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Pregledni članak
Review article

TEORETICAL AND PRACTICAL ASPECTS OF IMMUNOCASTRATION

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Summary

Due to animal welfare problems and strong public opposition a ban on surgical castration of male piglets is foreseen in EU until the end of 2018. To cope with this major change in pig production, intensive research of possible alternatives is carried out, in particular fattening of entire (EM) and immunocastrated (IC) males. Present publication is focused on the immunocastration; its physiological principles and impact on metabolism, growth performance, carcass and meat quality, animal welfare, economics and public acceptability. Although current trends indicate that rearing of EM is more likely to become a regular practice for the provision of fresh meat market, immunocastration offers a good alternative, especially interesting for fattening pigs to higher age and weight (e.g. more extensive systems) due to the efficient elimination of boar taint and advantages in animal welfare and meat quality.

Key words: pigs, immunocastration, welfare, performance, meat quality, public acceptance.

1. Introduction

Castration of male piglets is a standard practice in pig production worldwide. European Union (EU) currently allows surgical castration to be performed without analgesia/anaesthesia within first week after birth of piglet (Council Directive 2008). The main reason for the castration is to prevent the development of boar taint – an unpleasant odour of meat rejected by the majority of consumers (Weiler et al., 2000). Its formation is mainly defined by androstenone and skatole (Patterson, 1968; Walstra and Mars, 1970). Androstenone is a testicular steroid (with no anabolic effects) which has a distinctive urine-like odour. It is produced by the Leydig cells of the testes of sexually mature males. Due to its lipophilic character it accumulates in adipose tissue in much higher concentrations than other steroid hormones (Gower, 1972). Androstenoneis secreted through saliva and serves as a pheromone to promote sexual behaviour in sows. On the opposite, skatole has no known physiological function. It is toxic for most of the animals, but pigs are relatively resistant to it. Skatole is a product of bacterial degradation of the amino acid tryptophan in the large intestine. It is partly excreted through faeces, while the rest is absorbed in the blood and metabolised in the liver (Zamaratskaia and Squires, 2009). Its hepatic metabolism is inhibited by steroid hormones (also androstenone). As a result, the increased concentrations of androstenone result in higher levels of skatole (Doran et al., 2006). Due to its lipophilic nature, skatole also accumulates in the adipose tissue and causes faeces-like smell of the meat. With regard to the castration of piglets, major changes are foreseen in the EU. Castration of piglets as practiced nowadays without analgesia/anaesthesia is exposed to growing public criticism due to the induced pain (McGlone et al., 1993; Prunier et al., 2006).

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Actions have already been initiated in the EU to reach a ban on such practice[1]. At the same time, intensive research is carried out on the alternatives to surgical castration, diagnostics of boar taint and harmonization of analytical methods[2], supported by European Comission.

2. Alternatives to surgical castration

Possible alternatives to surgical castration as practiced nowadays are a) surgical castration with the use of anaesthesia and analgesia, b) sperm sorting and rearing of female pigs, c) rearing of entire males, and d) immunocastration. Rearing of entire males and immunocastration are the two most practical, short-term solutions, likely to prevail. Present paper focuses on the immunocastration, which has also been the objective of our studies in the last few years.

Surgical castration without anaesthesia and analgesia is currently allowed in newly born piglets until the age of 1 week. It consists of (after fixation of the piglet) cutting of the scrotum with a sharp blade, testicles are then drawn out and removed by cutting of spermatic cord. The castration is multipurpose; it prevents boar taint, excessive aggressiveness and sexual behaviour. For the piglets the procedure is painful and stressful (Marx et al., 2003; Rault et al., 2011). Additionally, post-surgical complications can include haemorrhages, excessive swelling, open wound increases the possibility for bacterial infections. From the productivity point of view, in later fattening phase, castrated animals have inferior production results (higher feed intake, increased carcass fatness) compared to entire males (Prunier et al., 2006).

