

**INVESTIGATION OF THE EFFICACY OF IMMUNOCASTRATION AIMED AT
THE PREVENTION OF SEX ODOUR IN BOAR MEAT**

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Disadvantage of meat known as boar taint is caused by steroids, androstenone being of particular importance, as well as indole and its derivatives, among which the best known is skatole. The common practice in Europe, in order to control these changes in meat, is castration without anesthesia. This intervention causes pain and stress to animals, thus undermining animal welfare. Many countries considering animal welfare try to find the alternative solutions in order to avoid castration. The alternative to surgical castration and possible solution to the problem of sex odour in the meat, as well as androstenone and skatole contents decrease in the boar's fat is immunological castration of boars (immunocastration). Average skatole content in fat tissue of boars was significantly higher ($0.21 \pm 0.03 \mu\text{g/g}$) compared to skatole content in fat tissue of the castrates, or immunocastrates ($0.12 \pm 0.02 \mu\text{g/g}$). In adipose tissue of the castrates and immunocastrates there was no significant difference in the average content of skatole. The content of androstenone in the adipose tissue of immunocastrates was below detection limits, and the average androstenone level in adipose tissue of boars was $0.66 \pm 0.13 \mu\text{g/g}$. The obtained results show that immunocastration is justified in consideration of the meat quality and can completely replace castration in male animals, which is in compliance with the preservation of animal welfare in rearing fattening young boars.

Key words: boars, boar taint, castration, immunocastration

INTRODUCTION

The production of uncastrated male pigs shows some advantages compared with castrated ones in terms of efficiency, because the carcasses are leaner, the production costs are reduced and the animals grow faster (Walstra, 1974; Desmoulin, 1983; Fortin *et al.*, 1983). However, castration of young male pigs is practiced in some European countries and in North America in order to avoid the occurrence of boar taint, an unpleasant odour present in meat from 5%

to 10% in the carcasses of uncastrated pigs (Malmfors and Lundstrom, 1983; Diestre *et al.*, 1990).

Boar taint, an undesirable odour from meat from entire male pigs, is caused by the naturally occurring compounds androstenone and skatole. The level of boar taint can be minimized by decreasing the concentrations of these compounds in the adipose tissue, e.g. via immunocastration, genetic selection, dietary manipulations and improved rearing conditions. Meat processing can probably reduce or mask boar taint, however, more studies are needed to investigate the possible processing techniques and consumers attitudes towards the final pork product. Genetic selection against high boar taint is probably the most attractive alternative, but is not realistic in the near future. At the moment, the best temporary solutions are "humane" castration using anaesthesia and analgesia, or immunocastration. The advantages and disadvantages of alternative methods should be carefully studied before the final decision is made about how to prevent boar taint without the need of stressful and painful surgical castration. It is generally believed that in future, surgical castration of male piglets can be avoided and replaced by practical and ethically acceptable alternatives (Chen, 2007; Zamaratskaia, 2009).

Tainted meat and its undesirable odour is perceived by nasal mucosa receptors especially when fat, meat and meat products are exposed to heating. For the appearance of boar taint numerous substances are responsible, and of primary importance are androstenone (5- α -androst-16-en-3-one) and skatole (3-methylindole) (Hansson *et al.*, 1980; Dijksterhuis *et al.*, 2000). Substances such as indole (Garcia-Requeiro and Diaz, 1989; Rius and García-Regueiro, 2001) and 4-phenyl-3-butene-2-one (Rius and García-Regueiro, 2001) also contribute to the appearance of this meat disadvantage. Of particular importance are indol compounds such as indole-methanol (indole-3-methanol), indole-propionic acid (indole-3-propionic acid), indole-acetonitrile (indole-3-acetonitrile) and indole-ethanol (indole-3-ethanol) (Hansen-Møller, 1998). Taint meat acceptability in the most European countries is at very low rate (Bonneau *et al.*, 2000).

