Animal Nutrition 12 (2023) 284–296

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Supplementing oat hulls to the diet of suckling piglets altered their intestinal tract and colonic microbiota development

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ARTICLE INFO

Article history:
Received 24 January 2022
Received in revised form
2 October 2022
Accepted 14 October 2022
Available online 20 October 2022

Keywords:
Oat hull
Insoluble fibre
Gastrointestinal development
Suckling piglet

ABSTRACT

Current study evaluated the effect of a fine and coarsely ground insoluble dietary fibre source on the gastrointestinal development of suckling pigs. Oat hulls (OH) were selected as a model feedstuff, rich in cellulose, lignin, and insoluble dietary fibre. Three experimental supplemental diets were formulated: a finely ground, low fibre and nutrient dense diet served as control (CON). For the 2 high fibre diets, 15% heat-treated starch in CON was exchanged with OH, either finely (OH-f) or coarsely ground (OH-c). Litters of 10 primi- and multiparous sows (mean litter size 14.6 ± 0.84) were used. Within a litter, experimental diets were allotted to triplets of 4 piglets. From approximately 12 d of age, piglets' individual feed intakes were recorded 2 times per day when separated from their dam for 70 min. Piglets could suckle with their dam for the remainder of the day. On d 24 and 25, from the total pool of 120 piglets, seven healthy well-eating piglets per treatment were selected for post-mortem evaluation, resulting in 14 replicates per treatment. Consumption of OH-c and OH-f did not impede clinical health and production performance of piglets. The full stomach weights tended to be greater for OH-c compared to OH-f whereas CON was intermediate ($P = 0.083$). Supplementing OH significantly increased ileal villus height and caecal dry matter concentration ($P < 0.05$). For the colon, OH increased its length, contents weight, short-chain fatty acid concentration and reduced total bacterial count as well as γ-proteobacteria count and proportion ($P < 0.05$). The OH-c treatment specifically increased full gastrointestinal tract weight and caecum contents weight compared to piglets fed CON and OH-f. Furthermore, OH-c reduced colonic crypt depth when compared to OH-f ($P = 0.018$). In conclusion, supplementing OH to a diet for suckling piglets exerted subtle developmental effects on gastrointestinal morphology and colonic microbial community. These effects were largely independent from the particle size of the OH.

1. Introduction

During the initial weeks after birth, when piglets are sucking the sow, their gastrointestinal tract (GIT) is morphologically and functionally immature (Cranwell and Moughan, 1989). During this period, its development follows an intrinsic trajectory, influenced by nutrition. First through sow milk, followed by increasing quantities of supplemented solid feed (Choudhury et al., 2021; Everaert et al., 2017). After weaning at 3 to 4 wk, piglets may benefit from a properly developed GIT able to digest and absorb nutrients

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.10.002

from non-milk feed material present in weaner diets (Pluske et al., 2003).

Diets for commercially kept piglets before weaning are usually formulated under the assumption that they should be highly digestible and palatable. Therefore, the inclusion of fibrous feedstuffs is commonly avoided. Moreover, these diets are milled down to a very fine particle size. In contrast, feral pigs are observed to manipulate and possibly consume non-milk feedstuffs, including particulate fibrous vegetable material already at a young age (Cox and Cooper, 2016; Jensen et al., 1991; Petersen, 1994). In weaned piglets, dietary fibre sources, especially the ones with a low viscosity and a high proportion of insoluble slowly fermentable fibre, support the development of morphological and functional aspects of the GIT. More specifically, insoluble dietary fibre increased stomach and colon development, improved the function of the small intestinal epithelium (greater villus-to-crypt ratio, digestive enzyme activity, barrier function), stimulated colonic fermentative activity (short-chain fatty acid [SCFA] production) while reducing protein fermentation, and reduced the abundance of pathogenic bacteria (Flis et al., 2017; Molist et al., 2014). Recently we observed that supplemental feeds with micronized insoluble cellulose fibre stimulated GIT mass, reduced the concentration of putative pathogenic bacteria, and modified hind gut microbial activity in suckling pigs (Van Hees et al., 2019). Oat hulls (OH), a commercially available ingredient rich in insoluble dietary fibre, have been reported to support gastrointestinal health in weaned pigs. For instance, it stimulated ileal and caecal SCFA production and modified the large intestinal microbiome resulting in lower ammonia-N and urea production (Kim et al., 2008; Ndou et al., 2018).

Besides the physicochemical characteristics of dietary fibre, its particle size may also be relevant for the effect on the immature GIT. It appears that insoluble dietary fibre loses part of its health promoting properties when its particle size is reduced compared to coarser particles (Maxwell et al., 1967; Millet et al., 2012; Molist et al., 2012). Additionally, fine milling of the diet is unfavourable for gastric and colonic epithelial structure and health (Betscher, 2010; Brunsgaard, 1998; Grosse Liesner et al., 2009; Liermann et al., 2017; Papenbrock et al., 2005). Moreover, the research of Cappai et al. (2015) showed that diet particle size affects the morphology of the ileocecal sphincter possibly affecting its function, which pertains to regulating the outflow of small intestinal contents into the caecum and avoiding back-flow and as such, retrograde contamination of pathogens ascending into the ileum. Similarly, in broilers the development of the pro-ventriculus and gizzard was impaired when fed over-processed diets lacking physical structure (Liermann et al., 2018). Collectively, these findings further support the concept that insoluble dietary fibre may be beneficial for GIT development and health in the pre-weaning piglet (Correa-Matos et al., 2003; Mu et al., 2017; Zhang et al., 2016). However, little experimental data is available on the relevance of particle size of dietary fibre sources on GIT health and development in suckling pigs.

Based on the above, we hypothesized that introducing particulate dietary fibre in the immature GIT of suckling piglets will stimulate its morphological and functional development. Oat hulls were selected as a model dietary fibre source, and we chose a broad array of response variables to assess its effect on GIT development. The specific objectives of the current study were to investigate the effects of fine and coarse OH on GIT macro morphology and bacterial metabolites, gastric functionality, small intestinal and colonic structure, and colonic bacterial groups, potentially beneficial and pathogenic to the host.

2. Materials and methods

2.1. Animal ethics

The housing and care of the experimental animals was in accordance with the European Union Directive 2010/63/EU. The Dutch central animal welfare committee approved specific experimental procedures under application number AVD2040020184665.

2.2. General sow and litter housing and management

Ten sows (average parity 3.1; ranging from 1 to 6) were housed in a climate-controlled farrowing room of the Swine Research Centre (Trouw Nutrition R&D, Sint Anthonis, The Netherlands) from d 109 of gestation onwards. Lights were switched on from 06:00 until 22:00. Sows were prolific Hypor Libra breed, artificially inseminated with Hypor Maxter boar semen (Hendrix Genetics, Boxmeer, The Netherlands) and gave birth to 16.4 ± 1.35 life-born piglets (minimum 15 piglets), within a 3-day period. We standardized all litters to 15 piglets within 4 d after farrowing by removing low-viable piglets in a similar manner across groups. In the farrowing crates, jute cloths were provided around expected farrowing as enrichment material. A calcium-carbonate based powder (Power-Cal, Power-Cal, Sint-Oedenrode, The Netherlands) was used during the first day after farrowing to dry-up the pen flooring. Sows had ad libitum access to water with a nipple-waterer while being fed daily rations through an elevated feed trough equipped with a drinker according to a step-up feeding scheme. The position of the sow feeder hindered access of piglets to the sow feed. Pens were equipped with a second nipple waterer suited for suckling piglets. The sows' pelleted diet was formulated to contain 8.98 MJ net energy (NE), 149 g crude protein, 7.7 g standard ileal digestibility (SID) Lys per kilogram as fed. Piglets were ear-tagged for identification and the following standard procedures were practised: tail docking and iron injection within 3 d after birth, vaccination against *Escherichia coli* serotype F18 on d 3 (Ecoporc Shiga, IDT Biologika GmbH, Dessau-Rosslau, Germany), 1 wk prior to weaning a triple-vaccination against *Mycoplasma hyopneumonia,* porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome (PRRS) (Ingelvac Mycoflex, Circoflex and PRRSflex, respectively, Boehringer Ingelheim Gmbh, Ingelheim, Germany). No teeth clipping was applied. Sows were vaccinated against PRRS, parvovirus combined with *Erysipelothrix rhusiopathiae* (Porcilis ERY + Parvo, MSD, Boxmeer, The Netherlands), the *E. coli* serotypes F4ab (K88ab), F4ac (K88ac), F5 (K99) of F6 (987 P; Porcoli Diluvac Forte, MSD, Boxmeer, The Netherlands) and dewormed (Zerofen 4%, Lifarma, Baexem, The Netherlands). Weaning took place when piglets were on average 26 d old. This day was set as reference day for other procedures.

