Effects of dietary supplementation with a laminarin-rich extract on the growth performance and gastrointestinal health in broilers

B. Venardou,* J.V. O’Doherty,† S. Vigors,‡ C.J. O’Shea,‡ E.J. Burton,§ M.T. Ryan,* and T. Sweeney*,†

*School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland; †School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland; ‡School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, United Kingdom; and §School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, NG25 0QF, United Kingdom

ABSTRACT Restriction in antimicrobial use in broiler chicken production is driving the exploration of alternative feed additives that will support growth through the promotion of gastrointestinal health and development. The objective of this study was to determine the effects of dietary inclusion of laminarin on growth performance, the expression of nutrient transporters, markers of inflammation and intestinal integrity in the small intestine and composition of the caecal microbiota in broiler chickens. Two-hundred-and-forty day-old male Ross 308 broiler chicks (40.64 (3.43 SD) g) were randomly assigned to: (T1) basal diet (control); (T2) basal diet + 150 ppm laminarin; (T3) basal diet + 300 ppm laminarin (5 bird/pen; 16 pens/treatment). The basal diet was supplemented with a laminarin-rich Laminaria spp. extract (65% laminarin) to achieve the two laminarin inclusion levels (150 and 300 ppm). Chick weights and feed intake was recorded weekly. After 35 days of supplementation, one bird per pen from the control and best performing (300 ppm) laminarin groups were euthanized. Duodenal, jejunal and ileal tissues were collected for gene expression analysis. Caecal digesta was collected for microbiota analysis (high-throughput sequencing and QPCR). Dietary supplementation with 300 ppm laminarin increased both final body weight (2033 vs. 1906 ± 30.4, P < 0.05) and average daily gain (62.3 vs. 58.2 ± 0.95, P < 0.05) compared to the control group and average daily feed intake (114.1 vs. 106.0 and 104.5 ± 1.77, P < 0.05) compared to all other groups. Laminarin supplementation at 300 ppm increased the relative and absolute abundance of Bifidobacterium (P < 0.05) in the caecum. Laminarin supplementation increased the expression of interleukin 17A (IL17A) in the duodenum, claudin 1 (CLDN1) and toll-like receptor 2 (TLR2) in the jejunum and IL17A, CLDN1 and SLC15A1/peptide transporter 1 (SLC15A1/PepT1) in the ileum (P < 0.05). In conclusion, supplementation with laminarin is a promising dietary strategy to enhance growth performance and 300 ppm was the optimal inclusion level with which to promote a beneficial profile of the gastrointestinal microbiota in broiler chickens.

Key words: laminarin, broiler, performance, microbiota, gastrointestinal health

INTRODUCTION

In the past, broiler production was heavily reliant on the routine use of antimicrobial growth promoters (AGP) which have established benefits in terms of performance and feed efficiency (Dibner and Richards, 2005). However, the EU ban of AGP in 2006 (Regulation (EC) No 1831/2003) and the implementation of further restrictions regarding the use of antimicrobials in food-producing animals (Regulation (EU) No. 2019/6 and 2019/4) from 2022 have increased the need for alternative feed additives. Prebiotic non-digestible polysaccharides are promising with regard to improving broiler growth and health (Gadde et al., 2017). Brown macroalgae or seaweeds are a source of wide ranging and novel bioactives, including laminarins. These polysaccharides are (1,3)-β-D-glucans with varying degrees of β-(1,6) branching that have exhibited prebiotic and immunomodulatory activities (O’Sullivan et al., 2010; Sweeney et al., 2012; Murphy et al., 2013; Kadam et al., 2015; Cherry et al., 2019; Vigors et al., 2020). In previous studies on broiler chickens, in ovo injection with a seaweed extract containing predominantly laminarin increased growth parameters, as well as Bifidobacterium spp. and Lactobacillus spp. counts (Bednarczyk et al., 2016; Maiorano et al.,
Furthermore, dietary supplementation with a highly purified laminarin extract derived from Laminaria digitata in chicks infected with Campylobacter jejuni not only improved overall performance but there were also improvements to villus architecture coupled with the upregulation of immunological markers (Sweeney et al., 2017). However, the concentration, structure and bioactivity of the macroalgal polysaccharides including laminarin varies with seaweed species, season of harvest and extraction method (García-Vaquero et al., 2017).

Recently, dietary inclusion of a laminarin-rich Laminaria spp. extract (65% laminarin) at 300 ppm led to improvements in growth performance through the modulation of nutrient absorption and markers of inflammation and intestinal integrity in newly weaned pigs (Rattigan et al., 2020). Furthermore, this laminarin-rich extract promoted a more beneficial bacterial composition in the caecal and colonic microbiota of the supplemented pigs characterized by a reduction in the potentially pathogenic Enterobacteriaceae family and stimulation of bacterial populations associated with improved growth (Vigors et al., 2020). To date, no optimal dietary inclusion level of laminarin has previously been established for broiler chickens. Therefore, the objective of this study was to explore the effects of two inclusion levels of a 65% purified laminarin Laminaria spp. extract on the growth performance of broiler chickens. The second objective of this study was to investigate the effects of the optimal inclusion level of laminarin on selected gastrointestinal parameters that influence performance such as the expression of nutrient transporters and markers of inflammation and intestinal integrity in the small intestine and the composition of the caecal microbiota. It was hypothesized that dietary supplementation with a laminarin-rich extract would enhance growth performance by increasing nutrient absorption, improving the composition of the caecal microbiota and modulating the immune response and intestinal integrity.

MATERIALS AND METHODS

All animal experimental procedures described in this study were approved by the Nottingham Trent University ethics committee (ARE856) and all animal care met the guidelines approved by the institutional animal care and use committee (IACUC) and the guidelines outlined in Directives 2007/526/EC and 2010/63/EU for animal experimentation.

**Experimental Design and Diets**

The experiment was a complete randomized design consisting of the following treatment groups: (T1) basal diet (Target Feeds, Whitchurch, UK) (control); (T2) basal diet + 150 ppm laminarin and (T3) basal diet + 300 ppm laminarin. Day old male Ross 308 broiler chicks (n = 240) with an average initial weight of 40.64 g (3.43 SD) were sourced from PD Hook Hatcheries Ltd (Cote, Bampton, UK). All chicks were vaccinated against Marek’s disease, infectious bronchitis and Newcastle disease at the hatchery. Chicks were allocated randomly in groups of five per pen. Each pen was assigned to one of the three treatments (16 replicates/treatment). Basal diets were mixed for the starter (d 0–21) and finisher (d 22–35) phases on a wheat-soya bean meal base and manufactured in house as mash. All diets were formulated to meet the nutrient requirements of broilers (National Research Council, 1994). The ingredient and nutrient composition of the starter and the finisher basal diet are presented in Table 1. The T2 and T3 diets were formulated from the basal diet through the addition of 150 and 300 ppm laminarin which were mixed for 5 min using a ribbon mixer (Rigal Bennett, Goole, UK). A laminarin-rich extract (BioAtlantis Ltd., Clash Industrial Estate, Tralee, Ireland) was obtained from Laminaria spp. using a hydrothermal-assisted extraction and pre-optimized conditions for maximum yield of laminarin as described previously (García-Vaquero et al., 2019). The crude extract was partially purified to contain 65% laminarin as described previously (Garcia-Vaquero et al., 2017).

