Title	Seeking to improve feed efficiency in pigs through microbial modulation via fecal microbiota transplantation in sows and dietary supplementation of offspring with inulin
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Publication date	2019-10-20
Original Citation	McCormack, U. M., Curião, T., Metzler-Zebeli, B. U., Wilkinson, T., Reyer, H., Crispie, F., Cotter, P. D., Creevey, C. J., Gardiner, G. E. and Lawlor, P. G. (2019) 'Seeking to improve feed efficiency in pigs through microbial modulation via fecal microbiota transplantation in sows and dietary supplementation of offspring with inulin', Applied and Environmental Microbiology, 85(22) e01255-19, (18 pp). doi: 10.1128/AEM.01255-19
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1128/AEM.01255-19
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Download date	2025-07-04 02:10:42
Item downloaded from	https://hdl.handle.net/10468/9755

1 **Seeking to improve feed efficiency in pigs through microbial modulation via fecal**

2 **microbiota transplantation in sows and dietary supplementation of offspring with inulin**

3

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18 Running title: Microbial modulation to alter feed efficiency in pigs

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22 **ABSTRACT**

23 As previous studies have demonstrated a link between the porcine intestinal microbiome and

24 feed efficiency (FE), microbiota manipulation may offer a means of improving FE in pigs. A

25 fecal microbiota transplantation procedure (FMTp), using fecal extracts from highly feed

26 efficient pigs, was performed in pregnant sows (n=11), with a control group (n=11) receiving

27 no FMTp. At weaning, offspring were allocated, within sow treatment, to 1) control (n=67; no

28 dietary supplement) or 2) inulin (n=65; 6-week dietary inulin supplementation) treatments. The

29 sow FMTp, alone or in combination with offspring inulin supplementation, reduced offspring

30 body weight by 8.1-10.6 Kg at ~140 days of age, but there was no effect on feed intake. It

31 resulted in better FE, higher bacterial diversity and higher relative abundances of potentially

32 beneficial bacterial taxa (*Fibrobacter, Prevotella*) in offspring. Due to FMTp and/or inulin

33 supplementation, relative abundance of potential pathogens (*Chlamydia*, *Treponema*) in the

34 ileum, and cecal concentrations of butyric acid were significantly lower. Maternal FMTp led

35 to a greater number of jejunal goblet cells in offspring. Inulin supplementation alone did not

36 affect growth or FE, but up-regulated duodenal genes linked to glucose and volatile fatty acid

37 homeostasis and increased mean platelet volume, but reduced ileal propionic acid, granulocyte

38 counts, and serum urea. Overall, FMTp in pregnant sows, with/without offspring dietary inulin

39 supplementation, beneficially modulated offspring intestinal microbiota (albeit mostly low

40 relative abundance taxa) and associated physiological parameters. Although FE was improved,

41 the detrimental effect on growth limits the application of this FMTp/inulin strategy in

42 commercial pig production.

43 **IMPORTANCE**

44 As previous research suggests a link between microbiota and FE, modulation of the intestinal

45 microbiome may be effective in improving FE in pigs. The FMTp in gestating sows, alone/in

46 combination with offspring post-weaning dietary inulin supplementation, achieved

47 improvements in FE, and resulted in higher relative abundance of intestinal bacteria associated

48 with fiber degradation, and lower relative abundance of potential pathogens. However, there

49 was a detrimental effect on growth, although this may not be wholly attributable to microbiota

50 transplantation, as antibiotic and other interventions were also part of the FMT regime.

51 Therefore, further work with additional control groups is needed to disentangle the effects of

52 each component of the FMTp in order to develop a regime with practical applications in pig

53 production. Additional research based on findings from this study may also identify specific

54 dietary supplements for promotion/maintenance of the microbiota transferred via maternal

55 FMTp, thereby optimizing pig growth and FE.

56 **INTRODUCTION**

57   Feed efficiency (FE) is of major importance in pig production, as feed accounts for the

58 majority cost associated with producing pigs (1). Previous work from our group and others,

59 have demonstrated an association between the intestinal microbiota and residual feed intake

60 (RFI; a metric for FE) in pigs (2-5). It may therefore be possible to improve FE through

61 manipulation of the intestinal microbiota. This could be achieved via fecal microbiota

62 transplantation (FMT) and/or dietary supplementation with feed additives.

63   To date, the use of FMT in pigs has mainly been limited to the establishment of the

64 human gut microbiota in pigs in order to develop a model for humans (6, 7). However, the pig

65 gut microbiota has also been transferred to rodents (8) and to a lesser extent other pigs (9-11).

66 One of these latter studies is from our group and used an inoculum derived from fecal extracts

67 collected from highly feed efficient pigs with a view to improving FE (11). The results showed

68 that FMT in pregnant sows and/or their offspring impacted lifetime growth of offspring, as pigs

69 were ~4-8 Kg lighter at slaughter (11). Intestinal microbiota composition and predicted

70 functionality, along with physiological parameters, were also impacted, and overall the results

71 indicated that FMT may not be a suitable approach to optimize FE in pigs. However, although

72 potentially beneficial FMT-associated modulation of the sow intestinal microbiota occurred,

73 with some evidence of microbiome transfer from the FMT-treated sows to their offspring, other

74 bacterial taxa were either not transferred to or did not colonize within the offspring

75 microbiome. Therefore, it is possible that dietary prebiotic supplementation of the offspring

76 might provide a substrate for transplanted microbiota, thereby encouraging their proliferation

77 and potentially improving FE.

78   A prebiotic is defined as "a substrate that is selectively utilized by host microorganisms

79 conferring a health benefit" (12). Inulin is a dietary fiber derived mainly from chicory which

80 is not digestible by the host (13). It has proved effective as a prebiotic in humans, but in pigs,

4

81   results have been more contradictory (14). Nonetheless, a number of studies have found

82   beneficial effects of inulin supplementation to pig diets, both in terms of improving growth

83   performance and modulating the intestinal microbiota (15-17). In particular, supplementing

84   weaner diets with inulin may be a useful way to counteract the susceptibility to infection and

85   reduced growth rate associated with the stress of weaning, and a number of studies have

86   demonstrated beneficial modulation of the intestinal microbiota and improved growth, gut

87   health, and FE (16, 18, 19). For example, Grela *et al.* found that dietary inulin addition

88   improved weight gain, reduced feed intake and improved FE in pigs between 10 and 84 days

89   of age (15). Inulin is fermented in the lower part of the digestive tract by enzymes produced by

90   certain types of bacteria, resulting in increased production of volatile fatty acids (VFA), mainly

91   butyrate (20). The addition of inulin to the diet of pigs (at various stages throughout their

92   productive life) has been shown to increase bacterial populations considered beneficial in the

93   small and/or large intestine (mainly the latter), while reducing potentially pathogenic bacteria

94   (14, 21). However, a recent meta-analysis showed that although strong negative relationships

95   were found between dietary inulin and colonic enterobacteria throughout all production phases,

96   the same was true for fecal lactobacilli, which are generally considered beneficial in the gut

97   (14).

98   The hypothesis here was that by promoting the proliferation and persistence of

99   amicrobial profile for improved FE early in life, lifetime FE in pigs would improve. The

100   objectives were to determine if FMT, using fecal extracts from highly feed efficient pigs, in

101   pregnant sows and/or dietary inulin supplementation to offspring post-weaning, would improve

102   offspring FE, and to determine if inulin supplementation would support the survival/growth of

103   any potentially beneficial bacteria transferred to offspring as a result of maternal FMT.

104   (This research was conducted by U.M. McCormack in fulfillment of the requirements for a

105   PhD from Waterford Institute of Technology (WIT), Waterford, Ireland in 2017 (22).)

5

106   **RESULTS**

107   This study comprised a total of 4 treatments: control sow and control offspring (CON/CON),

108   control sow and inulin-supplemented offspring (CON/INU), fecal microbiota transplant

109   procedure (FMTp)-treated sow and control offspring (FMTp/CON), and FMTp-treated sows

110   and inulin-supplemented offspring (FMTp/INU).

111   Due to the large number of significant sow × offspring treatment interactions observed, we

112   have focused on the effect of sow or offspring treatment, and have indicated if an interaction

113   was also observed, only if relevant. All significant interactions are summarized in Table S1.

114   Although there were several significant differences in the inulin-supplemented offspring at

115   weaning, and these are shown in the relevant tables and figures, they will not be outlined here,

116   as inulin was only supplemented to the diet from weaning. In addition, bacterial taxa and

117   predicted functional pathways present at relative abundances of <0.001% will not be discussed.

118

119   **Impact of FMT in sows and/or inulin inclusion in offspring diets on offspring growth**

120   The effect of maternal FMTp and post-weaning dietary inulin supplementation on

121   offspring lifetime growth is shown in Tables 1 and S1. At 100 days of age, FMTp/CON pigs

122   (51.2 Kg) had lighter body weight compared to CON/CON (59.0 Kg) and CON/INU (58.6 Kg),

123   and offspring from FMTp sows (52.5 Kg) were lighter compared to their control counterparts

124   (58.8 Kg; P<0.05). At ~140 days of age (slaughter), FMTp/CON and FMTp/INU offspring

125   were 10.6 and 7.1 Kg lighter (P<0.05) respectively, than control and inulin-supplemented

126   offspring from CON sows (P<0.05). Due to sow FMTp, offspring were also lighter at slaughter

127   (P<0.05). Consequently, the cold carcass weights of offspring from FMTp sows were 8.9 and

128   5.1 Kg lighter (P<0.05) than those of offspring from CON sows when offspring treatments

129   were control and inulin, respectively (P<0.05). The FMTp/INU pigs had a greater muscle depth

130   compared to CON/INU offspring (P<0.05). No treatment differences were observed for

131   average daily feed intake (ADFI), average daily gain (ADG) and feed conversion efficiency

132   (FCE) or other carcass traits measured in offspring.