2.3. Experimental feeding protocol

To allow for individual supplemental feed intake measurements and to avoid confounding litter-sow and treatment effects, a split-litter protocol was applied based on the previous description (Aherne et al., 1982) with the modification that piglets were separated from the sow 2 times per day for 70 min from 2 wk prior to weaning onwards. For this purpose, 4 identical pens were constructed adjoining the front of the farrowing crate. These separation pens' dimensions were 50 cm × 70 cm × 60 cm (length × width × height) and had solid rubber flooring with walls being partly wired and partly solid (the backside and both outer sides). Each pen contained a round feeder trough (diameter 20 cm).

This setup allowed continuous visual and olfactory contact between the sow and her piglets and in addition, visual and tactile contact with the neighbouring piglet (photos are provided in Appendix Fig. 1). Drinking water was only available through the gruel feed, i.e., no additional water was available in these pens. Before start of the experimental period all animals were accustomed to the experimental procedure. First, during d 3 to 7 after farrowing, small quantities (50 to 100 g) of the control diet mixed with water (water to feed 2:1) were presented to the whole litter. During the following 3 days, we separated pairs of piglets twice per day from the dam for an increasing period of time, i.e., 15, 30 and 70 min, respectively. Moreover, one day before start of the experimental period (d 11), the 12 selected piglets (see below) were housed individually instead of in pairs for 2 × 70 min. We maintained this daily separation protocol during the whole experiment, i.e., the period covering 2 wk from d 12 until the day of euthanasia (d 24 or 25).

On d 12 the median 12 pigs within a litter were assigned to 1 of 3 experimental groups. This resulted in 3 sub-groups of 4 piglets of similar average body weight and sex distribution. Approximately 1 h prior to the start of each feeding session, the crumbled pelleted experimental diets were mixed with lukewarm water (water-to-feed ratio = 2:1) and left to soak before feeding. Feeders were filled before piglets were put in the separation pens and feed disappearance was recorded as gruel (water and feed mixture) after removal of the animal. The 3 morning-feeding sessions commenced between 07:30 and 11:20, while the afternoon sessions started at 14:00 and ended at 17:50. The order of feeding the 3 sub-groups within a litter were changed daily to randomize the possible effect of feeding time within a day. During regular checks, fresh feed was added when needed, to assure that feed was always available ad libitum. During the feeding sessions, the remaining non-experimental piglets were left with the sow and allowed to suckle.

2.4. Dietary treatments

The 3 within-litter subgroups consisting of 4 piglets were fed 1 of 3 experimental diets during separation. The control diet was formulated as a palatable high nutrient-dense, low-fibre diet (Tables 1 and 2). Its total dietary fibre content was 120 g/kg of which 102 g/kg was insoluble fibre, originating from fibres in wheat, barley, extruded dehulled oats, full-fat toasted soybeans and soybean meal. Sucrose and palatability enhancers were added, next to organic acids as preservatives. The highly digestible protein and fat originated from dairy products, soy concentrate, extruded soybean meal, wheat gluten, potato protein, soyabean oil and salmon oil. The ingredients for the basal diet were pre-mixed and subsequently finely ground. For the 2 experimental diets, 150 g/kg extruded corn starch meal added to the control diet (CON) was substituted on a weight basis by either finely or coarsely ground OH (OH-f and OH-c, respectively). Oat hulls are the outer hulls of the oat grain that are encompassing the groats and were chosen as a source of insoluble dietary fibre (Table 3). They consist mainly of cellulose and lignin (Bach Knudsen, 1997). The inclusion rate of the OH was arbitrarily set as reference studies were lacking while we anticipated on the relatively low supplemental feed intakes of suckling piglets reported in literature and observed in the research facility. The unprocessed hulls were obtained from a commercial feed mill (ForFarmers, Heijen, The Netherlands). To arrive at a fine grind, the OH were subsequently milled over 3- and 1.5-mm sieves on a beater cross mill (Peppink Mills BV, Olst, The Netherlands), followed by sieving over a 500-μm screen to remove the remaining large particles (HeMe, Waddinxveen, The Netherlands). The coarse

Table 1
Composition of the experimental supplemental diets fed from 2 wk prior to weaning up to the day of post-mortem examination (d 24 or 25)[1].

Item	CON	OH-f	OH-c
Composition, g/kg as-fed basis			
Basal diet [2]	850	850	850
Corn starch, heat-treated	150	0	0
Oat hulls, finely ground	0	150	0
Oat hulls, coarsely ground	0	0	150
Total	1,000	1,000	1,000
Calculated nutrients, g/kg as-fed basis (unless stated otherwise)			
Moisture	99.8	95.6	95.6
Crude protein	169.8	164.2	164.2
Crude fat	51.8	48.0	48.0
Ash	49.5	54.0	54.0
Starch (Ewers method)	397.1	331.5	331.5
Lactose	50.0	50.0	50.0
Calcium	6.01	6.25	6.25
Phosphorus	5.82	5.64	5.64
Digestible phosphorus	4.16	4.13	4.13
6-Phytase, FTU/kg	366	366	366
Sodium	3.00	3.02	3.02
Potassium	6.04	6.12	6.12
Chloride	5.62	5.67	5.67
ME, MJ	13.38	12.12	12.12
NE, MJ	10.38	9.39	9.39
SID Lys	12.49	12.33	12.33
SID Met + Cys	7.60	7.27	7.27
SID Thr	8.31	8.04	8.04
SID Val	8.85	8.47	8.47
SID Trp	2.48	2.44	2.44

ME = metabolic energy; NE = net energy; SID = standard ileal digestibility.
[1] CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively. Diets were fed as a gruel (water-to-feed ratio = 2:1).
[2] Finely ground basal diet that consisted of wheat, barley (58.0%), extruded cereals (7.1%); soybean products (12.0%), including extruded soybean meal (Forcital, Trouw Nutrition, Gent, Belgium); dairy whey products (6.0%); fats and oils (3.6%); vitamin and minerals (3.2%); sucrose and palatability enhancers (2.5%); wheat protein (2.4%); potato protein (2.4%); synthetic amino acids (2.1%) and organic acids as feed preservatives (0.8%). Vitamins and minerals provided per kilogram total feed: 9,169 IU vitamin A, 2,002 IU vitamin D_3, 150 IU vitamin E-acetate, 1.5 mg menadione, 1.6 mg thiamine mononitrate, 4.3 mg riboflavin, 1.9 mg pyridoxine, 30.0 μg cyanocobalamine, 22.4 mg niacin, 12.0 mg calcium D-pantothenate, 683 μg folic acid, 43 μg biotin, 113.7 mg choline chloride, 50.1 mg betaine, 216.8 mg iron, 1.1 mg iodine, 144.2 mg copper, 52.0 mg manganese, 125.6 mg zinc, 0.35 mg selenite.

Table 2
Chemical composition and wet sieving analysis of the experimental diets prior to soaking[1].

Item	CON	OH-f	OH-c
Analyzed nutrients, g/kg as-fed basis (unless stated otherwise)			
Moisture	104	98	96
Crude protein (Dumas)	168	166	168
Crude fat (Soxhlet)	45	47	47
Ash	45	50	52
Total dietary fibre[2]	120	199	218
Soluble dietary fibre[2]	18	21	23
Insoluble dietary fibre[2]	102	178	195
Wet sieving analysis[3], % fraction			
3,500 μm	0.3	0.4	1.5
2,000 to 3,500 μm	0.6	0.6	1.9
1,600 to 2,000 μm	1.1	0.8	1.5
1,000 to 1,600 μm	2.9	2.7	4.0
400 to 1,000 μm	14.3	13.1	14.3
200 to 400 μm	10.4	11.2	7.3
<200 μm	70.4	71.2	69.5

[1] CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively.
[2] Association of official analytical chemists (AOAC) 991.43.
[3] Masterlab, Boxmeer, The Netherlands.

Table 3
Analysed nutrient composition (g/kg as-fed basis), water binding and purity of oat hulls.

