EFFECT OF ACTIVE IMMUNIZATION AGAINST GnRH ON "BOAR TAINT", TESTES AND ACCESSORY SEX GLANDS IN MATURED BOARS

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Abstract. The aim of the present study was to investigate the effect of active immunization with Improvac® (Pfizer Ltd, Louvain-la-Neuve, Belgium) on the concentration of boar taint substances, i.e., skatole and indole in the back fat and omentum tissues, testes, accessory sex glands and testosterone concentration in the blood serum of matured AI boars. Thirteen Danish Landrace clinically healthy mature boars from a commercial AI station were included in the study. The experiment lasted for 15 weeks. The animals were divided into 3 groups: not vaccinated animals (Control, n=7), twice vaccinated animals (Group II VAC, n=3), and 3 times vaccinated animals (Group III VAC, n=3). The mean concentrations of skatole in the back fat and in the omentum tissue of the Control group animals were by 0.62 ± 0.31 µg/g and 0.64 ± 0.43 µg/g higher than in Group III VAC ($P<0.05$). The total weight of testes and accessory sex glands after 2 or 3 vaccinations with Improvac was significantly lower compared with the Control group ($P<0.05$). The results of this study indicated that active immunization of matured boars against GnRH effectively reduced boar taint substances, i.e., indole and skatole levels, testosterone concentration and weight of testes and accessory sex glands.

Keywords: Improvac, boars, boar taint, testicles.

Introduction. Pig males are castrated in order to avoid aggressive behaviour, as well as to protect meat from boar taint. Boar taint has unpleasant odour, which is released by heating or cooking boar meat. This is due to the combined effect of androstenone derivatives (Bonneau, 1982) and such compounds in the fat tissue as skatole (Hansson, 1980; Dijksterhuis et al., 2000), indole (Garcia-Regueiro and Diaz, 1989; Moss et al., 1993; Rius Sole and Garcia-Regueiro, 2001) etc. Androstenone is a steroid formed in the testes, which causes a pronounced urine-like odour and flavour in meat. Other contributors to boar taint are indoles, especially 3-methyl indole or skatole. Almost all the consumers are sensitive to skatole, which gives meat a fecal-like odour and flavour (Batorek et al., 2012). Its presence in the fat tissue mostly depends on sexual maturity of male pigs (Bonneau et al., 1994) and on other factors such as diet composition (Claus and Raab, 1999; Claus et al., 2003), energy level of the diet (Claus et al., 1994), housing conditions (Hansen et al., 1995) etc. Indole is a structural analogue of skatole and its level in the fat tissue highly depends on testicular steroids. Indole is produced by bacteria in the colon from the breakdown of the amino-acid tryptophan. It is usually measured simultaneously with skatole (Haugen et al., 2012).

It is a common practice to castrate male piglets during their first days of life. Furthermore, adult boars, once they have completed their productive life in a breeding farm, should be castrated before slaughtering (Agudelo-Trujillo et al., 2011). Interest in animal welfare across Europe has encouraged the swine industry to reconsider its traditional approach to the control of boar taint and investigate alternatives (Lunström and Zamaratskaia, 2006; Gispert et al., 2010); according to "The European declaration on the Alternatives to Surgical Castration of Pigs", after January 1, 2012, no surgical castration will take place without recognized analgesia and/or anesthesia and after January 1, 2018, no surgical castration will take place at all (Haugen et al., 2012). There are some alternatives to surgical castration of young pig males, such as genetic selection, sperm sexing, immunization against gonadotropin-releasing hormone (GnRH), and slaughtering at lower age/weight to reduce the risk of boar taint, but for adult boars there is only one alternative to surgical castration, i.e., immunization against GnRH (Bonneau et al., 1994; Dunshea et al., 2001; Agudelo-Trujillo et al., 2011).

There are numerous reports on the effectiveness of immunocastration in young male pigs (Zamaratskaia et al., 2008; Einarsson et al., 2009), but there are a few studies that investigate the efficacy of immunocastration in adult boars. It has been shown that active immunization of sexually-matured boars against GnRH has a negative impact on testosterone concentration, sexual behaviour, volume of the ejaculate, and the total number of normal spermatozoa in the ejaculate (Bilskis et al., 2012). There is evidence that immunization prevented boar taint through testicle atrophy and resulted in no weight loss after castration (Agudelo-Trujillo et al., 2011).