133   Offspring from FMTp sows (FMTp/CON and FMTp/INU) had a lower RFI value

134   (better FE) compared to offspring from CON sows (P<0.05). This was reflected at sow

135   treatment level where pigs from FMTp sows had a lower RFI than those from CON sows

136   (P<0.05). Inulin supplementation alone however, did not influence offspring RFI (P>0.05).

137

138   **Influence of FMT in sows and/or inulin inclusion in offspring diets on offspring intestinal**

139   **microbial diversity**

140   In general, the offspring from FMTp sows had a greater number of OTU's in the feces

141   in the early post-weaning period, whereas inulin-supplemented offspring had less than their

142   control counterparts (Table S2). This was reflected to some extent in microbial diversity

143   measures (Fig. 1). At 130 days of age, all treatments had higher Shannon diversity (species

144   abundance and evenness, accounting for rare species) compared to CON/CON (P<0.05; Fig.

145   1A) and irrespective of offspring treatment, offspring from FMTp sows had a higher Shannon

146   diversity (4.2) than offspring from CON sows (3.8; P<0.05; data not shown). However, lower

147   Simpson diversity (species richness and evenness, which takes in to account number as well as

148   relative abundance of each species present) was observed in the ileum of inulin-supplemented

149   offspring (0.66) compared to control offspring (0.71; P<0.05; Fig. 1B and data not shown).

150   Microbial ß-diversity was also measured in all fecal and intestinal samples and is

151   depicted from OTUs using principle component analysis (PCA) plots using a Euclidean

152   distance metric, which is calculated from regularized log-transformed counts and plotted using

153   ggplot2 (Fig. S1). Throughout the lifetime, there was an influence of sow treatment on

7

154   offspring microbial diversity, with offspring from FMTp sows clustering away from offspring

155   born to CON sows in the feces at weaning (R^2: 0.45; P<0.05) and 130 days of age (just prior

156   to slaughter) (R^2: 0.32; P<0.05). On days 65 (R^2: 0.55) and 130 (R^2: 0.15), dietary

157   supplementation with inulin led to a clustering effect in the feces (P<0.05). Although there

158   were no significant differences in the ileum, CON/CON and FMTp/CON clustered separately

159   in the cecum (R^2: 0.51; P<0.05), and in the colon, CON/INU and FMTp/CON clustered away

160   from CON/CON offspring (R^2: 0.53; P<0.05).

161

162   **Effect of FMTp in sows and/or inulin supplementation of offspring on offspring intestinal**

163   **microbial composition**

164   Microbial composition, at the phylum and the genus levels, was investigated in

165   offspring feces throughout their lifetime and in the intestinal digesta collected at slaughter. The

166   number of OTUs present at each sampling time point/in each digesta type were as follows;

167   weaning: 542, day 50: 347, day 65: 75, day 100: 531, and day 130 of age: 585, ileum: 66,

168   cecum: 361, colon: 456. Composition at the phylum level for feces and digesta samples is

169   shown in Fig. S2. The number of phyla detected varied over time; 12 were detected in the feces

170   at weaning, eight at day 50 of age, six at day 65 of age, 15 at day 100 of age and 14 at day 130

171   of age, with eight detected in the ileum, and seven in both the cecum and colon, respectively.

172   However, many were detected at very low relative abundance.

173   A total of eight phyla and 25 genera differed significantly between treatments, and these

174   ranged in relative abundance from 0.004 - 18.6% and 0.002 – 18.0%, respectively, but were

175   mainly present at low relative abundance. Five phyla and 19 genera differed due to a sow ×

176   offspring treatment interaction, six phyla and 15 genera due to sow treatment, and 3 phyla and

177   10 genera due to offspring treatment. All bacterial taxa reported below are significantly

178   different (P<0.05) and are reported in Fig 2 and Table S1.

179   At weaning, *Lentisphaerae* and *Synergistetes* were higher in relative abundance in

180   offspring due to FMTp in sows. *Proteobacteria* was impacted throughout the lifetime of the

181   pig, mainly due to sow treatment. In the feces collected at day 50, FMTp in sows resulted in a

182   higher relative abundance of *Proteobacteria* in the offspring, but this phylum was lower in

183   relative abundance due to inulin supplementation. This FMTp effect was also observed in the

184   feces collected on day 100 and in the cecum as well. On day 100, *Fusobacteria* was higher in

185   relative abundance in offspring from FMTp sows, and 30 days later, *Fibrobacteres* was present

186   at a lower relative abundance due to FMTp , but was higher in relative abundance due to inulin

187   supplementation). In the ileum, *Spirochaetes* was lower in offspring due to FMTp in sows.

188   Furthermore, *Chlamydiae* was lower in relative abundance in all groups compared to

189   CON/CON offspring, and was also reduced due to dietary inulin supplementation.

190   Most of the treatment differences at the genus level occurred in the feces at weaning,

191   or just prior to slaughter, at day 130 of age, and in the ileal digesta at slaughter. Apart from

192   *Sphaerochaeta* (day 130 of age) all of the differences observed were for genera present at <5%

193   relative abundance. Throughout the lifetime of the pigs, several bacterial genera were impacted

194   at more than one fecal time point, as well as in the digesta collected at slaughter, with a strong

195   effect of sow treatment observed over time.

196   At weaning, due to FMTp in sows, *Faecalibacterium* was lower in offspring, whereas

197   S*treptococcus* was higher in relative abundance. Additionally, *Bifidobacterium*,

198   *Butyricimonas*, *Eubacterium*, *Lactobacillus*, and *Terrisporobacter* were higher in relative

199   abundance due to FMTp in sows. In the feces collected between days 28 - 130 of age a number

200   of bacterial genera were impacted; 10 due to an interaction effect, six due to sow treatment,

9

201   and four due to offspring treatment. All impacted genera were at a relative abundance of <5%,

202   except for *Sphaerochaeta*. At 50 days of age, *Butyricicoccus* and *Campylobacter* were lower

203   in relative abundance due to inulin supplementation. At 100 days of age, *Campylobacter* was

204   higher in relative abundance in FMTp/CON offspring compared to offspring from CON sows,

205   and this was reflected at sow treatment level also. *Sutterella* was also higher in relative

206   abundance due to all interventions compared to CON/CON pigs, and was higher due to FMTp

207   in sows also. Due to FMTp in sows, *Schwartzia* was present at a higher relative abundance in

208   offspring. Thirty days later (at ~130 days of age, just prior to slaughter), *Acetanaerobacterium*

209   was higher in relative abundance in FMTp/CON versus CON/INU, and pigs from FMTp sows

210   had a higher relative abundance also, but inulin supplementation lowered the relative

211   abundance. In addition, *Fibrobacter* was lower in relative abundance in FMTp/CON offspring

212   compared to all other groups, and offspring from FMTp sows had a lower relative abundance

213   also (Fig. 3C), but INU pigs had a higher relative abundance (Fig. 3D). Due to FMTp in sows,

214   *Turicibacter* was present at a lower relative abundance in offspring compared to those from

215   CON sows (Fig. 3C).

216   In the ileum, *Prevotella* was higher in relative abundance, whereas *Chlamydia* was

217   lower, in all groups compared to CON/CON. *Prevotella* was relatively more abundant and

218   *Chlamydia* was less so due to inulin supplementation (Table S1, Fig. 3D). Additionally,

219   *Prevotella* was higher in relative abundance due to FMTp in sows also (Fig. 3C). In the cecum,

220   *Bacteroides* was relatively more abundant due to FMTp/CON compared to all other groups,

221   and offspring born to FMTp sows had a higher relative abundance also (Table S1, Fig. 3C).

222

223 **Effect of FMTp in sows and/or inulin supplementation of offspring on predicted**

224 **functionality of the offspring intestinal microbiota**

225   The functionality of the intestinal microbiota was predicted in all offspring fecal and

226 digesta samples, and significant differences are shown in Fig S3. A total of 26 predicted

227 bacterial pathways in offspring were significantly impacted due to an interaction (Table S1).

228 As a result of FMTp in sows, 10 pathways were altered in the offspring, and these were mostly

229 related to lipid metabolism, carbohydrate metabolism and xenobiotics degradation and

230 metabolism (Fig. S3). Due to dietary inulin supplementation in offspring (Fig S3), 14 predicted

231 pathways, mostly related to carbohydrate metabolism and glycan biosynthesis and metabolism

232 were impacted. Overall, most of the effects were seen within the ileal microbiota. All pathways

233 that were significantly influenced by FMTp/inulin supplementation were present at <2.0%

234 relative abundance.

235   At 70 days of age, alpha-linolenic acid metabolism was predicted to be present at a

236 lower relative abundance due to inulin supplementation. In the ileum, porphyrin and

237 chlorophyll metabolism, and seleno-compound metabolism was lower in relative abundance

238 due to both intervention strategies, whereas the glycosphingolipid biosynthesis - ganglio series

239 pathway was higher in predicted relative abundance. The combination of FMTp and inulin

240 supplementation resulted in a higher predicted relative abundance of the glycosphingolipid

241 biosynthesis - globo series pathway compared to CON/INU offspring (Table S1), and inulin-

242 supplemented offspring had a higher relative abundance compared to their CON counterparts

243 also. Additionally, FMTp/INU resulted in a higher predicted relative abundance of a pathway

244 involved in biosynthesis of ansamycins compared to CON/INU offspring, and offspring from

245 FMTp sows had a higher relative abundance also. The FMTp resulted in a higher predicted

246 relative abundance of ether lipid metabolism, compared to offspring from CON sows.

