Purified natural pig immunoglobulins can substitute dietary zinc in reducing piglet post weaning diarrhoea

Hedegaard, Chris Juul; Lauridsen, Charlotte; Heegaard, Peter M. H.

Published in:
Veterinary Immunology and Immunopathology

Link to article, DOI:
10.1016/j.vetimm.2017.02.001

Publication date:
2017

Document Version
Peer reviewed version

Veterinary Immunology and Immunopathology

Volume 186, April 2017, Pages 9–14

Short communication

Purified natural pig immunoglobulins can substitute dietary zinc in reducing piglet post weaning diarrhoea

Chris J. Hedegaard[a], Charlotte Lauridsen[b], Peter M.H. Heegaard[a]

[a] Innate Immunology Group, Division of Vaccinology and Immunology, National Veterinary Institute, Technical University of Denmark, Frederiksberg, Denmark

[b] University of Aarhus, Faculty of Science and Technology, Department of Animal Science, Tjele, Denmark

Received 28 November 2016, Revised 1 February 2017, Accepted 3 February 2017, Available online 4 February 2017

Abstract

Enteric infectious disease in weaner piglets, including postweaning diarrhoea (PWD), are usually treated and/or prevented with antibiotics and/or zinc oxide in the piglet feed.

However extensive use of antibiotics and zinc oxide in intensive animal production is unwanted as it may promote microbial antibiotic resistance and pose environmental problems. Recently, in an experimental model of PWD, we observed that oral administration of purified porcine immunoglobulin G (ppIgG) from pooled natural pig plasma could reduce enteric infection. In the present study we were able to reproduce these results as it was observed that oral ppIgG accelerated clearance of faecal haemolytic bacteria in pigs challenged with E. coli in comparison with pigs not receiving ppIgG. This effect was observed upon feeding ppIgG for seven days postweaning suggesting that ppIgG does not have to be used prophylactically for several days preweaning. Furthermore, the effect of oral administration of ppIgG for seven days postweaning was equal to or better than that of dietary zinc oxide in reducing diarrhoea symptoms and in clearing faecal haemolytic bacteria for 14 days postweaning. These observations warrant future trials of dietary ppIgG in intensive swine production units to establish its performance as an alternative to dietary antibiotics and zinc oxide for preventing PWD.

Keywords

Postweaning diarrhoea; Immunoglobulins; IgG; Zinc oxide; Antibiotics; ETEC; E. coli F4; Feed supplement; Feacal score

1. Introduction

After parturition, lactogenic immunity provides offspring with oro-gastric protection against infectious pathogens (Hedegaard and Heegaard, 2016). In the conventional swine production systems piglets are weaned at an immunologically immature age (3–4

43 weeks) depriving them of the continued supply of protective lactogenic antibodies at the
44 same time as they are placed in a new environment with increased risk of enteric
45 bacterial infections that may lead to postweaning diarrhoea (PWD) (Madec et al., 1998).
46 PWD is characterised by diarrhoea caused by enteric infection by Enterotoxigenic
47 Escherichia coli (ETEC) usually within three days postweaning (Fairbrother et al., 2005).
48 PWD, like other enteric infections in pigs, can be treated with dietary antibiotics
49 (primarily tetracyclines, penicillins and macrolides (Becker, 2010; DANMAP, 2015 ;
50 Wageningen University, 2012)), and/or zinc oxide (Pluske, 2013). Indeed, antibiotics and
51 zinc can improve average daily growth (ADG) in weaner piglets (Cromwell, 2002 ; Molist
52 et al., 2011), and dietary zinc can reduce frequency of PWD in weaner piglets (Owusu-
53 Asiedu et al., 2003 ; Pluske, 2013). The mechanisms by which zinc oxide increases ADG
54 and reduces diarrhoea in weaner piglets are not fully understood but seem to involve
55 both restoring plasma zinc levels to normal in piglets after weaning (Davin et al., 2013)
56 and improving intestinal homeostasis (Liu et al., 2014a; Liu et al., 2014b ; Shen et al.,
57 2014).

58 However, the widespread use of dietary zinc oxide in intensive animal production can
59 result in pollution of farmlands and groundwater through repeated fertilisation with
60 zinc-containing residual manure (Hill et al., 2005). In addition, zinc oxide in combination
61 with antibiotics appears to accelerate microbial antibiotic resistance by increasing the
62 rate of the exchange of antibiotic-resistance-gene containing plasmids in the microbiota
63 community in both soil and piglet intestines (Lin et al., 2016; Pal et al., 2015 ; Vahjen et
64 al., 2015); thus, there is a need to reduce both kinds of interventions.

