Effect of vaccination against gonadotrophin-releasing hormone, using Improvac®, on growth performance, body composition, behaviour and acute phase proteins

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Abstract

The objective of this study was to evaluate the effect of vaccination against GnRH on performance traits, pig behaviour and acute phase proteins. A total of 120 pigs (36 non castrated males, NCM; 36 males to be vaccinated, IM; 24 castrated males, CM; and 24 females, FE) were controlled in groups of 12 in pens with feeding stations allowing the recording of individual feed intake. The two vaccinations (Improvac®) were applied at a mean age of 77 and 146 days. All pigs were individually weighed every 3 weeks from the mean ages of 74 to 176 days and backfat thickness (BT) and loin-muscle depth (LD) were also recorded ultrasonically. Twelve group-housed pigs for each treatment were video recorded during 2 consecutive days at weeks 9, 11, 20, 21, 23 and 25 of age to score the number of inactive or active pigs in each treatment group by scan sampling. Aggressive behaviour by the feeder and away from the feeder, and mounting behaviour was also scored by focal sampling. Blood samples from 12 NCM, 12 CM and 12 IM were taken to determine the concentration of circulating acute phase protein Pig-MAP at weeks 1, 2, 4, 11, 13, 21 and 25 of age. After slaughter, the number of skin lesions on the left half carcass was scored. IM presented overall a higher growth rate and daily feed intake compared to NCM ($P<0.05$), whereas their feed conversion ratios did not differ significantly. In comparison with CM, IM presented a better feed conversion ratio ($P<0.05$), since their overall daily weight gain did not differ significantly, but IM ate less. Final lean meat percentage of IM and CM was lower compared to that of NCM ($P<0.05$). Activity, mounting and aggressive behaviour of NCM was higher than in IM, CM and FE after the second vaccination. Pig-MAP concentrations were significantly elevated just after surgical castration and after both administrations of the vaccine ($P<0.05$), but concentrations subsequently decreased throughout time. Skin lesions of NCM were significantly higher compared to that of IM and FE ($P<0.05$). The effects of vaccination were especially remarkable after the second dose, when the higher feed intake and lower activity of IM compared to NCM might result in higher final body weight and more fat. Results from this study indicate that some welfare aspects such as a reduced aggression and mounting behaviour may be improved by vaccination against GnRH, together with productive benefits like adequate feed conversion ratio and daily weight gain.

Keywords: entire male pigs, boar taint, immunocastration, performance, behaviour

Introduction

Surgical castration of male pigs at an early age is carried out in most countries to prevent boar taint, increase intramuscular and subcutaneous fat content for certain quality products and prevent aggressive behaviour. However, welfare concerns of consumers are increasing the pressure on the pig industry to abandon this practice. In Norway, surgical castration was banned completely in 2009. In Switzerland, castration of young piglets without pain relief is prohibited from 2010. Banning surgical castration is also under consideration in other European countries. Therefore, the number of studies addressing the issue of alternative methods has increased as reviewed by Prunier et al. (2006). Moreover, the consumer acceptance of the meat from the different sexes has been recently reported (Font i Furnols, et al., 2008).

At present, three farm level alternatives to surgical castration are available: castration with anaesthesia, production of entire males with management practices to reduce boar taint and vaccination against gonadotrophin-releasing hormone (GnRH), also known as immunocastration. For the latter, one method is to inject a modified form of GnRH conjugated to protein (Improvac®, Pfizer Ltd., formerly CSL Limited, Parkville, Victoria, Australia) to induce the formation of antibodies against GnRH, which bind to endogenous GnRH, and prevents it from stimulating LH and FSH secretion by the pituitary gland. Vaccination comprises two administrations during the growing-finishing period, at least 4 weeks apart. Commonly, the second injection is applied 4-6 weeks before slaughter and most studies have focused on the response in a short period after vaccination (Cronin et al., 2003; Pauly et al., 2008), but Zamaratskaia et al. (2007) found that the product could have an extended effect compared with that currently implied by the directions of use. Rhydmer, Lundström and Andersson (2010) have recently suggested that the time span within which the vaccination could be performed is large, taking into account that it is not after the second injection that the full effect is observed and that the right time for this second injection may vary with pigsô genetic background and environment.

