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McDermott, K orcid.org/0000-0001-6618-5560, Icely, S, Jagger, S et al. (4 more authors) (2020) Supplementation with omega-3 polyunsaturated fatty acids and effects on reproductive performance of sows. *Animal Feed Science and Technology*, 267. 114529. ISSN 0377-8401

<https://doi.org/10.1016/j.anifeedsci.2020.114529>

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Supplementation with omega-3 polyunsaturated fatty acids and effects on reproductive performance of sows

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1 **1. Abstract**

2 In studies in both humans and farm animals, the inclusion of omega-3 polyunsaturated fatty
3 acids in the diet have been shown to have beneficial effects on many physiological processes
4 including reproduction. The aim of this study was to examine the effects of supplementary
5 omega-3 on sow reproductive performance and piglet survival. Salmon oil (1%) was fed to
6 sows throughout gestation and lactation as a source of omega-3 and sows were followed
7 through their subsequent parity when returned to a commercial gestation and lactation diet. It
8 was hypothesised that sows fed omega-3 would show improved piglet survivability (+2%) and
9 an increased litter size (one extra piglet born alive per litter) in the second experimental period
10 compared with a soya oil supplemented control. Supplementation of 1% salmon oil across one
11 parity increased the body weight of sows at weaning ($p=0.01$) and these sows maintained on
12 average $4 \text{ kg} \pm 2.3$ more over the lactation period than soya oil supplemented controls. Sows
13 that were followed across a second un-supplemented reproductive period were heavier at
14 farrowing ($p<0.01$) and weaning ($p<0.05$), had a higher condition score at farrowing and tended
15 to have a higher condition score ($p=0.063$) and back fat at weaning ($p=0.073$) when they had
16 received salmon oil in the previous reproductive cycle. However, salmon oil increased pre-
17 weaning mortality by 2.4% in the first reproductive period ($p<0.05$) and significantly reduced
18 litter weight at birth (*ca* 600g; $p<0.05$). Pre-weaning mortality was reduced by 3.4% in the
19 second experimental period when supplementation of both salmon oil and the soya oil control
20 had ceased ($p<0.001$). This effect tended to be greater for sows previously supplemented with
21 omega-3. There was no effect on litter size, or the number of piglets born alive.

22 Supplementation of 1% salmon oil improved sow body weight at weaning and increased
23 maternal stores across a second, un-supplemented reproductive cycle perhaps through effects
24 on maternal nutrient partitioning. The increased mortality in the first experimental period and
25 reduced mortality (across both treatment groups) when returned to a commercial diet suggests

26 a negative effect of omega 3 fatty acid supplementation on piglet survival when fed throughout
27 gestation.

28 **Key words:** Salmon oil, sow, reproduction, piglet mortality, omega-3

29

30 **2. Introduction**

31 The modern sow shows improved prolificacy resulting in an increased litter size however there
32 exists an antagonistic relationship with production metrics with decreased mean piglet birth
33 weight, increased occurrence of stillbirths and an increase in pre-weaning mortality (Tanghe
34 and De Smet, 2013). It is estimated that the proportion of low birth weight piglets (< 1 kg) in
35 large litters (> 16 piglets) equates to a quarter of all piglets born (*ca* 23%) of which more than
36 a tenth are still births (Quiniou et al., 2002). This represents both large economic losses to the
37 industry and raises welfare concerns (Rutherford et al., 2013). There is much interest, therefore,
38 in reducing the number of small birth weight piglets and improving both peri- and post-natal
39 survival.

40

41 It is widely recognised in nutritional research of both humans and farm animals, that inclusion
42 of omega-3 polyunsaturated fatty acids (PUFA), particularly those of marine origin (i.e. the
43 long chain PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), in the diet
44 can have beneficial effects on many physiological processes (Millet and Delezie, 2013, Tanghe
45 and De Smet, 2013). Omega-3 PUFA play a role in the molecular events that underpin
46 reproduction, and although the exact mechanisms are unknown, it is believed that these may
47 involve regulation of prostaglandin or cholesterol synthesis and endometrial gene expression
48 (Abayasekara and Wathes, 1999, Wathes et al., 2007, Coyne et al., 2008). Omega-3 PUFA also
49 play a role in immunity (Enke et al., 2008, Yaqoob, 2003). The supplementation of omega-3
50 PUFA to gestating sows may be able to enhance the general health of the sow and her piglets

51 as well as improve piglet survivability, factors which are profitable to the pig industry (Tanghe
52 and De Smet, 2013).

53

54 Previous studies have shown that supplementation of sows with omega-3 PUFA during mid-
55 to-late gestation and subsequent lactation offer the potential to increase the number of pigs born
56 alive per litter by 1 pig (Smits et al., 2011) and to reduce pre-weaning mortality by 2 % (Rooke
57 et al., 2001). As follicle development occurs prior to weaning (Soede et al., 2011), omega-3
58 supplementation throughout lactation has the potential to enhance future reproductive success.
59 The aim of this study was to examine the effects of supplementary omega-3 PUFA on sow
60 reproductive performance and piglet survival. Omega-3 PUFA was fed to sows throughout
61 gestation and lactation and sows were followed through their subsequent parity when returned
62 to a commercial gestation and lactation diet. It was hypothesised that sows fed omega-3 would
63 show improved piglet survivability in the first experimental gestation and an increased litter
64 size in the second experimental gestation.

