Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality

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ABSTRACT: In the present study, the effect of immunocastration on carcass traits, meat quality, reproductive organs development, and boar taint compounds was investigated. Male piglets (50% Duroc crosses) were randomly assigned to three treatment groups: entire males (EM; n = 19), surgical castrates (SC; n = 20) and immunocastrates (IC, vaccinated with Improvac® at the age of 79 and 142 days; n = 21). Pigs were fed *ad libitum* and weighed at the time of first and second vaccination and before slaughter (176 days of age). No differences between treatment groups were detected for carcass weight. In the case of backfat thickness, carcass lean meat content, and belly leanness score, IC were intermediate between EM (the leanest) and SC (the fattest), differing (P < 0.05) from both control groups. Regarding loin eye fat area, neck intermuscular fatness, ham leanness, and leaf fat weight, IC were similar to EM and were less fat than SC (P < 0.01). IC had lower intramuscular fat than SC (P < 0.01) and higher average pH 24 than both EM and SC (P < 0.01), resulting in darker colour. IC also demonstrated lower drip loss than EM (P < 0.05). Immunocastration caused a significant reduction of reproductive organs and concentrations of boar taint compounds (P < 0.01) which were comparable with the levels observed for SC.

Keywords: pigs; immunocastration; carcass; meat quality; reproductive organs; boar taint

Surgical castration of male animals is a routine and widely used practice in the pig breeding industry. It is aimed at preventing boar taint, an unpleasant smell of the meat attributed to the accumulation of skatole and androstenone in the fatty tissue of sexually mature male pigs (boars), causing negative perception and rejection of such meat by the consumers (Font i Furnols et al., 2003; Bonneau and Squires, 2004). Besides the absence of boar taint, castrates exhibit less aggressive behaviour (Cronin et al., 2003) than boars. There is, however, one important drawback. Due to lower feed efficiency and higher carcass fat deposition, the fattening of castrates is less cost-effective (Xue et al., 1997; de Roest et al., 2009). Moreover, surgical castration without anaesthesia has been severely criticised because of the pain inflicted to the animals during the procedure (Giershing et al., 2006). Several European countries have already prohibited surgical castration without anaesthesia and a gen-

eral ban on castration is envisaged in the EU by the year 2018 (EC declaration, 2010). In view of these changes, immunocastration is one of the possible alternatives. The method is based on vaccination against GnRH (gonadotropin releasing hormone) and may improve animal welfare, since no surgical procedure is needed (Thun et al., 2006). Two vaccinations are needed for a full response, causing reduction of testes and accessory reproductive organs and elimination of boar taint (Dunshea et al., 2001; Cronin et al, 2003; McCauley et al., 2003; Jaros et al., 2005; Zamaratskaia et al., 2008a, b). Notable changes in metabolism and behaviour of animals have also been reported (Cronin et al, 2003; Claus et al., 2007). Immunocastration enables exploiting boar-like growth potential until the second vaccination, when the castration is effective. In the last few years, several studies have investigated the effect of immunocastration in pigs (reviewed by Millet et al., 2011 and Batorek et al., 2012), however, they were mostly focused on growth performance and boar taint elimination, whereas there is much less information on carcass and meat quality. Moreover, because the results of the studies are not always consistent due to numerous factors interfering with the effect of immunocastration, there is a need for testing this alternative in the local conditions. The present paper presents the results of a study conducted in commercial rearing conditions, evaluating the effects of immunocastration on performance, carcass and meat quality traits, development of reproductive organs and boar taint elimination.