**Table 1. Ingredient and nutrient composition of the starter and finisher basal diets.**

| Ingredients (g/kg)       | Starter basal diet (d 0–21) | Finisher basal diet (d 22–35) |
|--------------------------|----------------------------|-------------------------------|
| Wheat                    | 630.4                      | 718.8                         |
| Soyabean meal (Hipro)    | 300.0                      | 206.0                         |
| Soya oil                 | 32.0                       | 44.0                          |
| Limestone flour          | 8.0                        | 7.2                           |
| Dicalcium phosphate (18%)| 13.1                       | 11.1                          |
| Salt                     | 1.6                        | 1.8                           |
| Sodium bicarbonate       | 2.5                        | 1.5                           |
| DL Methionine            | 3.9                        | 2.3                           |
| Lysine HCl               | 3.6                        | 2.8                           |
| L-Threonine              | 1.4                        | 1.0                           |
| TMV premix               | 3.5                        | 3.5                           |
| Phytase                  | 0.1                        | 0.1                           |

1Crude protein content 48%. 2TMV: Trace minerals and vitamins, TMV premix content (per kg diet): Mn, 100 mg; Zn, 88 mg; Fe, 20 mg; Cu, 10 mg; I, 1 mg; Mb, 0.48 mg; Se, 0.2 mg; Vitamin A, 45,000 IU (retinol, 13.5 mg); Cholecalciferol, 3 mg; Vitamin E 50% adsorbate, 25 IU (dl-α-tocopheroyl acetate, 25 mg); Manganese, 5 mg; Thiamine, 3 mg; Riboflavin, 10 mg; Pantothenic acid, 15 mg; Pyridoxine, 3.0 mg; Niacin, 60 mg; Cobalamin, 30 μg; Folic acid, 1.5 mg; Biotin, 125 μg. 3Quantum Blue Phytase (AB Vista Feed Ingredients, Marlborough, UK). 4Calculated for nutritional composition (Sauvant et al., 2004).
increase the relative polysaccharide content and to remove or reduce other constituents: proteins, polyphenols, mannitol and alginate. This was achieved through mixing the crude extract with pure ethanol (to remove polyphenols) followed by water (to remove protein) and calcium chloride (to remove alginites). The chemical composition (% w:w dry matter) of the laminarin-rich *Laminaria* spp. extract included 65% laminarin, 18.1% fucoidan, 6% mannitol, 2.8% alginate, 7.7% ash, and 0.4% phlorotannins. The basal diet was supplemented with the appropriate quantity of the laminarin-rich extract to achieve inclusion levels of 150 and 300 ppm.

**Housing and Animal Management**

Birds were housed in a 48-pen bird room similar to a commercial broiler setting at the Nottingham Trent University Poultry Research Unit (Brackenbury Campus, Southwell, UK). Each pen (0.64 m²) was bedded with clean wood shavings and equipped with a 30 cm trough and 2 nipple drinkers. Birds had *ad libitum* access to feed and water and care was taken to ensure birds ate and drank on d 1. Birds were kept under artificial light for 24 h on d 1 followed by an hour increase of darkness each day until d 6. Six hours of darkness were then maintained for the remainder of the study. The room was thermostatically controlled, and temperatures recorded daily from different areas of the unit. The initial temperature of 31°C on d 1 was gradually reduced to 21°C on d 21 and maintained at this temperature for the remainder of the study. Individual bird weight and feed consumption per pen was recorded on a weekly basis for the calculation of average daily gain (*ADG*), average daily feed intake (*ADFI*) and feed conversion ratio (*FCR*).

**Feed Analysis**

Dry matter of the feed was determined after drying for 5 d to stable weight at 105°C. Metabolizable and gross energy were determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). Ash content was determined after ignition of diet samples for 14 h at 650°C in a muffle furnace. (Nabertherm, Bremen, Germany). Nitrogen content of the diets was determined using a combustion analyzer (Dumatherm N Pro, Germany). This was snap frozen on dry ice and stored at −80°C for subsequent microbial analysis (QPCR and high-throughput sequencing).

**Sample Collection**

All birds from the control and the best performing laminarin group, 300 ppm inclusion level, were euthanized on d 35 by cervical dislocation and one bird per pen was randomly selected for sample collection. Euthanasia was completed by a trained individual in a separate room away from sight and sound of the other birds. For gene expression analysis, duodenal, jejunal and ileal tissue samples were rinsed in sterile phosphate buffer saline (Sigma-Aldrich, St. Louis, MO), cut into 1 cm² tissue sections using a sterile scalpel. These were stored overnight at room temperature in RNA later solution (Sigma-Aldrich, St. Louis, MO), and subsequently at −20°C prior to RNA extraction. Digesta were collected using gentle digital pressure from both caeca per bird and placed into sterile containers (Sarstedt, Nümbrecht, Germany). This was then snap frozen on dry ice and stored at −80°C for subsequent microbial analysis (QPCR and high-throughput sequencing).

**Gene Expression in the Small Intestine**

Total RNA was extracted from duodenal, jejunal and ileal tissue using TRI Reagent (Sigma-Aldrich, St. Louis, MO) and purified using GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO) and a DNase removal step using On-Column DNase I Digestion Set (Sigma-Aldrich, St. Louis, MO) according to the manufacturers’ instructions. The quantity and purity (260:280 nm absorbance ratio) of the total RNA, were determined using a Nanodrop-ND1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was synthesized from 2 μg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) with the total reaction volume (20 μL) adjusted to 400 μL using nuclease-free water. The QPCR reaction mix (20 μL) contained 10 μL GoTaq qPCR Master Mix (Promega, Madison, WI), 1.2 μL forward and reverse primers (5μM), 3.8 μL nuclease-free water and 5μL cDNA. All QPCR reactions were carried out in duplicate on the 7500 ABI Prism Sequence detection System (Applied Biosystems, Foster City, CA) with the following cycling conditions: 95°C for 10 min, 95°C for 15 s for 40 cycles and 60°C for 1 min. All primers were designed using the Primer Express software (Applied Biosystems, Foster City, CA) and synthesized by MWG Biotech UK Ltd (Milton Keynes, UK) and are presented in Table 2. The sequences of the forward and reverse primers have been described and validated previously (Sweeney et al., 2016; Sweeney et al., 2017) except for *SLC1A4, FABP2, AMY2A, SLC15A1/PepT1, SLC15A2/PepT2* genes which were newly designed and their specificity was verified *in silico* using Primer Basic Local Alignment Search Tool (Primer-BLAST, [https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi](https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi)). QPCR assays exhibiting 90 to 110% efficiency and single and specific products based on the generated dissociation curves were solely used in this study. Normalized relative quantities were obtained using the qbase PLUS software (Biogazelle, Ghent, Belgium) from two stable housekeeping reference genes; *GAPDH* and *PPA1* for duodenum and *GAPDH* and *ACTB* for jejunum and ileum. These genes were selected as reference genes due to their lowest stability M value (<1.5) generated by the GeNorm application.
Quantiﬁcation of Selected Bacterial Groups Using QPCR

Microbial genomic DNA was extracted using QIAamp PowerFecal Pro DNA Kit (Qiagen, West Sussex, UK) according to the manufacturer’s instructions. The DNA quantity and quality were evaluated using a Nanodrop ND-1000 Spectrophotometer. The domain-, function-, family- or genus-speciﬁc primers for the selected bacterial groups were available in the literature and are provided in Table 3. The 16S rRNA gene was targeted for most bacterial groups except for the butyrate-producing bacteria where the gene butyryl-CoA:acetate CoA-transferase (B-CoA) associated with this function was selected (Louis and Flint, 2007; Metzler-Zebeli et al., 2010). Primers were designed using two tools, Primer3 program (https://primer3.org/) for larger amplicons (>150 bp) and Primer Express software.