The differences in meat acceptability by consumers, i.e. large variations in the detection of tainted meat odour in different countries, are influenced by different genetic structures of pig populations, farm management, differences in culinary habits and methods of evaluation, a native origine of the consumers, age, gender and the fact that only a part of the population is sensitive to the smell of androstenone (Weiler *et al.*, 2000; Font and Furnols, 2000; Matthews *et al.*, 2000). Most of people show higher sensitivity to the smell of skatole, compared to the smell of androstenone (Baltić and Tadić, 1995). Skatole could be perceived by 99% of consumers (Weiler *et al.*, 1997). Androstenone could be perceived in very low concentrations by the consumers, while some consumers are anosmic and can not perceive it at all (Wysocki *et al.*, 1989). Anosmia is genetically determined (Wysocki and Beauchamp, 1984) and depends on the consumers gender (Elseley, 1968; Griffiths and Patterson, 1970) and country where it is performed (Gilbert and Wysocki, 1987). Perception ability varies, decreases in men and increases in women with age (Dorries *et al.*, 1989). It has been proven that meat acceptability varies among different populations. The reported proportions of

sensitive people in Germany (32 %), Spain (46–48%), Norway (39%) and Belgium (45%) were lower than it was observed in France which ranged between 63% and 74% (Bonneau and Chevillon, 2012). Because sensitivity to androstenone is known to be genetically determined (Keller *et al.*, 2007), it can be envisaged that the observed differences reflect real variations between populations. It seems more likely however that they result from differences in methodology.

Androstenone (5- α -androst-16-en-3-one) is a steroid hormone primarily synthesized in Leydig cells of boar testes, along with anabolic hormones of testis (Gower, 1972; Kwan *et al.*, 1985). Skatole (3-methylindole) is a metabolic product of the amino acid L-tryptophan in the colon of pigs (Yokoyama and Carlson, 1979). As the threshold level of sensitivity in evaluation of pig carcass acceptability for human consumption, different level of substances responsible for boar taint are taken in to consideration. Claus *et al.* (1994) and Rhodes (1971) showed that androstenone concentration level ranges from 0.5 to 1.0 μg per gram of fat. The limit for androstenone is 1.00 ppm and 0.25 ppm for skatole (Mortensen *et al.*, 1986). The limits are based upon these compounds concentrations in pigs adipose tissue, but also on consumers reaction after meat consumption.

In the most of countries there is an ambition to prohibit castration (Stevenson, 2000). This requires finding alternative solutions in order to eliminate tainted meat disadvantages. Legislation modifications and animal welfare growing concerns, force governments and industries to reconsider the traditional approach to solving these disadvantages of pork, and to find and introduce alternative methods. Surgical castration of pigs with anesthesia and analgesia and temporary suppression of testicular function by vaccination (immunocastration) are currently the most practical choices to avoid tainted meat (Fredriksen and Nafstad, 2006; Jaegglin and Kupper, 2008; Kluivers-Poodt and Spoolder, 2008; Svendsen, Strobeck and Forman, 2005; Zols *et al.*, 2008). ImprovacTM vaccine (Pfizer Ltd.) is used for immunological castration. Australia and New Zealand use this method since 1998 (Hennessy, 2006). Switzerland has started the use of this method since January 2007 (Bielefeld, 2006) and Belgium since October 2010. Vaccine ImprovacTM is approved in other countries: Brazil, Mexico, Korea, Thailand, Philippines, Guatemala, South Africa, Chile, Venezuela, Panama, Russia, El Salvador. ImprovacTM is registered in Serbia, Slovenia and Croatia. The vaccine producer has showed that the antigen has no hormonal activity (Clarke *et al.*, 2008), although efficiently produces specific antibodies against GnRH. Successful application of Improvac vaccine includes two doses. First dose is applied subcutaneously (2 mL) in the base region of the ear, and the second dose application is at least 4 weeks later. "Booster" injection application is 4-6 weeks before slaughtering (Evans, 2006). Usually the first vaccination is at eight weeks of age, and the second 4-6 weeks before slaughtering, although the risk of tainted meat, according to the manufacturer, is minimized up to 10 weeks after the second vaccination (EMA, 2010).

The object of this study was to assess the possibilities of immunocastration in the prevention of tainted boar's meat.

MATERIAL AND METHODS

Animals

The experiment included three groups of 30 male animals: surgically castrated pigs (at the age of less than seven days), boars and immunocastrates. All pigs are descendents of a single boar (a crossbred of Duroc and Pietrain) and sows of the same line (crossbreeds of Landrace and Yorkshire). They were all raised in identical conditions and were fed with the same feed.

