Evaluation of the efficacy of two postweaning colibacillosis vaccines in a field herd with PRRS circulation during postweaning stage.

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ABSTRACT

Postweaning diarrhea (PWD) and PRRS are two major concerns in swine production, which association has not been consistently explored. In the current scenario of restrictions in the use of antibiotics and ZnO, vaccination is more relevant to control PWD, but PRRS virus circulation may compromise the immune protection conferred by postweaning colibacillosis vaccines. We evaluated the efficacy of two postweaning colibacillosis vaccines (parenteral and oral) in a commercial herd affected by an outbreak of PWD and with PRRS circulation in postweaning. Five groups were studied during the postweaning period: one control (Group 1) and four vaccinated: two with each postweaning colibacillosis vaccine administered alone (Groups 2 and 3) or with sow vaccination against PRRS (Groups 4 and 5). We evaluated the effects on piglet weight, average daily weight gain and in the percentage of piglets with diarrhea, its duration, lethality and mortality. PRRS viremia and anti-PRRS antibodies were evaluated by qPCR and ELISA. Regarding control group, colibacillosis vaccination generally improved most of the measured parameters; but significant improvements were only observed in Groups 4 and 5 ($p < 0.05$). Moreover, cases of diarrhea occurred at different ages: in Groups 2 and 3 the peak of cases occurred just after ZnO was removed from the feed compared to Group 1, while in Groups 4 and 5 no peak was observed. This suggests that postweaning colibacillosis vaccination may be compromised by the PRRS circulation. In PRRS endemic herds an effective protection against PWD through vaccination may require PRRS vaccination to obtain a better performance.

KEYWORDS: diarrhea, *E. coli*, postweaning colibacillosis, PRRS, swine, vaccination, ZnO.

INTRODUCTION

Swine postweaning diarrhea (PWD) is a multifactorial disease responsible of significant economic losses worldwide as it is related with increased mortality, animal weight loss and growth retardation (Fairbrother et al., 2005). In addition, the occurrence of PWD also presents a risk for the emergence of resistances as farms with this problem present an increased use of antibiotics due to treatments. (García-Meniño et al., 2021). PWD is usually the result of environmental and husbandry factors that facilitate the infection with specific pathogens around weaning. Many pathogens can be involved in PWD, but Enterotoxigenic *Escherichia coli* (ETEC) is the most important among all of them (Fairbrother et al., 2005). In order to cause diarrhea, ETEC must present two specific virulence factors: fimbriae adhesins, which facilitate the bacteria attachment to the enterocytes (e.g., F4, F18 and less frequently F5, F6 or F41); and enterotoxins, which are responsible of the toxic effect and disrupt the intestinal homeostasis (heat-labile toxin (LT), heat-stable toxin a (STa) and heat-stable toxin b (STb)) (Fairbrother et al., 2005; Fairbrother and Nadeau, 2019). When ETEC is causing PWD, it generally affects to a high proportion of piglets, which typically show grey or yellowish watery diarrhea along with a characteristic smell around the 2-3 weeks after weaning. The disease usually lasts about one week, although sudden deaths can also be observed (Luppi, 2017). Secondary and immunosuppressive infections may also exacerbate the course of this disease (Drew, 2000; Segalés et al., 2004).

Several control strategies against PWD have been proposed: increasing the weaning age and performing creep-feeding during the lactation period, increasing the fiber levels and reducing the protein in the piglet diet, water acidification, the addition of organic acids or prebiotics to the feed, etc. (Zentek et al., 2012; McLamb et al., 2013; Jha and Berrocoso, 2016; Dubreuil, 2017; Rhouma et al., 2017; Lee et al., 2021). However, the preventive feed medication of piglets with zinc oxide (ZnO) has been the most successful and extended measures to control the proliferation of ETEC and incidence of PWD. Levels up to 3,000 parts per million (ppm) of ZnO in feed have shown to reduce clinical development of diarrhea, the mortality rate and improve growth (Luppi, 2017). However,

since July 2022 the use of ZnO as a feed additive in the European Union is prohibited due to environmental concerns (The European Medicines Agency, 2017). These new restrictions may increase the incidence and mortality rate of PWD in swine herds, as well as make other secondary intestinal disorders more important.

Despite different postweaning colibacillosis vaccines are commercially available, vaccination of piglets has been limitedly used on field due to the success provided using ZnO (Melkebeek et al., 2013), but this practice may gain now more importance under the new restricted scenario. However, the efficacy of vaccination against postweaning colibacillosis has been limitedly assessed and mostly in controlled conditions (Van der Stede et al., 2003; Fairbrother et al., 2017; Nadeau et al., 2017; Vangroenweghe and Thas, 2020; Correa et al., 2022; Ramis et al., 2022; Vangroenweghe and Boone, 2022), so studies in field conditions are highly required, for example, to evaluate interferences with other endemic infections. Most of the current commercial postweaning colibacillosis vaccines are for parenteral or oral administration and include the most important adhesins (F4 and F18). Parenteral vaccines stimulate the active immunity in piglets at systemic level, while the latter ones mainly active the production of secretory IgA antibodies at intestinal level (Melkebeek et al., 2013). The effectiveness of these vaccines against colibacillosis in field conditions can be compromised by low immunity status in the piglets or by the coinfection with immunosuppressive infections. In this regard, Porcine Reproductive and Respiratory Syndrome (PRRS) virus is an immunosuppressive infection that circulates endemically in most of the pork-producing countries and constitutes one of the major economic limiting factors in swine industry. The infection with PRRS virus may interfere in the development of an adequate immunity against other infections, reducing the effectiveness of the vaccines (Sang et al., 2011; Lunney et al., 2016). Moreover, it has been reported that PRRS infection induces intestinal damage, shortening the villus height and altering the jejunal function (Escobar et al., 2006; Helm et al., 2020); and that it causes the disruption of intestinal barrier by weakening tight junction barrier integrity (Zhao et al., 2021), which could also aggravate enteric process such as postweaning colibacillosis.