65 As a sustainable and economically feasible alternative to antibiotics and zinc for treating
66 PWD, we have previously investigated the use of natural purified porcine

immunoglobulin G (ppIgG) from pooled abattoir blood plasma (Hedegaard et al., 2016). First, it was established that natural ppIgG does indeed contain immunoglobulin dependent anti-E. coli activity. Secondly it was shown that a dietary ppIgG supplement in a model of PWD led to faster clearance of an ETEC challenge infection than seen in a comparable control group not provided with dietary ppIgG (Hedegaard et al., 2016). This prompted us to further investigate this effect of ppIgG in E.coli challenge models. A long term, low ppIgG dose experiment and a short term, high ppIgG dose experiment were performed. In both experiments dietary ppIgG resulted in reduction of diarrhoea and in number of faecal haemolytic bacteria; moreover, in the second trial we observed that ppIgG reduced diarrhoea and cleared the enteric infection faster than dietary zinc oxide. These observations warrant future experiments investigating the use of dietary ppIgG postweaning as an alternative to dietary zinc oxide and antibiotics in treating/preventing PWD.

2. Materials & methods

The study comprised two infection experiments, both using E. coli O149 challenge at two consecutive days post weaning. In experiment 1 (long term, low IgG dose pilot study) 750 mg/day of ppIgG was provided orally for five days before and 10 days post weaning. In experiment 2 (short term, high IgG dose) 1.9 g of ppIgG was given orally twice daily for seven days after weaning/challenge. Experiment 2 also comprised a group receiving dietary zinc oxide for 10 days after weaning.

2.1. Purified porcine immunoglobulin G (ppIgG)

90 The ppIgG was prepared from pooled pig plasma by expanded bed chromatography
91 (EBA) at Upfront Chromatography A/S (Copenhagen), as described previously
92 (Hedegaard et al., 2016). Concentrated porcine blood plasma was obtained from Daka
93 SARVAL A/S (Lunderskov, Denmark). The batch of ppIgG used in first experiment
94 (experiment 1, see below) was the same as in (Hedegaard et al., 2016), whereas a new
95 batch of ppIgG was prepared prior to the second (experiment 2, see below).

96

97 2.2. ELISA

98 The IgG concentration in the batches of ppIgG was measured by a sandwich ELISA
99 (Hedegaard et al., manuscript in preparation), utilising a goat anti-pig IgG (Fc) antibody
100 (AAI41, Nordic Biosite ApS, Copenhagen) both for capture and detection. The IgG-
101 concentration of ppIgG used in experiment 1 was 37.5 mg/ml and in experiment 2 the
102 ppIgG concentration was 75 mg/ml.

103 Anti-E. coli activity was found in both batches of ppIgG, used in this study, by indirect
104 whole-E. coli cell ELISA previously reported (Hedegaard et al., 2016); briefly 96 wells flat
105 bottom microtiter plates (Maxisorp, NUNC, Thermo Scientific, Denmark) were coated
106 with 100 µl fixed E. coli O138 (in-house strain isolated from piglet with diarrhoea) in 0.1
107 M sodium carbonate buffer pH 9.6 (OD546 = 0.25) at 4 °C overnight. All subsequent
108 operations were performed at room temperature. Next day wells were washed four
109 times in PBS with 0.05% Tween 20 (PBS-T), and blocked with 200 µl PBS-T with 1%
110 bovine serum albumin (BSA; Sigma-Aldrich, Brøndby, Denmark) for 30 min when shaking
111 then followed by four times wash as above. The ppIgG was added in 2-fold dilution
112 series (diluted in PBS-T 1% BSA from 10 to 0.02 mg/ml). After 1 h of incubation with
113 shaking and 4 washes in PBS-T, detection antibody HRP-conjugated goat anti-pig IgG

(GGHL-5P; ICL, SMS Gruppen; Rungsted, Denmark) diluted 1/2000 in PBS-T 1% BSA was added and incubated for 1 h with shaking. After washing, plates were developed by TMB Plus substrate (Kem-En-Tec, Taastrup, Denmark), 100 µl/well, and stopping colour development by 100 µl/well 0.5 M H2SO4 (VWR—Bie & Berntsen A/S). Optical density was measured by a Thermo Scientific Multiscan EX microplate reader at 450 nm subtracting background absorbance at 650 nm.

Using the antigen specific ELISA it was found that the ppIgG batch used in experiment 1 had lost 14% of activity in comparison to the original plasma pool, whereas the batch used in experiment 2 had lost no activity (data not shown).

