# Full-Fat Insect Meal as a Protein and Energy Source for Weaned Piglets: Effects on Growth Performance, Nutrient Digestibility, Gastrointestinal Function and Microbiota

**Ingrid Marie Håkenåsen**
Norwegian University of Life Sciences https://orcid.org/0000-0002-8499-6292
**Guro Holseth Grepperud**
Norwegian University of Life Sciences
**Jon Øvrum Hansen**
Norwegian University of Life Sciences
**Margareth Øverland**
Norwegian University of Life Sciences
**Ragnhild Martinsen Ånestad**
Norwegian University of Life Sciences
**Liv Torunn Mydland** ( liv.mydland@nmbu.no )
Norwegian University of Life Sciences https://orcid.org/0000-0002-9361-3687

**Research**

**Keywords:** Pig, Weaning, Insect meal, Black soldier fly larvae, Gut function, Microbiota

**Posted Date:** August 19th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-61552/v1

# Abstract

**Background:** Weaning is associated with growth decline and morphological changes in the gastrointestinal tract of the pig. Insects, such as the Black soldier fly larvae (BSFL), are suggested as a sustainable novel protein source in diets for pigs. They contain chitin, medium chained fatty acids, and anti-microbial peptides, which could improve the gastrointestinal function and health in the post-weaning pig. The objective of this study was therefore to investigate the effect of increasing inclusion of full-fat BSFL in diets for post-weaning pigs on growth performance parameters, digestibility, gut morphology, and the microbial community in the colon. Eighty crossbred weanling pigs were weaned at approximately 32 days of age, with an average weaning weight of 10.6 ± 0.8 kg. For four weeks, the pigs were fed one of four dietary treatments: a control diet or one of three diets containing an increasing amount of full-fat BSFL meal: 4.76% (BSFL5), 9.52% (BSFL10), and 19.06% (BSFL20).

**Results:** Increased level of full-fat BSFL in the diet did not affect growth performance or fecal consistency. A reduction in apparent total tract digestibility (ATTD) of crude protein ($P = 0.035$) was found for increased inclusion of BSFL, whereas the ATTD of crude fat increased ($P<0.001$). Jejunal, ileal, or colonic morphometry was not affected by the BSFL inclusion.

Some changes in the colon microbiota were observed, but no differences in the short-chain fatty acid concentrations were detected between the dietary treatments. At the phylum level, dietary treatment affected the relative abundance of Bacteroidota, Firmicutes, Campilobacteria, and Thermoplasmatota, but there was no clear pattern relationship with the BSFL inclusion level. At the genus level, the inclusion of BSFL in the diet reduced the relative abundance of *Lactobacillus* ($P = 0.015$) compared to the control.

**Conclusions:** Collectively, the results indicate that up to 19.06% of full-fat BSFL meal could be included in a balanced diet for PW pigs without affecting growth performance, gut function, or health.


# Background

The world is facing a growing food demand by an ever-increasing number of people. To meet this challenge, new resources must be considered by using innovative solutions. Insects have been proposed as a high quality, efficient, and sustainable alternative protein source [1]. Black soldier fly larvae (BSFL), *Hermetia illucens*, is an easily reared species, capable of efficient conversion of a wide range of organic materials [2], and do not accumulate pesticides or mycotoxins [3, 4]. The BSFL has an overall favorable amino acid profile but is limiting in sulfur-containing amino acids and contains a high amount of minerals, especially calcium [5].

A well-functioning gastrointestinal tract (GIT) is of importance to the overall growth performance and health of pigs in all stages, and especially for the newly weaned pigs. If not defatted, BSFL is high in fat, especially in lauric acid (12:0) [5] which is categorized as a medium-chain fatty acid (MCFA) with antimicrobial effects, especially against gram-positive bacteria [6]. Insects and insect larvae also contain chitin, a dietary polysaccharide that can function as a prebiotic and an immunostimulant [7]. Finally, anti-

microbial peptides, which are part of the insect immune system, are effective antimicrobial agents with low risk of development of bacteria resistance. Therefore, the anti-microbial peptides have good potential as health promoters in livestock, even though there is limited information about the *in vivo* effects [8].

Based on the content of these constituents, insects are interesting to investigate both as a feed ingredient and as a functional ingredient with potential health beneficial effects. Recently there has been a high focus devoted to the potential of insect meals in diets for monogastric farm animals [9–12], however, there is a scarcity of literature on the use of full-fat BSFL in diets for pigs. The objective of this study was to investigate the effect of increased inclusion of full-fat BSFL in diets for post-weaning (PW) pigs, focusing on growth performance parameters, digestibility, gut morphology, and microbial community in the colon.

## Materials And Methods

A 27 days experiment was performed in February 2019 at the Center for livestock production (SHF, NMBU, Ås, Norway), which is an animal experimental unit approved by the National Animal Research Authority (permit no. 174). All pigs were handled under the applicable laws and regulations controlling experiments with live animals in Norway (regulated by the “Animal Welfare Act” and “The Norwegian Regulation on Animal Experimentation” derived from the “Directive 2010/63/EU on the protection of animals used for scientific purposes.

# Animals and Housing

Eighty crossbred [Norwegian Landrace x Yorkshire z-line) x Duroc)] weanling pigs, selected from eleven litters (four or eight pigs from each litter) were included in the experiment. Pigs who had been under medical treatment in the nursing period were excluded. All piglets had access to the sows feed during the nursing period. The average weaning age was 32.8 ± 1.6 SD days and the average weaning weight 10.6 ± 0.8 SD kg. Pigs were blocked by litter, weight, and sex and assigned to one of four dietary treatments. Pigs were distributed in pens of four, and from a total of five pens per treatment, three of the pens per treatment were installed with rubber mats. The remaining pens had access to wood shavings as bedding material. The pen size was 1.6 $m^2$. The room temperature was logged every morning, and the average temperature for the experimental period was 21.6 ± 1.4 °C.

# Dietary treatments

BSFL meal was produced at HiProMine S.A., Poznan, Poland. The BSFL feed was normalized in terms of dry matter (DM) content by the addition of wheat middlings (17%) to fresh vegetables and fruit mix, consisting of apples (15%) carrots (50%) potatoes (15%) and cabbage (20%) and established at the level of 22% DM. Fresh vegetable pre-consumer waste was ground (2000 rpm/1 min, (HPM milling system, 55 kW, Poland) to pass a 2 mm screen and offered *ad libitum* to the BSFL. Substrates were not contaminated by any animal products in accordance with EC regulation (no 1069/09). At the prepupal

stage (10th day of rearing), larvae were harvested, sieved through a 3 mm screen, and washed with water on drum separator at 90 °C for 10 minutes (HPM cleaning system, Poland).

The dietary treatments included a control diet and three experimental diets with increased inclusion of BSFL; 4.76% (BSFL5), 9.52% (BSFL10), and 19.06% (BSFL20) (Table 1). Diets were formulated in collaboration with Felleskjøpet Fôrutvikling AS using their optimization least-cost program based on the Dutch energy evaluation system [13]. Diets were formulated on net energy and standardized ileal digestibility (SID) values to be isoenergetic, balanced for digestible amino acids, and to meet or exceed the nutritional requirements for this age pigs [14]. Literature values from Finke [5] for the amino acid content in the BSFL meal were used in the diet formulation. Digestibility coefficients for the amino acids in the BSFL meal were set to 83%. Yttrium oxide ($Y_2O_3$) was included as an inert marker in the diets for digestibility calculations. Pelleted diets were produced by the Center for Feed Technology (ForTek, NMBU, Ås, Norway). The feed mash was ground in a Münch hammer mill (HM 21.115, Wuppertal, Germany) fitted with a 3 mm screen before pelleting. The mash was steam conditioned at 82 °C in a double-pass pellet-press conditioner (Münch-Edelstahl, Germany) before pelleting (Münch-Edelstahl, Germany, 2 × 17 kW) through a 3.5 mm die with a production rate of 700 kg/h. The pigs had *ad-libitum* access to the experimental diets immediately PW through automatic feeders (FRH-2 Domino A/S, Tørring, Denmark) with 43 cm feeding space. The automatic feeders were checked daily and refilled when needed. Feed residues were registered weekly and average daily feed intake (ADFI) per pen was calculated. Clean drinking water was always available from a drinking nipple next to the feeder.

Table 1
Dietary composition of experimental diets, calculated total crude protein (CP) content in diets, and calculated CP and crude fat (CF) from black soldier fly larvae (BSFL) meal.

| | Dietary treatments | | | |
|---|---|---|---|---|
| **Ingredients, g/kg as fed** | Control | BSFL5 | BSFL10 | BSFL20 |
| Wheat[a] | 507.7 | 502.0 | 496.6 | 485.1 |
| Barley[b] | 200.0 | 200.0 | 200.0 | 200.0 |
| Oats[c] | 50.0 | 50.0 | 50.0 | 50.0 |
| BSFL meal[d] | □□ | 47.6 | 95.2 | 190.6 |
| Soybean meal[e] | 70.8 | 59.7 | 48.2 | 25.5 |
| Soy protein concentrate[f] | 36.1 | 27.1 | 18.1 | □□ |
| Fish meal[g] | 34.1 | 25.5 | 17.1 | □□ |
| Rapeseed oil | 32.8 | 25.1 | 17.4 | 1.3 |
| AkoFeed Gigant 60[h] | 11.0 | 8.1 | 5.1 | □□ |
| Rapeseed meal[i] | 10.0 | 10.0 | 10.0 | 10.0 |
| Monocalcium phosphate | 12.9 | 12.0 | 11.2 | 9.5 |
| Limestone | 8.2 | 6.7 | 5.1 | 2.1 |
| Sodium chloride | 5.3 | 5.4 | 5.5 | 5.8 |
| Selenium premix | 0.9 | 0.9 | 0.8 | 0.8 |
| Iron(II)fumarate | 0.4 | 0.4 | 0.4 | 0.4 |
| Micro-mineral premix[j] | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamins[k] | 3.0 | 3.0 | 3.0 | 3.0 |
| L-Lysin | 6.8 | 6.8 | 6.9 | 6.9 |
| L-Methionine | 2.4 | 2.5 | 2.6 | 2.8 |
| L-Threonine | 2.8 | 2.9 | 2.9 | 3.0 |
| L-Valin | 1.1 | 0.8 | 0.5 | 0.0 |
| L-Tryptophan | 0.9 | 0.8 | 0.7 | 0.6 |
| Betaine | 0.7 | 0.7 | 0.7 | 0.7 |

| | Dietary treatments | | | |
|---|---|---|---|---|
| Yttrium(III)oxide | 0.1 | 0.1 | 0.1 | 0.1 |
| Calculated CP content | 181.0 | 181.0 | 181.0 | 181.0 |
| Ratio CP from BSFL (% of total CP) | 0 | 10 | 20 | 39 |
| Ratio CF from BSFL (% of total CF) | 0 | 19 | 39 | 69 |

[a]Chemical composition per kg: 867 g DM, 14 g ash, 124 g CP, 488 g starch, 14 g CF, 116 g NDF, 45 g ADF, 16 MJ.

[b]Chemical composition per kg: 858 g DM, 17 g ash, 92 g CP, 564 g starch, 11 g CF, 206 g NDF, 82 g ADF, 16 MJ.

[c]Chemical composition per kg: 858 g DM, 23 g ash, 88 g CP, 408 g starch, 44 g CF, 270 g NDF, 150 g ADF, 17 MJ.

[d]HiProMine S.A., Poznanska Str, Poland. Chemical composition per kg: 905 g DM, 84 g ash, 380 g CP, 289 g CF, 17.4 g Ca, 2.4 g Mg, 7.8 g total P, 2.7 g. Amino acids per kg: 27.8 g alanine, 16.7 g arginine, 33.2 g aspartic acid, 2.7 g cysteine, 43.8 g glutamic acid, 18.2 g glycine, 9.0 g histidine, 16.4 g isoleucine, 30.4 g leucine, 21.9 g lysine, 6.5 g methionine, 15.0 g phenylalanine, 20.6 g proline, 14.2 g serine, 15.1 g threonine, 20.3 g tyrosine, 21.5 g valine. Lauric acid (C12:0): 86.7 g/kg.