Therefore, vaccination against ETEC *E. coli* is expected to gain demand after the prohibition of ZnO, but questions regarding the on-field efficiency of the current vaccines are still unresolved, especially when circulation of PRRS is happening simultaneously. Hence, we carried out an experimental study in the postweaning stage of a herd affected by an outbreak of PWD and with circulation of PRRS to evaluate the effect on the production parameters and mortality of two commercial vaccines against postweaning colibacillosis, applied either alone or together with a PRRS vaccination program.

MATERIAL AND METHODS

Farm description and nutrition

The present study was performed during 2020 on a conventional farrow-to-finish pig farm with 150 sows managed in a one-week batch-management system. Piglets were weaned at 21 days of age and housed in post-weaning facilities during approximately six weeks. Pigs were placed in pens with total-slated floor in groups of 13-14 piglets. Water and food were available *ad libitum* following a 3-phase feeding strategy: from 21 to 28 days old piglets were fed with a lacto-initiator (the same that had been used for creep-feeding), from 29 to 42 days a weaning starter supplemented with ZnO (3.100 ppm), and from 43 days onwards using a starter. Ventilation was performed by an automated air fan located in the roof of the room and fresh air entered the room directly from outside.

Herd health situation

The herd presented a history of PRRS circulation in postweaning piglets. One year before starting the present study, the herd had stopped its vaccination program against PRRS virus at weaning, so piglet vaccination only include vaccination against *Mycoplasma hyopneumoniae* and Porcine Circovirus Type 2 at 15 and 28 days old, respectively; piglets were never vaccinated against *E. coli*. Regarding the breeding-herd population, it was free of PRRS and had not been vaccinated before against this virus; the sow vaccination schedule includes a vaccination against *E. coli* and *Clostridium perfringens* Type C one month before farrowing.

Approximately 2-3 months before the beginning of the study, the farm suffered an outbreak of PWD affecting to piglets between 5 and 6 weeks old. Accordingly, rectal swabs and serum samples were taken from affected piglets (n=5) and analyzed by qPCR using a digestive diagnostic panel (F4, F5, F6, F41, F18, STa, STb, LT, Stx2, *eae*, AIDA and EAST genes from *E. coli*, *Salmonella* spp., Rotavirus A and Porcine Epidemic Diarrhea) and to detect PCV2 and PRRS viremia. Results from rectal swabs were positive to *E. coli* and included the detection of F4, F18, STa, STb, LT, *eae*, AIDA and EAST genes, as well as Rotavirus A (Cq 23.10 to 32.20). Regarding the results from serum samples, PRRS viremia was detected in all serum samples (Cq 26.11 to 30.52), while PCV2 viremia was discarded.

Study design

We carried out different vaccination schemes against PRRS and ETEC in five different groups of pigs (Table 1). The groups were created from five consecutive batches, so all the animals in each batch were assigned to each group. All the protocols and procedures followed during the study were approved by the bioethics committee of the University of Santiago de Compostela (project identification code: 2019-CE194). Individual piglets with clinical signs of PWD (watery feces and clinical signs of dehydration) were treated with a commercial injectable sulfadoxine-trimetoprim and were fasting while diarrhea was evident. Other disorders were also treated according to veterinarian recommendations.

Data collection and laboratory analysis

Piglets were individually ear tagged at weaning and weighted at 21, 28, 42 and 63 days old. The average daily weight gain (ADWG), food intake (total kg food/piglet), and feed conversion rate (FCR) (total kg food/total kg piglet weight gain) of piglets were calculated for each feeding period: lacto-initiator (between 21 to 28 days old), weaning starter (between 29 to 42 days old), starter (between 43 to 63 days old); and for all the study period (between 21 to 63 days old). Measurements from animals that dead during the study were registered if the piglet had started the

corresponding feeding period up to the moment of death and using their dead weight when was necessary for calculations.

Clinical signs of diarrhea (grey or yellowish watery feces) and the presence of dead were evaluated daily from 21 to 63 days old. We registered the week when clinical signs began and the duration of the diarrhea (days with clinical signs); dead piglets were individually weighed and registered in the post-weaning corresponding week.

The presence of antibodies and viremia against PRRS virus was monitored in Groups 1 and 4, as control and piglets from PRRS vaccinated sows, respectively. Serum samples were collected at 10, 25, 41 and 58 days old from a minimum of 20 random piglets from each group. These samples were frozen at -80 ºC until laboratory analysis. The serum samples were analyzed to detect the presence of antibodies against PRRS virus using the commercial kit Ingezim PRRS Universal (INGENASA, Madrid, Spain). A serum sample was considered positive to PRRS virus IgG antibodies if the ELISA sample/positive (S/P) ratio was higher than 0.25, then PRRS virus antibody titer was calculated according to manufacturer's instructions. For titer calculation of negative samples, a S/P ratio of 0.20 was considered. To detect viremia, serum samples at each sampling moment were pooled (5 pigs/pool) and pools were analyzed by qPCR. RNA extractions were performed using the commercial kit NucleoSpin® RNA (Macherey-Nagel, Düren, Germany) following manufacturer's instructions, using 200 µL of each blood pool as starting material and collecting the extracted RNA in 60 µL of molecular water. The isolated RNA was analyzed by qPCR using the commercial kit VetMAX PRRSV EU/NA (ThermoFisher Scientific, Waltham, Ma, USA). A sample was considered positive when Cq < 40. qPCR reactions were run on an Applied Biosystems ABI Prism 7500 thermocycler (ThermoFisher Scientific, Waltham, MA, USA).

Statistical Analysis

A linear regression analysis was used to evaluate the differences in piglet weight between groups at each sampling point. Only the piglets with all the weight measures were included for this analysis

160 excepting the weight evaluation at the beginning of the study, in which all piglets were included.
161 Linear regression analysis was also performed to evaluate differences between groups in the
162 ADWG in each feeding period as well as in all the study, the number of days with clinical diarrhea
163 and the PRRS antibody titer at each sampling age.