MATERIAL AND METHODS

Animals and fattening trial

The experiment took place in a commercial pig farm. Initially, seventy two male crossbred (progeny of Large White Landrace dams sired by Duroc boars) pigs were selected from 34 litters farrowed within two weeks. Within each litter, male pigs were either left entire or surgically castrated (SC, *n* = 24) during the first week of life. At the average age of 79.2 ± 3.3 days, when pigs entered the fattening unit, half of the entire male pigs (IC, *n* = 24) received the first vaccination against GnRH (2 ml of Improvac® vaccine per animal, Pfizer Animal Health), whereas the remaining half (EM, *n* = 24) was untreated. Pigs of each treatment group were

Table 1. Performance traits (mean ± standard deviation) in entire males, immunocastrates, and surgical castrates according to the phase of experiment

	EM *n* = 19	IC *n* = 21	SC *n* = 20
Weight (kg)			
V1	29.5 ± 1.7	29.8 ± 2.5	30.7 ± 3.2
V2	79.2 ± 7.6	77.3 ± 8.9	79.9 ± 8.7
Slaughter	111.2 ± 7.2	109.0 ± 7.8	110.5 ± 8.2
Daily gain (g/day)			
V1–V2	790 ± 118	753 ± 118	780 ± 128
V2-slaughter	966 ± 103	970 ±186	925 ± 136
V1-slaughter	852 ± 95	828 ± 94	830 ± 95

EM = entire males, IC = immunocastrates, SC = surgical castrates, V1 = at the time of the first vaccination, V2 = at the time of the second vaccination

group-housed in two pens of 12 pigs. The second vaccination of IC pigs was performed 9 weeks later (average age was 142.2 ± 3.3 days), i.e. 5 weeks prior to slaughter, as recommended by the vaccine producer. Pigs were fed *ad libitum* a commercial diet 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 not recorded. Pigs were weighed three times, at the first and second vaccination, and at the end of the experiment (three days prior to slaughter). Information on body weight and growth rate according to the phase of experiment is provided in Table 1. Pigs were slaughtered within the same week (at an average age of 175.5 ± 4.5 days) in two batches, with all pens equally represented in both batches. The slaughter took place in a commercial abattoir according to standard procedure including approximately 1 h transport, 2 h lairage, CO_2 stunning (86 vol.% in the air), vertical exsanguination, vapour scalding and evisceration. During transport and lairage the pigs were not mixed. At the slaughter line, testes and accessory sex glands were removed and taken to the laboratory for dissection and weighing. This was done for half of the EM and IC pigs, taken at random.

Blood sampling

At slaughter, blood samples from all experimental animals (approximately 9 ml) were taken into glass

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tubes (containing no anticoagulant) and immediately placed on ice. Within 1 h the blood samples were taken to the laboratory and left to coagulate overnight at 4°C. After 24 h, coagulum was removed, while serum was centrifuged at 1800 rpm for 10 min and the supernatant collected and stored at −20°C until analysed.

Carcass and meat quality measurements

At the slaughter line, after the evisceration, leaf fat (i.e. subperitoneal fat) was removed and weighed. The pig carcasses were weighed and classified according to SEUROP by official classification body, using a method approved for Slovenia (OJ EU L56/28, 2008) which consists of taking two measurements at the carcass split line: DM fat (minimal fat thickness over the *gluteus medius* muscle – GM) and DM muscle (shortest distance between cranial end of GM and dorsal edge of vertebral canal). Measurement of pH (pH_{45}) in *longissimus dorsi* muscle (LD) was taken 45 min *post mortem* using a MP120 Mettler Toledo pH meter (Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) fitted with a combined glass electrode (InLab427) and previously calibrated at pH 4.0 and 7.0. The carcasses were cooled overnight at 0–2°C until the internal carcass temperature dropped below 7°C.

The next day following slaughter, additional carcass traits were assessed. The hind leg was cut off the carcass between the 6th and 7th lumbar vertebra and the shank removed. The weight of the leg (ham) was recorded before and after the removal of the skin and subcutaneous fat. Ratio between ham weight and carcass weight (ham/CW, %) and ratio between ham muscle with bones and entire ham weight (ham leanness, %) were calculated. Two further cross-sections of carcass were made, one at the level of the last rib (cross-section A) and one between the 3rd and 4th cervical vertebra (cross-section B). A digital image of each cross-section was taken using a digital photo camera (Canon PowerShot G3, Canon Inc., Tokyo, Japan). Image analysis using LUCIA.NET 1.16.5 software (Laboratory Imaging s.r.o., Prague, Czech Republic) was used for determination of intermuscular fat of the neck (NIMF, in %) on cross-section B and LD surface (LEA) and the surface of the corresponding fat (LEA fat, in %) on cross section A. Additionally, belly leanness was visually assessed on cross-sec-

tion A using a 1–7 point scale (from 1 for extremely fat to 7 for extremely lean).

