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1 **The effect of post-farrowing ketoprofen on sow feed intake, nursing behaviour and**  
2 **piglet performance**

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## 14 **Abstract**

15 Farrowing is a critical time for sows and piglets. Poor post-farrowing sow recovery,  
16 and piglet mortality represent a welfare concern, as well as an economic loss to the pig industry.  
17 Providing a non-steroidal anti-inflammatory drug (NSAID) to the sow post-farrowing may  
18 improve sow welfare and productivity and thereby improve health status and welfare of the  
19 piglets, which would be of economic benefit to pig producers. This study investigated the  
20 production effects of providing the NSAID ketoprofen post-farrowing, to 24 primiparous (gilts)  
21 and 32 multiparous (sows) breeding pigs, in a randomised, blinded, placebo-controlled trial.  
22 Gilts and sows were allocated to receive ketoprofen (treated) or the equivalent volume of saline  
23 (control) by intramuscular injection 1.5 hours after the last piglet birth. Data collected included  
24 sow feed intake, immune transfer (colostrum and piglet serum immunoglobulin-G (IgG)),  
25 nursing behaviour and piglet weight, and mortality. An additional factor in this study was that  
26 13 individuals required additional treatment in the days after farrowing for post-farrowing  
27 illness. Therefore, data were analysed using mixed models, including treatment (treated or  
28 control), parity group (gilt or sow), and additional treatment (yes or no) as fixed factors.  
29 Stepwise binomial logistic regression was used to analyse the association between the  
30 experimental factors (treatment, additional treatment, gilt or sow), along with other gilt/sow,  
31 litter, and piglet-based measures, with piglet death before weaning. Few treatment effects were  
32 seen, with parameters being more affected by whether gilts and sows were treated for illness,  
33 or between gilts and sows. The only variable to differ by treatment was suckle grunt duration,  
34 which was greater for control compared with treated dams ( $P = 0.05$ ). Feed consumption was  
35 greater for sows compared with gilts on days 6 and 7 post-farrowing, and serum IgG was  
36 greater in piglets from sows than gilts ( $P < 0.05$ ). Feed consumption was reduced in dams  
37 needing additional treatment, from days 2-7 post-farrowing, and those developing illness  
38 consumed less feed overall ( $P = 0.004$ ). The best regression model for predicting the odds of a

39 piglet dying before weaning included number born alive ( $P = 0.03$ ), requiring additional  
40 treatment ( $P = 0.006$ ), being male ( $P = 0.0005$ ), and pre-farrowing gilt/sow back-fat ( $P <$   
41  $0.0001$ ), which increased the log-odds of death, whereas, piglet body weight decreased the log-  
42 odds of death ( $P < 0.0001$ ). This study did not demonstrate clear benefits to ketoprofen,  
43 however, high individual variation in piglet mortality, indicates potential for targeted NSAID  
44 use.

45

46 **Keywords:** farrowing; ketoprofen; nursing behaviour; pain; performance; sow

## 47 **Introduction**

48 Farrowing is a critical time in pig production. A common feature of modern pig  
49 production is increased litter size, and as the sow must produce enough milk to feed the litter,  
50 feed volume and composition must adjust to cope with the increased demand (Theil, 2015).  
51 Further, each piglet must have access to a functioning teat as soon as possible after birth to  
52 consume colostrum, followed by milk in order to survive (Baxter et al., 2013). Therefore, the  
53 sow must recover quickly following farrowing, including feeding and drinking. However, at  
54 that time the immunocompetence of the sow is impaired and as parturition is physically  
55 demanding, the vulnerability to illness in early lactation is increased (Friendship and  
56 O'Sullivan, 2015).

57 Post-partum dysgalactia syndrome (PPDS) describes any condition that affects milk  
58 production in the sow, including infections of the uterine tract (metritis) and udder (mastitis),  
59 but milk production can also decline with no obvious signs of infection (Klopfenstein et al.,  
60 2006). A number of non-infectious causes of PPDS have been discussed (Klopfenstein et al.,  
61 2006) and pain experienced by the sow could contribute to a decreased interest in the piglets

62 and a reduction in milk let down (Peltoniemi and Oliviero, 2015). This has resulted in recent  
63 research administering non-steroidal anti-inflammatory drugs (NSAIDs) post-farrowing and  
64 measuring the benefits to health, welfare and productivity (Homedes et al., 2014; Mainau et  
65 al., 2016, 2012; Sabaté et al., 2012; Tenbergen et al., 2014; Viitasaari et al., 2014, 2013).

66 A previous study, involving 15 commercial farms, investigated the production benefits  
67 of providing the NSAID ketoprofen post-farrowing to all sows, and demonstrated a reduction  
68 in piglet mortality and a greater number of piglets weaned (Homedes et al., 2014). Another  
69 study found no piglet performance benefits of administering ketoprofen, but did identify other  
70 sow health and welfare benefits including a reduced loss in back-fat, body condition and  
71 constipation, less severe shoulder sores, and a delay in feed refusal (Viitasaari et al., 2013).  
72 Two studies in which meloxicam was administered after farrowing found no mortality  
73 differences but did show an increased average daily weight gain of low birth weight piglets  
74 (Mainau et al., 2012) and a tendency for increased piglet weight gain of litters of 11 to 13  
75 piglets (Tenbergen et al., 2014). Another study using oral meloxicam, demonstrated  
76 improvements in piglet weaning weight, average daily gain, and plasma IgG concentrations  
77 measured on day 1 and 2 post-farrowing (Mainau et al., 2016). The administration of NSAIDs  
78 in addition to antibiotics has also been shown to aid in treatment of infectious causes of PPDS  
79 (e.g. Hirsch et al., 2003; Tummaruk and Sang-Gassanee, 2013) and on a farm with a high  
80 incidence of PPDS, piglet mortality was reduced and the number of piglets weaned increased  
81 in sows given ketoprofen and antibiotics (Sabaté et al., 2012).

82 Ketoprofen is an NSAID with anti-inflammatory, analgesic, and antipyretic properties,  
83 which was shown to reach maximum levels approximately one hour after intramuscular (IM)  
84 injection in pigs (Raekallio et al., 2008), and reduced nociceptive thresholds in piglets with  
85 kaolin-induced inflammation up to 24 hours after IM injection (Fosse et al., 2011). This study  
86 investigated the use of ketoprofen after farrowing for primiparous (hereafter referred to as gilts)

87 and multiparous (referred to as sows) breeding pigs. The aim was to evaluate the benefits of  
88 post-farrowing ketoprofen in terms of: i) gilt/sow feed intake; ii) immune transfer using IgG  
89 from colostrum and piglet serum; iii) piglet performance including growth and mortality; and  
90 iv) nursing behaviour. Based on previous studies, our hypothesis was that prompt post-  
91 farrowing treatment with ketoprofen improves sow recovery, including feed intake, and piglet  
92 performance through immune transfer and nursing behaviour.

### 93 **Materials and Methods**

94 This experiment was carried out under UK Home Office Licence, in compliance with  
95 EU Directive 2010/63/EU and following approval from the SRUC Animal Welfare and Ethical  
96 Review Body (AWERB).