247 Secondary bile acid biosynthesis was higher in relative abundance due to either/both

11

248   interventions. Due to FMTp in sows, phenylalanine metabolism was lower, but bisphenol

249   degradation and linoleic acid metabolism were higher in predicted relative abundance

250   compared to offspring from CON sows. Dietary supplementation of inulin in weaner offspring

251   resulted in a higher predicted relative abundance of two pathways related to glycan biosynthesis

252   and phenylpropanoid biosynthesis, but lowered the relative abundance of butanoate

253   metabolism.

254   In the cecum, FMTp/CON offspring had a higher relative abundance of fructose and

255   mannose metabolism but a lower relative abundance of D-alanine metabolism compared to the

256   other three groups, and sow FMTp resulted in a higher and lower predicted relative abundance,

257   respectively, whereas the opposite occurred due to inulin supplementation.

258

259   **Effect of FMTp in sows and/or inulin supplementation of offspring on offspring intestinal**

260   **volatile fatty acid concentrations**

261   Volatile fatty acid concentrations were measured in digesta from the ileum, cecum and

262   colon of the 32 selected offspring, and results are shown in Table 2 and S1. No differences

263   were observed between treatments for digesta pH in any of the intestinal segments. In the ileum,

264   offspring from FMTp/INU had higher concentrations of acetic acid compared to the other

265   groups, and CON/INU had lower propionic acid concentrations compared to CON/CON

266   offspring (P<0.05), and this VFA was also reduced in inulin-fed offspring (P<0.05).

267   In the cecum, butyric acid concentrations were lower for FMTp/INU compared to all

268   other groups, and for FMTp/CON compared to both offspring treatments from control sows,

269   (P<0.05). It was also lower due to FMTp in sows (P<0.05) and inulin supplementation in

270   offspring (P<0.05). Moreover, cecal valeric acid concentrations were lower in FMTp/INU

271   compared to all other groups, but CON/INU pigs had a higher concentration compared to

12

272   control offspring, regardless of sow treatment (P<0.05). Due to sow FMTp, valeric acid

273   concentrations were also lower (P<0.05). However, isovaleric acid concentrations were higher

274   in FMTp/CON, but lower in FMTp/INU compared to all other groups (P<0.05), and lower due

275   to inulin treatment also (P<0.05). In the colon, isobutyric acid concentrations were higher in

276   FMTp/CON pigs compared to all other groups (P<0.05), and higher due to FMTp in sows

277   (P<0.05).

278

279   **Influence of FMTp in sows and/or inulin supplementation of offspring on offspring**

280   **intestinal histology**

281   Histological analyses of the offspring small intestine (duodenum, jejunum, and ileum)

282   are shown in Table 3 and S1. In the duodenum, none of the parameters measured differed

283   between groups. However, FMTp offspring had a higher number of goblet cells per villus

284   compared to their control counterparts (P<0.05), and FMTp/CON had a higher number of

285   jejunal goblet cells (per villus and per µm villus height) compared to CON/CON, and this was

286   also observed due to FMTp in sows (P<0.05). The FMTp in sows resulted in shorter ileal villi

287   and a smaller villus area compared to CON sows (P<0. 05).

288

289   **Influence of FMTp in sows and/or inulin supplementation of offspring on offspring brush**

290   **border enzyme activity and gene expression in the duodenum**

291   Disaccharidase activity in the duodenum of offspring at slaughter (~140 days old) is

292   shown in Fig. 3A. Only maltase activity was impacted by a sow × offspring treatment

293   interaction, where CON/INU had lower activity compared to CON/CON and FMTp/INU

294   offspring, and the latter had higher activity compared to FMTp/CON offspring (P<0.05). No

295   differences at sow or offspring treatment level were observed (P>0.05).

296   Expression of three of the 11 genes measured in the duodenum was impacted as follows

297   (Fig. 3B): up-regulation of glucose-dependent insulinotropic peptide (*GIP*) was observed in

298   CON/INU compared to CON/CON offspring, and this was observed also in inulin-

299   supplemented compared to control offspring (P<0.05). In addition, glucagon-like peptide 1

300   (*GLP1*) and sodium-coupled monocarboxylate transporter (*SMCT*) were up-regulated in inulin-

301   supplemented offspring compared to their control counterparts (P<0.05).

302

303   **Influence of FMTp in sows and/or inulin supplementation of offspring on offspring blood**

304   **parameters**

305   The results of offspring hematological analysis at slaughter are shown in Table 4 and

306   S1. White blood cell counts were lower in CON/INU compared to CON/CON offspring

307   (P<0.05), and hemoglobin concentration was higher in FMTp/INU compared to FMTp/CON

308   offspring (P<0.05). Both granulocyte percentage (64 vs. 54) and number (17 vs. 11) were lower

309   in inulin-supplemented compared to control offspring (P<0.05) but platelet volume was higher

310   (10.3 vs. 9.5; P<0.05). In addition, mean corpuscular hemoglobin percentage was lower in

311   offspring from FMTp sows compared to their control counterparts (17.8 vs. 18.8; P<0.05).

312   Of all the serum biochemical parameters measured in offspring at slaughter (Table 4

313   and S1), only cholesterol and urea concentrations were impacted. Cholesterol tended to be

314   lower in both offspring treatments from FMTp sows compared to CON/CON (P=0.07),

315   whereas blood urea nitrogen tended to be lower due to inulin offspring supplementation (11.1

316   vs. 16.3 mg/dL; P=0.06).

14

317 **DISCUSSION**

318   Beneficial modulation of the intestinal microbiota may result in improved intestinal health

319 and nutrient utilization, and ultimately, improved growth and FE in pigs. Prebiotics, most

320 notably inulin, have been studied in pigs in order to achieve this (14, 21, 23, 24). Microbiota

321 transplantation may also be a useful approach, as it has been shown to transfer host

322 physiological traits, such as leanness, obesity, and immunological and gut characteristics, via

323 'reprogramming' of the intestinal microbiota (10, 25-28). However, previous work from our

324 group showed a depression in offspring body weight at slaughter as a result of FMT in sows

325 and/or offspring (11). Modulation of the intestinal microbiota also occurred in pregnant sows

326 receiving the FMTp, with some evidence of microbiome transfer from the FMT-treated sows

327 to their offspring. However, other bacterial taxa were either not transferred to, or did not

328 colonize, the offspring and so here we tested the hypothesis that dietary prebiotic (inulin)

329 supplementation of subsequent offspring might provide a substrate for transplanted microbiota,

330 thereby encouraging their proliferation.

331   Results showed that pigs born to FMTp sows (irrespective of post-weaning treatment) were

332 ~8.9 Kg lighter at slaughter, but were more feed efficient, given their lower RFI value. No

333 improvements in weight gain, or indeed FE were observed due to inulin inclusion in post-

334 weaning diets alone, contradictory to the findings of some previous studies (15), but in

335 agreement with others (14, 29). However, FE was improved when inulin was supplemented to

336 the diet of weaner pigs born to FMTp sows, and although body weight was reduced, it may

337 have a role in promoting the proliferation of beneficial bacterial populations implanted as a

338 result of modulation of the maternal microbiota. In some cases, there was an impact of FMTp

339 and/or inulin supplementation on offspring bacterial diversity, with a significant clustering

340 effect occurring within sample time points. However, although significant, the R^2 values are

341 low and therefore, these findings should be interpreted with caution. Due to the complexity of

15

342 the FMTp employed in the current study, it could be argued that the negative effects on pig

343 weight were due to *in utero* effects of the antibiotic and/or purgative and/or proton pump

344 inhibitor administered to sows as part of the regime or due to the fasting period, as control

345 animals were not given the same drug regimen. However, none of these interventions were

346 applied to FMT-treated offspring in a related study of ours (11), and similar FMT-associated

347 weight reductions were observed. Nonetheless, further studies with additional control groups

348 are warranted in order to fully elucidate the potential impact of these confounding factors on

349 the offspring microbiome.

350   A higher relative abundance of bacteria deemed 'beneficial' for host health was

351 observed in offspring feces due either to FMTp in sows (most pronounced) or offspring inulin

352 supplementation. However, for inulin treatment all of these were at weaning, which is

353 meaningless as inulin supplementation only commenced at that point, highlighting the

354 importance of biological vs. statistical significance. However, in later life, some bacterial

355 populations considered potentially beneficial were relatively more abundant in offspring from

356 FMTp sows supplemented with inulin compared to their unsupplemented counterparts; for

357 example, *Fibrobacter,* which is a fibre degrader (30). In the ileum, *Prevotella* was increased

358 by both FMT in sows and inulin supplementation of offspring, and is a key genus in pigs,

359 previously associated with weight gain (30), but also with poor FE (3). However weight gain

360 was not observed in the present study and FE was improved in offspring, highlighting the

361 difficulty in relating shifts in taxonomic composition to true functional differences. In general,

362 treatment effects were more evident within the fecal microbiome of pigs at the end of the

363 finishing period, at 100 and 130 days of age, even though inulin was removed from the diet 30-

364 60 days prior to this and FMTp was performed in the sows only, demonstrating that the effects

365 of both treatments persisted throughout the productive life of the pig. While the exact

366 mechanism by which the sow FMT influences offspring gut microbiome is unknown, it is most

16

367   likely through altered microbiome exposure both pre- and post-birth. Evidence of the effects

368   of pre-birth exposure comes from the fact that the microbiome of the offspring of sows

369   administered FMT during gestation only versus those not (FMT/CON vs CON/CON) differs.