65

66 **3. Methods**

67 The experiment described below was performed at two sites (University of Leeds Pig Research
68 Centre – Site 1 and Harper Adams University Farm Pig Unit– Site 2). Ethical approval for the
69 protocol was granted by the Animal Welfare and Ethical Review Body at both sites.

70

71 **3.1 Animals and Housing**

72 **3.1.1 Site 1**

73 A total of 200 Large White x Landrace sows with an average body weight of 205.6 ± 51.75 kg
74 (\pm SD) and parity of 4.1 ± 2.54 (range 1-10) were used in this study. Dry sows were housed in
75 straw pens with feeding stalls (Period 1) and straw pens with dump feed (Period 2). During

76 gestation, sows were fed individually (2.4 ± 0.2 kg) once daily in feeding stalls but when not
77 eating were kept in straw yards in groups of 8 to 10. Each pen contained sows from both
78 treatment groups. From approximately 3 days prior to farrowing until weaning each sow was
79 housed in an individual farrowing crate with fully slatted floor. Sows received their allocated
80 gestation ration until farrowing and then were fed twice daily according to a step up programme
81 following farrowing, through to weaning (7.4 ± 0.6 kg per day). At weaning sows returned to
82 a communal straw kennel area and were fed individually in stalls once daily until service. Sows
83 were then followed through a second parity during which all received the same standard
84 commercial diets and normal farm management. Cross fostering occurred within 24 hour of
85 birth and where possible, within a treatment.

86

87 **3.1.2 Site 2**

88 At Site 2, 219 Large White x Landrace sows with an average body weight of 227.3 ± 56.13 kg
89 and parity of 4.6 ± 3.03 (range 1-16) were used. Dry sows were housed in straw pens with open
90 access feeding stalls with dunging passage. During gestation, sows were fed 3.4 kg per day for
91 8 days post weaning and then 2.6 ± 0.1 kg per day to farrowing. From 7 days prior to farrowing
92 until weaning, sows were housed in an individual farrowing crate with fully slatted floor. Sows
93 received their allocated gestation ration until farrowing and then were fed twice daily according
94 to the Stotfold scale programme from farrowing, through to weaning (6.5 ± 0.7 kg per day). At
95 weaning sows returned to dry sow accommodation and were fed once daily following the same
96 regime as in Period 1. Sows were followed through a second parity where all sows received
97 the same commercial diet and normal farm management.

98

99 **3.2 Experimental design and treatments**

100 The same trial diets were fed at both experimental sites. All diets were formulated to meet or
101 exceed nutrient requirements of gilts and sows (BSAS, 2003). Sows were fed either a diet
102 supplemented with 1% omega-3 PUFA derived from salmon oil (Optomega-50 (Anpario); N
103 = 101 and 111 for Site 1 and Site 2 respectively) or a control diet containing 1% soya oil in the
104 same carrier matrix (N = 99 and 108 for Site 1 and Site 2 respectively), from the start of the
105 dry period (gestation), through lactation, to the next service (approximately 22 weeks; referred
106 to herein as Period 1). For the second parity (Period 2), sows all received the same commercial
107 gestation and lactation diets used in normal farm management at each site. Feed refusals were
108 weighed daily. At both sites, seven consecutive batches of sows were used with an average of
109 28.3 ± 1.70 and 31.3 ± 1.38 sows per batch for Sites 1 and 2 respectively. The composition of
110 the experimental gestation and lactation diets is shown in Table 1. Treatment groups were
111 balanced for live weight, parity, body condition and fat (P2) and previous litter history at the
112 start of the experiment.

113 **[Insert Table 1 here]**

114 **3.3 Measurements and sampling**

115 Sows were individually weighed and both body condition and P2 back fat (mm) were recorded
116 at the start of the experimental period, farrowing, weaning for Period 1 and service (Site 1 only)
117 farrowing and weaning for Period 2. Sow weights, body condition and P2 back fat were only
118 recorded at service at Site 1. Body condition was measured by the same trained personnel on
119 each site on a five-point scale, allowing for half point measures, where a higher number
120 indicates a fatter animal (AHDB, 2017). The number of piglets born, number of piglets born
121 alive, pre-weaning survival, weaning to service interval, litter weight at birth, litter weight at
122 weaning and number of piglets weaned per sow were recorded.

123

124

125 **3.4 Statistical analysis**

126 Data was initially analysed by Select Statistical Services (Exeter, UK). Analysis was repeated
127 and built upon in-house and the in-house analysis is shown below unless otherwise stated.

128 For Period 1, data were analysed using the mixed linear model procedure of IBM SPSS
129 Statistics 21 with sow as the experimental unit. A total of 174 sows were included from Site 1
130 and 217 from Site 2. Diet and parity were included as an interaction term with diet and parity
131 as main effects. A random effect of batch nested within herd was also included under the
132 assumption that sows within the same batch were likely to be more similar as well as sows
133 from the same herd. Where possible, all weights, condition and fat measurements included the
134 score at the previous time period (e.g. farrow weight at weaning) as a covariate in the model.
135 Weaning age was included as a covariate for weaning weight. For mortality, the total number
136 of piglets born and the change in the sow's body condition from farrowing to weaning were
137 included in the model. Odds-ratio for mortality was performed by Select Statistics after fitting
138 a logistic regression mixed-effects model to the data in R.

139

140 For Period 2, only sows which had successfully farrowed in both Period 1 and 2 were included.
141 This resulted in the inclusion of 125 sows from Site 1 and 152 sows from Site 2. A total of 114
142 sows were removed from this second part of the analysis with 49 sows removed from Herd 1
143 and 65 from Herd 2. These sows were either not in heat, returned or were culled from the
144 breeding herds. Data were again analysed using the mixed linear model procedure of IBM
145 SPSS Statistics 21 with sow as the experimental unit. Models were run as for Period 1 above
146 and the same covariates were used.

