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*Article*

# Dietary Probiotics Modulate Gut Barrier and Immune-Related Gene Expression and Histomorphology in Broiler Chickens under Non- and Pathogen-Challenged Conditions: A Meta-Analysis

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**Table S1.** List of references and the respective experimental variables included in the meta-analysis.

| | | | | Pathogen | | | | Gut Segments | | | | Variables | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study** | **Reference** | **Basal Diet[3]** | **Dietary Probiotics[1]** | **No** | **Yes** | **Types[2]** | **Route** | **D** | **J** | **I** | **C** | **BAR** | **IMU** | **HIS** | **GRO** |
| 1 | Zhen et al. (2018) | corn, soybean meal | *B. coagulans* | x | x | *S. enteritidis* | oral | | x | | | x | x | x | x |
| 2 | Kan et al. (2021) | corn, wheat, soybean meal | *B. licheniformis* | | x | *E. maxima, C. perfringens* | oral | | x | | | x | x | | x |
| 3 | Zhang et al. (2021) | corn, soybean meal, fish meal | *B. coagulans* | x | | | | | | x | | x | | | x |
| 4 | Li et al. (2015) | corn, soybean meal, corn gluten meal | *B. amyloliquefaciens* | | x | *Escherichia coli* | injection | | x | x | | x | x | x | x |
| 5 | Liu et al. (2021) | corn, soybean meal | *B. subtilis* | | x | *C. perfringens* | oral | | x | | | x | x | | |
| 6 | Wang et al. (2021) | corn, soybean meal, corn gluten meal | *B. subtilis* | | x | *C. perfringens* | oral | | x | | | x | | x | x |
| 7 | Wu et al. (2018) | corn, soybean meal | *B. coagulans* | x | x | *E. maxima, E. tenella, C. perfringens* | oral | | x | | | x | x | x | x |
| 8 | Musa et al. (2019) | corn, wheat, distiller grain, soybean meal, corn protein powder, peanut meal, | *B. subtilis, B. licheniformis* | | x | *C. perfringens* | oral | x | x | x | | x | | x | x |
| 9 | Bilal et al. (2021) | corn, soybean meal | *B. subtilis, B. pumilus* | x | | | | | | x | | x | | | x |
| 10 | Chaudhari et al. (2020) | corn, soybean meal | *B. licheniformis, B. amyloliquefaciens* | | x | *E. maxima, E. tenella, E. acervulina* | oral | | x | | x | x | x | | |
| 11 | Wu et al. (2021) | corn, soybean meal | *L. acidophilus* | x | x | *Escherichia coli* | oral | | x | x | | x | x | x | x |
| 12 | Xu et al. (2021) | corn, bran, soybean meal, cottonseed | *C. butyricum* | x | x | *E. maxima, C. perfringens* | oral | | | x | | x | x | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | meal, corn gluten meal | | | | | | | | | | | | |
| 13 | Wu et al. (2019) | corn, soybean meal | *E. faecium* | x | x | *C. perfringens* | oral | x | | | x | x | | |
| 14 | He et al. (2019) | corn, soybean meal, fish meal, corn gluten meal | *B. subtilis, B. licheniformis, S. cerevisiae* | x | | | | x | x | | x | | x | x |
| 15 | Memon et al. (2021) | corn, soybean meal | *B. subtilis* | | x | *E. tenella* | oral | | | x | x | | | |
| 16 | Mountzouris et al. (2019) | corn, soybean meal | *L. reuteri, L. salivarius, E. faecium, Bi. animalis, P. acidilactici* | x | | | | | x | | x | | | |
| 17 | Wu et al. (2019) | corn, wheat, soybean meal, fish meal | *L. plantarum, Pa. polymyxa* | x | | | | x | x | | x | | | |
| 18 | Huang et al. (2019) | corn, wheat, soybean meal | *E. faecium* | | x | *Escherichia coli* | injection | x | | | x | x | x | |
| 19 | Bai et al. (2013) | corn, soybean meal, rapeseed meal, cottonseed meal | *L. fermentum, S. cerevisiae* | x | | | | x | | | | x | | x |
| 20 | Yang et al (2021) | corn, bran, soybean meal, fish meal | *B. subtilis* | | x | *E. tenella* | oral | | | x | x | | | |
| 21 | Wang et al. (2016) | corn, soybean meal | *S. cerevisiae* | x | x | *Escherichia coli* | oral | x | x | | | x | x | x |
| 22 | Chang et al. (2020) | corn, soybean meal | *L. acidophilus, L. fermentum, L. casei, P. acidilactici* | | x | *S. enterica* | oral | x | | x | x | x | | |
| 23 | Lan et al. (2020) | corn, bran, soybean meal, rapeseed meal | *P. pentosaceus* | | x | *S. enteritidis* | oral | | x | x | | x | | |
| 24 | Wang et al. (2017) | corn, soybean meal | *L. johnsonii* | | x | *E. maxima, E. acervulina, C. perfringens* | oral | | x | | | x | | x |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Li et al. (2018) | corn, soybean meal | *L. acidophilus* | x | x | *C. perfringens* | oral | | x | | x | x | x | |