#### 97 Animal housing and husbandry

98 Thirty-two Large White × Landrace multiparous (mean parity  $4.63 \pm 0.43$ ) and 24  
99 primiparous sows were used in this study. The study was carried out at the SRUC pig research  
100 farm (Midlothian, UK), with gilts and sows farrowing in nine batches between February and  
101 October 2014. No more than five days before the expected farrowing date, gilts and sows were  
102 moved into individual farrowing crates (1.8 × 0.5 m), with solid concrete flooring (1.8 × 1.5  
103 m), a small slatted area at the back (0.5 × 0.5 m) and a water and feed trough at the front. Piglets  
104 had access to a heated creep area (1.5 × 0.65 m) in front of the water and feed trough. Gilts and  
105 sows were fed a standard pelleted lactation diet twice daily at 0745 and 1530 and had  
106 continuous access to fresh water. Gilt and sow crates were cleaned daily at the morning feed,  
107 and they were provided with fresh, long-stemmed straw. Additional straw was added and  
108 manure removed at the afternoon feed in the days preceding farrowing. Lights were switched  
109 on immediately before the morning feed, turned off at 1630 and an additional night-light was  
110 provided in the centre of each room of crates.

111 During the experiment and only after the six hour post-injection data collection, cross-  
112 fostering was conducted where necessary to even up litter sizes to maximise piglet survival as  
113 per normal farm practice. Cross fostering was conducted regardless of experimental treatments.  
114 When litter sizes were uneven, the largest piglet(s) were removed and placed on a gilt or sow  
115 with a smaller litter. Beyond the time of cross-fostering, data for individual foster piglets was  
116 then recorded against the foster sow. Piglets received an intramuscular injection of iron on day  
117 3 post-farrowing, and on the fourth week after farrowing (mean age  $26.39 \pm 0.20$ ), weaning  
118 took place. At weaning, piglets were ear tagged and vaccinated (CircoFLEX) as per farm  
119 practice.

#### 120 Blinding and treatments

121 This study was a randomised, blinded, placebo controlled trial, with gilts and sows  
122 allocated to receive a single intra-muscular (IM) injection of ketoprofen (Ketofen; Merial  
123 Animal Health Limited, Harlow, Essex, UK) or the equivalent volume of saline, 90 minutes  
124 following the birth of the last piglet. Gilts and sows in each batch were randomly allocated to  
125 receive either ketoprofen (**treated**; 3 mg per kg bodyweight or 1 ml per 33 kg pre-farrowing  
126 bodyweight rounded down to the nearest 0.5 ml) or the equivalent volume of saline as a placebo  
127 control (**control**). The 56 individuals were balanced as much as possible across batches and for  
128 parity over the two treatment groups, however, an error in the treatment allocation, resulted in  
129 unbalanced groups for gilts (gilts: treated, n = 11, control, n = 13; sows: parity 2 to 4; treated,  
130 n = 9, control, n = 8; parity 5 to 7; treated, n = 5, control, n = 6; parity 8+; treated, n = 2, control,  
131 n = 2). One experimenter allocated individuals to the two treatment groups and a second added  
132 the ketoprofen or saline to individual brown medicine bottles, sealed with rubber stoppers  
133 (Adelphi Healthcare Packaging, Haywards Heath, West Sussex, UK), which were labelled only  
134 with the individual gilt or sow ear tag for identification. Ketofen contains the active ingredient

135 ketoprofen at 100 mg/ml contained in a solution of l arginine, benzyl alcohol (10 mg/ml), citric  
136 acid monohydrate and water. It is a clear colourless solution, with low viscosity, making it  
137 indistinguishable from the saline placebo to the third experimenter administering the injection,  
138 who was unaware of the treatment.

139           Individuals were closely monitored for signs of farrowing, by observation at twice daily  
140 feeding and through remote monitoring using a CCTV digital surveillance system around the  
141 clock. Once the piglet expulsion phase began, the time of each piglet birth was recorded; and  
142 90 minutes after the last piglet birth and the gilt or sow appeared to have finished farrowing,  
143 ketoprofen or saline was administered by intra-muscular injection. Ketoprofen or saline were  
144 injected into the neck muscle, just behind the ear using an 18 gauge, 1.5 inch needle attached  
145 to a PVC extension tube and using a 10 or 20 ml syringe (Henry Schein Animal Health,  
146 Dumfries, Dumfries and Galloway, UK). Following treatment administration, individuals were  
147 left undisturbed.

#### 148 Piglet measurements

149           Six hours after the treatment administration, the litters were processed and three piglets  
150 per litter were blood sampled. All piglets were collected and shut into the heated creep area  
151 during processing. Each piglet was weighed, crown-rump length measured (from the tail base  
152 to the top of the crown, in between the ears) and were labelled numerically on the back with a  
153 permanent marker. Three piglets per litter were selected to be blood sampled for  
154 immunoglobulin-G (IgG), based on weight: one less than 1.3 kg, one between 1.31 and 1.63  
155 kg and one greater than 1.64 kg, balanced across litters for sex. If piglets at all weight ranges  
156 were not available, alternatives were selected as close as possible, and very weak piglets were  
157 avoided.



158 Selected piglets then had a topical local anaesthetic cream (EMLA) applied to their  
159 right ear. Each piglet was then held, while cotton wool soaked in hot water was applied to the  
160 right ear to promote vasodilation. A general purpose surgical steel lancet (HawksleyVet,  
161 Lancing, Sussex, UK) was used to make a small incision in the most prominent ear vein. Blood  
162 was allowed to pool briefly and collected into at least five 50 µl plain capillary tubes  
163 (HawksleyVet, Lancing, Sussex, UK). Blood was left to coagulate in the tubes for one hour at  
164 room temperature, before being sealed at one end using Cristaseal wax plates (HawksleyVet,  
165 Lancing, Sussex, UK), and then placed into a micro haemocrit centrifuge (HawksleyVet,  
166 Lancing, Sussex, UK) for 1.5 minutes at 13,000 g. The end of the tube containing the  
167 condensed cells was cut off and the serum was pushed out of the remaining section of tube  
168 using a clean needle and syringe into a clean, pre-labelled 1.5 ml tube. Samples were then  
169 stored at -70 °C to be assayed at a later date.

170 On day three post-farrowing, piglets were weighed when they were given a routine iron  
171 injection. At weaning, piglets were weighed and their crown-rump distance measured. All  
172 piglet deaths from birth to weaning were recorded and the cause of death identified by visual  
173 examination, and from video recording, including: still birth, crushing by the sow, low  
174 viability, starvation, savaged, 'greasy pig' (exudative epidermatis) and 'other' (unidentified  
175 causes). During the experiment, several litters were affected by exudative epidermatis, a  
176 bacterial skin infection, which was unrelated to the study, and was treated with long-acting  
177 antibiotics (amoxicillin).

#### 178 Gilt and sow measurements

179 On moving in before farrowing and out at weaning, all gilts and sows were weighed,  
180 body condition scored (1 = very thin, 2 = thin, 3 = not too thin, not too fat, 4 = fat, 5 = very fat)

181 and had their back-fat depth measured at the P2 position (Piglog 105; Carometec Food  
182 Technology, Smørum, Denmark).

183 At six hours after the treatment during piglet processing, a colostrum sample was  
184 collected from the dams. This was done by gently rubbing the udder, to ensure the dam was  
185 calm, then expressing colostrum from as many different teats as possible into a clean 30 ml  
186 plastic tube. Approximately 5 ml of colostrum was collected in the tube before pipetting into  
187 three 1.5 ml pre-labelled tubes, which were stored at -20°C to be assayed for IgG at a later date.

188 Gilt and sow feed intake was recorded on the day of farrowing, until seven days post-  
189 farrowing. Individuals were fed a standard pelleted lactation diet consisting of 16.4% crude  
190 protein, 6.8 % crude oils and fats, 4.0% crude fibre, 5.8% crude ash, 13.8% moisture, 0.8%  
191 calcium, 0.94% lysine, 0.25% methionine, 0.51% phosphorus and 0.22% sodium. Gilts and  
192 sows were fed, based on a feed chart, which was adjusted slightly according to the size, body  
193 condition and appetite of the individual (e.g. gilts were fed slightly less than sows and a reduced  
194 body condition score was given slightly more feed). Feed intake was restricted, and increased  
195 gradually from day 0 to day 7. The amount fed was marked on the feed chart (in kg) and the  
196 amount left over from the previous feed was removed, weighed and recorded at the next feeding  
197 time.

