Effects of an additional iron injection on growth and humoral immunity of weanling pigs

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Abstract

In a 5-week study, 120 crossbred weaning pigs (age 4 weeks) were used to examine the effects of an additional Fe-injection and immunization moment on the growth and humoral immune response. Pigs were allotted to one of eight treatments based on body weight and litter origin. Factors included: (1) single intramuscular injection (day 3 after birth) vs. double intramuscular injection (days 3 and 21 after birth) of 200 mg Fe; (2) antigen challenge (Keyhole limpet hemocyanin (KLH) and ovalbumin (OA) vs. placebo); and (3) immunization moment (day −1 vs. 1 after weaning). All pigs had free access to prestarter diets supplemented with 80 mg kg −1 Fe as FeSO4·7H2O. Pigs were housed in a room consisting of 12 pens (ten pigs per pen). During the experiment, blood hemoglobin (Hb) levels and body weight were determined weekly and total Ig, IgG and IgM titers to KLH and OA twice a week. At day 6 after weaning, the blood Hb levels of double injected pigs were higher (P < 0.01) than the blood Hb-levels of the pigs that had received a single Fe-injection. Prior to day 6 and thereafter, blood Hb levels were similar both for single and double injected groups. Throughout the experiment, the additional Fe-injection, and immunization moment had no effect (P > 0.1) on growth and total Ig response to KLH and OA. But the genotype of the pigs affected (P < 0.05) the kinetics of the total Ig response to KLH and OA. In conclusion, an additional injection of Fe one week prior to weaning does not affect body growth, and does not enhance or support the humoral immunity to a T-cell dependent antigen. Furthermore, the suppressing effect of weaning on humoral immunity was not affected by additional Fe. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The first 2 weeks after weaning are a critical period in the nursery phase of pigs. This period is often associated with depressed feed intake and
growth as well as health problems. Social, dietary and immunological stressors resulting from the process of weaning together with the occurrence of pathogens (e.g. specific Escherichia coli strains) are considered as main risk factors (Bourne, 1986; Nabuurs, 1991; Van Beers-Schreurs et al., 1992; Makkink, 1993).

Iron (Fe) is a nutrient related to health and immunity (Brock, 1994). Administration of iron (200 mg Fe as iron dextran per piglet) on day 2 or 3 after birth to prevent anemia is a common practice in modern pig husbandry. Kamphues et al. (1992) showed that an additional Fe-injection on day 21 after birth resulted in a higher growth post weaning. Furthermore, they suggested that the improved growth was associated with a lower incidence of diarrhoea. Recently, it was shown that a minor difference in Fe status has an effect on the metabolism (Gentry et al., 1997) and immunity (Schrama et al., 1997) of weanling pigs. The role of iron in maintaining health is, apart from anaemia prevention, still not completely clear. Hershko (1993) showed an optimal immune responsiveness at physiological normal levels of iron in human blood. An increase as well as a decrease in blood iron levels had negative effects on immune responsiveness (De Sousa et al., 1991; Brock, 1994). Additionally, it was shown (Gwazdauskas et al., 1978; Haye and Kornegay, 1979; Blecha and Kelley, 1981) that weaning resulted in reduced immunoglobulin production, probably due to the stress associated with weaning.

In this study, the effects of an additional iron injection were examined on growth and humoral immune response. A secondary aim was to examine whether the humoral immune responsiveness was affected by weaning.

2. Materials and methods

2.1. Animals

For this experiment, 120 weanling barrows and gilts of approximately 4 weeks of age with a mean BW of 7.6 kg (S.E.M. = 0.11) were used. Pigs originated from four Dutch Landrace (DL), five Finnish Landrace (FL), and six Great Yorkshire (GY-z) rotational bred sows and Great Yorkshire terminal boars (GY-s). Based on this rotational breeding system, pigs were divided into three groups that differed in major genotype: DL, FL and GY-z (Table 1). The sow line that contributed 28.1% to the genetic composition of the pig was considered the major genotype. All pigs received an intramuscular Fe injection (200 mg Fe, Fe dextran; AUV Cuijk) on day 3 of age. Pigs had no access to supplemental Fe (i.e. creep feeding, soil) during the suckling period.

