [130]. Vaccination against GnRH would therefore lead to transient infertility in both females and males. After an accidental self-vaccination, the user must not carry out further vaccinations to avoid high GnRH antibody production. In a study by Simms and co-authors [131] with prostate cancer patients, GnRH vaccination was tested to suppress testosterone-induced tumor growth in 12 patients with advanced prostate cancer. In five patients, a significant decrease in testosterone concentrations was shown. The suppression of testicular function was transient and testosterone returned to normal concentrations after 9 months.

4. Conclusions

Immunocastration is a technique to improve pork quality, animal welfare, economic profitability, and environmental protection, which can contribute to a more sustainable pork production in Europe. Nonetheless, conflicting aims within each pillar of sustainability as well as between the three pillars have to be balanced against each other in the production process (see **Figure 1**). High product quality with low boar taint levels and higher levels of intramuscular fat work well with production systems which optimize welfare aspects through an early second vaccination. These advantages have to be balanced against the higher anabolic potential of boars which can create economic and environmental benefits. The later the second vaccination is applied, the better its effects for the environment and for farm profitability. As demonstrated by this example, synergic aims exist between the pillar of economy and the pillar of environment. On the other hand, conflicting aims between these two pillars and the pillar of society also exist. Within the value chain, targeted communication about the impact of the timing of the second vaccination is essential in order to make use of this opportunity to produce meat quality tailored to various market segments with different impacts on sustainability.

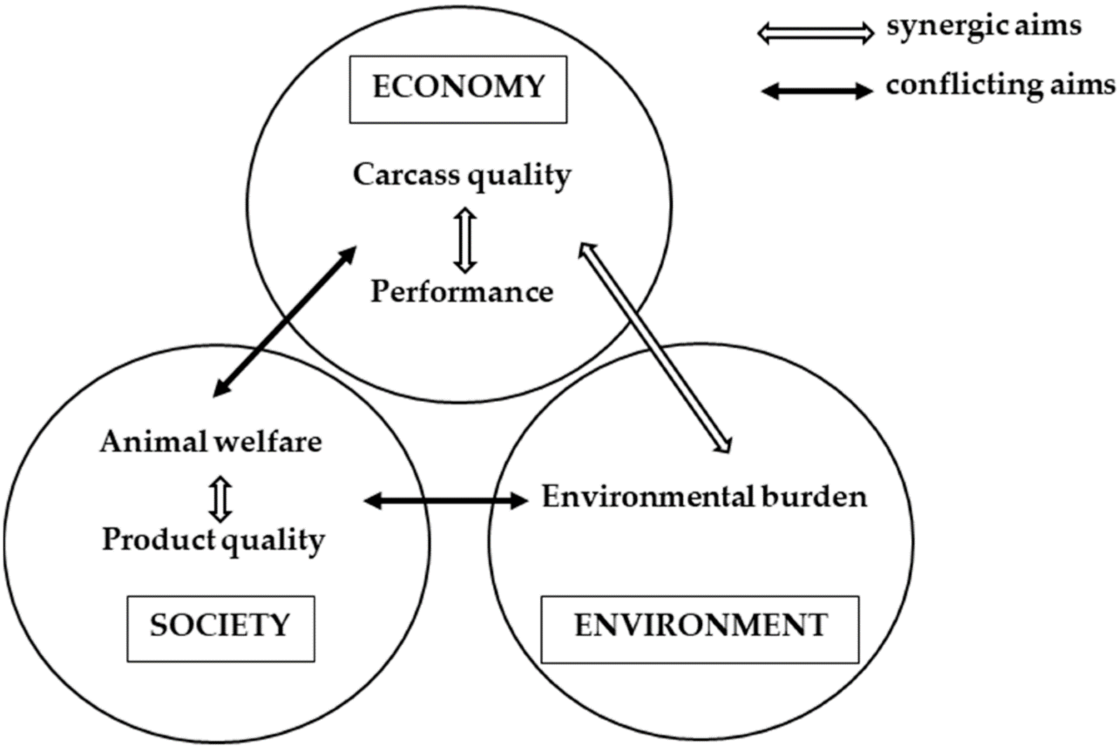

Figure 1. Relationships between the main criteria influenced by immunocastration within the frame of sustainability.

Author Contributions

Conceptualization, K.K.; Writing—original draft preparation, K.K., S.M., É.L., U.W., and V.S.; Writing—review and editing, K.K., S.M., É.L., U.W., and V.S.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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II

The Economic Impact of German Pig Carcass Pricing Systems and Risk Scenarios for Boar Taint on the Profitability of Pork Production with Immunocastrates and Boars

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Abstract

From 2021 onwards, surgical castration of male piglets without pain relief will be banned in Germany. In Europe, stakeholders have committed themselves to end piglet castration from 2018 onwards. Alternatives to surgical castration are pork production with boars or immunocastrates. The competitiveness of these production systems is required to increase their market acceptance. The aim of this study was to test the profitability of pork production with boars and immunocastrates under different carcass pricing systems and penalty systems linked to boar taint. The calculations were based on the performance parameters of 36 animals (n = 12 immunocastrates, n = 12 boars, n = 12 barrows) from an experimental study. In order to analyze the economic effects of both alternatives under different regional German production systems, the performance data were set in relation to the data of agri benchmark. Both boars and immunocastrates performed economically worse than barrows in all the scenarios tested. If immunocastrates are sold according to the boar pricing system, the profitability of this technique is even lower, but still more profitable than boar fattening. Pork production with boars is the most unprofitable alternative in this study and will be further devalued if a penalty system linked to boar taint will be introduced.

Keywords: immunocastration; boars; surgical castration; carcass pricing systems; boar taint; risk scenarios; pork production; profitability; pork market; androstenone; skatole

1. Introduction

For German pork production, about 80% of all male piglets are surgically castrated within their first week of life [1,2]. Consumers evaluate surgical castration without pain-relieving methods very critically [3]. In September 2009, this led German stakeholders of the pork chain to commit to the goal of ending surgical castration of piglets in the so-called ‘Düsseldorfer Erklärung’ [4]. These developments have also resulted in an amendment of the German animal protection law in 2013. From January 2019, piglet castration without anesthesia or analgesia was to be outlawed. Contrary to the planned change in the law, the government of the Federal Republic of Germany agreed in November 2018 to postpone the implementation of the amendment by 2 years [5]. As its reason, the German government stated that there are no competitive alternatives available, and that a prohibition of surgical castration without pain-relief could have a negative impact on German pig production [6].

Alternatives to surgical castration are pork production with boars or with immunocastrates [7,8]. Although these procedures are available in practice, their market shares are low. In

Germany, about 20% of male pigs are fattened as boars, and less than 1% as immunocastrates [1]. The acceptance of pork production with boars is limited because of the risk of unpleasant boar taint in the carcass, which can be ascribed to an excessive accumulation of the compounds androstenone and skatole [9,10]. A large part of the population is sensitive to skatole above a threshold of 0.25 µg/g liquid fat [10,11]. Because of a genetic polymorphism, only a lower proportion of the population is sensitive to androstenone above a threshold of 0.5–1.0 µg/g in liquid fat [10,12]. What both compounds have in common is that most consumers who are sensitive to these compounds dislike them [13]. In order to sort out boar-tainted meat, carcasses of boars are currently evaluated at the slaughter line by the human nose test [14]. However, under commercial conditions at the slaughter line, the reproducibility of valid results is only 23%, so it is highly likely that boar-tainted pork will be undetected and reaches consumers [15]. Objective at-line methods of detecting androstenone- and skatole-tainted carcasses have been developed and have a high potential for being implemented for commercial use at the slaughter line under real-time conditions [16]. However, commonly accepted thresholds for boar taint compounds to exclude tainted pork from the fresh meat market do not exist. Some research has been done to evaluate possibilities of using tainted pork with skatole levels up to 0.3 µg/g liquid fat and very high androstenone values above 3.5 µg/g liquid fat for processing, after blending it with meat from barrows or gilts [17]. However, the processing characteristics of pork from boars are unfavorable because of a higher proportion of polyunsaturated fatty acids, which makes it unsuitable for processing traditional dry-cured products [18].

Immunocastration is an active immunization against the hormone GnRH (Gonadotropin-releasing hormone) by vaccinating the boars twice with the vaccine Improvac® (Zoetis Inc., Parsippany, New Jersey, US). After the second vaccination, the secretion of LH (Luteinizing hormone) is reduced and testicular functions cease temporarily, so that from a physiological point of view the animals are barrows, with similar behavioral, metabolic, and meat quality characteristics. Immunocastration can therefore reliably prevent boar taint and can be regarded as a sustainable alternative to surgical castration and pork production with boars that meets animal welfare aspects as well as pork market requirements. Improvac® is licensed for commercial use in Europe with no technical or legal limitations and can be used for conventional as well as for organic pork production. Knowledge gaps on the potentials of this technique within the value chain prevent a more extensive market relevance [19]. Producing boars or immunocastrates can also be very attractive and cost-effective from an economic point of view [6], since the feed efficiency of boars and immunocastrates is higher than that of barrows [20,21]. In Germany, there are different carcass pricing systems for boars and barrows. It is currently unclear to which pricing system immunocastrates will be assigned, even though this is crucial for economic efficiency [6]. In addition, it is still unclear what effects a quantification of androstenone and skatole values at the slaughter line will have on the profitability of pork production with boars or immunocastrates, and on the use of boar-tainted meat. Penalty systems linked to boar taint are already used in France and Norway, and are likely to be also implemented in other European countries as market shares of boars and immunocastrates increase [14]. In order to enable a sustainable production of boars or immunocastrates in Germany, both alternatives must be critically analyzed under different economic scenarios by using risk scenarios for boar taint and evaluating immunocastration under different pricing systems.

2. Materials and Methods

2.1. Animal Performance Data

The study was performed at the experimental unit of the University of Hohenheim (Unterer Lindenhof 2572800 Eningen, Germany) between November 2017 and August 2018 as part of the SuSi project (ERA-NET SusAn). Two consecutive trials were conducted in total with 36 male pigs (F1 German Landrace × Pietrain; 18 animals per trial), which were assigned to three treatment groups: immunocastrates (IC, n = 12), boars (B, n = 12), and barrows (BA, n = 12). The animals were about 10 weeks of age at the beginning of the study and were housed in groups of six animals under standard conditions (1.2 m^2 per pig, solid floor). Animals were fed ad libitum with three different feed compositions, as given in **Table 1**. In all pens, 500 g of chopped straw and 1000 g of sawdust were supplied daily. Feed intake was recorded per pen. Individual weight was determined at day of birth, after 21 days, at the start of the study (age = 10 weeks), and three times during the fattening period corresponding to the end of the three feeding periods (week 17, 21, and 27/28). The animals were part of a physiological study with repeated blood sampling, and the experiment was approved by the ethical committee of the regional council of Tuebingen (Baden-Wuerttemberg, Germany) with number 47/17 TH. Thus, the number of animals in this study is lower than in field studies without frequent sampling. The number of animals and the assignment of individuals to different treatment groups and housing groups (standard and experimental) were carried out randomly according to the method of ‘Latin Squares’. For the present study, only the animals under standard housing conditions were analyzed.

Table 1. Feeding periods and feed compositions (ME: metabolizable energy; CP: crude protein; DM: dry matter).

Feeding Period	Age (weeks)	ME (MJ)	CP (%)	DM (%)
1	10–17	13.13	17.51	87.54
2	17–21	13.15	16.14	87.49
3	21–27/28	12.41	15.9	87.85

BA were surgically castrated during the first week of life without anesthesia but received 0.2 mL Metacam® (Meloxicam, 5 mg/mL) as post-surgery pain relief. IC received two applications of the vaccine Improvac® at an average age of 12 (V1—first vaccination) and 22 weeks (V2—second vaccination). The timeline of the experimental procedure is given in **Figure 1**. All pigs were slaughtered on two slaughter dates per trial at an age of either 27 or 28 weeks at an experimental slaughter facility (LSZ Boxberg, Seehöfer Straße 50, 97944 Boxberg). Hot carcass weights were recorded and fat samples from the neck area were collected for the measurements of androstenone (A) and skatole (S). Both were analyzed using HPLC as described by Batorek-Lukač and co-authors [22].

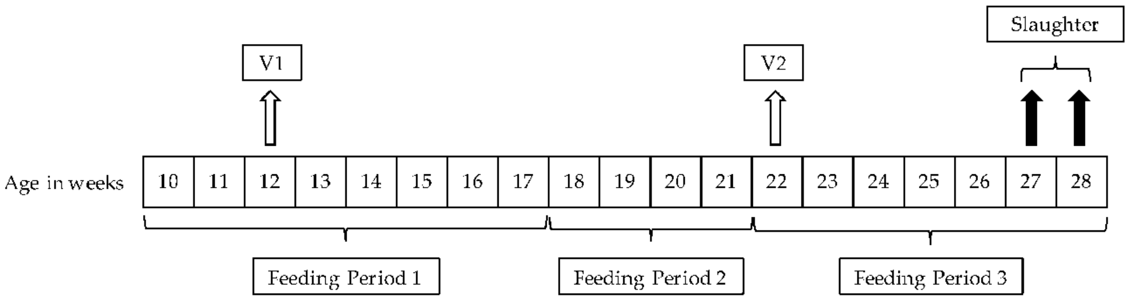

Figure 1. Generalized timeline of the trials (feeding periods, vaccination times (V1, V2), and slaughter dates according to the age (weeks) of the animals).

2.2. Generating Economic Data of Typical German Pig Fattening Farms for Modelling

The analysis of the economic effects of the different production systems with barrows, immunocastrates, or boars was conducted with data from so-called ‘typical farms’ of the international agri benchmark network [23]. This data concept describes representative regional farms, which are constructed from the data sets of several real farms, and evaluated for plausibility by an expert group [24]. The results show the typical economic situation of a common farming business type in a region [25]. For the calculations, all changes and effects on the existing production process had to be identified, specified, quantified and the economic effects analyzed in cooperation with the aforementioned expert group [6]. The evaluation of the economic indicators was based on the TIPI-CAL model. TIPI-CAL is a production and accounting model that provides a detailed representation of the production technology and the physical interrelationships in farms. It is a deterministic, recursive, and dynamic simulation model for various farm sectors and can basically map a 10 year period including trends of all output variables [26]. Thus, a full cost accounting for the business model of the typical regional farm is possible. On this basis, changes in the production process (e.g., boar fattening, immunocastration) can be predicted for the profitability of the whole farm. The typical farms were surveyed according to a standardized protocol, as described by Verhaagh and co-authors [23]. In short, a focus group consisting of a consultant and three to six participants from operating enterprises for each region were included to guarantee a valid data basis. The focus group was organized as a round table discussion in which all necessary operating data were collected on the basis of a standardized questionnaire by Verhaagh [23]. The focus group formed a consensus on each parameter in order to describe what a typical enterprise would look like, instead of adopting average values of participating producers. The data basis of typical German pig fattening farms is updated annually in cooperation with a focus group whose experts are familiar with the regional circumstances of pig producing farms. For this study, the five most important pig fattening regions of Germany were selected (see total number of pigs below) and the necessary operating parameters were included (see **Table 2**). All typical farms used in this study were specialized farms for pig fattening.

