Improvement of Feed Efficiency in Pigs through Microbial Modulation via Fecal Microbiota Transplantation in Sows and Dietary Supplementation of Inulin in Offspring

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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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Running title: Microbial modulation to alter feed efficiency in pigs

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ABSTRACT

As previous studies have demonstrated a link between the porcine intestinal microbiome and feed efficiency (FE), microbiota manipulation may offer a means of improving FE in pigs. A fecal microbiota transplantation procedure (FMTp), using fecal extracts from highly feed efficient pigs, was performed in pregnant sows (n=11), with a control group (n=11) receiving no FMTp. At weaning, offspring were allocated, within sow treatment, to 1) control (n=67; no dietary supplement) or 2) inulin (n=65; 6-week dietary inulin supplementation) treatments. The sow FMTp, alone or in combination with offspring inulin supplementation, reduced offspring body weight by 8.1-10.6 Kg at ~140 days of age, but there was no effect on feed intake. It resulted in better FE, higher bacterial diversity and higher relative abundances of potentially beneficial bacterial taxa (*Fibrobacter, Prevotella*) in offspring. Due to FMTp and/or inulin supplementation, relative abundance of potential pathogens (*Chlamydia*, *Treponema*) in the ileum, and cecal concentrations of butyric acid were significantly lower. Maternal FMTp led to a greater number of jejunal goblet cells in offspring. Inulin supplementation alone did not affect growth or FE, but up-regulated duodenal genes linked to glucose and volatile fatty acid homeostasis and increased mean platelet volume, but reduced ileal propionic acid, granulocyte counts, and serum urea. Overall, FMTp in pregnant sows, with/without offspring dietary inulin supplementation, beneficially modulated offspring intestinal microbiota (albeit mostly low relative abundance taxa) and associated physiological parameters. Although FE was improved, the detrimental effect on growth limits the application of this FMTp/inulin strategy in commercial pig production.

IMPORTANCE

As previous research suggests a link between microbiota and FE, modulation of the intestinal microbiome may be effective in improving FE in pigs. The FMTp in gestating sows, alone/in combination with offspring post-weaning dietary inulin supplementation, achieved improvements in FE, and resulted in higher relative abundance of intestinal bacteria associated with fiber degradation, and lower relative abundance of potential pathogens. However, there was a detrimental effect on growth, although this may not be wholly attributable to microbiota transplantation, as antibiotic and other interventions were also part of the FMT regime. Therefore, further work with additional control groups is needed to disentangle the effects of each component of the FMTp in order to develop a regime with practical applications in pig production. Additional research based on findings from this study may also identify specific dietary supplements for promotion/maintenance of the microbiota transferred via maternal FMTp, thereby optimizing pig growth and FE.

INTRODUCTION

Feed efficiency (FE) is of major importance in pig production, as feed accounts for the majority cost associated with producing pigs (1). Previous work from our group and others, have demonstrated an association between the intestinal microbiota and residual feed intake (RFI; a metric for FE) in pigs (2-5). It may therefore be possible to improve FE through manipulation of the intestinal microbiota. This could be achieved via fecal microbiota transplantation (FMT) and/or dietary supplementation with feed additives.

To date, the use of FMT in pigs has mainly been limited to the establishment of the human gut microbiota in pigs in order to develop a model for humans (6, 7). However, the pig gut microbiota has also been transferred to rodents (8) and to a lesser extent other pigs (9-11). One of these latter studies is from our group and used an inoculum derived from fecal extracts collected from highly feed efficient pigs with a view to improving FE (11). The results showed that FMT in pregnant sows and/or their offspring impacted lifetime growth of offspring, as pigs were ~4-8 Kg lighter at slaughter (11). Intestinal microbiota composition and predicted functionality, along with physiological parameters, were also impacted, and overall the results indicated that FMT may not be a suitable approach to optimize FE in pigs. However, although potentially beneficial FMT-associated modulation of the sow intestinal microbiota occurred, with some evidence of microbiome transfer from the FMT-treated sows to their offspring, other bacterial taxa were either not transferred to or did not colonize within the offspring microbiome. Therefore, it is possible that dietary prebiotic supplementation of the offspring might provide a substrate for transplanted microbiota, thereby encouraging their proliferation and potentially improving FE.