Some studies have reported that the performance traits of vaccinated males are similar to those of entire males until the administration of the second dose but subsequently resemble barrows (Cronin et al., 2003; Moore et al., 2005; Pauly et al., 2008). However, not all studies comparing vaccinated pigs, castrates and entire males have reported a similar pattern in performance. Zamaratskaia et al. (2008) found that there were no differences between these different genders in most performance parameters except weight gain, whereas Dunshea et al. (2001), Chumkam and Ravungsook (2003) or Pauly et al. (2009) presented significant differences. Different productive patterns of entire males compared to castrates or vaccinated pigs may be the result of the combination of endocrine controlled behaviours, such as higher physical and sexual activity or aggression of entires (Gray, 1971; Cronin et al., 2003) and their lower feed intake (Pauly et al., 2008). It has been suggested that the growth performance and carcass characteristics of vaccinated pigs may be considerably influenced by two factors: the growth potential of entire males before the second vaccination and the time interval between the second vaccination and slaughter (Dunshea et al, 2001). Moreover, group housing is known to affect the productive potential of individual pigs by introducing or mediating some of the previous factors such as social interactions, space allowance or food availability (Pauly et al., 2009).

The objectives of this study were: (1) to compare the performance traits of vaccinated pigs (IM), surgical castrates (CM), non castrated males (NCM) and females (FE) in a group-housing situation recording individual feed intake, (2) to relate these performance traits with behavioural patterns and (3) to evaluate the effect of vaccination on circulating acute phase proteins.

Material and Methods

Animals, housing and treatment

One hundred and fifty piglets (28 males castrated under 7 days of age (CM), 29 females (FE), and 93 non-castrated males (NCM)) were moved from a commercial farm with high health status to the weaning unit at IRTA-Monells at a mean age of 21 days. The piglets had a high meat yield potential, since they were crosses from Duroc x Landrace hybrid sows with Ryr(1) recessive homozygous (nn) Pietrain boars. The piglets were selected from a total of 33 litters born within a 10-day interval. No more than one FE and one CM were taken from each litter, and animals with extreme body weights were not selected. On the farm of origin, IRTA technicians individually identified the 150 piglets using a 4-digit printed ear tag.

At a mean age 59 days, 120 pigs were enrolled on the study ensuring the highest homogeneous body weight possible for all the treatment groups. Thirty-six NCM, 36 males to be vaccinated with Improvac® (IM); 24 CM and 24 FE were selected and identified using an 8-digit electronic chip that permitted the recording of individual feed intake.

The 120 selected pigs were transferred to the monitoring barn at a mean age of 67 days. Pigs were allocated per treatment to 10 fattening pens so that there were 12 pigs per pen whose liveweight was similar as possible (ie NCM, IM, FE and CM were in separated pens). As shown in Figure 1, there were three pens of pigs of NCM and of IM and two pens of CM and of FE.

Ventilation and temperature at the experimental barn were mechanically controlled. Each pen measured 3.7 x 3.6 m, had a partly slatted floor comprising 60% solid concrete and 40% slatted) and had one drinking bowl. Each pen was equipped with an IVOG®-station (INSENTEC, Marknesse, The Netherlands). The feeding station consisted of a single-space food hopper with a trough which weighed continuously and had an electronic identification system that was activated by ear responders as pigs entered the station. The feeding station was connected by a load cell to a computer and the trough refilled if the amount of food left after a completed pig visit was < 10kg. Each time a pig visited the feeder, time, the pig identification number and weight of the food at the beginning and at the end of the visit were recorded automatically (ie all food in the feeding trough at the beginning and at the end of each visit was weighed and consumption was calculated as the difference). Food consumption per visit was calculated with an accuracy of 10g. To enable competition for food, the entrance of the hopper was always open.

All pigs were fed the same commercial diet (14082.6 KJ DE/kg, 179g crude protein/kg, 70 g crude fat/kg, 19.5g lysine/kg, 65.5g ash/kg).