2.3. Experiments

2.3.1. Experimental procedure

Two separate factorial experiments at Aarhus University, Foulum, involving 12 (10.8 ± 1.8 kg BW, from 2 litters) and 18 (7.3 ± 1.0 kg BW, from 2 litters) pigs for the first and second experiments, respectively, were conducted (see Table 1). In both experiments, piglets were weaned from sows that had been tested to be homozygote carriers of the dominant gene encoding for intestinal F4 fimbriae receptors (Van Haeringen Laboratorium, b.v., Wageningen, The Netherlands) on DNA extracted from hair sample. Piglets were weaned from the sows at day 28 of age, and were challenged with E. coli O149:F4 on two consecutive days (d 29 and 30 of age). Within each experiment, piglets from different litters were equally distributed among treatments.

In the first experiment, half of the piglets (Table 1; Exp. 1, Group 1 + 2) received once daily for 15 days (5 days preweaning and 10 days postweaning) 20 ml (750 mg) of ppIgG,

137 provided by a 20 ml syringe; a small plastic tube was connected to the syringe and the
138 ppIgG was slowly dipped in and piglets willingly lapped up, ensuring that no ppIgG was
139 lost.

140 The other half of the pigs received 20 ml 0.9% NaCl (Table 1; Exp. 1, Group 3 + 4). Pigs
141 were provided the immunoglobulin product before feeding.

142 In the second experiment, pigs were allotted into three challenge-groups (Table 1; lower
143 part): two groups received no immunoglobulins but piglets were provided with 25 ml of
144 0.9% NaCl and provision of feed (from day of weaning) based on wheat, barley and
145 dehulled soybean, and with either 2500 ppm zinc oxide (Hammershøj Pharmacy,
146 Hammershøj, Denmark) for 14 days postweaning (Table 1; Exp. 2, Group 1), or no zinc
147 oxide (Table 1; Exp. 2, Group 2). The third group received ppIgG via 20 ml syringe on the
148 morning before weaning (day 27), and on the morning of weaning (day 28). The ppIgG
149 feeding was continued twice daily by drench gun for 7 days postweaning with a
150 provision of 25 ml (1.9 g) of ppIgG twice daily (Table 1; Exp. 2, Group 3).

151

152 The animal experiment was conducted according to the personal license (Charlotte
153 Lauridsen, J. nr. 2012-15-2934-00125) obtained by the Danish Animal Experiments
154 Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food
155 Administration, and animals were followed by proper veterinary surveillance throughout
156 the experiment.

157

158 2.3.2. Animals, feeding and housing

All piglets were tested susceptible to E. coli O149:F4 by using a DNA marker genotyping-based test (van Haeringen laboratorium b.v., Wageningen, The Netherlands) on DNA extracted from hair samples. From weaning and onwards, the pigs had ad libitum access to feed and water. The feed was a standard diet for weaners prepared at the facilities at Aarhus University, Foulum. Two littermates of similar weight were housed in pairs in 1.45 m × 1.7 m pens with concrete flooring and sawdust bedding. In experiment 1, the unchallenged (Groups 1 + 3) and challenged pigs (Groups 2 + 4) were housed in different rooms to avoid cross contamination with the challenge E. coli strain. A door separated the rooms between the challenged and unchallenged piglets, and personnel changed clothes/shoes before entering any of the rooms. Environmental conditions including temperature (~24 °C), humidity (~50%) and bedding (sawdust) were similar in the two rooms. Experiment 2 was conducted in the same rooms, but all pigs were challenged with E. coli, and there was free passage between the rooms throughout the entire experiment.

2.3.2.1. E. coli challenge

In both experiments, pigs were orally inoculated with 1.0 × 109 colony-forming units (cfu) of E. coli O149:F4 in 5 ml 0.9% NaCl on day 1 and 2 after weaning (day 0 was the day of weaning) using a syringe. After inoculation, the tube was flushed with approximately 10 ml 10% NaHCO3 in order to neutralize gastric acid and increase the survival rate of the challenge strain in the stomach, and to ascertain that all the E. coli suspension had been given to the piglets. The control pigs (exp. 1) received equivalent amounts (approximately 5 ml) of 0.9% NaCl and 10 ml 10% NaHCO3 in order to obtain

an equal level of stress associated with the oral inoculation as for the challenge treatment.

2.3.3. Performance and diarrhoea assessment

Diarrhoea assessment was based on the consistency of the faeces (1 = hard, dry and cloddy, 2 = firm, 3 = soft with shape, 4 = soft and liquid, 5 = watery and dark, 6 = watery and yellow, 7 = foamy and yellow) from the day prior to challenge until 7 days after. A faecal consistency score >3 was defined as a clinical sign of diarrhoea (Carstensen et al., 2005). Before the E. coli challenge, and on day 2 after challenge, and daily until day 5 after challenge, and thereafter every second day during the second week after weaning, faecal samples were collected from the rectum of the pigs and 1 g faeces was suspended in a (1:10, wt/wt) peptone solution and homogenized by bag mixer (BagMixer100, Interscience, St. Nom, France). Serial dilutions of the slurry were done prior to enumeration of haemolytic E. coli on blood agar (BA; Oxoid) after aerobic incubation at 37 °C overnight. From each BA plate, five haemolytic E. coli colonies were selected and tested for O149 and O138 type reactions by O-seroagglutination (Statens Serum Institut, Copenhagen, Denmark). In addition, faecal samples were analysed for dry matter by freeze-drying (ScanVac Coolsafe 55, Labogene Aps, Lynge, Denmark).