A prebiotic is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (12). Inulin is a dietary fiber derived mainly from chicory which is not digestible by the host (13). It has proved effective as a prebiotic in humans, but in pigs,

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).)

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

offspring microbial diversity, with offspring from FMTp sows clustering away from offspring born to CON sows in the feces at weaning (R^2: 0.45; P<0.05) and 130 days of age (just prior to slaughter) (R^2: 0.32; P<0.05). On days 65 (R^2: 0.55) and 130 (R^2: 0.15), dietary supplementation with inulin led to a clustering effect in the feces (P<0.05). Although there were no significant differences in the ileum, CON/CON and FMTp/CON clustered separately in the cecum (R^2: 0.51; P<0.05), and in the colon, CON/INU and FMTp/CON clustered away from CON/CON offspring (R^2: 0.53; P<0.05).

Effect of FMTp in sows and/or inulin supplementation of offspring on offspring intestinal microbial composition

Microbial composition, at the phylum and the genus levels, was investigated in offspring feces throughout their lifetime and in the intestinal digesta collected at slaughter. The number of OTUs present at each sampling time point/in each digesta type were as follows; weaning: 542, day 50: 347, day 65: 75, day 100: 531, and day 130 of age: 585, ileum: 66, cecum: 361, colon: 456. Composition at the phylum level for feces and digesta samples is shown in Fig. S2. The number of phyla detected varied over time; 12 were detected in the feces 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 of age, with eight detected in the ileum, and seven in both the cecum and colon, respectively. However, many were detected at very low relative abundance.

A total of eight phyla and 25 genera differed significantly between treatments, and these ranged in relative abundance from 0.004 - 18.6% and 0.002 – 18.0%, respectively, but were mainly present at low relative abundance. Five phyla and 19 genera differed due to a sow × 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,

and four due to offspring treatment. All impacted genera were at a relative abundance of <5%, except for *Sphaerochaeta*. At 50 days of age, *Butyricicoccus* and *Campylobacter* were lower in relative abundance due to inulin supplementation. At 100 days of age, *Campylobacter* was higher in relative abundance in FMTp/CON offspring compared to offspring from CON sows, and this was reflected at sow treatment level also. *Sutterella* was also higher in relative abundance due to all interventions compared to CON/CON pigs, and was higher due to FMTp in sows also. Due to FMTp in sows, *Schwartzia* was present at a higher relative abundance in offspring. Thirty days later (at ~130 days of age, just prior to slaughter), *Acetanaerobacterium* was higher in relative abundance in FMTp/CON versus CON/INU, and pigs from FMTp sows had a higher relative abundance also, but inulin supplementation lowered the relative abundance. In addition, *Fibrobacter* was lower in relative abundance in FMTp/CON offspring compared to all other groups, and offspring from FMTp sows had a lower relative abundance also (Fig. 3C), but INU pigs had a higher relative abundance (Fig. 3D). Due to FMTp in sows, *Turicibacter* was present at a lower relative abundance in offspring compared to those from CON sows (Fig. 3C).

In the ileum, *Prevotella* was higher in relative abundance, whereas *Chlamydia* was lower, in all groups compared to CON/CON. *Prevotella* was relatively more abundant and *Chlamydia* was less so due to inulin supplementation (Table S1, Fig. 3D). Additionally, *Prevotella* was higher in relative abundance due to FMTp in sows also (Fig. 3C). In the cecum, *Bacteroides* was relatively more abundant due to FMTp/CON compared to all other groups, and offspring born to FMTp sows had a higher relative abundance also (Table S1, Fig. 3C).

