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Original Scientific Article

EFFECT OF IMMUNOCASTRATION (IMPROVAC®) IN FATTENING PIGS I: GROWTH PERFORMANCE, REPRODUCTIVE ORGANS AND MALODOROUS COMPOUNDS

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Summary: The effect of the immunocastration (vaccination against gonadotropin releasing hormone using Improvac® vaccine) on growth performance, size of reproductive organs and levels of fat androstenone and skatole was studied in Slovenian pig fatteners. The pigs (50% crosses of Duroc), selected from 35 litters (2 castrates and 3-4 boars per litter) farrowed within two weeks period, were assigned to three experimental groups: boars (n=25), immunocastrated males (n=24) and surgically castrated males (n=25). The vaccinations with Improvac® were performed at the age of 10 and 19 weeks. The trial started when pigs were 12 weeks old, and lasted until 24 weeks of age, when pigs were slaughtered. During the trial, pigs were lodged individually and their daily feed intake (*ad libidum*) and weight (at 12, 19 and 24 weeks of age) were recorded. At the slaughter line, the testes and accessory reproductive glands were removed and weighed. Subcutaneous fat samples were taken for the determination of androstenone and skatole concentration. Results on androstenone and skatole showed that the immunisation was 100% successful. The levels of androstenone were below a detection limit of the laboratory method in all pigs. For skatole, the concentrations were at comparably low level in surgically and immunocastrated males, whereas higher levels were observed in boars. Vaccination strongly reduced the weight of testes and accessory reproductive glands. Regarding growth performance, the immunocastrated males showed comparable feed intake, feed efficiency and growth rate as boars in the period from the start of the experiment until the revaccination; thereafter they were closer to the surgical castrates. In the present study, the beneficial impact of the immunocastration on pig productivity and on the reduction of substances responsible for boar taint was confirmed in Slovenian local conditions.

Key words: pig; immunocastration; growth performance; androstenone; skatole

Introduction

Surgical castration is routinely practiced in male pigs destined for fattening. The aim is to prevent, an unpleasant off-flavour which develops in meat of sexually mature entire males and results in rejection of such meat by the consumers (1). The two main compounds held responsible for boar taint are androstenone (2) and skatole (3). Androstenone is a testicular steroid producing a urine-like smell, while skatole has a faecal-like odour and is a by product of bacterial metabolism of the tryptophan in the intestine. Although the origin of these two compounds is different, the elevated level of androstenone is accompanied by the elevated level of skatole, the correlation coefficient being around 0.3 (4). It seems that their metabolic pathways are interdependent, and it has been hypothesised that androstenone, along with other testicular steroids, affects the metabolism of skatole in the liver (5). Surgical castration has also a positive impact since castrated pigs

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exhibit less aggression and sexual behavior (mounting). However, surgical castration has also drawbacks. Fattening of castrates compared to the entire males is economically less efficient due to their lower feed efficiency and carcass leanness. Surgical castration without anaesthesia has also been criticized from the animal welfare viewpoint (6). A ban on surgical castration without anaesthesia is under consideration by the EU and other European countries. Therefore, the alternative methods for boar taint prevention are of prime interest. One of them is immunocastration, a non-surgical castration by vaccination in which male pigs are actively immunized against GnRH (gonadotropin-releasing hormone). It is a more welfare-friendly alternative to surgical castration without anaesthesia (7). Currently this method is extensively used in a number of countries, including Australia, New Zealand and Brazil. In Europe its use has been approved in Switzerland in 2008 and in the EU in 2009. Immunocastration reduces the weight of testes and accessory sex glands (8) and the production of the substances (androstenone and skatole) responsible for boar taint (9). Moreover, immunocastrated pigs have been shown to exhibit better growth performance as surgical castrates (10).

Due to the recent approval of the vaccine Improvac® in the EU, the tests in local conditions are currently going on in many European countries. The objective of the present study was to test the effectiveness of the vaccine on growth performance, weight of the reproductive organs and fat androstenone and skatole levels in pigs fattened in the controlled environment (individually housing with *ad libidum* feeding) for one of the main commercial hybrids in Slovenia.