| 26 | Wang et al. (2017) | corn, soybean meal, fish meal | *L. plantarum* | | x | *Escherichia coli* | oral | | x | x | | x | x | |
| 27 | Chen et al (2022) | corn, soybean meal | *L. salivarius* | | x | *S. pullorum*, Aflatoxin B1 | oral, diet | | | x | x | | | |
| 28 | Calik et al. (2019) | corn, soybean meal | *B. amyloliquefaciens* | | x | *E. maxima, E. tenella, E. acervulina* | oral | | x | x | | x | x | |
| 29 | Qiu et al. (2021) | corn, soybean meal | *B. subtilis* | x | | | | | x | x | x | | x | |
| 30 | Cao et al. (2019) | corn, soybean meal | *L. plantarum* | | x | *C. perfringens* | oral | | | x | | x | | |
| 31 | Li et al. (2017) | wheat, soybean meal | *B. subtilis* | x | x | *C. perfringens* | oral | | x | x | | x | x | |
| 32 | Aliakbarpour e al. (2012) | corn, soybean meal | *B. subtilis, L. casei, L. acidophilus, Bi. Thermophilium, E. faecium* | x | | | | | x | | x | | x | x |
| 33 | Gao et al. (2022) | corn, soybean meal, fish meal | *L. plantarum* | x | | | | | x | | x | | x | x |
| 34 | Hosseini et al. (2019) | corn, soybean meal | *B. subtilis, B. tequilensis* | x | | | | | | x | x | | x | x |
| 35 | Konieczka et al (2022) | corn, wheat, soybean meal | *B. licheniformis, B. amyloliquefaciens, B. subtilis* | x | x | *C. perfringens, E. maxima, E. tenella, E. acervulina, E. mitis, E. praecox* | oral | x | x | x | x | | x | x |
| 36 | Hosseini et al. (2018) | triticale, soybean meal, corn gluten meal | *B. subtilis, B. licheniformis* | x | | | | | x | | x | | | |
| 37 | Gong et al. (2021) | corn, wheat, soybean meal, fish meal | *L. plantarum, Pa. polymyxa* | | x | *C. perfringens* | oral | | | x | x | x | | |
| 38 | Zhao et al (2022) | corn, soybean meal | *B. licheniformis* | | x | *C. perfringens* | oral | | x | | x | | | |
| 39 | Liu et al. (2022) | corn, soybean meal, fish meal, rapeseed meal | *C. butyricum* | x | | | | | x | x | x | x | x | x |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | Guo et al. (2021) | barley, corn, soybean meal, fish meal | *L. fermentum, B. coagulans* | x | x | *C. perfringens* | oral | | x | | | | x | | |
| 41 | Emami et al. (2019) | corn, soybean meal | *L. acidophilus, L. casei, E. faecium, Bi. Bifidum* | | x | *C. perfringens, E. maxima, E. tenella, E. acervulina, E. mivati* | spray | | x | | | x | x | | x |
| 42 | Mohammed et al. (2021) | corn | *St. faecalis, C. butyricum, B. mesentericus* | | x | *S. minnesota* | oral | | | | x | | x | | |
| 43 | Mohsin et al. (2022) | corn, soybean meal | *L. plantarum* | | x | *E. tenella* | oral | | | | x | x | | | |
| 44 | Wu et al. (2018) | corn, soybean meal, corn gluten meal | *L. plantarum* | | x | *F. graminearum* | diet | | | x | | x | x | | |
| 45 | Wang et al. (2022) | corn, soybean meal | *B. subtilis* | | x | *S. pullorum* | oral | | | | x | x | | | |
| 46 | Deng et al. (2020) | corn, soybean meal, corn gluten meal | *L. acidophilus, L. plantarum* | | x | *L. monocytogenes* | oral | | | | x | | x | | |
| 47 | Ateya et al. (2019) | corn, soybean meal, corn gluten meal | *P. acidilactici* | | x | *Escherichia coli* | oral | | | x | | | x | | |
| 48 | Zhang et al. (2022) | corn, soybean meal, corn gluten meal | *B. amyloliquefaciens* | | x | *C. perfringens* | spray | | x | | | x | x | | x |
| 49 | Dong et al. (2016) | corn, soybean meal, cottonseed meal | *E. fecalis* | x | | | | | x | x | | x | | x | x |
| 50 | Xiao et al. (2022) | corn, soybean meal | *B. methylotrophicus* | x | | | | | x | x | | x | | x | x |
| 51 | Azizi et al. (2021) | corn, rice, soybean meal | *L. acidophilus, L. animalis, L. reuteri, L. fermentum, L. gallinarum, S. cerevisiae* | | x | *F. graminearum* | diet | x | x | x | | x | | x | |
| 52 | Emami et al. (2020) | corn, soybean meal | *B. licheniformis* | x | x | *C. perfringens, E. maxima, E. tenella, E. acervulina, E. mivati* | spray | | x | x | | x | x | x | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | Gharib-Naseri et al. (2020) | wheat, soybean meal, sorghum | *B. amyloliquefaciens* | x | x | *C. perfringens, E. maxima, E. acervulina, E. brunetti* | oral | x | | x | x |
| 54 | Bodinga et al. (2020) | corn, soybean meal | *B. subtilis* | | x | *C. perfringens* | oral | x | x | | x |

