VACCINATING MALE PIGS AT YOUNGER AGE WITH IMPROVAC®

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Abstract—The objective of this study was to investigate the effect of a gonadotropin-releasing hormone (GnRH) vaccine, Improvac[®] (Pfizer, Ltd.), with the second dose administered at a younger age than recommended, on the levels of serum anti-GnRH antibodies, skatole in plasma, indole, skatole and androstenone in fat, testes weight and bulbourethral gland length. This earlier vaccination regimen would provide greater flexibility for pig farmers and allow handling of pigs at lighter weights. Male pigs were assigned to four groups: surgical castrates, earlier and recommended vaccination and entire male pigs. The surgical castrates were castrated without anesthesia before the age of one week. Vaccinations were performed with two injections of Improvac[®] four weeks apart, at ages 10 and 14 weeks for earlier vaccination and at ages 16 and 20 weeks for recommended later vaccination. Blood samples were collected for all pigs on four occasions during the trial. All other sampling was performed after slaughter, which occurred between 23 and 29 weeks of age. Testes weight and length of the bulbourethral glands were significantly reduced for the vaccinated pigs (p<0.001) with more pronounced effect for the earlier vaccination. Both earlier and recommended later vaccination produced anti-GnRH antibodies shortly after the second vaccine injection and maintained significant levels to the end of the trial (p<0.001). Plasma skatole levels decreased for both vaccination regimens after a delay period. At the end of the trial, the skatole level had decreased for both regimens to the same low level found in surgically castrated pigs (p<0.001). The concentrations of skatole (p<0.01) and androstenone (p<0.001) in fat at slaughter had in vaccinated pigs also decreased to the same low levels found in surgically castrated pigs. In this study, earlier vaccination successfully managed to control the levels of skatole and androstenone in fat, thereby increasing the versatility of vaccination.

Index Terms-Immunocastration, Pig, Boar taint, Skatole, Androstenone.

I. INTRODUCTION

Vaccination against gonadotropin-releasing hormone (GnRH), so called immunocastration, is an attractive alternative to physical castration of entire male piglets to prevent boar taint (Bonneau, Dufour, Chouvet, Roulet, Meadus & Squires, 1994, Dunshea et al, 2001, Cronin, Dunshea, Butler, McCauley, Barnett, & Hemsworth, 2003 and Jaros, Bürgi, Stärk, Claus, Hennessy, & Thun, 2005). Vaccination in commercial herds is performed by two injections with Improvac[®] (Pfizer, Ltd.) during the growing-finishing period, with the second injection given 4-6 weeks prior to slaughter (although there is flexibility on the product label to slaughter up to 10 weeks after the second dose if necessary). However, there is a desire in Sweden to avoid group treatment of heavy pigs (Einarsson, 2006). Zamaratskaia et al (2008) demonstrated that the effects of immunization with Improvac[®] (Pfizer, Ltd.) on the levels of androstenone and skatole lasted up to 22 weeks after the second injection. In the present study, we examined the effects of giving the 2nd vaccine injection to male pigs at a younger age than the manufacturer's recommendation on boar taint compounds and reproductive characteristics. The objective of this study was to evaluate the possibility to vaccinate pigs earlier, at lower weights, and also as a step towards developing a practical carcass sorting tool.

II. MATERIALS AND METHODS

Animals, treatments and sampling

A total of 192 male pigs were used in this study, comprising two trials each with 96 pigs. Data are, at the time of submission, reported for the first trial only. The dams were Swedish Yorkshire (n=20) and the sires were either Swedish Landrace (n=5) or Swedish Yorkshire (n=2). At birth, the piglets within litter were randomly allocated to four equally sized groups. In one group, the piglets were surgically castrated without anesthesia before the age of one week. In two groups, the pigs were vaccinated with Improvac[®] (Pfizer Ltd) according to two different regimens (Fig. 1). Vaccination was performed twice, 4 weeks apart. In the first (earlier) vaccination group, the first injection was given at an age of 10 weeks (live weight (LW) 29.4 kg) and the second injection at an age of 14 weeks (LW 46.8 kg). In the second (later) vaccination group, the first injection was given at an age of 16 weeks (LW 81.8 kg). In the last group, the pigs were kept intact throughout the study.

The growing/finishing period started when the pigs were at an age of 71.5 days and had an initial LW of 29.8 kg. In each pen, there were eight pigs from the same treatment group. All pigs were fed a commercial diet (12.4 MJ ME per

kg, digestible CP 13.5%) twice a day according to the standard feeding regimen for growing/finishing pigs in Sweden (Andersson et al., 1997).