Table 2. Panel of target genes evaluated in the different regions of small intestine.

| Target gene | Accession No | Forward primer (5′-3′) | Reverse primer (5′-3′) | Amplicon length (bp) | Tm (°C) |
|-------------|--------------|------------------------|------------------------|----------------------|---------|
| Nutrient transporters and digestive enzymes |  |  |  |  |  |
| SLC2A1/ GLUT1 | NM_205209 | F: AACGCCAATGAGGAGAACAAA | R: GATCCGTCTGACATCTGTTG | 73 | 55.9 |
| SLC2A5/ GLUT5 | XM_00497446 | F: GGATCAATGCTCTCTCTTGAAT | R: CACCTATGGACACGGTGATGC | 81 | 61.3 |
| SLC5A1/ SGLT1 | NM_001293420 | F: AGCATTCCTCCTCTCTTCACT | R: GGCACTCCCTCCCTCACAT | 83 | 58.2 |
| FABP2 | NM_001060723 | F: CTTCGAAAATAGAAAAATGAGAATGAT | R: GGCTCTACAATTTTCTTTTCATCA | 83 | 59.9 |
| AMY2A | XM_02515293 | F: CACGGGCAACCACTCAAC | R: GGCAAGCAGGAAAATCTC | 66 | 60.5 |
| SLC15A1/ PepT1 | NM_204365.1 | F: GACAACTTTTCTCTACAGCCATTCACA | R: CATGAGGCTCCCAAGGAT | 76 | 61.6 |
| SLC15A2/ PepT2 | XM_02515260.1 | F: CGAAACTCTGTGGCTCCAACT | R: CCGTTGCGAAGATCGTTC | 67 | 59.8 |

Inflammmatory markers |  |  |  |  |  |
| IL6 | XM_015281283 | F: CTCTCTGCGCAATGGGAATGTC | R: CCCTCTGCTCTCTCTACAT | 100 | 59.4 |
| CXCL8 | NM_00105698.1 | F: CTGGAGTGCTCTAGTATG | R: TCTTACAGATTCTGCAGGTTAGATTTCT | 112 | 53.7 |
| IL10 | NM_001040414.1 | F: GCTTGCCCTTCTTTACAGCATG | R: GCCCCATGCTCTGCTGA | 73 | 62.1 |
| IL17A | NM_204460.1 | F: CCATTCACTGCAGGTGAPAAG | R: TTGTATGGGACAGGAGTT | 64 | 58.8 |
| LITAF | NM_204267.1 | F: CCCTCTACCTGCTCACA | R: GAGGGTACTGCCAGCAT | 67 | 61.0 |
| TGFB1 | JQ423909.1 | F: GTGTTATATGGCGCACTTCGCA | R: CCCCGGTTGGTTGGT | 102 | 58.9 |
| IFNG | FJ788637.1 | F: AAGCCGCGAATGAACTCTTC | R: CTTGAGACTTGGCTCTTTTTCTT | 80 | 57.3 |

Toll-like receptors |  |  |  |  |  |
| TLR2 | NM_001161650 | F: TCTGCAAAAGGCTGTGAACCT | R: CCAACGAGCTCTCTATCTATG | 78 | 57.9 |
| TLR4 | NM_001030693 | F: GACCTCTGTTGGTTGTTGTCATCA | R: CTTTGATGGTCACGGAGTTG | 91 | 60.4 |

Tight Junctions |  |  |  |  |  |
| CLDN1 | NM_001036112.1 | F: GCCCTTGGCCCAATACATTACA | R: TGGCAACCGAGGAGATAGC | 71 | 57.9 |
| OCLN | NM_205128.1 | F: CCCAGAAGACGCGCAGTAAAG | R: GCGCGGTGCCAGTAGATG | 61 | 61.4 |

Mucins |  |  |  |  |  |
| MUC2 | XM_001234581 | F: CTGGATGTCATCCTACGCGCTTAATC | R: GCCCGGCCCACTTG | 147 | 61.8 |

Cell apoptosis |  |  |  |  |  |
| TNSR5F1A | NM_001030779.1 | F: GACCCCATGCAACCCAAAAG | R: AAATCACCTGCTGCGCAAATCG | 75 | 58.8 |

Reference genes |  |  |  |  |  |
| GAPDH | NM_204305.1 | F: GGGTGCTAAGGCTGTTGATCTCA | R: CATGGTTGCAACCCATCACA | 70 | 61.0 |
| ACTB | NM_205518.1 | F: TGCTGCGCTGTTGGTTGA | R: TGTGCCTCCGGCGAAA | 60 | 56.0 |
| PPIA | NM_001166326.1 | F: CTTGCTCCTCCAGGATCAT | R: CCGTTGCGAAGATCGTTC | 64 | 58.8 |

Abbreviations: ACTB, actin beta; AMY2A, amylase alpha 2A; bp, base pairs; CLDN1, claudin 1; FABP2, fatty acid binding protein 2; IL6, interleukin 6; CXCL8, C-X-C motif chemokine ligand 8; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL10, interleukin 10; IL17A, interleukin 17A; IFNG, interferon gamma; LITAF, lipopolysaccharide induced TNF factor; MUC2, mucin 2; OCN, occludin; PPIA, peptidylprolyl isomerase A; SLC15A2/ PepT2, peptide transporter 2; SLC15A1/ PepT1, peptide transporter 1; SLC2A1/ GLUT1, glucose transporter 1; SLC2A5/ GLUT5, glucose transporter 5; SLC5A1/ SGLT1, sodium-glucose cotransporter 1; SLC1A4, glutamate and neutral amino acid transporter; TNFRSF1A, TNF receptor superfamily member 1A; TGFB1, transforming growth factor beta 1; TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; Tm, melting temperature.

Quantification of Selected Bacterial Groups Using QPCR

Microbial genomic DNA was extracted using QIAamp PowerFecal Pro DNA Kit (Qiagen, West Sussex, UK) according to the manufacturer’s instructions. The DNA quantity and quality were evaluated using a Nanodrop ND-1000 Spectrophotometer. The domain-, function-, family- or genus-specific primers for the selected bacterial groups were available in the literature and are provided in Table 3. The 16S rRNA gene was targeted for most bacterial groups except for the butyrate-producing bacteria where the gene butyryl-CoA:acetate CoA-transferase (B-CoA) associated with this function was selected (Louis and Flint, 2007; Metzler-Zebeli et al., 2010). Primers were designed using two tools, Primer3 program (https://primer3.org/) for larger amplicons (>150 bp) and Primer Express software.
for smaller amplicons (<125 bp), and their specificity was verified in silico using Primer-BLAST. For the absolute quantification of the selected bacterial groups using QPCR, specific plasmids (total bacteria, *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacteriaceae*) were prepared as described by Venardou et al. (2021). The plasmid containing the B-CoA gene was additionally prepared using the genomic DNA of *Faecalibacterium prausnitzii* (DSMZ 17677) that was purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

The primers and genomic locations of all targeted genes that were incorporated into plasmids are outlined in Table S1. The QPCR reaction volume (20 µL) included 3 µL template DNA, 1 or 2 µL (for B-CoA) of each primer (10 µM), 5 or 3 µL (for B-CoA) nuclease-free water and 10 µL of GoTaq qPCR Master Mix. All QPCR reactions were carried out in duplicate on the ABI 7500 Fast PCR System (Applied Biosystems, Foster City, CA) with the following cycling conditions; 95°C for 10 s, 95°C for 15 s for 40 cycles and 60°C for 1 min. The specificity of the resulting PCR products was confirmed by the generation of dissociation curves and visualization on an agarose gel stained with 1% ethidium bromide. To ensure a 90 to 110% QPCR reaction efficiency, the cycling threshold (CT) values derived from 5-fold serial dilutions of the plasmid were plotted against their arbitrary quantities. The primer sequences were determined using a standard curve derived from the mean CT values and the log transformed gene copy number of the plasmid and expressed as log transformed gene copy number per gram of digesta (logGCN/g digesta).