[1] *B, Bacillus; Bi, Bifidobacterium; C, Clostridium; E, Enterococcus; L, Lactobacillus; Pa, Paenibacillus; P, Pediococcus; S,* Saccharomyces*; St, Streptococcus.*

[2] *C, Clostridium; E, Eimeria; F, Fusarium; L, Listeria; S, Salmonella.*

[3] Main energy and protein feedstuffs of basal diet.

D, duodenum; J, jejunum; I, ileum; C, caeca; BAR, gut barrier-related gene expression; IMU, gut immunity-related gene expression; HIS, histomorphology; GRO, growth performance.

**Table S2**. Descriptive statistics for predictive and response variables of jejunal and ileal gene expression (fold-change) related to barrier function and immune response in broiler chickens at week 3 and 6 of life without pathogen challenge.

| Variable[1,2] | nTreat | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 17 | 4.8 | 1.16 | 0 | 11.0 | 6.6 |
| Dietary ME (MJ/kg) | 17 | 12.4 | 0.06 | 12.1 | 12.9 | 12.4 |
| Dietary CP (%) | 17 | 21.3 | 0.14 | 20.5 | 22.1 | 21.0 |
| *MUC2* | 10 | 1.2 | 0.13 | 0.9 | 2.3 | 1.0 |
| *ZO1* | 11 | 1.1 | 0.04 | 0.9 | 1.4 | 1.0 |
| *OCLN* | 13 | 1.4 | 0.20 | 0.9 | 3.5 | 1.0 |
| *CLDN1* | 11 | 1.2 | 0.10 | 0.9 | 1.9 | 1.0 |
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 22 | 4.7 | 0.96 | 0 | 11.0 | 6.8 |
| Dietary ME (MJ/kg) | 22 | 12.4 | 0.05 | 12.1 | 12.9 | 12.3 |
| Dietary CP (%) | 22 | 21.3 | 0.11 | 20.5 | 22.0 | 21.0 |
| *IL1B* | 11 | 1.0 | 0.04 | 0.6 | 1.1 | 1.0 |
| *IFNG* | 14 | 1.0 | 0.04 | 0.8 | 1.4 | 1.0 |
| *TLR2* | 11 | 1.1 | 0.12 | 0.7 | 2.1 | 1.0 |
| Jejunum, Week 6 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 24 | 5.8 | 0.95 | 0 | 11.0 | 8.7 |
| Dietary ME (MJ/kg) | 24 | 13.0 | 0.06 | 12.6 | 13.4 | 13.2 |
| Dietary CP (%) | 24 | 19.5 | 0.15 | 18.2 | 20.7 | 19.3 |
| *MUC2* | 10 | 1.8 | 0.37 | 1.0 | 4.5 | 1.3 |
| *ZO1* | 14 | 1.7 | 0.33 | 1.0 | 5.6 | 1.2 |
| *OCLN* | 16 | 1.4 | 0.18 | 0.6 | 3.1 | 1.0 |
| *CLDN1* | 12 | 1.4 | 0.18 | 0.5 | 2.6 | 1.3 |
| Ileum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 13 | 5.7 | 1.32 | 0 | 11.3 | 8.0 |
| Dietary ME (MJ/kg) | 13 | 12.3 | 0.05 | 12.1 | 12.6 | 12.2 |
| Dietary CP (%) | 13 | 21.5 | 0.18 | 21.0 | 22.4 | 21.0 |
| *MUC2* | 10 | 2.0 | 0.64 | 0.7 | 7.5 | 1.0 |
| *ZO1* | 11 | 1.2 | 0.19 | 0.1 | 2.3 | 1.0 |
| *OCLN* | 13 | 1.8 | 0.62 | 0.7 | 9.0 | 1.0 |
| *CLDN1* | 10 | 1.3 | 0.15 | 0.7 | 2.2 | 1.0 |
| Ileum, Week 6 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 19 | 6.2 | 0.99 | 0 | 10.4 | 8.5 |
| Dietary ME (MJ/kg) | 19 | 12.9 | 0.06 | 12.6 | 13.3 | 12.8 |
| Dietary CP (%) | 19 | 19.4 | 0.18 | 18.1 | 20.7 | 19.2 |
| *MUC2* | 15 | 1.9 | 0.39 | 0.7 | 5.2 | 1.0 |
| *ZO1* | 14 | 2.3 | 0.63 | 0.5 | 8.6 | 1.0 |
| *OCLN* | 16 | 1.3 | 0.17 | 0.7 | 3.4 | 1.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *CLDN1* | 11 | 1.5 | 0.26 | 0.6 | 3.1 | 1.0 |