Colour and pH (pH_{24}) were measured on a freshly cut surface of LD (cross-section A). Colour of LD was assessed using a 1–6 point Japanese colour scale (Nakai et al., 1975). Colour parameter measurements (CIE L^*, a^*, b^*) were taken in triplicate using a Minolta Chroma Meter CR-300 (Minolta Co. Ltd, Osaka, Japan) with an 11 mm aperture, D_{65} illuminant, calibrated against a white tile. Muscle ultimate pH (pH_{24}) was determined in two replicates in the central area of LD. Caudally from the level of the last rib two 2.5 cm thick slices of LD were removed from the loin for the determination of drip loss and intramuscular fat. Drip loss was determined according to the EZ drip loss method (Christensen et al., 2003). Shortly, two cylindrical samples were excised from the central part of LD, weighed, and placed in special plastic containers. The samples were reweighed after 24 h and after 48 h of storage at 4°C. Drip loss (drip loss 24 h and drip loss 48 h) was expressed as a difference (%) to initial sample weight. Samples of LD muscle were minced and intramuscular fat content (IMF, %) estimated using NIRS (NIR System model 6500 Spectrometer, Silver Spring, USA) (Prevolnik et al., 2005). On the next day (48 h after slaughter) the second LD slice was cleaned of fat and connective tissue, vacuum packed, and frozen at −20°C until further analysis. For determination of cooking loss and tenderness the LD samples were thawed overnight at 4°C, weighed, and cooked in a thermostatic water bath (ONE 7-45, Memmert, GmbH, Schwabach, Germany) until the internal temperature reached 72°C. Then the drained LD samples were weighed again (difference in weights was used for cooking loss % calculation) and cooled at 4°C overnight. The next day, two 2.5 cm wide cylindrical cores were excised and shear force (maximum cutting force) was measured using TA Plus texture analyser (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s.

Carcass lesions evaluation

The evaluation took place on the skin of the cooled carcasses, with the exception of the regions distally from the tarsal and carpal joints. The lesions were counted (only fresh lesions, inflicted

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during pre-slaughter period were included) and sorted according to aetiology i.e. as lesions caused either by teeth, hoofs, or hits (strokes).

Cortisol determination

The levels of cortisol were determined in collected serum samples using a commercially available ELISA kit (IBL International, GmBH, Hamburg, Germany). The intensity of the colour was read at 450 nm using a Varioscan Flash spectrophotometer and SkanIt Software Version 2.4.3. RE (Thermo Fisher Scientific Inc., Waltham, USA). Plasma concentrations of cortisol were expressed in ng/ml.

Androstenone, skatole, and indole determination

Samples of subcutaneous fat were taken at the level of the last rib for the determination of boar taint compounds. Androstenone, skatole, and indole concentrations were measured by HPLC as described by Pauly et al. (2008), and the levels were expressed as µg/g of liquid fat. The detection limits were 0.24 µg/g for androstenone and 0.03 µg/g for skatole and indole.

Statistical analysis

Analysis of variance was performed using the MIXED procedure of statistical package SAS (SAS Institute Inc., Cary, USA). For reproductive organs and boar taint compounds, the model included the fixed effects of treatment group and pen nested within treatment group. For carcass traits, the carcass weight was additionally included as a covariate. For meat quality traits, cortisol concentration, and carcass lesions, the model comprised also the slaughter batch as a random effect. When a significant effect of the treatment group ($P < 0.05$) was detected, the least square means (LSM) were compared using Tukey's test.