Table 2. Key figures for typical German pig fattening farms—baseline scenario [27].

Farm	Region	Number of Pigs Sold (per year)	Fattening Places	Production Principle (All in–All out)
DE_0_3600	Lower Saxony	3.628	1.320	Pen
DE_0_3800	Bavaria	3.758	1.472	Pen
DE_0_5000	North Rhine-Westphalia	5.220	1.850	Pen
DE_0_6000	Lower Saxony	5.941	2.100	Barn
DE_0_6300	Schleswig-Holstein	6.228	2.000	Barn
Farm	**Live Weight at Slaughter (kg)**	**Dressing Percentage (%)**	**Hot Carcass Weight (kg)**	**Price (EUR per kg Hot Carcass Weight)**
DE_0_3600	121	79	95.3	1.68
DE_0_3800	123	80	98.4	1.63
DE_0_5000	121	79	95.6	1.60
DE_0_6000	123	80	97.8	1.76
DE_0_6300	122	78	95.2	1.60

2.3. Economic Risk Scenarios and Pig Carcass Pricing Systems

The scenario specifications of typical German pig fattening farms were adjusted with the data from the experiment on the basis of different boar taint risk scenarios and different

German carcass pricing systems for pigs. First, performance parameters (average daily gain—ADG) from the trial were used in the calculations for the typical farms. The gradient curves of the three treatment groups (IC, B, and BA) were derived from the experimental data. As each typical farm had individual process ranges (weight at the beginning and end of the fattening period), the gradient curves of the trial were adjusted to the respective typical farms. This resulted in new gradient curves for all typical farms, as the process ranges of the trial were different to those of the baseline. In the next step, the ADG of BA was set in relation to the ADG of IC and B and then set in relation to the baseline, which resulted in the final gradient curves and performance parameters (ADG) for all typical farms. The relative changes in feed conversion ratio (FCR) was also derived from the trials and set in relation to the typical farms.

In addition, literature data was also used for estimating several cost factors, such as the additional working time changes (IC: +0.79 min per animal; B: +1.2 min per animal; additional costs for sex-separate housing, application of vaccination, and more intensive observation of animals), the costs for Improvac® (3.59 EUR per animal for both vaccinations), and the costs for removing the testes at the slaughter line (0.64 EUR per male pig) [28]. The costs are not offset by the value of the testicles for any other use. In order to analyze the impact of different pricing systems on the profitability of immunocastration and boar fattening, the pricing system for boars and barrows used was that of the German pork market leader, Tönnies Holding ApS & Co. KG [29]. The values for live weight at slaughter were taken from the baseline (**Table 2**) and the dressing percentages derived from the experiment (B: 80.27%, IC: 80.17%, and BA: 82.54%). They were then set in relation to the values from the baseline (**Table 2**). For the evaluation of carcasses, the parts of the carcass (ham, loin, and belly weight) were estimated on the basis of the Auto-FOM III formula [30]. Historical slaughter data of boars and barrows were used for the belly meat percentage (B: 61.72% and BA: 57.65%) [29]. For IC, the mean value of the belly lean meat percentage of boars and barrows was calculated (IC: 59.69%), as the lean meat content of immunocastrates lies between boars and barrows [21] and lean meat content correlates significantly with the belly lean meat percentage ($p = 0.92$) [31]. The base prices in EUR per kg hot carcass weight from **Table 2** were used for the calculations. BA were only priced based on the barrow pricing system, and for B only on the basis of the pricing systems for boars. IC prices were calculated both under the barrow pricing system with and without the additional costs for removing the testes, and again under the boar pricing system. In addition, the occurrence of boar taint was economically evaluated. For this, androstenone (A) and skatole (S) values were also included to create various risk scenarios depending on the intensity of boar taint (see **Table 3**). For the respective risk scenarios, carcasses with values of boar taint compounds above a certain threshold were valued at the 0 EUR minimum. Furthermore, a proportion of 3.5% was subtracted from the boar tainted carcasses above a certain threshold, as 3.5% of all carcasses are already discounted as being affected by boar taint in the boar pricing system [28].

Table 3. Risk scenarios and thresholds for boar taint compounds in adipose tissue from the neck area.

Scenario	**1**	**2**	**3**	**4**	**5**
Boar taint compounds	S[1]	A[1]	A[1]	A[1]	A[1]
Threshold	0.25	0.5	1	2	5

[1]µg per g liquid fat.

3. Results

3.1. Performance Data of the Trials in Relation to Typical German Pig Fattening Farms

In all typical German pig fattening farms, the ADG of immunocastrates is lower than the baseline scenario (see **Table 4**). Although after the second vaccination (ADG-Period 3) the ADG of IC was higher than that of BA (see **Table S1**), over the total fattening period, the ADG in BA was higher in all typical farms because feeding period 3 was too short to compensate ADG disadvantages of the previous feeding periods. The FCR of IC was more than 7% lower in all typical farms and therefore more efficient than the FCR of the baseline. Because of the higher ADG of BA compared to IC, the fattening period of IC was longer in all typical fattening farms than in the baseline.

Table 4. Impact of immunocastration on performance data of typical German pig fattening farms in relation to the baseline (barrows).

Farm	**ADG IC (g)**	**Δ ADG IC (%)**	**FCR IC**	**Δ FCR IC (%)**	**Fattening Period IC (days)**	**Δ Fattening Period IC (days)**
DE_0_3600	821	−1.18	2.61	−7.12	110.3	+1.3
DE_0_3800	763	−0.61	2.57	−7.22	120.7	+0.7
DE_0_5000	805	−1.33	2.61	−7.44	115.5	+1.5
DE_0_6000	788	−1.04	2.70	−7.22	121.3	+1.3
DE_0_6300	868	−0.91	2.53	−7.33	106.0	+0.7

The differences between B and BA were even more obvious (see **Table 5**). In B, ADG was more than 6% lower in all typical farms than in the baseline. The higher ADG of BA compared to B was a result of the higher daily feed intake of BA compared to B (see **Table S1**). FCR of B was lower in all typical farms and therefore more efficient compared to the baseline. The fattening period of B was about one week longer in all farms than in the baseline.

Table 5. Impact of boar fattening on performance data of typical German pig fattening farms in relation to baseline (barrows).

Farm	ADG B (g)	Δ ADG B (%)	FCR B	Δ FCR B (%)	Fattening Period B (days)	Δ Fattening Period B (days)
DE_0_3600	779	−6.20	2.69	−4.46	116.2	+7.2
DE_0_3800	721	−6.04	2.66	−3.97	127.7	+7.7
DE_0_5000	765	−6.24	2.70	−4.26	121.6	+7.6
DE_0_6000	748	−6.16	2.78	−4.47	127.9	+7.9
DE_0_6300	823	−6.12	2.61	−4.40	111.9	+6.9

3.2. Proportion of Treatment Groups (B, IC, and BA) above Thresholds of Boar Taint Compounds

All BA and IC were free of boar taint and were below the threshold for the respective androstenone and skatole scenarios. Accordingly, immunocastration was 100% successful in preventing boar taint. In B, only 8.33% of the animals were above the threshold of 0.25 µg per g liquid fat skatole. Androstenone levels in B were relatively high, and 83.33% of all B had androstenone levels of over 1 µg per g liquid fat. Very high levels of androstenone (above 5 µg per g liquid fat) were detected in 25% of all B (see **Table 6**).

Table 6. Proportion of animals above threshold of corresponding boar taint scenario.

Scenario	1	2	3	4	5
Group	**Proportion of Animals Above Thresholds in %**				
B	8.33	100.00	83.33	58.33	25.00
IC	0.00	0.00	0.00	0.00	0.00
BA	0.00	-	-	-	-

3.3. Profitability of IC and B in Relation to the Baseline (Barrows)

Table 7 shows the additional revenue required to reach the level of profitability of the baseline after implementing IC and B. On the basis of the full cost accounting, IC (priced according to the pricing system for barrows) as well as B (priced according to pricing system for boars without price reductions due to boar taint) were less profitable than the baseline in all typical German pig fattening farms (see **Table 7**). An improvement in FCR for B and IC cannot compensate for decreasing ADG compared to BA. Higher working time requirements for B and IC, additional costs for the vaccine in IC, and price reductions for B due to the pricing system for boars, all result in worse economic efficiency of B and IC compared to the baseline. The results of IC, however, are better than those of B for all farms. For IC, the additional revenue required to be as profitable as the baseline was found to be between EUR 1.44 and 3.20 per 100 kg hot carcass weight. The size of the farm had no direct influence on the change in profitability. The results for B were even more obvious. In the long run, an additional revenue of EUR 5.62 up to 7.38 per 100 kg hot carcass weight was necessary to be as competitive as the baseline scenario.

Table 7. Additional revenue (EUR) required per 100 kg hot carcass weight for IC and B to be on the same profitability level as the baseline scenario.

Farm		DE_0_3600	DE_0_3800	DE_0_5000	DE_0_6000	DE_0_6300
Region		**Lower Saxony**	**Bavaria**	**North Rhine-Westphalia**	**Lower Saxony**	**Schleswig-Holstein**
Group	IC	3.20 €	1.44 €	2.54 €	2.62 €	2.85 €
	B	6.90 €	5.62 €	5.71 €	7.38 €	6.95 €

In addition to **Table 7**, in **Table 8** the extra working time for removing the testes of IC at the slaughter line is calculated but the IC carcasses were still priced on the barrow pricing system. In addition, IC carcasses were also priced on the basis of the pricing system for boars. Unlike B, none of the IC had skatole or androstenone levels above the thresholds, so no further price reductions were applied. The additional costs for removing the testes reduced the profitability of IC compared to the baseline in all typical farms. An application of the boar pricing system for IC lowered the efficiency of IC to the level of B, but three out of five typical farms were still more profitable with IC than with B. If we concluded price reductions due to boar taint, the profitability of B declined even more. Discounts for B carcasses above a skatole threshold of 0.25 µg/g liquid fat reduced the profitability of B by around EUR 9.25 to 11.32 per 100 kg hot carcass weight. The effects of high androstenone levels were even more drastic. An androstenone threshold of 0.5 µg/g liquid fat worsened the operating profitability of B by EUR 75.23 to 84.92 per 100 kg hot carcass weight. As the threshold values continued to rise and the proportion of B carcasses above the thresholds decreased, these losses decreased as well. Above an androstenone threshold of 5.0 µg/g liquid fat, the profitability was still EUR 21.45 to 24.81 per 100 kg hot carcass weight lower compared to the baseline.

Table 8. Additional revenue required per 100 kg hot carcass weight for IC and B to be on the same profitability level as the baseline scenario, considering different carcass pricing systems and the occurrence of boar taint.

Farm	DE_0_3600	DE_0_3800	DE_0_5000	DE_0_6000	DE_0_6300
Region	**Lower Saxony**	**Bavaria**	**North Rhine-Westphalia**	**Lower Saxony**	**Schleswig-Holstein**
			IC		
BA pricing	3.20 €	1.44 €	2.54 €	2.62 €	2.85 €
+remove testes	3.54 €	1.79 €	2.88 €	2.95 €	3.20 €
B pricing	7.09 €	5.44 €	6.30 €	6.81 €	6.58 €
			B		
B pricing	6.90 €	5.62 €	5.71 €	7.38 €	6.95 €
+scenario 1	10.48 €	9.39 €	9.25 €	11.32 €	10.41 €
+scenario 2	76.77 €	78.41 €	75.50 €	84.92 €	75.23 €
+scenario 3	64.80 €	66.09 €	63.62 €	71.59 €	63.64 €
+scenario 4	46.54 €	47.41 €	45.70 €	51.57 €	46. 05 €
+scenario 5	22.81€	22.23 €	21.45 €	24.81 €	22.34 €

(+) – including further costs; scenarios: including the value of refused carcasses according to various thresholds (0 € per carcass above certain thresholds) – for details see **Table 3**.

4. Discussion

Surgical castration without pain relief is considered unacceptable by society. The fattening of boars and immunocastrates is regarded as animal friendly by some stakeholder groups and discussed as potential alternatives to the fattening of barrows. Both immunocastrates and boars have a better FCR than barrows [32], which is more efficient from an economic point of view, as less feed is needed to produce the same amount of pork. On the other hand, these production systems generate additional production costs due to extra working time and additional vaccination costs [28,33]. In addition, no objective boar taint detection systems are currently in use at slaughterhouses and potential reductions in the value of carcasses by boar taint may reduce the profitability. Because of the small market share of immunocastrates on the German pork market, it is unclear at the moment how these carcasses will be priced. This study therefore analyzed the economic impact of immunocastration and boar fattening under different pig carcass pricing systems, including the occurrence of boar taint.

In the present study, feed composition was based on the feed requirements of boars, which means that there is further optimization potential for immunocastrates and barrows, as a less expensive feed with reduced protein and energy content might be appropriate. Such corrections within the calculation would potentially worsen the profitability of pork production with boars even more when compared to immunocastrates or barrows. All animals, irrespective of treatment and weight, were slaughtered in two groups, either at 27 or 28 weeks of age, and not according to the optimal slaughter weight. Similarly, the feeding phases followed the same timeline and were not adapted to weight gain, treatment group, and live weight. This may mask group-specific effects, as the feeding strategy similarly to feed composition should differ between the groups (B, IC, and BA) to optimize performance data, as well as to avoid excessive nitrogen excretion [34,35]. Barrows of the used genotype in particular have a higher ADG and would switch earlier to a different feeding period than boars or immunocastrates [32]. In future research, optimal feeding strategies for respective groups should be considered.

The performance data of the experiment show that immunocastrates had higher ADG compared to barrows and boars. This is caused mainly by the performance of immunocastrates in the last feeding period after the second vaccination, which results in an increased feed intake and a higher growth rate [21,32]. Some studies confirm our results and show that immunocastrates grow faster over the entire fattening period than barrows and boars [20,21]. In another study [32], however, barrows revealed a higher ADG than immunocastrates and boars. Such differences may be explained in part by the genotype used in the study. Crossbreds with Belgian Pietrain, for example, have a reduced growth rate before and after the second vaccination than, for example, Duroc crossbreds. In both genotypes, however, the growth rate increased in the two weeks following the second vaccination compared to the growth rate between the first and second vaccination [36]. This may help to explain why, in typical German pig fattening farms, the ADG of barrows is higher than in immunocastrates. Moreover, the effect of slaughter weight has to be considered, as animals are slaughtered at a lower live weight compared to the experimental trials and thus the last feeding period is shorter than in our experiment. In this study, FCR is more efficient in immunocastrates as well as in boars, which is also illustrated by previous studies [20,21,32].