Immunization procedure

The pigs destined for immunocastration were immunised by s.c. injection of 2x2 ml Improvac vaccine (Pfizer Ltd.), in the dorsal part of the neck, behind the ear base. The vaccine immunogenic material is synthetic gonadotropin-releasing factor (GnRF) conjugated to a carrier protein in a water adjuvant. The first dose was administered at the age of eight weeks and the second one five weeks prior to slaughter.

Animals slaughtering and carcasses processing

After fattening and transport to the slaughterhouse, pigs slaughtering and carcasses processing were performed in the same way. From slitted carcass parts (loin with fat) samples were taken in order to determine androstenone and skatole concentrations. Determination of skatole content was carried out in samples of adipose tissue taken from all animals, and for measurements of androstenone content samples of all animals from groups of young boars and immunocastrates were used.

Skatole and androstenone content determination

For skatole determination in pigs' adipose tissue, the colorimetric method based on a modified Ehrlich reaction of indole with 4-dimethylaminobenzaldehydom-DMBA was used (Mortensen i Sørensen, 1984). For androstenone determination in pigs' adipose tissue, gas chromatography after extraction and purification of the fat sample (Garcia-Requeiro *et al.*, 1986; Garcia-Requeiro and Diaz, 1989) was used.

Statistical analysis

Results obtained during the experiment were statistically analysed using the basic statistical methods, descriptive statistical parameters, i.e. the arithmetic mean (\bar{X}), standard deviation (SD), standard error (Se) and coefficient of variation (Cv%). For measurements of the significance of differences between mean values of two groups t-test was used. To test the significance of differences among three or more of the observed treatment groups ANOVA, and Tukey test were used. Significance of differences was determined at the level of significance of 5%, 1% and 0.1%. Statistical analysis of the results was performed in the statistical package PrismaPad 5.00.

RESULTS

Skatole content in the adipose tissue of tested pigs

Average skatole content in boars adipose tissue was $0.21 \pm 0.03 \mu\text{g/g}$ and was significantly higher than the average content of skatole in the adipose tissue of the castrated, or immunocastrated pigs ($0.12 \pm 0.02 \mu\text{g/g}$).

Results of skatole content in the fat tissue of castrate, boars and immunocastrates are shown in Table 1.

Table 1. Skatole content ($\mu\text{g/g}$) in the adipose tissue of tested categories

Categories	\bar{X}	Sd	Se	Min	Max	Cv (%)
Castrates	0.12 ^á	0.02	0.004	0.09	0.18	16.7
Boars	0.21 ^á	0.03	0.005	0.17	0.28	14.3
Immunocastrate	0.12 ^á	0.02	0.003	0.10	0.16	16.7

Distribution patterns of skatole content in fat among categories of pigs are shown in Table 2.

Table 2. Distribution of skatole content in the adipose tissue according to content ($\mu\text{g/g}$)

Skatole content ($\mu\text{g/g}$)	Category of pigs					
	Castrates		Boars		Immunocastrate	
	Number	%	Number	%	Number	%
0.05-0.09	2	6.66	-	-	-	
0.10-0.14	25	83.33	-	-	27	90.00
0.15-0.19	3	10.00	10	33.00	3	10.00
0.20-0.24	-		15	50.00	-	
≥ 25	-		5	16.66	-	

Distribution of fatty tissue samples analyzed by categories of pigs skatole content ($\mu\text{g/g}$) were as follows: in two castrates were established values in the range from 0.05 to 0.09 $\mu\text{g/g}$, respectively, in 25 castrates skatole was from 0.10 to 0.14 $\mu\text{g/g}$, or 83.33% of the samples, in three castrates skatole was from 0.15 to 0.19 $\mu\text{g/g}$. Ten boars had skatole values ranging from 0.15 to 0.19 $\mu\text{g/g}$; in 15 boars skatole concentrations ranged from 0.20 to 0.24 $\mu\text{g/g}$. Concentrations higher than 25 $\mu\text{g/g}$ was determined in five boars, or 16.66% of samples.

In 27 immunocastrates values ranged from 0.10 to 0.14 $\mu\text{g/g}$ (90% of samples), and in three of them concentrations of skatole ranged from 0.15 to 0.19 $\mu\text{g/g}$ (10%).

If the acceptance limit for skatole in fat tissues was 0.20 $\mu\text{g/g}$, 50% of boars carcasses would be unacceptable because of altered sensory characteristics.