2.2. Experimental design

The experiment was conducted following a $2 \times 2 \times 2$ factorial arrangement of treatments. The first factor was an additional intramuscular injection with 200 mg Fe (FE; 2 ml Fe-dextran; AUV Cuijk) on day 21 of age or 2 ml of a placebo (SALINE; PBS solution). The second factor was an intramuscular injection with 2 ml of an antigen cocktail (IMMU; 1 mg Keyhole limpet hemocyanin (KLH)), 4 mg ovalbumin (OA), and 15 μg Lf tetanus toxoid (TT) in a 1:1 v/v Specoll:PBS solution (ID-Lelystad, Lelystad, The Netherlands) or 2 ml of a placebo (PLAC; 1:1 v/v Specoll:PBS solution). The third factor was immunization 1 day before weaning (D$^{-1}$) or 1 day after weaning (D1). The antigen cocktail was used to elicit a T-cell-dependent humoral immune challenge.

The experimental design resulted in eight treatment groups. The four control groups that received a placebo injection (PLAC) consisted each of ten pigs, whereas, the four experimental treatments consisted each of 20 pigs. Pigs were assigned according to weight, genotype, and blood Hb status to one of the eight treatment groups at the end of the third week in the suckling period.

<table>
<thead>
<tr>
<th>Major genotype (%)</th>
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<tr>
<td>GY-z</td>
</tr>
<tr>
<td>Finnish Landrace</td>
</tr>
<tr>
<td>Dutch Landrace</td>
</tr>
<tr>
<td>Great Yorkshire sow-line</td>
</tr>
</tbody>
</table>

$^a$ GY-z, percentage of Great Yorkshire sow-line; DL, Dutch Landrace; FL, Finnish Landrace; GY-s, Great Yorkshire terminal-boar-line.
2.3. Housing and feeding

After weaning pigs were housed in 12 pens (2.65 × 1.25 m; ten pigs per pen). All pens accommodated pigs of all treatment groups. The pens had partially solid, electrically heated, floors and tri-bar metal slats. The room was equipped with a computer-controlled heating and mechanical ventilation system. The first 2 weeks after weaning a room temperature of 27°C was maintained. Afterwards, room temperature was gradually decreased to 20°C (1.5°C per week). The first two weeks of the experimental period floor temperature was set at 32°C. Thereafter, floor heating was turned off.

Pigs had free access to water and pelleted commercial diets using nipple drinkers and dry feeders, respectively. The first two weeks after weaning pigs were fed a prestarter diet (14.3 MJ ME kg⁻¹ and 1.03% apparent ileal digestible lysine). Thereafter, all pigs were fed a starter diet (13.6 MJ ME kg⁻¹ and 0.93% apparent ileal digestible lysine). Both diets were supplemented with 80 mg Fe as FeSO₄·7H₂O per kg feed. All nutrients met or exceeded NRC (1998) recommended levels.

2.4. Measurements

Pigs were weighed 1 week before weaning (d −8) and on the day of weaning (d 0). After weaning, pigs were weighed on days 6, 13, 20, 27 and 34. On the days of weighing, blood samples were taken using an ear prick and capillary tube (5 μl) for analysis of blood hemoglobin (Hb) concentration. Blood Hb was determined using a miniphotometer (AMES™ Minilab; Miles Inc., Elkhart, IN) and a reagent kit (AMES™ Mini-pak hemoglobin test pack; Miles, Inc.).