[e]Non-GMO soybean meal, Denofa AS, Fredrikstad, Norway. Chemical composition per kg: 876 g DM, 53 g ash, 426 g CP, 11 g CF, 156 g NDF, 128 g ADF, 17 MJ.

[f]AX3 Gastric, TripleA a/s, Hornsyld, Denmark.

[g]Nordsildmel AS, Egersund, Norway. Chemical composition per kg: 920 g DM, 147 g ash, 679 g CP, 101 g CF, 19 MJ.

[h]AAK AB, Malmö, Sweden.

[i]Expeller-pressed rapeseed cake, Mestilla, UAB, Klaipeda Lithuania. Chemical composition per kg: 928 g DM, 53 g ash, 342 g CP, 100 g CF, 223 g NDF, 179 g ADF, 20 MJ.

[j]"Mikro-Svin"; provided per kilogram of diet: 475 mg Ca; 3.4 mg Mg; 13.2 mg S; 120 mg Fe; 60 mg Mn; 120 mg Zn; 26 mg Cu; 0.6 mg I.

[k]Provided per kilogram of diet: 0.7 g Vitamin A; 1.2 g Vitamin E v5; 0.8 g Vitamin ADKB mix; 0.3 g Vitamin C (Stay C 35%).

The farm had an ongoing issue with edema disease and symptoms resulted in antibiotic treatment of five pigs on days 8–10 PW. After one pig died without any registered symptoms on day 11 PW, all pigs were treated with intramuscular antibiotic injections (Borgal vet., Ceva Santé Animale, France) for three consecutive days (11–13 PW). After treatment, all pigs appeared healthy throughout the experiment.

# Registrations and sample collection during the experiment

Fecal consistency was assessed daily and registered for all pens based on the four category scale developed by Pedersen and Toft [15]. The daily fecal score was registered as a pen average with 0.25 intervals on the scale. All pigs were weighed weekly and average daily gain (ADG) and feed conversion ratio (FCR) was calculated per pen. Fecal samples were also collected every week for the determination of the fecal DM. An equal amount of feces from each pig was pooled for the pen before oven drying at 103 °C for 24 h. Individual fecal samples were collected on days 21, 22, 25, 26, and 27 for the determination of apparent total tract digestibility (ATTD). Individual samples from all days were pooled, freeze-dried, and ground at 0.5 and 1 mm (Retsch ZM 100 centrifugal mill, Haan, Germany) before chemical analysis. Apparent digestibility of nutrients was calculated as described by Maynard and Loosli [16].

# Terminal sample collection

Only pigs from the pens with rubber mats (n = 3 pens per treatment, a total of 47 pigs) were included in the terminal sampling. Pigs were fasting from the evening before but had access to feed three hours before euthanasia. Euthanasia was done using a captive bolt pistol followed by exsanguination. Immediately after exsanguination, the abdominal cavity was opened, and the GIT removed. Intestinal content was collected from the oral part of the jejunum and the top of the colon spiral. Tissue from oral jejunum, aboral ileum, and top of the colon spiral were collected for histological assessment. Intestinal content from the last two meters of the small intestine was collected and stored at -20 °C for the determination of apparent ileal digestibility (AID). This sample was unfortunately not collected from seven of the pigs (three control pigs, two pigs fed BSFL10, and two pigs fed BSFL20). The ileal contents were later freeze-dried, homogenized using a batch mill (A11 basic Analytical mill, IKA®, England) and chemically analyzed. Liver weight was recorded to calculate liver index (liver weight, kg / live body weight, kg * 100).

Table 2
Analyzed chemical composition of dietary treatments.

| | Dietary treatments | | | |
|---|---|---|---|---|
| **Nutrients, g/kg DM** | Control | BSFL5 | BSFL10 | BSFL20 |
| Dry matter, g/kg | 890.3 | 888.9 | 887.7 | 893.0 |
| Ash | 54.3 | 52.3 | 52.5 | 52.9 |
| Crude protein | 196.8 | 196.1 | 199.8 | 206.8 |
| Starch | 512.5 | 523.8 | 504.1 | 488.0 |
| Crude fat | 69.3 | 79.7 | 78.6 | 89.1 |
| NDF | 124.1 | 122.9 | 118.3 | 131.0 |
| ADF | 52.9 | 52.0 | 50.2 | 53.8 |
| Gross energy (MJ/kg) | 19.5 | 19.6 | 19.8 | 19.9 |
| Phosphorus | 7.4 | 7.3 | 7.6 | 7.0 |

# Chemical analyses

Pooled feed samples, collected during the experiment for each diet, were ground at 0.5 and 1 mm (Fritsch Pulverisette 19 cutting mill, Idar-Oberstein, Germany) before the chemical composition of nutrients were analyzed in triplicates. The analyzed chemical composition of the dietary treatments can be found in Table 2. Analyzed amino acid and fatty acid composition of the diets are shown in Table 3 and Table 4, respectively. The chemical analyses were performed by the LabTek group at the Department of Animal and Aquacultural Science, NMBU, Ås, Norway. In brief, DM was determined by drying to constant weight at 103 °C ± 2 °C [17], and ash was determined by complete combustion at 550 °C for at least 4 h [18]. Gross energy (GE) content was determined using a PARR 6400 Automatic Isoperibol Calorimeter (Parr Instruments, Moline, IL, USA) according to ISO 9831 [19]. Crude protein (CP) was analyzed with the Kjeldahl method (AOAC Official Method 2001.11), using a Digestor™ 2520 (FOSS Analytical, Hillerød, Denmark) and the Kjeltec™ 8400 analyzer (FOSS Analytical, Hillerød, Denmark). Crude fat (CF) was analyzed using Accelerated Solvent Extraction (ASE 350, Thermo Fisher Scientific Inc.). Extraction was conducted with 80% petroleum ether and 20% acetone at 125 °C. Starch was determined using an enzymatic-colorimetric method according to McCleary et al. [20], with some modifications. In brief, starch was degraded with heat-stable α-amylase and amyloglucosidase-enzymes to glucose. Glucose concentration was then determined using a spectrophotometer (RX Daytona +, Randox Laboratories Ltd., UK). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to the manufacturer's methods using the Ankom200 Fiber Analyzer (ANKOM Technology, Macedon, New York, USA). Amino acids in diets and ileal samples were analyzed according to Commission Regulation (EC) No 152/2009. Amino acids were analyzed on a Biochrom 30 + Amino Acid Analyzer with an autosampler (Biochrom Ltd., Cambridge, UK). Tryptophan was analyzed in diets on a Dionex Ultimate 3000 HPLC

system (Dionex Softron GmbH, Germering, Germany) with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). The fatty acid composition of the diets was analyzed according to O'fallon et al. [21] by synthesizing the fatty acids to fatty acid methyl esters (FAME), in which concentrations were determined using a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, US). Total phosphorus was analyzed after combustion and acid digestion (Commission Regulation (EC) No 152/2009) using a commercial spectrophotometric kit (PH8328, Randox laboratories, County Antrim, UK). Yttrium concentration was determined after acid decomposition in a microwave digestion system (Start D, Milestone Srl, Italy), using a microwave plasma atomic emission spectrometer (MP-AES 4200, Agilent Technologies, US).

Table 3
Analyzed amino acid composition of dietary treatments.

| | Dietary treatments | | | |
|---|---|---|---|---|
| **Indispensable AA, g/16 g N[a]** | Control | BSFL5 | BSFL10 | BSFL20 |
| Arginine | 4.9 | 4.9 | 4.6 | 4.2 |
| Histidine | 2.1 | 2.2 | 2.2 | 2.3 |
| Isoleucine | 3.2 | 3.3 | 3.2 | 3.3 |
| Leucine | 6.0 | 6.0 | 5.9 | 5.9 |
| Lysine | 6.7 | 7.2 | 6.6 | 6.9 |
| Methionine | 2.3 | 2.4 | 2.3 | 2.5 |
| Phenylalanine | 3.8 | 3.7 | 3.6 | 3.8 |
| Threonine | 4.1 | 4.3 | 4.0 | 4.3 |
| Tryptophan | 1.4 | 1.2 | 1.4 | 1.4 |
| Valine | 4.0 | 4.1 | 3.9 | 4.1 |
| **Dispensable AA, g/16 g N** | | | | |
| Alanine | 3.3 | 3.6 | 3.7 | 4.1 |
| Aspartic acid | 6.8 | 6.9 | 6.5 | 6.4 |
| Cysteine | 1.3 | 1.3 | 1.2 | 1.1 |
| Glutamic acid | 19.9 | 19.1 | 18.7 | 18.2 |
| Glycine | 3.3 | 3.4 | 3.3 | 3.3 |
| Proline | 5.9 | 5.9 | 6.0 | 6.3 |
| Serine | 3.7 | 3.7 | 3.6 | 3.5 |
| Tyrosine | 1.7 | 1.9 | 2.2 | 3.1 |
| Total amino acids, g/16 g N | 83.1 | 83.8 | 81.6 | 83.5 |

[a]Determined using water-corrected molecular weights

Table 4
Analyzed fatty acid composition of dietary treatments, and calculated sum of saturated-, monounsaturated-, and polyunsaturated fatty acids.

| | Dietary treatments | | | |
|---|---|---|---|---|
| **Fatty acids, g/kg DM** | Control | BSFL5 | BSFL10 | BSFL20 |
| C12:0 | 0.40 | 6.11 | 11.7 | 23.1 |
| C14:0 | 0.42 | 1.35 | 2.33 | 4.25 |
| C15:0 | 0.04 | 0.06 | 0.07 | 0.09 |
| C16:0 | 13.3 | 12.4 | 11.4 | 9.96 |
| C17:0 | 0.07 | 0.08 | 0.10 | 0.13 |
| C18:0 | 4.31 | 3.49 | 2.76 | 1.54 |
| C20:0 | 0.24 | 0.20 | 0.15 | 0.09 |
| C21:0 | 0.01 | 0.09 | 0.28 | 0.32 |
| C22:0 | 0.14 | 0.11 | 0.08 | 0.04 |
| C23:0 | 0.02 | 0.01 | 0.01 | - |
| C24:0 | 0.05 | 0.04 | 0.03 | 0.02 |
| Sum saturated fatty acids | 19.02 | 23.95 | 28.89 | 39.57 |
| C14:1 | - | 0.02 | 0.04 | 0.08 |
| C16:1 | 0.40 | 0.58 | 0.76 | 1.13 |
| C20:1 | 0.89 | 0.73 | 0.55 | 0.22 |
| C24:1 | 0.11 | 0.08 | 0.04 | 0.03 |
| Sum monounsaturated fatty acids | 1.40 | 1.41 | 1.39 | 1.46 |
| C18:1n9t | 0.46 | 0.37 | 0.21 | 0.04 |
| C18:1n9c | 25.9 | 22.8 | 19.0 | 12.2 |
| C18:2n6c | 21.1 | 22.0 | 21.7 | 21.1 |
| C18:3n3 | 4.56 | 4.15 | 3.56 | 2.39 |
| C18:3n6 | 0.13 | 0.11 | 0.08 | 0.02 |
| C20:2 | 0.13 | 0.11 | 0.09 | 0.05 |
| C20:4n6 | 0.02 | 0.02 | 0.01 | - |
| C20:5n3 | 0.30 | 0.23 | 0.16 | - |

| | Dietary treatments | | | |
|---|---|---|---|---|
| C21:1n9 | 0.05 | 0.05 | 0.04 | 0.02 |
| C22:2 | 0.04 | 0.02 | 0.02 | 0.02 |
| C22:5n3 | 0.03 | 0.02 | 0.01 | - |
| C22:6n3 | 0.36 | 0.30 | 0.20 | - |
| Sum polyunsaturated fatty acids | 53.06 | 50.17 | 45.04 | 35.89 |

Trypsin and lipase activities were analyzed in jejunal content. 1.5 ml (600 µl for lipase analysis) ice-cold Milli-Q water were added to approximately 100 mg of the jejunal content, homogenized using a bead mill (TissueLyser, Qiagen Retsch, Haan, Germany) and sonicated in an ice-cold bath for three minutes (T 460/H, Elma Schmidbauer GmbH, Ransbach-Baumbach, Germany). After centrifugation at 21,100 × g for 10 min at 4 °C, the supernatant was collected, aliquoted, stored at -80 °C, and used for the analysis of lipase, trypsin, and total protein. Total protein concentration was determined in microtiter assay according to the Quick Start™ Bradford Protein Assay protocol (Bio-Rad Laboratories, Oslo, Norway). Absorbance was measured using a SpectraMax M2e Microplate Reader (Molecular Devices, LLC., San Jose, USA). Lipase and trypsin activity were analyzed using commercial kits (Lipase Activity Assay Kit III, MAK048-1KT, fluorometric, Sigma-Aldrich, Merck KGaA; Trypsin Activity Assay Kit, ab102531, colorimetric, Abcam), according to the manufacturer's protocols.