147

148 Interactions between diet and period were also examined for those sows that successfully
149 farrowed in both Period 1 and Period 2 and the main effect of experimental time period were

150 also explored. These models included diet and period as an interaction, with diet, period and
151 parity as main effects. A random effect of sow nested within batch, nested within site was also
152 included. Only those sows that farrowed in both Period 1 and 2 were included in this analysis
153 resulting in the same number of sows as for the Period 2 analysis above.

154

155 Where an interaction, main effect or covariate was found to have no significant effect in the
156 model, it was removed, and the model was re-run. Results were considered significant if $p <$
157 0.05 and a trend if $p < 0.1$. Data are presented as least square means \pm SEM. All sows of parity
158 7 or over were grouped into the same category (7+) due to low replication.

159

160 **4. Results**

161 **4.1 The effect of supplementing omega-3 on sow reproductive performance (Period 1)**

162 In the first experimental period, during which sows were fed either an omega-3 supplement or
163 a soya oil control, it was found that sows fed omega-3 were significantly heavier at weaning
164 compared to the control group (234.8 vs 230.9 kg; ± 2.0 ; $p = 0.01$). The sows fed omega 3
165 retained on average *ca* 4 kg more live weight than those on the soya oil control ($p < 0.01$; Table
166 2). Although numbers born and born alive were similar for both treatments, the litter
167 birthweight from sows fed omega-3 was significantly lighter than that from sows on the control
168 treatment (19.3 vs 19.9 kg; ± 0.36 ; $p < 0.05$). Litter weight gain to weaning was similar for
169 both treatments (66.2 vs 65.9 kg; ± 2.15 for omega-3 and soya oil treatments respectively).
170 Litter weight gain increased with parity up to parity 3 and then decreased with each subsequent
171 parity thereafter.

172

173 There was a significant effect of omega 3 supplementation on piglet mortality (16.6 vs 14.2 %
174 ± 1.0 ; $p < 0.05$ for omega-3 and soya oil respectively). Supplementation with omega-3

175 increased mortality by 2.4% with both the number of piglets born ($p < 0.001$) and the change
176 in the sow's body condition from farrowing to weaning ($p < 0.018$) having significant effects
177 on the model. Higher numbers of piglets born increased the mortality rate and sows that
178 maintained their body condition showed increased piglet mortality to weaning. Higher parity
179 sows (7+) maintained body condition better than lower parity sows ($p < 0.05$) with the
180 exception of parities 4 and 5 (Figure 1). This difference in mortality between treatments
181 equated to an odds-ratio of 1.28. This means that if a sow not fed omega-3 would have a pre-
182 weaning mortality of 10%, a supplemented sow from this study would be expected to have a
183 pre-weaning mortality of around 12.5%. Effectively an increase of 25% (not percentage
184 points).

185

186 Parity had a significant effect on all measures with the exception of piglet mortality, gestation
187 length and wean to service interval. All data can be seen in Table 2. Parity tended to affect
188 farrowing live weight and farrowing back fat ($P < 0.1$) both measures increasing with each
189 subsequent parity. Supplementation of omega-3 did not affect feed intake during lactation with
190 both treatment groups consuming on average 6.9 ± 0.167 kg per day ($p = 0.752$).

191

192 **[Insert Table 2 here]**

193

194 **4.2 The carry-over effect of omega-3 supplementation into a second, un-supplemented,** 195 **experimental reproductive cycle (Period 2)**

196 Sows were followed through a second gestation and lactation after the treatment diets had been
197 withdrawn to determine if there was any carry over effect of omega-3 supplementation when
198 sows were returned to an un-supplemented commercial diet. Sows that were fed omega-3
199 during the previous reproductive cycle were found to be significantly heavier at farrowing than

200 soya oil supplemented controls (290.0 vs 282.3 kg \pm 3.13 respectively; $p < 0.01$; Table 3) and
201 also had a higher body condition score (3.5 vs 3.3 \pm 0.11; $p < 0.001$). These results, however,
202 should be interpreted with caution as weight and condition could not be included as co-variables
203 in their respective models as these were not measured for the second service period at Site 2
204 and body condition score is a subjective measure.

205

206 The sows that were previously supplemented with omega-3 were *ca* 3.5 kg heavier at weaning
207 ($p < 0.05$) and tended to have a higher body condition score (3.3 vs 3.1 \pm 0.07; $p = 0.063$).

208 When returned to a commercial diet, sows fed omega-3 in the previous period lost a similar
209 amount of live weight, back fat and body condition from farrowing to weaning as control sows.

210 All data can be seen in Table 3.

211

212 There was an interaction between diet and parity for both litter weight at birth ($p < 0.05$; Figure
213 2a) and pre-weaning mortality ($p < 0.05$; Figure 2b). Gilts that were supplemented with omega-
214 3 in Period 1 tended to have both a lower litter weight (19.5 vs 21.6 kg \pm 0.60; $p = 0.079$) and
215 higher piglet mortality (9.5 vs 5.7% \pm 1.27; $p = 0.052$) compared to controls following an un-
216 supplemented second parity. The opposite was shown for second parity sows, which showed
217 significantly higher litter weight at birth (21.7 vs 19.2 kg \pm 0.63; $p < 0.05$) and tended to show
218 lower mortality (5.6 vs 11.0% \pm 1.50; $p = 0.087$) when supplemented with omega-3 throughout
219 the previous reproductive cycle. No differences were observed for parities 3 to 7 ($p > 0.1$).