RESULTS AND DISCUSSION

During the experiment, six animals (four EM and two SC) were lost due to illness, death or due to balancing the number of pigs per pens. After slaughter and following preliminary data analysis, further six pigs were removed from the data set: one SC pig was actually a cryptorchid, two IC pigs did not respond to the vaccination (as shown by genital tract development), and three pigs (one from each treatment group) exhibited extremely slow growth.

Table 2. Carcass traits (LSM) in entire males, immunocastrates, and surgical castrates

	EM $n = 19$	IC $n = 21$	SC $n = 20$	RMSE	*P*-value
Carcass weight (kg)	79.4	76.6	79.0	6.0	0.29
DM fat (mm)	6.7[a]	8.4[b]	11.8[c]	2.0	< 0.001
DM muscle (mm)	68.9	68.1	69.3	4.2	0.65
DM meat (%)	64.3[c]	63.0[b]	60.6[a]	1.5	< 0.001
LEA (cm^2)	42.9	41.9	43.6	4.7	0.54
LEA fat (cm^2)	10.6[a]	11.6[a]	13.6[b]	2.0	< 0.001
Belly leanness (1–7)	5.9[c]	5.5[b]	4.6[a]	0.4	< 0.001
NIMF (%)	13.0[a]	15.5[a]	19.1[b]	3.3	< 0.001
Ham/CW (%)	23.6	23.6	23.7	1.0	0.94
Ham leanness (%)	86.5[b]	85.3[b]	81.1[a]	1.9	< 0.001
Leaf fat (kg)	0.48[a]	0.65[a]	0.91[b]	0.3	< 0.001

EM = entire males, IC = immunocastrates, SC = surgical castrates, RMSE = root mean square error, DM fat = minimal fat thickness over the *gluteus medius* muscle, DM muscle = shortest distance between cranial end of GM and dorsal edge of vertebral canal, DM meat = carcass lean meat content according to the DM5 method, LEA = loin eye area, LEA fat = fat corresponding to loin eye area, NIMF = neck intermuscular fat (%) surface relative to the entire surface of neck cross-section area, Ham/CW = ratio between ham weight and carcass weight
[a,b,c]LSM values within a row with different superscript letters are significantly different ($P < 0.05$)

Carcass traits

No difference was observed between treatment groups on carcass weight and most muscle development indicators including DM muscle, LEA and ham/CW weight ratio (Table 2). The differences between treatment groups were, however, significant for all traits associated with fat deposition. IC were intermediate and differed from both EM and SC in backfat thickness (DM fat), lean meat content (DM meat), and belly leanness score. The 2.4% point difference in carcass lean meat content (DM meat) between SC and IC shows an economically important advantage of the latter. In case of backfat corresponding to LEA, neck intermuscular fat, ham leanness, and leaf fat, the IC were closer to EM and both groups exhibited significantly lesser fat depots than SC.

In general, the available literature provides information about the immunocastration effect on backfat thickness or lean meat content, whereas more detailed carcass quality evaluation is rarely presented. In the present study, we evaluated the effect of immunocastration on various fat depots (subcutaneous, intermuscular, intramuscular, and subperitoneal fat) which are known to vary in deposition rate according to anatomical position, and age (Kouba et al., 1999; Stupka et al., 2008) and also to have different value for meat industry and consumers. In agreement with the results of the present

research, less subcutaneous fat depots than SC and more than EM were reported for IC by several studies (Pauly et al., 2009; Gispert et al., 2010; Morales et al., 2010). Others observed IC to be either similar to EM (Bonneau et al., 1994; Dunshea et al., 2001; Škrlep et al., 2010), closer to SC (Zeng et al., 2002; D'Souza and Mullan, 2003; Fabrega et al., 2010) or even not different from both EM and SC (Metz et al., 2002; Kim et al., 2007). The variability of the responses can be explained by the differences in experimental designs, slaughter weight and, most important, timing of the second vaccination. 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) and Lealiifano et al. (2011). In agreement with the present study, recently performed meta-analysis showed that in terms of carcass leanness IC are intermediate between SC and EM (Batorek et al., 2012).