Feed intake was recorded daily for each pen and body weight of the pigs was recorded at the beginning and weekly thereafter until the end of the experiment. Average daily feed intake (ADFI) and gain (ADG) were determined based on pen by dividing the total feed intake or total weight gain of pigs in each pen by days of feeding.

205 2.4. Statistics

206 The effects of ppIgG, dietary zinc oxide or no treatment for seven days postweaning on
207 diarrhoea symptoms and bacterial count in both experiments were statistically analysed
208 using either Mann-Whitney test or Two-way ANOVA followed by Tukey's post-test, in
209 GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA,
210 www.graphpad.com.

211

212 **3. Results and discussion**

213 In this study we performed two infection experiments using an E. coli O149 challenge
214 strain at both day one and two after weaning. The administration of ppIgG was different
215 in the two experiments: In experiment 1, 750 mg of ppIgG was provided orally once daily
216 prophylactically for five days preweaning and then 10 days postweaning, whereas in
217 experiment 2, 1.9 g of ppIgG was administered orally twice daily for seven days
218 postweaning. Two different ppIgG batches were used for the two experiments, for
219 experiment 1 a 37.5 mg/ml batch and for experiment 2 a 75 mg/ml batch.

220

221 3.1. Disease

222 In both experiments diarrhoea was observed within one day after inoculation due to the
223 O149 ETEC challenge as observed by faecal scoring (Fig. 1A + B) and faecal dry matter
224 measurement (Fig. 1C + D). We observed in both experiments that the +ppIgG
225 +challenge group (group 2 in exp. 1 and group 3 in exp. 2) had, at day five, significantly
226 less diarrhoea as compared to the control challenge groups (group 4 in exp. 1 and group

227 2 in exp. 2) that were not provided with ppIgG as observed by a lower faecal scoring and
228 a higher percentage of faecal dry matter ($p < 0.03$; Fig. 1, days 1–5). In experiment 1 the
229 O149 ETEC challenge strain began to cease at day 7, however an unintended enteral
230 infection, starting around day 5–7 after weaning and lasting the remainder of the study
231 period was observed (Fig. 1A + C, days 5–21), coinciding with the appearance of an O138
232 ETEC strain in faeces (detected by PCR; data not shown). The presence of this
233 unintended infection somewhat obscured experiment 1, however the data from day 0 to
234 five were promising and prompted us to conduct a second experiment in which the daily
235 IgG dose was quadrupled but then only provided seven days post weaning. This batch of
236 ppIgG had an IgG concentration of 75 mg/ml, and was administered twice daily,
237 amounting to a daily dose of 3.8 g.

238 Experiment 2 proceeded without any unintended infection, and group 3 (+ppIgG
239 +challenge group) had significantly less diarrhoea than both group 1 and 2 (+zinc
240 +challenge and no treatment groups) within the first week after weaning ($p < 0.02$ Two-
241 way ANOVA; Fig. 1B + D); demonstrating that ppIgG significantly reduced diarrhoea in
242 the PWD model within the first week post weaning to the same level or lower than
243 dietary zinc.

244 Moreover, our results indicate that it is not necessary to use ppIgG prophylactically for
245 several days preweaning as there was a clear effect of ppIgG in experiment 2 on
246 diarrhoea without the five days preweaning ppIgG treatment applied in experiment 1
247 (Fig. 1). This also corroborates a previous study (Hedegaard et al., 2016) where ppIgG
248 was mixed into the feed that was only available from weaning and decreased
249 diarrhoeagenic Enterobacteriaceae in comparison to a non-ppIgG diet. Also, it has been
250 shown (Foged et al., 1986) that monoclonal anti-E. coli F4 fimbriae antibodies

administered at as well as after challenge, but not prophylactically, protected neonatal piglets from an otherwise lethal challenge with F4+ ETEC. On the other hand, it seems to be important to maintain a high administration frequency and an adequately high antibody dose for limiting diarrhoea.