Item	Content
Moisture	80
Crude protein (Dumas)	<40[1]
Crude ash	49
Crude fat (Soxlet)	18
Soluble dietary fibre	16
Insoluble dietary fibre	731
Acid detergent lignin	65
Microscopic purity, %	100
Water binding capacity, g water/g oat hulls	2.7

[1] Below detection limit.

OH were fed over a 3-mm sieve mounted on a hammer mill only. All diets were pelleted at low temperature (max 62 °C), using a 5 mm × 35 mm dye (diameter × depth) and subsequently fed through a crumble device (35 mm gap setting). Diets were otherwise formulated in line with commercial practice for this category of piglets and did not contain antibiotics nor therapeutic levels of zinc. Dietary insoluble and soluble dietary fibre were analysed according to Method 991.43 association of official analytical chemists (AOAC) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA).

2.5. Performance measurements

Individual body weight of piglets was measured at birth, after 24 h, d 12 (start of the experimental period) and on d 24 or 25 at the moment of dissection. Piglet's weight increment during the first 24 h was used to estimate the colostrum intake based on the previous description (Theil et al., 2007).

2.6. Post-mortem examinations

One or 2 d prior to weaning, from the total group of 120 piglets, a subset of 14 piglets per treatment was subjected to post-mortem examination. To this aim, we ranked animals based on their recorded supplemental feed intake, within treatment. Sixty-one percent of the animals had negligible intakes (<120 g DM intake until d 23) and we decided to discard them, taking into account the objectives of this study. From the remainder, the best eating piglets were selected and euthanized by an intra-cardiac injection with 40% barbiturate pentobarbital and immediately exsanguinated. The internal organs from the abdomen were removed to determine empty body weight and to calculate carcass yield (empty body weight/life body weight × 100%). The different sections of the GIT were identified, and fissures were used to retain the intestinal contents. For the stomach, caecum and the full colon weight was determined after removal of mesentery ligaments and empty weight after removal of its contents. The length of the colon was measured while avoiding it to stretch. Furthermore, from the ileum, caecum and mid-colon, tissue samples were collected in a 4% formaldehyde solution for subsequent microscopic examination. Representative samples of the stomach and intestinal contents were collected in micro tubes containing 50% sulphuric acid for subsequent lactate, volatile fatty acids, and ammonia-N analysis. A second sub-sample was immediately put on dry ice for subsequent pH and dry matter content determination. The fluidity of its contents and the pars nonglandularis region of the stomach was clinically examined. Regarding the latter, no mucosal alterations were observed for any of the piglets. Fluidity was scored either as L (all liquid), IL (mainly liquid with some dry chunks), I (mainly dry chunks together with liquid) or S (solid).

The digesta samples were centrifuged. The supernatant was analysed for lactic acid and volatile fatty acids concentration (collectively: SCFA) by high-performance liquid chromatography (HPLC) on a BioRad Aminex HPX-87H using a 0.005-mol/L sulfuric acid eluent at a flow rate of 0.7 mL/min. Ammonia-N (nitrogen as NH_4^+ and NH_3) was analysed colorimetrically using the Berthelot reaction as described earlier (Van Hees et al., 2019).

After embedding in paraffin, 5 μm ileal wall sections were stained with hematoxylin-eosin (H&E) for light-microscopic examination (Nikon Eclipse Ni with DS-Fi3 camera; NIS-elements software, version 5.3). In 10 well-oriented villi, not surfacing a Peyer's patches region, we determined the distance between the basal lateral membrane and the villus tip as well as villus width and crypt depth. Paraffin-embedded intestinal sections of the caecum and colon were double-stained to allow identification of the different mucin subtypes present as mucin droplets within a goblet cell. Firstly, sections were stained by periodic acid-Schiff (PAS), which stains the neutral mucins magenta. Secondly, we used Alcian Blue at pH 2.5, to stain the (acid) sialomucins turquoise. With this staining the mixed mucins would colour purple. Images of representative cross-sections of the colon epithelium were examined manually (Nikon Eclipse Ni with digital camera DS-Fi3 and NIS-elements D version 5.3 software) while for the caecum computerized imaging software (Olympus cellSens Dimension version 1.12) connected to a digital camera (Olympus Dp26) mounted on a microscope (Olympus BX41) was used. The development of the gastroduodenal sphincter (*torus pyloricus*) was examined in H&E-stained cross-sections. Similarly, for the ileocecal sphincter we measured the width of the longitudinal and circular smooth muscles in a cross-section of the protrusion (Cappai et al., 2015). Moreover, crypt depth was determined for the mucosa covering this organ.

Lactase activity was determined for a 10-cm section taken 25 cm distal to the stomach, further referred to as the proximal jejunum. After cutting open the anti-mesenteric side and rinsing with phosphate buffered saline (PBS), approximately 0.15 g of mucosal scrapings were collected in a cryotube filled with PBS and immediately stored on dry ice. At the lab, lactase activity was determined colorimetrically in triplicate using commercial kits and following the manufacturers' instructions (Lactase Activity Assay Kit, Elabscience, Houston, Texas, USA; Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, Rockford, Illinois, USA). One unit of enzyme activity was defined as the amount of lactase that hydrolysed 1 nmol of lactose per minute at 37 °C and pH 6.0 (U/mg mucosal protein).

Small intestinal permeability was assessed at weaning using the everted gut sack method (EGS; De Greeff et al., 2016). Briefly, a 25-cm long section, cut from 35 cm proximal to the ileocecal junction was cleaned with PBS, everted and filled with glucose-Ringers solution and submerged in Ringers solution kept aeriated and at 39 °C. The submersion fluid contained cobalt ethylene-diamine-tetra-acetate (Co-EDTA; 18 g/L) as permeability marker. This marker is assumed to permeate mainly para-cellular based on its molecular weight (347 g/mol). The cobalt concentration was analysed in a 10-mL sample extracted from the gut segment after 1 h of incubation, by inductively coupled plasma-mass spectrometry (ICP-MS; NexION 350D, PerkinElmer Inc., Waltham, MA, USA).

Following a wet sieving procedure, we determined the particle size distribution of the diets and the contents of the proximal half of the colon. Briefly, material was flushed with water over a series of sieves with decreasing mesh size, collected and dried for 48 h at 65 °C. The size of the largest particle was determined with a calliper, based on Fritz et al. (2012).

To evaluate the impact of diet on the bacterial community of the mid-colon quantitative PCR (qPCR) targeted to specific 16 S rRNA gene sequences was applied. To this aim, 100 mg of digesta was lysed while shaken for 1 min at 7,000 rpm together with 0.1-mm

glass beads in a bead beater (MagNA Lyser, Roche, Burges Hill, UK). Subsequently, DNA extraction was performed with the MagAttract PowerMicrobiome kit (DNA/RNA EP, Qiagen, Hilden, Germany) following the manufacturer's instructions. For total counts and the γ-proteobacteria encompassing the Enterobacteriaceae, among which several pathogenic genera (e.g., *E. coli*, *Chigella* and *Salmonella*) we used the primers proposed by (Bacchetti De Gregoris et al., 2011). For the Lactobacillaceae and 2 groups within the *Clostridiales* order, i.e., *Ruminococcaceae* (cluster IV) and Lachnospiraceae (cluster XIV) we used the primers described elsewhere (Matsuki et al., 2002, 2004; Ten Bruggencate et al., 2005).

2.7. Statistical analysis

The experiment had a split-litter design in which groups of 4 piglets within a litter were assigned to 1 of 3 experimental treatments. The low supplemental feed consumption limited the number of piglets available for selection. As a result, we were not completely successful in balancing for the effect of sow across treatments. Piglets for group CON, OH-f and OH-c originated from 7, 10 and 9 litters, respectively. The raw data were screened, and outliers were removed when either higher or lower than 2 times standard deviation of that specific treatment group. We used a general linear model (PROC GLM procedure of SAS Studio (SAS Institute Inc., Cary, NC, USA, release 3.71) to evaluate the effect of dietary treatment on performance and GIT characteristics with the individual piglet as the experimental unit ($n = 14$ per treatment). The piglet' sex was included as class variable in the model for the performance data, but it did not contribute to the goodness of fit of the models used for other response variables. It was, therefore, omitted from the statistical model. A covariate was retained when its *P*-value was less than 0.200 and not affected by treatment. As a result, body weight at dissection and supplemental feed intake were included for several parameters as indicated in the respective tables. The time interval between the last feeding session and sampling, colostrum intake, birth weight, age and day of dissection were not included as covariate. A log10 data transformation was applied in case within group data were not normally distributed. This resulted in the following statistical model:

$$Y_{ij} = \mu + \text{Diet}_i + \text{COV} + e_{ij}$$

where Y_{ij} = response variable; μ = overall mean; COV = covariable(s); Diet = experimental diet ($i = 1$ to 3) using 14 experimental units (j); and e_{ij} = residual error.