$n_{Treat}$, number of treatments mean; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit; *MUC2*, mucin-2; *ZO1*, zonula occludens-1; *OCLN*, occludin; *CLDN1*, claudin-1; *IL1B*, interleukin-1beta; *TLR2*, toll-like receptor-2; *IFNG*, interferon-gamma.

[1]Probiotic genera included for these response variables were *Bacillus, Bifidobacterium, Lactobacillus, Clostridium, Enterococcus, Pediococcus, Paenibacillus,* and *Saccharomyces.*

[2]Data were calculated as $log_2$fold change between probiotic and control treatments and then expressed in fold-change using a logarithmic scale to base 2.

**Table S3**. Descriptive statistics for predictive and response variables of jejunal and ileal histomorphology (fold-change) in broiler chickens at week 3 and 6 of life without pathogen challenge.

| Variable[1,2] | nTreat | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 15 | 5.0 | 1.27 | 0 | 11.0 | 6.6 |
| Dietary ME (MJ/kg) | 15 | 12.5 | 0.07 | 12.1 | 12.9 | 12.5 |
| Dietary CP (%) | 15 | 21.3 | 0.19 | 19.9 | 22.1 | 21.5 |
| Villus Height | 15 | 1.1 | 0.04 | 1.0 | 1.5 | 1.0 |
| Crypt Depth | 15 | 1.0 | 0.04 | 0.7 | 1.3 | 1.0 |
| Villus Height/Crypt Depth | 15 | 1.2 | 0.08 | 0.9 | 1.9 | 1.0 |
| Jejunum, Week 6 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 19 | 5.9 | 1.08 | 0 | 11.0 | 9.0 |
| Dietary ME (MJ/kg) | 19 | 12.9 | 0.06 | 12.5 | 13.2 | 12.9 |
| Dietary CP (%) | 19 | 19.4 | 0.16 | 18.2 | 20.4 | 19.2 |
| Villus Height | 19 | 1.1 | 0.03 | 0.9 | 1.3 | 1.0 |
| Crypt Depth | 19 | 1.1 | 0.04 | 0.7 | 1.5 | 1.0 |
| Villus Height/Crypt Depth | 19 | 1.0 | 0.04 | 0.8 | 1.5 | 1.0 |
| Ileum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 11 | 5.1 | 1.50 | 0 | 11.0 | 8.5 |
| Dietary ME (MJ/kg) | 11 | 12.4 | 0.06 | 12.1 | 12.7 | 12.4 |
| Dietary CP (%) | 11 | 21.6 | 0.16 | 21.0 | 22.1 | 22.0 |
| Villus Height | 11 | 1.0 | 0.03 | 0.9 | 1.3 | 1.0 |
| Crypt Depth | 11 | 0.9 | 0.04 | 0.6 | 1.1 | 1.0 |
| Villus Height/Crypt Depth | 11 | 1.1 | 0.06 | 0.9 | 1.7 | 1.0 |
| Ileum, Week 6 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 17 | 6.1 | 1.14 | 0 | 11.0 | 9.0 |
| Dietary ME (MJ/kg) | 17 | 13.0 | 0.06 | 12.6 | 13.2 | 13.0 |
| Dietary CP (%) | 17 | 19.6 | 0.15 | 18.9 | 20.7 | 19.2 |
| Villus Height | 17 | 1.1 | 0.04 | 0.9 | 1.5 | 1.0 |
| Crypt Depth | 17 | 1.0 | 0.03 | 0.7 | 1.2 | 1.0 |
| Villus Height/Crypt Depth | 17 | 1.1 | 0.04 | 0.8 | 1.4 | 1.1 |