### Table 3. List of forward and reverse primers used for the bacterial quantification by QPCR.

| Target bacterial group | Forward primer (5' - 3') | Reverse primer (5' - 3') | Amplicon length (bp) | Tm (°C) | References |
|------------------------|--------------------------|--------------------------|----------------------|---------|------------|
| Total bacteria         | F: GTGCCACGCGCGCGGTAA     | R: GACTACGGGTATCTAAT     | 291                  | 64.2    | Frank et al. (2007) |
| *Lactobacillus* spp.   | F: AGCAGTAGGGAATCTTCCA    | R: CACCGTACACATGGAG      | 341                  | 54.5    | Metzler-Zebeli et al. (2010) |
| *Bifidobacterium* spp. | F: GCCTGTTAACACATGGAATGC | R: CACCGGTTCACAGGGGTATT | 125                  | 60.3    | Penders et al. (2005) |
| *Enterobacteriaceae*   | F: ATGGTACAAACAAACGGTACA | R: TTAACCTTGACGCTTAACTGC | 185                  | 54.0    | Takahashi et al. (2017) |
| Butyryl-CoA:acetate CoA-transferase (B-CoA) | F: GCGIGAICATTTCACITGGAAYW-SITGGCAYATG | R: | | |

**Bioinformatic and Statistical Analysis of 16S rRNA Gene Sequencing Data**

The bioinformatic analysis of the resulting sequences was performed by Eurofins Genomics (Ebersberg, Germany) using the open source software package (version 1.9.1) Quantitative Insights into Microbial Ecology (Qiime) (Caporaso et al., 2010). All raw reads passing the standard Illumina chastity filter were demultiplexed according to their index sequences (read quality score >30). The primer sequences were clipped from the starts of the raw forward and reverse reads. If primer sequences were not perfectly matched, read pairs were removed to retain only high-quality reads. Paired-end reads were then merged if possible, to obtain a single, longer read that covers the full target region using the software FLASH 2.2.00 (Magoc and Salzberg, 2011). Pairs were merged with a minimum overlap size of 10 bp to reduce false-positive merges. The forward read was only retained for the subsequent analysis steps when merging was not possible. Merged reads were quality filtered according to the expected length and known length variations of the V3–V4 region (ca. 445 bp). The ends of retained forward reads were clipped to a total read length of 285 bp to remove low quality bases. Merged and retained reads containing ambiguous bases were discarded. The filtered reads (merged and quality clipped retained forward reads) were used for the microbiome
profiling. Chimeric reads were identified and removed based on the de-novo algorithm of UCHIME (Edgar et al., 2011) as implemented in the VSEARCH package (Rognes et al., 2016). The remaining set of high-quality reads was processed using minimum entropy decomposition (MED) to partition reads to operational taxonomic units (OTU) (Eren et al., 2013; Eren et al., 2015). DC-MEGABLAST alignments of cluster representative sequences to the NCBI nucleotide sequence database were performed for taxonomic assignment (from phylum to species) of each OTU. A sequence identity of 70% across at least 80% of the representative sequence was the minimal requirement for considering reference sequences. Abundances of bacterial taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates (Angly et al., 2014).

The normalized OTU table combined with the phenotype metadata and phylogenetic tree comprised the data matrix. This matrix was then input into the phyloseq package within R (http://www.r-project.org; version 3.5.0). The dynamics of richness and diversity in the chicken’s microbiota were computed with the observed, Chao1, ACE, Shannon, Simpson, inverse Simpson and Fisher indices. The Simpson and Shannon indices of diversity account for both richness and evenness parameters. To estimate beta diversity measurements, which are a measure of separation of the phylogenetic structure of the OTU in one sample compared with all other samples, the data was normalized to make taxonomic feature counts comparable across samples. Several distance metrics were considered, in order to calculate the distance matrix of the different multidimensional reduction methods. These included weighted/unweighted UniFrac distance and non-phylogenetic distance metrics (i.e., Bray–Curtis, Jensen–Shannon divergence and Euclidian) using phyloseq in R (Hamady et al., 2010; McMurdie and Holmes, 2013). Taxonomy and diversity plots were produced using graphics tailored for phylogenetic analysis using the R package ggplot2 (Wickham, 2009). Differential abundance testing was performed on tables extracted from the phyloseq object at phylum, family, genus and species level. The data were analyzed using the PROC GLIMMIX procedure of Statistical Analysis Software (SAS) 9.4 (SAS Institute, Cary, NC). The model assessed the effect of treatment with the bird being the experimental unit. Fourteen birds per treatment group were used for the statistical analysis of the relative bacterial abundances. Results are presented using Benjamini–Hochberg (BH) adjusted P-values.

**RESULTS**

**Growth Performance**

The effects of increasing the inclusion level of laminarin on final body weight (BW), ADG, ADFI, and FCR are presented in Table 4. Dietary supplementation with 150 and 300 ppm laminarin had no effect on the final BW, ADG, ADFI, and FCR during d 0 to 21 (starter phase) and d 22 to 35 (finisher phase). However, during the overall 35-d experimental period, birds supplemented with 300 ppm laminarin had increased final BW and ADG compared to the control group (P < 0.05) and a higher ADFI compared to all other groups (P < 0.05). There was no difference in FCR between different treatment groups throughout the experiment.

**Effects of Laminarin Supplementation on the Caecal Microbiota**

**Bacterial Richness and Diversity.** Dietary supplementation with 300 ppm laminarin had no effect on the measures of alpha diversity (Table S2) and on beta diversity (Figure S1) in the caecal digesta. **Differential Bacterial Abundance Analysis.** There were three bacterial phyla identified in the caecal digesta with Firmicutes being the predominant (~94–97%) followed by Actinobacteria (~1.6–3.6%) and Proteobacteria (~1.3–2.7%). All data on bacterial abundances at phylum, family, genus and species level is provided in Tables S3 to 6. Only differentially abundant bacterial taxa at phylum, family, genus and species level in response to laminarin supplementation are presented in Table 5.

At the phylum level, dietary supplementation with 300 ppm laminarin increased the relative abundance of Actinobacteria and Proteobacteria in the caecal digesta (P < 0.05). At the family level, dietary supplementation with 300 ppm laminarin increased the relative abundance of Bifidobacteriaceae within the Actinobacteria

---

**Statistical Analysis of Data on Performance, Bacterial Populations by QPCR and Gene Expression**

All data were initially tested for normality using PROC UNIVARIATE procedure of SAS. The performance data was analyzed by repeated measures analysis using PROC MIXED procedure (Littell et al., 2006). The model included the fixed effects of treatment and time and their associated interactions with the initial weight used as a covariate and the pen being the experimental unit. The data on gene expression and bacterial populations by QPCR were analyzed using PROC GLM procedure. The model assessed the effect of treatment with the bird being the experimental unit. An outlier from the 300 ppm laminarin-supplemented group was removed from the analysis of the data on bacterial populations by QPCR as it consistently had lower logGCN/g digesta for each bacterial group tested that was associated with problematic bacterial DNA extraction. Probability values of <0.05 denote statistical significance. Results are presented as least-square mean values ± standard error of the means.
phylum in the caecal digesta ($P < 0.05$). At the genus level, dietary supplementation with 300 ppm laminarin increased the relative abundance of *Bifidobacterium* within the *Bifidobacteriaceae* family and decreased the relative abundance of *Sporobacter* within the Firmicutes phylum in the caecal digesta ($P < 0.05$). At the species level, dietary supplementation with 300 ppm laminarin increased the relative abundance of *Bifidobacterium pseudolongum* and *Streptococcus alactolyticus* and decreased the relative abundance of *Sporobacter termitidis* in the caecal digesta ($P < 0.05$).