Blood samples (5 ml) were taken from the jugular vein on days 0, 7, 11, 14, 18, 21, 25, 28, 32, and 35 after weaning for the analyses of total immunoglobin (Ig), IgM and IgG titers against KLH, OA and TT. Collected blood samples were immediately placed in warm water (37°C) for 1 h to stimulate blood coagulation. Thereafter, blood samples were centrifuged (2250–2500 rpm) for 20 min and serum samples were collected and stored at −20°C. Antigen-specific in vivo antibody titers were determined by an indirect two step ELISA procedure. Serial dilutions of serum were applied to antigen-coated wells (1 μg KLH, 1 μg OA, or 0.2 μg TT per ml) of a microtiter plate and incubated for 1 h at room temperature. Subsequently 1:8000 diluted horseradish-peroxidase (PO) conjugated rabbit anti-swine H + L (R·Sw-IgG (H + L)/PO; Cappel, Gaithersburg, MD) for total immunoglobulin (Ig), 1:8000 diluted rabbit anti-IgG Fc (R·Sw-IgG (Fc)/PO; Nordic, Tilburg, The Netherlands) for IgG, or 1:8000 diluted goat anti-swine IgM Fc (GaSW-IgM(Fc)/PO; Nordic) for IgM was added during 1 h at room temperature. After washing, tetramethylbenzidine (TMB; Sigma, St. Louis, MO) was added as a substrate. Color formation was stopped with 2.5 N sulfuric acid after 10 min. All absorbances were expressed relative to the absorbance of a standard positive control serum. The titer of sera against KLH, TT, or OA in a sample was that dilution that showed an extinction closest to 50% of E_MAX, where E_MAX represents the highest mean extinction of a standard positive (pooled) serum (Frankena, 1987).

2.5. Statistical analyses

All data were analyzed with a pig as the experimental unit. The effect of the additional Fe injection, immunization procedure and immunization moment on growth, blood Hb levels and antibody production were tested with the GLM procedures of SAS (1989) by means of F-tests using a split-plot model, with values in time of individual animals taken as repeated measurements:

\[ Y_{ijklmn} = \mu + F_i + I_j + T_k + G_l + \text{interaction} + e_{1,ijklmn} + D_n + \text{interaction} + e_{2,ijklmn} \] (1)

where \( Y_{ijklmn} \) = a specific trait per animal; \( \mu \) = overall mean; \( F_i \) = fixed effect of additional injection with iron \( (i=1,2) \); \( I_j \) = fixed effect of immunization procedure \( (j=1,2) \); \( T_k \) = fixed effect of immunization moment \( (k=1,2) \); \( G_l \) = fixed effect of major genotype \( (l=1,2,3) \); \( e_{1,ijklmn} \) = error term 1, which represented the random effect of animal \( m \) nested within the additional iron/saline injection \( i \), immunization procedure \( j \), immunization moment \( k \), and major genotype \( l \); \( D_n \) = fixed effect of sampling day \( n \); and \( e_{2,ijklmn} \) = error term 2, which represented the random
effect within animals between sampling days. The effects of the additional iron injection, immunization procedure, immunization moment, genotype and their interactions were tested against error term 1. The effect of sampling day and all interaction effects with sampling day were tested against error term 2.

3. Results

Preliminary analysis of the various antibody titres with model (1) showed the presence of several three way interaction effects between immunization procedure, sampling day and the other effects. Furthermore, preliminary analysis within the PLAC treatment showed that there were nearly no effects of an additional iron injection, immunization moment and genotype. Therefore, the effects of an additional iron injection, immunization moment and genotype were tested only in immunized animals using a similar model as (1) omitting the effect of immunization.

In general, immunized pigs mounted similar systemic antibody responses directed to KLH and OA. Therefore only the results of the KLH challenge will be presented. The antibody response to TT will not be shown because it was masked by TT resembling antigens at the time of analysis (cross-reactivity).

3.1. Blood hemoglobin concentration

The primary aim of this study was to assess the effect of an additional iron injection for weanling pigs on day 21 on their Hb status, growth and humoral immune response. Averaged over the total experimental period Hb concentration of FE pigs was higher ($P<0.05$, Table 2) than that of SALINE pigs (6.8 vs. 6.7 mmol l$^{-1}$). Averaged over the experimental period, Hb concentration was not affected by immunization procedure, immunization moment and genotype (Table 2). Although blood Hb level of FE pigs was higher ($P<0.01$; Fig. 1) at day 6 after weaning, there was no interaction ($P>0.1$) between an additional iron injection and time (sampling moment). Both immunization procedure and immunization moment did also not interact with time ($P>0.1$; Fig. 1). The pattern (kinetics) of the blood Hb concentration was different among the three major genotypes as indicated by the interaction effect between genotype and time ($P<0.05$; Fig. 1) concentration.