nTreat**,** number of treatments means; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit.

[1]Probiotic genera included for these response variables were *Bacillus, Bifidobacterium, Lactobacillus, Clostridium, Enterococcus*, and *Saccharomyces.*

[2]Data were calculated as $log_2$fold change between probiotic and control treatments and then expressed in fold-change using a logarithmic scale to base 2.

**Table S4**. Descriptive statistics for predictors and response variables of growth performance in broiler chickens at starter, finisher, and overall periods without pathogen challenge.

| Variable[1] | $n_{Treat}$ | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Starter (1-3 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 33 | 5.6 | 0.80 | 0 | 11.0 | 8.5 |
| Dietary ME (MJ/kg) | 33 | 12.4 | 0.03 | 12.1 | 12.8 | 12.5 |
| Dietary CP (%) | 33 | 21.5 | 0.13 | 21.0 | 23.0 | 21.1 |
| ADFI (g) | 33 | 50.2 | 1.52 | 35.9 | 65.3 | 49.6 |
| ADG (g) | 30 | 33.7 | 0.82 | 25.8 | 44.6 | 33.7 |
| FCR | 33 | 1.5 | 0.03 | 1.1 | 1.8 | 1.4 |
| Finisher (4-6 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 29 | 5.7 | 0.86 | 0 | 11.0 | 8.5 |
| Dietary ME (MJ/kg) | 29 | 12.8 | 0.04 | 12.5 | 13.2 | 12.8 |
| Dietary CP (%) | 29 | 19.5 | 0.11 | 18.9 | 20.7 | 19.2 |
| ADFI (g) | 29 | 151.8 | 4.69 | 105.9 | 199.7 | 153.0 |
| ADG (g) | 26 | 77.6 | 2.98 | 51.0 | 107.2 | 77.2 |
| FCR | 29 | 2.0 | 0.04 | 1.5 | 2.3 | 1.9 |
| Overall (1-6 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 32 | 5.7 | 0.80 | 0 | 11.0 | 8.5 |
| Dietary ME (MJ/kg) | 32 | 12.7 | 0.03 | 12.3 | 13.0 | 12.6 |
| Dietary CP (%) | 32 | 20.6 | 0.11 | 19.7 | 21.5 | 20.3 |
| ADFI (g) | 32 | 100.5 | 2.79 | 71.4 | 132.0 | 99.5 |
| ADG (g) | 26 | 56.5 | 1.57 | 42.1 | 71.3 | 56.7 |
| FCR | 32 | 1.7 | 0.02 | 1.4 | 2.0 | 1.7 |

$n_{Treat}$, number of treatments means included; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit; ADFI, average daily feed intake; ADG, average daily weight gain; FCR, feed conversion ratio.

[1]Probiotic genera included for these response variables were *Bacillus, Bifidobacterium, Lactobacillus, Clostridium, Enterococcus,* and *Saccharomyces.*

**Table S5**. Descriptive statistics for predictive and response variables of jejunal, ileal, and caecal gene expression (fold-change) related to barrier function in broiler chickens from week 2 to 5 with pathogen challenge.