## 198 Behaviour

199 Closed-circuit television (CCTV) cameras (LL20, infra-red cameras, FR concepts,  
200 Ireland) were mounted above each farrowing crate and were connected to a computer to record  
201 behaviour using GeoVision Digital Surveillance System software (ezCCTV Ltd, Herts, UK).  
202 This surveillance system was also set up to enable remote monitoring of individuals. Digital  
203 video footage was collected and stored to be observed later using The Observer XT 11.0  
204 (Noldus Information Technology, Wageningen, The Netherlands). Three hour observations

205 were made for suckling behaviour between 15 and 18 hours after the last piglet was born, to  
206 coincide with a regular pattern of milk let down and udder massage by the piglets, (Castren et  
207 al., 1989) which enabled obvious nursing bouts to be recognised on video. The frequencies and  
208 duration of suckle grunting (rapid flank movements indicating suckle grunting), whether more  
209 than 50% of piglets were active at the udder (performing udder massage/rapid suckling  
210 movements), as well as gilt and sow posture (stand, sit, kneel, lie lateral, lie ventral) and  
211 drinking behaviour (snout in the drinking trough with head movements indicating drinking  
212 behaviour) were recorded.

### 213 Analysis of Immunoglobulin G (IgG) concentrations

214 Sow colostrum and piglet serum samples were assayed for IgG using an enzyme linked  
215 immunosorbent assay (ELISA) kit (Bethyl Laboratories, Inc., Montgomery, Texas, USA).  
216 Colostrum and serum samples were removed from the freezer and allowed to thaw gradually  
217 at 4 °C overnight before the assay. On the day of the assay, samples were removed from the  
218 fridge, placed at room temperature for 30 minutes before further preparation.

219 Colostrum samples were centrifuged twice at 16,249 g for 2 minutes, removing the fat  
220 layer after each spin. Serum samples were centrifuged for one minute at 865 g. Assays were  
221 then conducted according to the manufacturer's instructions, with samples tested in duplicate.  
222 A test assay was run, indicating that a 1:500,000 dilution was best for both sample types. This  
223 dilution was created using serial dilution in, un-coated V-bottomed 96-well plates.

224 Quality control (QCs) samples were created using pooled colostrum samples to run  
225 across and between plates to measure drift within and between plates. To avoid drift in the time  
226 taken to add the samples to the coated plate, 130 µl of standards, blanks, samples and QCs were  
227 added to an uncoated 96-well plate according to the plate layout, before using a multi-channel  
228 pipette to transfer into the coated plate. The plate was read using a Multiskan<sup>TM</sup> FC Microplate

229 Photometer plate reader and results calculated using a 5 point logistic regression curve using  
230 Thermo Scientific SkanIt™ for Multiskan™ FC software (version 2.5.1) (Thermo Fisher  
231 Scientific Inc, Waltham, Massachusetts, USA). Samples were spread across nine assay runs,  
232 balanced as much as possible for treatment, sample type (colostrum or serum), for gilts and  
233 sows and between farrowing batches. Duplicate samples with a coefficient of variation (CV)  
234 above 10% were repeated and those that failed to reach a CV% of less than 10% were left as  
235 missing values. The assay range was 1.37 – 1000 ng/ml.

236 The lower and upper detectable limits of the samples analysed were 4.76 and 77.37  
237 ng/ml respectively. The average intra-assay CV was 6.66% (7.79, 6.91, 4.51, 6.69, 9.35, 6.17,  
238 6.58, 9.07 and 2.82 for assay runs 1 to 9 respectively) and the inter-assay CV was 8.69%.

#### 239 Data analysis

240 Unless stated at the start of each results section, data were available for all individuals.  
241 Due to an error in the treatment allocation for gilts, there were 11 gilts and 16 sows in the  
242 ketoprofen treated group and 13 gilts and 16 sows in the saline control group. An additional  
243 factor in this study was that 13 individuals; 5 gilts (4 treated and 1 control treatment) and 8  
244 sows (4 treated and 4 control treatments) required additional treatment in the days after  
245 farrowing for PPDS. Therefore, data were analysed by treatment (treated vs. control), parity  
246 group at the level of gilt vs. sow and whether additional treatment was needed (yes vs. no). All  
247 data were analysed and descriptive statistics calculated using R version 3.3.1 (R core team,  
248 2013). All figures were plotted using the ggplot2 function, and any correlations were conducted  
249 using the spearman.test function. Results were considered statistically significant at  $P < 0.05$ .

250 *Feed intake*

251 Feed consumed was analysed with linear mixed models, using the lmer function, with  
252 dam identity and batch in the random model. Initially, total feed consumed was analysed with  
253 treatment (treated or control), parity group (gilt or sow) and additional treatment (yes or no)  
254 and their interactions as fixed factors. Then each of the factor interactions with day was tested  
255 (0, 1, 2, 3, 4, 5, 6, and 7), including: day × treatment, day × gilt/sow and day × additional  
256 treatment. Post hoc analyses were conducted using the lsmeans function.

257 *Immunoglobulin-G (IgG)*

258 Colostrum IgG concentrations (mg/ml) were analysed using linear mixed models with  
259 the lmer function, with batch in the random model. Treatment (treated or control), parity group  
260 (gilt or sow) and additional treatment (yes or no), and their interactions, and the number of  
261 piglets born alive were added as fixed factors. Piglet serum IgG was also analysed using the  
262 lmer function, with dam identity and batch in the random model, also with treatment (treated  
263 or control), parity group (gilt or sow) and additional treatment (yes or no) and their interactions,  
264 and piglets born alive as fixed factors. A Spearman's rank correlation coefficient was  
265 calculated between piglet weight (kg) and IgG concentration (mg/ml), resulting in no  
266 significant correlation ( $\rho = 0.039$ ,  $P = 0.64$ ), therefore piglet weight was not included in the  
267 model.

268 *Production data*

269 The frequency of piglets born alive, still born, and number weaned, as well as live-born  
270 pre-weaning deaths were analysed at the litter level with a generalized linear mixed model,  
271 using the glmer function, using a Poisson distribution and log link function. Sow weights, bat-  
272 fat thickness, and piglet weights and crown rump distances were analysed using linear mixed

273 models with the lmer function. The number of piglets born alive was included as a random  
274 variable in the piglet mortality model. Gilt/sow identity and batch were included in the random  
275 model for the piglet measures, and batch for the sow measures. Treatment, additional treatment,  
276 gilt or sow and the interactions as fixed factors in all models. No piglets were fostered before  
277 the 6 hour post-injection sampling, therefore fostered piglets were analysed with their birth  
278 dam for the 6 hour post-injection measures, and with their foster dam for the other piglet  
279 measures. Sow weight and back-fat thickness was then analysed with moving in or post-  
280 weaning as a fixed factor, also with batch and ID in the random model. Body condition scores  
281 were analysed with ordinal logistic regression models using the polr function, with treatment,  
282 additional treatment, gilt or sow and the interactions, and batch as fixed factors, and with  
283 moving-in or post-weaning, and batch as fixed factors.

284 Piglets that were born alive were allocated as dead (yes) or alive (no) by weaning. A  
285 stepwise binomial logistic regression was conducted using the glm and AIC.step functions, to  
286 analyse associations between variables, and whether piglets died before weaning (yes or no).  
287 Variables included: treatment (treated or control), additional treatment (yes or no), gilt or sow,  
288 batch, litter size at birth, piglet gender, piglet post 6 hour weight, and whether the piglet was  
289 fostered (yes or no), as well as sow back-fat, body condition score, farrowing duration  
290 (previously obtained from video footage), and lie lateral duration from behavioural  
291 observations. Variables were chosen, based on available data, and including known risk factors  
292 for piglet mortality (e.g. Baxter and Edwards, 2015).