164 We used a logistic regression to evaluate the presence of differences in the proportion of piglets
165 with diarrhea and the accumulated mortality by group of vaccination.

166 Data analysis was performed in R v. 4.1.1 (R Core Team, 2021) (Vienna, Austria). In all analyses a
167 $p < 0.05$ was considered as significant.

168 **RESULTS**

169 **Animal weight and ADWG**

170 The weight of the piglets and the ADWG could be assessed in 375 out of 414 animals. The
171 remaining ones were excluded due to death. Regarding weight, no significant differences were
172 found in piglets at 21 and 28 days old between the groups of vaccination, but they were detected in
173 older piglets (Figure 1). In this case, animals with double protection against *E. coli* and PRRS
174 (Group 5) were the only ones that showed significantly higher weights than the control group
175 (Group 1), at 42 or at 63 days old. In contrast, animals with only *E. coli* IM vaccination (Group 2)
176 showed a worse final weight than the control group.

177 Regarding ADWG, we found statistically significant differences among groups in all the feeding
178 periods (Figure 2). It must be highlighted that during the first postweaning week the ADWG in
179 groups with PRRS vaccination (Group 4 and 5) was significantly higher than the control and the
180 other groups. Thereafter, the ADWG was not significantly higher in any vaccination group than in
181 the control group.

182 Results for food intake and FCR for each group are showed in Table 2. The most remarkable
183 observation occurred during the period of feeding with lacto-initiator, in which upon of having a

similar food intake, the FCR was slightly better in *E. coli* vaccinated groups and notoriously better in groups with PRRS vaccination.

Postweaning diarrhea, mortality and lethality

Compared to the control group, the vaccination programs significantly decreased the cumulative proportion of piglets affected by PWD in Groups 3 to 5, especially in Groups 4 and 5: Group 3 (OR = 0.394; 95% CI = 0.161 – 0.098; $p = 0.031$), Group 4 (OR = 0.101; 95% CI = 0.016 – 0.365; $p = 0.003$) and Group 5 (OR = 0.083; 95% CI = 0.013 – 0.299; $p = 0.001$) (Table 3). In contrast, Group 2 did not show a significant reduction of the proportion of piglets affected by PWD (OR = 0.903; 95% CI = 0.426 – 1.891; $p = 0.788$). The clinical duration of PWD was also reduced in all the vaccinated groups, although only significantly in Groups 2 and 3 compared to Group 1 (Table 3). However, Groups 4 and 5 only presented two diarrhea clinical cases.

In addition, while most of the affected piglets from Group 1 appeared in the three first postweaning weeks, the pattern was different in Groups 2 and 3, and the peak of cases was delayed to the fourth postweaning week, coinciding with the change of weaning starter feed with ZnO to the starter feed. Groups 4 and 5 did not suffer that peak after this feeding change (Figure 3).

Mortality rates were generally lower in vaccinated groups, though the difference was only significant for Group 4 (OR = 0.261; 95% CI = 0.058 – 0.852; $p = 0.042$). No significant differences were observed between vaccinated groups (Table 3). However, the number of casualties attributed to PWD in vaccinated piglets was low (ranging 1-2), as well as the lethality. The exceptions to these observations occurred in Groups in which denominators were very small (< 3), so the percentages may be unreliable. Due to the small number of observed cases, no statistical analysis was performed for the % of mortality due to diarrhea and to % of lethality.

PRRS monitoring

We did not detect PRRS viremia until 25 days of life (three positive pools, Cq ranged from 31.86 to 34.53) in piglets from unvaccinated sows. In piglets from vaccinated sows, the first detection

occurred later, at 41 days old (three positive pools, Cq ranged from 29.80 to 34.41). After that, PRRS virus circulation was detected in both groups with similar Cq values until the end of the study (Figure 4).

PRRS serology differed between those piglets from vaccinated and from unvaccinated sows (Figure 4). At the beginning of the monitoring, the piglets from vaccinated sows showed a significantly higher level of anti-PRRS antibodies ($p = 0.016$) than those from unvaccinated sows, however, at 25 and 41 days of life the levels were similar ($p > 0.05$). Instead, at 58 days old the level of anti-PRRS antibodies was significantly higher in those piglets from unvaccinated sows ($p = 0.036$) than those from vaccinated ones.

DISCUSSION

In the present study we evaluated the efficacy of two postweaning colibacillosis vaccines applied alone or combined with a reinforcement of PRRS immunity in a farm affected by an outbreak of PWD and PRRS circulation during postweaning. Upon the technification in swine production during the last decades, both PWD and PRRS remain as two of the major concerns in this livestock sector. However, a close association between PWD and PRRS was not historically considered. In a scenario of increasing restrictions on the use of antibiotics and products such as ZnO, which are widely used for the control of PWD, preventive measures, such as immunoprophylaxis, gain special relevance. However, the efficacy of vaccines may be limited when immunosuppressive agents such as PRRS virus are circulating (Drew, 2000; Kitikoon et al., 2009). Thus, the combination of vaccination schedules for *E. coli* and PRRS virus could be beneficial to improve the consequences of PWD but, as far the authors known, this has not been explored in the scientific literature.