In our study, the carcass data (weight of carcass parts) were adapted to the results obtained via the Auto-FOM III formula. In the case of immunocastrates and boars in particular, however, it can be assumed that this study underestimates the weight of carcass parts (especially the shoulder), since other studies show that carcass yields and the output of valuable meat in immunocastrates is higher than in boars or barrows. Compared to boars, immunocastrates also have higher belly weights [20]. Actual Auto-FOM III data of immunocastrates, however, which would be crucial for future calculations, are not currently available. The dressing percentages of boars and immunocastrates compared to barrows were even worse in this study than in previous studies [20,21], indicating higher economic losses in pork production with boars and immunocastrates compared to the baseline.

All immunocastrates responded well to the vaccine in this study, and no non-responders with boar-tainted carcasses were detected. However, several reviews assume a proportion of non-responders of up to 3% [18,37,38]. This would worsen the profitability of immunocastration, as it would result in a certain number of boar-tainted carcasses above the thresholds. Furthermore, the proportion of tainted boar carcasses is very high in this study and thus reduces the profitability of pork production with boars. An international study by Walstra and co-authors [39], with different genotypes produced under different conditions in Europe, revealed very high androstenone (>1 µg/g liquid fat) concentrations in 29% of the boars, whereas a higher proportion of boars were affected by skatole levels above 0.25 µg/g liquid fat compared to our study. Nonetheless, more objective boar taint detection systems at the slaughter line are essential in valuing carcasses with regard to boar taint, and would worsen the profitability of boar fattening also for the 29% of boars affected by boar taint.

By the end of 2018, Tönnies Holding ApS & Co. KG introduced a new boar pricing system in Germany. This has even further reduced the economic profitability of boar fattening compared to the baseline [6,28]. The impact of the new boar pricing system on the profitability of immunocastration is also negative and makes the technique economically unviable [28]. In a recent study by Verhaagh and Deblitz [28], the production of pork with immunocastrates was more profitable in all typical German pig fattening farms compared to the baseline (barrows). Although producing immunocastrates generate higher production costs, they were compensated by better FCR, higher ADG, and a shorter fattening period. In the study by Verhaagh and co-authors [28], however, the calculation was based on the ADG values of the entire fattening period, which resulted from higher ADG after the second vaccination. However, in this present study, it could be shown that the last feeding period had a positive effect on the ADG of the entire fattening period, but was economically not sufficient to compensate for the lower ADG of the earlier fattening periods. Furthermore, performance data of this trial declined in relation to the typical farms, as animals were fattened and slaughtered on fixed dates so that greater economic efficiency might be achieved through optimized management of feeding, fattening periods, and age at slaughter.

5. Conclusions

This study illustrates that pork production with immunocastrates or boars is economically less profitable under the assumed performance and market criteria compared to the pork production with barrows as the baseline. A change to pork production with boars or immunocastrates would worsen the competitiveness of all typical German pig fattening farms investigated. Better FCR of boars and immunocastrates cannot economically compensate for the higher ADG of barrows. The higher ADG of immunocastrates after the second vaccination is masked in the overall calculation by their lower ADG prior to the second vaccination. The application of the boar pricing system for immunocastrates would further worsen the profitability of immunocastration in comparison to barrows. Boars, however, tend to be less economically viable than immunocastrates, even if both are priced on the boar pricing system. More objective boar taint detection systems at the slaughter line, however, could lead to further price reductions for boars.

Supplementary Materials

Table S1. Mean values of animal performance data of the trials

	BA	IC	B
ADG (g) – Period 1	946.9	845.8	861.0
ADG (g) – Period 2	964.3	928.6	916.8
ADG (g) – Period 3	845.1	1,016.5	843.7
ADG (g) – total fattening period	921.7	930.3	873.8
FCR – Period 1	2.03	2.09	2.10
FCR – Period 2	3.84	3.58	3.38
FCR – Period 3	3.82	3.16	3.53
FCR – total fattening period	3.23	2.94	3.00
Daily feed intake (g) – Period 1	1,920.6	1,807.2	1,760.6
Daily feed intake (g) – Period 2	3,673.0	3,289.6	3,092.0
Daily feed intake (g) – Period 3	3,143.3	3,103.1	2,881.6
Daily feed intake (g) – total fattening period	2,770.2	2,618.6	2,458.0

Author Contributions

Conceptualization, K.K. and M.V.; methodology, K.K. and M.V.; formal analysis, K.K. and M.V.; data curation, K.K. and M.V.; writing—original draft preparation, K.K. and M.V.; writing—review and editing, K.K. and M.V.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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III

Influence of Housing Conditions on Reliability of Immunocastration and Consequences for Growth Performance of Male Pigs

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Simple Summary

Surgical castration of male piglets is societally criticized as it is painful and violates the integrity of the animals. Pork production with boars and immunocastrates are possible alternatives. Even if immunocastration is an animal-welfare-friendly alternative, its market share is low and the reliability of this technique is discussed controversially within the pork chain. Currently, the number and the reason for non-responders to vaccination are not clear. Various factors may contribute to impaired immune response including adverse and stressful housing conditions. This study, therefore, examines the influence of different housing conditions on the immune response after two Improvac® vaccinations. To determine vaccination success, testosterone concentrations, GnRH-binding, and boar taint compounds were evaluated. Furthermore, the growth performance of male pigs was compared. The results show that immunocastration is reliable under different housing systems and prevents boar taint. Moreover, the growth performance of immunocastrates is high and even superior to that of boars and barrows after the 2nd vaccination. Accordingly, immunocastration is not only animal-welfare-friendly but also economically attractive and suitable for different housing systems.

Abstract

Immunocastration is a sustainable alternative to piglet castration but faces limited market acceptance. The phenomenon of non-responders has not to date been examined in detail, but adverse and stressful housing conditions (e.g., mixing of groups) might impair the success of vaccinations. Therefore, we evaluated the influence of housing conditions on the immune response after two Improvac® vaccinations at an age of 12 and 22 weeks, respectively. Boars, immunocastrates and barrows (n = 48 each) were assigned to three different housing conditions (n = 36 enriched, n = 36 standard n = 72 repeated social mixing). Immune response was quantified by measuring GnRH-binding and its consequences for testosterone concentrations, development of the genital tract and boar taint. Growth performance was evaluated via average daily gain (ADG). GnRH-binding and testosterone levels revealed that immunocastration reliably suppressed testicular functions after the 2nd vaccination. Housing conditions did not modify testicular function but influenced ADG as animals under mixing grew slower than those under enriched conditions. Gonadal status had only a slight impact on ADG except in immunocastrates, which showed a temporarily higher ADG after the 2nd vaccination. The results show that immunocastration is a reliable procedure under different housing conditions and competitive in terms of growth performance.

Keywords: immunocastration; vaccination; Improvac; non-responder; immune response; housing conditions; surgical castration; boar taint; growth performance; genital tract

1. Introduction

Traditionally, male piglets designated for pork production have been surgically castrated within the first week of life to avoid boar taint and to prevent problems due to male-specific behavior [1]. As the surgical castration is mostly carried out without anesthesia and analgesia, the animals suffer due to the pain inflicted on them as a consequence of the surgical castration [2,3]. As a consequence, this technique is facing increasing societal criticism [4]. In 2010, these circumstances led European stakeholders of the pork chain to commit themselves voluntarily to stop surgical castration of male piglets by 2018 [5]. Today, however, about 60% of all male piglets produced in Europe are still surgically castrated [6] as pork production with boars still faces problems due to boar taint and other meat quality and animal welfare issues [7]. Quality problems result from the accumulation of the two main boar taint compounds, androstenone and skatole, in adipose tissue [8]. Furthermore, boar carcasses are leaner than those of barrows and have softer adipose tissue due to higher amounts of polyunsaturated fatty acids, which reduce their suitability for processing especially in the case of high-quality dry meat products [9]. From an animal welfare point of view, pork production with boars may lead to boar-specific welfare problems related to sexual behavior and aggression (e.g., injuries, lameness) due to boars' higher potential for agonistic behavior [10,11].

An alternative to both surgical castration and pork production with boars is immunocastration. Immunocastration is an active immunization against GnRH, a key hormone regulating testicular functions. The treatment consists of two consecutive vaccinations that trigger an immune response which results in the production of antibodies against the endogenous hypothalamic hormone GnRH [12]. Until the second vaccination, immunocastrates are from a physiological perspective similar to boars, with the same anabolic potential but also with the same welfare-associated problems. After the second vaccination, testicular hormone synthesis ceases and boar-specific behavioral problems decline within two weeks [13] as do the number of injuries (e.g., penile injuries) [14]. In Europe, only one vaccine (Improvac®) is currently available for commercial use [13]. Although Improvac® has been approved by the EMA (European Medicines Agency) in the European Union since 2009 [15], the market share of immunocastrates in Europe is only about 2.8% to date [6]. The reasons for the low market acceptance of this technique are diverse and are mainly due to knowledge gaps regarding the optimal use of immunocastration for various market demands. In order to expand the market share of immunocastrates, vaccination has to work reliably under different production systems, and the growth performance of immunocastrates has to be competitive [13]. However, some reports describe a lower vaccination reliability, with about 0%–3% of vaccinated animals responding poorly to the vaccinations. These are the so-called “non-responders”. The reasons for poor immune response are not clear and may be due to either accidentally missed vaccinations or due to health problems in animals during the vaccinations [9,13,16,17,18].

Another possible explanation might be stress-induced immunosuppression. Studies in humans and animals have amply shown that social stressors can impair the immune system [19,20]. Antibody response, for example, was found to be suppressed in defeated rats [21] or highly stressed human caregivers in response to influenza virus vaccination [22]. As stress can have a negative impact on the porcine immune system [23], stressful housing conditions might be a predisposing factor for non-responders. In pork production, unstable social environments cannot be avoided and social mixing is a common procedure (e.g., at the beginning of the fattening phase, while sorting into homogeneous groups etc.). It is well known that social mixing and unstable groups lead to social stress in pigs [24], with more aggressive behavior [25,26] and a negative impact on the pigs’ immune system [26,27]. Social mixing not only temporarily activates the hypothalamic–pituitary–adrenal (HPA) axis by increasing cortisol levels after a mixing event has occurred [25,27], but may also lead to chronic stress if frequent mixing persists over a longer period of time [25]. Alternative housing systems such as outdoor housing or organic farming can have both positive and negative effects on the welfare and health status of pigs. In alternative housing systems, pigs may be able to behave in a species-specific way with pen partners, but on the other hand are at an increased risk for exogenous factors such as sunburn or ecto- and endoparasites [28]. A study by de Groot et al. [29] showed that pigs housed under enriched housing conditions had higher salivary cortisol levels than pigs housed under barren housing conditions, whereas no differences existed in immune parameters such as proliferation of leukocytes or lymphocytes. Thus, housing conditions had no clear impact on the immune system. The formation of antibodies against a vaccine was however not studied. These results therefore do not exclude the possibility that a moderate impairment of animals’ antibody response due to housing conditions increase the risk for occurrence of non-responders to immunocastration. Social stress not only affects the pigs’ immune system but also their growth performance, which is lower in pigs exposed to social stress and mixing [30,31,32].

The aim of this study was to investigate whether housing conditions have an impact on the immune response after two Improvac® vaccinations against GnRH in male pigs, and on the creation of non-responders. The influence of housing conditions and castration status of male pigs on growth performance during the fattening period was also investigated.

2. Materials and Methods

2.1. Animals and Experimental Setup

The experimental protocol was approved by the ethical committee for animal experiments by the regional authority of Tuebingen, Germany, (ID HOH 47/17TH), and all procedures were conducted in accordance with the German Animal Welfare Act. In total, two subsequent trials were conducted with 72 pigs per trial. The trials were performed at the animal experimental unit of the University of Hohenheim (Unterer Lindenhof, Eningen, Germany). All pigs were a crossbreed of Pietrain x German Landrace. In this experiment, three different sex groups of male pigs (boars, n = 48; immunocastrates, n = 48 and barrows, n = 48) were housed under three different housing conditions (standard: n = 36; enriched: n = 36; mixing: n = 72). In the 'standard' scenario, the animals were housed in conventional housing conditions (1, 2 m^2 per pig). Under 'enriched' conditions, the animals had twice as much space (2, 6 m^2 per pig) as under 'standard' conditions and additional access to the outdoor area (3, 1 m^2 per pig). In the 'mixing' scenario, the animals were kept similar to 'standard' conditions, but the groups were mixed repeatedly to induce social stress. Mixing consisted of an exchange of two of 6 animals per pen with two unfamiliar animals from another pen of a similar sex group every third day of the mixing phase. For this reason, the animal number of the mixing scenario was twice as high as in the two other housing conditions. Mixing was assigned around vaccination time points to maximize probable effects of social stress on vaccination outcome. Thus, mixing started 7 days before the first vaccination at an age of 11 weeks, with a total number of 5 mixing events. The second mixing phase started at an age of 20 weeks and consisted of 8 mixing events over 24 days. The selection of animals which were mixed was randomized.

The animals for this experiment had been selected from a total of 48 litters (322 male piglets). The piglets were allocated randomly at an age of three days to 9 different experimental groups (sex group x housing) by the method of Latin Squares. Barrows were surgically castrated within the first week of life without anesthesia, but received 0.2 mL Metacam® (Meloxicam, 5 mg/mL) as post-surgery pain relief. Immunocastrates (IC) were vaccinated twice with Improvac® at an age of 12 (first vaccination – V1) and 22 weeks (second vaccination – V2) as shown in the timeline of the experiment in **Figure 1**. It was decided that full siblings were not to be assigned to the same interaction of sex group x housing condition. The assignment of the interaction of the sex group x housing condition to the respective pens was also randomized, and it was ensured that two mixing groups of the same sex group (e.g., boars) were not located directly next to each other.

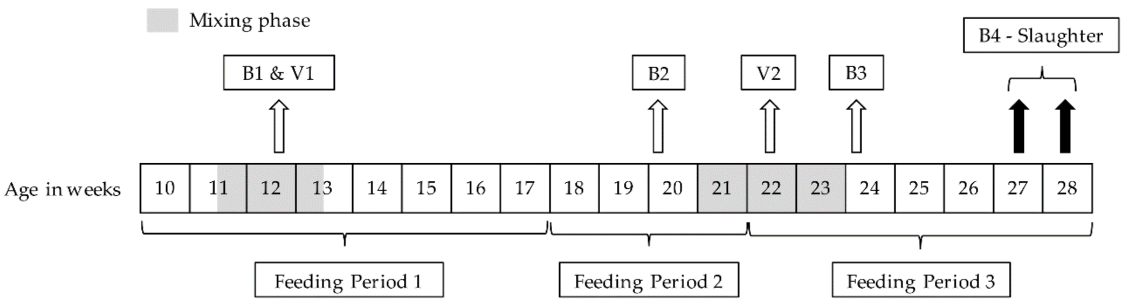

Figure 1. Generalized timeline of the trials (feeding periods, blood samples (B1–B4), vaccination times (V1—applied immediately after B1, V2), mixing periods, and slaughter dates according to the age (weeks) of the animals).