In case of belly leanness, the present study agrees with Fuchs et al. (2009) reporting IC to have leaner bellies than SC, but not with Škrlep et al. (2010) positioning IC closer to SC than EM. In the case of NIMF, IC pigs were similar to EM and lower than SC, in agreement with our previous observations (Škrlep et al., 2010). For leaf fat, Gispert et al. (2010) and Škrlep et al. (2010) showed the intermediate position of IC, differing from both EM and SC, whereas in the present study IC were similar to EM.

Table 3. Meat quality traits measured in *Longissimus dorsi* muscle (LSM) in entire males, immunocastrates, and surgical castrates

	EM $n = 19$	IC $n = 21$	SC $n = 20$	RMSE	*P*-value
IMF (%)	1.4[a]	1.5[a]	1.9[b]	0.4	< 0.001
pH$_{45}$	6.27[a]	6.31[ab]	6.40[b]	0.17	0.04
pH$_{24}$	5.42[a]	5.52[b]	5.44[a]	0.08	< 0.001
Colour (1–6)	3.3[a]	3.6[b]	3.5[ab]	0.4	0.04
*L**	54.2[b]	50.6[a]	53.5[b]	2.8	< 0.001
*a**	7.8[b]	6.8[a]	7.1[ab]	1.0	0.01
*b**	3.6[b]	2.9[a]	3.4[ab]	0.7	0.01
Drip loss 24h (%)	5.3[b]	2.9[a]	3.6[a]	1.9	0.001
Drip loss 48h (%)	8.5[b]	5.5[a]	6.4[a]	2.1	< 0.001
Cooking loss (%)	28.4	28.6	26.6	4.4	0.29
Shear force (N)	139	144	136	23	0.54

EM = entire males, IC = immunocastrates, SC = surgical castrates, RMSE = root mean square error, IMF = intramuscular fat content, pH$_{45}$ = muscle pH value 45 min after slaughter, pH$_{24}$ = muscle pH value 24 h after slaughter, Colour = subjective LD muscle colour evaluated according to a 6-point Japanese colour scale
[a,b]LSM values within a row with different superscript letters are significantly different (*P* < 0.05)

Meat quality traits

IC had lower LD intramuscular fat content than SC but similar to EM (Table 3). This is in agreement with our previous study (Škrlep et al., 2010), whereas Gispert et al. (2010) and Morales et al. (2010) reported intermediate position of IC compared to SC and EM for IMF in *semimembranosus* and *gluteus medius* muscle, respectively. Studies comparing only IC and SC reported either no difference (Morales et al., 2011) or lower LD intramuscular fat in IC (Boler et al., 2011). If the deposition of IMF is related to other fat depots, such as leaf fat, LEA fat, and neck intermuscular fat, a similar pattern is observed, i.e. IC similar to EM and lower than SC.

The rate of post-mortem pH decline was not affected by immunocastration, but IC had higher pH_{24} than EM and SC, resulting in darker (higher subjective colour score and lower Minolta L^*), less red (lower Minolta a^*), and less yellow (lower Minolta b^*) meat. IC had also lower drip loss than EM (making up a difference of 3.0% points for drip loss 48 h). The higher pH_{24} in IC is not consistent with the usually observed higher pH_{24} in entire males, due to more aggressive behaviour and higher level of physical activity (Fernandez et al., 1994; Sather et al., 1995; D'Souza et al., 1998, 1999;

Cronin et al., 2003). There are not many studies dealing with the immunocastration effect on meat quality. They show for the most part the absence of any effect on pH_{24} (Pauly et al., 2009; Gispert et al., 2010; Škrlep et al., 2010; Boler et al., 2011; Morales et al., 2011;), drip loss or colour (Pauly et al., 2009; Škrlep et al., 2010; Boler et al., 2011). On the other hand, some reports indicate IC to have darker meat than SC (Silveira et al., 2008), lighter than EM (Gispert et al., 2010) or lower drip loss than SC (Miclat-Sonaco et al., 2008).