220

221

[Insert Table 3 here]

222

223 **4.3 The interactive effect of omega-3 supplementation across two reproductive cycles**

224 The interactive effect of diet and time period, and the effect of time period alone were also
225 examined. Only those sows that farrowed in both reproductive periods were included in this
226 analysis. All data is shown in Table 4. There was a significant interaction between diet and
227 period ($p < 0.01$) for weight at farrowing with sows fed omega 3 heavier than control sows at
228 Period 2 (291 vs 283 kg \pm 2.1 respectively; Table 4). Both groups had had similar weights
229 during farrowing at Period 1 (258 kg \pm 2.1). A similar pattern was shown for the sow's body
230 condition at farrowing whereby significant interaction was also observed ($p < 0.05$). The body
231 condition of the previously omega-3 supplemented sows was significantly higher than that of
232 soya supplemented controls during Period 2 (3.6 vs 3.3 \pm 0.06 respectively; $p < 0.05$). Again,
233 these were similar in Period 1 (3.1 vs 3.0 \pm 0.06).

234

235 There was a trend towards an interaction between diet and period for pre-weaning mortality (p
236 = 0.089; Figure 3). There appeared to be a detrimental effect of omega-3 supplementation on
237 piglet mortality in the first time period (15.5 vs 13.9 % \pm 0.9) but at the second time period,
238 when sows were returned to a commercial diet, mortality was similar, if not improved for the
239 sows that had received omega-3 in the first reproductive period (10.7 vs 11.9 % \pm 0.9 for omega
240 3 and the control diet respectively). Piglet mortality was significantly greater in the first
241 experimental time period than in the second (14.7 vs 11.3 respectively; $p < 0.001$) irrespective
242 of experimental diet.

243

244 Total litter weight was lighter in the first time period than in the second (19.5 vs 20.5 kg
245 respectively; $p < 0.001$) with a trend towards an interaction between diet and period ($p = 0.066$).
246 Omega-3 supplemented sows produced litters *ca* 700g lighter than control sows in the first
247 reproductive period with similar weights in the second time period (20.5 kg for both groups).

248 Period had a significant effect on gestation length (and by association, wean to service interval)
249 with an additional 0.3 days of gestation in Period 2 ($p = 0.01$). Sows maintained significantly
250 more live weight between farrowing and weaning in Period 1 when compared to Period 2 (-
251 25.5 vs - 36.5 kg \pm 1.65; $p < 0.001$). However, they also lost more back fat (- 4.3 vs - 1.5 mm
252 \pm 0.24; $p < 0.001$). This is likely due to the fact that the P2 back fat measurement was
253 significantly higher at farrowing for the sows in Period 1 than Period 2 to begin with (20.0 vs
254 19.2 mm \pm 0.25; $p < 0.05$).

255

256 **[Insert Table 4 here]**

257 **5. Discussion**

258 The inclusion of omega-3 polyunsaturated fatty acids derived from salmon oil in the diet of
259 sows is thought to improve reproductive performance but reports in the literature present
260 conflicting results, some indicating benefit (Rooke et al., 2001, Smits et al., 2011) and others
261 not (Posser et al., 2018). In addition, when a performance benefit was observed the type of
262 benefit varied between studies. This may be due to the considerable variation in the level and
263 period of feeding between different experiments making it difficult to identify the optimum
264 period or inclusion level for feeding omega-3 PUFA. In farming practice, it is often only
265 possible to feed one diet through gestation and one through lactation therefore in this
266 experiment multiparous sows were fed omega-3 salmon oil at 1% of the diet throughout both
267 periods for one parity and then followed for a further parity after the omega-3 feeding had
268 ended. The salmon oil fed was incorporated onto a carrier for ease of mixing into the diets and
269 therefore the control diet contained an equivalent amount of soya oil incorporated onto the
270 same carrier to ensure that the carrier itself was not a confounding factor.

271

272 The initial hypotheses behind this experiment were that supplementation of sows with omega-
273 3 PUFA from salmon oil throughout gestation and the subsequent lactation would increase
274 number of pigs born alive per litter by 1 pig and reduce pre-weaning mortality by 2 %. In this
275 experiment the number of piglets born alive was similar for both treatments, 13.6 versus 13.5
276 for omega-3 fed sows versus control fed sows respectively in the first parity and 13.5 versus
277 13.4 respectively in the second parity and therefore the first hypothesis must be rejected.
278 Feeding omega-3 PUFA supplemented diets from service through gestation and then through
279 to the following service did not improve numbers born alive in either that or the subsequent
280 parity. This is in contrast to the findings of Smits et al. (2011) who found that supplementing
281 sows with 3g omega-3 PUFA per kg diet during the last part of gestation and lactation increased
282 the number of pigs born alive in the subsequent litter by an average of one pig, thought to be
283 due to improved oocyte quality and embryo survival. However, omega-3 supplementation in
284 the form of linseed oil has been shown to have a detrimental effect on both the quality of
285 embryos and on related hormonal and fatty acid metabolism in the uterus of gilts prior to
286 placentation (Chartrand et al., 2003). Prostaglandin (PG) E₂ and PGF_{2α} were both lower in the
287 uterine fluid of gilts fed linseed oil compared to a hydrogenated tallow control which the
288 authors suggested may be due to interference of the *n-3* PUFA with desaturase and/or
289 cyclooxygenase enzymes required for PG synthesis. Bilby et al. (2006) observed no difference
290 in oocyte quality when supplementing PUFA (vegetable or linseed oil) or MUFA (sunflower
291 oil) to dairy cattle.