### 293 *Behaviour*

294 Postures (stand, sit, kneel, lie lateral, lie ventral), suckle grunting and the duration when  
295 there were more than 50 % of piglets active at the udder, were converted to percentages of the  
296 three hour observation duration. The frequency of posture changes during the three hour

297 observation period was also calculated. Individual bouts of suckle grunting were exported from  
298 The Observer for each gilt or sow, to calculate the frequency of bouts, the mean duration of  
299 each bout, and the mean inter-bout intervals. These behavioural variables were analysed using  
300 linear mixed models with the lmer function, including treatment (treated or control), parity  
301 group (gilt or sow) and additional treatment (yes or no) and their interactions as fixed factors,  
302 with batch in the random model.

## 303 **Results**

### 304 Feed intake

305 Total feed consumed did not differ by treatment  $\times$  gilt/sow ( $t = -0.49$ ,  $P = 0.62$ ),  
306 treatment  $\times$  additional treatment ( $t = 1.39$ ,  $P = 0.17$ ), or gilt/sow  $\times$  additional treatment ( $t =$   
307  $1.19$ ,  $P = 0.23$ ), by treatment ( $t = 0.33$ ,  $P = 0.74$ ), or between gilts and sows ( $t = 1.37$ ,  $P = 0.17$ )  
308 (Fig.1). However, total feed consumed differed by day  $\times$  additional treatment ( $t = -3.65$ ,  $P =$   
309  $0.0003$ ), day  $\times$  gilt/sow ( $t = 3.20$ ,  $P = 0.002$ ), and overall by additional treatment ( $t = -2.92$ ,  $P$   
310  $= 0.004$ ). Post hoc analysis revealed that sows consumed more feed compared with gilts on  
311 days 6 and 7 post-farrowing (Fig.1 b) and that although individuals requiring additional  
312 treatment consumed less feed throughout, the difference was not significant until day 2 post  
313 farrowing (Fig.1 c).

### 314 Immunoglobulin-G (IgG)

315 Colostrum IgG concentrations were available for 52 of the 56 gilts and sows. No  
316 significant interactions (treatment  $\times$  gilt/sow:  $t = 0.40$ ,  $P = 0.69$ ; treatment  $\times$  additional  
317 treatment:  $t = 0.85$ ,  $P = 0.40$ ; gilt/sow  $\times$  additional treatment:  $t = -0.32$ ,  $P = 0.75$ ) were found,  
318 or differences for treatment ( $t = -0.81$ ,  $P = 0.42$ ), between gilts and sows ( $t = 0.73$ ,  $P = 0.47$ ),  
319 or with additional treatment ( $t = -0.14$ ,  $P = 0.89$ ) (Fig.2, A-C).

320 Of the 168 piglets that were blood sampled, serum IgG concentrations were available  
321 for 147 piglets. There were no differences by treatment  $\times$  gilt/sow ( $t = -0.75$ ,  $P = 0.46$ ),  
322 treatment  $\times$  additional treatment ( $t = 1.03$ ,  $P = 0.31$ ), or gilt/sow  $\times$  additional treatment ( $t = -$   
323  $0.78$ ,  $P = 0.44$ ). Piglets from sows had greater IgG concentrations than those from gilts ( $t =$   
324  $2.10$ ,  $P = 0.04$ ), but piglet serum IgG, did not differ by treatment ( $t = -0.15$ ,  $P = 0.88$ ), or  
325 additional treatment ( $t = -0.22$ ,  $P = 0.82$ ) (Fig.2, D-F).

### 326 Production data

327 Table 1 presents production information, including litter, gilt/sow- and piglet-based  
328 measures, by treatment, for gilts and sows, and by additional treatment. Table 2 presents the  
329 total frequencies and causes of death, and frequencies of piglets fostered on and off treated and  
330 control gilts and sows, to illustrate the total numbers of piglet deaths by treatment for gilts and  
331 sows, and the imbalance in piglet fostering between treatments. Figure 3 is a dot plot showing  
332 the number of live-born deaths for individual treated and control gilts and sows, which shows  
333 the individual variation in piglet pre-weaning deaths. There were no significant treatment  $\times$   
334 gilt/sow, treatment  $\times$  additional treatment, or gilt/sow  $\times$  additional treatment interactions for  
335 any of the results presented in Table 1 ( $P > 0.05$ ). As shown, none of the results presented  
336 differed by treatment, or additional treatment ( $P > 0.05$ ). However, pre-farrow and post-wean  
337 weight differed between gilts and sows, as did the piglet weight and crown-rump measurements  
338 for piglets from gilts and sows (see Table 1). In addition, gilt or sow weight ( $t = -12.25$ ,  $P <$   
339  $0.001$ ), back-fat ( $t = -10.66$ ,  $P < 0.001$ ), and body-condition ( $t = -5.12$ ,  $P < 0.001$ ) were greater  
340 overall pre-farrowing, compared with post-weaning.

341 Of the 705 piglets born alive, any row with missing values for any of the variables was  
342 excluded, leaving 659 rows of data for analysis. The best logistic regression model included  
343 the variables piglets born alive, additional treatment, piglet gender, sow back-fat, and piglet 6



344 hour post-injection weight, which were significant predictors of death before weaning. For  
345 every increase in piglet born alive in the litter, the log odds of dying before weaning increased  
346 (log-odds = 0.11,  $P = 0.03$ ). Requiring additional treatment (log-odds = 0.87,  $P = 0.006$ ), as  
347 well as being male (log-odds = 0.97,  $P = 0.0005$ ) increased the log odds of dying before  
348 weaning. For every mm increase in gilt or sow back-fat, the log-odds of piglet death increased  
349 (log-odds = 0.16,  $P < 0.0001$ ). Every kg increase in piglet 6 hour post-injection bodyweight,  
350 decreased the log-odds of dying before weaning, (log-odds = -4.18,  $P < 0.0001$ ).

### 351 Behaviour

352 Behaviour was observed for 53 of the 56 individuals and results are shown in Table 2.  
353 There were no significant interactions for treatment  $\times$  gilt/sow, treatment  $\times$  additional  
354 treatment, or gilt/sow  $\times$  additional treatment, for any of the behaviours shown in Table 3 ( $P >$   
355 0.05). For nursing behaviour, ketoprofen treated dams suckle grunted less ( $t = -2.02$ ,  $P = 0.05$ )  
356 than the controls, but there were no other differences between treatment groups, gilts and sows  
357 and those requiring additional treatment or not ( $P > 0.05$ ). For the postures observed, sitting  
358 and kneeling behaviour differed between gilts and sows ( $t = 2.08$ ,  $P = 0.04$  and  $t = 2.49$ ,  $P =$   
359 0.02 respectively), with greater values for sows compared with gilts. Lying lateral also differed  
360 ( $t = -2.38$ ,  $P = 0.02$ ) with greater values for gilts than sows. There were no differences in  
361 drinking behaviour between treatment groups, gilts and sows or those requiring additional  
362 treatment or not ( $P > 0.05$ ).

### 363 **Discussion**

364 This study investigated effects of the provision of the NSAID ketoprofen to gilts and  
365 sows following farrowing. Few effects of the treatment were seen, with production parameters  
366 being more affected by whether individuals were treated for disease, or between gilts and sows.