**Selected Bacterial Populations Quantified by QPCR.** The effects of dietary supplementation with 300 ppm laminarin on selected bacterial populations in the caecal digesta of broiler chickens are presented in Table 6. Birds supplemented with 300 ppm laminarin had increased caecal *Bifidobacterium* spp. counts compared to the control group ($P = 0.05$). The inclusion of 300 ppm laminarin had no effects on the counts of total bacteria, *Lactobacillus* spp., *Enterobacteriaceae* and butyrate-producing bacteria in the caecum.

**Table 5.** Differential relative abundance of bacterial taxa at the phylum, family, genus and species level in the caecal digesta of chickens supplemented with 300 ppm laminarin (mean % relative abundance ± standard errors).

| Laminarin inclusion level (ppm) | Phylum | 0 | 300 | SEM | Adjusted $P$-value |
|--------------------------------|--------|---|-----|-----|-------------------|
| Actinobacteria                | 1.60   | 3.56 | 0.419 | 0.005 |
| Proteobacteria                | 1.29   | 2.66 | 0.368 | 0.020 |
| Family                        |        |     |     |     |                   |
| *Bifidobacteriaceae*          | 1.53   | 3.53 | 0.416 | 0.003 |
| Genus                         |        |     |     |     |                   |
| *Bifidobacterium*             | 1.48   | 3.48 | 0.410 | 0.004 |
| *Sporobacter*                 | 2.76   | 1.51 | 0.389 | 0.034 |
| Species                       |        |     |     |     |                   |
| *Bifidobacterium pseudolongum*| 1.22   | 2.71 | 0.367 | 0.011 |
| *Sporobacter termitidis*      | 3.06   | 1.63 | 0.404 | 0.023 |
| *Streptococcus alactolyticus* | 1.06   | 2.23 | 0.337 | 0.026 |

1A total of 14 replicates were used for the control and 300 ppm laminarin treatment respectively (replicate = bird).

**Table 6.** Effects of 300 ppm laminarin on selected caecal bacterial populations measured by QPCR (Least-square mean values ± standard errors).

| Bacterial group (logGCN/g digesta) | Laminarin inclusion level (ppm) | 0 | 300 | SEM | $P$-value |
|-----------------------------------|---------------------------------|---|-----|-----|----------|
| Total bacteria                    | 11.24                          | 11.37 | 0.049 | 0.075 |
| *Lactobacillus* spp.              | 10.22                          | 10.34 | 0.095 | 0.409 |
| *Bifidobacterium* spp.            | 8.88                           | 9.36  | 0.163 | 0.050 |
| *Enterobacteriaceae*              | 7.64                           | 7.81  | 0.166 | 0.462 |
| B-CoA                             | 6.30                           | 6.46  | 0.167 | 0.505 |

Abbreviations: GCN, gene copy number; B-CoA, Butyryl-CoA:acetate CoA-transferase.

1A total of 16 and 13 replicates were used for the control and 300 ppm laminarin treatment respectively (replicate = bird).
Table 7. Differential expression of nutrient transporters, immunological markers and intestinal integrity markers in the small intestine of chickens supplemented with 300 ppm laminarin (Least-square mean values ± standard errors).

| Region                        | Target gene       | Laminarin inclusion level (ppm)¹ | SEM   | P-value |
|-------------------------------|-------------------|-----------------------------------|-------|---------|
| Nutrient transporters         |                   |                                   |       |         |
| Ileum                         | SLC15A1/PepT1     | 0.90                              | 1.14  | 0.061   | 0.009  |
| Markers of immune response    |                   |                                   |       |         |
| Duodenum                      | IL17A             | 0.88                              | 1.43  | 0.176   | 0.035  |
| Jejunum                       | TLR2              | 0.93                              | 1.17  | 0.123   | 0.036  |
| Ileum                         | IL17A             | 0.86                              | 1.54  | 0.222   | 0.041  |
| Markers of intestinal integrity|                  |                                   |       |         |
| Jejunum                       | CLDN1             | 0.94                              | 1.13  | 0.060   | 0.029  |
| Ileum                         | CLDN1             | 0.93                              | 1.19  | 0.089   | 0.042  |

Abbreviations: CLDN1, claudin 1; IL17A, interleukin 17A; SLC15A1/PepT1, peptide transporter 1; TLR2, toll-like receptor 2.

¹A total of 16 and 14 replicates were used for the control and 300 ppm laminarin treatment respectively (replicate = bird).

Gene Expression in the Small Intestine

The effects of laminarin supplementation on the expression of genes associated with the functionality and health in the small intestine of broiler chickens were evaluated. All gene expression data is presented in Table S7. The differentially expressed genes in the different parts of the small intestines are presented in Table 7 and highlighted in this section:

**Nutrient Transporters.** Dietary supplementation with 300 ppm laminarin upregulated the expression of SLC15A1/peptide transporter 1 (SLC15A1/PepT1, $P < 0.05$) in the ileum.

**Immunological Markers.** Dietary supplementation with 300 ppm laminarin upregulated the expression of interleukin 17A (IL17A, $P < 0.05$) in the duodenum and ileum and the expression of toll-like receptor 2 (TLR2, $P < 0.05$) in the jejunum.

**Intestinal Integrity.** Dietary supplementation with 300 ppm laminarin upregulated the expression of claudin 1 (CLDN1, $P < 0.05$) in the jejunum and ileum.

DISCUSSION

In this study, it was hypothesized that dietary supplementation with a laminarin-rich extract would enhance the performance of broiler chickens by upregulating the expression of nutrient transporters, promoting a beneficial caecal microbiota and enhancing intestinal integrity. The optimal inclusion level identified was 300 ppm laminarin as it led to the greatest broiler performance. Further investigation of the effects of 300 ppm laminarin on the gastrointestinal system identified an increase in the absolute and relative abundance of the caecal *Bifidobacterium* spp. and an increase in the expression of a protein transporter gene (SLC15A1/PepT1), a tight junction protein gene (CLDN1) and the inflammatory genes IL17A and TLR2.

Laminarin supplemented at 300 ppm increased feed intake, final BW and ADG during the 35-d supplementation period, whereas that was not evident at the lower inclusion level of 150 ppm. A supplement that increases feed intake is of major significance to the broiler industry as it directly influences growth rate, nutrient uptake and utilization, and subsequently gastrointestinal integrity and development (Ferket and Gernat, 2006; Maiorka et al., 2006; Yegani and Korver, 2008). Interestingly, an earlier study has provided some supporting evidence to suggest that laminarin ingestion stimulates feed intake; as chicks that were supplemented with seaweed extracts (containing $>30\%$ laminarin) in water for the first 7 d post-hatching compared to *in ovo* had an increase in feed intake (Bednarczyk et al., 2016). Based on the above, the treatment group supplemented with 300 ppm laminarin was solely selected for further analysis along with the control group. In the current study, an increase in the expression of SLC15A1/PepT1 in the ileum was identified in the laminarin-supplemented birds. PepT1 is a proton-dependent, low-affinity/high capacity di- and tripeptide transporter that is predominantly expressed in the small intestine in chickens (Daniel and Kottra, 2004; Zwarycz and Wong, 2013). The upregulation of SLC15A1/PepT1 expression may have led to improved absorption of the oligopeptides produced after the digestion of dietary protein. Thus, the enhanced growth in the laminarin-supplemented chicks in the current study may be attributed to increased feed intake and the concomitant increased absorption of dietary protein. Interestingly, SLC15A1/PepT1 was also among the nutrient transporters that were stimulated in weaned pigs supplemented with the same laminarin-rich extract (Rattigan et al., 2020).