3.2. Microbiology

In both experiments ppIgG helped clear the challenge strain within one week of challenge (Fig. 2, days 1–7). However, as noted above, all groups in experiment 1 experienced an unintended O138 ETEC infection from day 4–9, as seen by an increase in numbers of faecal haemolytic bacteria (Fig. 2A, days 4 + 7 + 9). Group 1 (+ppIgG no challenge) experienced this infection at day 4 while it was observed in group 2 (+ppIgG +challenge) on day 9. Thus, the daily administration of 750 mg of natural IgGs did not provide protection against the unintended infection in neither of the two +ppIgG groups (Fig. 2A, groups 1 + 2, days 4–21), even though in vitro data support that ppIgG binds to O138 (and O149) ETEC and can inhibit their adhesion to intestinal epithelial cells in vitro (Hedegaard et al., 2016). This might indicate that the unintended infection was multifactorial, and/or that the daily dose of 750 mg natural ppIgG used in this experiment was not adequate to prevent this type of infection. Data from dose-response field trials are needed before any further conclusions can be made on this matter.

In experiment 2, only infection with the challenge-strain was observed. Confirming previous results (Hedegaard et al., 2016), ppIgG caused a faster clearance of diarrhoeagenic (haemolytic) bacteria in group 3 than was observed in the other two groups (Fig. 2B). Thus day 7 was the last day on which faecal haemolytic bacteria were

detected in group 3 (+ppIgG), while these bacteria could still be detected on day 9 in group 1 (+zinc group), and in group 2 (control) one piglet still had faecal haemolytic bacteria on day 15 (Fig. 2B). Taken together, ppIgG appears to intervene with the colonization by the ETEC challenge strain shortening the period of infection significantly (Fig. 2B, Day 5, $p < 0.02$) and was as efficient as dietary zinc in reducing the infection with the ETEC challenge strain. Although this suggests that ppIgG can shorten the duration of diarrhoea in weaner piglets by decreasing the number of faecal diarrhoeagenic haemolytic bacteria, this should ideally take place without a perturbing the composition of the normal intestinal microbiota. In experiment 1, no change in the faecal non-haemolytic bacteria were initially observed (Fig. 2C, days 1–5) however, as the unintended infection emerged the number of faecal non-haemolytic bacteria began decreasing (Fig. 2C, days 7–21). In group 2 (+ppIgG +challenge) the faecal non-haemolytic bacteria actually were not recovered for 3 days at the height of the unintended infection (Fig. 2C, days 11–14), which coincided with the termination of administration of ppIgG. However in experiment 2, no changes in the count of faecal non-haemolytic bacteria in any of the three other groups were observed (Figs. 2D), indicating that ppIgG does not intervene with the intestinal non-haemolytic commensal microbiota and that the microbiota changes observed in experiment 1 was probably due to the unintended infection. These observations are supported by preliminary next generation sequencing data on the faecal microbiota composition of piglets fed ppIgG showing no change in non-haemolytic commensals (unpublished data).

Disease (diarrhoea) frequency, growth and feed conversion are primary end points for swine producers however the two experiments described here comprised low numbers of piglets (n = 12/18) and pens (n = 4/3) making it very difficult to analyse growth data statistically. Also, the data on growth and feed intake showed a very large pig-to-pig

variation. For example, in experiment 2 half of the piglets in group 3 (+ppIgG), for unknown reasons, became anorexic during the third week resulting in almost no weight gain for the group. Therefore it will be interesting to observe how ppIgG supplementation will influence disease resistance, growth and feed conversion in field trials incorporating an adequate number of pigs to allow for appropriate statistical data analysis to be performed. In spite of being a small preliminary study the results shown here do however demonstrate the ability of dietary ppIgG to clear an enteric ETEC infection and thus ppIgG could be used as an alternative to dietary zinc.

Acknowledgements

The project was supported by Green Development and Demonstration Programme (Ministry of Food, Agriculture and Fisheries, The Danish AgriFish Agency, Journal number: 34009-12-0471). Henriette Vorsholt is thanked for excellent technical assistance with the laboratory analysis and Inger Marie Jepsen is thanked for excellent technical assistance during the animal experiments.

References

Becker, 2010

G.S. Becker

Antibiotic Use in Agriculture: Background and Legislation

Congressional Research Service, MA, USA (2010)

Carstensen et al., 2005

L. Carstensen, A.K. Ersboll, K.H. Jensen, J.P. Nielsen

Escherichia coli post-weaning diarrhoea occurrence in piglets with monitored exposure to creep feed

Vet. Microbiol., 110 (2005), pp. 113–123

Cromwell, 2002

G.L. Cromwell

Why and how antibiotics are used in swine production

Anim. Biotechnol., 13 (2002), pp. 7–27

DANMAP, 2015

DANMAP,

DTU

National Food Institute, Søborg, Denmark (2015)