The present study was designed to investigate the efficacy of active immunization with Improvac applied as double or triple injections on concentration of skatole and

indole in the porcine adipose tissue, testosterone concentration in the blood serum, and on testes and accessory sex glands in matured AI boars.

Materials and methods

Animals and experimental design

Thirteen Landrace clinically healthy mature boars from a commercial AI station were included in the study. The animals were approximately 25-43 months old and were used for semen production for AI. All the boars were kept in separate pens, fed a commercial boar diet (Jančienė, 2005), and had free access to water. The experiment lasted for 15 weeks. The animals were divided into 3 groups: not vaccinated animals (Control group, n=7), twice vaccinated animals (Group II VAC, n=3), and 3 times vaccinated animals (Group III VAC, n=3). For the vaccination of the experimental animals, the gonadotropin-releasing hormone Improvac® (Pfizer Ltd, Louvain-la-Neuve, Belgium) was used. Group II VAC was vaccinated on weeks 3 and 7, and Group III VAC on weeks 3, 7, and 11 from the beginning of the experiment. The vaccinations were performed according to the manufacturer's instructions (2 mL/pig injected subcutaneously behind and below the base of the ear). The animals were observed daily for general health status. At the end of the experiment, 4 weeks after the last vaccination, all the boars were slaughtered for the analysis of samples of meat, testicles, and accessory sex glands.

Serum analysis

Blood samples for the analysis of testosterone were taken 1 week before slaughter from the ear vein. The samples were transported to the laboratory within ½–1 hour. The blood was centrifuged at 3000 rpm for 5 minutes and 2 mL of the serum were transferred to the Eppendorf test tube, using a 1-mL Paster pipette (Einweg-Pasteurpipetten, Carl Roth GmbH, Germany). The tubes were immediately frozen and stored at -20°C for further analysis. Testosterone concentrations (ng/mL) were analyzed by a computerized Multi-Detection Microplate Reader Synergy™ HT (Bio-Tek® Instruments, Inc., USA, 2004) with a DIAsource TESTO-EASIA Kit (DIAsourse ImmunoAssays S.A., Belgium).

Analysis of testicles

The testes with epididymis and accessory sex glands were examined macroscopically, their weight was measured, and the form, position, sectional view color, and consistency were estimated. Slices of the testicle samples were fixed in Bouin´s solution for 24 hours and thereafter washed in 70% methanol. The samples were processed and embedded in paraffin. They were cut to 2-µm-thick sections, stained routinely with Hematoxylin and Eosin (H&E), and evaluated under the light microscope (Laurusevičienė and Smaliukienė, 2007).

Fat analysis

The back fat samples for the analysis of indole and skatole were taken from the animals 36 hours after carcass cooling. The omentum samples for the analysis of indole and skatole were taken from the animals just after slaughter. Afterwards, the samples were frozen at -80°C until analysis. Concentrations of skatole and indole in the back fat and omentum tissues were assessed using a modified HPLC method (Denhard et al., 1993; Claus et al., 2003).

Statistical analysis

Statistical analysis was performed using the SPSS statistical package No. 15 for Windows (SPSS for Windows 9.0, SPSS Inc., Chicago, IL, USA, 1989–1995). The data included in the model were analyzed using descriptive statistics (mean ± SD) and 1-way ANOVA analysis. The differences between the investigated groups were analyzed by the LSD method (α = 5 %). The data were considered to be statistically reliable when: * $P<0.05$; ** $P<0.01$; and *** $P<0.001$. The correlation between the dependent variables and the strength of the direct relation was evaluated by Pearson correlation coefficients.

Results. All the animals tolerated vaccine injections well and were clinically healthy during the entire experiment. The testosterone concentration correlated with the testicles weight ($r = 0.58$; $P<0.05$) and with the concentration of skatole in the back fat ($r = 0.66$; $P<0.05$) and in the omentum ($r = 0.6$; $P<0.05$). The mean of the testosterone concentration in the serum of the Control group was by 2.35 ± 1.35 ng/mL higher compared with Group II VAC ($P<0.05$) and by 2.98 ± 1.48 ng/mL higher compared with Group III VAC ($P<0.01$). The mean of the testosterone concentration and the mean of the testicles weight in all the groups are shown in Fig. 1.