Effect of FMTp in sows and/or inulin supplementation of offspring on predicted functionality of the offspring intestinal microbiota

The functionality of the intestinal microbiota was predicted in all offspring fecal and digesta samples, and significant differences are shown in Fig S3. A total of 26 predicted bacterial pathways in offspring were significantly impacted due to an interaction (Table S1). As a result of FMTp in sows, 10 pathways were altered in the offspring, and these were mostly related to lipid metabolism, carbohydrate metabolism and xenobiotics degradation and metabolism (Fig. S3). Due to dietary inulin supplementation in offspring (Fig S3), 14 predicted pathways, mostly related to carbohydrate metabolism and glycan biosynthesis and metabolism were impacted. Overall, most of the effects were seen within the ileal microbiota. All pathways that were significantly influenced by FMTp/inulin supplementation were present at <2.0% relative abundance.

At 70 days of age, alpha-linolenic acid metabolism was predicted to be present at a lower relative abundance due to inulin supplementation. In the ileum, porphyrin and chlorophyll metabolism, and seleno-compound metabolism was lower in relative abundance due to both intervention strategies, whereas the glycosphingolipid biosynthesis - ganglio series pathway was higher in predicted relative abundance. The combination of FMTp and inulin supplementation resulted in a higher predicted relative abundance of the glycosphingolipid biosynthesis - globo series pathway compared to CON/INU offspring (Table S1), and inulin-supplemented offspring had a higher relative abundance compared to their CON counterparts also. Additionally, FMTp/INU resulted in a higher predicted relative abundance of a pathway involved in biosynthesis of ansamycins compared to CON/INU offspring, and offspring from FMTp sows had a higher relative abundance also. The FMTp resulted in a higher predicted relative abundance of ether lipid metabolism, compared to offspring from CON sows. Secondary bile acid biosynthesis was higher in relative abundance due to either/both

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

control offspring, regardless of sow treatment ($P<0.05$). Due to sow FMTp, valeric acid concentrations were also lower ($P<0.05$). However, isovaleric acid concentrations were higher in FMTp/CON, but lower in FMTp/INU compared to all other groups ($P<0.05$), and lower due to inulin treatment also ($P<0.05$). In the colon, isobutyric acid concentrations were higher in FMTp/CON pigs compared to all other groups ($P<0.05$), and higher due to FMTp in sows ($P<0.05$).

Influence of FMTp in sows and/or inulin supplementation of offspring on offspring intestinal histology

Histological analyses of the offspring small intestine (duodenum, jejunum, and ileum) are shown in Table 3 and S1. In the duodenum, none of the parameters measured differed between groups. However, FMTp offspring had a higher number of goblet cells per villus compared to their control counterparts ($P<0.05$), and FMTp/CON had a higher number of jejunal goblet cells (per villus and per µm villus height) compared to CON/CON, and this was also observed due to FMTp in sows ($P<0.05$). The FMTp in sows resulted in shorter ileal villi and a smaller villus area compared to CON sows ($P<0. 05$).

Influence of FMTp in sows and/or inulin supplementation of offspring on offspring brush border enzyme activity and gene expression in the duodenum

Disaccharidase activity in the duodenum of offspring at slaughter (~140 days old) is shown in Fig. 3A. Only maltase activity was impacted by a sow × offspring treatment interaction, where CON/INU had lower activity compared to CON/CON and FMTp/INU offspring, and the latter had higher activity compared to FMTp/CON offspring ($P<0.05$). No 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).

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

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

likely through altered microbiome exposure both pre- and post-birth. Evidence of the effects of pre-birth exposure comes from the fact that the microbiome of the offspring of sows administered FMT during gestation only versus those not (FMT/CON vs CON/CON) differs. There will also likely be residual effects on the microbiome of these pigs post-birth as a result of exposure to the altered intestinal and colostrum microbiome of FMTp-treated sows (information on the sow microbiome is presented in our related publication (11). Indeed, there was some evidence of microbiome transfer from the FMT-treated sows to their offspring. Additional evidence of the influence of post-birth effects on the microbiome also come from this associated study which showed that offspring from untreated mothers administered FMT themselves have an altered microbiome (11).