3.2. Growth

Averaged over the total experiment, ADG was not affected ($P>0.1$) by an additional iron injection, immunization procedure and immunization moment (Table 2). With regard to genotype, the highest ADG was found in the predominantly Finnish Landrace pigs (359 g day$^{-1}$; Table 2). Averaged over all treatments, ADG increased over time ($P<0.001$) from 131 g day$^{-1}$ in week 1 to 471 g day$^{-1}$ in week 5. This increase in ADG with time was not affected ($P>0.1$) by an additional iron injection, immunization procedure or immunization moment. Growth pattern was affected ($P<0.01$) by genotype. During the fifth week after weaning, pigs that had pre-

Table 2
Least squares means for the effects of an additional iron injection, major genotype and immunization moment on average daily gain (ADG), average blood hemoglobin concentration (Hb), average Keyhole limpet hemocyanin (KLH) specific antibody titer (total Ig, IgM, and IgG) of immunized newly weaned pigs during the experimental period

<table>
<thead>
<tr>
<th>Item</th>
<th>Iron injection</th>
<th>Major genotype</th>
<th>Immunization moment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron</td>
<td>Saline</td>
<td>S.E.M.$^a$</td>
</tr>
<tr>
<td>Number of pigs</td>
<td>60</td>
<td>60</td>
<td>–</td>
</tr>
<tr>
<td>ADG (g d$^{-1}$)</td>
<td>343</td>
<td>329</td>
<td>10.5</td>
</tr>
<tr>
<td>Hb (mmol l$^{-1}$)</td>
<td>6.8</td>
<td>6.7</td>
<td>0.06$^c$</td>
</tr>
<tr>
<td>IgTotal</td>
<td>6.7</td>
<td>6.6</td>
<td>0.18</td>
</tr>
<tr>
<td>IgM</td>
<td>4.6</td>
<td>4.6</td>
<td>0.19</td>
</tr>
<tr>
<td>IgG</td>
<td>3.8</td>
<td>3.8</td>
<td>0.19</td>
</tr>
</tbody>
</table>

$^a$ S.E.M., pooled standard error of the mean.
$^b$ Major genotype main effect, $P<0.05$.
$^c$ Additional iron injection main effect, $P<0.05$. 
dominantly the FL genotype achieved the highest ADG (550 g day\(^{-1}\)), whereas, pigs that had predominantly the DL genotype achieved the lowest ADG (378 g day\(^{-1}\)). The ADG (485 g day\(^{-1}\)) of pigs that had predominantly the GY-z genotype was intermediate (Fig. 2).

### 3.3. Keyhole limpet hemocyanin

Averaged over the total experiment, total Ig, IgM and IgG titers against KLH were not affected \((P > 0.1)\) by an additional iron injection or immunization moment (Table 2). Immunized pigs that had predominantly the GY-z genotype had a higher total Ig and IgG response to KLH than pigs that had predominantly the FL or DL genotype \((P < 0.05);\) Table 2). Genotype had no effect on the IgM response to KLH.

Total antibody titers and titers of the IgM and IgG isotypes were affected by time \((P < 0.001)\). The patterns of the total Ig, IgM and IgG titers against KLH were not affected \((P > 0.1)\) by additional iron administration. Immunization moment did affect \((P < 0.05)\) the pattern of total Ig, IgM and IgG

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**Fig. 1.** Least-square means of blood hemoglobin (Hb) concentration in newly weaned pigs during the experimental period. Panel A: the main effect of iron treatment (pigs injected with additional iron [FE] vs. placebo pigs [SALINE]); panel B: the main effect of immunization procedure (immunized pigs [IMMU] vs. placebo pigs [PLAC]); panel C: main effect of immunization moment (pigs immunized 1 day before [D\(^{-1}\)] or 1 day after [D\(^{1}\)] weaning); and panel D: the main effect of major genotype (predominantly Finnish Landrace pigs [FL] vs. predominantly Dutch Landrace pigs [DL] vs. predominantly Great Yorkshire pigs [GY-z]).
responses (Figs. 3A, 4A and 5A). At day 7 the total Ig titer to KLH of pigs immunized 1 day before weaning (D−1) was higher than of pigs immunized 1 day after weaning (D1). The pattern of total Ig response to KLH was also affected (P<0.005) by genotype (Fig. 3B). At day 11, the total Ig titer for GY-z pigs was approximately 1.4 units (log-scale) higher than that of FL pigs. This difference was reduced to approximately 1.1 units from day 14 onwards. The development of the total Ig titer against KLH of DL pigs was intermediate to GY-z and FL pigs. In contrast, the kinetics of IgM and IgG responses to KLH were not affected by genotype (P>0.1; Figs. 4B and 5B).