Davin et al., 2013

R. Davin, E.G. Manzanilla, K.C. Klasing, J.F. Perez

Effect of weaning and in-feed high doses of zinc oxide on zinc levels in different body compartments of piglets

J. Anim. Physiol. Anim. Nutr. (Berl), 97 (Suppl. 1) (2013), pp. 6–12

Fairbrother et al., 2005

J.M. Fairbrother, E. Nadeau, C.L. Gyles

Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies

Anim. Health Res. Rev., 6 (2005), pp. 17–39

Foged et al., 1986

N.T. Foged, P. Klemm, F. Elling, S.E. Jorsal, J. Zeuthen

Monoclonal antibodies to K88ab, K88ac and K88ad fimbriae from enterotoxigenic Escherichia coli

Microb. Pathog., 1 (1986), pp. 57–69

Hedegaard and Heegaard, 2016

C.J. Hedegaard, P.M. Heegaard

Passive immunisation, an old idea revisited: basic principles and application to modern animal production systems

Vet. Immunol. Immunopathol., 174 (2016), pp. 50–63

Hedegaard et al., 2016

C.J. Hedegaard, M.L. Strube, M.B. Hansen, B.K. Lindved, A. Lihme, M. Boye, P.M. Heegaard

Natural pig plasma immunoglobulins have anti-Bacterial effects: potential for use as feed supplement for treatment of intestinal infections in pigs

PLoS One, 11 (2016), p. e0147373

Hill et al., 2005

D.D. Hill, W.E. Owens, P.B. Tchoounwou

Impact of animal waste application on runoff water quality in field experimental plots

Int. J. Environ. Res. Public Health, 2 (2005), pp. 314–321

Lin et al., 2016

H. Lin, W. Sun, Z. Zhang, S.J. Chapman, T.E. Freitag, J. Fu, X. Zhang, J. Ma

Effects of manure and mineral fertilization strategies on soil antibiotic resistance gene levels and microbial community in a paddy-upland rotation system

Environ. Pollut., 211 (2016), pp. 332–337

Liu et al., 2014a

P. Liu, R. Pieper, J. Rieger, W. Vahjen, R. Davin, J. Plendl, W. Meyer, J. Zentek

Effect of dietary zinc oxide on morphological characteristics, mucin composition and gene expression in the colon of weaned piglets

PLoS One, 9 (2014), p. e91091

Liu et al., 2014b

P. Liu, R. Pieper, L. Tedin, L. Martin, W. Meyer, J. Rieger, J. Plendl, W. Vahjen, J. Zentek

Effect of dietary zinc oxide on jejunal morphological and immunological characteristics in weaned piglets

J. Anim. Sci., 92 (2014), pp. 5009–5018

Madec et al., 1998

F. Madec, N. Bridoux, S. Bounaix, A. Jestin

Measurement of digestive disorders in the piglet at weaning and related risk factors

Prev. Vet. Med., 35 (1998), pp. 53–72

Molist et al., 2011

F. Molist, R.G. Hermes, A.G. de Segura, S.M. Martin-Orue, J. Gasa, E.G. Manzanilla, J.F. Perez

Effect and interaction between wheat bran and zinc oxide on productive performance and intestinal health in post-weaning piglets

Br. J. Nutr., 105 (2011), pp. 1592–1600

Owusu-Asiedu et al., 2003

A. Owusu-Asiedu, C.M. Nyachoti, R.R. Marquardt

Response of early-weaned pigs to an enterotoxigenic Escherichia coli (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody, zinc oxide, fumaric acid, or antibiotic

J. Anim. Sci., 81 (2003), pp. 1790–1798

Pal et al., 2015

C. Pal, J. Bengtsson-Palme, E. Kristiansson, D.G. Larsson

Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential

BMC Genomics, 16 (2015), p. 964

Pluske, 2013

J.R. Pluske

Feed- and feed additives-related aspects of gut health and development in weanling pigs

J. Anim. Sci. Biotechnol., 4 (2013), p. 1

Shen et al., 2014

J. Shen, Y. Chen, Z. Wang, A. Zhou, M. He, L. Mao, H. Zou, Q. Peng, B. Xue, L. Wang, X. Zhang, S. Wu, Y. Lv

Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets

Br. J. Nutr., 111 (2014), pp. 2123–2134

435 Vahjen et al., 2015

436 W. Vahjen, D. Pietruszynska, I.C. Starke, J. Zentek

437 High dietary zinc supplementation increases the occurrence of tetracycline and
438 sulfonamide resistance genes in the intestine of weaned pigs

439 Gut Pathog., 7 (2015), p. 23

440

441 Wageningen University, 2012

442 Wageningen University

443 Antibiotic Usage in Pigs

444 Wageningen University & Research (2012) (Wageningen, Netherlands,
445 http://www.wur.nl/en/Research-Results/Projects-and-programmes/MARAN-Antibiotic-
446 usage/Trends-in-use-per-species/Antibiotic-usage-in-pigs.htm)