Our results confirm that postweaning colibacillosis vaccination aids to reduce the incidence and magnitude of PWD, though differently depending on if it was performed in groups with PRRS sow vaccination. We generally found fewer animals affected by diarrhea and for less time in groups with piglets vaccinated against postweaning colibacillosis. This vaccination also reduced the impact of PWD in the survival of piglets. This is evidenced first, by a reduction of the mortality rate in

vaccinated groups, which was statistically significant in Group 4, and second, by a general reduction of the mortality attributed to diarrhea, except in Group 4, in which the result could be an artifact due to the very small number of casualties (Table 3). Lethality also tended to be lower, though we could not statistically assess it due to the low number of counts. Groups with PRRS-vaccination reported more lethal diarrhea cases, but considering that only 1-2 animals were affected, sporadic conditions such as bad vaccinations, weak animals, etc. cannot be ruled out. It must also be noted that the number of antibiotic treatments is likely to be lower in vaccinated groups due to the lower incidence of PWD cases. However, treating PWD with antibiotics could also secondarily reduce the incidence of other bacterial diseases. Thus, the constant reduction in antibiotic treatments in consecutive batches due to vaccine efficacy might be correlated with a sustained intensification in the direct transmission of some secondary infections, experiencing in vaccinated groups an increase in their incidence and/or mortality rates. For example, Group 5 presented a similar mortality to the control group, but the percentage of animals affected by PWD was markedly lower (2.33% *vs* 22.22%). Most of casualties in this group consisted of sudden deaths with polyserositis and *Streptococcus suis* was diagnosed as the responsible. The increase of *S. suis* incidence may have been favored by a reduced antibiotic treatment against PWD used in this vaccinated group. Nevertheless, changes in the incidence of other diseases after ETEC vaccination are not well clarified. For example, Vangroenweghe and Boone, 2022 reported the exact opposite situation, a lower incidence of streptococcal meningitis in pigs vaccinated for ETEC. Overall, further studies are needed to assess the incidence of other pathologies after controlling PWD.

Postweaning colibacillosis vaccination was also positive to improve the productive parameters in most of the analyzed scenarios. First, it is notable the FCR values observed in the first postweaning week: in Group 1, despite the ingestion of lacto-initiator, the whole of piglets lost weight probably as consequence of PWD outbreak and PRRS circulation, in addition, fasting animals due to PWD probably contributed to magnify this value; in Groups 2 and 3 the postweaning colibacillosis vaccination was able to reduce the magnitude of PWD although the piglets were not able to convert

261 the feed energy in meat, which explains the high values observed in both groups; in Groups 4 and 5,
262 with the reinforcement against PRRS infection, the FCR achieved normal values. Second, apart
263 from Group 2, food intake per piglet for each feeding phase and ADWG in the lacto-initiator and
264 weaning starter periods were better in all the vaccinated groups, and remarkably in Groups 4 and 5,
265 than in the control group. These results are consistent with those reported by Correa et al. (2022),
266 who indicated that vaccination against postweaning colibacillosis can promote gut health specially
267 in the two first postweaning weeks. Third, same as these authors, we observed that unvaccinated
268 piglets used more efficiently the starter feed than vaccinated piglets. We hypothesize that the lower
269 FCR may be mainly related with the absence of immune response in unvaccinated piglets. The
270 vaccination involves an energy consumption in the development of antibody and mucosal
271 immunity, which may be remarkable in oral vaccinations (Van der Weken et al., 2021); thus, it
272 could influence in the lower FCR observed in control group. In addition, the higher antibiotic
273 treatment requirement in this group could reduce the influence of secondary infections and
274 contribute to the better FCR observed in unvaccinated piglets. Regarding Group 2, it must be noted
275 that 8 – 10 % of the piglets presented a slightly swollen neck. This has been notified as a possible
276 secondary effect of the vaccine by the manufacturer; however, we did not observe it in the other
277 group that used the same vaccine. We hypothesize that repeated injections in the neck may have
278 favored this finding, as it was more frequent in animals treated by the intramuscular route due to
279 PWD. Despite being a minor lesion, it could have caused some discomfort that had an impact on the
280 food intake and therefore, on the final weight of the piglets in this group.

281 We also found a peak in the percentage of piglets with PWD just in the first week without ZnO in
282 the feed but only in groups without PRRS vaccination (Figure 3). This phenomenon has been also
283 notified by other authors, even naming it as “post-ZnO diarrhea” (Vangroenweghe and Thas, 2020).
284 On the contrary, in groups with PRRS vaccination, no piglets showed diarrhea from the third
285 postweaning week onwards. Thus, in field conditions, the vaccination against postweaning

colibacillosis itself may not be enough to totally prevent the diarrhea when the ZnO at therapeutic levels withdraws from the feed.

The improvement observed with colibacillosis vaccination was significantly better when both types of *E. coli* vaccines were applied to piglets from PRRS vaccinated sows. This suggests that PRRS infection interferes with the vaccination against *E. coli* and compromises the success of the measure. Regardless the type of *E. coli* vaccine administered, we detected viral circulation later in the groups with PRRS vaccination of sows (around three weeks postweaning) along with a significant reduction in the proportion of piglets with diarrhea. The later circulation of PRRS in piglets is a consequence of the sow's vaccination. It is known that the vaccination and revaccination of sows with the employed PRRS vaccine induces a booster effect in their immunity (Díaz et al., 2013; Geldhof et al., 2013), and a higher level of PRRS virus maternal derived antibodies in their offspring, as it was observed in the present study. This can result in high titers of neutralizing antibodies from passive transfer that contribute to a low susceptibility of piglets to PRRS and to a delayed age of infection (Lopez et al., 2007; Hsueh et al., 2021; Martín-Valls et al., 2022), which is consistent with our results.