Promoting a beneficial microbial composition in the caecum of broiler chickens via dietary interventions may improve productivity and health and confer colonization resistance to pathogens (Gaglia et al., 2010; Gadde et al., 2017). The major change in the overall composition of the caecal microbiota, as determined by 16S rRNA gene sequencing, in response to laminarin supplementation was the increase in the relative abundance of *Bifidobacterium* spp. (Actinobacteria phylum, *Bifidobacteriaceae* family). This result was subsequently confirmed by QPCR. In previous studies, laminarin supplementation increased the counts of *Bifidobacterium* spp. and selected strains of this genus in batch fermentation and pure cultures (Zhao and Cheung, 2011; Seong et al., 2019), while an increase in this bacterial group was also observed in day-old chicks after *in ovo* injection of a seaweed extract (containing $>30\%$ laminarin) (Bednarczyk et al., 2016). The ability of
Bifidobacterium spp. to utilize laminarin as a substrate is further supported by the presence of relevant carbohydrate-degrading enzymes such as β-glycosidases in their genome (Zhao and Cheung, 2011; Seong et al., 2019). In ovo injection with various Bifidobacterium spp. strains, particularly B. bifidum, resulted in improved performance and villus architecture in the ileum, stimulation of humoral immunity and beneficial changes in the composition of the ileal microbiota (Abdel-Moneim et al., 2020; El-Moneim et al., 2020). A positive correlation of bird weight and Bifidobacterium spp. relative abundance in ileum, caecum and trachea early in the life of broiler chicks has previously been reported (Johnson et al., 2018). Furthermore, anti-C. jejuni activity was observed for several Bifidobacterium spp. strains in vitro with B. longum also reducing C. jejuni counts in the feces of broiler chickens following oral supplementation (Santini et al., 2010; Baffoni et al., 2012). Thus, laminarin supplementation may have simultaneously promoted growth and gastrointestinal development and increased colonization resistance to pathogens by stimulating the beneficial Bifidobacterium spp. population, particularly during the first weeks post-hatching.

Laminarin supplementation also led to an increase in the relative abundance of Proteobacteria, which is considered an indicator of dysbiosis (Shin et al., 2015). However, the relative abundance of Proteobacteria was below 3% and no specific families, genera, or species linked to compromised health or zoonotic pathogens were differentially abundant following laminarin supplementation. Furthermore, members of the Proteobacteria phylum such as Sutterella have previously been correlated with improved body weight (Johnson et al., 2018). Additionally, there was a reduction in the relative abundance of Sporobacter genus and Sporobacter termitidis, and an increase in the relative abundance of Streptococcus alactolyticus in response to laminarin supplementation. The observed reduction in Sporobacter may be indirectly affected by laminarin supplementation as a result of the increases in Bifidobacterium spp. In a separate study, the relative abundance of this genus was reduced following supplementation with a mixture of Bifidobacterium spp. strains in mice (Wang et al., 2019). Sporobacter higher abundance in pathological conditions such as constipation in mice (Wang et al., 2019) and ETEC infection in weaned pigs (Zhang et al., 2017) indicates that it possibly acts as an opportunistic pathogen in animals. Streptococcus alactolyticus is the dominant species within the commensal streptococcal population in the chicken caecum (Czerwinski et al., 2010). Streptococcus alactolyticus (Streptococcus bovis/ Streptococcus equinus complex) can degrade β-glucans including laminarin, which supports the observed increase in relative abundance (Schlegel et al., 2003; Beckmann et al., 2006).

Markers of intestinal integrity were also influenced by laminarin supplementation with increased expression of CLDN1 in the jejunum and ileum, an effect that supports a similar observation in a previous study (Sweeney et al., 2017). Claudins are major components of the tight junctions that control the paracellular permeability between adjacent epithelial cells and, thus, control the diffusion of dietary antigens and nutrients and the translocation of microorganisms (Ulluwishewa et al., 2011). For instance, upregulation of claudin 1 and occludin in the jejunal of Salmonella enterica subsp. enterica serotype Typhimurium-infected chickens was associated with reduced pathogen translocation (Shao et al., 2013).

Nutritional immunomodulation is a promising strategy to confer resistance to important pathogens while minimizing the negative impact of the inflammatory response on broiler performance (Korver, 2012; Suresh et al., 2018; Kim and Lillehoj, 2019). In this study, laminarin supplementation upregulated the expression of IL17A and TLR2 in the small intestine. β-glucans have previously been identified as a stimulant of genes within the IL17 family, via cytokine signaling (Werner et al., 2011; Kamiya et al., 2018). IL17A is among the major cytokines associated with a protective inflammatory response against pathogens (Iwakura et al., 2011). IL17A is highly expressed in the small and large intestine in chickens (Kim et al., 2012) and is involved in the immune response against various infectious agents such as Eimeria spp. (Kim et al., 2008; Zhang et al., 2012), C. jejuni (Connerton et al., 2018), S Enteritidis (Chranova et al., 2011; Karaffova et al., 2015) and Clostridium perfringens (Enami et al., 2019). Previously, oral supplementation of mice with laminarin increased TLR2 expression in the small intestine, like the current study (Rice et al., 2005). TLR2 is a member of the transmembrane Toll-like receptors involved in pathogen recognition and immune response initiation, that is predominantly involved in the recognition of Gram-positive bacteria but also Gram-negative bacteria, fungi, viruses, and parasites (Keestra et al., 2013; Nawab et al., 2019). S. Enteritidis infection in chickens led to the suppression of the inflammatory response including TLR2 downregulation that was associated with increased S. Enteritidis counts in the caecum and crop, S. Enteritidis invasion in extraintestinal tissues and susceptibility to infection (Gou et al., 2012; Quinteiro-Filho et al., 2017). Therefore, the observed upregulation of IL17A and TLR2 solely due to laminarin supplementation might be indicative of a more controlled and potentially protective immune response without compromising the growth performance of the laminarin-supplemented birds.

**CONCLUSION**

Dietary supplementation with a laminarin-rich extract at 300 ppm was associated with increased BW, ADG and ADFI. Further analysis of various aspects of gastrointestinal functionality and health identified an upregulation of genes associated with nutrient absorption, intestinal integrity and protective immune responses against pathogens and an alteration in the composition of the microbiota characterized
predominantly by a beneficial increase in *Bifidobacterium* spp. Thus, laminarin supplementation is a promising dietary supplement to improve performance and support gastrointestinal development and health in broiler chickens.

**ACKNOWLEDGMENTS**

This work was supported by the Science Foundation Ireland (SFI) [grant number: 14/IA/2548].

**DISCLOSURES**

The authors have no conflict of interest to declare.

**SUPPLEMENTARY MATERIALS**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j. psj.2021.101179.

**REFERENCES**

Abdel-Moneim, A. E., A. M. Elbaz, R. E. Khidr, and F. B. Badri. 2020. Effect of in ovo inoculation of Bifidobacterium spp. on growth performance, thyroid activity, ileum histomorphometry, and microbial enumeration of broilers. Probiotics Antimicrob. Proteins. 12:873–882.

Angly, F. E., P. G. Dennis, A. Skarszewski, I. Vanwonterghem, P. Hugenholtz, and G. W. Tyson. 2014. CopyRighter: a rapid tool for improving the accuracy of microbial community profiles through lineage-specific gene copy number correction. Microbiome. 2:11.

Baffoni, L., F. Gaggia, D. Di Gioia, C. Santini, L. Mogna, and B. Biavati. 2012. A Bifidobacterium-based symbiotic product to reduce the transmission of C. jejuni along the poultry food chain. Int. J. Food Microbiol. 157:156–161.

Beckmann, L., O. Simon, and W. Vahjen. 2006. Isolation and identification of mixed linked beta-glucan degrading bacteria in the intestine of broiler chickens and partial characterization of respective 1,3-1,4-beta-glucanase activities. J. Basic Microbiol. 46:175–185.

Bednarczyk, M., K. Stadnicka, I. Kozłowska, C. Abioso, S. Tavaniello, A. Dunkowiakowska, A. Slawinska, and G. Maiorano. 2016. Influence of different prebiotics and mode of their administration on broiler chicken performance. Animal 10:1271–1279.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Penna, J. K. Goodrich, J. I. Gordon, A. G. Hutten, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Weidman, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335–336.