Material and methods

Animals and fattening trial

The pigs used in the present study were 50% Duroc crosses raised on commercial pig farm. For the experiment, the pigs were selected from 35 litters farrowed within two weeks period. Pigs (2 surgical castrates and 3-4 boars per litter) were individually marked (with plastic mark and with a tattoo). At the age of ten weeks they were transferred to the experimental stable and individually housed. At that time the first vaccination against GnRH (2 ml of Improvac® vaccine per animal, Pfizer Animal Health) was applied by veterinarian and pigs were assigned to three treatment groups: boars (n=25), immunocastrated males (n=24) and surgically castrated males (n=25, due to the illness, one animal from this group was subsequently removed from the experimental dataset). The second vaccination of immunocastrated males (2 ml of vaccine) was performed at 19 weeks of age (5 weeks prior to slaughter). The trial started when pigs reached 12 weeks of age and lasted for 12 weeks. Pigs were individually housed and fed *ad libitum* a commercial diet BEK-1 (Jata Emona d.o.o., Ljubljana, Slovenia) containing 13.0 MJ/kg of metabolisable energy, 17% crude protein, 2.6% crude fat, 4% crude fibre, 6% crude ash and 1% lysine. Feed intake was individually recorded and pigs were weighed at 12, 19 and 24 weeks of age, when they were sent to slaughter. Additionally, daily gain and feed conversion ratio were calculated. Animals were slaughtered in a commercial abattoir according to standard procedure (app. 1 hour transport, 2 hours of lairage, CO_2 stunning). At the slaughter line, testes and accessory male reproductive glands were removed and taken to the laboratory for dissection and weighing.

Skatole and androstenone determination

Samples of subcutaneous fat were taken at the level of last rib and sent to the laboratory (CCL Nutricontrol, Veghel, Netherlands) accredited according to SIST EN 17025 for the determination of androstenone and skatole concentration. Briefly, the fat of the samples (25 g) was liberated by heating the sample in a microwave oven, and the fat extracted with an organic solvent. For skatole determination, the extracts were analysed by HPLC on a reversed phase column with fluorescence detection using an external standard. For androstenone, the fat was extracted using organic solvent with known amount of internal standard of androstenone, and the extracts analysed by GSMS in SIM mode. The lower limit of sensitivity was 0.04 μg androstenone/g fat.

Statistical analysis

One-way analysis of variance (procedure GLM of SAS, SAS Inc., Cary, NC, USA) was used to evaluate the effect of treatment group (*i.e.* surgically castrated, immunocastrated males or boars) on growth performance, weight of the reproductive organs, and skatole and androstenone concentrations. When a significant group effect ($P<0.05$) was detected, means were compared using a *Tukey* test.

Results and discussion

Reproductive organs

Vaccination strongly reduced testes and accessory male reproductive glands (Table 1, Figure 1) in immunocastrated males compared to the entire males (3 to 15 times lighter in case of testes with epididymides and vesicular gland, respectively). In surgical castrates the accessory reproductive glands were even more severely atrophied.

Table 1: Weight of the reproductive organs (mean ± se) in surgically castrated, immunocastrated males and boars

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
Number of animals	24	24	25	
Testes with epididymides, g		223.2 ± 18.5[a]	701.3 ± 38.8[b]	<0.000
Epididymides, g		23.6 ± 1.92[a]	88.9 ± 4.05[b]	<0.000
Bulbo-urethral gland, g	6.9 ± 0.32[a]	36.6 ± 3.34[b]	117.8 ± 6.2[c]	<0.000
Vesicular gland, g	1.7 ± 0.06[a]	12.2 ± 0.76[a]	175.4 ± 13.6[b]	<0.000
Prostate, g	1.3 ± 0.05[a]	2.1 ± 0.12[b]	11.0 ± 0.38[c]	<0.000

Means with different letters within one row are significantly different (P<0.05).