The proportions of goblet cells sub-categories, particle size distribution of the diets and colonic bacterial groups were analysed using generalised linear mixed models (PROC GLIMMIX in SAS) with a beta distribution and logit link function. For bacterial groups the day of sampling was included as a random variable. The ordinal data obtained for stomach contents fluidity scores were evaluated with a generalized linear model for categorical data by comparing the scores using a multinomial distribution (PROC GENMOD in SAS Studio). For all analysis we applied the Tukey–Kramer correction for *post hoc* multiple comparison. Moreover, the inclusion of OH (CON versus OH-f and OH-c) was also evaluated. Differences were considered significant if $P < 0.05$ while a tendency was considered when $0.05 < P \leq 0.10$.

3. Results

3.1. General results

The wet sieving analysis of the diet largely reflected the anticipated contrasts in dietary fibre concentration and particle size distribution (Table 2). Still, it is important to note that needle shaped OH particles were able to escape the larger sieve meshes (Appendix Fig. 2). As a result, the OH-c diet contained particles that were probably coarser than would be expected based on Table 2.

Four sows were treated with meloxicam (non-steroid anti-inflammatory drug; Novem, Boehringer Ingelheim, Ingelheim, Germany) and 2 of them required additional antibiotic treatment for health disorders in the peri-parturient period (Depocillin, MSD, Boxmeer, The Netherlands). All sows recovered and remained clinically healthy during the experimental period. The sows ate their designated amounts resulting in a lactation feed intake of 7.49 ± 1.73 kg per day on average. The litter size on d 26 was on average 14.6 ± 0.84, including the piglets sacrificed for the study. In general, during the study period, piglets remained healthy, and mortality was low (2.7%). Five piglets needed medical treatment because of locomotory issues and were excluded from the study. Still, one piglet that received antibiotics combined with an anti-inflammatory agent was accidently selected. We decided to exclude its microbiota data from further analysis. For the other GIT variables, it was maintained, as its data did not deviate from treatment average.

The interval between the last feeding session and the moment of sampling was on average 4 h and 12 min and did not differ between treatments ($P = 0.289$). The supplemental feed intake was characterized by a large variation and a skewed distribution, including one piglet eating notably higher amounts represented in each group. The experimental diets did not affect performance variables ($P > 0.05$; Table 4).

3.2. Carcass yield and gastrointestinal morphology

The calculated carcass yield was on average 82.3% and was similar between diets (Table 5). None of the *pars nonglandularis* of

Table 4
Production performance data of the piglets selected for post-mortem evaluation (LSMeans and pooled standard error)[1].

Item	CON	OH-f	OH-c	SEM	*P*-value	
					Diet	Sex
n	14	14	14	–	–	–
Litters of origin	7	10	9	–	–	–
Boar-to-gilt ratio	5:9	8:6	4:10	–	–	–
Birth weight, kg	1.50	1.42	1.45	0.072	0.714	0.495
Colostrum intake, g/kg birth weight	292	356	315	18.8	0.066	0.345
Body weight d 12, kg	3.99	4.14	3.98	0.207	0.828	0.310
Average daily gain experimental period, g/d	233	246	242	12.7	0.729	0.045
Supplemental feed intake[2], g	649	666	544	113.1	0.466	0.012

[1] CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively. Suckling piglets had access to one of the 3 supplemental diets. No diet × sex interaction was observed.
[2] Total intake from d 12 until 23 as gruel (water-to-feed ratio = 2:1).

Table 5
Carcass yield and gastrointestinal morphometrics (LSMeans and pooled standard error)[1].

Item	CON	OH-f	OH-c	SEM	*P*-value
n	14	14	14	–	–
Age at dissection, d	24.2	24.1	23.9	0.27	0.736
Body weight at dissection[2], kg	6.87	7.57	7.08	–	–
Carcass yield, %	82.3	82.8	82.3	0.53	0.792
Stomach, empty weight, g	53.9	48.4	53.8	1.76	0.055
Stomach wet contents[3], g	69.9	67.9	97.2	13.07	0.251
Stomach, full weight[3], g	122.7	115.3	153.1	12.59	0.083
Caecum, empty weight, g	15.5	15.3	15.3	0.61	0.956
Caecum wet contents, g	17.9[b]	19.1[b]	26.2[a]	1.95	0.012
Colon, empty weight, g	45.0	46.3	47.0	1.88	0.753
Colon wet contents[3], g	32.3	43.4	45.0	4.62	0.078
Colon length, cm	124.3[b]	133.3 [ab]	143.1[a]	4.81	0.030
Colon weight/length[3], g/cm	0.36	0.35	0.33	0.012	0.112
Total GIT full weight[3], g	237.5[b]	234.4[b]	293.3[a]	17.44	0.036
Total GIT empty[3], g	115.3	110.0	116.7	3.18	0.312

GIT = gastrointestinal tract (stomach + caecum + colon).
[a,b] Values within a row with different superscripts differed significantly at $P < 0.050$.
[1] Suckling piglets had access to 1 of the 3 supplemental diets. CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively. Dissection was performed 1 or 2 d prior to anticipated weaning at d 26.
[2] Used as covariate.
[3] Creep feed intake as additional covariate.

Table 6
Concentration of metabolites (mmol/kg contents), dry matter (DM%) and pH of gastrointestinal contents in suckling piglets (LSMeans and pooled standard error)[1].

Item	CON	OH-f	OH-c	SEM	*P*-value
n	14	13	14	–	–
Stomach[2]					
pH, proximal region	4.07	4.17	4.23	0.261	0.910
pH, distal region	3.90	3.74	3.88	0.325	0.933
pH, gradient	0.17	0.43	0.34	0.225	0.709
DM, proximal region	24.6	22.3	24.8	1.40	0.385
DM, distal region	21.5	20.9	23.3	1.37	0.446
DM, gradient	2.36	1.39	1.45	0.857	0.676
Lactic acid	30	22	29	6.0	0.583
Acetic acid	10	9	10	1.2	0.862
Succinic acid	3.6	3.3	3.8	0.47	0.763
Total SCFA[3]	42	28	39	5.9	0.224
Ammonia-N	126	140	150	12.4	0.380
Caecum[4]					
pH	6.65	6.64	6.60	0.075	0.895
DM[5]	13.1[b]	15.9[a]	15.2[ab]	0.81	0.045
Acetic acid[5]	86	90	93	11.4	0.890
Propionic acid[5]	25	24	22	3.5	0.893
Butyric acid[5]	6.8	7.7	7.1	1.31	0.885
Succinic acid	3.5	4.2	4.2	0.68	0.731
Total SCFA[3,5]	115	124	130	16.8	0.832
Ammonia-N[5]	110	104	102	11.3	0.862
Mid-colon[6]					
pH	6.76	6.72	6.77	0.103	0.936
DM	30.4	30.6	31.6	1.92	0.891
Acetic acid[5]	22[b]	36[a]	34[ab]	4.0	0.046
Succinic acid	2.7	4.0	4.3	0.58	0.131
Total SCFA[3,5]	29	44	41	4.8	0.083
Ammonia-N	26	27	28	2.7	0.925

[a,b] Values within a row with different superscripts differed significantly at $P < 0.050$.
[1] Suckling piglets had access to 1 of the 3 supplemental diets. CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively. Dissection was performed 1 or 2 d prior to anticipated weaning at d 26.
[2] Propionic, butyric, valeric acid and BCFA were mostly below detection limit.
[3] SCFA = sum of lactic, acetic, propionic, butyric, valeric and succinic acid + branch-chained fatty acids (BCFA) concentrations.
[4] Lactic, valeric acid and BCFA were mostly below detection limit.
[5] Supplemental feed intake as covariate.
[6] Lactic, Propionic, butyric, valeric acid and BCFA were mostly below detection limit.