In all pens, chopped straw (500 g per pen) and sawdust (1000 g per pen) were supplied daily. Feed was supplied twice per day between 7:45 am and 8:00 am and between 4:00 pm and 4:30 pm. The pigs were fed ad libitum. Feed composition varied during the fattening period (three phase feeding) and was based on the recommendations for intact boars (see Kress and Verhaagh [33]). In order to avoid a feeding effect, all animals were fed the same diet. Individual weights of all animals were recorded at birth, at an age of 3 weeks, 10 weeks, 17 weeks and at slaughter to characterize the growth performance via average daily gain (ADG).

The experiment covered the period between an age of 10 weeks and 27 or 28 weeks (slaughter). Due to the limited capacity of the experimental slaughter unit (LSZ Boxberg, Boxberg, Germany), the animals were slaughtered on two different occasions to ensure standardized conditions and complete data collection.

In total, 4 blood samples were collected from each pig to measure antibody titer against GnRH, as well as testosterone and cortisol levels at an age of 12 weeks (B1, to analyze differences between sex groups and housing conditions immediately before V1), 20 weeks (B2, after V1 and before V2, to measure the immune response after V1 and the impact of sex group on testosterone concentrations) and 24 weeks (B3, two weeks after V2 and immediately after the second mixing phase to analyze the antibody response after V2 and the corresponding testosterone concentrations and to analyze the impact of social mixing on cortisol concentrations) and at slaughter line (B4, to measure the final antibody titer against GnRH and testosterone concentrations at slaughter to confirm that testicular functions are suppressed until slaughter and to analyze differences in sex groups and housing conditions on transport and slaughter stress) (as given in **Figure 1**). For blood sampling during the experiment, the animals were separated individually and fixed by a snare pole and blood was collected by puncture of the vena jugularis externa into heparinized vials. Plasma was removed after centrifugation and stored at −20 °C until further analyzed.

2.2. GnRH Binding in Plasma

Success of immunocastration was assessed by measuring GnRH binding in plasma with an in-house assay, based on ^{125}I-GnRH. GnRH-Iodination was carried out with the solid phase Iodogen-method according to Salacinski et al. [34], using 1 µg Iodogen/cup, 200 µCi ^{125}I (Na^{125}I, Hartmann Analytik GmbH, Braunschweig, I-RB-31.) and 200 ng GnRH (Fisher Scientific, PEP-168) diluted in 0.5 M phosphate buffer (pH 7,4). After an incubation period of 3 min the free iodine was separated from the iodinated peptide with an anion-exchange resin column. The specific activity was about 200 nCi/ng GnRH. In order to determine the GnRH binding, 15,000 cpm ^{125}I-GnRH (corresponding to 17.5 pg GnRH) in 100 µl in 0.1 M phosphate buffer were incubated with 5 µl of plasma and 200 µl of 0.1 M phosphate buffer with the addition of bovine serum albumin (BSA, 0.1%) at 4 °C for 24 h. Afterwards, bound free separation was carried out with dextran-coated charcoal (0.5%) in 1 mL H_20 and subsequent centrifugation. The supernatant was counted for one minute in a gamma counter. As controls, a pool sample of vaccinated animals with a good response (pool A) and a pool sample of non-vaccinated boars (pool B) were measured within each assay. The absolute binding of the biological samples was calculated (counts/total counts). The specific binding of pool A was 39.38% ± 6.29%; (CV: 16%; range 35.16% to 61.02%) in trial 1, and 38.79% ± 2.66%; (CV: 7%; range 39.22% to 56.74%) in trial 2. The non-specific binding determined with pool B was 4.44% ± 1.02%, (CV: 23%; range 1.35% to 2.67%) in trial 1, and 5.65% ± 0.76% (CV: 13%; range 4.20% to 6.33%) in trial 2.

2.3. Testosterone Levels in Plasma

Testosterone concentrations in plasma were determined in duplicate with a direct in-house radioimmunoassay (RIA). In brief, 20 µL plasma were incubated with [1,2,6,7-^{3}H]-testosterone (95.5 Ci/mmol, PerkinElmer, Boston, MA, USA) and antiserum. The antiserum had been raised in a rabbit against testosterone-3CMO-BSA and was used at a final dilution of 1:144,000. Cross reactivity was 67% with 5αDHT, and below 2% for other tested steroids. Charcoal-treated plasma (20 µL) was added to the calibration curve to compensate for substrate effects in case of measurements in plasma. Bound free separation was carried out with 0.5 mL ice cold solution of dextran coated charcoal (0.5%) in H_20 and subsequent centrifugation. The supernatant was transferred into counting vials with scintillation fluid and counted in a beta-counter. To determine the precision of the tests, plasma samples from barrows were spiked with defined concentrations of 0.5 to 10.0 ng/mL (precision 100%–125% recovery in each trial). In addition, biological samples were included to determine the repeatability of the measurements (coefficient of variation: intra-assay 1.99% (trial 1) and 5.22% (trial 2); inter-assay 8.46% (trial 1) and 6.87% (trial 2)).

2.4. Cortisol Levels in Plasma

In order to determine the cortisol concentrations of the respective experimental animals, a radioimmunoassay (RIA) was carried out as described by Engert et al. [35]. A polyclonal antibody against cortisol-3-BSA (MBS316242, MyBioSource, San Diego, CA, USA) at a final dilution of 1:112,000 in 0.1% BSA buffer was added and as a tracer [1,2,6,7-3H] cortisol (93 Ci/mmol, PerkinElmer, Boston, MA, USA) used. All samples of each animal were measured in a single assay. Intra-assay variance for a biological sample was 4.87% and inter-assay variance was 8.87%.

2.5. Boar Taint Compounds in Adipose Tissue

For the determination of boar taint compounds, samples of subcutaneous fat were vacuum packed at slaughter and stored at −20 °C until the start of the analyses (within 14 days after sampling). Androstenone and skatole concentrations were determined with high-performance liquid chromatography (HPLC, HP 1200, Agilent Technologies, Waldbronn, Germany) equipped with a fluorescence detector according to Pauly et al. [36]. Adipose tissue samples (10–20 g) were put in a microwave oven for 2 × 1 min at 350 W. Afterwards, the liquefied lipid fraction was removed and centrifuged for 20 min at 11,200 g and ambient temperature. After centrifugation, fat was heated to 50 °C and 0.5 ± 0.01 g water-free liquid fat transferred into 2 mL Eppendorf tubes adding 1 mL of internal standards diluted in methanol (0.496 mg/L androstenone and 0.050 mg/L 2-methylindol for androstenone and skatole determination, respectively). After stirring for 30 s, the tubes were incubated for 5 min at 30 °C in an ultrasonic water bath, kept on ice for 20 min and centrifuged for 20 min at 11,200 g at 4 °C. For androstenone determination, 50 µL of the supernatant was submitted to derivatization with dansylhydrazine and boron trifluoride (BF3) for 2 min. An aliquot of 10 µL of the derived mixture was then injected into the column (SunFire C18 3.5 µm 4.6 × 75 mm equipped with 20 mm precolumn) and analyzed using fluorescence (at λex = 346 nm, λem = 521 nm). For skatole determination, 20 µL of the supernatant was injected into the column and analyzed using fluorescence (λex = 285 nm, λem = 340 nm). Concentrations were expressed per g of the liquid fat. The detection limits were 0.24 µg/g for androstenone and 0.03 µg/g for skatole. For androstenone concentrations below detection limit, a value of 0.21 µg/g was assumed, and for skatole concentrations of 0.02 µg/g (half of lowest value). Inter- and intra-assay variation for both compounds was below 10%. Carcasses were classified with a threshold for androstenone of 1 µg/g fat and skatole of 0.25 µg/g fat [33].

2.6. Genital Tract Measurements

The efficacy of immunocastration was further assessed by genital tract measurements as follows: reproductive organs/accessory sex glands and the pelvic part of the urogenital tract were excised and weighed at slaughter line as described by Fazarinc [37]. For this purpose, the pelvic part of the urogenital tract was first separated from the rectum and anus and the urinary bladder emptied through an incision at its apex. The pelvic urogenital tract was then cleaned of excessive adipose and connective tissue and the penis removed by cutting off close to the caudal end of the bulbourethral glands. The dissected pelvic urogenital tract consisting of the accessory reproductive glands (i.e., paired vesicular and bulbourethral glands, and prostate) was then weighed. Subsequently, accessory reproductive glands and testes with epididymes from each boar and immunocastrate were dissected and weighed individually.

2.7. Statistical Analysis

Data were analyzed with SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA), using a linear mixed model of the MIXED (mixed linear model) procedure with degrees of freedom determined by the method of Kenward-Roger. Variance components were estimated using the restricted maximum likelihood (REML) method. The linear mixed model included sex group, housing conditions and the interaction of sex group x housing as fixed effect. As the interaction of sex group x housing condition was mainly insignificant (except in two cases, skatole and cortisol B3), only p-values of sex group and housing conditions are presented in the tables.

Furthermore, trial, pen and the interaction of trial x pen, slaughter date, dam, sire and the interaction of dam x sire were used as random effects. Residuals were tested on normal distribution and variance homogeneity by visual check of residuals plots [38]. If the residuals were not normally distributed (androstenone and skatole), the data were logarithmically transformed and the results then retransformed. Differences between groups were adjusted by a Bonferroni correction. Paired Student's t-tests (two-tailed) with Bonferroni correction were used to analyze differences between a priori specified blood samples (B1 vs. B2; B2 vs. B3; B3 vs. B4) within one sex group with SPSS Version 24 (IBM Corp., Armonk, NY, USA). p-values with $p < 0.05$ were considered as significant and $p < 0.10$ as a tendency. The results are presented as LS-means (last mean square) ± SEM (standard error of the mean).

3. Results

3.1. Characterization of Testicular Functions in Male Pigs

Mixed linear model analysis indicated that sex group had a significant impact on testosterone concentrations, whereas housing conditions and the interaction of sex group and housing condition did not modify testicular functions. **Figure 2** shows plasma testosterone concentrations of boars, immunocastrates and barrows during blood sampling (B1–B4) throughout the fattening period. The first blood sample B1, collected before V1, revealed similar testosterone concentrations in boars and immunocastrates (about 0.3 ng/mL). Both sex groups, however, had higher testosterone levels than barrows (about 0.13 ng/mL). Using Bonferroni corrected t-tests, changes in testosterone concentration were further analyzed in detail within each sex group. Results indicate that in barrows, testosterone levels remained at low levels between B1–B3, but were slightly higher at B4 ($p < 0.001$). In contrast, testosterone concentrations in boars increased during the fattening period and reached considerably high levels at slaughter (about 39 ng/mL). Testosterone concentrations in boars differed significantly ($p < 0.01$) between all three specified comparisons (B1 vs. B2; B2 vs. B3; B3 vs. B4). Immunocastrates had similar testosterone concentrations as boars until B2 (V2). Two weeks after V2 (B3), testosterone concentrations dropped to pre-vaccination levels, similar to that of barrows (about 0.11 ng/mL). At slaughter (B4), testosterone levels in immunocastrates tended to be marginally higher than in barrows (immunocastrates: 0.3 ng/mL vs barrows: 0.2 ng/mL; $p = 0.056$), but substantially lower than in boars ($p < 0.001$). This indicates that immunocastration successfully suppressed testicular functions. In immunocastrates, testosterone concentrations differed between all specified comparisons ($p < 0.001$).

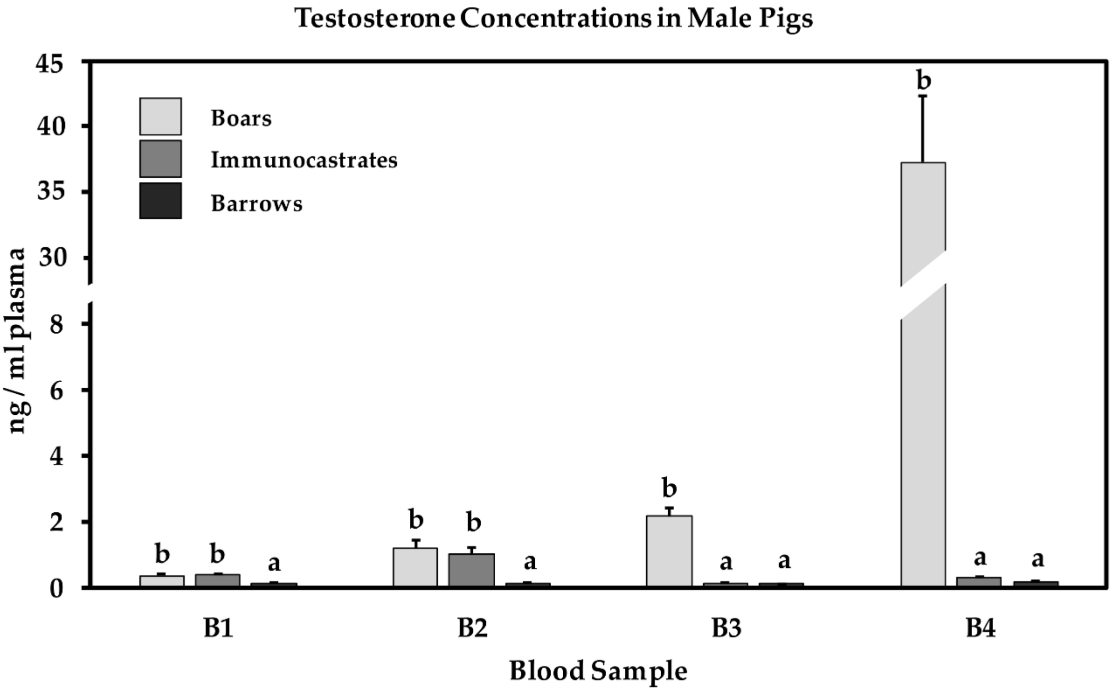

Figure 2. Plasma testosterone concentrations of boars, immunocastrates and barrows (n = 48 per sex group) at four blood sample time points (B1: before V1, B2: before V2, B3: 2 weeks after V2, B4 at slaughter). Testosterone concentrations between different sex groups with different superscripts differ significantly ($p < 0.05$).

3.2. GnRH Antibody Formation and Testicular Functions in Immunocastrates during the Investigation Period

In **Figure 3**, GnRH-binding of immunocastrates is given for the four blood samplings (B1–B4). Before V1 (B1), all immunocastrates revealed low unspecific GnRH-binding which corresponds to the low, unspecific binding of boars (B1: 3.71 ± 1.31%; B2: 3.04 ± 1.05%; B3: 2.63 ± 1.21%; B4: 2.58 ± 1.17%) and barrows (B1: 3.54 ± 1.32%; B2: 3.13 ± 1.03%; B3: 3.94 ± 1.31%; B4: 3.64 ± 1.24%). In immunocastrates, 2 weeks after V1, GnRH-binding increased markedly (B2, $p < 0.001$), followed by a further increase after V2 (B3, $p < 0.001$). This high GnRH-binding was maintained in all immunocastrates until B4 (B3 vs. B4, $p = 0.478$). Housing conditions had no significant influence on the level of antibody formation against GnRH at any time of sampling.