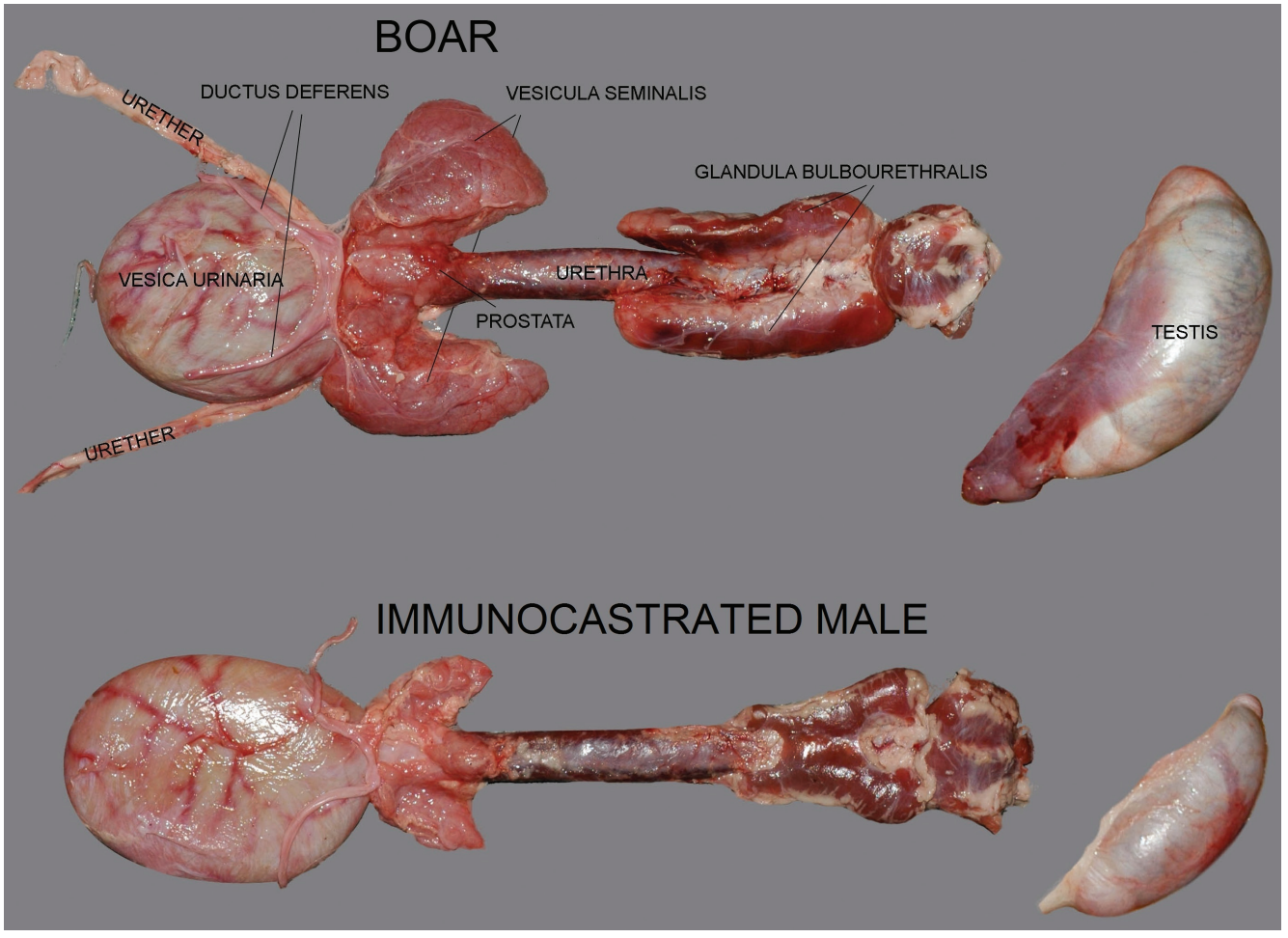

Figure 1: Representative image of testes and accessory reproductive glands belonging to a boar or an immunocastrated male

The effectiveness of anti-GnRH vaccine is known from numerous studies, and for the efficient inhibition of the testes function, two applications of vaccine are needed (9, 10). It was demonstrated (9, 10) that plasma testosterone level (an indicator of testes function) remained unaffected until the second vaccination, whereas at the time of the slaughter (only 15 days later), the level of testosterone was seven- to twenty five-fold lower in immunised pigs, which was also consistent with the rise of anti-GnRH antibody titres at that time. Along with a decline of the androgenic hormone level, the literature acquaints a reduction of reproductive organs. In various studies (8, 9, 10, 11, 12, 13, 14, 15, 16) a drastic reduction was reported for the testes (16% to over 60%), bulbourethral glands (50% to over 90%) and seminal vesicles (36% to over 90%) in immunocastrated males when compared to entire males. The results in the present study show a similar degree of reduction of the reproductive organs as reported in the literature. The difference in the weights reported is, however, greatly related to the onset of the second vaccination and the time elapsed until the slaughter.

Boar taint compounds

Consistent with a reduction of the size of reproductive organs observed in the present experiment, the levels of fat tissue androstenone and skatole were strongly affected (Table 2). The concentration of androstenone in fat was above the laboratory method detection limit (*i.e.* 0.04 μg/g of fat) only in the case of boars. Regarding the skatole, its level was fairly low and not statistically different between surgically castrated and immunocastrated males, whereas its concentration was almost six-fold higher in boars. These results prove the efficiency of vaccination against GnRH. Moreover, we can consider that the vaccination was successful in the prevention of boar taint, since, proposed sensory threshold (4) concentrations of androstenone (0.5 or 1.0 μg/g fat) and skatole (0.2 or 0.25 μg/g fat) were not exceeded in the present experiment. It has been shown, that fat androstenone concentration drops very sharply after the cessation of testes function (17), its half life being in the range of a few days. However, a complete clearance of androstenone from the fat tissue requires a period of at least 3 weeks (18). Recent study (19) interested in the effect of different timing of immunocastration has shown, that a revaccination performed only two weeks prior to slaughter should be sufficient for the prevention of boar taint (*i.e.* decline of androstenone and skatol below threshold levels). In agreement with our results, the effectiveness of the anti-GnRH vaccine has been proven by numerous studies (9, 10, 11, 12, 13, 14, 15). However, the absolute values of the concentrations of malodorous compounds reported are, again, difficult to compare due to the differences in the experimental designs but also due to the differences in analytical procedures which need harmonisation (20).