Alternatives to surgical castration are:

a) Surgical castration with the use of anaesthesia and analgesia uses pharmacologic agents during the procedure to prevent and reduce the pain. It is possible to use the local anaesthetic (with application under the skin, in testes or spermatic cord) or the use of general anaesthesia (usually CO_2). Despite reducing the pain the procedure has several disadvantages. It is laborious, expensive (pharmacological agents, instruments), complicated (especially for the breeder) and does not ensure the control over efficiency of reducing the pain (de Roestet al., 2009).
b) *Sperm sorting* is a method for separation of male and female gametes based on the difference in electric charge. It gives the possibility to rear females only. At present, the method is technologically poorly developed and thus unsuitable for commercial use in pig breeding (Hofmo, 2006), especially as, compared to other species, boar's sperm is very sensitive for manipulation.
c) *Rearing of entire males (boars)* is already a common practice. In some EU countries such as Great Britain, Ireland (also Spain and Portugal) boars are slaughtered before they reach puberty. In future a genetic selection of animals with low boar taint seems realistic. Due to the initiative rearing of boars is being gradually introduced in some pig production chains (e.g. in the Netherlands, Germany, Belgium and France). From the economic point of view, fattening of entire males is more cost effective compared to castrates (no costs for castration, no loss due to the infections of castration wounds, better feed conversion and improved lean meat percentage). However, due to higher level of aggressiveness and sexual behaviour, rearing of

[1]http://ec.europa.eu/food/animal/welfare/farm/initiatives_en.htm; http://boars2018.com
[2]http://eur-lex.europa.eu/ legalcontent/EN/TXT/? uri = uriserv: OJ.C_.2011.243.01.0005 .01. ENG

boars is more laborious for farmers and animals. Other weaknesses are the possibility to develop boar taint (which is rejected by the majority of consumers), lower meat quality (Pauly et al., 2012) and inferior suitability for processing of dry-cured meat products (Bonneau, 1998; de Roestat al., 2009).

d) *Immunocastration,* a method which blocks the testicular function by inducing an immune response against gonadotropin releasing hormone (GnRH) and consequently prevents development of boar taint.

3. Immunocastration

Immunocastration is based on the vaccination against gonadotropin releasing hormone (GnRH). The method uses natural immune system of the animal to achieve the effects of surgical castration. Male reproductive activity is controlled by mutual function of three main endocrine glands: the hypothalamus, the pituitary gland and testicles (known also as hipotalamic-pituitary-gonadal axis). GnRH, which is secreted from hypothalamus, passes through the portal veins to the pituitary gland where it stimulates releasing of follicle stimulating (FSH) and luteinizing (LH) hormone whose target organs are testicles. FSH is known to stimulate the development of male gametes (spermatogenesis) and LH stimulates the secretion of steroid hormones (androgens, estrogens and androstens), which are essential for normal reproductive function, but also affect metabolism, behaviour and development of the sexual odour. The first studies on immunocastration were performed more than twenty years ago (Caraty and Bonneau, 1986; Falvo et al., 1986; Awoniyi et al., 1988), but were not suitable for practical use due to the utilization of strong adjuvants. Commercial vaccine (Improvac®) was first introduced in Australia and New Zealand in the nineties. Currently, the vaccine is registered in more than 50 countries around the world, including Brazil (where it is used the most extensively), Switzerland and EU countries (since 2009).

The vaccine itself does not contain the hormone only its physiologically inactive substitute, bound to a protein carrier, which triggers the formation of antibodies against endogenous GnRH. Antibodies neutralize the hormone and thereby break the stimulation of the HHG (hypothalamus-hypophysis-gonades) axis. This prevents the formation of gonadal steroid hormones, causes regression of reproductive organs and some metabolic changes, which ultimately leads to changes in behaviour (reduced aggression, increased appetite and feed intake), body composition (higher fatness, faster growth) and most importantly, eliminates androstenone formation and consequently boar taint. Effect of immunocastration is comparable to surgical castration, where similar effects are achieved by physically removing the testes. To reach the proper results the vaccine had to be applied twice with a minimum interval of four weeks. Although there is no withdrawal period, vaccine producer recommends to perform the second vaccination at least four to six weeks before slaughter[3] to be sure that all substances of boar taint are eliminated from the adipose tissue. Fear of negative public opinion, the risk of autoimmunization (the same effect in humans) can be considered as disadvantages of immunocastration.