367 Feed intake

368           In contrast to a previous study (Viitasaari et al., 2013), there was no difference in feed  
369 consumption by gilts or sows given ketoprofen compared with controls. The previous study  
370 administered ketoprofen for three consecutive days following farrowing, which could have had  
371 a greater effect on sows, and overall feed refusal rather than consumption was measured  
372 (Viitasaari et al., 2013). In another study where the NSAID meloxicam was administered for  
373 three days post-farrowing, feed intake was not affected by drug treatment, but a difference  
374 between primiparous and multiparous sows was found, as multiparous sows had consumed a  
375 greater number of meals within an hour of feeding on days one, two and three post-farrowing  
376 (Mainau et al., 2012). In the current study, sows consumed more feed than gilts on days six and  
377 seven post-farrowing, as sows increased their feed intake at a greater rate than gilts. The feed  
378 that was not consumed was only measured at the next feeding time in this study, whereas the  
379 previous study scored feed as being completely consumed or not, one hour after it was given  
380 (Mainau et al., 2012). From day two after farrowing, and overall, there was a difference in the  
381 amount of feed consumed by individuals that required additional treatment compared to those  
382 that did not. This is not surprising as reduced feed intake is a good indicator of illness. In future  
383 studies, it would be interesting to measure the latency to feed and the time taken to fully  
384 consume the meal, as this could be an early indicator of subclinical PPDS and prompt treatment  
385 could produce a better outcome for the sow and litter.

386 Immune transfer

387           Piglets obtain passive immunity through the ingestion of immunoglobulin from sow  
388 colostrum (Rooke and Bland, 2002), and those with low concentrations of immunoglobulin are  
389 less likely to survive (Cabrera et al., 2012). Therefore, this is an important measure in  
390 identifying the benefits of administering post-farrowing NSAIDs. No differences in colostrum

391 or piglet serum IgG concentrations were detected in this study with drug treatment or whether  
392 additional treatment was required. A previous study found greater colostrum concentrations of  
393 piglets on day one and two post-farrowing from sows given oral meloxicam at farrowing  
394 (Mainau et al., 2016). As piglets were numerically heavier at six hours post-injection in this  
395 study, which could indicate greater colostrum intake, a difference may have been found if  
396 piglets were sampled at later time points.

397 Some studies have shown a link between colostrum intake and piglet birth weight  
398 (Devillers et al., 2007; Fraser and Rushen, 1992; Nguyen et al., 2013; Quesnel, 2011), although  
399 the link between colostrum consumed and piglet plasma IgG concentration plateau over a  
400 certain value, i.e. the link is stronger at lower concentrations (Devillers et al., 2011). No  
401 association between piglet weight and IgG at the point of sampling was found in this study,  
402 which was similar to a previous study (Cabrera et al., 2012), however, this could be explained  
403 by excessively small and/or weak piglets not being selected for blood sampling in the current  
404 and previous study (Cabrera et al., 2012). In addition, Fraser and Rushen, (1992) suggest that  
405 the failure to find a link between birth weight and IgG could be because of differences in blood  
406 volume (affecting the concentration) between large and small piglets.

407 Sow colostrum had a numerically greater IgG concentration than gilt colostrum, and  
408 piglet serum IgG was greater for piglets from sows compared with gilts. No link between piglet  
409 plasma IgG concentration and parity was detected at birth in one study (Quesnel, 2011), and  
410 another study showed a similar result, although it was not mentioned whether primiparous sows  
411 were included (Nguyen et al., 2013). Other studies measuring sow colostrum have found  
412 differences by parity, including lower concentrations measured 24 hours after birth in lower  
413 parity sows (Quesnel, 2011) and lower colostrum IgG concentrations in primiparous compared  
414 with multiparous sows 48-72 hours after birth (Cabrera et al., 2012).

415 Production data

416           There were no overall significant differences in pre-weaning piglet deaths, weight or  
417 size by treatment, or between those requiring additional treatment or not. However, it is worth  
418 discussing that numerically fewer piglets died in the ketoprofen compared with the saline-  
419 treated group, especially for gilts. High individual variation in piglet mortality was seen in this  
420 study, which possibly resulted in this difference not reaching significance. As piglet weight six  
421 hours after the injection was also numerically greater in ketoprofen-treated gilts and sows, it is  
422 also possible that piglet birth weight was greater for treated gilts and sows, resulting in the  
423 mortality difference. It is also possible that ketoprofen treatment increased piglet weight at six  
424 hours through increased colostrum intake, however, based on previous studies measuring early  
425 piglet weight gain, this may not have accounted for all of this weight difference (e.g. de Passillé  
426 and Rushen, 1989; Fraser and Rushen, 1992; Quesnel, 2011). This cannot be confirmed, since  
427 piglets were not weighed before the injection was given, and in a previous study, where 16  
428 sows were randomly allocated to be given butorphanol tartrate or a saline placebo post-  
429 farrowing, Haussmann et al., (1999) found a significant difference in birth weight of the piglets,  
430 with those from control sows being significantly heavier. So this may be an accidental outcome  
431 in this study and an important consideration for the piglet mortality difference between  
432 treatment groups.

433           A reduction in piglet mortality with the use of ketoprofen post-farrowing has been  
434 demonstrated previously in a study of 15 commercial farms (Homedes et al., 2014) and on a  
435 farm with a high incidence of PPDS (Sabaté et al., 2012), but another study reported no  
436 difference in mortality with the use of ketoprofen (Viitasaari et al., 2013). The individuals  
437 responsible for the care of the animals in the current study were blind to the treatments, and  
438 cross-fostering was performed to even litter size, resulting in more piglets being fostered off

439 the ketoprofen-treated gilts and more piglets being fostered onto the control gilts. This meant,  
440 despite a difference in mortality, no difference in the numbers of piglets weaned was detected  
441 between treatment groups for gilts, which is a result found in previously, where fostering was  
442 only conducted within treatment groups (Homedes et al., 2014; Sabaté et al., 2012). If  
443 ketoprofen does have an influence on piglet mortality, given the individual variation in the  
444 number of deaths, early identification to enable targeted use of drugs to those that could benefit  
445 the most would be the best use of drugs. No difference in mortality between treatment groups  
446 was detected the post-farrowing administration of the NSAID meloxicam (Mainau et al., 2012;  
447 Tenbergen et al., 2014) or with the opioid butorphanol tartrate (Hausmann et al., 1999).  
448 However, average daily weight gain of low birth weight piglets (<1180g) was increased  
449 (Mainau et al., 2012), growth rate of medium sized litters (11 to 13 piglets) tended to be greater  
450 (Tenbergen et al., 2014), and average daily gain and weaning weight was greater (Mainau et  
451 al., 2016) for multiparous sows treated with meloxicam compared with a placebo.

452 Piglet mortality in this study was most influenced by previously demonstrated risk  
453 factors, including piglet weight, sow back-fat, piglet gender, sow post-farrowing illness and  
454 the number of piglets born alive (for a review see Baxter and Edwards, 2015). It is widely  
455 agreed that birth weight is the most important factor in neonatal piglet survival and lower  
456 average piglet weight at six hours post-injection in this study was most strongly associated with  
457 pre-weaning death. Larger litter sizes come at the expense of reduced piglet viability, as well  
458 as increased competition for colostrum and milk (Baxter and Edwards, 2015). Interestingly,  
459 greater sow back-fat was associated with an increase in the odds of a piglet dying before  
460 weaning. A previous study using a high number of sows found a quadratic effect of sow back-  
461 fat at farrowing on the number of piglets weaned, with low and high back-fat being associated  
462 with fewer piglets weaned (Kim et al., 2015). Male-biased pre-weaning mortality has been  
463 found elsewhere, where piglets born were male-biased, and males were heavier at birth (Baxter

464 et al., 2012). This demonstrates a life-history strategy in domestic pig populations, with greater  
465 pre-natal maternal investment and an over-supply of more vulnerable males, in expectation of  
466 greater mortality (Baxter et al., 2012). Litter from sows developing PPDS suffer greater  
467 mortality (Klopfenstein et al., 2006), and treatment with NSAIDs in addition to antibiotics, can  
468 aid in the treatment of infectious causes of PPDS (Sabaté et al., 2012; Tummaruk and Sang-  
469 Gassanee, 2013).