Short-chain fatty acids (SCFA) were analyzed in colon content. Samples were thawed on ice and 500 mg of the colon content were mixed with 500 µl ice-cold internal standard solution (2-methyl valeric acid in 5% formic acid), before sonication for 5 min in cold water. After centrifugation for 15 min at 4 °C with 15000 × g, the supernatant was transferred to a spin column (45 kDa; VWR International, USA), and again centrifuged with the same parameters. SCFA concentration was determined by capillary gas chromatography on a stabilwax-DA, 30 m × 0.25 mm × 0.25 µm capillary column (Restek corporation, PA, USA) installed on a Trace 1300 gas chromatograph equipped with an AS 1310 autosampler, split injection, a flame ionization detector and Chromeleon software (Thermo Scientific, MA, USA). The initial oven temperature was 90 °C, held for 2 min, followed by a temperature increase of 10 °C/min to 150 °C and 50 °C/min to 250 °C and then held for 1 min. Helium was used as the carrier gas at a flow rate of 3 mL/min. The injector temperature was set at 260 °C and the detector temperature was set at 275 °C.

# Morphology and morphometry

Jejunal, ileal, and colon morphology were blindly assessed and villus heights (VH) and crypt depths (CD) measured by Aquamedic AS, Oslo, Norway. Gross pathological observations were recorded before tissue sampling. Samples were fixated in 10% formalin up to 48 h before processing following standard histological methods for gut tissue. Sections were stained with hematoxylin and eosin (H&E) and evaluated by light microscopy, where the evaluation was done on morphological characteristics such as

epithelial cell and barrier morphology and integrity, crypt changes such as hyperplasia, dilation or abscessation, degenerative and inflammatory mucosal changes including increased numbers of intraepithelial lymphocytes (IELs) and infiltration by leucocytes. Methodologies of the evaluation protocol are described by Day et al. [22] and Pérez de Nanclares et al. [23]. The morphological characteristics evaluated were graded using a semi-quantitative scoring system where score 0 is normal, score 1 represent mild changes, and score 2, 3, and 4 represent moderate, marked, and severe changes, respectively. VH and CD measurements were made on scanned whole-section images of the respective tissues captured using the PreciPoint M8 Microscope and Scanner (PreciPoint, Freising, Germany), and obtained using the ViewPoint software (PreciPoint, Freising, Germany). A minimum of three well-oriented crypts and villi were measured from each of the sections.

# Extraction of DNA and 16S rRNA sequencing

Total DNA was extracted from bacteria in approximately 190 mg of colon content using QIAamp Fast DNA Stolen Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. The DNA concentration was determined using NanoDrop™ 8000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). All samples were stored at -20 ºC until further analysis. Before preparing for 16S rRNA sequencing, samples were normalized to 10 ng/µL. The V3-V4 regions of the bacterial 16S rRNA gene were amplified using the primers Pro341f (5'-CCTACGGGNBGCASCAG-3') and Pro805r (5'-GACTACNVGGGTATCTAATCC-3'). The library preparation was conducted using the Miseq Reagent Kit V3 (Illumina, San Diego, California, USA) according to the Illumina 16 S Metagenomic Sequencing Library Preparation protocol (Illumina, San Diego, California, USA). For indexing, Nextera XT index kit V2 was used (Illumina, San Diego, California, USA). After the indexing reaction, all samples were measured by Qubit Fluorometer (Invitrogen, Carlsbad, California, USA) using Qubit™ 1X dsDNA HS Assay Kit (Invitrogen, Carlsbad, California, USA). An equal amount of each sample was pooled together, and spiked with 5% PhiX Control (Illumina, San Diego, Waltham, MA, USA). For sequencing, 10 pm of the pooled sample was loaded to a flow cell. The sequencing analysis was performed on the Miseq System (Illumina, San Diego, California, USA). The clustering density was 1256 k/mm2 and 88.3% of clusters were passing filter.

Raw sequence data were analyzed using DADA2 v. 1.12.1 [24] in R v. 3.5.0 [25]. Default parameters were used if other is not specified. In brief, primers were removed, and the forward reads were truncated at 280 bp and the reverse reads at 250 bp. Max expected errors were set to 7. This allowed 73% of the reads to pass the quality control. Error rates were estimated, and the core sample inference algorithm applied. The denoised read pairs were merged, and chimeras removed. The Silva v. 138 database [26, 27] was used as a reference database for the taxonomy assignment. A phyloseq object was built with the phyloseq v.1.26.1 for further analyses [28]. Figures were made with ggplot2 v.3.2.1 [29].

# Statistical analysis

Outliers in the data were identified using the interquartile range (IQR) method. Outliers were defined as > Q3 + 3* IQR or < Q1 + 3*IQR. Statistical analysis was performed using R v.3.6.1 [25] in Rstudio v.1.2.5001

[30]. A mixed procedure was run, using the lme4 1.1–21 [31] and lmerTest 3.1-1 [32] packages. The following model was used for all individual data: $Y_{ijklmn} = \mu + diet_i + sex_j + bedding_k + litter_l + pen_m + \varepsilon_{ijklmn}$ where Y is one observation on pig n; μ is the intercept; $diet_i$ is the fixed dietary treatment effect (i = 1:4); $sex_j$ is the fixed effect of the sex of the pig (j = 1,2); $bedding_k$ is the fixed effect of bedding material, rubber mat or wood shavings (k = 0,1); $litter_l$ is the random effect of the lth litter (l = 1:11); $pen_m$ is the random effect of the pen (m = 1:20) and $\varepsilon_{ijklmn}$ is a random residual. The bedding was not included in the model when analyzing the terminal sampling parameter, as it was constant. For all pen-level parameters, the following model was used with the lm function in the stats package [25]: $Y_{ikn} = \mu + diet_i + bedding_k + \varepsilon_n$ where Y now is one observation on pen n. Results are presented as mean values and standard error of the mean (SEM). In addition, linear regression with the dietary inclusion level of BSFL as the only explanatory variable was run with the lm function for all dependent variables. For the effects of sex and bedding material, only significant results are presented. Effects are considered statistically significant when $P \leq 0.05$ and tendencies are defined as P-values between 0.05 and 0.10.

To assess diversity in the microbial communities within pigs, Shannon alpha-diversity indices were calculated at the amplicon sequence variant (ASV) level. A comparison of the Shannon indices between dietary treatments was done using the Kruskal-Wallis Rank Sum Test in the stats package [25]. The beta-diversity (between pig variation in microbial communities) was assessed by principal coordinate analyses (PCoA) with the Bray-Curtis, unweighted, and weighted UniFrac distance matrices. Beta dispersion (variances) was calculated within each dietary group and a permutation-based test of multivariate homogeneity applied (vegan package v.2.6-6; Oksanen et al. [33]). Fulfilling the assumption of homogeneity in group variance, a PERMANOVA test was performed on the distance matrices with the dietary group as the independent variable. Pairwise PERMANOVA tests were performed to compare beta diversity between dietary treatments. P-values were adjusted for multiple testing with the method by Benjamini and Hochberg [34], also known as the false discovery rate.

The relative abundance was calculated, and the statistical difference in relative abundance between dietary treatments was analyzed with the Kruskal-Wallis test with dietary treatment as the explanatory variable.

To test for a statistical difference in relative abundance between dietary treatments, the Kruskal-Wallis test with dietary treatment as the explanatory variable was applied. Two-sample Wilcoxon tests, also known as the Mann-Whitney test, was used for pairwise comparison between the diets if the Kruskal Wallis test gave a significant effect of dietary treatment. P-values were corrected for multiple testing with the Benjamini and Hochberg [34] method.

To test for covariation between the microbiota and SCFA profile, dissimilarity indices with the Bray-Curtis indices were calculated separately for the microbiota and SCFA data with the vegdist function, and the covariation tested with the mantel function in the vegan package [33].

# Results

# Growth performance

Inclusion of BSFL meal did not significantly affect ADG, ADFI, or FCR in the experimental period (Table 5). The ADFI ranged from 650 to 712 g, whereas the ADG ranged from 496 to 552 g for pigs fed increased BSFL during the total period (0–27 days). There was a tendency for lower FCR for day 0–14 PW with increasing inclusion levels of the BSFL meal ($R^2$ = 0.194). Pigs in pens with wood shavings had significantly higher ADG (P = 0.040) for day 14–27 PW.

Table 5
Effect of dietary treatment on growth performance parameters (N = 20).

| | | Dietary treatments | | | | | P-value | |
|---|---|---|---|---|---|---|---|---|
| **Day PW** | | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| 0–14 | ADFI | 485 | 448 | 459 | 464 | 9 | 0.644 | 0.714 |
| | ADG | 432 | 392 | 425 | 427 | 7 | 0.177 | 0.702 |
| | FCR | 1.15 | 1.14 | 1.08 | 1.09 | 0.01 | 0.097 | 0.059 |
| 14–27 | ADFI | 943 | 867 | 918 | 917 | 15 | 0.389 | 0.941 |
| | ADG | 683 | 608 | 661 | 636 | 15 | 0.262 | 0.559 |
| | FCR | 1.39 | 1.44 | 1.39 | 1.45 | 0.01 | 0.538 | 0.426 |
| 0–27 | ADFI | 712 | 650 | 680 | 682 | 11 | 0.324 | 0.775 |
| | ADG | 552 | 496 | 538 | 528 | 9 | 0.114 | 0.758 |
| | FCR | 1.27 | 1.31 | 1.26 | 1.29 | 0.01 | 0.490 | 0.838 |

# General intestinal function

There were no significant differences for pH in stomach (N = 47; ANOVA P = 0.920; regression P = 0.658; SEM = 0.2) or pH in jejunum (N = 47; ANOVA P = 0.603; regression P = 0.612; SEM = 0.1) between the dietary treatments. Numerical means for pH measurements were; control: 4.5 and 4.7; BSFL5: 4.7 and 4.5; BSFL10: 4.3 and 4.8; BSFL20: 4.4 and 4.4, for stomach and jejunum, respectively.

There were no significant differences in fecal DM (Table 6) or fecal score (Table 7) between the dietary treatments. BSFL5 had the overall highest fecal score and BSFL10 the overall lowest fecal scores. The graph of the overall development in the fecal score during the experimental period (Supplementary Fig. 1) shows an increased score (> 2) in the first week PW, which seems to stabilize below score two after day 12, but again increasing to score > 2 on day 23–25. Pens with wood shavings had significant lower fecal scores in week 3 (P = 0.049), and numerical lower average fecal score all experimental weeks.