Improvac® which contains 200µg GnRH-protein conjugate/ml in an aqueous adjuvant system was administered twice by technical staff in accordance the manufacturerõs instructions. Each pig in group IM was injected 2ml injected subcutaneously just behind and below the base of the ear, at a mean age of 77 days and 146 days.

Productive and body composition measurements

All pigs were individually weighed every 3 weeks from the start of the study at the mean ages of 74 days until the mean age of 176 days. Pigs were slaughtered at the mean age of 180 days. In addition, backfat thickness (BT) and loin-muscle depth (LD) were also recorded ultrasonically every three weeks using the portable equipment PIGLOG 105 version 3.1 (SFK-Tehcnology, SØborg, Denmark). BT (mm) and LD (mm) were both measured at the level of the last rib, 4cm away from the mid line, on both the left and the right sides.

Daily feed intake (DFI) was determined by summing all partial consumptions per pig per day. Individual feed intake was recorded from the day of first monitoring to the day of the last BW and ultrasound measurements.

Average daily gain (ADG, g/day) and feed conversion ratio (FCR) of each pig were calculated for the entire study period. In addition, percentage of protein and percentage of lipids were estimated from BT and LD using the following equations (Gonzàlez, 2002).

Protein %= 15,5595 ï 0,18197 * BT (mm) + 0,05517* LD (mm)

Lipid %= 1,339 + 0,994 * BT (mm) + 0,0918*LD (mm)

The 120 pigs were slaughtered in five batches of 25 pigs, so that pigs from all treatment groups were slaughtered in the same proportions at each slaughtering, with selection priority given to the heaviest pigs in each treatment group.

Behavioural studies, skin lesions and acute phase proteins sampling

One pen of 12 pigs in each treatment was video recorded during 2 consecutive days during week 9 of age after transfer to monitoring barn; week 11, on the second and third day after administration of the first dose of Improvac®; week 20, two days before administration of the second dose of Improvac®; week 21, on the second and third day after administration of the second dose of Improvac®; week 23, two weeks after administration of the second dose of Improvac®; and week 25, 48 hours before slaughter. At week 23, only IM and NCM were filmed. To obtain a more representative sample of the behaviour, films were obtained during 2-hour sessions on each recording day (i.e. 9ï11h; 14ï16h and 18ï20h). Scan sampling at 5 min intervals was used to record the number of inactive and active pigs. A pig was considered active if it was standing, drinking or eating. Pigs sitting or laying down were recorded as inactive. In addition, during the last 10 minutes of every hour of each recording session continuous focal sampling was used to record the number of aggressive interactions by the feeder and away from the feeder and the number of sexual interactions. Mounting behaviour, i.e., when an animal placed one or two forelimbs on another animalôs loin at any point, was regarded as a sexual interaction.

Skin lesions were evaluated at IRTA-Monells experimental processing plant after slaughter. The number of skin lesions on the left half carcass of 23 CM, 24 FE, 35 NCM and 36 IM were counted following an adaptation of the Meat and Livestock Commission scale (MLC, 1985).

Blood samples from 12 NCM, 12 CM and 12 IM were taken to determine the concentration of acute phase protein Pig-MAP. Blood samples were obtained on seven occasions throughout the experimental period for NCM and IM: at week 1 on the farm of origin, week 2, two days after surgical castration of the piglets in group CM, week 4, one week after arrival at the experimental weaning unit, week 11, two days after the first vaccination, week 13, two weeks after the first vaccination, week,21, 2 days after the second vaccination and week 25, at slaughter. No samples were taken from pigs in group CM during weeks 11 and 21. Blood samples were taken from the upper vena cava in 10 ml test tubes without anticoagulant. Blood was then centrifuged at 3000 rpm for 10 minutes and serum was collected in Eppendorf tubes then frozen at ï 21ºC until they were analysed. Samples were analysed for Pig-MAP concentration using a commercial ELISA kit (pigMAP kit ELISA, PigCHAMP Pro Europa S.A.), based on the method described by Piñeiro et al. (2009).