However if the norm value of skatole was $0.25 \mu\text{g/g}$ fat, then 16.66% of carcasses would be unacceptable.

The content of androstenone in boars and immunocastrates adipose tissue

The content of androstenone in boars and immunocastrate adipose tissue is shown in Table 3. The obtained results show that the content of androstenone in adipose tissue from immunocastrates was below the limit of detection. The average content of androstenone in boars adipose tissue was $0.66 \pm 0.13 \mu\text{g/g}$, and ranged from 0.40 to $0.94 \mu\text{g/g}$.

Table 3. The content of androstenone in boars and immunocastrates adipose tissue

Category	\bar{X}	Sd	Se	Min	Max	Cv (%)
Boars	0.66	0.13	0.02	0.40	0.94	19.7
Immunocastrate	nd*					

nd* below detection limit

Table 4. shows the distribution of androstenone content in the boar fat samples with an interval value of $0.25 \mu\text{g/g}$

Table 4. The distribution patterns of androstenone content in boar's fat tissue

Androstenone content ($\mu\text{g/g}$)	Fat sample	
	Number	%
0.25-0.49	4	13.33
0.50-0.74	18	60.00
0.75-1.00	8	26.66

Androstenone content ($\mu\text{g/g}$) in immunocastrate adipose tissue was below the detection limit, and in boars androstenone average content was $0.66 \pm 0.13 \mu\text{g/g}$. By analyzing the distribution of androstenone content in samples of fat in boars (interval value of $0.25 \mu\text{g/g}$ androstenone) in 4 boars was found to be 0.25 to $0.49 \mu\text{g/g}$, 18 samples ranged from 0.50- $0.74 \mu\text{g/g}$ androstenone (60%) and in 8 boars from 0.75 to $1.00 \mu\text{g/g}$. The most of adipose tissue samples (60.00%) had androstenone content from 0.50 to $0.75 \mu\text{g/g}$. 26.66% of samples had androstenone content of 0.75 to $1.00 \mu\text{g/g}$, and 13.33% of the samples had less than $0.50 \mu\text{g/g}$ androstenone content.

DISCUSSION

Skatole content in immunocastrates was lower than in boars, and boars had higher skatole level compared to castrates. There was no difference in skatole content between castrates and immunocastrates. The results are in compliance

with results obtained by Škrlep *et al.* (2010). In studies by Škrlep *et al.* (2010), skatole level was low and not statistically different between castrates and immunocastrates. In boars skatole concentration was almost 6 times higher, thus confirming the effectiveness of vaccination against GnRH. Skatole concentration was higher in the adipose tissue of boars ($p < 0.01$) compared to castrates and immunocastrates. The concentration of skatole in 4 boars was high (0.21 and 0.24 $\mu\text{g/g}$) or very high (1.23 $\mu\text{g/g}$) (Pauly *et al.*, 2009). In studies by Morales *et al.* (2010) skatole concentration was low and below the accepted level for consumers sensory (0.2 $\mu\text{g/l}$), while boars had skatole concentrations almost two times higher than the immunocastrates ($p < 0.001$). Between immunocastrates, castrates and gilts were not observed statistically significant differences in the content of skatole (Morales *et al.*, 2010). By analyzing the distribution of fatty tissue samples analyzed by categories of pigs skatole content ($\mu\text{g/g}$) in 27 immunocastrates were determined concentration from 0.10 to 0.14 $\mu\text{g/g}$, while in 3 immunocastrates skatole concentration was in the range from 0.15 to 0.19 $\mu\text{g/g}$. The acceptability threshold for androstenone in the absence of skatole has still to be established clearly. To revise the acceptability threshold for skatole could also be recommended in the light of the results of Bañón *et al.* (2003) and Lunde *et al.* (2009) suggesting that the currently agreed threshold of 0.20-0.25 $\mu\text{g/g}$ might be too high. Finally an increased knowledge of the interaction between androstenone and skatole in determining the consumer response to entire male pork is also needed. According to research results, 60.00% of adipose tissue samples had androstenone content from 0.50 to 0.75 $\mu\text{g/g}$. Androstenone content of 0.75 to 1.00 $\mu\text{g/g}$ was in 26.66% of samples while the content of androstenone less than 0.50 $\mu\text{g/g}$, was present in 13.33% of samples.