The present study suggests that the PRRS passive protection during the first postweaning weeks prevents piglets from PRRS circulation and, as consequence, favors the development of immune system which may have important effects in terms of prevention of PWD and in productive parameters. Thus, this delay of PRRS circulation may explain the lower percentage of piglets with diarrhea in groups with PRRS vaccination (Groups 4 and 5) regarding groups without PRRS vaccination (Groups 2 and 3). As postweaning colibacillosis vaccines are usually administered around weaning, delaying the circulation of PRRS virus also delays any potential interference that the infection may have with the colibacillosis vaccination and the age in which the virus causes immunosuppression and intestinal damage (Escobar et al., 2006; Helm et al., 2020; Zhao et al., 2021). Piglets that are susceptible of PWD at an older age are less likely to suffer from severe clinical signs as they have a more mature immune system, as well as a greater ability to feed, which

implies having more energy reserves and, therefore, coping better with PWD (Thomson and Friendship, 2019; Ming et al., 2021). Moreover, it also should be highlighted that this delay in the PRRS circulation also entails a reduction in the risk of iatrogenic transmission of this virus, which was remarkable in those groups from PRRS vaccinated sows (Groups 4 and 5). In these groups, the need of antibiotic administration to treat PWD was considerably reduced and, secondarily, can contribute to reduce PRRS incidence in postweaning.

Overall, our results show that the performance of colibacillosis vaccination can be influenced by the existence of other active infections. In particular, farms with endemic PRRS and unstable. If the virus cannot be controlled, delaying PRRS infection can be key to ensure the *E. coli* vaccines can confer protection against clinical PWD. Based on our results, vaccination against both pathogens seems to protect piglets specially during the first postweaning weeks. Thus, a period of at least 2-3 weeks between completing the postweaning colibacillosis vaccination schedule and becoming PRRS infected seems to be required to guarantee that these vaccines provide an adequate immune protection. Further studies are required to characterize more in detail this observation as the demand and use of vaccines against postweaning colibacillosis seems that will increase in the forthcoming years.

CONCLUSION

The employed vaccines against postweaning colibacillosis reduced the lethality and the duration of PWD and tended to improve the production parameters. However, its effectiveness is restricted by the PRRS circulation which, at same time, seems to contribute to the appearance of clinical cases of PWD. The reinforcement in the piglet immunity against PRRS through sow vaccination seems required and beneficious to optimize the protection conferred by postweaning colibacillosis vaccines in herds with PRRS circulation during the postweaning stage.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The procedures which involved live animals (vaccination and blood sampling) were approved by the bioethics committee of the University of Santiago de Compostela (project identification code: 2019-CE194).

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

The authors declare that they did not use generative artificial intelligence (AI) and AI- assisted technologies in any part of the study development and in the paper redaction.