447

448

449

450

451

452

454 **Tables**

455 Table 1: Study setup

Experiment 1	Group 1 +ppIgG	Group 2 +ppIgG+E.coli	Group 3 Control	Group 4 +E.coli
Number of piglets	3	3	3	3
E. coli F4+ challenge	No	Yes	No	Yes
750 mg ppIgG (20 ml)	Yes	Yes	No	No
20 ml 0.9% NaCl solution	No	No	Yes	Yes
Avg. weight at weaning (kg)	11.2±3.1	10.6±1.7	10.4±1.9	11.0±1.2
Experiment 2	Group 1 + E.coli +Zn	Group 2 +E.coli	Group 3 +E.coli +ppIgG	
Number of piglets	6	6	6 (5)[1]	
E. coli F4+ challenge	Yes	Yes	Yes	
2x1.9 g ppIgG (2x25 ml)	No	No	Yes	
25 ml 0.9% NaCl solution	Yes	Yes	No	
Zinc oxide in feed	Yes	No	No	
Avg. weight at weaning (kg)	6.9±1.1	7.9±0.9	7.0±0.9	

456 [1] One piglet was euthanized on day 3 post infection due to serious illness.

457

458

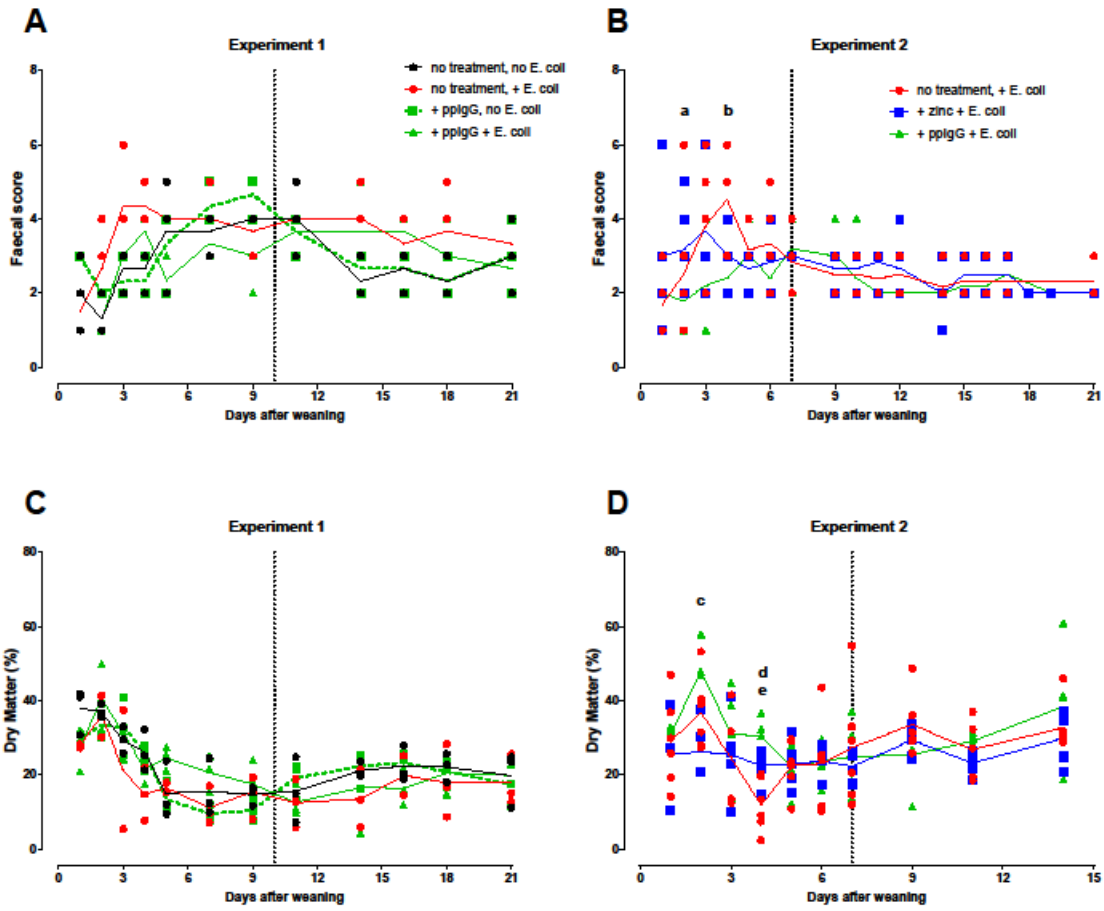
Figure legends

Figure 1. Faecal analysis: Faecal diarrhoea score (**A+B**) and faecal dry matter (**C+D**); (●) no treatment, no E. coli; (●) no treatment, +E. coli: (-■-) +ppIgG, no E. coli; (▲)ppIgG