Table 2: Subcutaneous fat skatole and androstenone concentrations (mean ± se)

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
N	24	24	25	
Skatole (ng/g)	38.2 ± 4.44[a]	39.2 ± 3.08[a]	231.4 ± 76.1[b]	0.006
Androstenone (μg/g)	*bd*	*bd*	1.48 ± 0.19	

bd – below detection limit (0.04 μg/g fat); means with different letters within one row are significantly different (P<0.05).

Growth performance

Results on growth performance (Table 3) show no difference between experimental groups in the average body weight at the beginning of the experiment (12 weeks) and at the time of the revaccination (19 weeks). However, the differences became significant at the end of the experiment, when we could note lower body weight of surgically castrated as compared to the immunocastrated males or boars. As a consequence, we noted lower daily gain for surgically castrated than immunocastrated males or boars, however the differences were insignificant. Feed intake and feed efficiency (considering the whole experimental period from the first vaccination until slaughter) differed between surgically cas-

trated males and boars. Surgically castrated males had higher overall feed intake (10%) and lower feed efficiency (15%) compared to boars. The immunocastrated males took intermediate position in both cases, but were not significantly different from either of the control groups. Considering the separate experimental phases, feed intake and feed efficiency of the immunocastrated males resembled those of boars in the first part of the experiment (until revaccination), whereas after the second vaccination a shift was noted and the immunocastrated males were more similar to the surgical castrates.

Table 3: Body weight, daily gain, feed intake and feed efficiency (mean ± se) in surgically castrated, imunocastrated males and boars, according to the phase of the experiment

	SURGICALLY CASTRATED MALES	IMMUNO-CASTRATED MALES	BOARS	*P*-value
Number of animals	24	24	25	
Body weight, kg				
12 weeks	29.8 ± 0.84	31.6 ± 0.89	31.3 ± 0.89	0.309
19 weeks	82.6 ± 0.87	85.4 ± 1.26	85.6 ± 1.25	0.146
24 weeks	117.1 ± 0.96	121.0 ± 1.37	121.9 ± 1.77	0.049
Daily gain, g/day				
Birth-12 weeks	364 ± 8	385 ± 11	381 ± 10	0.279
12-19 weeks	1010 ± 19	1031 ± 20	1038 ± 20	0.584
19-24 weeks	978 ± 23	1012 ± 23	1034 ± 27	0.273
12-24 weeks	998 ± 13	1022 ± 13	1038 ± 18	0.174
Birth-24 weeks	697 ± 6	719 ± 10	725 ± 12	0.112
Feed intake, kg/day				
12-19 weeks	2.77 ± 0.035^{a}	2.6 ± 0.026^{ab}	2.58 ± 0.065^{b}	0.032
19-24 weeks	3.77 ± 0.058^{a}	3.72 ± 0.090^{a}	3.37 ± 0.077^{b}	<0.000
12-24 weeks	3.21 ± 0.035^{a}	3.06 ± 0.036^{ab}	2.93 ± 0.061^{b}	0.008
Feed efficiency, kg feed/kg gain				
12-19 weeks	2.76 ± 0.062^{a}	2.54 ± 0.026^{b}	2.49 ± 0.036^{b}	<0.000
19-24 weeks	3.89 ± 0.089^{a}	3.72 ± 0.090^{a}	3.28 ± 0.061^{b}	<0.000
12-24 weeks	3.23 ± 0.057^{a}	2.99 ± 0.036^{b}	2.82 ± 0.032^{c}	<0.000

Means with different letters within one row are significantly different (P<0.05); 12 weeks - start of experiment; 19 weeks - revaccination; 24 weeks - end of experiment, when animals were slaughtered.