[3]http://www.improvac.co.nz /sites/improvac/en-NZ/Pages/technicalinformation.aspx

3.1. Physiological response to immunocastration (reproduction organs, boar taint)

Immunocastration is highly effective in elimination of boar taint. In most animal species the effect of vaccine is observed soon after the revaccination. Within a week after the treatment, a rapid increase of antibodies against GnRH occurs which causes, together with breakdown of HHG axis, a fast decrease of gonadal steroids including plasma androstenone (Claus et al., 2007). A complete clearence of boar taint requires a longer period. According to vaccine producer, a minimum of four weeks between second treatment and slaughter is recommended. Recent studies (Lealiifano et al., 2011; Kubale et al., 2013) demonstrated a decline of androstenone and skatole in fat tissue below sensory detection already two weeks after the successful immunization. However, these results were obtained on pigs slaughtered under commercial conditions at the age of about 6 months (*i.e*. pubertal animals) and it is thus impossible to give firm conclusions about the time needed for complete elimination of boar taint in adult animals. The decrease of steroid hormones is reflected in size reduction of testes and accessory glands (Bonneau, 2010). The immunization has the strongest effect on vesicular gland followed by testes and bulbourethral gland. Changes on histological level concern theatrophy of Leydig cells and germinative epithelium, cessation of spermatogenesis (testes), reduced size of glandular acini and secretion of accessory glands (Kubale et al., 2013). According to the literature, the effect of immunocastration persists up to 22 week (Zamaratskaia et al., 2008); however, it seems that it does not have a permanent effect. Studies show progressive restoration of testicular activity and increase of androstenone level after 12 weeks (Claus et al. 2008). According to Einarsson et al. (2009) irreversible loss of reproductive ability is most likely associated with earlier vaccination. It should be noted that some animals do not react to immunization (*i.e*. non-responders) which has been shown in some foreign (Zeng et al., 2002 Jaros et al., 2005), as well as in our studies (Škrlep et al., 2012a; Kubale et al., 2013). The number of non-responders is relatively small (1-3%) and is similar to the number of cryptorchids in case of surgical castration. The reasons can be poor immunological response or technically improperly performed vaccination. Non-responders are basically entire males and can develop boar taint. Whether immunocastration or rearing of entire males is employed in practice, a control system for the detection of boar taint on slaughter line needs to be established. Some producers rely on olfaction of adipose tissue by trained assessors (the method called "human nose") after being heated with a hot iron (Mathur et al., 2012; Bekaert et al., 2013). To recognize the unsuccessfully immunized animals at slaughter line fast and simple but effective method is needed. The estimation based on the size of testicles is not certain; for a more reliable prediction, it is better to assess thesize of accessory glands (Bonneau, 2010; Čandek-Potokar et al., 2014).

3.2. The effect of immunocastration on productivity, growth performance and carcass quality

Results of the meta-analysis (Batorek et al., 2012a) showed that when taking into consideration the entire fattening period (from first vaccination to slaughter) immunocastrates (IC) grow faster than surgical castrates (SC) and even faster than boars (EM), but in the same time consume more feed than EM and less than SC. Regarding the feed conversion ratio, the IC proved to be superior to SC and only somewhat lower than EM. The reason for such productivity of IC lies in the fact that these animals are physiologically equal to boars until the second (i.e. effective) vaccination and therefore exploit boar-like growth potential. After the second vaccination there are