At the genus level, the cellulolytic genus *Fibrobacter* was less relatively abundant in offspring due to maternal FMT but more abundant due to offspring inulin supplementation in the feces just prior to slaughter. However, the opposite was true for *Bacteroides*, a genus known to be hemicellulolytic, which was increased in relative abundance in the cecum of offspring as a result of FMT in sows. Interestingly, *Bacteroides* was previously found to be associated with better FE in finisher pigs (4), thus the higher relative abundance of *Bacteroides* in the cecum of offspring from FMTp sows may explain the improved FE observed in these animals in the present study.

The effect of the combination of maternal FMT and inulin supplementation on offspring microbiota was evident throughout the current study, not only in terms of composition, but also potential function, most notably carbohydrate and lipid metabolism. In agreement with the fact that inulin is a plant-storage glycan, the microbiota of inulin-supplemented offspring had an enhanced predicted relative abundance of glycan biosynthesis and metabolism pathways and a lower relative abundance of other carbohydrate metabolism pathways. However, a concomitant

increase in VFA concentrations was not observed, in contrast to previous findings in humans (31).

Genes involved in glucose homeostasis, in particular the secretion of insulin, such as *GIP* and *GLP1* were more relatively abundant in the duodenum of inulin-supplemented pigs. This is likely indicative of inulin fermentation in the upper gastrointestinal tract (GIT), or perhaps a compensatory mechanism for nutrient digestion in the small intestine, potentially leading to a better metabolic capability of inulin-supplemented pigs. Furthermore, a higher utilization of protein/nitrogen by the microbiota may have occurred, as indicated by lower serum urea concentrations in inulin-fed offspring (32). Inulin has also been linked with possible lipid-modulatory effects in humans and piglets (15, 33), which is in accordance with the reduced serum cholesterol concentrations found in the present study. Furthermore, the reduced cholesterol concentration observed may be due to the higher ileal concentrations of acetic acid, as dietary acetic acid has been found to reduce serum cholesterol in rats (34).

Inulin has been shown to modulate not only growth and FE, but also immunological features in pigs (15). Interestingly, white blood cell and granulocyte counts decreased due to FMTp in sows and/or inulin supplementation of offspring, and the lower counts of these immune cells may be linked to the lower relative abundance of potential pathogens (*Campylobacter*, *Chlamydia*) observed in the feces and digesta of these pigs. This in turn may be linked to the higher relative abundance of lactic acid bacteria in these animals, as these are known to reduce pathogens in the GIT (35). Moreover, offspring from FMT sows may have over-enhanced mucin production in the small intestine, as more goblet cells were present in the jejunum and mucin is a physical barrier which prevents pathogen adherence to the epithelial lining (36).

CONCLUSIONS

We provide evidence that maternal FMT alone or in combination with dietary inulin supplementation of offspring, as a strategy to modulate the intestinal microbiota of pigs has a beneficial impact on FE, but a detrimental effect on body weight, throughout the pig's productive lifetime. These effects were accompanied by influences on both intestinal microbiota composition and predicted functionality in the offspring. Although dietary supplementation with inulin alone had a similar impact on the intestinal microbiota, effects were not as pronounced and improvements in offspring growth or FE were not observed. Bacterial taxa considered potentially beneficial such as *Prevotella*, albeit mainly present at low relative abundance, were increased in the offspring, mainly due to FMT. Dietary inulin supplementation of offspring from FMTp sows led to a higher relative abundance of *Fibrobacter* than in non-supplemented counterparts, suggesting a possible role of inulin in supporting maternally-derived microbiota in the offspring. Pigs supplemented with inulin had lower levels of blood urea nitrogen, and granulocytes, indicating an improved health status. Taken together, the hematological, biochemical and gene expression data suggest improved health in offspring from FMT-treated sows, and/or those supplemented with inulin. Overall, the results from this study show that the maternal FMT regime used in the present study, either alone or in combination with post-weaning inulin supplementation, is not suitable for use in pig production, due to the detrimental impact on lifetime growth. However, possible *in utero* effects of the antibiotic and other interventions used as part of the FMT regime cannot be ruled out, and further work with additional control groups is needed to unravel the influence of the different components used. Additional research based on the findings from this study may also identify specific prebiotic or other dietary supplements for promotion/maintenance of the microbiota transferred via maternal FMTp, thereby optimizing pig growth and FE. Further studies on the exact mechanism(s) of action of the FMT are also warranted.