the stomachs revealed alterations from score 0. All stomachs had fluid contents, ranging from fully fluid to intermediate and no diet effects were observed ($P > 0.10$; Appendix Fig. 3). The full GIT weight increased by almost 24% in piglets fed OH-c compared to the 2 finely ground diets ($P = 0.036$; Table 5). This was due to an increase in weight of its contents, as empty weight was not affected. Specific sections of the GIT responded differently to the experimental diets. The caecum showed a higher contents weight for OH-c compared to CON and OH-f ($P = 0.012$). The weight of colonic contents tended to differ between CON and OH-c ($P = 0.078$) whereas stomach contents weight was not significantly affected, despite a 39% difference between these 2 groups. The weight of the colonic contents was increased for piglets fed the diets containing OH compared to the control diet (33.2 vs. 44.2 g; $P = 0.025$). The supplemental feed intake correlated positively with colonic contents weight, particularly for the OH-diets (Pearson correlation coefficient [r] = 0.49; $P = 0.001$), whereas it was absent for CON. On the other hand, caecum contents weight did not correlate, whereas stomach fill only weakly correlated with feed intake ($r = 0.30$; $P = 0.053$). The latter correlation was mainly observed for the OH-c group, largely due to one piglet with a high intake. A positive correlation was also observed between supplemental feed intake and empty stomach weight ($r = 0.58$; $P < 0.001$), while stomachs tended to be lighter for OH-f compared to the other treatments ($P = 0.055$; Table 5). The length of the colon was increased for OH-c ($P = 0.030$) as well as for OH inclusion (124.2 vs. 138.2 cm; $P = 0.024$) compared to CON.

3.3. Metabolic profile and particle size distribution of GIT contents

The pH in the consecutive sections of the GIT differed between the proximal and distal halves of the stomach (4.33 and 3.89, respectively; $P = 0.033$), whereas the DM concentration was similar (23.9% and 21.9%; $P > 0.10$). The DM concentration decreased to 14.3% in the caecum and increased again to 30.0% in the mid-colon region ($P < 0.001$). In general, diet composition had little impact on DM and metabolite concentrations of the GIT with some exceptions (Table 6). For instance, caecal DM concentration increased in piglets fed the OH diets compared to the control diet (15.6% vs. 13.1%; $P = 0.017$) and differed between CON and OH-f ($P = 0.045$; Table 6). In the mid-colon, SCFA concentrations were increased in piglets fed the OH diets compared to CON (42.4 vs. 25.9 mmol/kg; $P = 0.004$), mainly through increased acetic acid concentrations (34.8 vs. 22.3 mmol/kg; $P = 0.014$) and succinic acid concentration (4.15 vs. 2.71 mmol/kg; $P = 0.046$). For the different GIT sections, ammonia-N concentration did not differ, and branched-chain fatty acids (BCFA) levels were mostly below detection limits. Finally, we detected the largest particles in the digesta collected from the proximal half of the colon in piglets fed OH diets. However, particle size distribution did not differ (Appendix Fig. 4A and B).

3.4. Mucosal microscopic morphology, small intestinal lactase activity and permeability

The immature state of the gastroduodenal sphincter (*torus pyloricus*) in most piglets did not allow reliable measurements of its dimensions (no records for 7, 4 and 3 out of 14 piglets, for CON, OH-f and OH-c, respectively). The ileal microscopic structure was affected by diet. The villus height was increased for OH-f in comparison with CON and OH-c ($P = 0.041$; Table 7). Moreover, villus height increased by 15.2% ($P = 0.015$), while crypt depth and width tended to increase by 11.1% and 16.7%, respectively ($P < 0.100$) in piglets fed diets containing OH compared to CON. On the other hand, ex-vivo intestinal permeability revealed no treatment effect (Table 7). Mucosal lactase activity was also not affected by diet

Table 7
Intestinal permeability and microscopy (LSMeans and pooled standard error)[1].

Item	CON	OH-f	OH-c	SEM	*P*-value
n	14	14	14		
Jejunum[2]					
Permeability ex vivo, mg cobalt/L	25.39	27.33	26.63	4.334	0.960
Ileum[2]					
Villus height, μm	309[b]	370[a]	346[ab]	17.4	0.041
Villus width, μm	96	114	110	7.2	0.206
Crypt depth, μm	100	109	113	4.8	0.172
Ileocecal sphincter					
Crypt depth, μm	505	567	501	33.1	0.278
Longitudinal muscle[3], μm	187	189	177	14.6	0.854
Total muscle width[3], μm	764	778	807	39.4	0.752
Caecum					
Crypt depth, μm	328	328	309	12.4	0.456
Crypts per mm epithelium	14.2	14.1	14.3	0.54	0.944
Area covered by goblet cells, %	18.3	16.3	21.6	4.65	0.828
Goblet cell count	1,361	1,224	1,035	133.8	0.239
Mid-colon					
Crypt depth, μm	343[ab]	384[a]	319[b]	15.5	0.018
Crypts per millimetre epithelium	15.4	15.2	16.6	0.51	0.108
Goblet cells per square millimetre epithelium	915	858	953	61.8	0.534

[a,b] Values within a row with different superscripts differed significantly at $P < 0.050$.
[1] Suckling piglets had access to 1 of the 3 supplemental diets. CON stands for low fibre control diet; OH-f and OH-c contained finely or coarsely ground oat hulls, respectively. Dissection was performed 1 or 2 d prior to anticipated weaning at d 26.
[2] Supplemental feed intake + as covariate.
[3] Body weight as covariate.

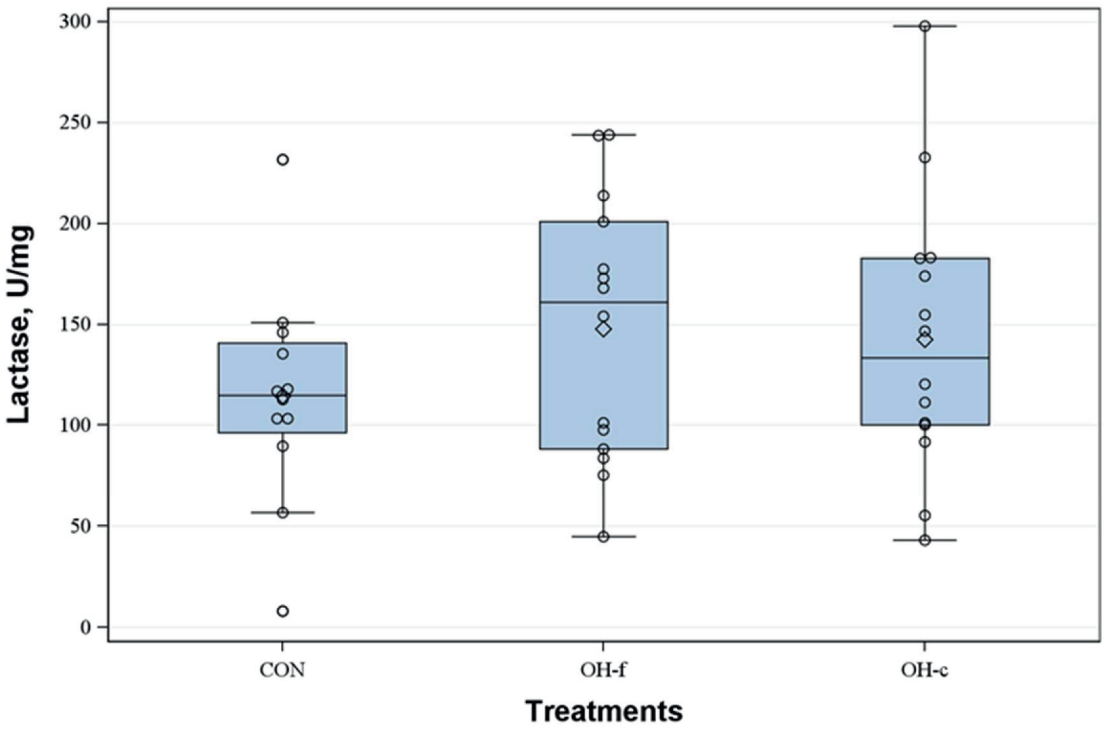
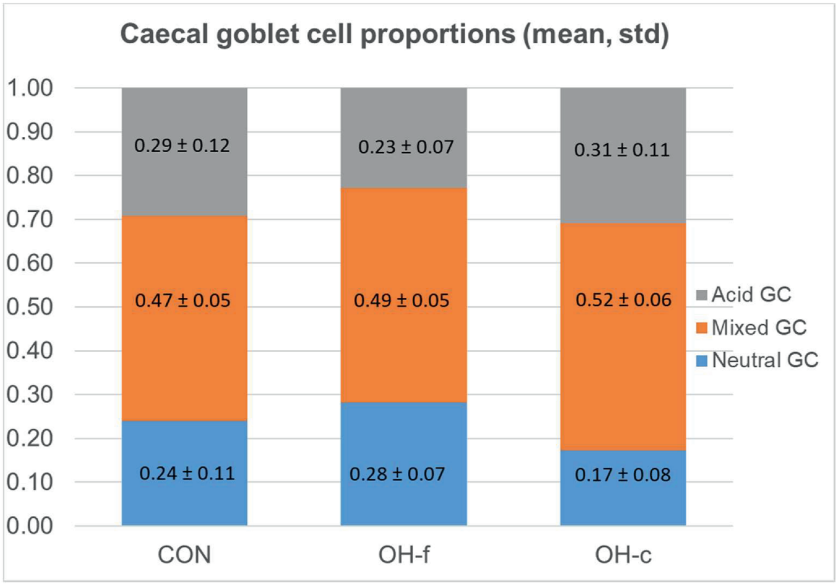