292

293 Soya oil was supplemented as a control in the current study. Soya oil contains PUFA, which
294 can be classified into a subgroup of PUFA (including sunflower oil and corn oil) that are made
295 up of MUFA and linoleic acid (LA) and has a *n6-n3* ratio of 6.7 (Dubois et al., 2007). The lack
296 of difference in litter size could perhaps be attributed to a similar action of the two oils.

297 Alternatively, it may be that the concentration of PUFA supplemented to the sow was
298 unsuitable. Indeed, there are limited dose-response studies using fish oil in the literature with
299 inconsistent experimental design (Tanghe et al., 2015) which makes determining a suitable
300 inclusion rate difficult. Therefore, more dose response studies in this area may be of use. In
301 addition to this, EPA and DHA are usually considered together as one entity. As there is
302 evidence that they have different effects on cell function including gene expression and
303 intracellular signalling pathways (Gorjão et al., 2009) future work in this area should consider
304 separately the absolute and relative concentration of these omega-3 PUFAs. The availability
305 and distribution of different PUFA sources to maternal and foetal tissue has also been shown
306 to vary (Gázquez et al., 2017) and this is something that should be considered in future work.
307

308 Supplementation of omega-3 was also shown to have a negative effect on pre-weaning survival
309 therefore the second hypothesis of this study must also be rejected. Supplementation of 1%
310 salmon oil to the diet significantly increased pre-weaning mortality by *ca* 2.4% compared with
311 a soya oil control in the first experimental period. This stands to directly influence the number
312 of pigs weaned per sow per batch per farm per year, which is not only a critical performance
313 metric for breeding units but a key influencer of a unit's financial viability. Interestingly, when
314 sows were returned to a standard commercial diet pre-weaning survival increased significantly
315 by *ca* 3.4% irrespective of diet. The effect was especially pronounced for those sows fed
316 omega-3 in the previous reproductive period. The increased pre-weaning survival of piglets in
317 Period 2 was observed across all sow parities which may suggest an un-controlled
318 environmental or husbandry effect, or alternatively, the higher mortality in Period 1 may be a
319 general effect of the carrier used to aid mixing of the oils into the diet. It is also possible that
320 PUFA supplementation (irrespective of source) throughout gestation may have consequences
321 for piglet survival.

322

323 Indeed, Shen et al. (2015) also showed a higher pre-weaning mortality when sows were fed
324 omega-3 supplementation when compared to a control (additional corn starch), whereas olive
325 oil supplementation reduced mortality. The authors also showed that fish oil increased
326 susceptibility to oxidative stress in both sows and piglets compared to both olive oil and the
327 control diet. Soya oil has also been shown to increase mortality compared to supplementation
328 of medium chain triglycerides or coconut oil, thought to be due to lower glycogen stores (liver
329 and muscle) in piglets from sows fed this treatment (Jean and Chiang, 1999). Replication,
330 however, was low in both studies (n = 8 and n = 16 to 18 per treatment respectively). It is also
331 of interest to consider whether the anti-inflammatory effects of PUFA are beneficial in a
332 pathogen rich environment such as a commercial farm.

333

334 The effect of omega-3 supplementation on pre-weaning mortality did not reach significance
335 when considering only those sows which farrowed across both experimental periods. Instead,
336 there was a trend towards an interaction between diet and the experimental time period. The
337 difference in the effect of omega-3 supplementation between the two sets of analysis is likely
338 due to the removal of sows from both herds that were not followed through to the second
339 experimental period when returned to commercial diets either because these sows were not in
340 heat, returned or were culled from the breeding herds.

341

342 An interaction was observed during Period 2 between diet and parity for both pre-weaning
343 mortality and average litter weight at birth whereby previous supplementation with omega-3
344 tended to be more detrimental for gilts and had a positive effect for sows that were
345 supplemented during their second parity. No effect was seen for other sow parities. It must be
346 taken into account that replication was low when considering each parity individually (57 gilts

347 and 42 parity 2 sows continued through to Period 2) however, it would be of interest to explore
348 the effect of omega-3 supplementation on gilts further.

349

350 Sows which received the omega-3 diets during the first trial parity tended to be fatter than sows
351 receiving soya oil at weaning, and showed a better body condition score at both the first trial
352 parity and the second trial parity where sows had been restored to the normal commercial diets
353 fed on the unit. This suggests that the omega-3 supplementation may have changed sow
354 metabolism and/or nutrient partitioning to allow more efficient use of nutrients and hence
355 increased energy storage compared to the control sows. This effect continued even after omega-
356 3 supplementation had been withdrawn with supplemented sows heavier at both weaning
357 periods and at farrowing in Period 2 than those sows which had received the control diet.
358 Omega-3 supplemented sows maintained significantly more body weight from farrowing to
359 weaning. During lactation, sows mobilise body fat to meet the high associated energy demand.
360 However, loss of body fat can have negative effects on subsequent reproductive performance
361 (Thaker and Bilkei, 2005). In this case however the improved body condition and fatness of
362 sows supplemented with omega-3 did not translate to improved piglet performance. It is likely
363 that energy was partitioned from conceptus growth to maternal reserves during gestation hence
364 resulting in lower litter weights at birth for sows supplemented with fish oil as observed in this
365 experiment and as previously observed by other authors (Rooke et al., 2001, Eastwood et al.,
366 2014).