4. Discussion

The additional iron injection on day 21 after birth did increase the blood Hb concentrations during the nursery period (Table 2). The highest difference (0.4 mmol l−1) was found at day 6 after weaning. In a study of Kamphues et al. (1992), pigs were also given an additional injection with iron at day 21 after birth. Blood Hb was measured at weaning and day 49 after weaning. The difference found at weaning (1.5 g dl−1 = 0.9 mmol l−1) suggests that the magnitude of the increase in blood Hb was higher in a study of Kamphues than in our study.

4.1. Effect of iron status on growth and immunity

Although the iron status was improved in our study, no effect on growth (329 vs. 343 g day−1) was observed in the 5-week nursery period. Both
Kamphues et al. (1992) and Schrama et al. (1997) reported an improvement in growth resulting from an increase in iron status. Like in our study, Kamphues et al. (1992) injected pigs with additional iron at day 21 after birth. The difference in iron status in the study of Schrama et al. (1997) was created by injection of 100 or 200 mg of Fe (Fe-dextran) at day 3 after birth. Pigs that received 100 and 200 mg of Fe had an average blood Hb concentration of 6.3 and 7.6 mmol l⁻¹, respectively, during a 6-week experimental period. The lack of growth response to additional iron in this study may be due to either the small difference in iron status or the relatively low growth response of the pigs (Table 2). The latter is supported by Kamphues et al. (1992) who found that the growth response to an additional iron injection was most profound in the heaviest pigs.

In this study, no effects of iron status on humoral immune response were found. These results confirm
the results of Schrama et al. (1997) and Kleinbeck and McGlone (1999). They also found no effects of iron status on, respectively the humoral immune response to KLH and plasma IgG concentrations using non-anemic pigs. However, reduced serum IgG levels in iron deficient children were reported by Galán et al. (1988). This might suggest that iron status only affects humoral immunity of pigs which are anemic.

In contrast to the humoral immune response, cellular immune response is known to be affected by the iron status of animals (De Sousa et al., 1988, 1991; Brock, 1994). They demonstrated decreased T-cell-dependent immune responses during iron deficiency. It remains questionable, however, that a decrease in cellular immune response would have been demonstrated with the difference in iron status achieved in this study using pigs without iron deficiency.

4.2. Effect of immunization moment

Weaning can be considered a stressful event for pigs that is accompanied with reduced performance (Le Dividich and Herpin, 1994) and decreased immune responsiveness (Blecha et al., 1983; Blecha and Charley, 1990). In the present study, all pigs experienced the stressful weaning process. Although both immunization moments were near this stressful experience, the results of the present study suggest that immunization of pigs following a stressor can affect the development of a humoral immune response (Figs. 3A, 4A and 5A). However, it should be realized that in this experiment there was a 2-day difference in the development of the immune responses that could be responsible for the observed differences between both immunization moments. The absence of the three-way interaction between immunization moment, day and iron status suggests that additional iron did not affect the immune responsiveness even under stressful conditions.

4.3. Effect of genotype

In this study, antibody responses to KLH were affected by major genotype of the pig (Table 2). Genotype effects on immune responses of purebred pigs were also reported by Joling et al. (1993). However, they found that Dutch Landrace pigs showed the highest antibody response to KLH. The Finnish Landrace and Norwegian Landrace pigs showed intermediate antibody responses, whereas Yorkshire pigs showed the lowest antibody response to KLH.

In addition to antibody response to KLH, growth was also affected by major genotype (Table 2). The genotype with the lowest antibody response had the highest growth (Finnish Landrace), whereas the genotypes with the highest antibody responses to KLH had the lowest growth (Great Yorkshire and Dutch Landrace).

References