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486

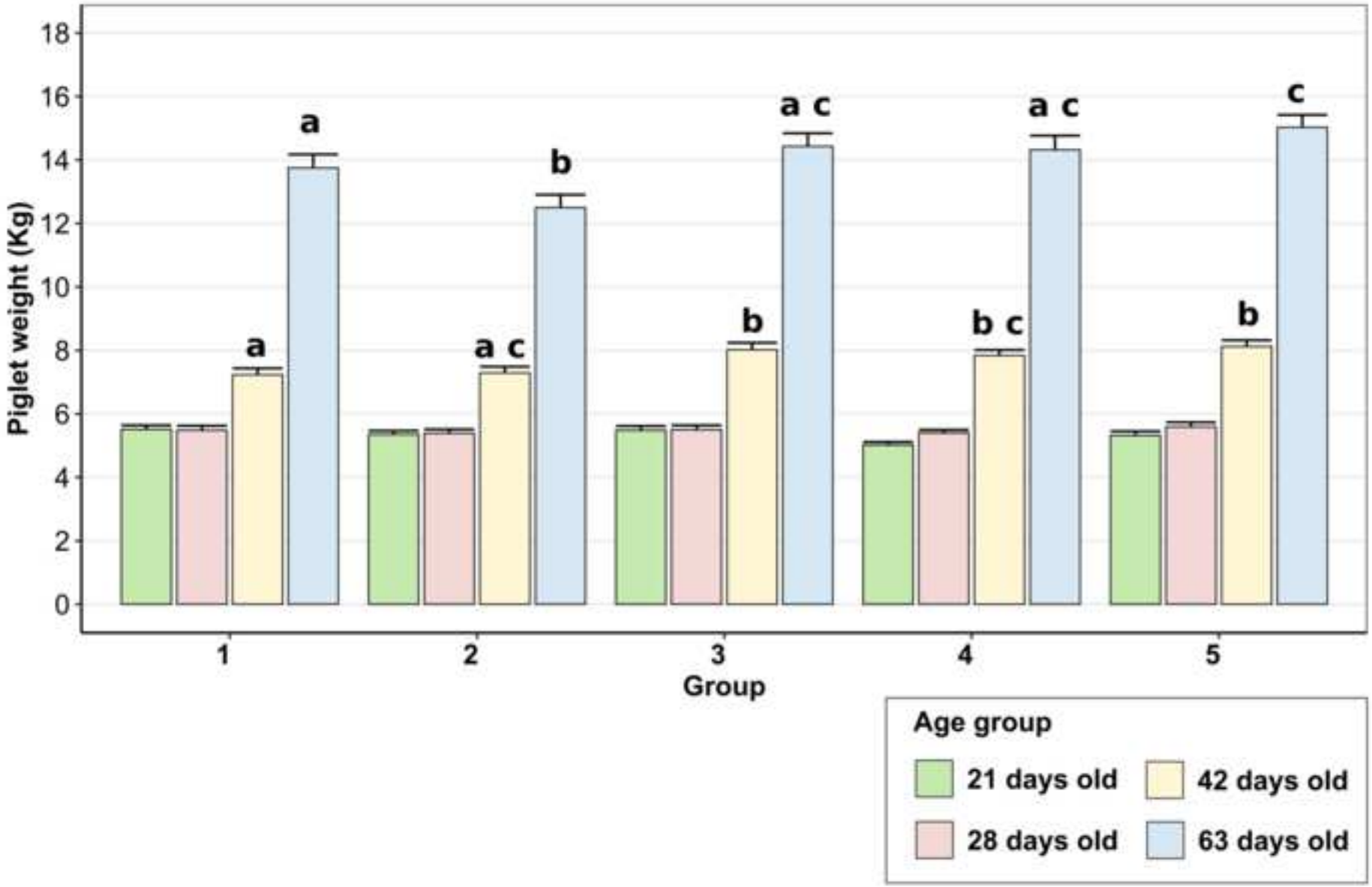
Figure captions

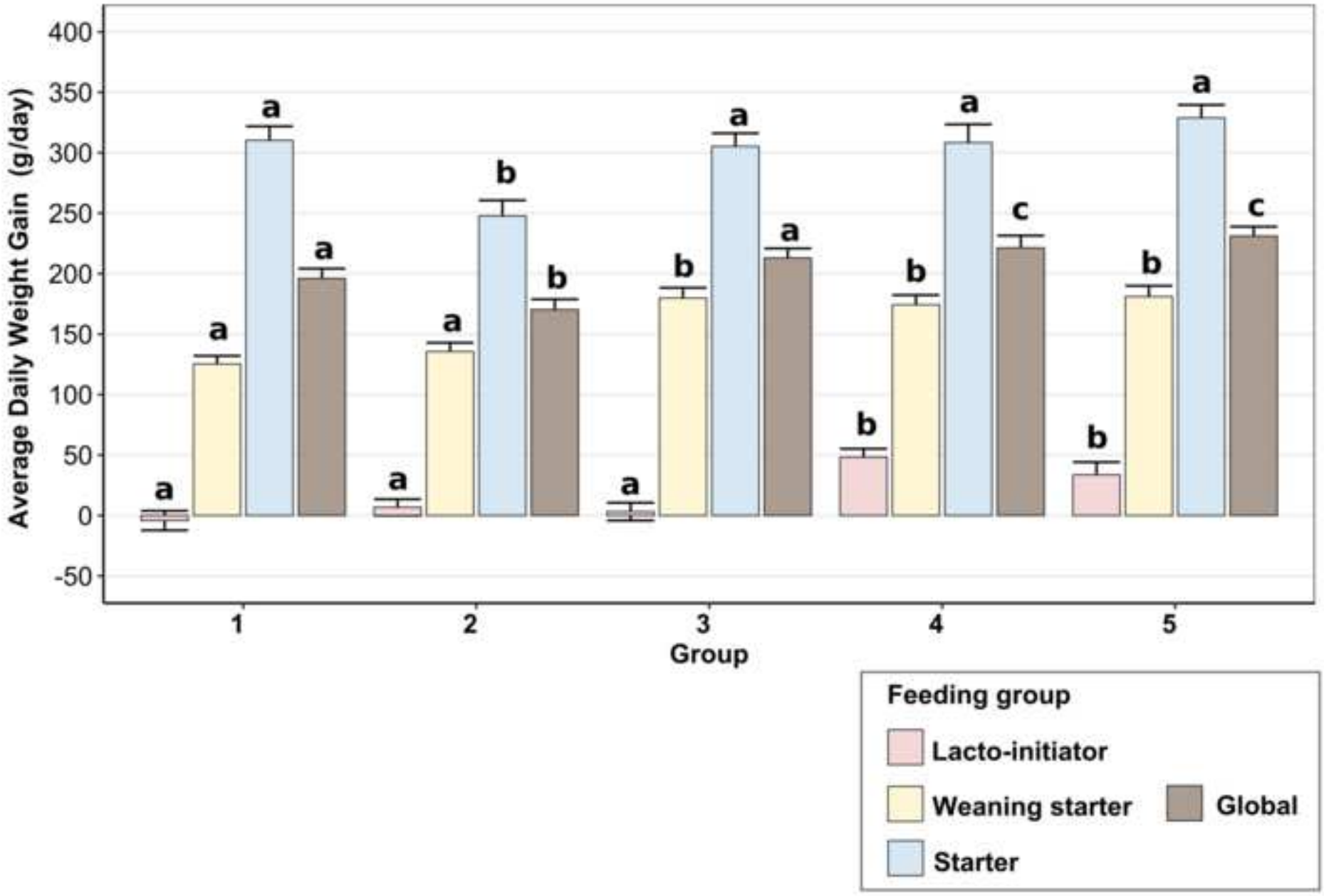
Figure 1. Average of piglet weight (expressed as mean ± standard error mean) for piglets in each group and age. Different letters indicate significant differences among groups at each age.