Fig. 1. Mucosal lactase activity of the proximal jejunum did not differ between suckling piglets fed different supplemental diets (LSMeans ± standard error, SE; $P = 0.454$), i.e., a low fibre-high nutrient dense control diet (CON; 116.8 ± 18.29 U/mg), a diet containing 15% fine oat hulls (OH-f; 147.1 ± 16.84 U/mg) or coarse oat hulls (OH-c; 140.9 ± 16.88 U/mg). Diets were fed as a gruel (water-to-feed ratio = 2:1).

(Fig. 1) nor OH inclusion (118 vs. 143 U/mg protein for CON and OH diets, respectively; $P > 0.100$). The latter variable correlated positively with bodyweight gain ($r = 0.48$; $P = 0.002$). As for the gastroduodenal sphincter, the ileocecal sphincter was poorly developed in approximately one-third of the piglets. Based on these limited data, no differences between diets could be detected (Table 7). Microscopic examinations of the ceca did not reveal a diet effect (Table 7), except for a shift in goblet cell sub-types. The proportion of goblet cell producing neutral mucins was decreased for OH-c compared to OH-f ($P = 0.015$; Fig. 2A). On the other hand, OH-c and inclusion of OH tended to increase the proportion of the mixed goblet cells compared to CON ($P < 0.01$) whereas the proportion of acid mucins was not altered. In the colon, mucosal crypts were deeper for OH-f compared to OH-c ($P = 0.018$; Table 7). The colonic goblet cell density was not affected by diet. The proportion of colonic goblet cell sub-types differed between treatments. The

A



B

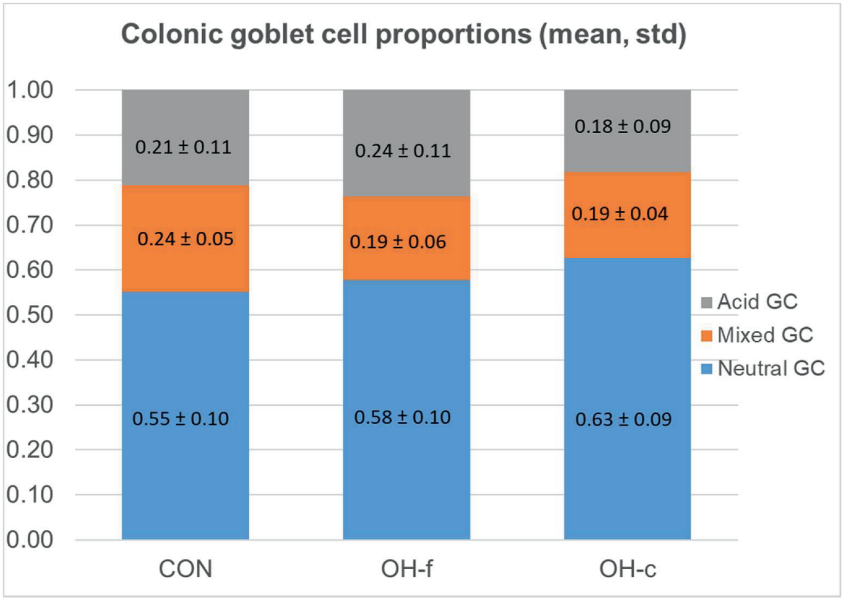


Fig. 2. Proportions of different goblet cell (GC) types based on their periodic acid-Schiff-alcian blue (PAS-AB) colouring. Data of the caecum (A) and mid-colon (B). CON = low fibre control diet; OH-f containing 15% fine oat hulls; OH-c containing 15% coarse oat hulls. Caecum: Proportion of neutral GC differed between OH-f and OH-c ($P = 0.015$). The caecal mixed GC tended to differ between CON and OH-c ($P < 0.100$: Contrast CON vs. both OH-diets: $P = 0.057$). Colon: Proportions of mixed GC differed between CON and OH fine ($P = 0.025$) and CON and OH-c ($P < 0.100$: Contrast CON vs. both OH-diets: $P = 0.007$).

control piglets had a higher proportion of mixed goblet cell in comparison to OH-f ($P = 0.025$) and both OH diets ($P = 0.007$; Fig. 2B).

3.5. Microbiology of the mid-colon contents

The colonic contents had a lower total bacterial count for piglets fed the OH-f diet compared to the control diet ($P < 0.05$; Fig. 3). Moreover, bacterial counts were lower for piglets fed diets containing OH compared to CON ($P = 0.048$). In addition, the OH diets reduced γ-proteobacteria counts ($P = 0.012$; Fig. 3) and its proportion (0.0136 vs. 0.008; $P = 0.042$) compared to CON. No differences in counts (Fig. 3) or proportions were detected for the other bacterial groups.

4. Discussion

In piglets selected for consuming reasonable amounts of supplemental feed, the inclusion of 15% OH exerted subtle effects in the GIT of suckling piglets when compared to a finely ground, low fibre control diet. Among the most pronounced effects were the increased ileal villus height and a higher DM concentration of the caecum contents. Besides, for the colon, an increased length, contents weight, elevated SCFA concentration and a reduction of total

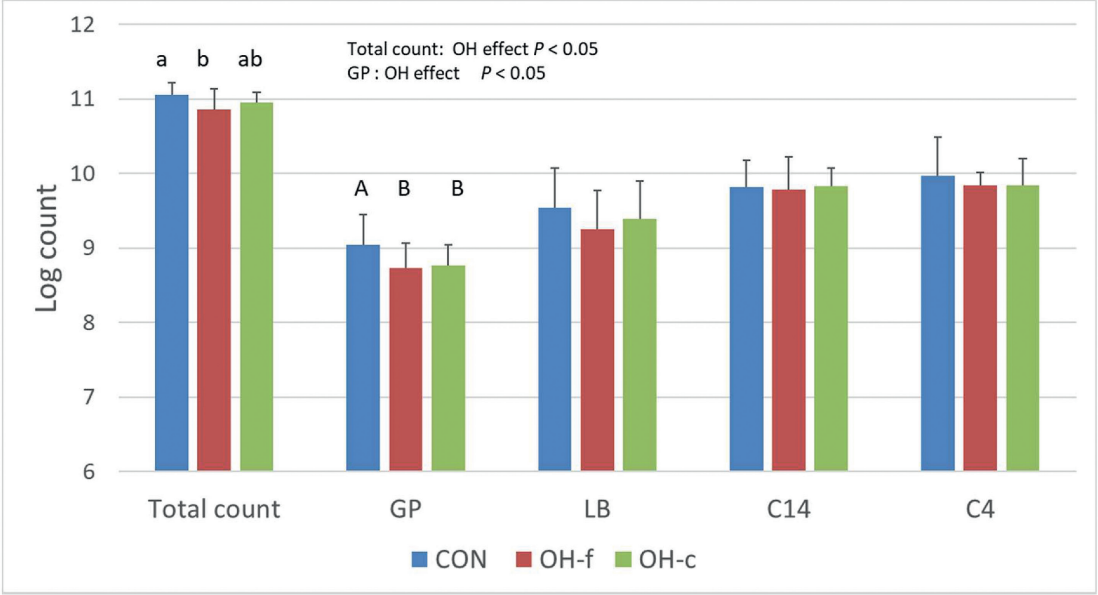


Fig. 3. Bacterium groups of the mid-colon in suckling piglets detected by qPCR (log counts per gram wet contents; means ± standard deviation), i.e., total counts, γ-proteobacteria (GP); Lactobacillaceae (LB); Lachnospiraceae (cluster XIV: C14) and *Ruminococcaceae* (cluster IV: C4). a, b: $P < 0.05$; A, B: $P < 0.10$. CON = low fibre control diet; OH-f containing 15% fine oat hulls; OH-c containing 15% coarse oat hulls.

bacterial counts as well as γ-proteobacteria were observed. The coarse OH specifically increased full GIT weight and the weight of caecal contents compared to piglets fed the control diet and the finely ground OH diet. Furthermore, coarse OH reduced colonic crypt depth when compared to the finely ground OH.