rapid changes in the hormonal status, particularly a drop in the levels of steroids. At the same time, concentrations of residual IGF-1 and somatotropin remain on the relatively high level, whereas serum leptine does not reach the values as high as in SC (Batorek et al, 2012b; Kubale et al., 2013), resulting in higher feed intake and growth rate. A detailed metabolic research (Batorek et al., 2013a) revealed that after efficient immunization the IC increase fat tissue deposition at the expense of lower heat production, while protein deposition remains similar to EM. Contrary to IC, SC deposit fat instead of protein (i.e. muscles). It is however worth noting that the mentioned studies refer to the so-called "late" immunocastration, where the first vaccination is performed at the start of the fattening period and the second vaccination very late, usually 4-6 weeks prior to slaughter. Published studies, dealing with "early" immunocastration, are rare, as such practice is not economically interesting. In general, the level of fat deposition in IC depends on the timing of immunisation. The longer the time that elapses between the second vaccination and slaughter, the higher is the fat deposition, as demonstrated by Turkstra et al. (2002), Lealiifano et al. (2011) and Škrlep et al. (2012b). Although moderate degree of fatness (especially intramuscular) seems favourable for meat sensory quality, it negatively influences the economics of rearing (higher fatness leads to lower lean meat %, governing the carcass price). Based on 30 available studies the meta-analysis of Batorek et al. (2012a) showed that compared to EM, IC pigs exhibit greater backfat thickness, resulting in lower carcass lean meat percentage. Concerning carcass prime cuts, IC seem to resemble EM, except for belly weight (higher in IC). On the other hand, a comparison of IC to SC shows advantages of the former (lower carcass fatness, heavier ham and shoulder). One of the efficient ways to control fat deposition in IC after the second vaccination is the manipulation of the pig diet. Our recent research has shown that the restricted feed intake (80% of *ad libitum* intake, Batorek et al., 2012b) or energy dilution (5 or 9%, Batorek et al, 2013b) improves carcass leanness due to lower fat deposition.

3.3. The effect of immunocastration on meat quality

As regards meat quality, the results of meta-analysis show that for the most part IC are superior to EM (Batorek et al., 2012a). They exhibit higher levels of intramuscular fat and higher meat tenderness, which is beneficial for sensory quality; however, a tendency of lower water holding capacity was also noted. On the other hand, meta-analysis did not find any major differences between IC and SC.

According to the trends in EU, the most likely alternative to be implemented after the ban on surgical castration is rearing of EM. Besides their inferior meat quality (i.e. lower IMF and tenderness, inferior WHC compared to surgical castrates; Pauly et al, 2012), entire males need to be slaughtered at younger age to minimise the occurrence of boar taint. Such drawbacks make the meat of EM meat less suitable for processing into seasoned/dry-cured products, where raw material of specific quality is required (Čandek-Potokar and Škrlep, 2012). Although there is a considerable lack of information/literature regarding this topic, the use of immunocastration could prove beneficial in the case of fattening to higher age or weight intended to get raw material for high-quality meat products (also in more extensive conditions like free range, organic farming). Available studies (Boleret al., 2011; Font i Furnols et al., 2012) which evaluated the suitability of IC for dry-cured ham processing, concluded that IC are similar to SC in regard to meat and fat quality (including quantity and fatty acid composition). In our recent study (Škrlep et al.,

2014a, b) green hams originating from IC and EM were compared for dry-cured ham; it was concluded that IC hams were more suitable due to lower seasoning losses, lower salt intake and softer end product with more intramuscular fat. Despite androstenone and skatole levels being above what is considered as threshold for sensory perception, there were no differences observed between IC and EM in foreign or off tastes (data not yet published).

3.4. The effect of immunocastration on animal welfare

Immunocastration is considered as a relatively welfare friendly alternative. Compared to surgical castration without anaesthesia, it excludes acute pain associated to the procedure, and the pain is limited only to the needle insertion during the application of the vaccine (Prunier et al., 2006). Study of Einarsson (2006) showed that vaccinated animals did not develop any visible skin reactions or these were only transient ones. Compared to EM, immunocastration reduces aggressive and sexual/mounting behaviour in the period, when this normally starts to occur (***i.e*** at the age of 5-6 months). Soon after the effective immunisation, the behaviour of IC becomes similar as in SC; IC reduce aggressive and mounting behaviour, while increasing SC-like feeding behaviour (Cronin et al., 2003; Zamaratskaia et al., 2008; Rydhmer et al., 2010). Calmer behaviour is important for carcass quality because it is related to lower incidence of skin lesions, a consequence of fighting and mounting prior to slaughter (transport, lairage). In this period the animals are often mixed which triggers aggression related to reestablishment of hierarchy between pigs (Bolhuis et al., 2005; Turner et al., 2006). Our recent research (Škrlep et al., 2011) showed IC intermediate between EM and SC regarding the incidence of skin lesions caused by teeth (i.e. direct aggression). Another study, dealing with restrictive feeding of immunocastrates (Batorek et al, 2012b) pointed out a negative aspect of such practice. Restrictively fed IC exhibited similar level of aggression (i.e incidence of carcass skin lesions) as EM, whereas IC fed *ad libitum* did not show any difference compared to SC.