#### 470 Behaviour

471 Posture was observed during nursing behaviour observations, with no differences by  
472 treatment. Previous studies investigating the administration of ketoprofen (Viitasaari et al.,  
473 2014) and meloxicam (Mainau et al., 2012) for three consecutive days post-farrowing showed  
474 differences in the level of activity between individuals given the NSAID or a saline placebo  
475 only on the third day post farrowing. This included a decrease in the time spent lying by  
476 meloxicam treated gilts and sows (Mainau et al., 2012) and an increased activity in younger  
477 (parity 2 -3) sows treated with ketoprofen, compared with their placebo treated counterparts,  
478 although older sows did not differ (Viitasaari et al., 2014). Greater activity suggests an  
479 improvement in the speed of recovery following parturition with the use of NSAIDs. By  
480 contrast, another study, using the opioid analgesic butorphanol tartrate post-farrowing showed  
481 a reduced number of posture changes 48 hours post farrowing (Hausmann et al., 1999).

482 Sows showed more sitting and kneeling behaviour compared with gilts, which could be  
483 related to the difference in size, weight and fitness between these two groups and the ease of  
484 changing body position. The gilts in this study spent more time lying lateral, in contrast to a  
485 previous study that showed younger sows to be more active (Viitasaari et al., 2014). This could  
486 be due to genetic improvements, as the gilts in this study were acquired directly from a breeding  
487 company, whereas the sows were home bred from an older genetic line of the same breed.

488 Modern breeding programs have focused on maternal traits to improve productivity, which  
489 could be reflected in greater lateral lying, allowing piglets access to the udder. Although there  
490 were no significant differences in posture between individuals that required additional  
491 treatment for PPDS, numerical differences for postures and the frequency of posture changes  
492 indicate PPDS individuals appear less active and, as with a reduction in feed intake, could be  
493 used as an early indication of PPDS to provide prompt treatment.

494 For the nursing behaviours observed, there was greater suckle grunting in control,  
495 compared with ketoprofen-treated dams. These data could indicate that ketoprofen dams had  
496 settled into a pattern of milk let-down sooner, providing support for the fact that the weight  
497 difference between ketoprofen and control-treatment dams could be due to greater colostrum  
498 intake. No previous studies have recorded nursing behaviour in relation to the use of post-  
499 farrowing NSAIDs.

## 500 **Conclusion**

501 This study did not demonstrate production benefits to the immediate post-farrowing  
502 administration of ketoprofen. However, in this study, as with others, high individual sow  
503 variation in piglet mortality was seen, with some performing well and the majority of piglet  
504 mortality often coming from a low number of sows (Baxter et al., 2015; Hales et al., 2013).  
505 Investigating whether pain is a component of decreased performance in these sows, could  
506 enable the targeted use of drugs. Additionally, identifying sows that could benefit from pain  
507 relief using measures of farrowing ease (e.g. Mainau et al., 2010), feed intake, activity and  
508 other behaviour measures, could assist with targeted drug treatment.