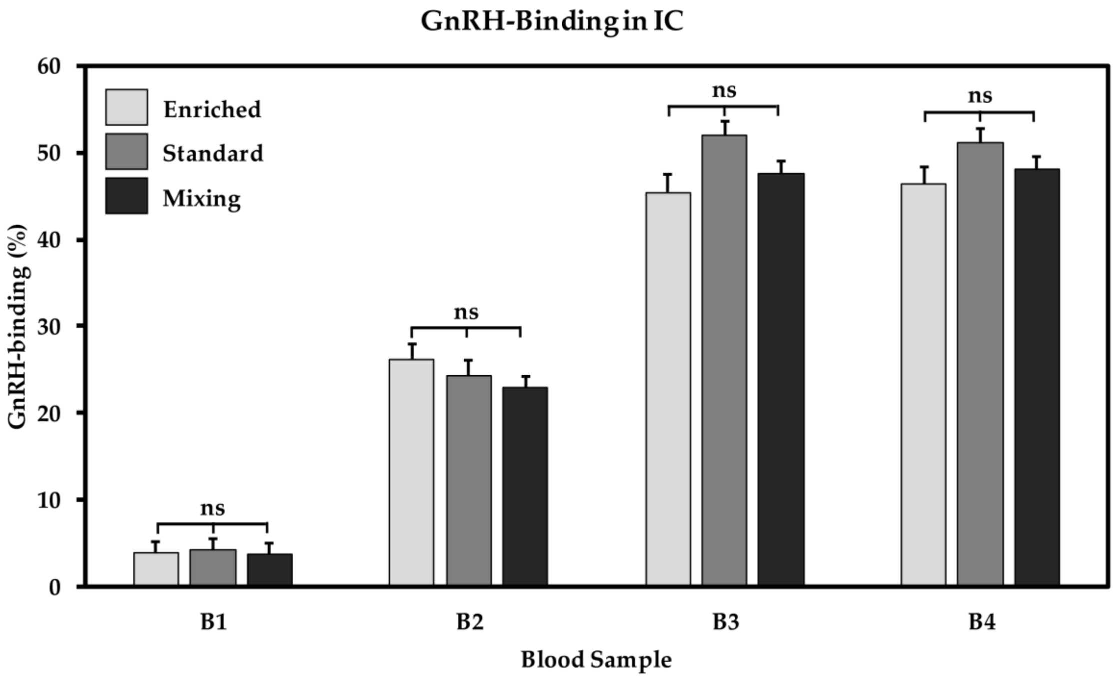

Figure 3. GnRH-binding in immunocastrates under three different housing conditions (enriched – n = 12, standard – n = 12 and mixing – n = 24) at four blood sample time points (B1: before V1, B2: before V2, B3: 2 weeks after V2, B4: at slaughter). Differences between housing groups marked with ns are not significant.

3.3. Evaluation of Reproductive Organs in Male Pigs

Furthermore, the differences between boars and immunocastrates in the characterization of genital tract weights were used to evaluate the efficacy of immunocastration (**Table 1**). In all the parameters tested (weight of testes with epididymes, vesicular glands, bulbourethral glands, prostate and pelvic part of the urogenital tract), differences between boars and immunocastrates were significant, which shows that immunocastration induced a regression of reproductive organs compared to boars. Similar to other parameters, housing conditions had no impact on the weight of reproductive organs.

Table 1. Genital tract weight (g) of boars, immunocastrates and barrows (n = 48 per sex group) housed under three different housing conditions (enriched – n =12, standard – n = 12 and mixing – n = 24).

Parameter	Boars (n = 48)	Immunocastrates (n = 48)	Barrows (n = 48)	p-Value	Enriched (n = 36)	Standard (n = 36)	Mixing (n = 72)	p-Value
Testes *	722.57 ± 21.43 [b]	288.41 ± 20.77 [a]	-	<.0001	490.97 ± 26.65	521.02 ± 24.23	504.48 ± 23.91	0.7001
Vesicular gl.	274.15 ± 16.26 [b]	38.94 ± 15.59 [a]	-	<.0001	140.04 ± 19.89	175.95 ± 19.15	153.63 ± 15.79	0.3026
Bulbourethral gl.	158.36 ± 10.87 [b]	58.95 ± 10.87 [a]	-	<.0001	105.42 ± 11.51	115.65 ± 11.37	104.90 ± 10.67	0.3930
Prostate	9.24 ± 0.47 [b]	3.38 ± 0.46 [a]	-	<.0001	6.18 ± 0.53	6.85 ± 0.53	5.89 ± 0.50	0.4648
Urogenital tract	540.61 ± 20.46 [c]	214.01 ± 18.16 [b]	115.41 ± 17.85 [a]	<.0001	281.00 ± 18.63	310.67 ± 18.68	278.36 ± 15.98	0.2729

Parameters within a row with different superscripts differ significantly ($p < 0.05$); * both testes weighed with epididymes.

3.4. Influence of Treatment and Housing Conditions on Boar Taint

Significant differences in boar taint compounds occurred between sex groups (**Table 2**). All immunocastrates had androstenone levels below the limit of detection (<0.24 µg/g fat) and consequently below the threshold of 1 µg/g fat. Compared to immunocastrates, 79.17% of all boars had androstenone levels above 1 µg/g fat. Housing conditions had no effect on androstenone levels in boars and immunocastrates. Androstenone is testis-derived and was not analyzed in barrows.

Table 2. Androstenone and skatole concentrations in boars, immunocastrates and barrows (n = 48 per sex group) housed under three different housing conditions (enriched – n = 12, standard – n = 12 and mixing – n = 24).

Parameter	Boars (n = 48)	Immunocastrates (n = 48)	Barrows (n = 48)	p-Value	Enriched (n = 36)	Standard (n = 36)	Mixing (n = 72)	p-Value
Androstenone	2.53 ± 0.50 [b]	<0.24 [a]	-	<.0001	0.64 ± 0.15	0.67 ± 0.15	0.74 ± 0.14	0.7184
Skatole	0.037 ± 0.005 [b]	0.020 ± 0.003 [a]	0.021 ± 0.003[a]	<.001	0.021 ± 0.003	0.029 ± 0.005	0.025 ± 0.003	0.1179

Parameters within a row with different superscripts differ significantly ($p < 0.05$). All immunocastrates had androstenone levels below the limit of detection (<0.24 µg/g fat). Androstenone is testis-derived and was not analyzed in barrows.

The differences in skatole levels were also significant between sex groups. All immunocastrates and barrows had skatole concentrations below 0.25 µg/g fat. In contrast to immunocastrates and barrows, 6.25% of boars (3 out of 48 animals) had skatole levels above the threshold of 0.25 µg/g fat. All 3 boars with increased skatole levels had concomitant androstenone levels above 1 µg/g fat. While housing conditions had no influence on the skatole values, sex group x housing condition (p = 0.0293) had an influence on skatole levels with boars housed under enriched conditions exhibiting lower skatole levels (0.023 ± 0.005 µg/g fat) than boars housed under standard conditions (0.063 ± 0.013 µg/g fat; p = 0.0105). It can be concluded that immunocastration was effective in preventing boar taint, and that housing conditions had no modifying influence on boar taint compounds in immunocastrates and barrows, whereas in boars, enriched conditions significantly reduced skatole levels.

3.5. Cortisol Levels in Male Pigs

Table 3 shows plasma cortisol concentrations of boars, immunocastrates and barrows throughout the fattening period at B1–B4. Cortisol levels did not differ between sex groups and housing conditions in the first two blood samples. At B3, the interaction of sex group and housing condition was significant (p = 0.0405), with no significant differences found in post-hoc testing.

Table 3. Cortisol levels in boars, immunocastrates and barrows (n = 48 per sex group) housed under three different housing conditions (enriched – n = 12, standard – n = 12 and mixing – n = 24).

Parameter	Boars (n = 48)	Immunocastrates (n = 48)	Barrows (n = 48)	p-Value	Enriched (n = 36)	Standard (n = 36)	Mixing (n = 72)	p-Value
Cortisol - B1	27.96 ± 2.77	26.37 ± 2.75	28.93 ± 2.74	0.7025	31.76 ± 2.94	26.34 ± 2.87	25.16 ± 2.49	0.1353
Cortisol - B2	23.82 ± 3.04	21.37 ± 3.03	27.31 ± 3.03	0.0584	23.44 ± 3.19	23.82 ± 3.13	25.23 ± 2.81	0.7137
Cortisol - B3	19.49 ± 1.47	18.56 ± 1.40	17.77 ± 1.34	0.6867	17.63 ± 1.46	21.49 ± 1.78	16.97 ± 0.99	0.0629
Cortisol - B4	59.15 ± 4.41 [b]	45.29 ± 4.36 [a]	49.74 ± 4.34 [ab]	0.0049	53.57 ± 4.66	48.26 ± 4.61	52.36 ± 3.86	0.4735

Blood Samples – B1: before V1, B2: before V2, B3: 2 weeks after V2, B4 at slaughter; parameters within a row with different superscripts differ significantly ($p < 0.05$).

Differences between sex groups were, however, evident at slaughter (B4). Boars had higher cortisol concentrations than immunocastrates ($p < 0.01$), and a tendency towards higher cortisol concentrations than barrows ($p < 0.1$). Immunocastrates and barrows did not differ significantly ($p = 0.899$). Within all sex groups, cortisol concentrations increased significantly from B3 to B4 ($p < 0.001$), which clearly shows the influence of slaughter and transport stress on cortisol levels in male pigs. There was no effect of housing conditions on cortisol concentrations at B4.

3.6. Growth Performance of Male Pigs

Growth performances of the three sex groups varied throughout the fattening period (**Table 4**). At the beginning of the fattening period (feeding phase 1), barrows showed a tendency towards higher ADG than boars (911 g vs. 854 g respectively; $p = 0.0569$). Immunocastrates were between boars and barrows in their growth performance in this period. In the second feeding phase, no differences between the sex groups were obvious. Growth performance of immunocastrates changed after V2, in feeding phase 3, and reached the highest ADG (967 g) of all sex groups. Growth performance was significantly higher in boars than in barrows in feeding phase 3 ($p = 0.0262$). Over the entire fattening period, differences between sex groups were less pronounced and did not reach the level of significance ($p = 0.069$).

Table 4. Growth performance (average daily gain, ADG, in g) in boars, immunocastrates and barrows (n = 48 per sex group housed under three different housing conditions (enriched—n = 12, standard—n = 12 and mixing—n = 24).

Parameter	Boars (n = 48)	Immunocastrates (n = 48)	Barrows (n = 48)	p-Value	Enriched (n = 36)	Standard (n = 36)	Mixing (n = 72)	p-Value
ADG - Phase 1	854 ± 23	864 ± 22	911 ± 22	0.0417	862 ± 23	887 ± 23	880 ± 21	0.4747
ADG - Phase 2	923 ± 46	905 ± 46	963 ± 46	0.0625	969 ± 47 [b]	926 ± 47 [ab]	894 ± 44 [a]	0.0099
ADG - Phase 3	869 ± 20 [b]	967 ± 20 [c]	816 ± 20 [a]	<.0001	911 ± 21 [b]	882 ± 21 [ab]	859 ± 18 [a]	0.0438
ADG - Total Fattening	855 ± 20	906 ± 20	879 ± 20	0.0694	898 ± 21	885 ± 21	886 ± 19	0.1220

Feeding phases: Phase 1—at an age of 10 to 18 weeks; Phase 2: at an age of 18 to 22 weeks; Phase 3—at an age of 22 to 27/28 weeks; Total fattening period: ADG (g) of total fattening period (age of 10 to 27/28 weeks); parameters within a row with different superscripts differ significantly ($p < 0.05$).

In contrast to other parameters, housing conditions had a significant impact on the growth performance of pigs. Animals from the mixing group revealed lower growth rates in the second and third feeding phase than animals from the enriched group, whereas animals from the standard group were in between. In the second feeding phase, animals from the enriched group had an 8% higher growth rate than animals from the mixing group. In the third feeding phase, the ‘enriched’ animals also had a 6% better growth performance than animals from the mixing group.

4. Discussion

Immunocastration and pork production with boars are possible alternatives to surgical castration of male piglets [13]. Pork production with boars has advantages when compared to pork production with barrows, as the feed conversion ratio (FCR) and growth performance are improved [7]. On the other hand, the risk of animal welfare-related problems is increased, as boars have a higher potential for agonistic behavior [13]. In addition, the quality of meat from boars is lower because of boar taint, a reduced intramuscular fat content, and increased amounts of unsaturated fatty acids in the fat which limits its suitability for traditional dry cured meat products [39]. From a scientific point of view, immunocastration has the potential to reduce these problems markedly, but the market relevance of immunocastrates is globally very low and the reliability of this procedure is often questioned as knowledge gaps exist on the market [13]. Therefore, the study analyzed the reliability of immunocastration in different housing conditions and compared the growth performance of immunocastrates with boars and barrows to evaluate whether the technique is competitive. To our best knowledge, this is the first study to test vaccination against GnRH with Improvac® under various housing conditions in experimental trials, measuring antibody response on the basis of GnRH binding and testosterone concentrations. Full siblings were allocated to different sex groups and housing conditions in order to reduce variability due to age and genotype.

In contrast to the literature reports [9,13,17] and the concerns of pork chain actors, our study found no evidence for non-responders. In fact, after two Improvac® vaccinations, the immune response was sufficient in all immunocastrates to fully suppress testicular functions. Zeng et al. [16] described that health problems during the vaccinations were linked to an insufficient immune response to Improvac® vaccinations, and thus resulted in non-responders. In the literature [9,13,17], wrong handling or missed vaccinations are often assumed to be the reasons for non-responders. In the present experiment, we ensured that the animals were healthy during the vaccinations and correct handling and careful vaccinations were ensured by experienced veterinarians. We can therefore conclude from this experiment that if vaccinations against GnRH (Improvac®) are carried out correctly, the technique is reliable even under more challenging housing conditions.

Testosterone concentrations in all immunocastrates decreased to the level of barrows after the second immunization against GnRH and remained at this basal level until slaughter. In comparison, intact boars had testosterone levels of 3–5 ng/mL plasma at this age, as similarly described by Zamaratskaia et al. [40]. These effects clearly show, that testicular functions were successfully suppressed in all immunocastrates after two vaccinations with Improvac®. In our study, boars revealed considerable testosterone concentrations at slaughter. A previous study by Wesoly et al. [41] describes the influence of transport time on the testicular functions of boars. Testosterone concentrations were increased by 2.2 ng/mL plasma per hour transport time, which shows an impact of pre-slaughter conditions on testicular functions in boars. In this study the transport time from the animal experimental unit to the slaughterhouse was about 3 h, the pre-unloading time about 1 h, followed by another hour until the pigs were actually slaughtered.