Cherry, P., S. Yadav, C. R. Strain, P. J. Allsopp, E. M. McSorley, R. P. Ross, and C. Stanton. 2019. Prebiotics from seaweeds: an ocean of opportunity? Mar. Drugs. 17:327.

Connerton, P. L., P. J. Richards, G. M. Lafontaine, P. M. O’Kane, N. Ghaflar, N. J. Cummings, D. L. Smith, N. M. Fish, and I. P. Connerton. 2018. The effect of the timing of exposure to Campylobacter jejuni on the gut microbiome and inflammatory responses of broiler chickens. Microbiome 6:68.

Chranova, M., H. Hradecká, M. Falkynová, M. Matulová, H. Havlíckova, F. Sisak, and I. Rychlík. 2011. Immune response of chicken gut to natural colonization by gut microflora and to Salmonella enterica serovar enteritidis infection. Infect. Immun. 79:2755–2763.

Czerwiński, J., O. Højberg, S. Smmulikowska, R. M. Engberg, and A. Miezczkowska. 2010. Influence of dietary peas and organic acids and probiotic supplementation on performance and caecal microbial ecology of broiler chickens. Br. Poult. Sci. 51:258–269.

Daniel, H., and G. Kottra. 2004. The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. Pfugers Arch 447:610–618.

Dünn, J. J., and J. D. Richards. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84:634–643.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200.

El-Moneim, A. E. A., I. El-Wardany, A. M. Abu-Taleb, M. M. Wakwak, T. A. Ebeid, and A. A. Saleh. 2020. Assessment of in ovo administration of Bifidobacterium bifidum and Bifidobacterium longum on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics Antimicrob. Proteins. 12:439–450.

Emami, N. K., A. Calik, M. B. White, M. Young and R. A. Dalloul. 2019. Necrotic enteritis in broiler chickens: the role of tight junctions and mucosal immune responses in alleviating the effect of the disease. Microorganisms. 7:231.

Eren, A. M., L. Maingien, W. J. Sui, L. G. Murphy, S. L. Grim, H. G. Morrison, and M. L. Sogin. 2013. Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. Methods Ecol. Evol. 4:1111–1119.

Eren, A. M., H. G. Morrison, P. J. Lescault, J. Reveillaud, J. H. Vineis, and M. L. Sogin. 2015. Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J. 9:968–979.

Ferket, P., and A. Gernat. 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci. 5:905–911.

Frank, D. N., A. L. St. Amand, R. A. Feldman, E. C. Boeckner, N. Harpaz, and N. R. Pace. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. PNAS 104:13780.

Gadée, U., W. H. Kim, S. T. Oh, and H. S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18:26–45.

Gaggia, F., P. Mattarelli, and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. Int. J. Food Microbiol. 141:(Suppl 1), S15–S28.

Garcia-Vaquero, M., J. V. O'Doherty, B. K. Tiwari, T. Sweeney, and G. Rajauria. 2019. Enhancing the extraction of polysaccharides and antioxidants from macroalgae using sequential hydrothermal-assisted extraction followed by ultrasound and thermal technologies. Mar. Drugs. 17:457.

Garcia-Vaquero, M., G. Rajauria, J. V. O’Doherty, and T. Sweeney. 2017. Polysaccharides from macroalgae: Recent advances, innovative technologies and challenges in extraction and purification. Food Res. Int. 99:1011–1020.

Gou, Z., R. Liu, G. Zhao, M. Zheng, P. Li, H. Wang, Y. Zhu, J. Chen, and J. Wen. 2012. Epigenetic modification of TLRs in leukocytes is associated with increased susceptibility to Salmonella enteritidis in chickens. PLoS One 7:e3627.

Hamaidy, M., C. Lozupone, and R. Knight. 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. ISME J 4:17–27.

Iwakura, Y., H. Ishigame, S. Saijo, and S. Nakae. 2011. Functional specialization of interleukin-17 family members. Immunity 34:149–162.

Johnson, T. J., B. P. Youmans, S. Noll, C. Cardona, N. P. Evans, T. P. Karnezos, J. M. Ngunjiri, M. C. Abundo, and C. W. Lee. 2018. A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl. Environ. Microbiol. 84:doi:10.1128/AEM.00362-18. e00362-18.

Kadum, S. U., B. K. Tiwari, and C. P. O’Donnell. 2015. Extraction, structure and biofunctional activities of laminarin from brown algae. Int. J. Food Sci. Technol. 50:24–31.

Kamiya, T., C. Tang, M. Kadoki, K. Oshima, M. Hattori, S. Saijo, Y. Adachi, N. Ohno, and Y. Iwakura. 2018. β-Glucans in food modify colonic microflora by inducing antimicrobial protein,
calproctin, in a Dectin-1-induced-IL-17F-dependent manner. Mucosal Immunol 11:763–773.

Karaffova, V., K. Bobikova, E. Husakova, M. Levkut, R. Herich, V. Revajova, M. Levkutova, and M. Levkut. 2015. Interaction of TGFBeta1 and IL-17 with IgA secretion in the intestine of chickens fed with E. faecium AL41 and challenged with S. Enteritidis. Res. Vet. Sci. 100:75–79.

Keestra, A. M., M. R. de Zoete, L. I. Bouwman, M. M. Vaezirad, and Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wadkins. 2018. Immune-related gene expression in two B-complex disparate genetically inbred Fayoumi chicken lines following Eimeria maxima infection. Poult. Sci. 87:433–443.

Kim, W. H., J. Jeong, A. R. Park, D. Yim, Y. H. Kim, K. D. Kim, H. H. Chang, S. H. Lillehoj, B. H. Lee, and W. Min. 2012. Chicken IL-17F: identification and comparative expression analysis in Eimeria-infected chickens. Dev. Comp. Immunol. 38:401–409.

Kim, W. H., and H. S. Lillehoj. 2019. Immunity, immunomodulation, and antibiotic alternatives to maximize the genetic potential of poultry for growth and disease response. Anim. Feed Sci. Technol. 250:41–50.

Korver, D. R. 2012. Implications of changing immune function through nutrition in poultry. Anim. Feed Sci. Technol. 173:54–64.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wollinger, and O. Schabenberger. 2006. SAS for Mixed Models (2nd ed.). SAS Publishing.

Louis, P., and H. J. Flint. 2007. Development of a semiquantitative degenerate real-time PCR-based assay for estimation of numbers of butyryl-CoA-CoA transferase genes in complex bacterial samples. Appl. Environ. Microbiol. 73:2009–2012.

Majoč, T., and S. L. Salzberg. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963.

Maiozani, G., K. Stadnicka, S. Tavaniello, C. Abiúso, J. Bogucka, and M. Bednarczyk. 2017. In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. Poult. Sci. 96:511–518.

McMurdie, P., and S. Holmes. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217.

Metzler-Zebeli, B. U., S. Hooda, R. Pieper, R. T. Zijlstra, R. T. Zijlstra, R. Pieper, and M. G. Ganzle. 2010. Non-starch polysaccharides modulate bacterial microbiota, pathways of short reads to improve genome assemblies. Bioinformatics 27:2957–2963.

National Research Council. 1994. Nutrient Requirements of Poultry: Ninth Revised Edition. 9th rev. Natl. Acad. Press, Washington, DC ed.

O'Sullivan, L. B., B. Murphy, P. McLoughlin, P. Duggan, P. G. Lawlor, H. Hughes, and G. E. Gardiner. 2010. Prebiotics from marine macroalgae for human and animal health applications. Mar. Drugs. 8:2058–2064.