Reproductive organs and boar taint compounds

The vaccination was not successful for all the immunocastrates. Two of the vaccinated pigs had boar-like weights of reproductive organs (Figure 1). These pigs were considered non-responders and therefore excluded from the dataset used for the statistical analysis. Non-responders have also been identified in previous studies (Zeng et al., 2002; Jaros et al., 2005; Hilbe et al., 2006). The non-respondance to vaccination has been ascribed either to the lack of immunological response or to acute illness.

Disregarding the non-responders, the immunocastration caused notable reduction of the size of
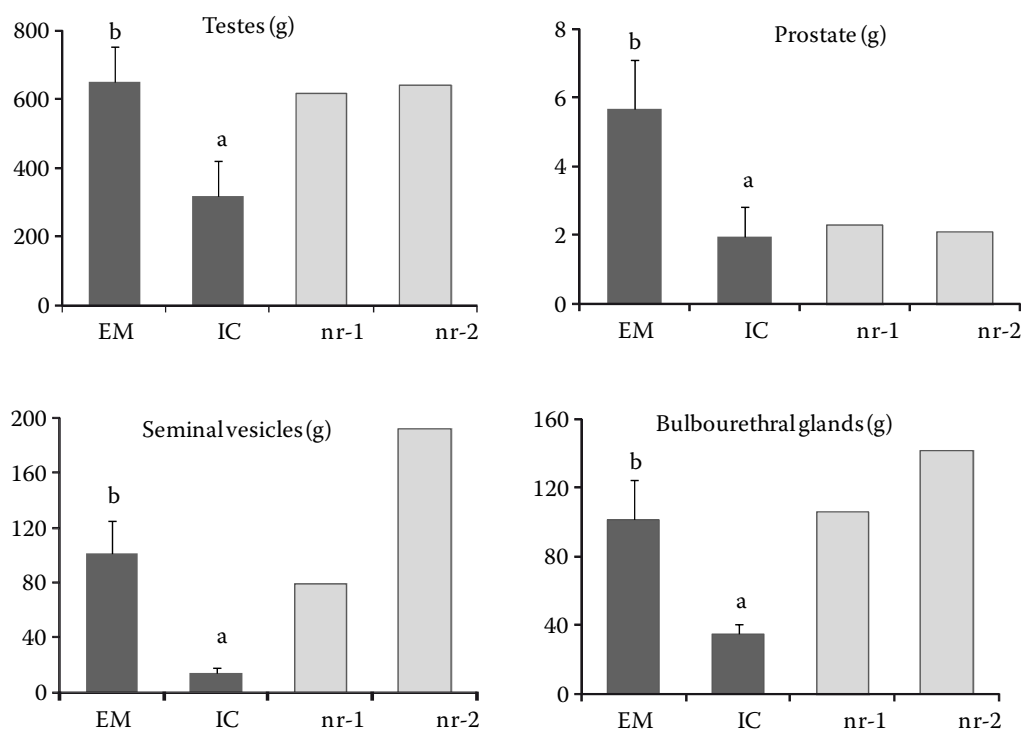

Figure 1. Weight of reproductive organs (mean ± standard deviation) in the case of entire males (EM), immunocastrates (IC), and two non-responders (nr-1, nr-2)

testes and accessory reproductive organs (Figure 1). In IC the weight of testes was reduced to 50%, bulbourethral gland to 34%, seminal vesicles to 14%, and prostate to 35% of that measured in EM. In consistence with the reduction of reproductive organs, immunocastration significantly reduced the amount of boar taint compounds (Figure 2). The concentration of androstenone in IC dropped to around one third of the level found in EM and was similar to that found in SC. The situation was similar for skatole, while concentrations of indole were below detection limit (0.03 μg/ml liquid fat) regardless of the treatment group (data not shown). Because none of the IC or SC pigs had androstenone concentrations higher than the proposed sensory threshold (0.5 to 1.0 μg/g fat tissue, i.e. 0.9 to 1.7 μg/g liquid fat; Walstra et al., 1999), it can be concluded that vaccination was efficient in preventing androstenone-related boar taint. None of the pigs had skatole concentration above the proposed sensory threshold (0.20 to 0.25 μg/g fat tissue, i.e. 0.32 to 0.35 μg/g liquid fat).