In the present study, immunocastration was also effective in the prevention of boar taint. Androstenone levels were below the limit of detection in all immunocastrates, all skatole levels below the defined thresholds for skatole (0.25 µg/g fat), indicating that immunocastration is also reliable in preventing boar taint. Skatole concentrations in barrows and immunocastrates were significantly lower than in boars, as high androstenone, testosterone and estradiol levels in boars inhibit the activity of hepatic skatole-degrading enzymes CYP2E1 and CYP2A [42,43,44,45]. This also agrees with the meta-analysis by Batorek et al. [46] and Nautrup et al. [47]. In our study, housing conditions had no effect on either androstenone nor or skatole levels. However, the opposite was shown in a study by Škrlep et al. [48] in which individually housed animals had lower skatole levels than group-housed animals, but no differences occurred in androstenone concentrations. Furthermore, a higher stocking density resulted in higher skatole levels but lower androstenone levels than in animals housed in a lower stocking density and slaughtered at higher ages.

In comparison to boars, all reproductive organs of immunocastrates were significantly lighter. These findings are in full agreement with several studies [49,50,51] which show a significant impact of treatment with Improvac® on the development of the male genital tract. The different compartments, however, were affected to a various degree. Above all, the glandula vesicularis, which is known to reflect testosterone levels in size and secretory activity [52], might be suggested as an additional parameter for determining the success of the vaccination [50]. However, for practical reasons it is difficult to excise the vesicular gland at slaughter line and to determine its weight. Even if testes weight differs significantly between boars and immunocastrates, this parameter is not recommended for detection of non-responders at slaughter line as there is no clear cut between the testes weight of boars and immunocastrates [53]. Therefore, we do not recommend this parameter to determine the success of an adequate immune response after immunocastration.

Similar to this study, previous reports in pigs showed that challenging housing conditions such as mixing must not necessarily lead to a pronounced influence on the plasma cortisol concentrations in pigs [26,32]. Sutherland et al. [30] found even lower cortisol concentrations in mixed than in control pigs. Notably, animals from enriched (and presumably less stressful) environments also show higher cortisol concentrations during daytime than animals from barren housing conditions [29]. However, it has to be considered that single cortisol measurements cannot reflect changes in the daily pattern of cortisol levels. De Jong et al. [54] have shown that pigs housed under barren condition show a blunted circadian rhythm in cortisol compared to pigs housed in enriched conditions and that a blunted rhythm may indicate decreased welfare. Moreover, it has been shown that CBG (corticosteroid-binding globulin) concentration can decrease under stressful conditions [55], making conclusions based only on plasma cortisol levels a complex task. Thus, more detailed investigation on circadian rhythm and the free (= active) vs. bound (= inactive) ratio of cortisol is needed to draw comprehensive conclusions regarding the effect of housing conditions and gender on hypothalamic–pituitary axis (HPA) activity.

On the other hand, behavioral observations in our study revealed that mixed animals more often showed severe agonistic behavior and were thus probably more stressed than animals from the standard or enriched housing environments [56]. Moreover, the poorer growth performance of mixed pigs in the present study can also be taken as an indicator of stressful housing. Studies by Ekkel et al. [57] showed that housing with social mixing of pigs has a negative impact on growth performance and on welfare when compared to "specific stress-free" housing environments. These results are also consistent with those from other reports on the effect of mixing on ADG [30,31,32]. Here, it is important to note that, although mixing stress most likely caused higher stress levels in immunocastrates as well, the intensity of the stressor 'mixing' stressor was not sufficient to negatively affect antibody response to GnRH or even to cause non-responders.

In the present study, the growth performance of immunocastrates was significantly higher after the second vaccination than that of boars and barrows, which agrees with the meta-analysis of literature reports [46]. A study by Pauly et al. [58], however, had shown that after the second vaccination, only differences between immunocastrates and boars remain, but not between immunocastrates and barrows. Until the second vaccination is applied, the growth performance of immunocastrates is identical to that of boars [46,58]. Over the entire fattening period of the present experiment, no differences in growth performance between the sex groups occurred. However, this does not exclude the possibility, that immunocastrates could be superior to barrows and boars over the entire fattening period. The extent to which the growth performance after the second vaccination or throughout the entire fattening period differs between the sex groups mainly depends on the genetically determined level of feed consumption of a genotype [33] and on the timing of the second vaccination [13]. The economic relevance of the higher growth rates of immunocastrates after the second immunization against GnRH gains relevance only if the feed conversion ratio is also competitive and the fattening duration decreases compared to barrows and boars [33].

5. Conclusions

This study shows that immunocastration is a reliable technique to supresses testicular functions under different housing conditions. Regardless of housing conditions, testosterone concentrations drop after the second Improvac®-vaccination to a low level, comparable to barrows. Furthermore, all carcasses of immunocastrates were free of boar taint, whereas a considerable number of boar carcasses were affected by boar taint. Reproductive organs, as well, react to the vaccinations and result in lower weights than in boars. In our study, based on Pietrain x German Landrace and two vaccinations at the age of 12 and 22 weeks, the growth performance between sex groups did not differ throughout the total fattening period. On the other hand, immunocastrates had a higher growth performance after the second vaccination which can be used in economic terms by choosing the optimal time for the second vaccination. Mixing had been applied as a standardized method to increase stress. A negative influence on growth performance by this was obvious and points to a moderate stress. However, this social stressor did not modify the immune response upon vaccination against GnRH or cause any non-responders in our study.

Author Contributions

Conceptualization, K.K., U.W., S.S., M.Č.-P. and V.S.; methodology, K.K., U.W.; S.S., M.V.; G.F., M.Š., N.B.-L. and V.S.; formal analysis, K.K. and U.W.; data curation, K.K.; writing—original draft preparation, K.K.; writing—review and editing, K.K., U.W., S.S., M.Č.-P., M.V., G.F., M.Š., N.B.-L. and V.S.; funding acquisition, V.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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3 GENERAL DISCUSSION

Although in 2010 many European stakeholders voluntarily committed to completely end surgical castration of male piglets by 2018 [1], today 63% of the male pigs designated for pork production are surgically castrated, most of them without adequate pain relief as described in chapter 1 [2]. The efforts of the pork chain in the past years to change the production system is reflected by increasing numbers of boars used for pork production in Europe [2,3]. However, the main limiting factors for a further market expansion, the quality problems due to boar taint and animal welfare associated problems of raising boars have not been resolved yet and alternatives have not reached the public acceptance required [4]. Immunocastration is currently the alternative that combines the advantages of pork production with boars and with barrows to the highest degree, and has positive effects on animal welfare, growth performance, environment, product quality and economic profitability [5]. Although this technique has been assessed very positively from a scientific point of view, and although the global market share of immunocastrates has increased by 100% in the last 3 years [5], in absolute figures the proportion of immunocastrates within the European Union is still only 2.8% [2]. Knowledge gaps which lead to uncertainties within the pork chain and thus to a low market acceptance of immunocastration are the overall reliability of immunocastration and its economic viability, as described in chapter 1. One potential approach to the issue of non-responders was to examine whether social stress by mixing groups may lead to a higher risk of non-responders. Regarding profitability, the main concern was that pricing carcasses from immunocastrates like boars and introducing fines on the pork market for boar taint may decrease the competitiveness of pork production with boars and immunocastrates. The aim of this doctoral thesis was therefore to identify and to reduce the knowledge gaps that contribute to the current low market acceptance of immunocastration.

3.1 Key findings

One major knowledge gap is the reliability of immunocastration and the background of non-responders after Improvac® vaccinations, as 0-3% non-responders are reported within the pork chain [6]. In the experiment further described in MANUSCRIPT III, we experimentally investigated to which extent housing conditions have an impact on the success of immunocastration in male pigs as it is well known that stress can impair the immune system and thus lead to an insufficient immune response after vaccination [7–10]. In the present thesis, we tested the hypothesis that stressful housing conditions may contribute to an insufficient immune response after Improvac® vaccinations under field conditions [5]. The effect of stress by social instability was studied as a relevant stressor, as this can be frequently observed under field conditions. As a consequence, we investigated whether animals from more socially challenging housing conditions showed a poorer vaccination response than the control groups. The timing of the stressful mixing phases was placed around the respective vaccinations in order to cause a stress reaction during the time span when the full immune response develops. Antibody titers against GnRH, testosterone as a marker of testicular functions and weights of reproductive organs were chosen as key parameters to evaluate the immunization success. In this experiment, housing conditions had no influence on the antibody titers against GnRH or on the testosterone concentrations in immunocastrates even though growth performance was significantly impaired by the stressful housing conditions. We therefore concluded, that immunocastration works reliably even under more challenging housing conditions and repeated social mixing [11]. In other studies, testosterone and the weights of reproductive organs were used to determine the success of the vaccination based on the suppression of testicular functions [12–14]. These studies also revealed that the weights of the reproductive organs are significantly lower in immunocastrates than in boars [12,15,16], and that testosterone concentrations are at the same level as those of barrows [12–14]. The reliability of immunocastration, as tested in the experiment described in MANUSCRIPT III, can similarly be assumed for pigs housed under field conditions. Social instability of group compositions can also be found in commercial pork production, for example at the beginning of the fattening period or towards the end, when the groups are harmonized according to similar live weights [17]. However, in the experiment, which is described in MANUSCRIPT III, a higher number of mixing events (continuous social mixing with five mixing events in mixing phase 1 and eight mixing events in mixing phase 2) were carried out than would occur under field conditions (single events) to ensure that the experimental results are transferable to field conditions. The experimental organic housing conditions were similar to those used in common organic pig farms. The animals had access to an outdoor area and were exposed to the same environmental stressors (sun, ecto- and endoparasites) [18]. The fact that immunocastration can also be used in organic pork production, as it reliably avoids boar taint and produces good meat quality, has already been shown by a comprehensive study with organic pigs by Grela et al. [19]. We therefore assume, that the reliability of immunocastration is also robust under field conditions. Thus, the reasons for up to 3% of non-responders under field conditions are more likely caused by incorrect handling (missed vaccinations, wrong storage of vaccine) during vaccinations [5,6] or a suppressed immune system at the time of vaccinations (e.g. diseases) [20] than by housing conditions.

Until the second vaccination, testosterone concentrations of immunocastrates were similar to those of boars. After the second vaccination, a drop of testosterone concentrations to the level of barrows could be observed until slaughter [11]. Successfully immunocastrated boars were defined as animals that had testosterone concentrations at slaughter below 0.5 ng/ml plasma. All immunocastrates revealed similarly low concentrations in this experiment [11]. The limit of <0.5 ng/ml plasma was chosen according to a previous study by Claus et al. [13] and reflects the fact that barrows have similar low testosterone concentrations [21–26], while boars at an age of 6 months have approx. 3-5 ng/ml plasma [24,27,28] - a concentration that is about 10 times higher than in barrows. Since testosterone is not only synthesized by the testes but also to a small extent in the adrenal glands, barrows still show low testosterone concentrations during the fattening period [29]. A significant increase in testosterone concentrations at slaughter was found in all male pigs, with the highest increase being found in boars. However, testosterone concentrations in immunocastrates and barrows were still below 0.5 ng/ml plasma, revealing successfully suppressed testicular functions in immunocastrates. The considerably elevated testosterone concentrations in boars at slaughter can be explained by stress caused by transportation and in the lairage after unloading. A study by Wesoly et al. [30] showed that a prolonged transport time increases testosterone concentrations by 2.2 ng/ml plasma per hour. Similarly, Escribano et al. [31] also showed that the transport to the slaughter house served as a stressor for the pigs and led to an increase in both testosterone and cortisol levels. The authors concluded from these results that in boars, blood testosterone concentrations can also be used as an additional marker for acute stress.

The weight of the reproductive organs served as a further parameter for validating the success of the vaccination, as growth, development and secretory activity of accessory glands depend on testicular hormones [32]. All reproductive organs of immunocastrates weighed significantly less than those of boars, which serves as further evidence for a successful immunocastration [11]. Evidence for similar effects on accessory glands and on the development of the genital tract has been obtained in previous studies [12,15,16].

As boar taint is the main critical factor for the market acceptance of pork from boars [4], the reliable suppression of androstenone and skatole accumulation is crucial for the acceptability and application of immunocastration. In our experiment, both, immunocastrates and barrows revealed significantly lower concentrations of the two boar taint compounds androstenone (only analyzed in immunocastrates) and skatole. These concentrations remained below the critical threshold of 1 µg/g fat for androstenone and 0.25 µg/g fat for skatole defined above for adverse consumer reactions [33,34]. In contrast to immunocastrates, 80% of the boars in our study exceeded androstenone concentrations of 1 µg/g fat. In this experiment, immunocastration thus proved to be as effective as surgical castration in preventing boar taint and supports the conclusions of meta-analyses that immunocastration is an effective tool in the prevention of boar taint [35,36]. The considerable high androstenone concentration of boars in our experiment further illustrates how robustly immunocastration avoids boar taint even in genotypes with high potential for androstenone synthesis. In an international study with different genotypes from 6 European countries at the usual slaughter weights, about 29% of boars had androstenone concentrations above this threshold. This study, however, also found that androstenone levels varied considerably between the countries, which was mainly explained by different genotypes and ages at slaughter [37]. Bonneau also describes in his review that a range of 10-75% of boars exhibit boar taint [38].

Not only product quality, but also growth performance must be competitive to make pork production with immunocastration attractive for farmers [39]. In our study, no differences in growth performance among different sex groups could be observed in feeding period one and two (both before the second vaccination). In the last feeding period (after the second vaccination), however, immunocastrates showed a higher average daily gain than boars and barrows [11]. This higher growth performance had already been shown by other studies [35,36] and is mainly related to a higher feed intake after the second vaccination [40], which could also be observed in our trial.

Cortisol levels in blood were measured as one parameter for stress in order to evaluate the impact of housing conditions and different sex groups on its concentration. As the blood collection of all pigs took no longer than 63 seconds, cortisol concentrations in the present study should not be influenced by blood sampling itself, as already described in other studies [31,41,42]. A blood sample was taken by no earlier than one day after a mixing event occurred. During the entire fattening period, no influence of housing conditions on cortisol concentrations could be detected. Possible effects of mixing on cortisol levels might have been masked in the present study by the timing of the sampling, as the first blood sample after each individual mixing event was collected after one day. Changes in cortisol might have been obvious if blood would had been collected shortly after a mixing event as cortisol levels might have returned to normal concentrations after one day [43]. Therefore, our data suggest that it might not be sufficient to analyze stress by measuring cortisol concentrations. For this reason, a continuous monitoring of other parameters, such as behavioral observations and growth performance, should also be taken into account when evaluating stress [44]. At slaughter, all sex groups revealed significantly higher cortisol concentrations than during the fattening period. In addition, sex differences became obvious in these samples as boars had higher cortisol concentrations at slaughter than immunocastrates and barrows. A similar difference between sex groups was not obvious in samples obtained during the fattening period [11]. The increase of cortisol concentrations in the animals at slaughter was to be expected as it reflects the increased stress response to transportation and slaughter and coincides with the increased testosterone concentrations described above. The differences in the magnitude of the reaction further suggests, that boars seem to react with higher testicular activity to new environmental stimuli and stressors. This could also lead to more agonistic behavior of boars in the lairage, which was already described by Wesoly et al. [30]. pCBG could also be measured in plasma to determine the proportion of free cortisol in plasma, as in addition to plasma cortisol, pCBG also reacts to acute stressors and can therefore influence the biologically active cortisol [45].