370   There will also likely be residual effects on the microbiome of these pigs post-birth as a result

371   of exposure to the altered intestinal and colostrum microbiome of FMTp-treated sows

372   (information on the sow microbiome is presented in our related publication (11). Indeed, there

373   was some evidence of microbiome transfer from the FMT-treated sows to their offspring.

374   Additional evidence of the influence of post-birth effects on the microbiome also come from

375   this associated study which showed that offspring from untreated mothers administered FMT

376   themselves have an altered microbiome (11).

377   At the genus level, the cellulolytic genus *Fibrobacter* was less relatively abundant in

378   offspring due to maternal FMT but more abundant due to offspring inulin supplementation in

379   the feces just prior to slaughter. However, the opposite was true for *Bacteroides*, a genus known

380   to be hemicellulolytic, which was increased in relative abundance in the cecum of offspring as

381   a result of FMT in sows. Interestingly, *Bacteroides* was previously found to be associated with

382   better FE in finisher pigs (4), thus the higher relative abundance of *Bacteroides* in the cecum

383   of offspring from FMTp sows may explain the improved FE observed in these animals in the

384   present study.

385   The effect of the combination of maternal FMT and inulin supplementation on offspring

386   microbiota was evident throughout the current study, not only in terms of composition, but also

387   potential function, most notably carbohydrate and lipid metabolism. In agreement with the fact

388   that inulin is a plant-storage glycan, the microbiota of inulin-supplemented offspring had an

389   enhanced predicted relative abundance of glycan biosynthesis and metabolism pathways and a

390   lower relative abundance of other carbohydrate metabolism pathways. However, a concomitant

17

391   increase in VFA concentrations was not observed, in contrast to previous findings in humans

392   (31).

393   Genes involved in glucose homeostasis, in particular the secretion of insulin, such as

394   *GIP* and *GLP1* were more relatively abundant in the duodenum of inulin-supplemented pigs.

395   This is likely indicative of inulin fermentation in the upper gastrointestinal tract (GIT), or

396   perhaps a compensatory mechanism for nutrient digestion in the small intestine, potentially

397   leading to a better metabolic capability of inulin-supplemented pigs. Furthermore, a higher

398   utilization of protein/nitrogen by the microbiota may have occurred, as indicated by lower

399   serum urea concentrations in inulin-fed offspring (32). Inulin has also been linked with possible

400   lipid-modulatory effects in humans and piglets (15, 33), which is in accordance with the

401   reduced serum cholesterol concentrations found in the present study. Furthermore, the reduced

402   cholesterol concentration observed may be due to the higher ileal concentrations of acetic acid,

403   as dietary acetic acid has been found to reduce serum cholesterol in rats (34).

404   Inulin has been shown to modulate not only growth and FE, but also immunological

405   features in pigs (15). Interestingly, white blood cell and granulocyte counts decreased due to

406   FMTp in sows and/or inulin supplementation of offspring, and the lower counts of these

407   immune cells may be linked to the lower relative abundance of potential pathogens

408   (*Campylobacter*, *Chlamydia*) observed in the feces and digesta of these pigs. This in turn may

409   be linked to the higher relative abundance of lactic acid bacteria in these animals, as these are

410   known to reduce pathogens in the GIT (35). Moreover, offspring from FMT sows may have

411   over-enhanced mucin production in the small intestine, as more goblet cells were present in the

412   jejunum and mucin is a physical barrier which prevents pathogen adherence to the epithelial

413   lining (36).

CONCLUSIONS

414   We provide evidence that maternal FMT alone or in combination with dietary inulin

415 supplementation of offspring, as a strategy to modulate the intestinal microbiota of pigs has a

416 beneficial impact on FE, but a detrimental effect on body weight, throughout the pig's

417 productive lifetime. These effects were accompanied by influences on both intestinal

418 microbiota composition and predicted functionality in the offspring. Although dietary

419 supplementation with inulin alone had a similar impact on the intestinal microbiota, effects

420 were not as pronounced and improvements in offspring growth or FE were not observed.

421 Bacterial taxa considered potentially beneficial such as *Prevotella*, albeit mainly present at low

422 relative abundance, were increased in the offspring, mainly due to FMT. Dietary inulin

423 supplementation of offspring from FMTp sows led to a higher relative abundance of

424 *Fibrobacter* than in non-supplemented counterparts, suggesting a possible role of inulin in

425 supporting maternally-derived microbiota in the offspring. Pigs supplemented with inulin had

426 lower levels of blood urea nitrogen, and granulocytes, indicating an improved health status.

427 Taken together, the hematological, biochemical and gene expression data suggest improved

428 health in offspring from FMT-treated sows, and/or those supplemented with inulin. Overall,

429 the results from this study show that the maternal FMT regime used in the present study, either

430 alone or in combination with post-weaning inulin supplementation, is not suitable for use in

431 pig production, due to the detrimental impact on lifetime growth. However, possible *in utero*

432 effects of the antibiotic and other interventions used as part of the FMT regime cannot be ruled

433 out, and further work with additional control groups is needed to unravel the influence of the

434 different components used. Additional research based on the findings from this study may also

435 identify specific prebiotic or other dietary supplements for promotion/maintenance of the

436 microbiota transferred via maternal FMTp, thereby optimizing pig growth and FE. Further

437 studies on the exact mechanism(s) of action of the FMT are also warranted.

439

440   **MATERIALS AND METHODS**

441   **Ethical approval**

442   The pig study was approved by the animal ethics committees of Teagasc (TAEC9/2013)

443   and Waterford Institute of Technology (13/CLS/02) and performed according to European

444   Union regulations outlining minimum standards for the protection of pigs (91/630/EEC) and

445   concerning the protection of animals kept for farming purposes (98/58/EC). An experimental

446   license (number AE1932/P032) was obtained from the Irish Health Products Regulatory

447   Authority.

448

449   **Animal management, recording and sampling**

450   Feces were collected from four highly feed efficient finisher pigs, anaerobically

451   processed and the resultant fecal extracts prepared for use as FMT inoculum as previously

452   described by McCormack et al. (11). The same 22 sows used in the McCormack et al. study

453   were used here; on day 60 of gestation, sows were assigned to one of two treatment groups; 1)

454   Control (n=11; CON), and 2) antibiotic treatment, purgative and FMT on days 70 and 100 of

455   gestation (n=11; FMTp). On day 61 of gestation, FMTp sows received a 7-day course of a

456   broad spectrum antibiotic cocktail [20 mg/Kg/day Amoxicillin Trihydrate (amoxinsol®;

457   Vetoquinol UK Ltd., Buckingham, UK), 10 mg/Kg/day lincomycin-spectinomycin (Linco-

458   Spectin® 100; Pfizer, Cork, Ireland) and 100,000 IU/Kg/day of colistin (Coliscour®; Ceva

459   Sante Animale, Libourne, France)], followed by two doses of a purgative (sodium picosulfate,

460   magnesium oxide and citric acid; Picolax powder, Ferring Ltd., Dublin, Ireland) to clear the

461   GIT of resident microbiota, followed by a fasting period of 36 h. On days 70 and 100 of

462   gestation, sows received the FMT (200 mL; which delivered a dose of ~2.6×10^{11} CFU) via

463   gastric intubation along with a proton-pump inhibitor (Omeprazole; Romep, Rowex Ltd., Cork,

21

464 Ireland) to prevent possible inhibition of the bacteria in the inoculum by the acidity of the

465 stomach.

466   A schematic depicting sow and offspring treatments and details of sampling is shown

467 in Fig. S4. At farrowing, the number of pigs born alive, stillbirths and mummies were recorded,

468 as well as individual piglet birth weights and gender. All viable piglets were tagged for

469 identification purposes, and litters remained intact in so far as possible between farrowing and

470 weaning. A commercial non-medicated starter diet (Table S3) was creep-fed between day 12

471 and weaning at ~day 28 of age.

472   At weaning, 132 pigs were selected across all litters, blocked by sow treatment, piglet

473 gender and body weight, and randomly assigned to single-gender pens, with 8-12 pigs per pen.

474 Within sow treatment, pens of pigs were randomly assigned to: 1) control (6 pens; n=67 pigs;

475 CON) or 2) inulin for the first six weeks post-weaning (6 pens; n=65 pigs, INU). Once weaned,

476 piglets in both CON and INU groups were provided with the same sequence of diets (Table S3;

477 starter for 1 week, followed by link for 2 weeks, followed by weaner for 3 weeks, followed by

478 finisher to slaughter at ~140 days of age) except that for the INU group starter and link diets

479 contained 2% inulin (Orafti Synergy 1, 50:50 chain length, Beneo Animal Nutrition, Belgium)

480 and the weaner diet contained 3% inulin. Pigs were provided with *ad-libitum* access to feed

481 using the Feed Intake Recording Equipment (FIRE) feeding system (Schauer Agrotronic, Wels,

482 Austria). The first week on the trial diets was regarded as a training period for the piglets, so

483 feed intake for this period was not included in data analysis.

484   From weaning to ~78 days of age, pigs were housed in 12 fully slatted concrete (80 mm

485 solid width, 18 mm slots) pens (2.4 m × 2.0 m). A canopy (2.4 m × 1.2 m) with 2 heat lamps

486 was placed at the back of each pen to create a micro-climate and a suitable lying area was

22

487   created using a solid rubber mat (2.4 m × 1.2 m) under the canopy. From ~78 days of age, the

488   size of each pen was increased to 2.4 m × 4.8 m and the canopy and rubber mat were removed.