Statistical analysis

Data were analysed using SAS v9.1. (SAS Institute, Cary, NC, USA). The effect of vaccination on growth and body composition was analysed for each pig before second vaccination at mean age 146 days age, from the second vaccination to slaughter and for the entire study period. The MIXED procedure was used for these analyses. For the first analysis, before and after second vaccination measurements were considered repeated measurements and analysed as such. The model included sex and measurement and its interaction as fixed effects, pen as a random effect and initial body weight. For the entire study period data, the fixed effect of the MIXED model was sex and pen the random effect. Initial body weight was included as a covariable in both analysis.

For the behavioural data, only descriptive statistics were generated as only one pen per treatment was examined. For general activity behaviour, the mean percentage of active animals in each period was calculated.

The number of skin lesions was analyzed using the GLM procedure with gender as a fixed effect. The day of slaughter was treated as a blocking effect for the analysis. Pig-MAP serum concentrations were analyzed using the MIXED procedure with sample number as a repeated measure, and gender, sample number, and gender*sample number interaction as fixed effects and animal as a random effect.

Differences were regarded as significant if $P<0.05$. Least squares means were compared using the PDIFF option.

Results

Performance results

Details of body weight and average daily gain are shown in Table 1. There was no significant difference in least squares mean body weight between pigs in the four treatment groups at the beginning of the fattening period. At the time of second vaccination, least squares mean bodyweight of females (corrected by initial body weight) was significantly higher than CM, showing IM and NCM an intermediate weight. At slaughter, there was no significant difference between CM and IM in body weight.

Until second vaccination, average daily gain was highest in castrated pigs ($P<0.05$). However, after second vaccination, least squares mean average daily gain was highest in vaccinated pigs ($P<0.05$) and, over the entire study period, there was no significant difference in least squares mean average daily gain between castrated males and vaccinated pigs.

Until the time of second vaccination, least squares mean feed consumption of castrated pigs was significantly higher than in the other three groups (*P*<0.05). However, after second vaccination least squares mean feed consumption was significantly higher in vaccinated pigs than the other three groups (*P*<0.05). The overall feed consumption of CM and IM did not differ significantly.

Least squares mean feed conversion ratio was significantly lower in the IM group from the beginning of the fattening period until second vaccination, from second vaccination to slaughter and during the fattening period as a whole compared with castrated pigs (*P*<0.05); and significantly lower than in females from second vaccination until slaughter and throughout the fattening period as a whole (*P*<0.05). Over the entire fattening period, the least squares mean feed conversion ratio of vaccinated pigs was 9% lower compared with castrated pigs, and 4% lower than females.

Body Composition

Table 2 shows the backfat depth, loin depth, protein percentage and lipid percentage of carcases.

At the time of second vaccination, least squares mean backfat and carcase lipid percentage were significantly higher in castrated males than in the other groups (P<0.05), but there was no difference between the other three groups. However, at the end of the fattening period, backfat thickness and carcase lipid percentage were both significantly higher in castrated and vaccinated pigs compared with entire males and females. (*P*<0.05), and least squares carcase lipid percentage was lowest in entire males (*P*<0.05). Therefore, the evolution (mm/kg) of backfat and lipid percentage over this period for the NCM was significantly lower ($P<0.05$), compared to the CM and IM, with no significant differences amongst those two.

There were no significant differences in loin depth between groups at any point in the study. At the time of second vaccination, least squares mean carcase protein percentage was significantly lower in castrated pigs compared with the other three groups ($P<0.05$). At the end of the fattening period, least squares mean carcase protein percentage was lower in castrated males and vaccinated pigs compared with entire males ($P<0.05$), but there was no significant difference between vaccinated pigs and females. This indicates that the gain in protein percentage for this period in the IM was significantly ($P<0.05$) lower compared to the other groups.

Behavioural studies and Pig-MAP concentrations

Before first vaccination, the incidence of active pigs was higher in IM and NCM than in CM, but similar to FE (Figure 2). In the 2-3 days after the first administration, IM had lower activity compared with NCM. The activity of IM increased again 9 weeks after the first vaccination (week 20 of age). Two weeks after the second administration (week 23 of age), the incidence of active pigs was lower in IM compared with NCM. Two days before slaughter IM, CM and FE groups all had lower activity compared with NCM.