Our design allowed to measure the individual supplemental feed intake of suckling piglets, which was low and highly variable as is typical for this age category (reviewed by Middelkoop, 2020). The quantities of intake of the selected pigs were comparable to recent data in litter-fed piglets (Choudhury et al., 2021; Clouard et al., 2018; Van Hees et al., 2019). In addition, the split-litter design separated the effect of diet from the effect of the dam, known to be significant (Craig et al., 2019). Still, many variables were characterized by a high within-group variation, which may have obscured diet effects. The implications of our findings per GIT section are discussed below.

4.1. Impact on the stomach

We did not observe major effects of diet on stomach variables. According to Pluske et al. (2003), both relative and absolute stomach mass increases from 2 wk in life onwards. Gastric mass and secretory capacity are further stimulated by the ingestion of solid food (Cranwell, 1985; Cranwell and Stuart, 1984). In line with this, feed intake in our study correlated positively with stomach tissue weight. Data in older pigs showed that the bulking effect of insoluble fibres increased stomach weight (Flis et al., 2017; Rijnen et al., 2001). Moreover, a coarse diet could exert a trophic effect on the stomach. This may be explained by a longer stomach retention and an increased DM concentration requiring higher propulsive action from the stomach musculature (Betscher et al., 2010; Mößeler et al., 2014; Regina et al., 1999). However, current results in suckling piglets do not confirm these effects reported for older pigs. This corroborates with a study supplementing purified cellulose (Van Hees et al., 2019). Similarly, Choudhury et al. (2021) did not observe an effect of a fibrous supplemental diet on empty stomach weight when compared to piglets consuming sow milk only. Also, in line with our findings, Molist et al. (2012) observed no effect on stomach weight when comparing diets containing either finely or coarsely ground wheat bran in weaned piglets of a similar age as current study. Beside age, the low feed intake and a limited contrast in particle size distribution may explain the absence of an effect on stomach development.

Based on data in weaned piglets, we further hypothesized that a diet containing larger particles would stimulate the formation of 2 distinct gastric compartments based on acidity and DM concentration (Betscher et al., 2010; Guerin et al., 2001; Mößeler et al., 2014). Achieving a lower pH for the distal stomach would favour protein digestion and create a protective barrier against pathogens invading the lower intestinal tract. However, only a minor pH and DM gradient between the proximal and distal stomach was observed, not affected by diet. Although care was taken when handling the stomachs in our study, the fluidity of the contents may have caused unintended mixing between the 2 sections of the stomach. We were not able to demonstrate higher gastric fermentative activity with a coarser diet, as was reported for post-weaning piglets (Canibe et al., 2005; Mikkelsen et al., 2004, 2007; Papenbrock et al., 2005). This may be explained by nature of the coarse particles (i.e., coarse fibres only versus whole diets). Alternatively, the availability of readily fermentable substrates in sow milk (lactose, milk oligosaccharides) as well as in the basal diet (lactose, amongst other simple sugars), may have led to substantial basal concentrations of SCFA for all experimental groups. Another explanation may be come from Regina et al. (1999) who observed the largest differences in SCFA concentrations between a coarse and fine diet in the proximal gastric region as compared to the distal region, whereas our SCFA data were obtained from the mid-gastric region. The relatively high gastric ammonia concentrations were notable and unexpected. Pieper et al. (2016a) reported ammonia concentrations of 2.5 mmol/L in 14 d suckling piglets only consuming sow milk. The high concentration found in our study (138.4 mmol/kg) may have originated from proteolytic fermentation of the finely ground dietary proteins present in the basal diet. This may incur a potential risk for gastric epithelial health as it could lead to mucosal damage (Pieper et al., 2016a).

Based on numerous studies in older pigs, diet particle size reduction imposes a risk for keratinization and ulceration of the *pars nonglandularis* (reviewed by Flis et al., 2014). The absence of a stratified layer and the highly-fluid contents, hence allowing easy mixing of the more acidic distal contents with the proximal contents, is considered a major risk factor in this respect (Mößeler et al., 2014). Nevertheless, no alterations of the *pars nonglandularis* were detected in current study. Given the young age of the pigs in our study, lesions may not have developed yet. Moreover, the relatively high pH (>4) measured in the proximal gastric region, was presumably not damaging (Mößeler et al., 2014). Alternatively, results may include false negatives because alterations at the microscopic level may have been unnoticed (Cappai et al., 2013).

4.2. Impact on the small intestine

The early life small intestinal maturation is responsive to dietary influences. For instance, supplemental feeding increased small intestinal mass and cylindrical growth through expression of growth factors, increased cell proliferation and deepening of mucosal crypts (Choudhury et al., 2021; De Greeff et al., 2016; Schokker et al., 2018). The current study revealed extended ileal villi in piglets fed diets supplemented with OH compared to CON piglets. Also, villi growth was most pronounced in piglets fed the finely ground OH. An increased villus length was observed earlier in newly weaned piglets fed barley hulls, a fibre source with similar characteristics as OH (Hedemann et al., 2006). It may be indicative of an adaptive response of the epithelium to the abrasive effect of dietary fibre or to altered luminal conditions (Kim et al., 2012).

The specific lactase activity and mucosal permeability were not affected in current study, suggesting similar digestive capacity and small intestinal barrier across diets, respectively. The general believe that small intestinal permeability diminishes with age (Rådberg, 2001) was recently disputed by Arnaud et al. (2020) and may be applicable to the immediate post-natal days only. Moreover, little information is available on the association between early life nutrition and this parameter. An absence of a supplementary diet effect on intestinal permeability concurs with other reports (De Greeff et al., 2016; Van Hees et al., 2019). On a similar note, small intestinal lactase activity is considered a maturation parameter as it decreases with age (Manners and Stevens, 1972) and responds to nutrition (Hampson and Kidder, 1986; Pieper et al., 2016b; Radberg et al., 2001). The ontogenetic decrease in lactase activity may be counteracted by dietary fibre, as increased levels of pectin and barley hulls resulted in an increase in brush border enzyme activity (lactase and maltase) in newly weaned piglets (Hedemann et al., 2006).