Table 6
Effect of dietary treatment on fecal dry matter (DM) (N = 20).

| | Dietary treatments | | | | P-value | |
|---|---|---|---|---|---|---|
| **Fecal DM, %** | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| Day 7 | 26.6 | 25.7 | 25.3 | 24.3 | 0.7 | 0.725 | 0.235 |
| Day 14 | 27.8 | 26.1 | 26.9 | 26.3 | 0.3 | 0.212 | 0.230 |
| Day 21 | 26.3 | 26.2 | 26.1 | 24.7 | 0.4 | 0.414 | 0.105 |
| Day 27 | 26.7 | 26.3 | 27.3 | 26.7 | 0.4 | 0.897 | 0.872 |
| Total average | 26.8 | 26.1 | 26.4 | 25.5 | 0.3 | 0.500 | 0.159 |

Table 7
Effect of dietary treatment on fecal score (N = 20).

| | Dietary treatments | | | | P-value | |
|---|---|---|---|---|---|---|
| **Fecal Score** | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| Week 1 | 1.82 | 1.87 | 1.87 | 1.84 | 0.06 | 0.992 | 0.952 |
| Week 2 | 1.83 | 1.99 | 1.88 | 1.91 | 0.06 | 0.800 | 0.855 |
| Week 3 | 1.73 | 1.78 | 1.56 | 1.72 | 0.06 | 0.586 | 0.817 |
| Week 4 | 1.86 | 2.00 | 1.86 | 1.95 | 0.05 | 0.760 | 0.775 |
| Overall period | 1.82 | 1.92 | 1.80 | 1.86 | 0.05 | 0.811 | 0.960 |

# Digestibility and enzymatic activity

There were no significant differences in jejunal trypsin (N = 42; ANOVA P = 0.856; regression P = 0.380; SEM = 0.14) or lipase (N = 45; ANOVA P = 0.988; regression P = 0.769; SEM = 0.01) activity between the dietary treatments. The highest numerical enzymatic activities were seen in BSFL10, and the lowest in BSFL20. Numerical means for trypsin activity were 1.04, 0.90, 1.06, 0.68 mU/µg protein, and for lipase activity 0.07, 0.07, 0.08 and 0.06 mU/mg protein, for the control, BSFL5, BSFL10 and BSFL20 diet, respectively.

No significant differences between dietary treatments were found in AID of the main nutrients given by the ANOVA (Table 8). The mean AID of CP ranged from 65.9 to 69.7 among the dietary groups, but no linear relationship with increased BSFL was seen. A significant linear regression was, however, found between increased inclusion of BSFL and increasing AID of CF ($R^2$ = 0.108) and ash ($R^2$ = 0.106). A significant effect of sex was found for AID of CP (P = 0.026), CF (P = 0.007) and ash (P = 0.025), which all where higher for gilts compared to barrows.

Table 8
Effect of dietary treatment on apparent ileal digestibility (AID) coefficients.

| | | Dietary treatments | | | | | P-value | |
|---|---|---|---|---|---|---|---|---|
| **AID, %** | N | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| Dry matter | 39 | 71.1 | 67.4 | 68.0 | 68.0 | 1.0 | 0.536 | 0.507 |
| Crude protein | 39 | 69.7 | 65.9 | 66.6 | 67.6 | 1.4 | 0.728 | 0.838 |
| Starch | 40 | 97.4 | 94.9 | 95.3 | 95.8 | 0.5 | 0.434 | 0.586 |
| Crude fat | 40 | 81.7 | 81.7 | 84.8 | 86.8 | 1.0 | 0.491 | 0.038 |
| Ash | 37 | 30.9 | 28.1 | 26.6 | 40.7 | 2.0 | 0.147 | 0.049 |

There was a significant difference in AID of tyrosine between the dietary treatments. A tendency for increased AID with increasing inclusion of BSFL was found for alanine ($R^2$ = 0.085) and proline ($R^2$ = 0.079), whereas AID of lysine ($R^2$ = 0.087) and tyrosine ($R^2$ = 0.080) tended to decrease with increased inclusion of BSFL. For the AID of the other amino acids and total amino acids, no significant differences were found between the dietary treatments (Table 9). A significant effect of sex was found for alanine (P = 0.009), arginine (P = 0.004), aspartic acid (P = 0.004), cysteine (P = 0.046), histidine (P = 0.005), isoleucine (P = 0.003), leucine (P = 0.004), lysine (P < 001), methionine (P = 0.007), phenylalanine (P = 0.005), serine (P = 0.003), threonine (P = 0.005), valine (P = 0.007) and total amino acids (P = 0.020). All higher for gilts compared to barrows.

Table 9
Effect of dietary treatment on apparent ileal digestibility (AID) coefficients for amino acids (AA).

| | | Dietary treatments | | | | P-value | |
|---|---|---|---|---|---|---|---|
| **AID of AA[a], %** | N | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| **Indispensable AA** | | | | | | | | |
| Arginine | 40 | 81.1 | 80.2 | 78.4 | 78.2 | 0.8 | 0.295 | 0.164 |
| Histidine | 39 | 78.3 | 76.3 | 74.1 | 75.7 | 0.6 | 0.174 | 0.190 |
| Isoleucine | 40 | 77.8 | 75.7 | 73.8 | 77.5 | 0.8 | 0.476 | 0.918 |
| Leucine | 40 | 79.0 | 76.8 | 75.2 | 78.1 | 0.8 | 0.476 | 0.906 |
| Lysine | 40 | 86.5 | 80.2 | 78.4 | 78.2 | 0.8 | 0.234 | 0.065 |
| Methionine | 40 | 87.3 | 87.6 | 85.5 | 88.4 | 0.5 | 0.252 | 0.502 |
| Phenylalanine | 40 | 79.5 | 76.0 | 73.6 | 77.8 | 0.8 | 0.185 | 0.740 |
| Threonine | 40 | 75.8 | 75.2 | 73.8 | 76.4 | 0.9 | 0.784 | 0.775 |
| Valine | 40 | 79.2 | 76.3 | 74.6 | 77.2 | 0.9 | 0.431 | 0.628 |
| **Dispensable AA** | | | | | | | | |
| Alanine | 40 | 69.2 | 67.3 | 68.3 | 74.0 | 1.2 | 0.330 | 0.068 |
| Aspartic acid | 40 | 67.5 | 65.5 | 66.7 | 69.9 | 1.3 | 0.687 | 0.331 |
| Cysteine | 40 | 63.7 | 56.4 | 60.4 | 60.0 | 1.9 | 0.558 | 0.865 |
| Glutamic acid | 40 | 81.1 | 77.0 | 79.7 | 81.3 | 1.1 | 0.480 | 0.519 |
| Glycine | 40 | 42.5 | 39.7 | 43.2 | 53.1 | 3.2 | 0.453 | 0.152 |
| Proline | 39 | 63.5 | 66.7 | 73.2 | 74.2 | 2.2 | 0.246 | 0.083 |
| Serine | 40 | 73.5 | 70.9 | 70.6 | 71.5 | 1.0 | 0.681 | 0.692 |
| Tyrosine | 40 | 57.6 | 50.3 | 41.1 | 48.1 | 1.7 | 0.008 | 0.078 |
| Total amino acids | 40 | 75.0 | 73.3 | 73.4 | 75.6 | 1.0 | 0.745 | 0.655 |

[a]Determined using water-corrected molecular weights

The ATTD of CP, starch, CF, phosphorus, and ash significantly differed between the dietary treatments (Table 10). The ATTD of CP decreased with increasing inclusion of BSFL ($R^2$ = 0.056), whereas by contrast, the ATTD of CF increased with increasing inclusion of BSFL ($R^2$ = 0.147). The starch ATTD was overall high for all diets (> 99.5%) and pigs fed the BSFL20 obtained the highest ATTD of starch. The

ATTD of phosphorus increased with increasing inclusion of BSFL ($R^2$ = 0.153). A linear increase in ATTD of ash was also found for increasing inclusion levels of BSFL ($R^2$ = 0.067).

Gilts had significantly higher ATTD of CP (P = 0.035), starch (P = 0.015) and phosphorus (P = 0.005). Using wood shavings as bedding material in the pens gave significantly higher ATTD of CP (P = 0.035), CF (P = 0.016), ADF (P = 0.016) and ash (P = 0.030).

Table 10
Effect of dietary treatment on apparent total tract digestibility (ATTD) coefficients.

| | | Dietary treatments | | | | | P-value | |
|---|---|---|---|---|---|---|---|---|
| **ATTD, %** | N | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| Dry matter | 79 | 83.4 | 82.8 | 83.0 | 82.7 | 0.2 | 0.743 | 0.308 |
| Crude protein | 79 | 79.6 | 78.4 | 77.5 | 77.3 | 0.4 | 0.045 | 0.035 |
| Starch[a] | 79 | 99.7 | 99.7 | 99.8 | 99.8 | 0.02 | 0.045 | 0.079 |
| Crude fat | 79 | 75.7 | 78.0 | 79.4 | 80.0 | 0.4 | 0.026 | < 0.001 |
| ADF | 78 | 30.3 | 27.9 | 28.4 | 29.2 | 1.1 | 0.897 | 0.858 |
| NDF | 77 | 39.9 | 36.9 | 37.5 | 39.4 | 0.7 | 0.565 | 0.870 |
| Phosphorus | 79 | 51.3 | 51.5 | 55.3 | 56.4 | 0.6 | < 0.001 | < 0.001 |
| Ash | 79 | 58.7 | 57.4 | 57.6 | 61.2 | 0.5 | 0.043 | 0.022 |
| Energy | 79 | 82.6 | 82.0 | 82.3 | 81.8 | 0.2 | 0.676 | 0.274 |

[a]26 feces samples had starch levels below lower detection limit

# Intestinal morphology, morphometry and liver index

Macroscopic evaluation of the intestinal tissue during sampling revealed some changes, mostly in jejunum and colon, with reddening of the mucosa, and low muscle tone and no mucosal folding in the jejunum (Table 11, Fig. 1). The macroscopic appearance was distributed equally with no clear differences between the pigs fed the control diet and those fed increasing levels of BSFL. However, most macroscopic changes were found in the pigs fed BSFL5.

In the jejunum, morphological changes such as lamina propria lymphocyte infiltration, lamina propria edema, submucosal edema, intraepithelial lymphocyte numbers, and enterocyte vacuolization were present in several samples. The distribution of these main morphological changes showed no notable differences between the dietary treatments.

In the ileum, tissue sections were observed to have a normal and healthy mucosal appearance.

In the colon, mild to moderate inflammatory morphological changes were observed. The inflammation was largely characterized by an increased content of lymphocytes and plasma cells in the inter-crypt compartment (Fig. 2). Other notable changes observed were mild crypt dilatation as well as a few samples showing crypt abscessation characterized by an accumulation of sloughed-off epithelial cells and neutrophils. Pigs fed the control diet were all scored “mild” or “moderate” changes for inter-crypt area lymphocyte infiltration, whereas pigs fed the highest BSFL inclusion level had the least number affected with 6 out of 12 scored “normal”. For colon crypt dilatation, some pigs were scored “mild”, but no remarkable differences between the dietary treatments were found. Dietary treatment did not influence VH, CD, or VH:CD ratio in jejunum, ileum, or colon, as shown in Table 12.

Table 11
Summary of the main macroscopic findings.

| | **Dietary treatments** | | | |
|---|---|---|---|---|
| **Macroscopic findings**[a] | Control | BSFL5 | BSFL10 | BSFL20 |
| Reddening of the jejunal mucosa | 3 | 3 | 1 | 2 |
| Jejunum with low muscle tone and no mucosal foldings | 2 | 4 | 2 | 1 |
| Reddening of the ileal mucosa | | 1 | 2 | 1 |
| Reddening of the colonic mucosa | 5 | 6 | 4 | 6 |

[a]Numbers represent total samples observed with specific changes (N = 47).