367

368 Litter weight at weaning was not significantly different between treatments in this study, in
369 contrast to that of Eastwood et al. (2014) but in agreement with Rooke et al. (2001) again
370 highlighting that the improved body reserves of the sows at farrowing did not result in
371 improved piglet performance. The lack of difference in weaning weight may be attributable to

372 the treatments used. Both fish oil and soybean oil have been shown previously to increase
373 weaning weight of piglets compared to an isoenergetic control diet that contained no oil (Jin et
374 al., 2017). Therefore, the two treatments may both act on the same, currently unknown,
375 mechanisms associated with piglet growth. Fish oil and soybean oil fed in combination (3%
376 and 1% respectively) tended to increase piglet average daily gain in the first 7 days of life but
377 were found to have no effect on gene expression in the liver or blood suggesting that feeding
378 PUFA to gestating sows had no effect on metabolic programming of piglets (de Greeff et al.,
379 2015) which may explain the lack of difference in litter weight at weaning observed in this
380 experiment.

381

382 Arachidonic acid (ARA) is a precursor to the 2 series prostaglandins (PG) and has been shown
383 to have a much higher concentration in foetal than maternal blood due to preferential selection
384 between PUFAs for placental transfer (Haggarty, 2002, Tanghe and De Smet, 2013). Both EPA
385 and DHA can decrease the synthesis of 2 series PG through competition for the same enzyme
386 (prostaglandin H synthase) and/or by selective incorporation into the phospholipid membrane
387 (Allen and Harris, 2001). It has been suggested that omega-3 supplementation may increase
388 gestation length by reducing prostaglandins required for the induction of labour.
389 Supplementation of omega-3 PUFA during pregnancy in humans has been definitively shown
390 to reduce the incidence of pre-term labour through inhibition of PG synthesis (Middleton et al.,
391 2018). However, in this study, there was no difference in gestation length between sows fed
392 salmon oil and those fed soya oil although somewhat surprisingly gestation length increased
393 by 0.3d in both treatment groups in the second experimental period when all sows were
394 receiving commercial diets. This increase in gestation length in the second time period cannot
395 be attributed to the cessation of omega-3 supplementation.

396

397 Interestingly, although the evidence is conflicting in the literature, the predicted reduction in
398 ARA associated with omega-3 supplementation may reduce birthweight of piglets (reviewed
399 by Tanghe and De Smet (2013)). Litter weight at birth was significantly lower for sows
400 supplemented with omega-3 in Period 1 and significantly increased during the second observed
401 period (for both omega-3 and soya oil supplemented sows) when sows were returned to a
402 commercial diet. ARA concentration alongside the concentration of PGE₂ and PGF₂α may be
403 something to be explored in future studies investigating the influence of omega-3 PUFA on
404 sow reproductive performance, to determine the effect on prostaglandin synthesis and piglet
405 birthweight as PGE₂ has previously been suggested to have a key role during the early stages
406 of pregnancy on embryo development, reduced levels of which may impact both litter size and
407 birth weight (Giguère et al., 2000). Supplementation of PUFA in the first half of gestation has
408 been shown previously to increase the incidence of low birth weight piglets whereas
409 supplementation of monounsaturated fatty acids (MUFA) has been shown to do the opposite
410 (Laws et al., 2009) thought to be due to the partitioning of nutrients to placental development.
411 Rooke et al. (2001) found that although supplementing salmon oil at 1.65% throughout
412 gestation and lactation resulted in reduced individual pig birthweights it increased neonatal
413 vitality resulting in a reduction in pre-weaning mortality (98 sows per treatment). In contrast,
414 in the current experiment reduced litter weight was accompanied by a 2.4% increase in pre-
415 weaning mortality in sows which had received omega-3 during gestation compared with sows
416 which had received soya oil.

417

418 **7. Conclusion**

419 Feeding omega-3 fatty acids to sows during gestation negatively affected piglet survival and
420 did not increase subsequent litter size but improved the body weight, condition and fatness of
421 sows at weaning compared to controls.

422 **Ethics approval and consent to participate**

423 Ethical approval for the protocol was granted by the Animal Welfare and Ethical Review
424 Body at both sites (University of Leeds and Harper Adams University).

425 **Consent for publication**

426 Not applicable

427 **Competing interests**

428 The authors declare that they have no competing interests

429 **Funding**

430 This work was funded by AHDB. The funding body had a role in the design of the study and
431 in analysis and interpretation of the data.

432 **Authors' contributions**

433 KMcD contributed to analysing the data, interpreting the results and co-wrote the manuscript,
434 SI provided the experimental data and specific management information from site 2, SJ
435 formulated the experimental diets and contributed to study design, LJB contributed to the
436 study design, development of the 2 oil products and contributed to writing of the manuscript,
437 DC contributed to the study design, CE contributed to the study design and HMM led study
438 design, data analysis, interpretation of the data and co-writing the manuscript. All authors
439 read and approved the final manuscript

440 **Acknowledgements**

441 The authors would like to acknowledge Select Statistics for performing the initial statistical
442 analysis of the data and the research technicians and farm staff on both sites for conducting the
443 trial.