489   Body weight was recorded weekly and feed disappearance daily between ~35 and ~140

490   days of age from which ADFI, ADG and FCE were determined, and used to calculate RFI, as

491   previously outlined (11). A total of 11 pigs were removed due to health issues; CON/CON:

492   rectal prolapse (n=1), CON/INU: shoulder injury (n=1), navel rupture (n=2), FMTp/CON:

493   lameness (n=1), navel rupture (n=3), FMTp/INU: lameness (n=1), navel rupture (n=2).

494   At ~140 days of age, all pigs were slaughtered by CO_2 stunning followed by

495   exsanguination. Following evisceration, hot carcass weight was recorded, and multiplied by

496   0.98 to obtain cold carcass weight. Kill-out percentage was calculated as [(carcass weight/body

497   weight at slaughter) × 100] and back-fat and muscle depth were measured at 6 cm from the

498   edge of the split back at the third and fourth last ribs using a Hennessy Grading probe

499   (Hennessy and Chong, Auckland, New Zealand). Lean meat yield was estimated according to

500   the following formula: Lean meat yield = 60.30 – 0.847 X1 + 0.147 X2 [where X1= back-fat

501   depth (mm) and X2= muscle depth (mm)].

502   Fecal sampling was conducted by rectal stimulation at 28 (weaning), 50, 65, 100 and

503   130 days of age on the same subsample of 32 pigs (n=16 per sow treatment and n=16 per

504   offspring treatment; Fig. S4). Digesta from the ileum, cecum and colon was also collected at

505   slaughter from the same 32 selected pigs, as previously described (11). All samples were snap-

506   frozen in liquid nitrogen and stored at -80 °C for microbiota and VFA analyses. Additionally,

507   tissue from the duodenum, jejunum and ileum were collected from the same 32 selected pigs

508   for histological analysis and duodenal tissue scrapings were taken for both brush border

509   enzyme and gene expression analyses, as previously described (11).

510

23

511   **DNA extraction, 16S rRNA gene sequencing and data analysis**

512   Total DNA was extracted from fecal, ileal, cecal and colonic samples using the QIAamp

513   DNA stool minikit (Qiagen, Crawley, United Kingdom) according to the manufacturer's

514   instructions, apart from adding a beat beating step, and increasing the lysis temperature to 95°C

515   to increase DNA yield (37).

516   The V3-V4 region of the 16S rRNA gene (~ 460 bp) was sequenced (2×250 bp paired

517   end reads) on an Illumina MiSeq platform following the standard protocol with alterations, as

518   previously outlined. (4) Sequence reads were checked for quality using FastQC and trimmed

519   to 240 bp in length at the end of the sequence using Trimommatic version 0.36 (38) with

520   adapters removed (Illumina CLIP software). Forward and reverse reads were merged using

521   Flash Version 1.2.11 (39) and quality checks were performed to guarantee maximum read

522   coverage. Reads were then clustered into operational taxonomical units (OTUs) using a 97%

523   sequence identity threshold and chimeras were removed and reads were aligned to the CD-

524   HIT-OTU specific database (version 111) and then the Ribosomal Database Project classifier

525   (RDP) own database (version 11.5) was used for taxonomy assignments (40), with any samples

526   containing reads <80% labelled 'unclassified'. Samples with <1,000 total reads were excluded

527   from the analysis. The OTU data were scaled to the minimum number of total reads for each

528   sample type (feces at weaning: 67,236, day 50: 51,458, day 65: 38,887, day 100: 70,095, ileum:

529   4,242, cecum: 78,276, and colon: 41,924) and filtered to remove OTUs present at <100 reads.

530   As an alternative to rarefaction of the data, data were scaled before Alpha-diversity indices i.e.

531   Shannon and Simpson's diversity indices (measure OTU richness and evenness) and beta-

532   dispersion estimates were calculated by dividing each number of OTU counts by the sample

533   total count, by the minimum total OTU counts across samples in order to normalize to equal

534   depths and using the Adonis2 and beta permutation functions of the Vegan package in R, each

535   with 999 permutations. The Adonis2 function performs the PERMANOVA test in vegan on a

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536   Bray-Curtis dissimilarity/distance matrix, and the betadisper function assesses the

537   homogeneity of dispersion among the groups. The PCA plots were generated using the OTU

538   data and calculated on the inter-sample distance in the distance/dissimilarity matrix, with the

539   bioconductor package DESeq2 Version 1.24.0 (41) and ggplot in R Version 3.4.0 . Heatmaps

540   depicting relative abundance were generated in GraphPad Prism7.

541

542   **Prediction of microbial function**

543   The functionality of the microbiota for each sample based on 16S rRNA gene sequences

544   and the 13_5 version of the Greengenes database for taxonomy and OTU assignments was

545   predicted *in silico* using the Phylogenetic Investigation of Communities by Reconstruction of

546   Unobserved Species (PICRUSt) software (42) version 1.1.0. Prediction of functions was

547   inferred based on Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations, using level

548   3 pathways from the KEGG database. Pathways not related with bacteria, not relevant to

549   porcine studies and for which the relative abundance was <0.001% in samples were dismissed.

550

551   **Volatile fatty acid concentrations and pH**

552   Concentrations of acetic, propionic, butyric, isobutyric, valeric and isovaleric acids

553   were measured in the ileal, cecal and colonic digesta as previously described (4) Briefly, ~8 g

554   of sample was weighed and pH-recorded, diluted with Trichloroacetic acid (x 2.5 times sample

555   weight), and centrifuged (1,800 × *g* at 4 °C for 10 min). The resultant supernatants were mixed

556   with equal volumes of internal standard (1.5 mL) filtered into vials, and stored at -80 °C until

557   analysis by gas chromatography (Agilent 5890 gas chromatograph) using hydrogen (30 psi)

558   and helium (50 psi) as carrier gases, and temperatures of 80 °C (oven), 280 °C (detector), and

559   250 °C (injector).

560

25

Intestinal histology, disaccharidase activity and gene expression analysis

561

562   Intestinal tissue from the duodenum, jejunum and ileum (~3 cm sections) collected at

563   slaughter was rinsed in PBS, placed in No-Tox fixative (Scientific Device Lab, Des Plaines,

564   IL, USA) and put on a shaker for 48 h. Samples were then removed from the shaker and stored

565   at room temperature in the fixative until processing, which was performed as outlined

566   previously (11). Ten villi were examined per sample slide for villus height and width, crypt

567   depth and goblet cell number using a light microscope at 400X magnification.

568   Duodenal mucosal scrapings were collected over a length of 10 cm for the analysis of

569   disaccharidase activity and relative gene expression. Preparation of duodenal homogenates

570   (20%, w/v) and mucosal enzyme activity measurements were performed as previously

571   described by Metzler-Zebeli et al. (43). Target genes included intestinal alkaline phosphatase

572   (*IAP*), facilitated glucose transporter member 2 (*GLUT2*), *GIP*, *GLP1*, monocarboxylate

573   transporter 1 (*MCT1*) and *SMCT*, sodium/glucose cotransporter member 1 (*SGLT1*), tight

574   junction proteins [occludin (*OCLN*) and zonula occludens 1 (*ZO1*)], and toll-like receptors

575   (*TLR2* and *TLR4*). Total RNA was isolated from 20 mg duodenal mucosal scrapings using

576   mechanical homogenization and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Samples

577   were homogenized using the FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA, USA)

578   [3 x 60 s (speed 6.5 m/s), with cooling on ice for 1 min between runs]. After isolation, genomic

579   DNA was removed by treating samples with the Turbo DNA kit (Life Technologies Limited,

580   Vienna, Austria). The RNA was quantified using the Qubit HS RNA Assay kit on the Qubit

581   2.0 Fluorometer (Life Technologies Limited, Vienna, Austria) and the quality of extracted

582   RNA evaluated with the Agilent Bioanalyzer 2100 (Agilent RNA 6000 Nano Assay, Agilent

583   Technologies, Waghaeusel-Wiesental, Germany). Complementary DNA was synthesized from

584   2 µg RNA using the High Capacity cDNA RT kit (Life Technologies Limited) and 1 µL of

585 RNase inhibitor (Biozym, Hessisch Oldendorf, Germany) was added to each reaction. Primers

586 used for qPCR are listed in Table 5.

587 The primers were verified with PrimerBLAST (www.ncbi.nlm.nih.gov/tools/primer-

588 blast/) and tested for efficiencies and specificity using melting curve analysis. Amplifications

589 were performed on a real-time PCR Mx3000P (Agilent Technologies) thermocycler using the

590 following conditions: 95°C for 5 min, followed by 95°C for 10 s, 60°C for 30 s and 72°C for

591 30 s for 40 cycles, followed by the generation of melting curves. Negative controls and reverse

592 transcription controls (RT minus) were included in order to control for residual DNA

593 contamination. Each 20 µL reaction consisted of 50 ng cDNA, 10 µL Fast Plus Eva Green

594 master mix with low ROX (Biotium, Hayward, CA, USA), 100 nM each of forward and reverse

595 primers and 10 µL DEPC-treated water in a 96 well plate (VWR, Vienna, Austria). All

596 reactions were performed in duplicate as previously described by Metzler-Zebeli et al. (43).