Table 12
Effect of dietary treatment on intestinal morphometry (N = 45).

| | Dietary treatments | | | | | P-value | |
|---|---|---|---|---|---|---|---|
| **Morphometry, µm** | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| **Jejunum** | | | | | | | |
| VH | 454 | 457 | 472 | 465 | 13 | 0.976 | 0.783 |
| CD | 413 | 389 | 368 | 398 | 12 | 0.717 | 0.736 |
| VH:CD ratio | 1.23 | 1.25 | 1.37 | 1.30 | 0.06 | 0.916 | 0.631 |
| **Ileum** | | | | | | | |
| VH | 232 | 233 | 250 | 247 | 7 | 0.669 | 0.334 |
| CD | 134 | 148 | 150 | 137 | 4 | 0.588 | 0.954 |
| VH:CD ratio | 1.92 | 1.64 | 1.75 | 1.96 | 0.6 | 0.276 | 0.420 |
| **Colon** | | | | | | | |
| CD | 486 | 479 | 480 | 482 | 9 | 0.990 | 0.927 |

There were no significant differences in liver index (N = 44; ANOVA P = 0.557; regression P = 0.221; SEM = 0.04) between the dietary treatments. Numerical means for liver index were 2.65, 2.58, 2.64 and 2.74 for the control, BSFL5, BSFL10 and BSFL20, respectively. Barrows had higher liver index compared to gilts (P = 0.007).

# SCFA and microbial community in the colon

There was no difference in the SCFA profile in the colon between the dietary treatments (Table 13). However, the control diet had the numerical highest level of butyric acid and the highest numerical level of total SCFA. The SCFA profile differed between sexes. Barrows had higher levels of acetic- (P = 0.035) and propionic acid (P = 0.002), but lower levels of isobutyric acid (P = 0.045), isovaleric acid (P = 0.047) and of total SCFA (P = 0.030) compared to gilts.

Table 13
Effect of dietary treatment on short-chain fatty acid (SCFA) and ammonia concentration in colon content.

| | | Dietary treatments | | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| **SCFA, µmol/g** | N | Control | BSFL5 | BSFL10 | BSFL20 | SEM | ANOVA | Regression |
| Acetic acid | 47 | 56.5 | 51.9 | 52.7 | 52.1 | 1.63 | 0.708 | 0.474 |
| Butyric acid | 47 | 13.5 | 10.4 | 12.4 | 12.6 | 0.62 | 0.221 | 0.978 |
| Isobutyric acid | 47 | 0.87 | 0.69 | 0.87 | 0.78 | 0.06 | 0.643 | 0.839 |
| Isovaleric acid | 47 | 1.05 | 0.79 | 1.11 | 0.92 | 0.08 | 0.400 | 0.860 |
| Propionic acid | 47 | 25.6 | 21.8 | 24.2 | 24.5 | 0.84 | 0.438 | 0.921 |
| Valeric acid | 46 | 1.96 | 1.75 | 1.70 | 1.77 | 0.11 | 0.889 | 0.622 |
| Total SCFA | 47 | 99.8 | 87.4 | 93.0 | 92.3 | 2.68 | 0.437 | 0.602 |
| Ammonia, mM | 47 | 3.00 | 2.72 | 2.87 | 2.70 | 0.08 | 0.574 | 0.327 |

There was no difference in alpha diversity indices in the colon of piglets fed the different dietary treatments (Fig. 3a; N = 45, P = 0.355) as measured by the Shannon diversity index. Beta-diversity was assessed by several distance methods (Bray-Curtis, unweighted and weighted UniFrac). There were no differences in colon microbiota variance (beta dispersion) between the dietary treatments with neither of the distance methods. The PERMANOVA test showed significant effect of dietary treatment for the Bray-Curtis ($R^2$ = 0.094; P = 0.029) and unweighted UniFrac ($R^2$ = 0.082; P = 0.041) distances, but not for the weighted UniFrac distances ($R^2$ = 0.084; P = 0.203). However, when adjusted for multiple testing, the pairwise PERMANOVA tests gave no significant differences between any of the dietary treatments. The PCoA plot using Bray-Curtis distances (Panel b in Fig. 3) shows no clear grouping of dietary treatments.

At the phylum level, the colon microbiota was dominated by Bacteroidota and Firmicutes (Fig. 3, Panel c). The relative abundance of Bacteroidota significantly differed between the dietary treatments (P = 0.026), whereas a tendency was found for the Firmicutes (P = 0.055). The Bacteroidota and Firmicutes were contributing 46.3% and 44.2% to the control group, 51.0% and 39.4% to the BSFL5 group, 50.0% and 42.1% to the BSFL10 group, and 48.1% and 38.9% to the BSFL20 group, respectively. There was also a significant effect of dietary treatment on Campilobacteria (P = 0.042) and Thermoplasmatota (P = 0.002), but the relative abundance of these two phyla were low in all groups (Campilobacteria: 1.0% in Control, 1.5% in BSFL5, 0.6% in BSFL10 and 0.5% in BSFL20; Thermoplasmatota: <0.01% in all groups).

Figure 4 shows the relative abundance of the top 10 most abundant genera in the colon. *Prevotella* was the most abundant genus, contributing 15.2%, 18.2%, 14.0% and 13.6% to the colon microbiome in the pigs fed control, BSFL5, BSFL10, and BSFL20, respectively. A significant effect of dietary treatment was found for the relative abundance of *Lactobacillus*, where the control pigs had a significantly higher

relative abundance of *Lactobacillus* compared to the pigs fed the BSFL20 diet. A significant effect of dietary treatment was also found for the *Rikenellaceae RC9* gut group, where higher relative abundance was found in the colon of pigs fed the BSFL10 diet compared to pigs fed the BSFL5 diet. No covariation was found between the colonic SCFA concentrations and the microbiota composition (i.e. the overall ASV data) (P = 0.366).

# Discussion

The interest of using insects in animal feed is increasing [35, 36], and inclusion of BSFL have been investigated in diets for pigs [9], chicken [12] and fish [11, 37], but to this date, there is limited information about the effect of high dietary inclusion levels. The present study demonstrated that up to 19.06% full-fat BSFL meal can be included as an alternative protein and energy source in pelleted diets for weaned piglets without affecting growth performance.

Dietary inclusion of BSFL did not alter the ADG, ADFI, or FCR for a four-week period PW. There was, however, a tendency for decreased FCR with increased inclusion of BSFL the first 14 days PW. These results are partly consistent with Yu et al. [38], which reported a linear improvement in both ADG and FCR when feeding increasing BSFL inclusion from 0 to 4% in the two first weeks PW, whereas no differences were found for a four-week feeding period. Contrary, another study showed no differences in performance when feeding up to 8% full-fat BSFL for 15 days, after weaning at 21 days of age [9].

Insects contain varying levels of chitin, depending on both types of insects and life stage [5]. This polysaccharide is the major component of the insect's cuticle and creates a strong skeleton together with minerals and proteins. The exoskeleton of insects is different from crustaceans by having more amino acids strongly bound to the chitin [39, 40] and the degree of chitin deacetylation is dependent on the insect life stage [41]. Inclusion of chito-oligosaccharide (derivates from chitosan) in a corn-soybean meal diet has been shown to reduce the incidences and score of diarrhea in piglets weaned at 16 days of age [42], and in piglets weaned at 21 days of age and challenged with *Escherichia coli* [43]. However, no difference in fecal score or fecal DM were found between the dietary treatments in this study and may be due to the complexity of the chitin in the insect cuticle and the low degree of acetalization and solubility compared to the chito-oligosaccharides [44]. The increased fecal score in the first week PW is in accordance with the critical period for PW diarrhea [45]. The increase in fecal score in the last experimental week could be related to increased stress from sampling, as individual fecal samples for digestibility were collected daily in this period.

There is scarce information regarding the nutrient digestibility of insects and only a few studies including BSFL. Newton et al. [46] found similar ATTD of CP in five weeks old barrows fed 33% BSFL meal compared to a soybean meal control. In the present study, there was reduced ATTD of CP with increased inclusion of BSFL, but no difference in trypsin activity was found. A decline in ATTD of CP was also reported by Yu et al. [38] when including low amounts of BSFL (< 4%). Reduced CP digestibility with increased dietary inclusion of BSFL has also been reported for broiler chickens [47], Atlantic salmon [37,

48], and rainbow trout [49]. The CP digestibility is found to negatively correlate with chitin content in BSFL, *in vitro* [50]. Because of the nitrogen-rich chitin, the Kjeldahl-N method overestimates the digestible protein content of insects [51]. This is supported by the fact that the AID of total amino acids were not affected by dietary treatment. Janssen et al. [52], suggested using a conversion factor of 5.6 to avoid the overestimation of protein content in BSFL.

Limited information is available about the amino acid digestibility of BSFL in pig diets. However, the amino acid digestibility of full-fat BSF prepupae was recently investigated by Tan et al. [53]. The AID coefficients for amino acids were found to be between 0.641 and 0.821, and the SID coefficients to be between 0.767 and 1.177. During the diet optimization in the present study, the SID coefficients of all amino acids were set to 0.83. AID of lysine tended to decrease with increased inclusion of BSFL. Tan et al. [53] reported a SID coefficient of 0.776 for lysine in BSFL. Overestimation of the SID coefficient in the diet formulation of lysine might be the cause of the tendency of reduced AID of lysine with increased BSFL. Moreover, AID of alanine and proline tended to increase with increased inclusion of BSFL. The SID values for BSFL published by Tan et al. [53] were close to the one used in the diet formulation, but Tan et al. [53] also reported a higher AID coefficient for alanine in BSFL than soybean meal. Proline is high in endogenous losses, attributed to low reabsorption of mucin [54], but no information about BSFL affecting endogenous losses was found in the literature. In general, the AID of amino acids in the control diets was slightly lower compared with the control diet in a previous experiment with this age pigs [55]. Especially the AID of tyrosine was in general low and differed between the dietary treatments. The reported SID coefficient for tyrosine in BSFL (82.4%; Tan et al. 2020) is only slightly lower than the coefficient used in the diet formulation (83%). In the method used for amino acid analysis, glucosamine, resulting from hydrolyzed insect chitin, give a ninhydrin-positive peak close to the tyrosine peak in the chromatogram, which may cause a higher uncertainty for accurate tyrosine analyses. It is important to remember that even with standardized feeding time before sampling, the AID calculations with the slaughtering method are snapshots compared to methods with continuous collections or pooled collections over several days. This might contribute to why the trend in AID and ATTD are the same, but only the ATTD results are significant.

There was a linear increase in AID and ATTD of CF with increased inclusion of BSFL, whereas lipase activity measured in the oral part of jejunum showed no difference. The fat from BSFL amounted to be 19–69% of the total dietary fat and was significantly affecting the fatty-acid profile of the diets. Saturated fatty acids increased with increased BSFL inclusion, whereas the total amount of polyunsaturated acids decreased. The increase in saturated fatty acids was dominated by lauric acid (C12:0). The MCFA lauric acid is a major constituent of the BSFLs fat. It is synthesized by the larvae, and therefore present at a high level independent of diet [56, 57]. Finke [5] reported that lauric acid constituted 42% of the total fatty acids in the BSFL. The MCFAs are rapidly absorbed in the mucosa and, unlike long-chain fatty acids, directly into the portal vein for transportation to the liver [6, 58]. The differences in fatty acid composition between the diets are therefore believed to be the cause of differences in ATTD of CF. Higher digestibility of fat was also reported with 8% [9] and 33% inclusion of BSFL [46]. Contrary, Yu et al. [38] found a lower ATTD of CF when including 4% full-fat BSFL. Their reported ATTD of CF was overall

low (< 70%). Different feed ingredients causing a different fatty acid profile of the diets, and low inclusion level of BSFL limiting the differences in fatty-acid profiles between diets could explain the contradictory result by Yu et al. [38].