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Table 1 The composition and nutrient specifications of both the gestation and lactation diets for the control and omega 3 diets (% as-fed basis)

Treatment	B	A	B	A
	Dry sow Control	Dry sow Omega 3	Lactating sow Control	Lactating sow Omega 3
Raw Material	%	%	%	%
Barley	15.00	15.00	.	.
Wheat	35.52	35.52	53.43	53.43
Wheatfeed	36.86	36.86	4.42	4.42
Maize Germ	.	.	10.00	10.00
Bakery Meal	3.01	3.01	2.61	2.61
Soya Hipro GM	.	.	7.10	7.10
Sunflower meal	.	.	10.00	10.00
Phytase	0.25	0.25	0.25	0.25
Optomega 50 Salmon Oil ^a	.	2.00	.	2.00
Soy50 Fat Premix ^a	2.00	.	2.00	.
L-Lysine	0.13	0.13	0.66	0.66
DL-Methionine	.	.	0.02	0.02
Threonine	.	.	0.14	0.14
L-Tryptophan	.	.	0.01	0.01
Choline Chloride Sol 75%	0.03	0.03	0.03	0.03
Vitamin E 100	0.15	0.15	0.08	0.08
Sow premix ^b	.	.	0.10	0.10
Limestone Coarse	1.24	1.24	1.02	1.02
DCP	.	.	0.72	0.72
Salt	0.35	0.35	0.34	0.34
Sodium Bicarbonate	0.22	0.22	0.04	0.04
Soya Oil GM	0.25	0.25	2.03	2.03
Rouxminate	5.00	5.00	5.00	5.00
	100.00	100.00	100.00	100.00
Nutrient Composition				
Oil B	4.62	4.62	6.46	6.46
Protein	11.50	11.50	15.21	15.21
Fibre	4.43	4.43	4.06	4.06
Ash	5.73	5.73	6.19	6.19
Salt	0.52	0.52	0.50	0.50
Calcium	0.83	0.83	0.96	0.96
Phosphorous	0.44	0.44	0.55	0.55
Sodium	0.25	0.25	0.20	0.20
Digestible energy	12.28	12.28	13.48	13.48
Vit A	10000.0	10000.0	10000.0	10000.0
Vit D3	1875.0	1875.0	1875.0	1875.0
Vit E	200.0	200.0	200.0	200.0
Lysine	0.45	0.45	0.89	0.89

^a Salmon and soya oil supplements contained 50% oil and 50% carrier matrix so that each oil was supplemented as 1% of the diet.

^b Vitamin and mineral premix included the following (per kg of diet): 10,000 IU Vitamin A (retinyl acetate), 1875 IU Vitamin D₃ (cholecalciferol), 200 IU Vitamin E (alpha tocopherol acetate), 4mg Vitamin K (Hetrazeen), 1.5mg Vitamin B1 (Thiamine), 4mg Vitamin B2 (Riboflavin), 3.5mg Vitamin B6 (Pyridoxine hydrochloride), 15ug Pantothenic acid (calcium pantothenate), 20mg Nicotinic acid, 200ug Biotin, 2mg Folic acid, 15 mg Copper (sulphate), 1mg Iodine, 80mg Iron (sulphate monohydrate), 50mg Manganese (sulphate monohydrate), 0.25mg selenium (selenite), 100mg Zinc (sulphate monohydrate) and 100mg OXY-NIL® BPC Dry (BHT, propyl gallate, citric acid)

Table 2 Sow reproductive performance across the first experimental period where sows received a diet containing either salmon oil (omega-3, n = 199) or soya oil (control, n = 192) from service through to the subsequent service. Values presented are estimated marginal means. Significant values ($p < 0.05$) and trends ($p < 0.1$) are highlighted in bold.

	Diet			P value		
	Omega 3	Control	SEM	Diet	Parity	Diet*Parity
Farrowing live weight ^a (kg)	258.9	261.1	3.26	0.123	0.068	0.294
Farrowing back fat ^a (mm)	20.5	20.1	1.17	0.365	0.070	0.930
Farrowing condition score ^a	3.1	3.0	0.16	0.743	0.002	0.243
Weaning live weight ^a (kg)	234.8	230.9	2.04	0.010	< 0.001	0.238
Weaning P2 fat ^a (mm)	16.2	15.7	0.83	0.252	< 0.001	0.416
Weaning condition score ^a	2.9	2.8	0.11	0.426	< 0.001	0.179
Change in live weight (kg)	-23.7	-27.8	2.28	0.008	0.046	0.469
Change in back fat (mm)	-3.8	-4.6	0.96	0.152	0.005	0.330
Change in condition score	-0.22	-0.27	0.14	0.473	< 0.001	0.110
Gestation length (d)	115.5	115.2	0.12	0.324	0.488	0.451
Total born	14.9	14.9	0.28	0.895	< 0.001	0.940
Born alive ^b	13.4	13.4	0.15	0.803	0.044	0.817
Total litter wt ^b (kg)	19.3	19.9	0.36	0.047	< 0.001	0.258
Number weaned	11.1	11.2	0.18	0.342	0.003	0.458
Weaning age (d)	26.6	26.6	0.13	0.956	0.002	0.742
Litter wean wt ^c (kg)	85.1	86.0	2.88	0.517	< 0.001	0.317
Litter gain ^d (kg)	66.2	65.9	2.15	0.811	< 0.001	0.916
Mortality ^e (%)	16.6	14.2	1.00	0.046	0.332	0.695
Wean to service (d)	5.9	6.3	0.43	0.503	0.503	0.619

^a Controlled for weight, condition or fat at the start of the experiment, ^b Controlled for total born, ^c Controlled for weaning age, ^d Controlled for total born and total weaned ^e Controlled for total born and the change in sows body condition

Table 3 Sow reproductive performance across the second experimental period where sows returned to an un-supplemented commercial diet that had previously received salmon oil (omega-3, n = 139) or a soya oil control (n = 138) throughout the prior parity. Values presented are estimated marginal means. Significant values ($p < 0.05$) and trends ($p < 0.1$) are highlighted in bold.