597

598 **Hematology and blood biochemistry analyses**

599 Blood was collected during exsanguination at slaughter for hematology and

600 biochemistry analyses from the same 32 selected pigs. For hematological analysis, blood was

601 collected in vacuette tubes (Labstock, Dublin, Ireland) containing EDTA to prevent clotting,

602 and analyzed within 4 h using a Beckman Coulter Ac T Diff analyzer (Beckman Coulter Ltd.,

603 High Wycombe, UK).

604 For biochemical analysis, blood was collected in vacuette tubes (Labstock, Dublin,

605 Ireland) and allowed to clot at room temperature, followed by centrifugation at $1,500 \times g$ for

606 10 min. The serum was then collected and stored at -80 °C for subsequent analysis.

607 Concentrations of total protein, blood urea nitrogen, glucose, triglycerides, cholesterol,

608 creatinine and creatine kinase were measured using an ABS Pentra 400 clinical chemistry

609 analyzer (Horiba, ABX, North Hampton, UK). The analyzer was calibrated according to the

27

610   manufacturer's instructions and every fifth sample was analysed in duplicate to determine

611   analyzer accuracy.

612

613   **Statistical analysis**

614   Growth performance parameters recorded throughout the study were analysed for

615   repeated measures using PROC MIXED in SAS 9.3 (44), with gender, boar, and treatment

616   (sow/offspring) used as fixed effects. Pig nested within pen was used as a random effect to

617   account for variability regarding pen assignment. The RFI was calculated between day 35 and

618   ~140 days of age (at slaughter) as the residuals from a least squares regression model of ADFI

619   on ADG, metabolic live-weight, gender and all relevant two-way interactions, as well as the

620   effects of back-fat and muscle-depth which were recorded at slaughter.

621   Intestinal histology, gene expression, brush border enzymatic activity, and blood

622   parameters (hematology and serum biochemistry) were also analysed using the MIXED

623   procedure in SAS 9.3, with similar models as for growth performance used. A generalized

624   linear mixed model using PROC GLIMMIX in SAS 9.3 was used to analyze VFA

625   concentrations, which were deemed "not-normal", following log transformation.

626   Microbial composition and predicted functionality data were analysed using

627   generalized linear mixed model equation methods in PROC GLIMMIX of SAS 9.3. A gamma

628   distribution was assumed for all data. Models for offspring bacterial relative abundance for the

629   fecal time points and digesta included sow treatment, offspring treatment, fecal sampling time

630   point and their interactions as fixed effects. Additionally, a random intercept for each fecal

631   time point was included to account for the repeated measurements. Microbial composition and

632   predicted functionality for which relative abundance was present at <0.001% were dismissed.

633   The PCA plots were calculated from regularized log-transformed counts and plotted using

634   ggplot 2 and the DESEq2 package was used to calculate the differential abundance, which used

635 negative binomial generalized liner models. In all models, data were back-transformed to the

636 original distribution using the *ilink* option in PROC GLIMMIX. Multiple comparisons were

637 corrected for using the Benjamini-Hochberg method in SAS also.

638   For all analyses, statistical significance was set at P<0.05. Heatmaps used to depict

639 relative abundance differences between treatments (for microbial composition and predicted

640 functionality) were generated in GraphPad prism 7.

641

642 **ACCESSION NUMBER**

643 The raw 16S rRNA gene sequence data generated from this study are available in the European

644 Nucleotide Archive under accession number PRJEB22233.

645

646 **ACKNOWLEDGEMENTS**

656

657 **AUTHOR CONTRIBUTIONS**

29

658   P.L., G.G., B.M.Z. and P.C. conceived and designed the study. U.M.M., and P.L. conducted

659   the animal study and together with G.G. and H.R. collected intestinal samples. U.M.M., H.R.,

660   B.M.Z., and F.C. performed laboratory analysis. C.C., T.W., and T.C. performed

661   bioinformatics analyses. U.M.M. statistically analyzed all the data, and together with T.C, P.L.

662   and GG, interpreted the data and drafted the manuscript. H.R., F.C., P.C., B.M.Z, G.G. and

663   P.L. revised the manuscript. All authors read and approved the final version of this manuscript.

664   **COMPETING INTERESTS**

665   The authors declare that they have no competing interests.

666   **FUNDING INFORMATION**

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804

33

805 **Tables**

806 **Table 1. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days**

807 **post-weaning on pig growth performance and carcass traits[1]**

Parameter	Sow effect				Offspring effect			
	Control	FMTp	S.E.M	P	Control	Inulin	S.E.M	P
Weight (kg)								
Birth	1.50	1.30	0.893	0.85	1.39	1.41	0.898	0.99
Weaning	9.1	7.5	0.89	0.18	8.3	8.3	0.90	0.97
Day 100	90.5	82.7	0.89	<0.001	86.3	87.0	0.90	0.41
Day 140	104.4	95.5	0.89	<0.001	99.6	100.3	0.90	0.59
ADFI[6] (g/day)	1999	1930	29.8	0.13	1963	1965	27.4	0.96
ADG[7] (g/day)	819	814	11.1	0.63	816	817	10.2	0.93
FCE[8] (g/G)	2.38	2.34	0.055	0.63	2.35	2.37	0.051	0.77
RFI[9] (g/day) day 35 – 140	19.5	-17.4	10.96	0.05	-0.07	2.19	11.25	0.88
Carcass traits								
Weight (kg)	80.5	73.5	1.15	0.01	76.6	77.5	1.15	0.54

34

Kill out yield (%)	76.6	77.3	0.45	0.25	76.9	77.0	0.44	0.83
Fat depth (mm)	13.6	13.8	0.29	0.36	13.9	13.5	0.28	0.41
Muscle depth (mm)	52.8	53.2	0.51	0.05	53.0	53.0	0.49	0.92
Lean meat yield (%)	56.6	56.5	0.25	0.93	56.4	56.7	0.25	0.47

808 [1]Least square means and pooled standard error of the mean are presented. Parameters in bold depict a significant sow x offspring interaction

809 (details given in Table S1).

810 Sows: [2]Control (n=11) and [3]FMT procedure (FMTp; n=11); Piglets: [4]Control (n=62), [5]Inulin (n=59) for the first 6 weeks post-weaning.

811 Days in the table correspond to days of age. [6]ADFI: average daily feed intake (between weaning and ~ day 140 of age); [7]ADG: average daily gain

812 (between weaning and ~ day 140 of age); [8]FCE: feed conversion efficiency (between weaning and ~ day 140 of age); [9]RFI: residual feed intake.

813 [a,b,c] Within each row, values that do not share a common superscript are significantly different (P≤0.05).

814

815 **Table 2. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days**

816 **post-weaning on pH and volatile fatty acid concentrations of the intestinal digesta (µmol/g digesta)**

Parameter	Sow effect				Offspring effect			
	Control	FMTp	S.E.M	P	Control	Inulin	S.E.M	P
pH								
Ileum	6.5	6.5	0.10	0.75	6.5	6.5	0.10	0.76
Cecum	5.8	5.8	0.10	0.67	5.9	5.7	0.10	0.32
Colon	5.9	5.9	0.10	0.88	5.8	6.0	0.10	0.40
Total								
Ileum	26.1	28.0	2.05	0.52	27.5	26.6	2.08	0.76
Cecum	130.2	128.2	9.85	0.89	132.4	126.1	9.92	0.66
Colon	99.5	94.8	7.45	0.66	102.1	92.4	7.46	0.36
Acetic								
Ileum	12.8	13.1	1.14	0.85	12.5	13.5	1.15	0.52
Cecum	44.8	46.8	4.03	0.73	48.0	43.7	4.06	0.46
Colon	41.7	37.4	3.47	0.39	41.0	38.1	3.45	0.55
Propionic								
Ileum	3.15	3.97	0.563	0.28	4.7	2.7	0.58	0.01
Cecum	42.27	43.51	6.282	0.89	44.7	41.2	6.29	0.69
Colon	37.83	33.99	5.258	0.61	37.3	34.5	5.26	0.71

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Butyric								
Ileum	4.06	4.29	0.836	0.85	3.86	4.52	0.838	0.57
Cecum	9.64	3.24	1.327	<0.001	8.10	3.86	1.229	0.01
Colon	6.54	4.35	1.089	0.15	5.41	5.26	1.065	0.92
Valeric								
Ileum	1.78	1.61	0.168	0.48	1.74	1.65	0.170	0.69
Cecum	7.38	5.56	0.641	0.04	6.35	6.46	0.634	0.89
Colon	7.74	6.77	0.718	0.34	7.06	7.41	0.717	0.73
Isobutyric								
Ileum	2.18	2.64	0.509	0.52	2.67	2.16	0.511	0.47
Cecum	22.60	23.40	4.821	0.91	20.39	25.92	4.827	0.42
Colon	4.16	9.40	1.489	0.01	7.74	5.05	1.339	0.15
Isovaleric								
Ileum	1.32	1.67	0.176	0.19	1.47	1.51	0.175	0.86
Cecum	2.66	2.76	0.345	0.83	3.58	2.06	0.368	0.003
Colon	1.48	1.69	0.186	0.45	1.50	1.67	0.190	0.51

817 Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTp) n=16; Offspring treatment level: Control (CON) n=16;

818 Inulin (INU) n=16. Standard error of the means are depicted.

819 The intestinal segments shown in bold represent those at which the indicated VFA was also impacted due to a sow x offspring interaction (details

820 given in Table S1).