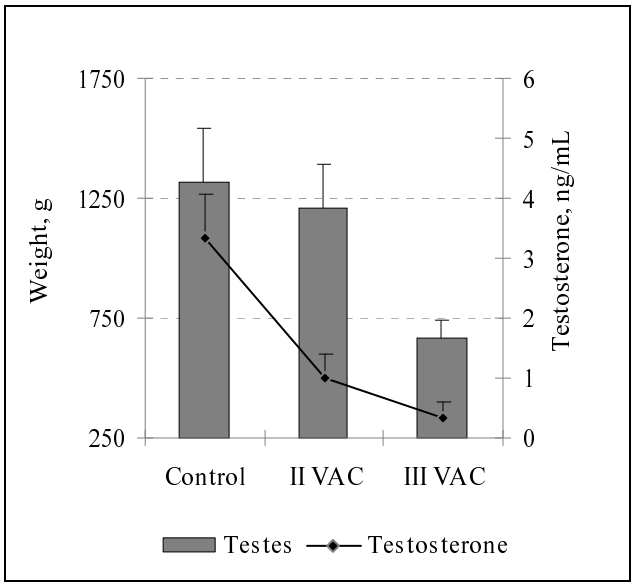

Fig. 1. **The mean testosterone concentration and the mean total weight of testes in 3 experimental groups ± SD** (n=13)

The mean of the testes weight correlated with the concentration of skatole in the back fat ($r = 0.62$; $P<0.05$) and in the omentum ($r = 0.57$; $P<0.05$), with the concentration of indole in the back fat ($r = 0.61$; $P<0.05$) and in the omentum ($r = 0.56$; $P<0.05$), and also with the weight of the vesicular gland ($r = 0.67$; $P<0.05$). The testes weight in Group III VAC after the third vaccination was by 652.47 ± 151.07 g lower compared with the

Control group (P<0.001) and by 541.0 ± 112.22 g lower compared with Group II VAC (P<0.05) (Figure 1).

Normal spermatogenesis in the seminiferous tubuli was observed in the testicular tissue of the Control group animals. Layers of germinative cells were present including spermatocytes and spermatids (Fig. 2). Marginal differences were observed in histological sections between the immunized specimens from Group 2 VAC and the control group. But after the third vaccination, marked changes in the testicular tissue were noted. Spermatogenesis was arrested, marked spermatocyte loss, decrease of the normal number of layers of germ cells, and in some tubuli only sustentacular Sertoli cells were present (Fig.3).

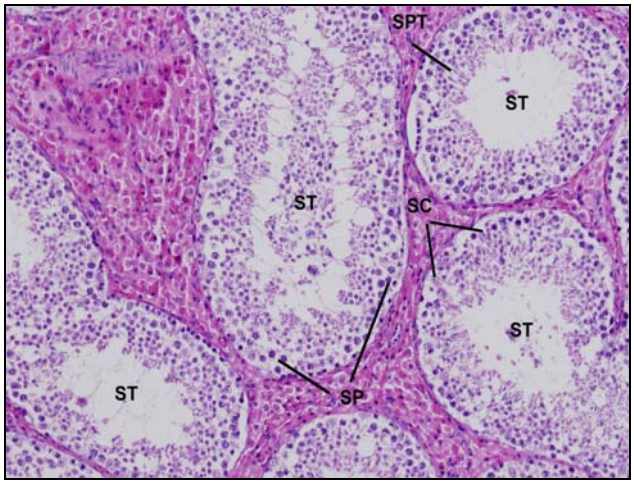

Fig. 2. **Histological view of normal spermatogenesis in control boar testicles**

ST – seminiferous tubules; SP – spermatocytes; SPT – spermatids, SC – Sertoli cells (H&E; 200x magnification).

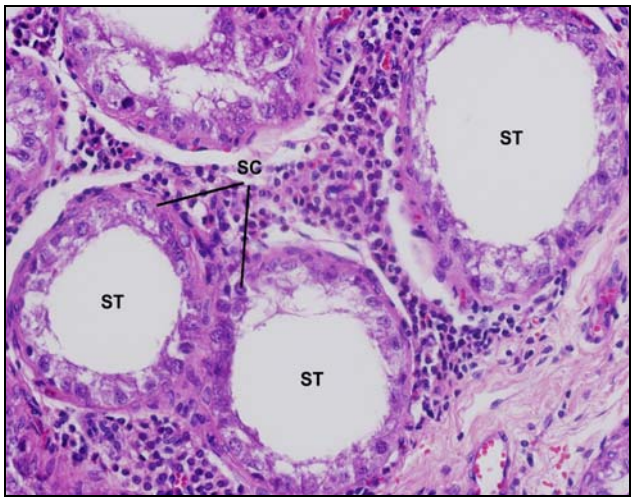

Fig. 3. **Histological view of arrested spermatogenesis and empty tubules in III VAC boar testicles**

ST – seminiferous tubules, SC - Sertoli cells (H&E; 400x magnification).