	Diet			P value		
	Omega 3	Control	SEM	Diet	Parity	Diet*Parity
Farrowing live weight ^a (kg)	290.0	282.3	3.13	0.006	< 0.001	0.497
Farrowing back fat ^a (mm)	19.1	18.2	1.28	0.100	< 0.001	0.260
Farrowing condition score ^a	3.5	3.3	0.11	< 0.001	< 0.001	0.398
Weaning live weight ^b (kg)	247.2	243.7	2.03	0.043	< 0.001	0.719
Weaning P2 fat ^b (mm)	18.0	17.5	0.46	0.188	< 0.001	0.692
Weaning condition score ^b	3.3	3.1	0.07	0.063	< 0.001	0.101
Change in live weight (kg)	-36.0	-38.0	1.90	0.264	0.047	0.573
Change in back fat (mm)	-1.14	-1.31	0.73	0.693	0.263	0.991
Change in condition score	-0.18	-0.20	0.10	0.752	< 0.001	0.060
Gestation length (d)	115.4	115.5	0.13	0.629	0.671	0.835
Total born	14.7	14.7	0.36	0.955	0.210	0.852
Born alive ^c	13.4	13.3	0.10	0.562	0.009	0.364
Total litter wt ^c (kg)	20.4	20.3	0.26	0.718	0.049	0.045
Number weaned	11.4	11.3	0.26	0.393	< 0.001	0.157
Weaning age (d)	26.4	26.4	0.15	0.716	0.087	0.827
Litter wean wt ^d (kg)	88.1	87.7	1.46	0.749	0.001	0.733
Litter gain ^e (kg)	68.4	67.9	1.33	0.700	< 0.001	0.470
Mortality ^f (%)	10.8	12.5	1.12	0.201	0.020	0.019
Wean to service (d)	5.2	5.2	0.20	0.882	0.760	0.437

^a Not controlled for weight, condition or fat at service as this was not recorded for site 2 ^b Controlled for weight, condition or fat at farrowing, ^c Controlled for total born, ^d Controlled for number weaned and weaning age, ^e Controlled for total born and total weaned ^f Controlled for total born

Table 4 Sow reproductive performance across two experimental periods when provided with a diet supplemented with salmon oil (omega-3, n = 139) or soya oil (control, n = 138) throughout the first time period (Period 1). Values presented are estimated marginal means. Significant values ($p < 0.05$) and trends ($p < 0.1$) are shown in bold.

	Period 1			Period 2			P value		
	Omega 3	Control	<i>SEM</i>	Omega 3	Control	<i>SEM</i>	Diet	Period	Diet * Period
Farrowing live weight ^a (kg)	258.1	258.1	2.07	290.9	283.1	2.10	0.130	< 0.001	0.003
Farrowing back fat ^a (mm)	20.3	19.7	0.54	19.4	18.9	0.54	0.460	0.016	0.240
Farrowing condition score ^a	3.1	3.0	0.06	3.6	3.3	0.06	0.036	< 0.001	0.011
Weaning live weight ^b (kg)	241.0	236.9	1.25	237.7	233.5	1.38	0.008	0.012	0.945
Weaning back fat ^b (mm)	16.0	15.3	0.32	18.4	17.7	0.31	0.073	< 0.001	0.791
Weaning condition score ^b	2.9	2.8	0.05	3.2	3.0	0.05	0.024	< 0.001	0.750
Change in live weight (kg)	-23.9	-27.1	1.23	-34.8	-38.1	1.25	0.031	< 0.001	0.401
Change in back fat (mm)	-4.11	-4.58	0.38	-1.27	-1.75	0.37	0.277	< 0.001	0.621
Change in condition score	-0.27	-0.33	0.06	-0.21	-0.27	0.06	0.382	0.297	0.695
Gestation length (d)	115.2	115.1	0.12	115.5	115.4	0.11	0.814	0.010	0.334
Total born	15.1	14.8	0.29	14.9	14.6	0.29	0.502	0.456	0.610
Born alive ^c	13.6	13.5	0.10	13.5	13.4	0.10	0.418	0.573	0.962
Total litter weight ^c (kg)	19.1	19.8	0.25	20.5	20.5	0.25	0.250	< 0.001	0.066
Number weaned ^c	11.3	11.3	0.12	11.4	11.4	0.12	0.762	0.421	0.256
Weaning age (d)	26.6	26.5	0.13	26.5	26.4	0.13	0.490	0.099	0.502
Litter wean weight ^d (kg)	88.1	87.7	1.02	88.6	88.3	1.02	0.777	0.508	0.835
Litter gain ^e (kg)	69.3	68.3	0.96	68.5	67.5	0.96	0.405	0.319	0.649
Mortality ^f (%)	15.5	13.9	0.88	10.7	11.9	0.93	0.838	< 0.001	0.089
Wean to service (d)	5.8	6.1	0.28	5.1	5.4	0.28	0.481	0.031	0.397

^a Not controlled for starting weight, condition or fat as this data was not recorded for period 2 at Site 2, ^b Controlled for farrowing weight, condition or fat ^c Controlled for total born ^d Controlled for wean age and number weaned ^e Controlled for total born and total weaned, ^f Controlled for total born and the change in condition score