37

821 **Table 3. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days**

822 **post-weaning on intestinal histology**

Parameter	Sow effect				Offspring effect			
	Control	FMTp	S.E.M	P	Control	Inulin	S.E.M	P
Villus height								
Duodenum	469	483	10.1	0.34	472	480	10.1	0.56
Jejunum	192	191	10.3	0.93	192	190	10.3	0.91
Ileum	463	425	10.1	0.001	438	451	10.0	0.36
Villus width								
Duodenum	163	162	4.1	0.78	163	161	4.0	0.77
Jejunum	28	27	4.0	0.85	26	29	4.0	0.58
Ileum	162	162	4.2	0.42	160	160	4.1	0.98
Villus area								
Duodenum	1024	1056	22.1	0.32	1031	1049	22.1	0.56
Jejunum	1201	1191	22.5	0.76	1199	1193	22.4	0.84
Ileum	1046	965	22.0	0.01	992	1019	22.1	0.39
Crypt depth								
Duodenum	457	415	20.5	0.14	446	426	20.5	0.49
Jejunum	121	117	20.9	0.87	122	116	20.8	0.82
Ileum	329	332	20.6	0.91	353	308	20.6	0.12

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Villus height : crypt depth								
Duodenum	1.09	1.24	0.097	0.28	1.16	1.18	0.098	0.89
Jejunum	1.64	1.75	0.100	0.44	1.64	1.75	0.100	0.44
Ileum	1.49	1.32	0.098	0.23	1.28	1.52	0.099	0.09
Number of goblet cells per villi								
Duodenum	36	37	1.2	0.51	37	36	1.2	0.71
Jejunum	26	31	1.3	0.01	29	28	1.3	0.55
Ileum	33	32	1.2	0.79	31	33	1.2	0.13
Number of goblet cells per μm villus height								
Duodenum	0.08	0.08	0.004	0.93	0.08	0.07	0.004	0.60
Jejunum[1]	0.13	0.016	0.004	<0.001	0.15	0.05	0.004	0.93
Ileum	0.07	0.07	0.004	0.47	0.07	0.07	0.004	0.72

823   Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTp) n=16; Offspring treatment level: Control (CON)

824   n=16; Inulin (INU) n=16.

825   Standard error of the means are depicted.

826   [1]This was also impacted due to a sow x offspring interaction (details given in Table S1).

827 **Table 4. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days**

828 **post-weaning on hematological and blood biochemical parameters in pigs[1]**

Parameter	Sow effect				Offspring effect			
	Control	FMTp	S.E.M	P	Control	Inulin	S.E.M	P
White blood cells (×10³ cells/µL)	25.0	25.5	1.019	0.78	26.6	23.9	1.10	0.09
Lymphocytes								
%	35.7	33.9	1.93	0.52	32.5	37.1	1.93	0.11
no. ×10³ cells/µL	8.4	8.5	0.42	0.95	8.6	8.3	0.42	0.64
Monocytes								
%	3.8	2.8	0.47	0.16	3.2	3.4	0.46	0.69
no. x 10³ cells/µL	0.91	0.74	0.142	0.39	0.84	0.81	0.141	0.90
Granulocytes								
%	60.9	7.4	2.58	0.35	64.3	54.0	2.60	0.01
no. × 10³ cells/µL	15.4	13.7	1.05	0.26	17.1	11.9	1.04	0.001
Red blood cells (×10⁶ cells/µL)	7.4	7.3	0.14	0.73	7.4	7.2	0.14	0.52
Red cell distribution width (fL)	19.1	20.3	0.57	0.14	19.7	19.7	0.57	0.93
Hemoglobin (g/dL)	13.8	13.4	0.27	0.31	13.3	13.8	0.27	0.16
Hematocrit (%)	0.42	0.39	0.010	0.13	0.41	0.40	0.010	0.87
Mean corpuscular volume (fL)	56.6	54.9	0.71	0.11	55.3	56.3	0.71	0.37
Mean corpuscular hemoglobin								

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%	18.8	17.8	0.32	0.03	18.0	18.6	0.31	0.16
pg	32.9	32.1	0.35	0.09	32.3	32.8	0.35	0.34
Platelets (×10^6 cells /µL)	257	256	27.9	0.98	274	240	28.0	0.42
Mean platelet volume (fL)	9.8	9.9	0.20	0.63	9.5	10.3	0.20	0.01
Blood urea nitrogen (mg/dL)	15.5	11.9	1.92	0.20	16.3	11.1	1.92	0.06
Total protein (g/L)	66.3	54.9	6.57	0.23	58.1	62.9	6.57	0.60
Triglycerides (mmol/L)	0.46	0.47	0.039	0.91	0.44	0.49	0.040	0.32
Glucose (mmol/L)	4.9	5.0	0.52	0.91	5.1	4.9	0.51	0.79
Cholesterol (mmol/L)	2.74	2.34	0.266	0.29	2.75	2.33	0.265	0.28
Creatine (µmol/L)	147	129	11.9	0.28	142	135	11.9	0.66
Creatinine kinase (µmol/L)	75.2	34.3	11.9	0.26	63.1	46.5	11.9	0.39

829 [1]Least square means and pooled standard error of the mean are presented. Sows: [2]Control (n=11) and [3]FMT procedure (FMTp; n=11); Piglets:

830 [4]Control (n=16), [5]Inulin (n=16) for the first 6 weeks post-weaning.

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832

833 **Table 5. Forward and reverse primers used for quantitative PCR, PCR efficiency, and coefficient correlation of standard curves used in**

834 **gene expression analysis**

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr.[5]
ACTB	XM_003357928.2	Beta-actin	GGGCATCCTGACCCTCAAG	TGTAGAAGGTGTGATGCCAGATCT	89	1	97.3	0.99
B2M	NM_213978.1	Beta-2-microglobulin	CCCCCGAAGGTTCAGGTT	GCAGTTCAGGTAATTTGGCTTTC	66	1	102.2	0.99
GAPDH	NM_001206359.1	Glyceraldehyde-3-phosphate dehydrogenase	GGCGTGAACCATGAGAAGTATG	GGTGCAGGAGGCATTGCT	60	1	96.5	0.99
HPRT1	NM_001032376.2	Hypoxanthine guanine phosphoribosyl transferase	AGAAAAGTAAGCAGTCAGTTTCATATCAGT	ATCTGAACAAGAGAGAAAATACAGTCAATAG	131	1	92.1	0.99
OAZ1	NM_001122994.2	Ornithine decarboxylase antizyme 1	TCGGCTGAATGTAACAGAGGAA	GAGCCTGGATTGGACGTTTAAA	70	1	99.2	0.99
OCLN	NM_001163647.2	Occludin	TTGTGGGACAAGGAACGTATTT	TGCCTGCCGACACGTTT	76	1	95.4	0.98
ZO1	XM_013993251.1	Zona occludin 1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	131	1	109.2	0.98
SGLT1 (SLC5A1)	NM_001164021.1	Sodium-dependent glucose transporter 1	TGTCTTCCTCATGGTGCCAA	AGGAGGGTCTCAGGCCAAA	149	1	108.0	0.99
GLUT2 (SLC2A2)	NM_001097417.1	Facilitated glucose transporter member 2	TACGGCATCTGCTAGCCTCAT	CCACCAATTGCAAAGATGGAC	66	2	89.3	1.00
MCT1 (SLC16A1)	AM286425.1	Monocarboxylate transporter 1	GGTGGAGGTCCTATCAGCAG	AAGCAGCCGCCAATAATCAT	74	1	96.4	1.00
SMCT (SLC5A12)	XM_003122908.1	Sodium-coupled monocarboxylate cotransporter	AGGTCTACCGCTTTGGAGCAT	GAGCTCTGATGTGAAGATGATGACA	77	2	82.3	0.99

42

Gene symbol[1]	Accession number[2]	Gene name	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Ref[3]	Eff. (%)[4]	Corr. [5]
GIP	NM_001287408.1	Glucose-dependent insulinotropic peptide	GGATGGTGGAGCAGTTGGA	CCAATCCTGAGCTGGGTTTG	71	2	98.1	0.99
GLP1	NM_001256594.1	Glucagon-like peptide-1	GCTGATGGTGGCGATCTTGT	TCCCAGCTCTTCCGAAACTC	69	2	98.1	0.99
TRL2	NM_213761.1	Toll-like receptor 2	AATAAGTTGAAGACGCTCCCAGAT	GTTGCTCCTTAGAGAAAGTATTGATCGT	97	1	92.7	0.99
TRL4	AB188301.2	Toll-like receptor 4	TGTGGCCATCGCTGCTAAC	GGTCTGGGCAATCTCATACTCA	124	1	105.8	0.98
ALPI	XM_003133729.3	Intestinal alkaline phosphatase	AGGAACCCAGAGGGACCATTC	CACAGTGGCTGAGGGACTTAGG	83	2	97.1	0.99

835 [1]Gene symbol: alternate gene names are shown in brackets; [2]Accession number: National Center for Biotechnology Information (NCBI) Entrez

836 Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene); [3]Ref: references for oligonucleotide primer sequences- 1) Metzler-Zebeli BU, Mann

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838 balance and tight junction proteins expression in jejunum of weaned pigs. Br J Nutr. 2015; 113(7):1019-31. doi: 10.1017/S0007114515000380.;

839 2) Metzler-Zebeli BU, Ertl R, Grüll D, Molnar T, Zebeli Q. Enzymatically modified starch up-regulates expression of incretins and sodium-

840 coupled monocarboxylate transporter in jejunum of growing pigs. Animal 2016; 11(7):1180-1188. Doi: 10.1017/S175131116002615; [4]Eff: PCR

841 efficiency: $E = 10^{(-1/slope)}-1$; [5]Corr: Correlation coefficient of standard curve.

43

842   **Figure legends**

843   **Fig. 1. Variations in A. the Shannon diversity index of the offspring microbiota in feces**

844   **at 130 days of age and in B. the Simpson diversity index of ileal digesta as a result of fecal**

845   **microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring**

846   **with inulin for 42 days post-weaning**

847   Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTp) n=16;

848   Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

849   *Indicates significant differences at sow × offspring treatment level (P≤0.05); φ indicates sow

850   treatment effect (P≤0.05); λ indicates offspring treatment effect (P≤0.05).