The relative contribution of androstenone and skatole to boar taint is under discussion. An international study involving seven European countries was carried out to determine the contributions of androstenone and skatole to boar taint and their possible variations according to production systems and consumer populations (Bonneau *et al.*, 2000a; 2000b). The obtained results showed that the contribution of both compounds to boar taint could be influenced by such factors as the concentration of androstenone and skatole, different methodologies used for the sensory evaluation (Dijksterhuis *et al.*, 2000), different consumption habits (Matthews *et al.*, 2000) and different human responses to androstenone (Weiler *et al.*, 2000).

Vaccination preventing boar taint in pigs was the subject in numerous studies that have shown that the vaccine ImprovacTM is very effective in reducing the incidence of boar taint in entire males (Fuchs *et al.*, 2009; Zamaratskaia *et al.*, 2008; Jaros *et al.*, 2005; McCauley *et al.*, 2003; Cronin *et al.*, 2003; Dunshea *et al.*, 2001). Compounds that contribute the most to the appearance of boar taint, androstenone and skatole, are metabolized in the period after the second vaccination (Škrlep *et al.*, 2010; Hemonic *et al.*, 2009; Lealiifano *et al.*, 2009; Jaros *et al.*, 2005). In pigs slaughtered just two weeks after the second vaccination, androstenone and skatole concentrations were comparative to the concentrations of these compounds in castrated pigs and significantly below the level of detection (Lealiifano *et al.*, 2009).

CONCLUSION

The results of our study showed that skatole content in boars adipose tissue was significantly higher than the average level of skatole in the castrates and immunocastrates adipose tissue. The difference in the average content of skatole in the fat tissue of immunocastrates and castrates was not determined. In adipose tissue of immunocastrates androstenone content was below the limit of detection, and the average content of androstenone in the adipose tissue of boars was $0.66 \pm 0.13 \mu\text{g/g}$.

Immunocastration is proved to be efficient in preventing boar taint and can completely replace the procedure of male surgical castration, which supports animal welfare during breeding pigs for fattening.

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ISPITIVANJE MOGUĆNOSTI PRIMENE IMUNOKASTRACIJE U CILJU SPREČAVANJA MANE POLNOG MIRISA MESA NERASTOVA

ALEKSIĆ JELENA, DOKMANOVIĆ MARIJA, ALEKSIĆ Z, TEODOROVIĆ V, STOJIĆ V, TRBOVIĆ DEJANA i BALTIC MŽ

SADRŽAJ

Mani mesa poznatoj kao polni miris mesa doprinose polni steroidi, od kojih je androstenon od posebnog značaja, kao i indol i njegovi derivati, među kojima je najpoznatiji skatol. Najčešća praksa u Evropi u cilju kontrole ove mane mesa je izvođenje kastracije bez anestezije. Izvođenjem ove intervencije prouzrokuje se bol i stres i narušava dobrobit životinja, što je predstavljalo podsticaj da se u mnogim zemljama, gde je poslednjih godina dobrobit životinja od velikog interesa, odustane od kastracije. Napuštanje ove metode zahteva nalaženje alternativnih rešenja u cilju otklanjanja ove mane mesa. Jedna od obećavajućih alternativa hirurškoj kastraciji i potencijalno rešenje problema polnog mirisa mesa, odnosno smanjenja sadržaja androstenona i skatola u masnom tkivu nerastova je imunološka kastracija (imunokastracija). Prosečan sadržaj skatola u masnom tkivu nerastova bio je značajno veći ($0.21 \pm 0.03 \mu\text{g/g}$) u odnosu na prosečan sadržaj skatola u masnom tkivu kastrata, odnosno imunokastrata ($0.12 \pm 0.02 \mu\text{g/g}$). U masnom tkivu imunokastrata i kastrata nije utvrđena razlika u prosečnom sadržaju skatola. Sadržaj androstenona u masnom tkivu imunokastrata je bio manji od granice detekcije metode, a u masnom tkivu nerastova prosečan sadržaj androstenona bio je $0.66 \pm 0.13 \mu\text{g/g}$. Naši rezultati ukazuju da je postupak imunokastracije opravdan sa stanovišta prihvatljivosti mesa i da u potpunosti može da se zameni postupak kastracije muških jedinki, što ide u prilog očuvanju dobrobiti životinja kod uzgoja svinja za tov.