In contrast to the short term changes of endocrine parameters, our data suggest from growth performance data and behavioral observations that stress was applied by mixing in our experiment. The growth performance of the mixed animals was lower, especially in the second half of the fattening period (after the first and second mixing phase) than of the animals from the enriched group as described in MANUSCRIPT III. The effects of stress on reduced growth performance have already been shown in several studies [46–48]. Similar to differences in growth performance, the animals in the mixing group displayed significantly more severe agonistic behavior than animals in the enriched group [49]. Other studies already showed that stress negatively influences behavior in pigs and stimulates agonistic behavior [50,51].

Another decisive aspect for the pork chain and especially for pig producers besides the reliability of immunocastration is whether the technique is profitable or whether applying it may lead to higher costs and lower profit margins [5]. The alternatives to surgical castration of male piglets without pain relief are very sensitive to changes in the production system with regard to its profitability [52,53]. Whether or not a technique is accepted on the market depends primarily on its economic feasibility or on legal regulation [39]. In comparison to barrows, immunocastrates remain intact until slaughter, but must be vaccinated twice during the fattening period [5]. Thus the costs related to castration are saved (i.e. fewer personnel costs [53] and fewer piglet losses [54]), but different personnel costs for vaccinating the animals and costs for the vaccine apply [55]. Additional costs for pork production with immunocastrates can also result at the slaughterhouse or later during processing. As slaughterhouses have to identify boar-tainted carcasses, they may check all uncastrated male pigs (boars as well as immunocastrates) via human nose test for boar taint [56]. Furthermore, tainted carcasses are allocated to less valuable sales channels or even completely removed for further processing [57]. When conducting an economic evaluation of immunocastration, meat quality should also be taken into account as it determines potential sales channels and opportunities. Depending on the timing of the second vaccination, meat quality of immunocastrates is either more comparable to boars (late second vaccination) or more comparable to barrows (early second vaccination) [5]. A meta-study by Batorek et al. [35] showed that pork quality of immunocastrates resembles that of barrows due to similar values in lean meat content, intramuscular fat content and fatty acid composition. If the second vaccination is only applied four weeks before slaughter, however, the meat from immunocastrates is not suitable for the production of traditional dry-cured ham products, as it shares certain pork quality characteristics of boars (high lean meat content, more unsaturated fatty acids) [58].

However, immunocastration can also realize a higher growth performance and a more efficient feed conversion [35,36], thus lowering feeding costs during the fattening period compared to barrows [53,55]. In our study presented in MANUSCRIPT II, we set the performance data of male pigs (from the experiment described in MANUSCRIPT III) in relation to an economic dataset from agri benchmark and analyzed the profitability according to various carcass pricing systems and risk scenarios for boar taint. The study therefore simulated different market conditions and tested the economic sensitivity of pork production with boars and immunocastrates. The results showed that pork production with immunocastration was almost as profitable as with surgical castration (performed without adequate pain relief) as long as carcasses from immunocastrates are priced as barrows and all carcasses are free of boar taint. For 3 out of 5 farms, pork production with immunocastrates is still more profitable than with boars. In a study by Verhaagh and Deblitz [53], immunocastration was the most profitable alternative under current market conditions (no fines for boar taint, immunocastrates priced on standard pricing system). These results show that if the pork market accepts immunocastration and farmers optimizes the production of immunocastrates in terms of growth performance and feed conversion, immunocastration can be competitive with the traditional surgical castration.

The extent to which immunocastration is profitable depends in particular on the timing of the second vaccination. Due to the higher feed intake of immunocastrates after the second vaccination (even higher than in barrows) [40], the growth performance of immunocastrates after the second vaccination is higher than in boars and barrows [35,36]. Consequently, immunocastrates are reaching their final slaughter weights earlier than boars, thus reducing the duration of the fattening period. The duration of the fattening period is a decisive factor in the profitability of a production system, as it determines how many animals can be produced within a certain time [57]. Nevertheless, the consequences of a late second vaccination must also be mentioned. On the one hand, a more efficient feed conversion ratio can result in economic advantages [53], but animal welfare may be impaired as immunocastrates display more boar-specific agonistic behavior over a longer time period [59]. Moreover, a late second vaccination has negative effects on pork quality. Both are critical for consumer acceptance, since consumers demand both high standards of animal welfare and a high pork quality [39].

As the key objective in the experiment (MANUSCRIPT III) the data was derived from, was to test the reliability of immunocastration under different housing conditions and not to optimize growth performance of male pigs, immunocastrates were economically undervalued as the performance data was set in relation to the process limits of the data set of agri benchmark. The last period after the second vaccination, which is crucial for the economic profitability, was therefore underrepresented in this study und would lead to a higher economic output if the study design would be adjusted. This conclusion supports an earlier second vaccination time than applied in this experiment, which would also have a positive impact on animal welfare and product quality [5]. As described in MANUSCRIPT III, immunocastration is very reliable when used correctly and prevents boar taint. But even with an assumed proportion of 3% non-responders [6], only 0.9% of the animals would probably be affected by boar taint [5]. Therefore, boar taint detection for each individual immunocastrate is neither economical nor necessary, but rather a corporate risk decision of each slaughterhouse [57].

3.2 Implications for the practical use of immunocastration

The results of this doctoral thesis show that immunocastration works reliably under different housing conditions and therefore is a possible alternative to surgical castration and pork production with boars for all production systems. The main problem with boar fattening so far is the occurrence of boar taint [4]. This can be reliably suppressed by immunocastration and a product quality similar to that of barrows can be achieved, if the timing of the second vaccination is scheduled accordingly [5,58]. Depending on the timing of the second vaccination, immunocastration also has the potential to be competitive in terms of growth performance [5]. Lower testosterone concentrations of immunocastrates and barrows could also lead to less agonistic reactions to new environmental stimuli during transport to the slaughterhouse and in the lairage. This not only has a positive impact on animal welfare, but also on pork quality [60].

The economic viability of immunocastration depends primarily on the extent to which immunocastrates are accepted on the market, both by consumers and by pricing [55] or by legal regulation. Various consumer studies show that immunocastration can indeed be accepted by consumers if there is targeted communication within the pork chain [39,61]. The advantages and disadvantages associated with the timing of the second vaccination in particular have to be openly communicated and discussed, so that production can be targeted to product quality, animal welfare and market requirements [5].

The price at which carcasses of immnocastrates are sold is also crucial for the acceptance of this technique, especially among farmers [39]. As immunocastration works reliably, routine boar taint detection at the slaughter line is not necessary [57]. If immunocastration is further accepted in international sales channels, there is no reason why immunocastrates should be priced inferior to barrows or gilts, as from a product quality perspective the technique has the potential to produce the same quality as barrows, if the second vaccination is applied accordingly [5,58]. Instead, the market has to develop alternative systems to detect non-responders before slaughter. On the farms, a reliable visual check of testes size and behavioral observations should be carried out and at the slaughterhouse, whether all immunocastrates have been successfully immunized against GnRH (testes size, behavior, boar taint detection). Immunocastration provides the industry with a technique that produces good pork quality, has a lower environmental impact, has positive effects on animal welfare and is also economically feasible without receiving governmental subsidies and therefore a sustainable alternative to surgical castration und pork production with boars [5,11,57].

Even when employing immunocastration, the technique's advantages and disadvantages must be balanced depending on the respective market requirements. Advantages deriving from the timing of the second vaccination (as described in MANUSCRIPT I) result in disadvantages in other areas. For this reason, immunocastration is always a compromise between advantages and disadvantages of either pork production with boars or barrows [5].

3.3 Suggestions for future research

In the study described in MANUSCRIPT II, we observed that immunocastration works reliably if it is carried out correctly. Nonetheless, other studies describe a proportion of 0-3% non-responders after two Improvac® vaccinations. In order to obtain further information about the phenomenon of non-responders, data from slaughterhouses and farms must be collected. As described in MANUSCRIPT I, wrong handling or missed vaccinations can also lead to boar-tainted carcasses in immunocastrates. Reliable vaccination protocols and assurance systems that minimize the risk for non-responders must therefore be developed.

It can also be derived from MANUSCRIPT II and MANUSCRIPT III that a routine boar taint detection of all immunocastrates is neither economical nor necessary. Nevertheless, quality assurance systems must be developed that identify non-responders or incorrectly vaccinated animals before slaughter. A potential two-stage system could be developed. A first step would be an effective vaccination control on the farms, the second step, an appropriate detection control of non-responders at the slaughterhouse. Parameters such as behavior (less agonistic behavior) and testes size/weight can be used in order to detect non-responders or incorrectly vaccinated pigs. In critical cases, boar taint detection at the slaughter line could then be performed. Taking these aspects into account, a reliable system can be developed and implemented that is more cost-effective than the routine boar taint detection of all immunocastrates.

In order to estimate more precisely the economic viability of immunocastration, a consistent performance study should be undertaken with data from animals of different sexes (boars, immunocastrates, barrows and gilts) from the same fattening period/farm (growth performance and feed conversion) until slaughter (dressing percentage and carcass characteristics). On this basis, a consistent relation between revenue at slaughter and costs during the fattening period can be connected for each individual. In a next step, the optimal slaughter weight and time for each sex can then be determined based on sex-specific growth performance, slaughter weight and carcass characteristics, in order to optimize the processes accordingly. In this context, the effects of a possible third Improvac® vaccination and higher requirements for animal welfare and product quality can also be analyzed economically.

As the economic performance of alternatives to surgical castration without pain-relief also depends on trade and other factors, it would be necessary to carry out elasticity studies to explain the effects of a change in the production system (e.g. surgical castration vs intact male piglets) on the entire pork chain. A potential scenario, for example, is that when male piglets are intact, the production costs of piglet production decrease and thus the prices of piglets decrease as well. However, it is also possible that a limited market of intact male piglets will even further lower their prices. A simulation analysis along the entire pork chain is therefore necessary in order to estimate the overall economic consequences when changing the production systems.

3.4 Conclusion

The end of surgical piglet castration without pain relief or to completely end with surgical castration can be a sustainable chance for the European pork chain. If immunocastration is used correctly, consumer protection is as efficient as with surgical castration. It is competitive for producers, ensures improved animal welfare because animals remain intact and the aggression potential of boars is minimized, and it can also have advantages by making use of the feed conversion of boars, which leads to a lower environmental impact than pork production with barrows. Nevertheless, a high market acceptance along the pork chain must be ensured, so that there is no need for the implementation of government subsidies, as immunocastration can be economically viable on its own. However, immunocastration would considerably benefit from government incentives to implement an information system where practical experiences are exchanged and uncertainties regarding immunocastration within the market are eliminated.

3.5 References

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4 SUMMARY

In Europe, male piglets have been surgically castrated for centuries in order to avoid boar-tainted carcasses and to eliminate boar-specific aggressive and sexual behavior. Surgical castration of male piglets is still legal within the European Union during the first week of life, even without anaesthesia or analgesia. These circumstances have led to increasing societal criticism, as the castration is painful and violates the physical integrity of the animals. In 2010, European stakeholders of the pork chain committed themselves voluntarily to ending surgical castration from 2018 onwards, but at present, more than 2 years later, the majority of male piglets are still castrated surgically without adequate pain relief. Immunocastration is one alternative to surgical castration or pork production with boars. Although this technique is approved for commercial use in Europe since 2009, the market shares of immunocastrates within the European pork market are very low. The main reasons for this low market acceptance are uncertainties whether immunocastration is reliable, competitive and accepted along the pork chain. The main objective of the present doctoral thesis was therefore to investigate the advantages and disadvantages of immunocastration with regard to the three pillars of sustainability aspects. The various sustainability aspects of immunocastration were summarized and reviewed (as part of the present thesis). In addition, the effects of different housing conditions on the reliability of immunocastration were experimentally tested, as was the impact of housing conditions and sex group on the growth performance of male pigs. Finally, the consequences of pork production with immunocastrates and boars on the profitability of German pig production were analyzed with a simulation model using different carcass pricing systems for immunocastrates and risk scenarios for boar taint. The results are described in three peer-reviewed scientific papers and the main results are summarized below.

Immunocastration is an active immunization against GnRH and consists of two consecutive vaccinations to induce antibodies which temporarily suppress testicular functions and prevent boar taint. It is a method which ensures both a high product quality and a high level of animal welfare. The impact of immunocastration on the three pillars of sustainability has been studied extensively, although a contemporary global overview of its different aspects has been missing. Performance results in immunocastrates are better than in barrows, but worse than in boars. The environmental impact of pork production with immunocastrates is lower than with barrows, but higher than with boars. The level of aggression is considerably

lower in immunocastrates compared to boars. Societal concerns are mainly related to food safety, and are not supported by scientific evidence. After the second vaccination, immunocastrates switch physiologically from a boar-like to a barrow-like status. Therefore, the timing of the second vaccination is a fine-tuning tool to balance advantages of boars with their environmental and economic benefits against the increased risk of welfare problems and boar taint. Nevertheless, both the synergic and the conflicting relationships between the pillars of sustainability must be communicated along the value chain to produce tailored pork products.

The literature analysis revealed that one significant aspect that might lead to a low market acceptance is the reliability of immunocastration. Various studies and experiences of slaughterhouses described the phenomenon of non-responders, immunocastrates which, despite being twice vaccinated with Improvac®, revealed boar taint. The reasons leading to non-responders were unclear, but might be related to management failure (e.g. inappropriate application of the vaccine) or poor antibody response because of stress. Social stress due to unstable group compositions occurs regularly under field conditions, and scientific studies have shown that social stress can impair the immune system. For this reason, we investigated in an experimental study whether different housing conditions, and especially socially unstable group compositions, might lead to non-responders at the time of vaccination. Therefore, the influence of housing conditions on the immune response after two Improvac® vaccinations at an age of 12 and 22 weeks was evaluated. Boars, immunocastrates and barrows (n=48 each) were assigned to three different housing conditions (n=36 enriched, n=36 standard n=72 repeated social mixing). Immune response was quantified by measuring GnRH-binding and its consequences for testosterone concentrations, weight of reproductive organs and boar taint. Growth performance was evaluated via average daily gain (ADG). GnRH-binding and testosterone levels revealed that immunocastration reliably suppressed testicular functions after the 2nd vaccination. Housing conditions did not modify testicular functions, but influenced ADG as animals exposed to mixing grew more slowly than those under enriched conditions. Sex group had an impact on ADG in immunocastrates, who showed a temporarily higher ADG after the 2nd vaccination than boars and barrows. The results show that immunocastration is a reliable procedure under differing housing conditions and competitive in terms of growth performance.

Another aspect that leads to market uncertainty with regard to immunocastration is the pricing of immunocastrates and the question of whether fines for boar taint might be introduced once boars and immunocastrates gain increasing market shares. The

competitiveness of production systems is required to increase their market acceptance. Thus the profitability of pork production with boars and immunocastrates was evaluated under different carcass pricing and penalty systems linked to boar taint. The calculations were based on the performance parameters of 36 animals (n=12 immunocastrates, n=12 boars, n=12 barrows) from the experimental study mentioned above. In order to analyze the economic effects of both alternatives under different regional German production systems, the performance data were set in relation to the data of agri benchmark. Both boars and immunocastrates performed economically worse than barrows in all the scenarios tested. If immunocastrates are sold according to the boar pricing system, the profitability of this technique is even lower, but still more profitable than boar fattening. Pork production with boars is the most unprofitable alternative in this study and will be further devalued if a penalty system linked to boar taint should be introduced.