439

MATERIALS AND METHODS

Ethical approval

The pig study was approved by the animal ethics committees of Teagasc (TAEC9/2013) and Waterford Institute of Technology (13/CLS/02) and performed according to European Union regulations outlining minimum standards for the protection of pigs (91/630/EEC) and concerning the protection of animals kept for farming purposes (98/58/EC). An experimental license (number AE1932/P032) was obtained from the Irish Health Products Regulatory Authority.

Animal management, recording and sampling

Feces were collected from four highly feed efficient finisher pigs, anaerobically processed and the resultant fecal extracts prepared for use as FMT inoculum as previously described by McCormack et al. (11). The same 22 sows used in the McCormack et al. study were used here; on day 60 of gestation, sows were assigned to one of two treatment groups; 1) Control (n=11; CON), and 2) antibiotic treatment, purgative and FMT on days 70 and 100 of gestation (n=11; FMTp). On day 61 of gestation, FMTp sows received a 7-day course of a broad spectrum antibiotic cocktail [20 mg/Kg/day Amoxicillin Trihydrate (amoxinsol®; Vetoquinol UK Ltd., Buckingham, UK), 10 mg/Kg/day lincomycin-spectinomycin (Linco-Spectin® 100; Pfizer, Cork, Ireland) and 100,000 IU/Kg/day of colistin (Coliscour®; Ceva Sante Animale, Libourne, France)], followed by two doses of a purgative (sodium picosulfate, magnesium oxide and citric acid; Picolax powder, Ferring Ltd., Dublin, Ireland) to clear the GIT of resident microbiota, followed by a fasting period of 36 h. On days 70 and 100 of gestation, sows received the FMT (200 mL; which delivered a dose of ~2.6×10^{11} CFU) via gastric intubation along with a proton-pump inhibitor (Omeprazole; Romep, Rowex Ltd., Cork,

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

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

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

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

Intestinal histology, disaccharidase activity and gene expression analysis

Intestinal tissue from the duodenum, jejunum and ileum (~3 cm sections) collected at slaughter was rinsed in PBS, placed in No-Tox fixative (Scientific Device Lab, Des Plaines, IL, USA) and put on a shaker for 48 h. Samples were then removed from the shaker and stored at room temperature in the fixative until processing, which was performed as outlined previously (11). Ten villi were examined per sample slide for villus height and width, crypt depth and goblet cell number using a light microscope at 400X magnification.

Duodenal mucosal scrapings were collected over a length of 10 cm for the analysis of disaccharidase activity and relative gene expression. Preparation of duodenal homogenates (20%, w/v) and mucosal enzyme activity measurements were performed as previously described by Metzler-Zebeli et al. (43). Target genes included intestinal alkaline phosphatase (*IAP*), facilitated glucose transporter member 2 (*GLUT2*), *GIP*, *GLP1*, monocarboxylate transporter 1 (*MCT1*) and *SMCT*, sodium/glucose cotransporter member 1 (*SGLT1*), tight junction proteins [occludin (*OCLN*) and zonula occludens 1 (*ZO1*)], and toll-like receptors (*TLR2* and *TLR4*). Total RNA was isolated from 20 mg duodenal mucosal scrapings using mechanical homogenization and the RNeasy Mini Kit (Qiagen, Hilden, Germany). Samples were homogenized using the FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA, USA) [3 x 60 s (speed 6.5 m/s), with cooling on ice for 1 min between runs]. After isolation, genomic DNA was removed by treating samples with the Turbo DNA kit (Life Technologies Limited, Vienna, Austria). The RNA was quantified using the Qubit HS RNA Assay kit on the Qubit 2.0 Fluorometer (Life Technologies Limited, Vienna, Austria) and the quality of extracted RNA evaluated with the Agilent Bioanalyzer 2100 (Agilent RNA 6000 Nano Assay, Agilent Technologies, Waghaeusel-Wiesental, Germany). Complementary DNA was synthesized from 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 × *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

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

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656

657 **AUTHOR CONTRIBUTIONS**

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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.