Gilts showed higher AID and ATTD of CP, CF, and ash, as well as for AID of several amino acids, compared to barrows. Higher ATTD of CP in gilts is previously reported by [59], including a nonsignificant increase in ATTD of ether extract. The use of wood shavings as bedding material also increased the numerical ATTD except for NDF. This is probably a result of the pigs eating the wood shavings. Dietary fiber is known to decrease ATTD [60], but when not included in the dietary composition it can disturb digestibility calculations by reducing the relative amount of the nutrient in the digesta.

The MCFA can directly supply the enterocytes with energy and thereby improve morphological changes in periods with nutrient deficiency such as weaning [6]. It is well known that weaning causes villus atrophy. However, both in this study and the study by Spranghers et al. [9], no effect of BSFL inclusion was found on intestinal morphometry. The effect of MCFA on morphology could be more evident the first days PW as the VH in the small intestine seems to recover within two weeks PW [61]. Biasato et al. [10] also reported no difference in intestinal morphometric indices after feeding pigs diets with up to 10% defatted BSFL. In accordance with our results, they also observed greater morphometric indices in the jejunum than the ileum.

The gut health was mildly suboptimal due to the observation of inflammation and edema in the jejunum and colon, but the morphological disorders did not appear to have a strong association with any of the dietary treatments. The morphological changes in edema and inflammation could be related to the edema disease outbreak. The results are consistent with Biasato et al. [10] which replaced defatted BSFL with soybean meal, concluding that up to 10% BSFL inclusion does not negatively affect gut morphology or histological features.

The microbiota of the gut is involved in the nutritional, physiological, and immunological functions of the pig [62]. Variation in the diet composition is one of the most important factors affecting the GIT microbial ecosystem of the pig [63]. The microbial community in the colon was dominated by Bacteroidota and Firmicutes, also reported by Yu et al. [64]. Yu and coworkers found no differences in phyla abundances when including 4 or 8% full-fat BSFL in diets for finishing-pigs. By contrast, the relative abundance of Bacteroidota, Firmicutes, Campilobacteria, and Thermoplasmatota differed between the dietary treatments in the present study, but there was no clear dose-effect of the dietary BSFL inclusion. However, the highest relative abundance of Firmicutes and lowest relative abundance of Bacteroidota was found in the pigs fed the control diet. In accordance with Yu et al. [64], no significant differences in Shannon indices were observed.

*Prevotella* was the most abundant genus in the colon of the pigs regardless of dietary treatment. This is in correspondence with several authors reporting *Prevotella* to be the most abundant genus in the fecal microbiome in the PW period [65–67]. *Lactobacillus* was more abundant in the colon of the control piglets. This result is contrary to what recently was reported by Yu et al. [64], where inclusion of 4% BSFL

increased the abundance of *Lactobacillus*. In the study by Yu et al. [64], finishing-pigs were fed corn-based diets contrary to our PW pigs, which were fed wheat and barley-based diets. Also, when they increased the inclusion to 8% BSFL, Yu et al. [64] did not find the same beneficial effects on the colonic microbiota as when feeding the 4% inclusion of BSFL. *Lactobacillus* is a genus of gram-positive lactic acid-producing bacteria in general considered favorable in the gut microbiome. It is known that lauric acid, abundantly found in BSFL fat, has antimicrobial effects, especially against gram-positive bacteria [6]. Spranghers et al. [9], observed inhibition of the growth of lactobacilli in an *in vitro* study with BSFL fat but did not observe differences *in vivo* in the small intestine. They also observed that a high amount of the lauric acid was absorbed already in the stomach and therefore had little opportunity to modulate the colon microbiome. In rainbow trout, it is shown that the BSFL life stage and lipid content of the BSFL are important factors influencing the gut microbiome [68].

It is important to mention the treatment with antibiotics of all pigs in the second week of the experiment. The effect of the antibiotic on the gut microbiota is antibiotic specific [62], and it is therefore difficult to know what impact the antibiotic treatment of the pig in this experiment had on their gut microbiota. Indeed, the antibiotic treatment could have affected microbiota results and be the reason for the small differences in the colon microbiome between the dietary treatments. Because of the antibiotic treatment in the second experimental week, it might be that the feeding period was too short to cause significant changes in the gut microbiome. Also, therapeutic doses of antibiotics might suppress the innate immune defenses and contribute to increased disease susceptibility [62]. Almost half of the pigs, independent of dietary treatment, had reddening of the colonic mucosa, indicating some inflammation in the colonic intestinal wall. However, the overall Shannon diversity indices reported were similar to what was reported by Yu et al. [64] in finishing pigs.

# Conclusion

To conclude, up to 19.06% of full-fat BSFL meal could be included in a balanced diet for PW pigs without affecting performance results. Increased inclusion of BSFL decreased ATTD of CP and increased ATTD of CF but did not affect the general gut function, as assessed by enzyme activity, morphometry, and histological evaluation. Some changes in the colon microbiota composition were observed, such as decreased relative abundance of *Lactobacillus* with dietary inclusion of BSFL. However, there are limited information and contradictive results on the effect of BSFL on the gut microbiome in pigs, thus further investigations are needed.

# Abbreviations

ADF: Acid detergent fiber

ASV: Amplicon sequence variant

AID: Apparent ileal digestibility

ATTD: Apparent total tract digestibility

ADF: Average daily gain

ADFI: Average daily feed intake

BSFL: Black soldier fly larvae

CF: Crude fat

CP: Crude protein

CD: Crypt depth

DM: Dry matter

FCR: Feed conversion ratio

GIT: Gastrointestinal tract

MCFA: Medium-chain fatty acid

NDF: Neutral detergent fiber

PW: Post-weaning

PCoA: Principal coordinate analyses

SCFA: Short-chain fatty acid

SEM: Standard error of the mean

SID: Standardized ileal digestibility

VH: Villus height

## Declarations

# Ethics approval and consent to participate

The experiment was conducted at an animal experimental unit approved by the National Animal Research Authority (permit no. 174). The authors state that all piglets were handled under the applicable laws and regulations controlling experiments with live animals in Norway, and in accordance with the EU Directive 2010/63/EU for animal experiments.

# Consent for publication

Not applicable

# Availability of data and materials

The datasets analyzed in the present study are available from the corresponding author on reasonable request.

# Competing interests

The authors declare that they have no competing interests.

# Funding

This work was supported by Foods of Norway, a Center for Research-based Innovation (The Research Council of Norway; grant number 237841/030) and FeedMileage, Efficient use of Feed Resources for a Sustainable Norwegian Food Production (The Research Council of Norway; grant no. 310 233685/E50).

# Authors' contributions

IMH, JØH, MØ, and LTM contributed to the conceptualization and study design. IMH, JØH, LTM, GHG, and RMÅ contributed to sample collections. RMÅ conducted enzyme activity analyses, 16s rRNA extraction, and sequencing. IMH and GHG performed statistical analysis on growth performance, liver index, fecal consistency, and digestibility data. IMH performed statistical analysis on pH, enzyme activities, short-chain fatty acids, and raw sequence data. IMH wrote the original draft. All authors critically reviewed the original draft and approved the final manuscript.

# Acknowledgement

The authors would like to thank the staff at the Center for Livestock Production (SHF), NMBU, Ås, for taking care of the daily animal management during the experiment, and members of the Foods of Norway team for their help during sampling. The authors also want to thank Elvis, Amir, and Thijs for contributing to sample collection and histological evaluation of intestinal tissue.

## References

1. Veldkamp T, Van Duinkerken G, Van Huis A, Lakemond C, Ottevanger E, Bosch G, et al. Insects as a Sustainable Feed Ingredient in Pig and Poultry Diets: a Feasibility Study. Wageningen UR Livestock

Research; 2012.
2. Wang Y-S, Shelomi M. Review of black soldier fly (Hermetia illucens) as animal feed and human food. Foods. 2017;6(10):91.
3. Purschke B, Scheibelberger R, Axmann S, Adler A, Jäger H. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (Hermetia illucens) for use in the feed and food value chain. Food Addit Contam Part A. 2017;34(8):1410-20.
4. Leni G, Cirlini M, Jacobs J, Depraetere S, Gianotten N, Sforza S, et al. Impact of Naturally Contaminated Substrates on Alphitobius diaperinus and Hermetia illucens: Uptake and Excretion of Mycotoxins. Toxins. 2019;11(8):476.
5. Finke MD. Complete nutrient content of four species of feeder insects. Zoo Biol. 2013;32(1):27-36.
6. Zentek J, Buchheit-Renko S, Ferrara F, Vahjen W, Van Kessel A, Pieper R. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim Health Res Rev. 2011;12(1):83-93.
7. Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, et al. Prebiotics as immunostimulants in aquaculture: a review. Fish Shellfish Immunol. 2014;40(1):40-8.
8. Wang S, Zeng X, Yang Q, Qiao S. Antimicrobial peptides as potential alternatives to antibiotics in food animal industry. Int J Mol Sci. 2016;17(5):603.
9. Spranghers T, Michiels J, Vrancx J, Ovyn A, Eeckhout M, De Clercq P, et al. Gut antimicrobial effects and nutritional value of black soldier fly (Hermetia illucens L.) prepupae for weaned piglets. Anim Feed Sci Technol. 2018;235:33-42.
10. Biasato I, Renna M, Gai F, Dabbou S, Meneguz M, Perona G, et al. Partially defatted black soldier fly larva meal inclusion in piglet diets: Effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. J Anim Sci Biotechnolog. 2019;10(1):12.
11. Nogales-Mérida S, Gobbi P, Józefiak D, Mazurkiewicz J, Dudek K, Rawski M, et al. Insect meals in fish nutrition. Rev Aquac. 2019;11(4):1080-103.
12. Józefiak A, Kierończyk B, Rawski M, Mazurkiewicz J, Benzertiha A, Gobbi P, et al. Full-fat insect meals as feed additive–the effect on broiler chicken growth performance and gastrointestinal tract microbiota. J Anim Feed Sci. 2018;27(2):131-9.
13. Blok MC, Brandsma G, Bosch G, Gerrits WJ, Jansman AJ, Everts H. A new Dutch net energy formula for feed and feedstuffs for growing and fattening pigs. Wageningen UR Livestock Research; 2015.
14. NRC. Nutrient Requirements of Swine: Eleventh Revised Edition. Council NR, editor. Washington, DC: The National Academies Press; 2012. 420 p.
15. Pedersen KS, Toft N. Intra-and inter-observer agreement when using a descriptive classification scale for clinical assessment of faecal consistency in growing pigs. Prev Vet Med. 2011;98(4):288-91.
16. Maynard LA, Loosli JK. Animal Nutrition 6th ed. New York: McGraw-Hill Book Company; 1969.

17. ISO 6496. Animal feeding stuffs–Determination of moisture and other volatile matter content. Institute for Standardization of Serbia, Belgrade. 2001.
18. ISO 5984. Animal feeding stuffs–Determination of crude ash. Institute for Standardization of Serbia, belgrade. 2002.
19. ISO 9831. Determination of Gross Caloric Value: Bomb Calorimeter Method (ISO 9831). 65120 Animal feeding stuffs. Geneva, Switzerland: International Organization for Standardization 1998.
20. McCleary B, Solah V, Gibson T. Quantitative measurement of total starch in cereal flours and products. J Cereal Sci. 1994;20(1):51-8.
21. O'fallon J, Busboom J, Nelson M, Gaskins C. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. J Anim Sci. 2007;85(6):1511-21.
22. Day M, Bilzer T, Mansell J, Wilcock B, Hall E, Jergens A, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. J Comp Pathol. 2008;138:S1-S43.
23. Pérez de Nanclares M, Trudeau MP, Hansen JØ, Mydland LT, Urriola PE, Shurson GC, et al. High-fiber rapeseed co-product diet for Norwegian Landrace pigs: Effect on digestibility. Livest Sci. 2017;203:1-9.
24. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-3.
25. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.
26. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(D1):D590-D6.
27. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. Nucleic Acids Res. 2014;42(D1):D643-D8.
28. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One. 2013;8(4):e61217.
29. Wickham H. ggplot2: Elegant Graphics for Data Analysis: Springer-Verlag New York; 2016.
30. RStudio Team. RStudio: Integrated Development for R. Boston, MA: RStudio, Inc.; 2019.
31. Bates D, Maechler M, bolker B, Walker S. Fitting Linear Mixed-Effects models Using lme4. J Stat Softw. 2015;67:1-48.
32. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: tests in linear mixed effects models. J Stat Softw. 2017;82(13).
33. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. 2019.