Pender, M., C. Vink, C. Driessen, N. London, C. Thijis, and E. E. Stoberling. 2005. Quantification of Bifidobacterium spp., Escherichia coli and Clostridium difficile in faecal samples of breast-fed and formula-fed infants by real-time PCR. FEMS Microbiol. Lett. 243:141–147.

Quintino-Filho, W. M., A. S. Calefi, D. S. Cruz, T. P. A. Aloi, A. Zager, C. S. Astolfi-Ferreira, J. A. Piantino Ferreira, S. Sharif, and J. Palermo-Neto. 2017. Heat stress decreases expression of the cytokines, avian beta-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. Vet. Immunol. Immunopathol. 186:19–28.

Rattigan, R., T. Sweeney, S. Mahler, K. Thornton, G. Rajairaja, and J. V. O’Doherty. 2020. Laminarin-rich extract improves growth performance, small intestinal morphology, gene expression of nutrient transporters and the large intestinal microbial composition of piglets during the critical post-weaning period. Br. J. Nutr. 123:255–263.

Rice, P. J., E. L. Adams, T. Ozment-Skelton, A. J. Gonzalez, M. P. Goldman, B. E. Lockhart, L. A. Barker, K. F. Breuel, W. A. Bluett, J. H. Kaldiliebsch, E. E. Ensey, G. D. Brown, S. Gordon, and D. L. Williams. 2005. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. J. Pharmacol. Exp. Ther. 314:1079–1086.

Rogues, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.

Santini, C., L. Baffoni, F. Gaggia, M. Granata, R. Gasbarri, D. Di Gioia, and B. Biavati. 2010. Characterization of probiotic strains: an application as feed additives in poultry against Campylobacter jejuni. Int. J. Food Microbiol. 141:(Suppl 1), S98–108.

Sauvant, D., J. M. Perez, and G. Tran. 2004. Table of Composition of Fresh Organic Materials. 9th rev. Natl. Acad. Press, Washington, DC ed.

Schlegel, L., F. Grimont, E. Ageron, P. A. D. Grimont, and A. Bouvet. 2003. Reappraisal of the taxonomy of the Streptococcus bovis/Streptococcus equinus complex and related species: description of Streptococcus galgaliticus subsp. galgaliticus subsp. nov., S. galgaliticus subsp. macrodonicus subsp. nov. and S. galgaliticus subsp. pasteurianus subsp. nov. Int. J. Syst. Evol. Microbiol. 53:631–645.

Seong, J.-H. Bae, J. S. Seo, S.-A. Kim, T.-J. Kim, and N. S. Han. 2019. Comparative analysis of prebiotic effects of seaweed polysaccharides laminaran, porphyran, and ulvan using in vitro human fecal fermentation. J. Funct. Foods. 57:408–416.

Shao, Y., Y. Guo, and Z. Wang. 2013. beta-1,3-1.6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with Salmonella enterica serovar Typhimurium. Poult. Sci. 92:1764–1773.

Shin, N. R., T. W. Whon, and J. W. Bae. 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33:496–503.

Sleş, G., R. K. Das, S. Kaur Brar, T. Rouissi, A. Avalos Ramirez, Y. Chorro, and S. Godbout. 2018. Alternatives to antibiotics in poultry feed: molecular perspectives. Crit. Rev. Microbiol. 44:318–335.

Sweeney, T., C. B. Collins, P. Reilly, K. M. Pierce, M. Ryan, and J. V. O’Doherty. 2012. Effect of purified beta-glucons derived from Laminaria digitata, Laminaria hyperborea and Saccharomyces cerevisiae on piglet performance, selected bacterial populations, volatile fatty acids and pro-inflammatory cytokines in the gastrointestinal tract of pigs. Br. J. Nutr. 108:1226–1234.

Sweeney, T., H. Meredith, M. T. Ryan, V. Gath, K. Thornton, and J. V. O’Doherty. 2016. Effects of Ascophyllum nodosum supplement on Campylobacter jejuni colonisation, performance and gut health following an experimental challenge in 8-day old chicks. Innov. Food Sci. Emerg. Technol. 37:247–252.

Sweeney, T., H. Meredith, S. Vigors, M. J. McDonnell, M. Ryan, and K. Thornton, J. V. O’Doherty. 2017. Extracts of laminarin and laminarin/fucoidan from the marine macroalgal species Laminaria digitata improved growth rate and intestinal structure in young chicks, but does not influence Campylobacter jejuni colonisation. Anim. Feed Sci. Technol. 232:71–79.

Takahashi, H., R. Saito, S. Miya, Y. Tanaka, N. Miyamura, T. Kuda, and H. Kimura. 2017. Development of quantitative real-time PCR for detection and enumeration of Enterobacteriaceae. Int. J. Food Microbiol. 246:92–97.

Tavaniello, S., G. Maiorano, K. Stadnicka, R. Mucci, J. Bogucka, and M. Bednarczyk. 2018. Prebiotics offered to broiler chicken exert positive effect on meat quality traits irrespective of delivery route. Poult. Sci. 97:2979–2987.

Ulluwisheva, D., R. C. Anderson, W. C. McNabb, P. J. Moughan, J. M. Wells, and N. C. Roy. 2011. Regulation of tight junction
permeability by intestinal bacteria and dietary components. J. Nutr. 141:769–776.
Venardou, B., J. V. O’Doherty, M. J. McDonnell, A. Mukhopadhyya, C. Kiely, M. T. Ryan, and T. Sweeney. 2021. Evaluation of the in vitro effects of the increasing inclusion levels of yeast beta-glucan, a casein hydrolysate and its 5 kDa retentate on selected bacterial populations and strains commonly found in the gastrointestinal tract of pigs. Food Funct., doi:10.1039/d0fo02269a.
Venardou, B., J. V. O’Doherty, M. J. McDonnell, A. Mukhopadhyya, C. Kiely, M. T. Ryan, and T. Sweeney. 2021. Evaluation of the in vitro effects of the increasing inclusion levels of yeast beta-glucan, a casein hydrolysate and its 5 kDa retentate on selected bacterial populations and strains commonly found in the gastrointestinal tract of pigs. Food Funct., doi:10.1039/d0fo02269a.

Wang, L., C. Chen, S. Cui, Y. K. Lee, G. Wang, J. Zhao, H. Zhang, and W. Chen. 2019. Adhesive Bifidobacterium induced changes in cecal microbiome alleviated constipation in mice. Front. Microbiol. 10:1721.
Werner, J. L., M. A. Gessner, L. M. Lilly, M. P. Nelson, A. E. Metz, D. Horn, C. W. Dunaway, J. Deshane, D. D. Chaplin, C. T. Weaver, G. D. Brown, and C. Steele. 2011. Neutrophils produce interleukin 17A (IL-17A) in a dectin-1- and IL-23-dependent manner during invasive fungal infection. Infect. Immun. 79:3966–3977.
Wickham, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, Berlin/Heidelberg, Germany.
Vegani, M., and D. R. Korver. 2008. Factors affecting intestinal health in poultry. Poult. Sci. 87:2052–2063.
Zhang, L., L. Ma, R. Liu, Y. Zhang, S. Zhang, C. Hu, M. Song, J. Cai, and M. Wang. 2012. Eimeria tenella heat shock protein 70 enhances protection of recombinant microneme protein MIC2 subunit antigen vaccination against E. tenella challenge. Vet. Parasitol. 188:239–246.
Zhao, J., and P. C. Cheung. 2011. Fermentation of beta-glucans derived from different sources by bifidobacteria: evaluation of their bifidogenic effect. J. Agric. Food Chem. 59:5986–5992.
Zwarycz, B., and E. A. Wong. 2013. Expression of the peptide transporters PepT1, PepT2, and PHT1 in the embryonic and posthatch chick. Poult. Sci. 92:1314–1321.