It is well known that boars exhibit better growth performance in comparison with the surgically castrated males (*i.e.* lower feed intake, higher feed efficiency, higher weight gain), which was reconfirmed in the present study. Concerning growth performance of the immunocastrated males, the literature reports are not completely coherent, which we can ascribe to different experimental conditions. The early experiments (8) demonstrated that immunoneutralization of GnRH in boars resulted in the castration-like effects, without any major influence on growth performance (average daily gain, body weight). Some studies, comparing only the immunocastrated males and surgically castrated males (13, 21, 22) also found no difference in growth rate between surgically and immunocastrated males, although some trends for better average daily weight gain (13, 22) and better overall feed efficiency (21) of the immunocastrated males were observed. Several studies (10, 23, 24, 25) reported Improvac® treated pigs to grow faster and present higher feed intake as boars in the period following a revaccination (besides being also superior to the surgical castrates). This could be related to the relatively high (boar-like) levels of growth hormone in immunocastrated males (26) and the fact that the experimental animals were not housed individually. Namely, boars housed in groups waste more energy due to their social behaviour (aggressiveness, mounting), which could, combined with lower feed consumption, be responsible for lower performance as compared to their castrated counterparts (15, 24, 27, 28). The key reason for the observed differences in the literature is related mainly to the timing of the immuniza-

tion; the longer the time elapsed between the second vaccination and slaughter, the greater the difference between the immunocastrated males and boars. It was, for example, shown (11) that pigs who responded earlier to the anti-GnRH immunisation exhibited growth performance which was comparable to the surgically castrated males, whereas late responders were more similar to the boars. It is only after the second vaccination that an immune reaction with antibody titres against GnRH is elicited (29). As a consequence a production of gonadal steroids is reduced, leading the immunocastrated males to start acting like surgical castrates in regard to the social behaviour, feed consumption and consequently growth performance (24). This is supported by the results of the present study, where the feed intake of immunocastrated males increases (along with a decrease of feed efficiency) from that similar to the boars to the one similar to surgical castrates after the revaccination.

Conclusions

Present study on the individually housed Slovenian commercial fatteners confirmed the efficiency of Improvac® vaccine in regard to the elimination of malodorous compounds. The study also confirmed the benefits of the immunocastrated males as compared to the surgical castrates in growth performance, but mainly feed efficiency.

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UČINEK IMUNOKASTRACIJE (IMPROVAC®) PRI PRAŠIČIH PITANCIH: I. RASTNOST, REPRODUKTIVNI ORGANI IN SUBSTANCE, ODGOVORNE ZA VONJ PO MERJASCU

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Povzetek: Preučevali smo vpliv imunokastracije (cepljenja proti gonadotropin sproščajočemu hormonu s cepivom Improvac®) na rastnost, velikost reproduktivnih organov ter na raven androstenona in skatola v maščobnem tkivu. Poskusni prašiči (50% križanci pasme durok) so bili izbrani iz 35 gnezd (po 2 kastrata in 3-4 merjasci na gnezdo), rojeni v obdobju dveh tednov. Živali smo razdelili v tri poskusne skupine: merjasce (n=25), imunokastrate (n=24) in kirurške kastrate (n=25). Cepljenje z Improvacom ® smo opravili pri starosti 10 in 19 tednov. S poskusom smo pričeli pri starosti 12 tednov in je trajal do 24. tedna starosti, ko so bili prašiči zaklani. Med poskusom so bili prašiči individualno vhlevljeni in so dobivali hrano po volji. Zapisana je bila dnevna poraba krme, prašiči so bili med poskusom tehtani pri 12, 19 in 24 tednih starosti. Na klavni liniji so jim bili odvzeti in stehtani testisi in akcesorne spolne žleze. Dan po zakolu so bili odvzeti vzorci podkožnega maščevja za določitev koncentracije androstenona in skatola. Rezultati so pokazali, da je bilo cepljenje 100% uspešno, saj so bile koncentracije androstenona pod mejo detekcije laboratorijske metode. Koncentracije skatola so bile nizke in primerljive pri kirurških in imunokastratih, medtem ko so bile pri merjascih znatno višje. Cepljenje je močno vplivalo tudi na zmanjšanje teže mod in akcesornih spolnih žlez. Kar zadeva pitovne lastnosti so bili imunokastrati v prvem obdobju (do revakcinacije) po zauživanju in porabi krme ter dnevnem prirastu podobni merjascem, v drugi fazi rasti (po revakcinaciji) pa bolj podobni kirurškim kastratom. Pričujoča raziskava, narejena v slovenskih pogojih prašičereje, je pokazala pozitivne učinke imunokastracije na proizvodne rezultate. Prav tako je bila uspešna v smislu preprečitve tvorbe substanc odgovornih za nastanek vonja mesa po merjascu.

Ključne besede: prašiči; imunokastracija; rastnost; androstenon; skatol