The present doctoral thesis shows that immunocastration can balance the advantages and disadvantages of pork production with boars or barrows, and thus serve as a sustainable alternative for the European pork chain. If used correctly, immunocastration is reliable in preventing boar taint and can be economically competitive with traditional surgical castration. Based on this thesis, future studies might investigate quality assurance systems that reliably detect non-responders, or animals that are incorrectly vaccinated, before slaughter or at slaughter line. In addition, the economic impact of switching from traditional pork production with barrows to pork production with immunocastrates along the entire pork chain should be further analyzed.

5 ZUSAMMENFASSUNG

Seit Jahrhunderten werden in Europa männliche Ferkel chirurgisch kastriert, um Ebergeruch und eberspezifisches Aggression- und Sexualverhalten zu vermeiden. Die chirurgische Kastration von Eberferkeln erfolgt dabei meistens ohne Anästhesie oder Analgesie und darf so in der Europäischen Union innerhalb der ersten Lebenswoche durchgeführt werden. Diese Praxis wird von der Gesellschaft zunehmende kritisiert, da die Kastration schmerzhaft ist und die körperliche Unversehrtheit der Tiere verletzt. Im Jahr 2010 verpflichteten sich daher europäische Stakeholder der Wertschöpfungskette freiwillig dazu, die chirurgische Ferkelkastration ab 2018 zu beenden. Mehr als zwei Jahre nach dieser Frist, wird die Mehrheit der Eberferkel nach wie vor chirurgisch kastriert, die meisten weiterhin ohne Narkose und Schmerzausschaltung. Die Immunkastration ist eine Alternative zur chirurgischen Kastration und zur Jungebermast. Obwohl diese Technik in Europa seit 2009 für den kommerziellen Gebrauch zugelassen ist, sind die Marktanteile von Immunkastraten auf dem europäischen Schweinefleischmarkt sehr gering. Die Hauptgründe, die zu dieser geringen Marktakzeptanz führen sind Unsicherheiten, ob die Immunkastration zuverlässig und wettbewerbsfähig ist und ob sie von der gesamten Wertschöpfungskette akzeptiert wird. Daher war das Hauptziel der vorliegenden Doktorarbeit zu untersuchen, welche Vor- oder Nachteile sich im Hinblick auf Nachhaltigkeitsaspekte aus der Immunkastration ergeben. Die Analyse verschiedener Nachhaltigkeitsaspekte der Schweinefleischerzeugung mit Immunkastraten wurde in einem Review zusammengefasst. Zusätzlich wurde in einem experimentellen Ansatz der Einfluss verschiedener Haltungsbedingungen auf die Zuverlässigkeit der Immunkastration untersucht. In dieser Studie wurde zudem der Einfluss der Haltungsbedingungen und des Gonadenstatus auf die Wachstumsleistung männlicher Schweine erfasst. Basierend auf den Leistungsdaten dieser Studie wurde außerdem die Rentabilität der Mast von Immunkastraten, chirurgischen Kastraten und Jungebern mit einem Simulationsmodell für deutsche Betriebe unter Verwendung verschiedener Schlachtkörperpreissysteme für Immunkastraten und Risikoszenarien für Ebergeruch analysiert. Die Fragestellungen führten zu drei referierten wissenschaftlichen Publikationen deren wichtigsten Ergebnisse im Folgenden zusammengefasst sind.

Die Immunkastration ist eine aktive Immunisierung gegen GnRH und besteht aus zwei aufeinanderfolgenden Impfungen zur Induktion von Antikörpern, die die Hodenfunktionen vorübergehend unterdrücken und damit die Bildung von Ebergeruch sowie der

Hodenhormone verhindern. Es handelt sich um eine Methode, die sowohl eine hohe Produktqualität als auch ein hohes Maß an Tierschutz gewährleistet. Die Auswirkungen der Immunkastration auf die drei Säulen der Nachhaltigkeit sind bereits ausführlich untersucht worden, jedoch fehlte ein aktueller globaler Überblick über verschiedene Nachhaltigkeitsaspekte des Verfahrens. Durch die Hodenhormonbildung vor der zweiten Impfung ist die Mastleistung von Immunkastraten – insbesondere die Futterverwertung – besser als die von Börgen, aber schlechter als die von Ebern. Die Umweltbelastung bei der Mast mit Immunkastraten ist geringer als bei Börgen, aber höher als bei Ebern. Das Aggressionspotential ist bei Immunkastraten nach der zweiten Impfung im Vergleich zu Ebern wesentlich geringer. Verbraucherbedenken hinsichtlich der Erzeugung von Schweinefleisch mit Immunkastraten beziehen sich hauptsächlich auf die Lebensmittelsicherheit. Solche Bedenken sind aus wissenschaftlicher Sicht unbegründet, da die Unbedenklichkeit des Verzehrs von Fleisch aus dieser Produktion belegt ist. Vor der zweiten Impfung sind Immunkastraten aus physiologischer Sicht wie Eber. Daher ist der Zeitpunkt der zweiten Impfung ein Instrument zur Feinsteuerung, um die ökologischen und wirtschaftlichen Vorteile von Ebern partiell zu nutzen, ohne das erhöhte Risiko von Tierschutzproblemen und Ebergeruch einzugehen. Dennoch müssen sowohl synergistische als auch antagonistische Beziehungen zwischen den Säulen der Nachhaltigkeit entlang der Wertschöpfungskette kommuniziert werden, um marktorientierte Schweinefleischprodukte anzubieten.

Die Analyse der Literatur ergab, dass ein Aspekt, der aktuell zu einer geringen Marktakzeptanz führt, die Zuverlässigkeit der Immunkastration ist. Verschiedene Studien und Erfahrungen von Schlachthöfen beschreiben das Phänomen der Impfversager (Non-Responder), also von Immunkastraten, die trotz zweimaliger Impfung mit Improvac® Ebergeruch aufweisen. Die Gründe, die zu Non-Respondern führten, waren unklar, könnten aber mit Fehlern im Handling (z.B. unkorrekte Anwendung der Impfung) oder einer schlechten Antikörperreaktion aufgrund von Stress zusammenhängen. Sozialer Stress durch instabile Gruppenzusammensetzung tritt unter Praxisbedingungen regelmäßig auf und wissenschaftliche Studien haben gezeigt, dass sozialer Stress das Immunsystem beeinträchtigen kann. Aus diesem Grund wurde in einer experimentellen Studie untersucht, ob unterschiedliche Haltungsbedingungen und insbesondere sozial instabile Gruppenzusammensetzungen zum Zeitpunkt der Impfungen zu Non-Respondern führen. Daher wurde der Einfluss der Haltungsbedingungen auf die Immunreaktion nach zwei Improvac®-Impfungen im Alter von 12 bzw. 22 Wochen untersucht. Eber, Immunkastraten und Börge (jeweils n=48) wurden drei verschiedenen Haltungsbedingungen zugeordnet (n=36 angereichert entsprechend EU-Öko-Verordnung, n=36 Standard, n=72 belastende Haltungsbedingungen durch wiederholtes soziales Mixing). Die Immunreaktion wurde durch Messung der GnRH-Bindung und der Konsequenzen für die Testosteronkonzentration sowie das Gewicht des Genitaltraktes und die Konzentrationen der Komponenten, die den Ebergeruch verursachen, quantifiziert. Die Wachstumsleistung wurde durch die durchschnittlichen täglichen Zunahmen charakterisiert. Die erhöhte GnRH-Bindung und die niedrigen Testosteronkonzentrationen (vergleichbar mit Börgen) zeigten, dass die Immunkastration die Hodenfunktionen zuverlässig unterdrückte. Die Haltungsbedingungen hatten keinen Einfluss auf die Immunreaktion, aber sie beeinflussten die täglichen Zunahmen, da die Tiere der Mixing-Gruppe geringere Wachstumsleistungen aufwiesen als Tiere die unter angereicherten Bedingungen gehalten wurden. Der Kastrationsstatus hatte einen Einfluss auf die Wachstumsleistungen, da Immunkastraten nach der zweiten Impfung höhere tägliche Zunahmen aufwiesen als Eber und Börge. Die Ergebnisse zeigen, dass die Immunkastration unter verschiedenen Haltungsbedingungen zuverlässig funktioniert und in Bezug auf die Mastleistung konkurrenzfähig ist.

Ein weiterer Aspekt, der zu Marktunsicherheiten in Bezug auf die Immunkastration führt, ist die Frage, wie Schlachtkörper von Immunkastraten bepreist werden und ob bei steigenden Marktanteilen von Ebern und Immunkastraten eine Sanktionierung für Ebergeruch am Markt eingeführt wird. Die Wettbewerbsfähigkeit der Jungebermast und der Schweinefleischproduktion mit Immunkastraten ist erforderlich, um die Marktakzeptanz dieser Verfahren zu steigern. Daher wurde die Rentabilität der Schweinefleischproduktion mit Jungebern und Immunkastraten unter verschiedenen Preismasken und Sanktionen für geruchsauffällige Schlachtkörper bewertet. Die Berechnungen basierten auf Leistungsparametern von 36 Tieren (n=12 Immunkastraten, n=12 Eber, n=12 Börge) aus der oben erwähnten experimentellen Studie. Um die wirtschaftlichen Auswirkungen der beiden Alternativen unter verschiedenen regionalen deutschen Produktionssystemen zu analysieren, wurden die Versuchsdaten mit einem ökonomischen Datensatz von agri benchmark vernetzt. Sowohl Eber als auch Immunkastraten schnitten in allen getesteten Szenarien wirtschaftlich schlechter ab als Börge. Werden Immunkastraten nach der Ebermaske bepreist, ist die Rentabilität dieses Verfahrens noch geringer, aber immer noch profitabler als die Jungebermast. Die Schweinefleischproduktion mit Jungebern ist in dieser Studie die unrentabelste Alternative und wird weiter abgewertet, wenn Ebergeruch am Markt sanktioniert wird.

Die vorliegende Doktorarbeit zeigt, dass der Ersatz der chirurgischen Ferkelkastration durch die Immunkastration die Vorteile der Jungebermast und der Mast von Börgen partiell vereint und somit eine nachhaltige Alternative für die europäische Schweinefleischerzeugung darstellen kann. Bei korrekter Anwendung ist die Immunkastration zuverlässig bei der Verhinderung von Ebergeruch und kann wirtschaftlich mit der traditionellen chirurgischen Kastration konkurrieren. Ausgehend von dieser Doktorarbeit könnten zukünftige Studien Qualitätssicherungssysteme untersuchen, die zuverlässig Non-Responder oder falsch geimpfte Tiere vor der Schlachtung oder am Schlachtband detektieren. Darüber hinaus sollten die wirtschaftlichen Auswirkungen der Umstellung von der chirurgischen Kastration auf die Mast von Immunkastraten auf die gesamte Wertschöpfungskette analysiert werden.

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Stuttgart, 18.02.2020

Ort, Datum

Kevin Benjamin Kress

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List of Peer-Reviewed Publications

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ORAL PRESENTATIONS AT INTERNATIONAL SCIENTIFIC MEETINGS

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POSTER PRESENTATIONS AT INTERNATIONAL SCIENTIFIC MEETINGS

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Wiesner, L.; Kress, K.; Weiler, U.; Stefanski, V. Welfare of Entire Males, Immunocastrates and Surgical Castrated Pigs in Socially Unstable Groups. *70th Annual Meeting of the European Federation of Animal Science (EAAP)*, Ghent, Belgium, August 29, **2019**.

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Škrlep, M.; Pokluka, K.; Batorek Lukač, N.; Kress, K.; Čandek-Potokar, M. Myofibrillar fragmentation in entire male, immunocastrated and surgically castrated pigs. *60th International Meat Industry Conference*, Kopaonik, Serbia, September 24, **2019**.

CURRICULUM VITAE

PERSONAL DATA

Name:	Kevin Benjamin Kress
Date of Birth:	25.10.1988
Place of Birth:	Karlsruhe, Germany
Nationality:	German

PROFFESIONAL EXPERIENCES

Since 07/2017	University of Hohenheim – Institute of Animal Science – Behavioral Physiology of Livestock | Research Assistant and PhD Candidate | Stuttgart – Germany
05/2016 – 06/2017	Pestalozzi Kinder- und Jugenddorf Wahlwies e.V. | Head of Production and Marketing of Organic Food | Stockach – Germany
06/2015 – 11/2015	University of Hohenheim – Institute of Animal Science – Behavioral Physiology of Livestock | Student Research Assistant | Stuttgart – Germany
12/2013 – 04/2015	Blättchen Financial Advisory GmbH | Equity & Debt Capital Markets – Small & Mid Cap’s | Working Student | Leonberg - Germany
07/2014 – 12/2014	University of Hohenheim – Institute of Agricultural Policy and Markets – Agricultural Markets and Marketing | Student Research Assistant | Stuttgart – Germany
04/2011 – 07/2011	UBS Deutschland AG | Private and Institutional Wealth Management | Internship | Wiesbaden / Frankfurt am Main - Germany
10/2010 – 03/2011	MIBAV Consulting KG | Business Consultancy | Project Manager | Cologne – Germany
09/2008 – 05/2009	Bayerisches Rotes Kreuz KdöR | Haus der Senioren | Civilian Service | Oberstdorf – Germany

EDUCATION

04/2013 – 04/2016	University of Hohenheim | M.Sc. Agribusiness | Stuttgart – Germany
09/2009 – 03/2013	University of Cologne | B.Sc. Economics | Cologne – Germany
08/2010	University of Basel | Summer School – International Financial Regulation | Basel – Switzerland
09/2000 – 06/2008	Ludwig-Marum Gymnasium | Abitur | Karlsruhe – Germany

FURTHER EDUCATION

Sensory Evaluation of Boar Taint (IPEMA Training School – CA15215) – 2018 | FELASA (Category B) – 2018 | Harmonisation of methods in entire male and immunocastrate research (IPEMA Training School – CA15215) – 2017 | TÜV Quality Management Specialist (HACCP) - 2013 | Hunting License - 2012 | Techniques of Scientific Working for Economists - 2010 | Basic Level Ski Instructor - 2010 | Microsoft Excel - 2010 | Business English Course - 2010

PUBLICATIONS

Weiler, U.; Isernhagen, M.; Stefanski, V.; Ritzmann, M.; Kress, K.; Hein, C.; Zöls, S. Penile Injuries in Wild and Domestic Pigs. *Animals* **2016**, 6, 25.

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Kress, K. Fleischkonsum - Eine Frage von Gesundheit und Haltung. *Sendung Marktplatz – Deutschlandfunk*, Berlin, Germany, January 24, **2019**.

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Stuttgart, 18.02.2020

Place, Date

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