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671

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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

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

[1]Least square means and pooled standard error of the mean are presented. Parameters in bold depict a significant sow x offspring interaction (details given in Table S1).

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.

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

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

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

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).

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

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 ($\times 10^3$ 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. $\times 10^3$ 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 10^3 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. $\times$ 10^3 cells/µL	15.4	13.7	1.05	0.26	17.1	11.9	1.04	0.001
Red blood cells ($\times 10^6$ 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								

%	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 ($\times 10^6$ 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.

831

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	TTGTGGGACAAGGAACGTATTTA	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

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

[1]Gene symbol: alternate gene names are shown in brackets; [2]Accession number: National Center for Biotechnology Information (NCBI) Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene); [3]Ref: references for oligonucleotide primer sequences- 1) Metzler-Zebeli BU, Mann E, Ertl R, Schmitz-Esser S, Wagner M, Klein D, Ritzmann M, Zebeli Q. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. Br J Nutr. 2015; 113(7):1019-31. doi: 10.1017/S0007114515000380.; 2) Metzler-Zebeli BU, Ertl R, Grüll D, Molnar T, Zebeli Q. Enzymatically modified starch up-regulates expression of incretins and sodium-coupled monocarboxylate transporter in jejunum of growing pigs. Animal 2016; 11(7):1180-1188. Doi: 10.1017/S175131116002615; [4]Eff: PCR efficiency: $E = 10^{(-1/slope)}-1$; [5]Corr: Correlation coefficient of standard curve.

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.

Predicted bacterial pathways in bold depict those also affected by a sow x offspring treatment interaction. Additional sow treatment × offspring treatment interactions not shown in either panel A or B are shown in Table S1.

Fig. 3. Effect of fecal microbiota transplantation (FMT) in sows and/or dietary supplementation of offspring with inulin for 42 days post-weaning on A. brush border enzyme activity and on B. expression of 11 selected genes in the duodenal mucosa of 140 day-old offspring

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

*Indicates significant differences at sow × offspring treatment level ($P \leq 0.05$); λ indicates offspring treatment effect ($P \leq 0.05$).

[1]Bars represent $\log_{10}$-fold changes relative to Control sow × Control offspring treatment after normalization to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), Beta-actin (ACTB) and Beta-2 microglobulin (B2M) gene expression.

Candidate genes measured: sodium-dependent glucose transporter 1 (SGLT1), monocarboxylate transporter 1 (MCT1), sodium-coupled monocarboxylate transporter (SMCT), intestinal alkaline phosphatase (ALPi), tight-junction proteins [zona occludens 1 (ZO1) and occludin (OCLN)], toll-like receptor 2 (TLR2) and 4 (TLR4), facilitated glucose transporter member 2 (GLUT2), glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP1).

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).

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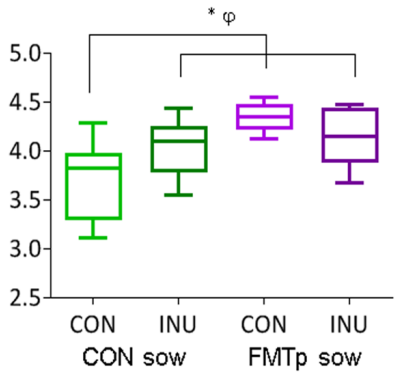
A. Shannon index: offspring feces at 130 days of age



B. Simpson index: offspring ileal digesta