Blood samples were taken by jugular venipuncture from all pigs on four occasions: prior to the first Improvac[®] injections of both vaccination regimens, thereafter at an age of 155.5 days, and one day prior to slaughter. To obtain plasma, blood samples were collected into vacutainer tubes with heparin, separated by centrifugation at 2000 x g and stored at -80° C prior to analysis. To obtain serum, blood samples were taken in vacutainer tubes without heparin, separated by centrifugation at 2000 x g and stored at -20° C until determination of anti-GnRH antibodies.

The pigs were slaughtered at an average age of 178.9 days (25¹/₂ weeks) and an average LW of 116.4 kg. On the transport to the slaughterhouse, pigs from different pens were mixed, in order to simulate normal transport procedures.

Samples of adipose tissue were taken from the neck region of the carcass and kept at -20° C prior to analysis. Testes and bulbourethral glands were removed from entire male and immunized pigs at slaughter, and dissected from extraneous tissue. The length of both bulbourethral glands was measured to record the average length, and testes were weighed as pairs. Vaccination schedules and sampling are graphically represented in Figure 1

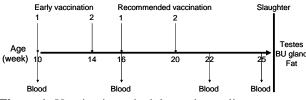


Figure 1: Vaccination schedules and samplings.

Chemical analyses

Analysis of skatole in plasma was performed according to the procedure described by Zamaratskaia, Babol, Andersson and Lundström (2004), modified by Brunius and Zamaratskaia (2010). Analyses of indole, skatole and androstenone in fat were performed according to the procedure described by Chen, Zamaratskaia, Madej and Lundström (2006). Antibodies against GnRH were measured by Frontage Laboratories Co., Ltd., Shanghai, P.R. China, using an in-house validated ELISA method (SHAM-043-R0)

Statistical analyses

Data were analysed with the Statistical Analysis System, version 9.2 (SAS Institute, Cary, NC, USA). The effects of treatment on testes weight, bulbourethral gland length, levels of androstenone, skatole and indole in adipose tissue and levels of skatole in plasma were evaluated using mixed procedure. The model included the fixed factor of treatment (surgical castration, early and recommended vaccination with Improvac[®] and entire males), and the random factors of pen and litter. For the levels of skatole in plasma, the mixed procedure also included sampling occasion as by-statement. The effect of treatment on anti-GnRH titers in serum was evaluated by two-tailed t-tests of immuno- or surgically castrated pigs vs. entire males.

III. RESULTS AND DISCUSSION

Vaccination against GnRH effectively reduced both testes weight (p<0.001, Fig. 2a) and bulbourethral (BU) gland length (p<0.001, Fig. 2b). The reductions were more pronounced for earlier vaccination (EV) than for recommended later vaccination (RV). For RV and entire males (EM) pigs, there were apparent overlaps in the distributions of testes weight and BU gland length. The overlaps between EV and EM distributions were substantially smaller and for testes, the overlap was virtually absent.

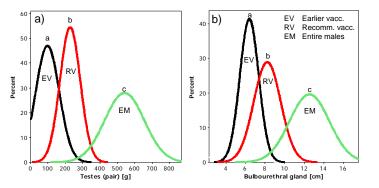


Figure 2: a) Testes weight and b) bulbourethral gland length distributions. Letters a-c indicate significantly different group means (P < 0.05).

The anti-GnRH antibody titer in serum displayed a substantial increase shortly after the second injection of Improvac[®] for both vaccination regimens (Fig. 3). The anti-GnRH titer decreased seemingly exponentially after the initial response. The entire males were included as control and, as expected, did not exhibit any measurable anti-GnRH titer. Noticeable is the highly significant level of anti-GnRH antibodies in the earlier vaccinated pigs 11 weeks after the 2nd injection. An extrapolation of the antibody count (not shown) would indicate that antibodies, and therefore the desired effects of the vaccine, are present longer than the reported safety limit of 10 weeks reported by the manufacturer under our conditions.

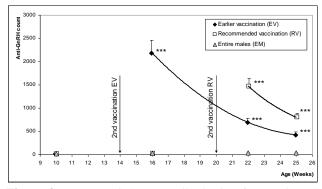


Figure 3: Serum anti-GnRH antibody titer for vaccinated (earlier and recommended later regimens) and entire male pigs. For reference, the dates of the 2^{nd} injection for the two vaccination regimens have been accentuated. *** indicate values significantly different from control/entire males (p<0.001).