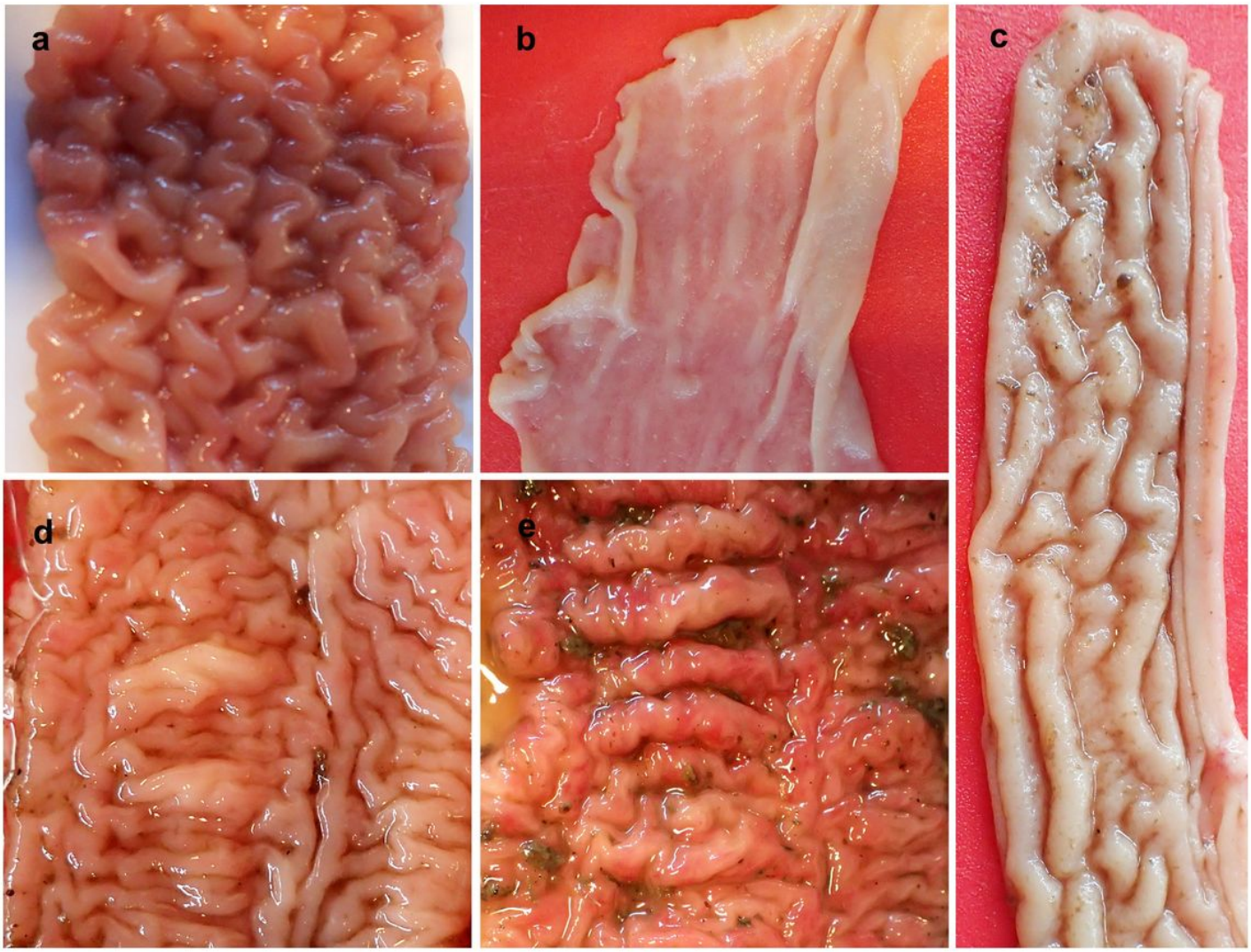
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc Ser B (Stat Method). 1995;57(1):289-300.
35. DiGiacomo K, Leury B. Insect meal: a future source of protein feed for pigs? Animal. 2019;13(12):3022-30.
36. Varelas V. Food wastes as a potential new source for edible insect mass production for food and feed: A review. Fermentation. 2019;5(3):81.
37. Weththasinghe P, Hansen J, Nøkland D, Lagos L, Rawski M, Øverland M. Full-fat black soldier fly larvae (Hermetia illucens) meal and paste in extruded diets for Atlantic salmon (Salmo salar): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. Aquac Asia. 2021;530:735785.
38. Yu M, Li Z, Chen W, Rong T, Wang G, Wang F, et al. Evaluation of full-fat Hermetia illucens larvae meal as a fishmeal replacement for weanling piglets: Effects on the growth performance, apparent nutrient digestibility, blood parameters and gut morphology. Anim Feed Sci Technol. 2020:114431.
39. Finke MD. Estimate of chitin in raw whole insects. Zoo Biol. 2007;26(2):105-15.
40. Andersen SO. Insect cuticular sclerotization: a review. Insect Biochem Mol Biol. 2010;40(3):166-78.
41. Smets R, Verbinnen B, Van De Voorde I, Aerts G, Claes J, Van Der Borght M. Sequential Extraction and Characterisation of Lipids, Proteins, and Chitin from Black Soldier Fly (Hermetia illucens) Larvae, Prepupae, and Pupae. Waste Biomass Valori. 2020:1-12.
42. Liu P, Piao X, Kim S, Wang L, Shen Y, Lee H, et al. Effects of chito-oligosaccharide supplementation on the growth performance, nutrient digestibility, intestinal morphology, and fecal shedding of Escherichia coli and Lactobacillus in weaning pigs. J Anim Sci. 2008;86(10):2609-18.
43. Xiao D, Wang Y, Liu G, He J, Qiu W, Hu X, et al. Effects of chitosan on intestinal inflammation in weaned pigs challenged by enterotoxigenic Escherichia coli. PloS One. 2014;9(8):e104192.
44. Guan G, Azad M, Kalam A, Lin Y, Kim SW, Tian Y, et al. Biological effects and applications of chitosan and chito-oligosaccharides. Front Physiol. 2019;10:516.
45. Madec F, Bridoux N, Bounaix S, Jestin A. Measurement of digestive disorders in the piglet at weaning and related risk factors. Prev Vet Med. 1998;35(1):53-72.
46. Newton GL, Booram CV, Barker RW, Hale OM. Dried Hermetia Illucens larvae meal as a supplement for swine. J Anim Sci. 1977;44(3):395-400.
47. Cullere M, Tasoniero G, Giaccone V, Miotti-Scapin R, Claeys E, De Smet S, et al. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal. 2016;10(12):1923-30.
48. Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, et al. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar). Aquac Asia. 2019;503:609-19.
49. Renna M, Schiavone A, Gai F, Dabbou S, Lussiana C, Malfatto V, et al. Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout

(Oncorhynchus mykiss Walbaum) diets. J Anim Sci Biotechnolog. 2017;8(1):57.

50. Marono S, Piccolo G, Loponte R, Di Meo C, Attia YA, Nizza A, et al. In vitro crude protein digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with chemical composition traits. Ital J Anim Sci. 2015;14(3):3889.
51. Jonas-Levi A, Martinez J-JI, Analysis. The high level of protein content reported in insects for food and feed is overestimated. J Food Compos. 2017;62:184-8.
52. Janssen RH, Vincken J-P, van den Broek LA, Fogliano V, Lakemond CM. Nitrogen-to-protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. J Agric Food Chem. 2017;65(11):2275-8.
53. Tan X, Yang H, Wang M, Yi Z, Ji F, Li J, et al. Amino acid digestibility in housefly and black soldier fly prepupae by growing pigs. Anim Feed Sci Technol. 2020:114446.
54. Stein H, Trottier N, Bellaver C, Easter R. The effect of feeding level and physiological status on total flow and amino acid composition of endogenous protein at the distal ileum in swine. J Anim Sci. 1999;77(5):1180-7.
55. Cruz A, Håkenåsen IM, Skugor A, Mydland LT, Åkesson CP, Hellestveit SS, et al. Candida utilis yeast as a protein source for weaned piglets: Effects on growth performance and digestive function. Livest Sci. 2019;226:31-9.
56. Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, et al. Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. J Sci Food Agric. 2017;97(8):2594-600.
57. Ewald N, Vidakovic A, Langeland M, Kiessling A, Sampels S, Lalander C. Fatty acid composition of black soldier fly larvae (Hermetia illucens)–Possibilities and limitations for modification through diet. Waste Manage Res. 2020;102:40-7.
58. Odle J. New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. J Nutr. 1997;127(6):1061-7.
59. Sheikh GG, Baghel R, Nayak S, Fatima B, Ganie AA. Effect of sex on growth performance, nutrient utilization and carcass characteristics in cross bred pigs. Indian J Anim Res. 2017;51(1):175-8.
60. Wilfart A, Montagné L, Simmins PH, van Milgen J, Noblet J. Sites of nutrient digestion in growing pigs: Effect of dietary fiber. J Anim Sci. 2007;85(4):976-83.
61. Håkenåsen IM, Øverland M, Ånestad R, Åkesson CP, Sundaram AYM, Press CM, et al. Gene expression and gastrointestinal function is altered in piglet small intestine by weaning and inclusion of Cyberlindnera jadinii yeast as a protein source. J Funct Foods. in press.
62. Fouhse J, Zijlstra R, Willing B. The role of gut microbiota in the health and disease of pigs. Anim Front. 2016;6(3):30-6.
63. Rist V, Weiss E, Eklund M, Mosenthin R. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 2013;7(07):1067-78.