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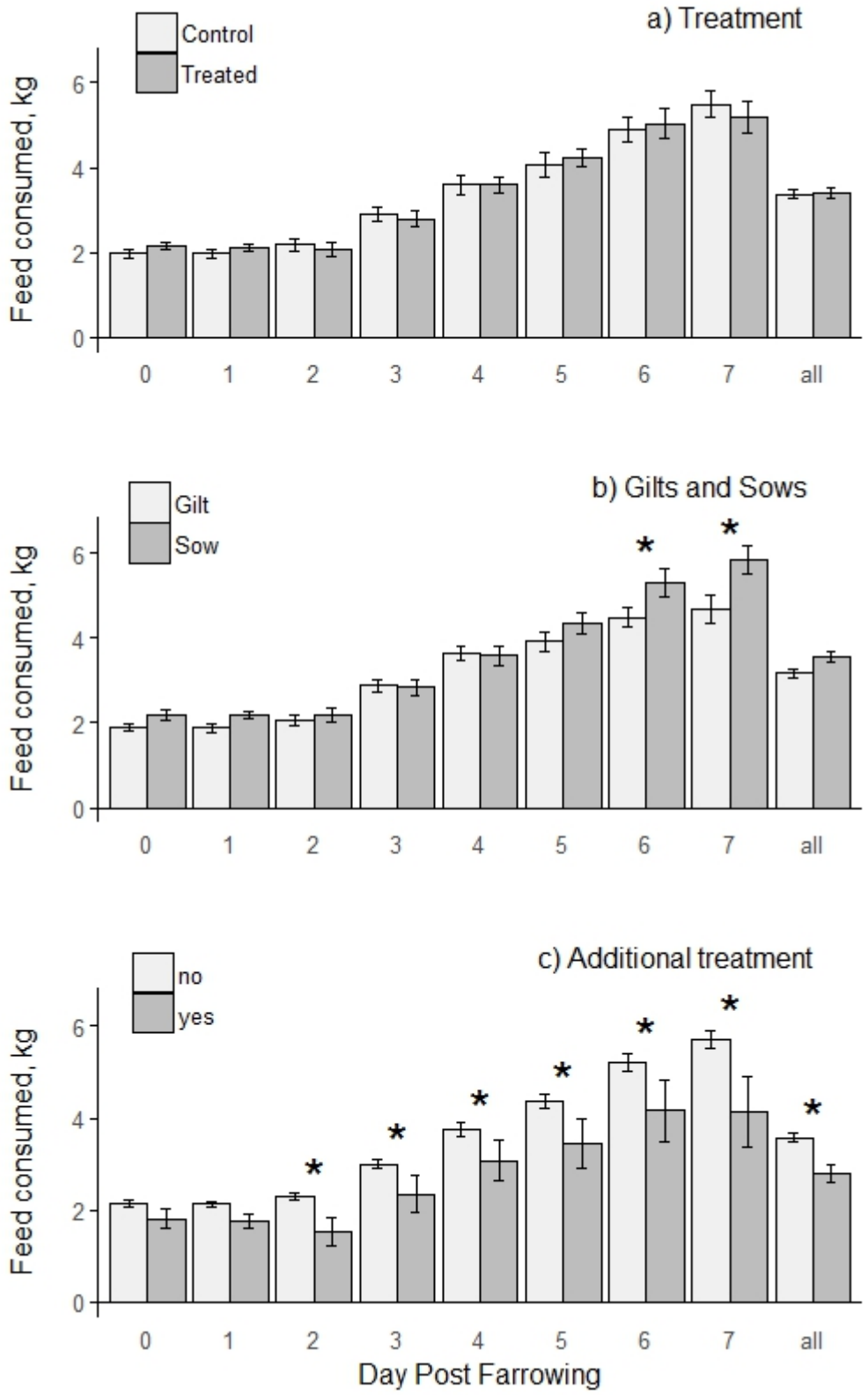
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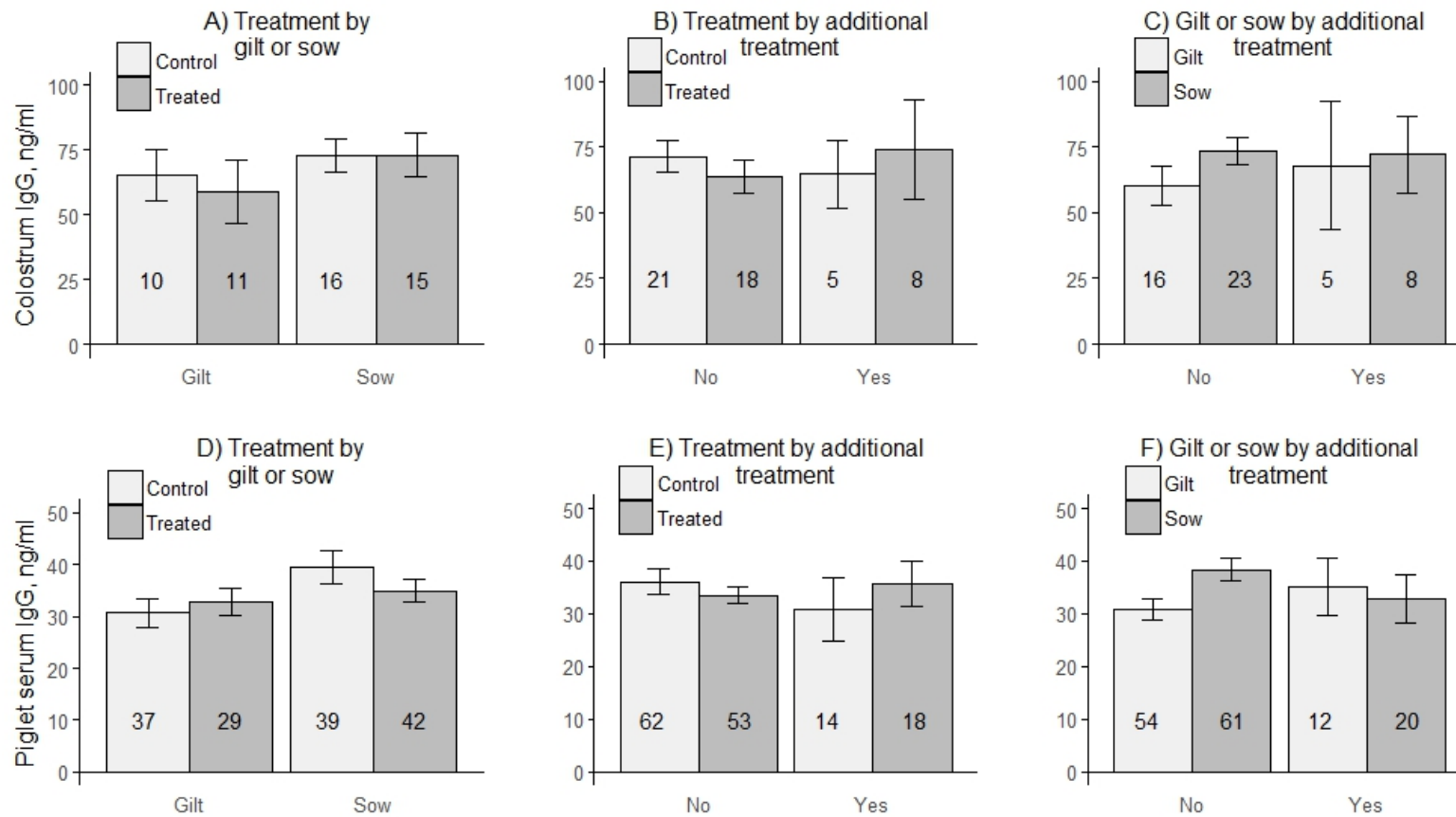
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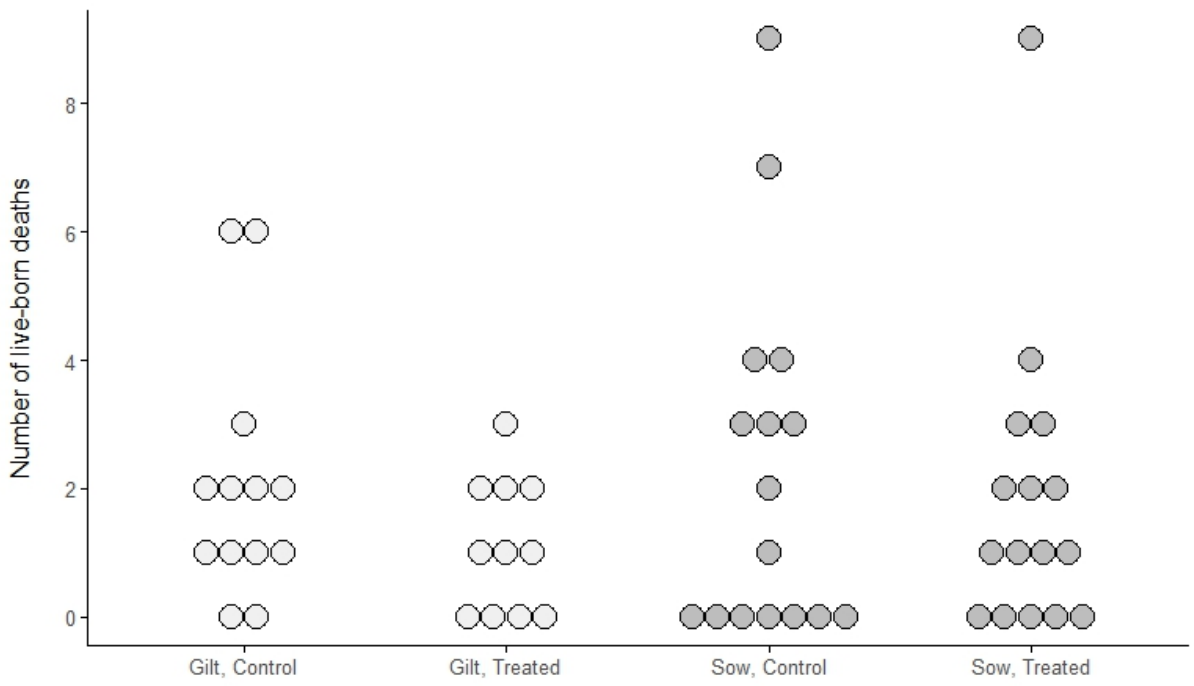
612 Fig.1. Mean  $\pm$  SEM of the total feed consumed (kg) per day by a) treatment (treated or  
 613 control); b) gilts and sows and; c) additional treatment (yes or no). Bars with a \* indicate a  
 614 significant difference ( $P < 0.05$ ).



616 Fig.2. Mean  $\pm$  SEM for colostrum immunoglobulin-G concentrations (mg/ml) for A) gilts and sows  $\times$  treatment; B) additional treatment (yes or  
 617 no)  $\times$  drug treatment and; C) additional treatment (yes or no)  $\times$  gilts and sows. Mean  $\pm$  SEM for piglet serum immunoglobulin-G concentrations  
 618 (mg/ml) for D) gilts and sows  $\times$  treatment; E) additional treatment (yes or no)  $\times$  drug treatment and; F) additional treatment (yes or no)  $\times$  gilts  
 619 and sows. Labels on the bars indicate the number of samples represented



621 Fig.3. Dot plot of individual gilt or sow live-born piglet deaths by treatment.



622

623 **Table 1.** Production information presented by treatment, gilts and sows, and additional treatment, including litter-based measures, gilts/sow based  
624 measures taken before moving in and at weaning, and piglet-based measures. Body condition was scored from 1 to 5 (1 = very thin, 5 = very fat)  
625 Gilt/sow data with different letters, represents an overall difference pre-farrowing, compared with post-weaning ( $P < 0.001$ ). \*One sow weaning  
626 weight is missing.