3.5. Economics of immunocastration

The costs associated with the immunocastration include the price of the vaccine and the expenses of extra labour at vaccination. However, these costs are compensated by more efficient feed utilisation, faster growth and leaner carcasses (as compared to SC). According to de Roest et al. (2009), direct costs of immunocastration are 3.00–3.65 EUR per pig. This does not include the costs ofscreening for non-responders on the slaughter line or the loss of income due to possible boar taint presence and additional carcass trimming (remains of testicles). On the other hand, benefits can be expected from better growth and feed conversion (as compared to SC). According to Hennessy (2006) IC are superior to SC (depending on the experiment) in performance e.g. 7.7% to 16.9% better feed conversion ratio, 3.5% to 10.6% higher weight gain. Overall economic merit of immunocastration is however variable according to the country, depending on efficiency, labour price, ranging from -0.02 EUR per kg carcass (Italy) to +0.007 EUR per kg carcass (Denmark). Research on Slovenian commercial pig fatteners has also shown benefits of IC over SC in terms of performance. For the entire fattening period (12 to 24 weeks of age) 7.5% better feed conversion ratio was observed in the case of Duroc crosses (Škrlep et al., 2010), whereas in the case of Pietrain crosses (Batorek et al., 2012b) feed conversion ratio was improved for 18% in IC compared to SC.

3.6. Immunocastration and public acceptability

In PIGCAS project survey (Bonneau et al., 2009) the stakeholders (breeders, meat processing industry, governmental bodies, consumers and animal welfare organisations) rated low the prospects of the immunocastration, giving priority to surgical castration with anaesthesia/analgesia. According to this study, one of the main drawbacks is the fear from negative public response. However, public opinion about immunocastration has been poorly investigated. As shown by some available studies (cited latter on), in general, the consumers are not well informed about boar taint and the methods used in rearing to avoid it. According to the literature, consumers expect meat to be safe, healthy and tasty. Thus boar taint can represent a potential problem. As mentioned, studies on the acceptability of immunocastration are relatively scarce; however, the existing ones show differences between European consumers. For the majority of consumers in Switzerland, the most acceptable alternative was the use of surgical castration with anaesthesia, whereas immunocastration was disfavoured (Huber-Eicher et al., 2011). On the opposite, Swedish consumers evaluated immunocastration more positively than rearing of entire males or standard surgical castration (Lagerkvist et al., 2006). Belgian consumers favoured immunocastration to surgical castration, after being well informed about all alternatives (Tuyttens et al., 2011). Extensive research, which included over 4000 participants from France, Germany and the Netherlands (Vanhonacker and Verbeke, 2011) showed that the fear of negative response to immunocastration might be overestimated, since the method was acceptable for more than 70% of the respondents. It should be mentioned, that immunocastration is acceptable for non-governmental organisations for animal welfare, although they give priority to rearing of EM. According to the PIGCAS project survey, scientific experts perceive immunocastration as much better alternative than the SC with anaesthesia/analgesia (Edwards et al, 2009). According to them it improves animal welfare, is more economical and easier to put in practice.

4. Conclusions

Immunocastration provides a solution to majority of drawbacks including economic yield, meat sensory quality and animal welfare. On the other side, fear of public reaction remains its strongest weak point, despite several encouraging results from the latest consumer studies. According to current trends in the EU, the rearing of entire males is more likely to become a predominant practice, at least for supplying the market of fresh meat. It looks that immunocastration will not be largely used, because it is less practical for farmers than rearing of entire males. On the other hand, it can be convenient, when higher quality of meat is required (i.e. rearing to higher weight or age, for dry-curing, with boar meat being less suitable), or in the case of castration of culled breeding boars, as a more welfare friendly and economically favourable solution.

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