The mean of the concentration of skatole in the back fat in the Control group was by 0.58 ± 0.34 µg/g higher compared with Group II VAC (P<0.05) and by 0.62 ± 0.31 µg/g higher compared with Group III VAC (P<0.05). The mean concentration of skatole in the omentum in the Control group was by 0.64 ± 0.43 µg/g higher than in Group III VAC (P<0.05) and by 0.6 ± 0.45 µg/g higher than in Group II VAC (P<0.05). The mean of the concentration of indole in the back fat and the omentum between all the 3 groups was statistically insignificant; however, the level of indole in the Control group was slightly higher compared with the experimental groups (Table 1).

Table 1. **The mean** (±SD) **of concentration of skatole and indole in back fat and omentum tissues** (n=13)

Parameter	Boar groups		
	Control [a]	II VAC [b]	III VAC [c]
Skatole (back fat), µg/g	1.03 ± 0.37 [b, c]	0.45 ± 0.03 [a]	0.41 ± 0.06 [b]
Skatole (omentum), µg/g	0.93 ± 0.51 [b, c]	0.33 ± 0.06 [a]	0.29 ± 0.08 [b]
Indole (back fat), µg/g	1.98 ± 0.69	1.67 ± 0.16	1.34 ± 0.42
Indole (omentum), µg/g	2.11 ± 0.36	2.0 ± 0.37	1.97 ± 0.33

[a, b, c,] Means with different letters in columns are significantly different (P<0.05)

The mean weight of the accessory sex glands in all the groups is shown in Figure. 4. Though the weight of the vesicular and the prostate glands in Group III VAC was markedly lower than in the Control group (204.81 ± 81 g and 15.14 ± 13.12 g, respectively), the differences were statistically insignificant (P>0.05). The mean of the bulbourethral gland weight in the Control group was by 148.33 ± 10.61 g higher than in Group II VAC (P<0.05) and by 161.67 ± 6.13 g higher than in Group III VAC (P<0.01). It was shown that from the accessory sex glands only the weight of the prostate gland correlated with boar taint. The prostate gland weight correlated positively with the concentration of skatole in the back fat (r = 0.77; P<0.01) and in the omentum tissue (r = 0.78; P<0.01) and with the concentration of indole in the back fat (r = 0.77; P<0.01).

Discussion. Negative consumer perception of meat from entire male pigs is anticipated in many traditionally–castrating markets. Some authors have reported that boar taint compounds are important factors affecting consumer buying habits of fresh pork, as well as processed products such as bacon and dry-cured ham (Matthews et al., 2000; Font i Furnols et al., 2003). A vaccination scheme using 2 doses of Improvac® is recommended by the manufacturer.

In the present study, 3 doses of the vaccine were used to monitor effects of prolonged exposure of the vaccine on the adult animals.

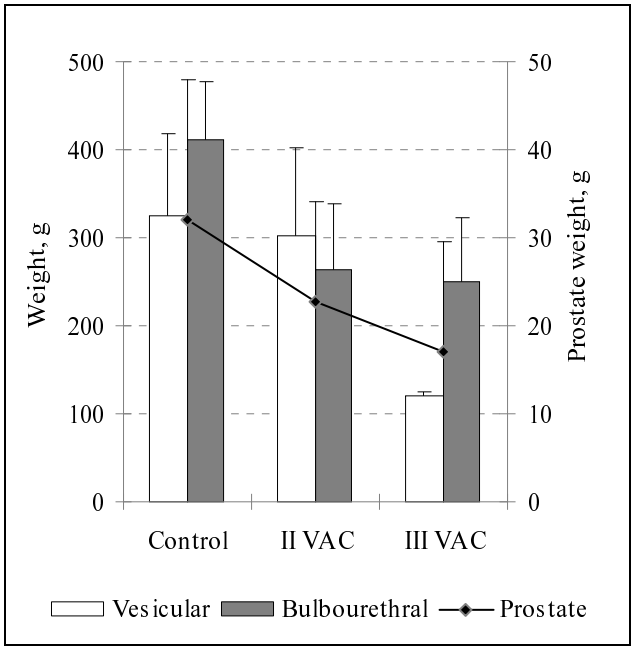

Figure 4. **The mean weight of accessory sex glands in 3 experiment groups** (n=13)