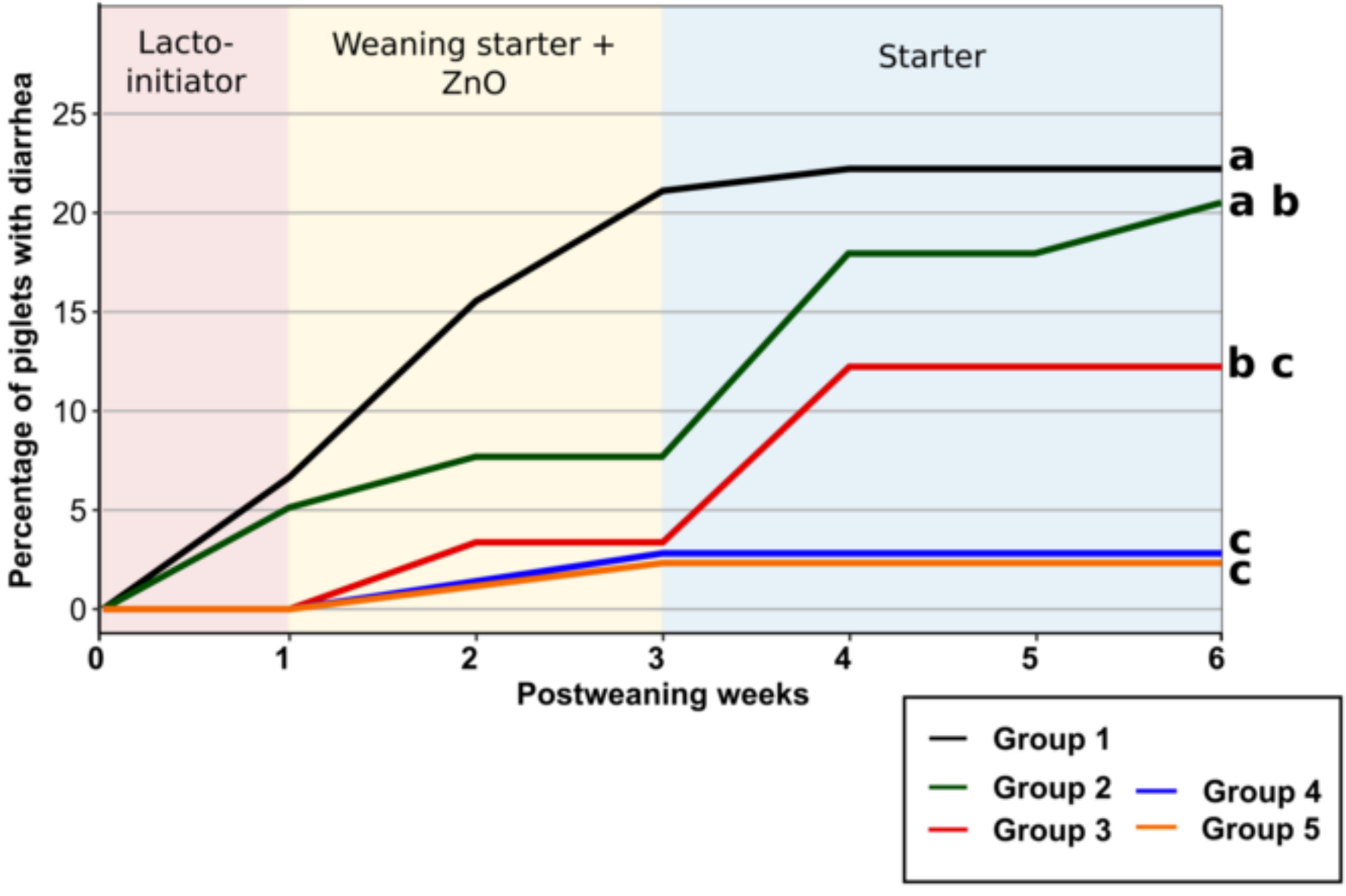
Figure 2. Average of piglet ADWG (expressed as mean ± standard error mean) for piglets in each group and feeding period. Different letters indicate significant differences among groups at each feeding period.

Figure 3. Evolution of the percentage of piglets with diarrhea in each group. Different letters indicate significant differences in the final proportion of piglets with diarrhea.

Figure 4. Dynamic of PRRS antibody titer and PRRS viremia in piglets from unvaccinated and vaccinated sows. The lines represent the evolution of the mean of PRRS antibody titer (individual samples) and the dots indicate the Cq value obtained in the positive pools. Different letters indicate significant differences in the PRRS antibody titer in each sampling age.


Piglet weight (Kg)
18
16
14
12
10
8
6
4
2
0
a
a
b
a c
b
a c
b c
a c
b
c
1
2
3
4
5
Group
Age group
21 days old
28 days old
42 days old
63 days old


Average Daily Weight Gain (g/day)
400
350
300
250
200
150
100
50
0
-50
a
a
a
a
a
b
a
b
b
a
b
a
a
b
a
b
b
a
b
c
b
a
b
c
1
2
3
4
5
Group
Feeding group
Lacto-initiator
Weaning starter
Starter
Global


Lacto-
initiator
Weaning starter +
ZnO
Starter
Percentage of piglets with diarrhea
0
5
10
15
20
25
0
1
2
3
4
5
6
Postweaning weeks
a
a b
b c
c
c
Group 1
Group 2
Group 3
Group 4
Group 5