851

852   **Fig. 2. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary**

853   **supplementation of offspring with inulin for 42 days post-weaning on median relative**

854   **abundance (%) of bacterial phyla in feces and digesta of offspring at A. sow treatment**

855   **level and B. offspring treatment level and of bacterial genera at C. sow treatment level**
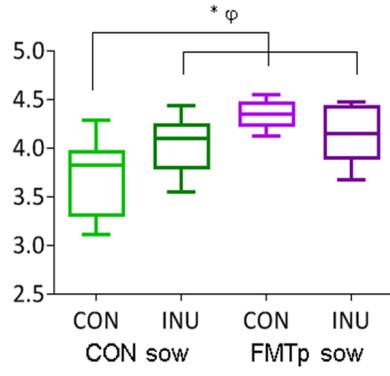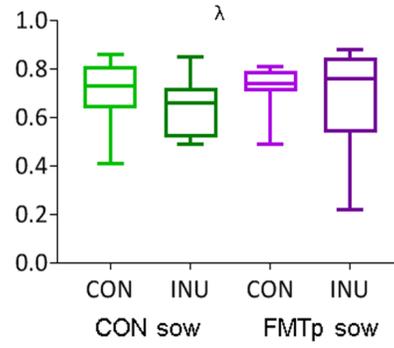
856   **and D. offspring treatment level**

857   Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTp) n=16;

858   Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

859   Heat maps are split by relative abundance with higher abundance phyla/genera shown in the

860   upper heat maps, and lower abundance phyla/genera shown in the lower heat maps.

861   Phyla and genera in bold depict those also affected by a sow x offspring treatment interaction.

862   Additional sow treatment × offspring treatment interactions not shown in either panel A, B, C

863   or D are shown in Table S1.

44

864

865 Predicted bacterial pathways in bold depict those also affected by a sow x offspring treatment

866 interaction. Additional sow treatment × offspring treatment interactions not shown in either

867 panel A or B are shown in Table S1.

868

869 **Fig. 3. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary**

870 **supplementation of offspring with inulin for 42 days post-weaning on A. brush border**

871 **enzyme activity and on B. expression of 11 selected genes in the duodenal mucosa of 140**

872 **day-old offspring**

873 Data from 32 pigs: Sow treatment level: control (CON) n=16; FMT procedure (FMTp) n=16;

874 Offspring treatment level: Control (CON) n=16; Inulin (INU) n=16.

875 *Indicates significant differences at sow × offspring treatment level (P≤0.05); λ indicates

876 offspring treatment effect (P≤0.05).

877 [1]Bars represent $\log_{10}$-fold changes relative to Control sow × Control offspring treatment after

878 normalization to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Beta-actin (ACTB)

879 and Beta-2 microglobulin (B2M) gene expression.

880 Candidate genes measured: sodium-dependent glucose transporter 1 (SGLT1),

881 monocarboxylate transporter 1 (MCT1), sodium-coupled monocarboxylate transporter

882 (SMCT), intestinal alkaline phosphatase (ALPi), tight-junction proteins [zona occludens 1

883 (ZO1) and occludin (OCLN)], toll-like receptor 2 (TLR2) and 4 (TLR4), facilitated glucose

884 transporter member 2 (GLUT2), glucose-dependent insulinotropic peptide (GIP) and glucagon-
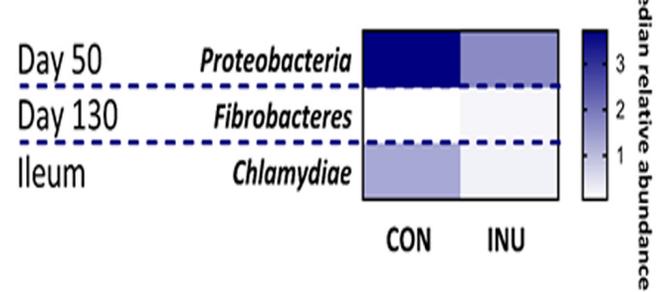
885 like peptide-1 (GLP1).

45

886   Gene expression affected by offspring treatment: *GLP1* (CON: 0.94, INU: 1.38 fold-change);

887   *GIP* (CON: 1.05, INU: 1.19 fold-change); *SMCT* (CON: 0.91, INU: 1.77 fold-change); and

888   *ZO1* (CON: 0.99, INU: 1.23 fold-change; P=0.06).

A. Shannon index: offspring feces at 130 days of age

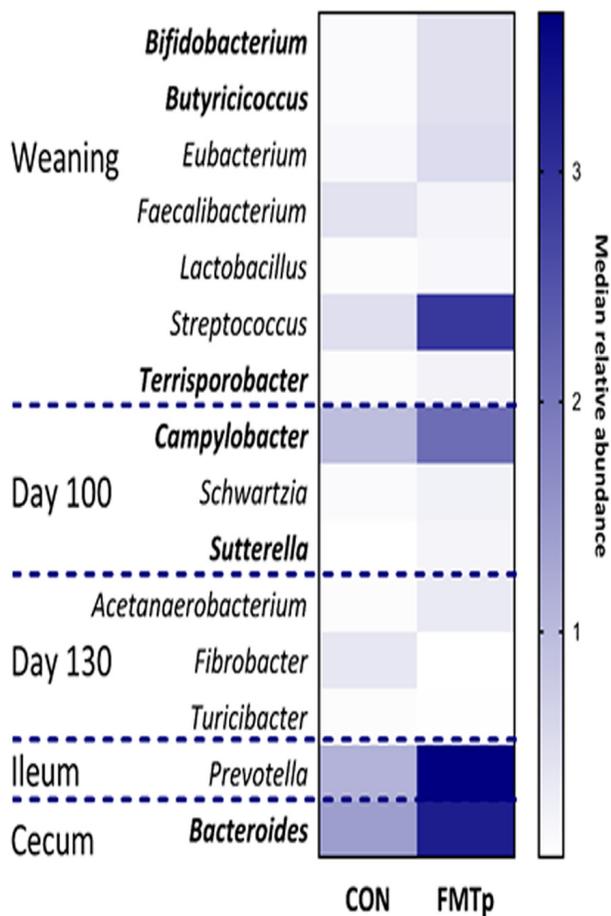**B. Simpson index: offspring ileal digesta**
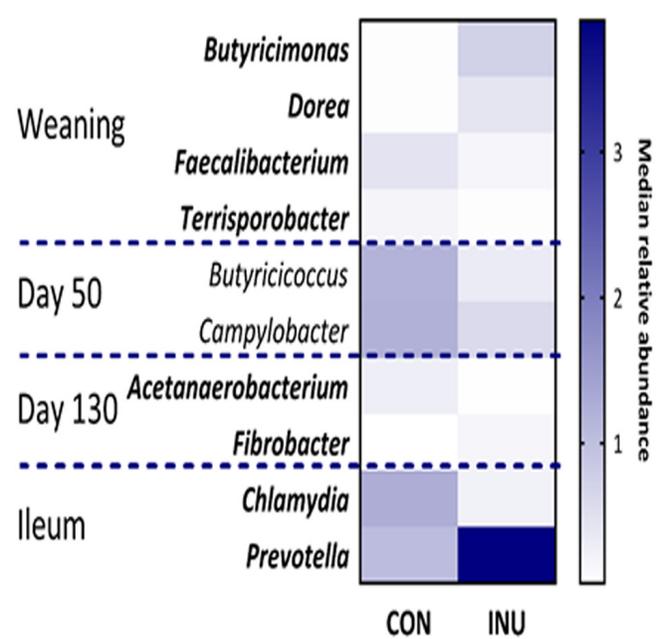
A. Phyla at sow treatment level
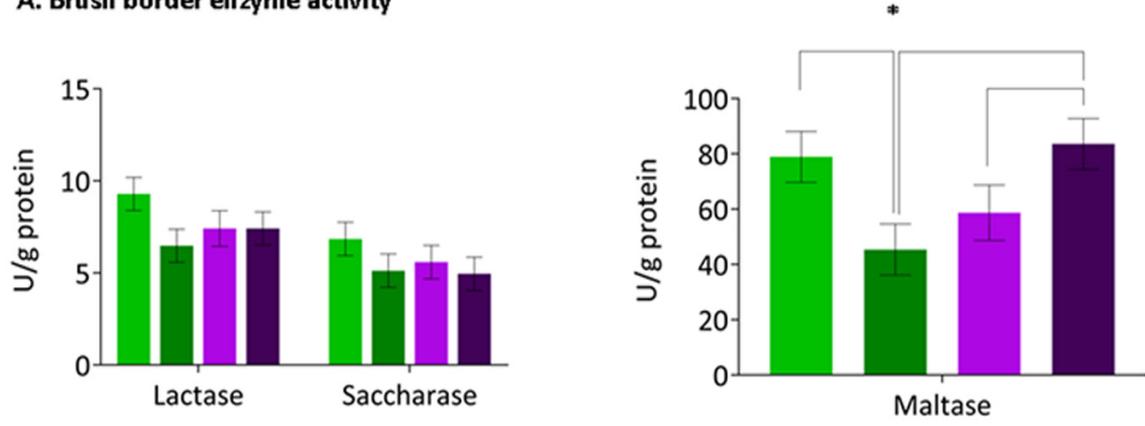

B. Phyla at offspring treatment level

C. Genera at sow treatment level

D. Genera at offspring treatment level

A. Brush border enzyme activity

B. Gene expression[1]