After slaughter, the concentrations of indole, skatole and androstenone in fat (Fig. 4) exhibited similar patterns, with low levels in surgical castrates and vaccinated pigs (earlier and recommended regimens) and no difference between these groups. For skatole and androstenone, the concentrations in entire males were significantly higher. In the case of indole, this difference was not significant, due to the large variations within the treatment groups. With threshold values of 0.2 and 1 ppm for skatole and androstenone, respectively, it is of interest to notice that only entire males contained these compounds in concentrations above threshold.

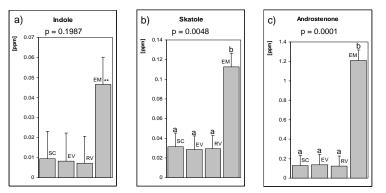


Figure 4: Levels of a) indole, b) skatole and c) androstenone in fat. SC, EV, RV and EM denote surgical castrates, earlier vaccination, recommended later vaccination and entire males. Letters a-b indicate significantly different group means (P < 0.05).

After vaccination, plasma concentrations of skatole do not decrease rapidly (measurement two weeks after 2^{nd} injection), but in later measurements, the levels are comparable to those of surgical castrates (Fig. 5). In fact, at the end of the trial, there is no difference between immuno- and surgically castrated pigs, but a significant leap up to the entire males (p=0.0007) The exact mechanism behind this delayed response is not clear.

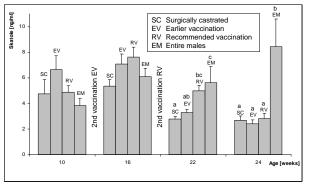


Figure 5: Skatole levels in plasma. For reference, the 2^{nd} injections for the two vaccination regimens have been accentuated. Letters a-c indicate significantly different group means within sampling occasion (P < 0.05). NB: The age axis is not linear.

IV. CONCLUSION

Under our experimental conditions, an early vaccination regimen decreased the concentrations of androstenone, skatole and indole in fat at slaughter to the same low levels found in surgical castrates. Testes weight and bulbourethral gland length were lower in earlier vaccinated pigs compared to pigs vaccinated later according to manufacturer's recommendations. Thus, in this study, the effect of Improvac[®] at earlier vaccination was shown to comfortably exceed the 10 week safety limit reported by the manufacturer, suggesting the possibility of greater flexibility in the application of immunocastration, should this be desired. However, to ensure that pork supplied to consumers is free of boar taint further data are needed, covering a range of breeds and production systems.

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REFERENCES

Andersson, K., Schaub, A., Andersson, K., Lundström, K., Thomke, S. & Hansson, I. (1997). The effects of feeding system, lysine level and gilt contact on performance, skatole levels and economy of entire male pigs. *Livestock Production Science*, 51, 131-140.

Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, J. (1994). The effects of immunization against luteinizing hormonereleasing hormone on performance, sexual development and levels of boar taint-related compounds in entire male pigs. *Journal of Animal Science*, 72, 14-20.

Brunius, C. F. & Zamaratskaia, G. (2010). Improved high-performance liquid chromatographic method for the determination of skatole and indole in porcine plasma. Presentation at EAAP Working Group meeting, 18-19 March, Bristol, UK, *Manuscript in preparation*.

Chen, G., Zamaratskaia, G., Madej, A. & Lundström, K. (2006). Effect of hCG administration on the relationship between testiocular steroids and indolic compounds in fat and plasma in entire male pigs. *Meat Science*, 72, 339-347.

Cronin, G. M., Dunshea, F. R., Butler, K. L., McCauley, I., Barnett, J. L. & Hemsworth, P. H. (2003). The effects of immuno- and surgical-castration on the behaviour and consequently growth of group-housed, male finisher pigs. *Applied Animal Behaviour Science*, 81, 111-126

Dunshea, F. R., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K. A., Lopaticki, S., Nugent, E. A., Simons, J. A., Walker, J. & Hennessy, D. P. (2001). Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science*, 79, 2524-2535.

Einarsson, S. (2006), Vaccination against GnRH: pros and cons. Acta Veterinaria Scandinavica, 48 (Suppl. 1), S10.

Jaros, P., Bürgi, E., Stärk, K. D. C., Claus, R., Hennessy, D. & Thun, R. (2005). Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. *Livestock Production Science*, 92, 31-38.

Zamaratskaia, G., Babol, J., Andersson, H., & Lundström, K. (2004). Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. *Livestock Production Science*, 87, 91-98.

Zamaratskaia, G., Rydhmer, L., Andersson, H. K., Chen, G., Lowagie, S., Andersson, K. & Lundström, K. (2008). Long-term effect of vaccination against gonadotropin-releasing hormone, using ImprovacTM, on hormonal profile and behaviour in male pigs. *Animal Reproduction Science*, 108, 37-48.