4.3. Impact on the large intestine

In the suckling piglet, the absolute and relatively large intestinal weight increases from 14 to 28 d in life and is followed by a massive increase the first 2 wk following weaning (Pluske et al., 2003). It is proposed that a well-developed large intestine may serve resilience after weaning and reduces diarrhoea in its capacity to absorb fluids and electrolytes (Heo et al., 2013; Van Beers-Schreurs, 1996). Based on Bach Knudsen et al. (2012), insoluble fibres exert a bulking effect in the large intestines and possibly stimulates large intestinal development. In our study, a bulking effect of OH was observed coinciding with an increased length, most pronounced for the coarse type, whereas empty colon weight was not increased. In contrast, the greater large intestinal fill with insoluble fibre supplementation resulted in a greater organ weight and length (Van Hees et al., 2019). Other, yet unidentified characteristics of the insoluble fibre source, and inclusion level may explain the difference in effect on colon weight between both studies. More specifically, Van Hees et al. (2019) reported a butyrogenic effect with cellulose whereas this was absent in current study. Butyric acid is a major energy source for colonic enterocytes and stimulates cell proliferation (Scheppach, 1994). Choudhury et al. (2021) fed a high (soluble and insoluble) fibre diet to suckling pigs and reported greater colonic contents mass and increased colonic length without a change in empty weight when compared to piglets only fed sow milk. Apparently, the colonic response to a greater fill can be either an increase in weight or increase in length or both. With respect to the microbial community harbouring the large intestines of suckling piglets, consumption of supplemental feed changes its composition and stimulates its activity (Choudhury et al., 2021; De Greeff et al., 2016). Moreover, Choudhury et al. (2020) documented that consumption of a high fibre supplemental diet led to an accelerated maturation of the faecal bacterial community, closely resembling the post-weaning pig. In addition, dietary fibre composition further modifies the microbial community and activity (Mu et al., 2017; Schokker et al., 2018; Van Hees et al., 2019). In line with this, increased SCFA concentrations in our study indicated that supplementing OH affected the substrate arriving in the hind gut, thus modulating its metabolic activity. The SCFA are considered to be beneficial for the epithelium as they are used as a metabolic fuel, stimulate cell proliferation, act anti-inflammatory, improve the barrier function and inhibit pathogen proliferation (Richter et al., 2014; Scheppach, 1994). These SCFA may not originate from the added OH fibres, as they are difficult to ferment and an adaptation period of at least 2 wk is required to stimulate large intestinal fermentation (Zervas and Zijlstra, 2002). Specifically, the increased acetic acid concentration may indicate a general stimulation of fermentation of endogenous protein (e.g., mucus) and simple carbohydrates structures, like starch or lactose originating from the basal diet and sow milk, as suggested earlier (Van Hees et al., 2019). Oat hull particle size differentially affected colonic epithelial microscopic structure, as deeper crypts were observed for the fine compared to coarsely ground OH. Deeper crypts may indicate increased cell proliferation and restoration of the epithelium, for instance due to the abrasive action of dietary fibre. Based on Brunsgaard (1998), Jin et al. (1994) and Hedemann et al. (2005), current findings were unexpected. These authors reported deeper crypts in weaned piglets fed insoluble dietary fibre and coarsely ground cereal-based diets compared to diets with a fine particle size. On the other hand, the deeper crypts found for piglets fed the finely ground OH is consistent with the higher SCFA concentrations found for this group compared to CON, as SCFA are known to stimulate epithelial growth (Scheppach, 1994). However, this association was reversed in piglets fed the coarse OH. Apparently, the combined effect of OH fibre and its larger-sized particles modulated the epithelial adaptive response differently compared to fine particles.

A mucus gel covering the intestinal epithelial lining is present from stomach to rectum. It consists mainly of glycoproteins produced by goblet cells. Being part of the intestinal barrier, it protects epithelial cells from mechanical, chemical, and microbial damage. Goblet cell numbers and activity are under the influence of diet, including dietary fibre, in monogastric animals (Lien et al., 2001). For instance, supplementing the diet of grower pigs with wheat bran and beet pulp increased ileal goblet cell density, *mucin 2* expression and faecal mucus excretion (Wellington et al., 2020). In current study, however, no effect on goblet cell density in the large intestines was observed, suggesting similar mucus secretion capacity across diets. This agrees with Hedemann et al. (2005) but seems to contradict data of Brunsgaard (1998). The young age of pigs in current study together with the nature of the coarse

particles and a smaller contrast in particle size between the control and the OH-c diet may explain this. Nevertheless, diet modified mucus composition in the large intestine. During maturation of goblet cells, the mucin staining characteristics change from neutral to more acid type mucins (Hedemann et al., 2005). Our data showed a decreased caecal proportion of neutral mucins for coarse compared to fine OH, coinciding with a trend for increasing mixed mucins compared to the control diet, which may suggest the formation of a more mature mucus (Deplancke and Gaskins, 2001). A reduction in the proportion of caecal neutral goblet cells was observed in weaned piglets fed a coarse meal when compared to a finely ground pelleted diet, possibly reducing the ability of Salmonella to adhere (Betscher et al., 2010). This phenomenon could offer an additional explanation for the reduced proportion of putative pathogens as discussed below. Unfortunately, diet effects on mucus staining were not consistent between caecum and mid-colon, hampering interpretation of the results regarding intestinal health and piglet's resilience. A site-dependent effect of diet on mucus characteristics has also been reported by others (Betscher et al., 2010; Hedemann et al., 2005).

In contrast to Pieper et al. (2016a) but substantiating our earlier findings, including insoluble dietary fibre did not affect indices of proteolytic fermentation (BCFA and ammonia concentrations). This suggests an unaltered protein-to-carbohydrate ratio of the digesta reaching the caecum and the colon. Consistent with the absence of an effect on butyrogenic groups (Ruminococcaceae and Lachnospiraceae) we could not confirm a butyrogenic effect by insoluble dietary fibre reported by others (Mu et al., 2017; Van Hees et al., 2019). Further to the microbial composition, a reduction in putative pathogenic groups (i.e., γ-proteobacteria) in the colonic contents of piglets fed OH was observed in agreement with earlier findings (Gerritsen et al., 2012; Molist et al., 2012; Van Hees et al., 2019; Zhang et al., 2016). On the other hand, our data do not support the earlier postulates (e.g., Mikkelsen et al., 2004) that this effect was caused by an improved barrier function of the stomach by feeding coarser diets, as discussed above. Alternatively, the higher luminal SCFA concentrations seen with OH inclusion may have created an environment less favourable for this bacterium group and bacteria in general (Williams et al., 2001). The OH may also, through their abrasive action, increased the wash-out of pathogens that adhere to the epithelium. On a similar note, increased intestinal fill may have decreased retention time by stimulating peristaltic muscular activity, thus reducing intestinal stasis (Molist et al., 2014). Another explanation for a reduced pathogenic load could come from a changed interaction with the large intestinal mucosal lining, as suggested by the observed changes in goblet cell mucus sub-types (Hedemann et al., 2005).

In general, the observed gastrointestinal modifications brought about by adding an insoluble dietary fibre source to a low-fibre supplemental feed were subtle and less pronounced compared to effects reported for older pigs. They must be evaluated in the context of a developing gastrointestinal system, yet unacquainted with particulate solid feed material. The poorly developed gastroduodenal and ileocecal sphincters observed in many piglets is exemplary in this context. Proper development of these sphincters may support processing of solid feeds and maintain intestinal health upon weaning, in line with Boudry et al. (2004) and Cappai et al. (2015, 2020). Furthermore, we may have observed changes generated in a relatively short time frame with sow milk as the major nutrient source. Notably, the relatively high level of OH, independent of its particle size, did not impede production performance and piglets remained clinically healthy throughout the study. Although fine particle size is commonly applied in practice for creep feed, particle size has not been evaluated in suckling piglets, whereas studies in other species and older pigs (Flis et al., 2014; Liermann et al., 2018) demonstrated that particle size has a protective and promoting effect on the GIT. As such, there are no reasons to avoid coarser high fibre diets in this category of pigs, although further optimization is warranted. Whether the observed changes provide health advantage for piglets exposed to the weaning event requires further experimental proof.

5. Conclusion

In conclusion, current data show that supplementing OH to a diet for suckling piglets had subtle developmental effects on the ileum, caecum, and colon as well as on the colonic microbial community. These effects were largely independent from the particle size of the OH.

Author contributions

All authors contributed to the conceptualization of the study. **Hubèrt M.J. van Hees**; investigation, methodology, data curation, visualization, formal analysis, writing - original draft. **Koen Chiers**; methodology, supervising the microscopic evaluations performed by HvH, writing - reviewing and editing. **Leo A. den Hartog**; writing - reviewing and editing. **Theo A.T.G. van Kempen**; writing - reviewing and editing. **Dominiek Maes**; writing - reviewing and editing. **Sam Millet**; writing - reviewing and editing. **Geert P.J. Janssens**; supervision, writing - reviewing and editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hubert M.J. van Hees reports financial support was provided by Trouw Nutrition R&D. Hubert M.J. van Hees reports a relationship with Trouw Nutrition R&D that includes: employment. Leo A. den Hartog and Theo A.T.G. van Kempen are employed by Trouw Nutrition, a global producer of piglet feeds. The current study was performed in a collaborative PhD project between the University of Gent and Trouw Nutrition Research and Development.

Acknowledgements

We are grateful to Kelly Venrooij, Carlijn de Bruijn, Awot Teklu Mebratu, Herman de Rycke and the staff of the swine research unit of Trouw Nutrition for providing help and expertise during the execution of the experiment. Laboratory analysts Orhan Daud, Marc Reijersen, Pascal van Lith and Ralph Litjens are acknowledged for their expertise on biochemical and microbiota analyses. Naomi de Bruijn DVM, Dipl. ECVP at the Animal Health Service (Deventer, The Netherlands) is acknowledged for her microscopic work on the caecum tissue samples.

Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.10.002.

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