The results of our study showed that the general status of health was not affected by vaccination and in the injection site no inflammatory response was present, indicating that the vaccine was very well tolerated. This confirms the results of other authors, who did not observe any side reactions of vaccinated animals at slaughter (Agudelo-Trujillo et al., 2011).

The results of our study showed significant differences on testosterone concentration between the Control and the immunized animal groups. These observations are in concert with the results of the studies where testosterone levels decreased after the second vaccination (Zamaratskaia et al., 2008) and were significantly lower in immunocastrated males (Killian et al., 2006; Bilskis et al., 2012). We observed that testosterone remained at high concentration of approximately 3.33 ng/mL in boars and decreased to 0.98 ng/mL after the second and to 0.35 ng/mL after the third vaccination. Low or even undetectable levels of plasma testosterone and decreased weight of testes and accessory sex glands of immunocastrates indicate pronounced suppression of reproductive performance of male pigs. The testosterone concentration in our study correlated with the testes weight ($P=0.038$) and with the concentration of skatole in the back fat and in the omentum ($P<0.05$). As expected, immunocastration affected the weight of the testes and the accessory sex glands, being heavier in entire male pigs compared with that of the immunized animals ($P<0.05$). The testicles of the immunized animals were lighter by 8.45% in Group II VAC and by 49.5% in Group III VAC ($P<0.05$) compared with the testes of the Control group. This is in agreement with the results obtained by Dunshea et al. (2001) and Pauly et al. (2009), Agudelo-Trujillo et al. (2011). Furthermore, the bulbourethral glands of the Control male pigs were heavier by 3% and 39.24% ($P<0.05$) compared with those of the pigs of Groups II VAC and III VAC, respectively. These findings are in agreement with recently performed studies (Pauly et al., 2009; Gispert et al., 2010). Zamaratskaia et al. (2008) found that vaccination dramatically decreased weight and size of testicles and bulbourethral glands at slaughter, both in pigs vaccinated at ages of 15 and 21 weeks, slaughtered 16 weeks after the second vaccination, and in pigs vaccinated at ages of 14 and 18 weeks and slaughtered 22 weeks after the second vaccination. Based on these findings, size and weight of testes and accessory sex glands could be used to assess efficiency of vaccination. On the basis of our results and results described in literature, regarding the effect on the development of accessory sex glands and testes, weight of seminal vesicles might be used as an indicator of evaluation of the efficacy of immunocastration because their size decreases more rapidly compared with that of testicles (Bonneau, 2010; Batorek et al., 2012).

From the point of view of meat quality, the most important effect of immunization is that testosterone and androstenone synthesis is arrested. Furthermore, reduced levels of testicular steroids in immunocastrates accelerate the metabolic clearance of indolic compounds, thus, lowering skatole and indole concentrations at the tissue level (Zamaratskaia et al., 2008; Batorek et al., 2012). In the present study, - very low (0.46 µg/g) to high (1.48 µg/g) skatole concentrations were estimated in the adipose tissue of entire male pigs. Also, the clearance from the adipose tissue is age-dependent, and it may take more than 6 weeks in older boars (Claus et al., 2007). In the present study, the boars were slaughtered 4 weeks after the last vaccination. The concentrations of indole and skatole in our studies were significantly lower in the vaccinated boars than in the Control group. Reduction in testosterone concentration leads to reduction in skatole and indole concentration in the adipose tissue. Several studies have demonstrated a very consistent effect of Improvac on androstenone as well as skatole and indole levels (Dunshea et al., 2001; Zamaratskaia et al., 2008; Pauly et al., 2009; Batorek et al., 2012). Our findings confirmed this effect, and the skatole and the indole concentrations in the adipose tissue of the vaccinated pigs were comparable with the results described by Walstra et al. (1999).

In conclusion, this study indicated that active immunization of matured boars against GnRH effectively reduced boar taint substances, i.e., indole and skatole levels, testosterone concentration, and weight of testicles and accessory sex glands.

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