treatment, +E. coli; (■) dietary zinc, +E. coli. Each data point is plotted and curves outline the mean for each group. Vertical dotted line indicates the last day of ppIgG administration. Pairwise comparisons between groups on each day were tested for statistical significance using Mann-Whitney test: a = IgG vs. Zinc ($p<0.03$); b = IgG vs. Control ($p<0.03$); c = IgG vs. Zinc ($p<0.02$); d = IgG vs. Control ($p<0.01$); e = Zinc vs. Control ($p<0.02$).

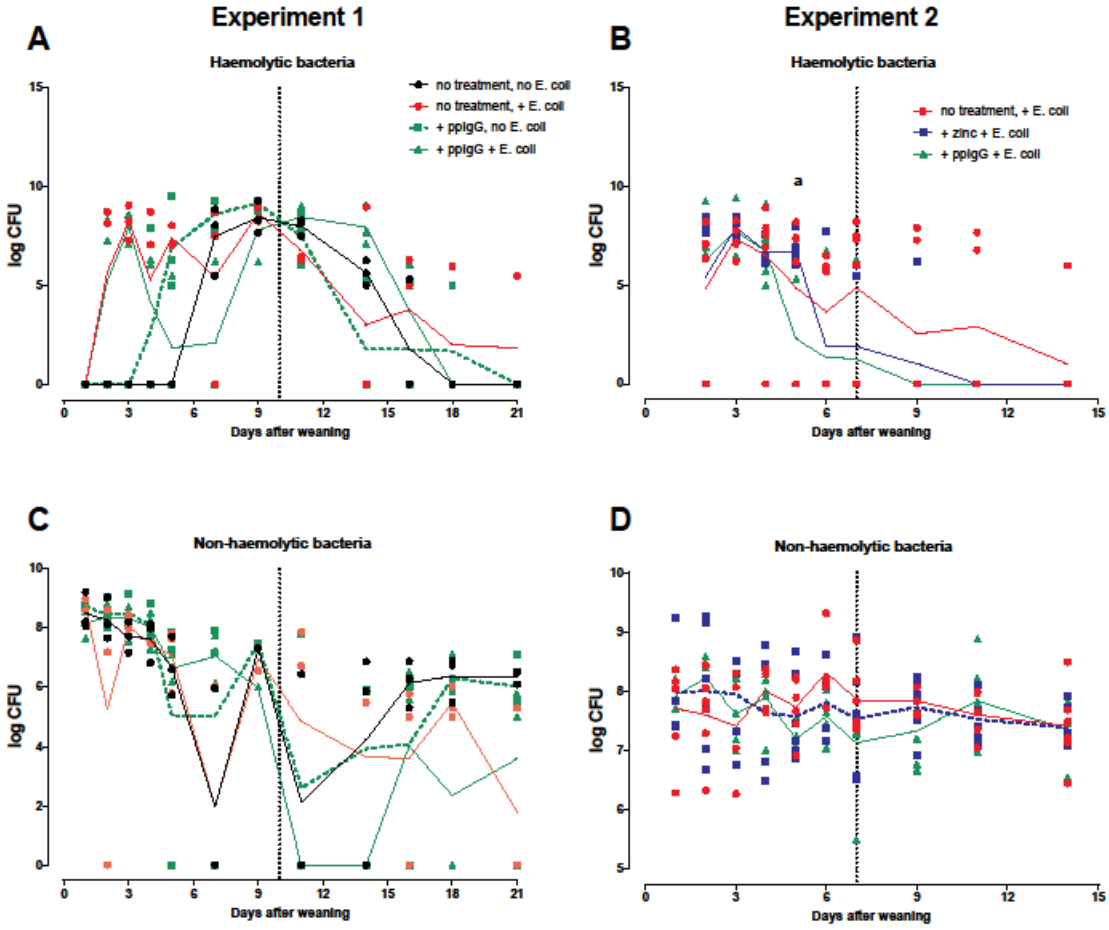
Figure 2. Faecal bacterial analysis: Content (CFU/ml) of haemolytic bacteria (**A+B**) and non-haemolytical bacteria (**C+D**). Symbols as for Figure 1. Each data point is plotted and curves outline the mean for each group. Vertical dotted line indicates the last day of ppIgG administration. Pairwise comparisons between groups on each day were tested for statistical significance using Mann-Whitney test: a = IgG vs. control ($p<0.02$).

Figures

Figure 1



490 Figure 2



491
492