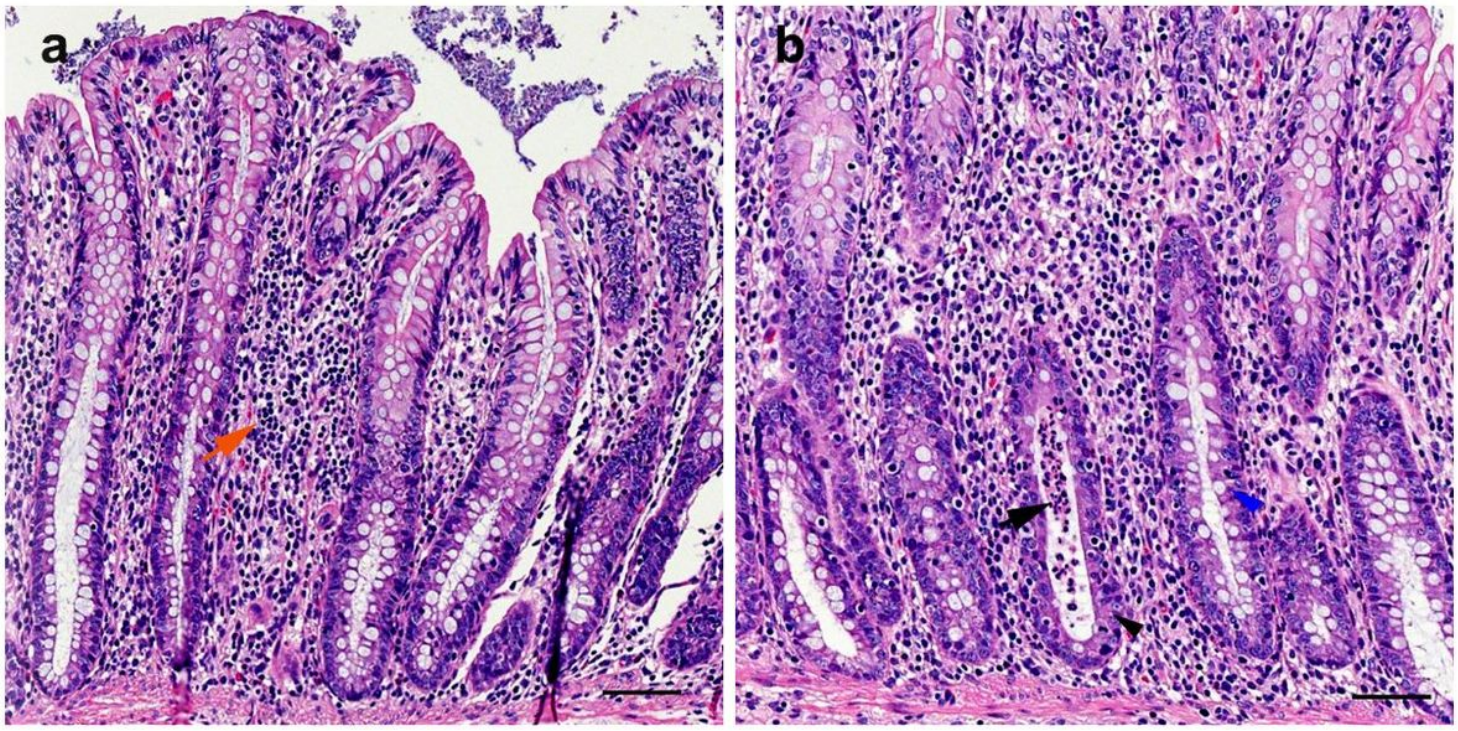
64. Yu M, Li Z, Chen W, Rong T, Wang G, Ma X. Hermetia illucens larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. J Anim Sci Biotechnolog. 2019;10(1):50.
65. Isaacson R, Kim HB. The intestinal microbiome of the pig. Anim Health Res Rev. 2012;13(1):100-9.
66. Guevarra RB, Hong SH, Cho JH, Kim B-R, Shin J, Lee JH, et al. The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. J Anim Sci Biotechnolog. 2018;9(1):54.
67. Wang X, Tsai T, Deng F, Wei X, Chai J, Knapp J, et al. Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. Microbiome. 2019;7(1):109.
68. Huyben D, Vidaković A, Hallgren SW, Langeland M. High-throughput sequencing of gut microbiota in rainbow trout (Oncorhynchus mykiss) fed larval and pre-pupae stages of black soldier fly (Hermetia illucens). Aquaculture. 2019;500:485-91.

# Figures



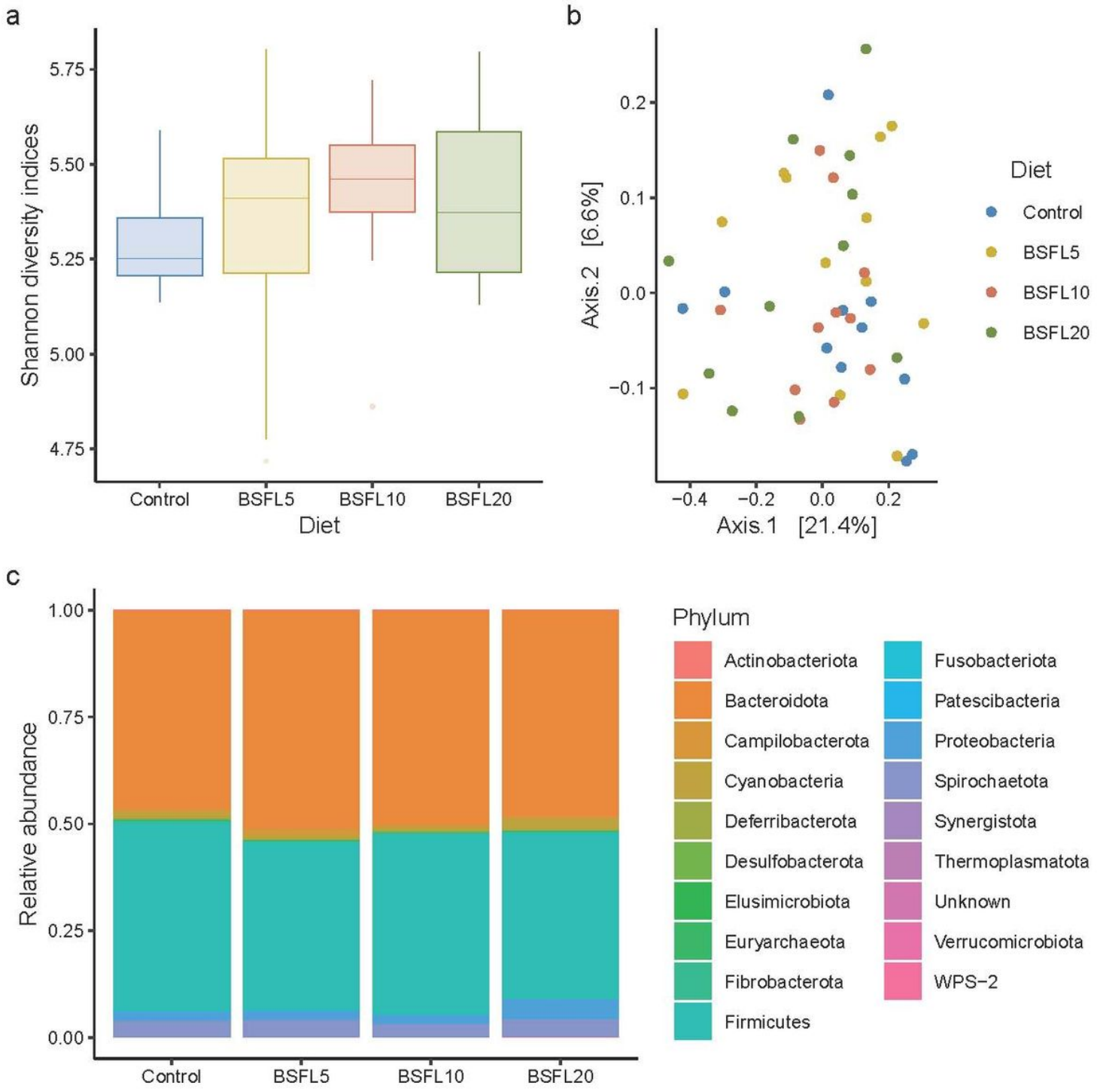

## Figure 1

Main findings from the visual inspection of intestinal tissue prior to the collection of tissue for histology. Panel a: Normal jejunum mucosa. Panel b: Jejunal mucosal with low muscle tone and complete loss of mucosal folds. Panel c: Normal appearance of ileum mucosa as observed for most of the samples. Panel d: Normal and healthy appearance of the colon. Panel e: Colon mucosa with mild mucosal reddening.




## Figure 2

Representative images of the morphological appearance of the colon tissue showing the histopathological changes of a: lymphocytic and plasma cell infiltration of the inter-crypt area (orange arrow), and b: crypt abscessation (black arrow) characterized by an accumulation of neutrophils in the crypt lumen and surrounded by crypt epithelial cells that have reduced numbers (black arrowhead) of goblet cells compared to healthy crypt epithelium (blue arrowhead). Scale bars in both images represent a distance of 100 μm.




## Figure 3

Colon microbiota diversity in pigs fed an increased amount of BSFL. Panel a: Boxplot of Shannon diversity indices. The median value is represented as the lines inside the boxes. Panel b: Principal coordinate analysis plot with Bray-Curtis distances of colon microbiota colored by diet. Panel c: Average relative abundance of the microbial populations at the phylum level for each of the dietary treatments.

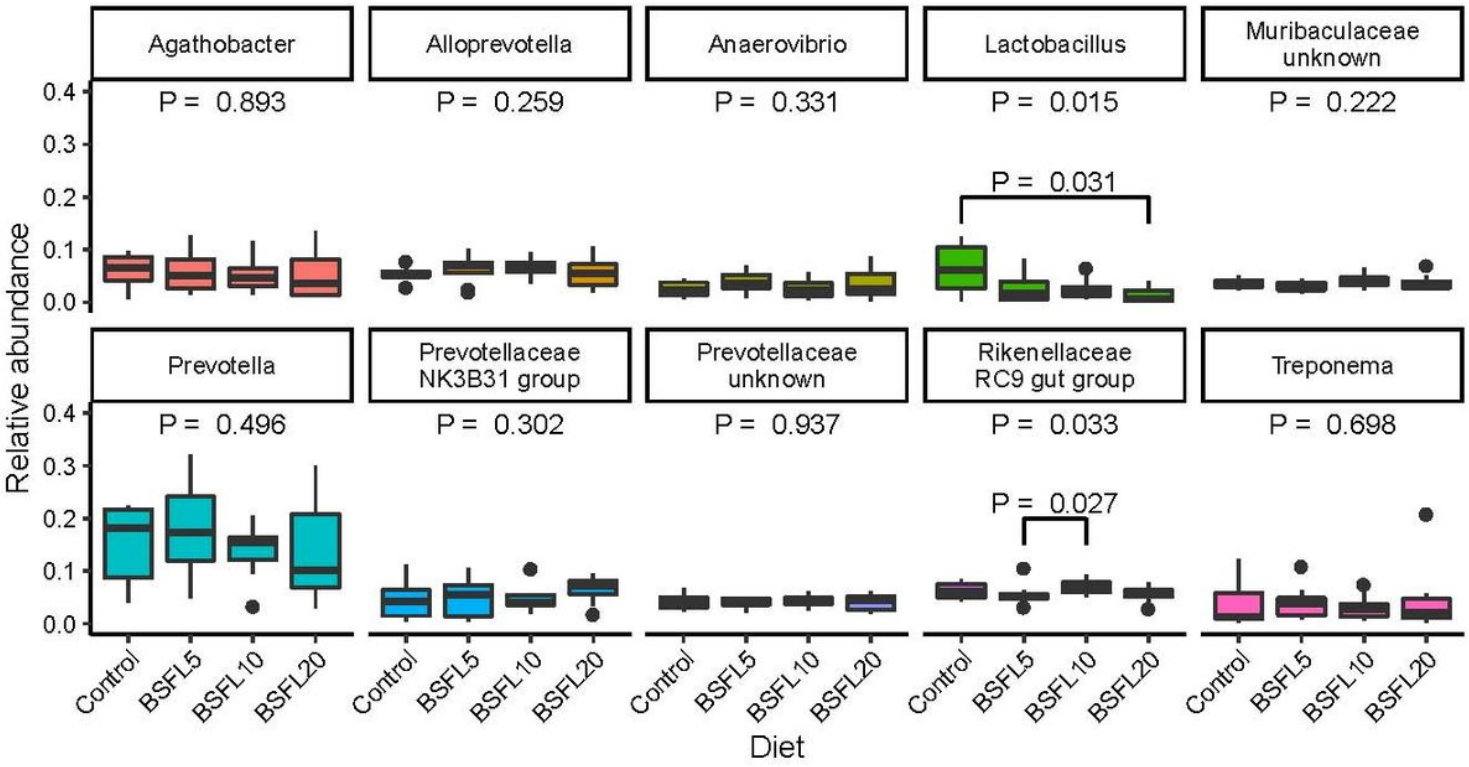



**Figure 4**

Relative abundance of top 10 abundant genera. Brackets denote significant differences between dietary treatments.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfigure1.pdf