Both at-feeder and off-feeder aggressive behaviour were similar between treatments until week 25. Prior to slaughter, at-feeder aggression was only observed in NCM compared to the other three genders (mean number of aggressions: 0.83 vs. 0.00, respectively). Off the feeder, NCM were more aggressive than IM and FE (4.00 vs. 0.25 and 1.25 respectively).

The number of mounts was similar among treatments until the first vaccination (Figure 3). At week 11 of age, the number of mounts was higher in NCM than in CM but similar to IM and FE, but this pattern disappeared 9 weeks after first vaccination at week 20 of age. Two days after the second vaccination (week 21), NCM showed more mounts than CM and FE. However, two weeks after the second vaccination, the number of mounts in NCM was higher in NCM than in IM. During the days prior to slaughter, the number of mounts in NCM was also higher than in MC, IM and FE.

After slaughter, the group with the significantly highest number of skin lesions was NCM, higher than IM and FE (6.00 vs. 4.05 and 3.98, respectively, $P<0.05$). The number of skin lesions of CM (4.49) did not differ significantly from the other three genders.

Figure 4 shows the Pig-MAP concentrations over time for CM, IM and NCM. Comparisons were made both across samplings and between genders. Pig-MAP concentrations significantly increased after surgical castration (0.94 vs. 1.37). Nevertheless, during subsequent samplings this value gradually reduced and it became significantly lower at 12 weeks of age and at slaughter, compared with the level after surgical castration. For IM, Pig-MAP concentration was significantly increased after the administration of the vaccine, more remarkably after the first dose. However, Pig-MAP concentrations decreased after both vaccinations and values obtained two weeks and four weeks after first and second vaccination, respectively, did not differ significantly from those found before. For NCM, no major changes occurred among any of the samplings. When comparing genders, a significantly higher Pig-MAP concentration was observed in IM compared with NCM and CM in samples collected both the first and second vaccination. No significant differences were observed in the reminder of samples.

Discussion

The main objectives of this study were to evaluate the effect of vaccination against GnRH on growth performance, behaviour and some physiological parameters, trying to determine if behavioural responses were in accordance with performance results and could partly explain them.

Effect of Vaccination Against GnRH on Productive Traits and Body Composition

Overall, results for the entire growing period showed that IM had a higher ADG, DFI but similar FCR compared with NCM. These results agree with those presented by Moore et al. (2005) and Dikeman (2007). As suggested by previous studies, pigs vaccinated with Improvac may be regarded as entires until the second vaccination (Dunshea et al., 2001; Pauly et al., 2009) which is consistent with results found in the present study. The differences between NCM and IM in ADG and DFI were especially remarkable after second vaccination, presenting, however, both genders a non different feed efficiency during this period (Table 1). Vaccination against GnRH suppresses production of testosterone and oestrogens, reducing aggression and sexual activity through reduced male activity (Cronin et al., 2003; Mackinnon and Pearce, 2007) and enhancing feed intake (Cronin et al., 2003). Both effects were found in the present study, the feed intake of IM being particularly increased after the second vaccination.
For the entire experimental period, growth rate was not significantly different between IM and CM but DFI was lower for IM. Again, these differences were mostly associated with the performance patterns of IM after the second administration of the product. Similar to results presented by Jaros et al. (2005) and Pauly et al. (2009), IM grew more

and were more efficient than CM after second vaccination. Pauly et al. (2009) suggested that the known anabolic potential of entire males compared with castrates, positively affected the productive results for IM and that these benefits were not lost after the second vaccination.

Body composition results obtained in the present study agree with previous investigations (Dunshea et al., 2001; Pauly et al., 2009; Zamaratskaia et al., 2008). IM were leaner compared with CM, but had a higher lipid content compared to NCM. This again was mainly related to the effect of the second administration, since no differences in body composition were observed between IM and NCM before that. The higher feed intake combined with the lower social and sexual activity of IM after the second vaccination would have provided the large demand of energy supply for fat deposition, resulting in this higher lipid percentage of IM compared to NCM.