Production data	Treatment			Gilt or sow			Additional treatment		
	Treated	Control	<i>P</i>	Gilt	Sow	<i>P</i>	Yes	No	<i>P</i>
<b>Litter data</b>									
Born alive, frequency	12.6±0.7	13.0±0.7	0.92	12.3±0.8	13.2±0.6	0.65	13.5±0.9	12.6±0.5	0.65
Still born, frequency	0.4±0.2	0.5±0.2	0.66	0.2±0.1	0.7±0.2	0.16	0.3±0.2	0.5±0.1	0.99
Number weaned, frequency	10.7±0.4	10.9±0.3	0.91	10.8±0.4	10.9±0.3	0.62	10.5±0.4	10.9±0.3	0.72
Live-born deaths, frequency	2.4±0.3	3.0±0.4	0.37	2.5±0.3	2.9±0.4	0.83	3.4±0.6	2.5±0.3	0.23
<b>Gilt/sow data</b>									
<sup>a</sup> Pre farrow weight, kg	260.2±7.7	261.5±7.7	0.87	223.4±5.8	289.0±3.5	0.00003	266.5±10.9	259.2±6.3	0.89
<sup>b</sup> Post wean weight, kg	228.5±7.9	231.7±7.9	0.96	199.2±5.96	254.2±5.1*	0.01	228.2±12.5	230.8±5.9	0.52
<sup>a</sup> Pre farrow back-fat, mm	19.0±0.8	18.8±0.9	0.44	17.4±0.9	20.0±0.8	0.33	19.1±1.4	18.8±0.7	0.48
<sup>b</sup> Post wean back-fat, mm	14.0±0.8	14.2±0.7	0.93	13.3±0.9	14.7±0.6	0.85	13.5±0.9	14.3±0.6	0.79
<sup>a</sup> Pre farrow body condition score	3.1±0.1	3.2±0.1	0.69	3.3±0.1	3.1±0.1	0.34	3.2±0.1	3.2±0.04	0.54
<sup>b</sup> Post wean body condition score	2.6±0.1	2.7±0.1	0.18	2.7±0.1	2.7±0.1	0.35	2.7±0.1	2.7±0.1	0.87
<b>Piglet data</b>									
Piglet 6 hour weight, kg	1.5±0.02	1.4±0.02	0.19	1.3±0.02	1.51±0.02	0.002	1.5±0.03	1.4±0.02	0.87
Piglet 6 hour crown-rump, cm	27.1±0.1	26.4±0.1	0.34	25.8±0.1	27.37±0.12	0.002	26.9±0.2	26.7±0.1	0.74
Piglet day 3 weight, kg	1.8±0.02	1.7±0.02	0.25	1.7±0.02	1.86±0.02	0.009	1.8±0.03	1.8±0.02	0.57
Piglet wean weight, kg	8.00±0.1	7.6±0.1	0.24	7.2±0.1	8.16±0.09	0.008	7.6±0.2	7.8±0.1	0.75
Piglet wean crown-rump, cm	50.3±0.3	49.5±0.2	0.62	48.7±0.3	50.72±0.25	0.06	49.2±0.4	50.1±0.2	0.74

628 **Table 2.** Frequencies of pre-weaning deaths, including totals and separated by suspected cause  
 629 of death, and the frequencies of piglets that were fostered on and off the litter for the 11 treated  
 630 and 13 control gilts and 16 treated and 16 control sows.

	GILT		SOW		Totals
	Treated (n = 11)	Control (n = 13)	Treated (n = 16)	Control (n = 16)	
Crushed	4	12	7	10	<b>33</b>
Low viability	2	8	8	8	<b>26</b>
Starve	1	1	6	7	<b>15</b>
Savage	0	1	4	1	<b>6</b>
Greasy pig	2	2	2	10	<b>16</b>
Other	0	3	1	2	<b>6</b>
<b>Total deaths</b>	<b>9</b>	<b>27</b>	<b>28</b>	<b>38</b>	<b>102</b>
Fostered on	4	13	5	7	<b>29</b>
Fostered off	14	5	11	12	<b>42</b>

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632 **Table 3.** Behaviour results (mean  $\pm$  SEM) by treatment, gilts or sows and additional treatment, for three hour observations between 15 and 18  
633 hours after the last piglet was born. Results are displayed as a percentage of time in the three hour observation (% of time), frequency of events in  
634 the observation, duration in seconds or minutes. Columns with a different letter indicate a difference ( $P < 0.05$ ).

Behaviour	Treatment		Gilts vs. Sow		Additional treatment	
	Treated	Control	Gilt	Sow	Yes	No
<b>Sow behaviour</b>						
Stand, % of time	8.4 $\pm$ 1.5	9.2 $\pm$ 1.9	7.6 $\pm$ 1.6	9.8 $\pm$ 1.8	6.1 $\pm$ 1.6	9.4 $\pm$ 1.4
Sit, % of time	1.1 $\pm$ 0.3	2.2 $\pm$ 0.5	1.1 $\pm$ 0.2 <sup>a</sup>	2.2 $\pm$ 0.5 <sup>b</sup>	1.8 $\pm$ 1.1	1.6 $\pm$ 0.3
Kneel, % of time	0.1 $\pm$ 0.04	0.1 $\pm$ 0.03	0.1 $\pm$ 0.01 <sup>a</sup>	0.2 $\pm$ 0.04 <sup>b</sup>	0.1 $\pm$ 0.04	0.1 $\pm$ 0.03
Lie lateral, % of time	79.7 $\pm$ 3.3	77.2 $\pm$ 3.6	83.3 $\pm$ 2.9 <sup>a</sup>	74.4 $\pm$ 3.7 <sup>b</sup>	86.1 $\pm$ 3.5	76.6 $\pm$ 2.8
Lie ventral, % of time	10.7 $\pm$ 2.9	11.3 $\pm$ 2.9	8.0 $\pm$ 1.9	13.5 $\pm$ 3.3	5.9 $\pm$ 2.4	12.2 $\pm$ 2.4
Posture changes, frequency	12.8 $\pm$ 1.8	13.3 $\pm$ 2.1	11.1 $\pm$ 1.7	14.7 $\pm$ 2.1	9.6 $\pm$ 2.7	13.9 $\pm$ 1.6
Drinking, seconds	121.2 $\pm$ 25.0	122.1 $\pm$ 26.6	124.5 $\pm$ 31.0	119.3 $\pm$ 21.4	142.0 $\pm$ 38.4	116.9 $\pm$ 20.6
<b>Nursing behaviour</b>						
> 50 % of piglets active at udder, % of time	16.9 $\pm$ 1.3	18.7 $\pm$ 1.2	17.8 $\pm$ 1.2	17.8 $\pm$ 1.3	16.9 $\pm$ 1.8	18.1 $\pm$ 1.0
Suckle grunt duration, % of time	11.9 $\pm$ 0.9 <sup>a</sup>	14.5 $\pm$ 1.0 <sup>b</sup>	13.8 $\pm$ 1.1	12.8 $\pm$ 0.9	11.2 $\pm$ 0.9	13.7 $\pm$ 0.8
Suckle grunt bouts, frequency	5.2 $\pm$ 0.4	5.9 $\pm$ 0.4	5.4 $\pm$ 0.4	5.7 $\pm$ 0.5	5.0 $\pm$ 0.5	5.7 $\pm$ 0.4
Mean suckle grunt bout duration, seconds	254.9 $\pm$ 8.9	276.7 $\pm$ 10.5	280.8 $\pm$ 11.6	253.7 $\pm$ 8.0	245.6 $\pm$ 15.7	270.7 $\pm$ 7.8
Inter bout interval, minutes	34.0 $\pm$ 2.3	30.6 $\pm$ 1.9	32.3 $\pm$ 2.3	32.2 $\pm$ 2.0	33.3 $\pm$ 2.2	32.0 $\pm$ 1.8

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