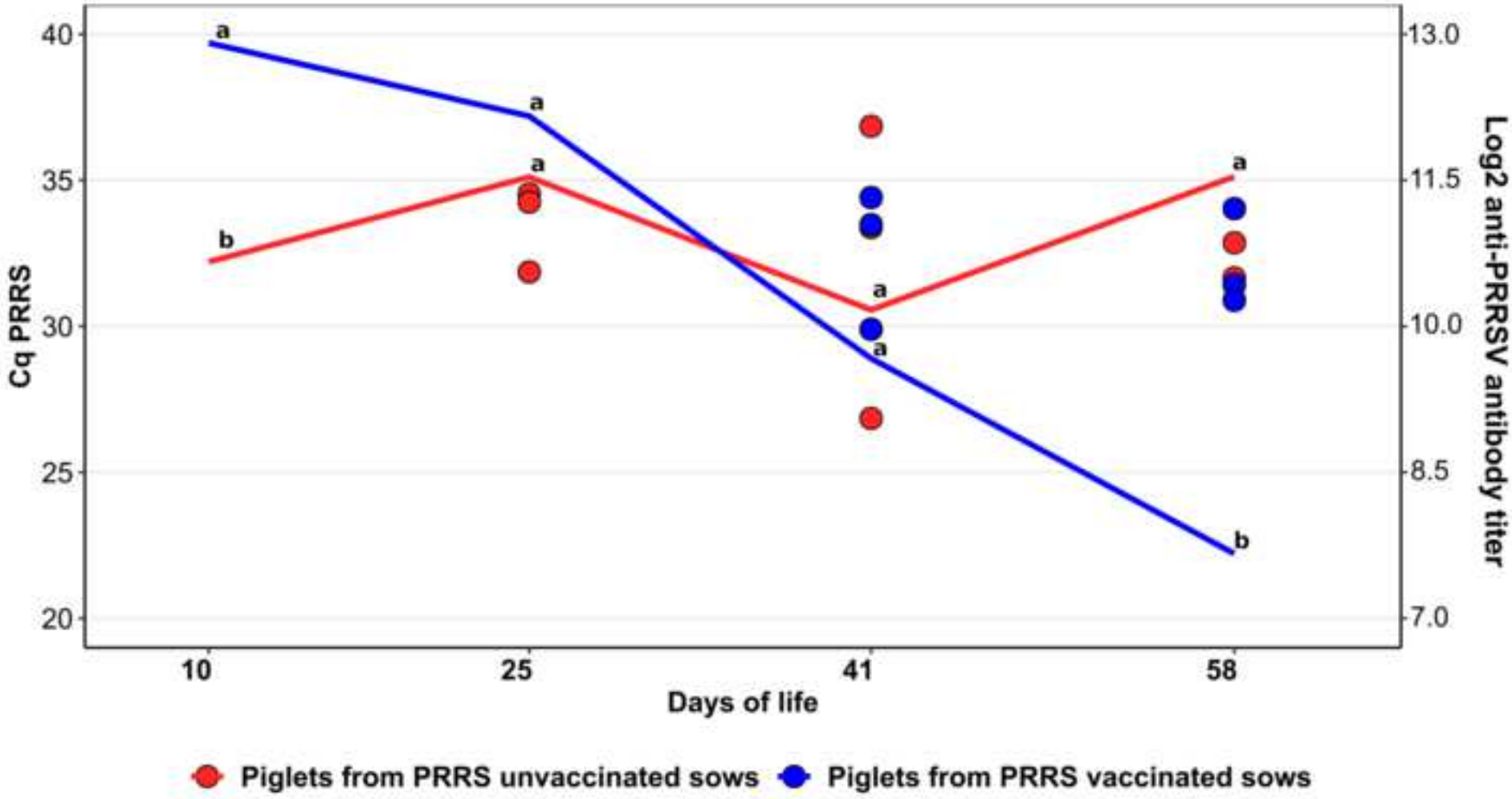

40
35
30
25
20
Cq PRRS
13.0
11.5
10.0
8.5
7.0
Log2 anti-PRRSV antibody titer
a
a
b
a
a
a
a
b
10
25
41
58
Days of life
Piglets from PRRS unvaccinated sows
Piglets from PRRS vaccinated sows

Table 1. Groups included in the study and *E. coli* and PRRSV vaccination programs.

Group	Pathogen	Route	Product	Schedule
Group 1 – Control (n = 90).	*E. coli*	Piglets not vaccinated		
	PRRSV	Piglets from PRRS non-vaccinated sows		
Group 2 – IM *E. coli* vaccination (n = 78)	*E. coli*	Intramuscular	Colidex-C [1]	0.5 and 1 mL at 10 and 21 days old, respectively
	PRRSV	Piglets from PRRS non-vaccinated sows		
Group 3 – oral *E. coli* vaccination (n = 89).	*E. coli*	Oral	Coliprotec F4/F18 [2]	One dose: 2 mL at 21 days old
	PRRSV	Piglets from PRRS non-vaccinated sows		
Group 4 – IM *E. coli* vaccination + PRRSV vaccination (n = 71).	*E. coli*	Intramuscular	Colidex-C [1]	0.5 and 1 mL at 10 and 21 days old, respectively
	PRRSV	Piglets from PRRS vaccinated sows	Progressis [3]	2 mL at 60 and 21 days before farrowing, respectively
Group 5 – oral *E. coli* vaccination + PRRSV vaccination (n = 86).	*E. coli*	Oral	Coliprotec F4/F18 [2]	One dose: 2 mL at 21 days old
	PRRSV	Piglets from PRRS vaccinated sows	Progressis [3]	2 mL at 60 and 21 days before farrowing, respectively

[1] Vetia Animal Health, San Sebastián de los Reyes, Spain.

[2] Elanco GmbH, Heinz-Lohman-Str, Germany.

[3] Ceva Salud Animal, Barcelona, Spain.

Table 2. Food intake and FCR for each phase of the feeding strategy.

Group	Lacto-initiator		Weaning starter		Starter	
	Food intake (kg/piglet)	FCR (kg food)	Food intake (kg/piglet)	FCR (kg food)	Food intake (kg/piglet)	FCR (kg food)
Group 1	0.489	-5.15	2.72	1.80	10.40	1.65
Group 2	0.421	131.4	3.11	1.77	9.06	1.84
Group 3	0.532	150.2	3.66	1.56	11.44	1.78
Group 4	0.903	2.56	3.76	1.60	11.00	1.76
Group 5	0.674	2.66	4.08	1.83	12.18	1.87

Table 3. Postweaning diarrhea outcomes and percentage of accumulated mortality

Group	Piglets with clinical diarrhea/Total piglets (%)	Days with clinical diarrhea ($\bar{x}$ ± sd)	Total antibiotic injections to treat diarrhea (injections per piglet with clinical diarrhea)	Dead piglets affected by diarrhea/total dead piglets (%)	Dead piglets/total piglets with diarrhea (% diarrhea lethality) *	Dead piglets /total piglets (% accumulated mortality)
Group 1	20/90 (22.22) [a]	2.53 ± 1.50 [a]	50 (2.50)	7/13 (53.85)	7/20 (35.00)	13/90 (14.44) [a]
Group 2	16/78 (20.51) [a,b]	1.63 ± 0.72 [b]	26 (1.63)	2/5 (40.00)	2/16 (12.50)	5/78 (6.41) [a,b]
Group 3	9/89 (10.11) [b,c]	1.56 ± 0.53 [b]	14 (1.56)	2/7 (28.57)	2/9 (22.22)	7/89 (7.86) [a,b]
Group 4	2/71 (2.81) [c]	1.50 ± 0.71 [a,b]	3 (1.50)	2/3 (66.67)	2/2 (100)	3/71 (4.23) [b]
Group 5	2/86 (2.33) [c]	1.50 ± 0.71 [a,b]	3 (1.50)	1/11 (9.09)	1/2 (50.00)	11/86 (12.79) [a,b]

[a] Different superscript letters indicate statistically significant differences ($p < 0.05$)