Effect of Immunisation Against GnRH on Behaviour and Acute Phase Proteins Response

Prunier et al. (2006) suggested that the absence of testicular hormones after surgical castration has an influence on behaviour. In accordance with our observations, one of these changes is a decrease in general activity in the group. In CM, this reduced activity compared with NCM was observed from age 10 weeks and it remained higher throughout the production process.

A general decrease in activity among IM was observed during the three days after the first vaccination. During this period, the percentage of animals that were inactive was lower in IM than in NCM, but similar to CM and FE. However, the activity of IM increased again by the time of the second observation nine weeks later. These results agree with those published by Cronin et al. (2003) which showed that two weeks after first vaccination IM were more active than CM and the same as NCM. However, these authors did not observe the pigs just after the first vaccination. A possible hypothesis to explain this reduced activity after the first administration might be a temporary acute pain response caused by an inflammatory reaction after the subcutaneous injection. Nevertheless, Improvac® is an aqueous suspension, and previous authors have suggested that it results in a very small reaction at injection site (Dunshea et al., 2001; Einarsson, 2006). The significant difference in activity between NCM and IM was not observed immediately after the second vaccination. However, as reported by Cronin et al. (2003), a decreased activity of IM was observed two weeks after the second vaccination and two days before slaughter. In these measurements, the behaviour of IM was similar to that of CM being likely influenced, as Prunier et al. (2006) suggested, by the inactivation of the endogenous hormones. Recent results presented by Rydhmer et al. (2010) have also found a reduced level of aggressive and sexual behaviour, one week after the second injection.

Differences in at-feeder and off-feeder aggression between genders were observed during the late finishing period. NCM demonstrated the highest incidence of aggressive and sexual behaviours, both of which are influenced by gonad steroids. As found in previous studies (Lundström et al., 1987; Giersing et al., 1998; Cronin et al., 2003), sexual hormone inhibition following vaccination against GnRH and surgical castration results in decreased aggression and mounting behaviour. Aggression and mounts are an animal welfare problem: first, because they cause injuries, pain and, in extreme cases, death; secondly, this behaviour together with increased general activity may stress pigs, depress the immune system and decrease feed intake (Fraser and Rushen, 1987). In this study, a decreased number of carcass skin lesions in IM and CM compared with NCM were observed, thus improving carcass quality.

Increased serum Pig-MAP concentrations were observed in IM two days after the two vaccinations. Plasma release of this acute phase protein is stimulated by stress related hormones such as adrenocorticotropic hormone (ACTH) and glucocorticoids (Gruys et al., 1994), by non-specific reactions after tissue damage that can also appear as a result of infectious, inflammatory or neoplastic processes, or together with an immune response (Le Floc'h, 2003). The increase in Pig-MAP concentrations observed after surgical castration by Geers et al. (2003) could be associated with stress or an infectious and inflammatory process. Given that Improvac is an aqueous suspension and results in a limited reaction at the injection site (Dunshea et al., 2001), and that the vaccination with Improvac stimulates the immune system to produce GnRH-specific antibodies, it is most likely that the immune reaction induced in this study accounts for the increased serum Pig-MAP concentration observed.

Conclusions

The results of this study suggest that vaccination against GnRH could be associated with welfare improvements during sexual maturity by reducing sexual and aggressive activity, together with productive benefits such as better growth rate and feed conversion ratio.

Acknowledgments

The authors would like to thank Albert Fontquerna and Toni Muñoz at Centre dôAvaluació del Porcí for their technical assistance. Furthermore, the authors also thank Pfizer GmbH for the financial, technical support and comments on the first draft of the paper.

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Figure captions

Figure 1. Per-pen animal distribution in the experimental barn (pigs distributed by treatment and assigned by weight)

Figure 2. Percentage of active pigs for the different genders and weeks

Figure 3. Number of mounts for the different genders and weeks

Figure 4. Pig-MAP concentrations for the IM, NCM and CM during all monitoring period