In accordance with the present study, numerous studies have proven the effectiveness of anti-GnRH vaccine in elimination of boar taint compounds and reduction of reproductive organ size (Bonneau et al., 1994; Dunshea et al., 2001; Metz et al., 2002; Turkstra et al., 2002; Jaros et al., 2005; Zamaratskaia et al., 2008a, b; Pauly et al., 2009; Gispert et al., 2010). In the mentioned studies a reduction from 16% to 60% for the testes, 50% to 90% for the bulbourethral glands, and 36% to 90% for the seminal vesicles is reported. The comparison with the absolute values reported in the literature data is, however, difficult due to the differences in the experimental design (timing of the second vaccination, type of animals used) and differences in analytical procedures. In the present study the recommendations of the vaccine producer were respected concerning the delay between the immunisation and slaughter. The second vaccination gives a rise of anti-GnRH antibody titres, causing then a rapid decrease of plasma testosterone levels (Bonneau et al., 1994; Dunshea et al., 2001; Zamaratskaia et al., 2008a). Consequently, a relatively sharp drop of fat androstenone concentration occurs due to the cessation of testes function. According to Claus et al (1994) at least three weeks are needed for complete clearance of androstenone from fat tissue. However, a recently published study (Lealiifano et al., 2011) suggests that only two weeks after the second vaccination might be enough for sufficient clearance (below sensory thresholds) of skatole and androstenone.

Carcass lesions and cortisol

There were no differences between treatment groups in the level of cortisol and incidence of carcass skin lesions (data not shown). Incidence of skin lesions was proposed as indicator of aggressiveness between pigs prior to slaughter (Turner et al., 2006). According to the literature, entire males show more aggressive behaviour in comparison to their castrated counterparts (either surgical or immunocastrates) (Cronin et al., 2003; von Borell et al., 2009; Rydhmer et al., 2010) which was not observed in the present study. The reason for the absence of any difference may be due to the fact that the pigs of different groups were not mixed even during pre-slaughter handling. Namely mixing of unfamiliar animals is one of the reasons triggering the aggression (Bolhuis et al., 2005).
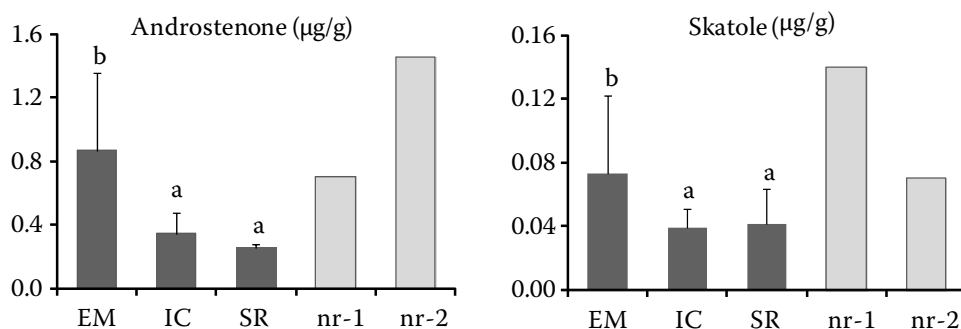

Figure 2. Fat androstenone and skatole concentrations (mean ± standard deviation) in the case of entire males (EM), immunocastrates (IC), surgical castrates (SC), and two non-responders (nr-1, nr-2)

CONCLUSIONS

The elimination of boar taint using the immuno-castration in group-housed Slovenian commercial fatteners was efficient for the majority of vaccinated pigs. Improved leanness in comparison to surgical castrates and lower meat drip loss in comparison to entire males was found proving the benefits of this new alternative to surgical castration.

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