| Variable[1-3] | nTreat | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Jejunum, Week 2 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 18 | 4.9 | 1.07 | 0 | 10.7 | 8.2 |
| Days post-infection | 18 | 6.6 | 0.78 | 3.0 | 12.0 | 6.0 |
| Dietary ME (MJ/kg) | 18 | 12.5 | 0.05 | 12.1 | 12.8 | 12.6 |
| Dietary CP (%) | 18 | 21.8 | 0.24 | 20.0 | 23.0 | 21.8 |
| *ZO1* | 14 | 1.1 | 0.04 | 0.7 | 1.3 | 1.0 |
| *OCLN* | 16 | 1.6 | 0.62 | 0.6 | 10.8 | 1.0 |
| *CLDN1* | 14 | 1.1 | 0.17 | 0.6 | 3.3 | 1.0 |
| *CLDN3* | 10 | 1.5 | 0.17 | 1.0 | 2.3 | 1.2 |
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 19 | 5.3 | 1.07 | 0 | 10.7 | 8.7 |
| Days post-infection | 19 | 7.0 | 0.59 | 4.0 | 12.0 | 7.0 |
| Dietary ME (MJ/kg) | 19 | 12.5 | 0.06 | 12.1 | 12.9 | 12.4 |
| Dietary CP (%) | 19 | 20.9 | 0.12 | 20.0 | 22.0 | 21.0 |
| *MUC2* | 10 | 1.2 | 0.19 | 0.7 | 2.8 | 1.0 |
| *ZO1* | 17 | 1.2 | 0.08 | 0.9 | 2.0 | 1.0 |
| *OCLN* | 17 | 1.3 | 0.14 | 0.7 | 3.1 | 1.0 |
| *CLDN1* | 14 | 1.6 | 0.45 | 0.4 | 7.3 | 1.0 |
| Jejunum, Week 4 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 12 | 5.0 | 1.28 | 0 | 9.2 | 7.7 |
| Days post-infection | 12 | 12.0 | 1.69 | 7.0 | 21.0 | 10.0 |
| Dietary ME (MJ/kg) | 12 | 12.6 | 0.07 | 12.4 | 13.0 | 12.5 |
| Dietary CP (%) | 12 | 20.0 | 0.28 | 19.0 | 21.2 | 19.8 |
| *ZO1* | 12 | 1.3 | 0.10 | 0.9 | 2.0 | 1.1 |
| *OCLN* | 12 | 1.3 | 0.20 | 0.6 | 2.8 | 1.0 |
| Jejunum, Week 5 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 13 | 6.9 | 1.10 | 0 | 9.6 | 9.0 |
| Days post-infection | 13 | 28.8 | 2.26 | 17.0 | 34.0 | 34.0 |
| Dietary ME (MJ/kg) | 13 | 12.7 | 0.03 | 12.5 | 12.8 | 12.8 |
| Dietary CP (%) | 13 | 19.0 | 0.02 | 19.0 | 19.2 | 19.0 |
| *MUC2* | 13 | 1.1 | 0.20 | 0.2 | 2.7 | 1.0 |
| Ileum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 16 | 5.6 | 1.12 | 0 | 9.7 | 8.4 |
| Days post-infection | 16 | 14.3 | 1.62 | 4.0 | 20.0 | 16.0 |
| Dietary ME (MJ/Kg) | 16 | 12.3 | 0.04 | 12.1 | 12.6 | 12.2 |
| Dietary CP (%) | 16 | 21.4 | 0.13 | 21.0 | 22.4 | 21.2 |
| *ZO1* | 16 | 1.3 | 0.15 | 0.4 | 2.7 | 1.0 |
| *OCLN* | 16 | 1.3 | 0.16 | 0.4 | 2.7 | 1.0 |
| *CLDN1* | 11 | 1.0 | 0.14 | 0.5 | 2.1 | 1.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ileum, Week 4 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 11 | 5.1 | 1.50 | 0 | 11.3 | 8.0 |
| Days post-infection | 11 | 11.6 | 0.85 | 8.0 | 14.0 | 14.0 |
| Dietary ME (MJ/kg) | 11 | 12.8 | 0.15 | 12.1 | 13.2 | 13.2 |
| Dietary CP (%) | 11 | 20.5 | 0.17 | 20.0 | 21.2 | 20.0 |
| *ZO1* | 11 | 1.2 | 0.11 | 1.0 | 2.2 | 1.1 |
| *OCLN* | 11 | 1.3 | 0.18 | 0.8 | 2.9 | 1.0 |
| *CLDN1* | 11 | 1.3 | 0.21 | 0.5 | 2.7 | 1.0 |
| Caeca, Week 4 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 10 | 4.6 | 1.29 | 0 | 8.7 | 6.5 |
| Days post-infection | 10 | 7.6 | 0.16 | 7.0 | 8.0 | 8.0 |
| Dietary ME (MJ/Kg) | 10 | 12.7 | 0.08 | 12.6 | 13.2 | 12.6 |
| Dietary CP (%) | 10 | 19.2 | 0.13 | 19.0 | 20.0 | 19.0 |
| *ZO1* | 10 | 2.6 | 1.05 | 1.0 | 11.9 | 1.5 |

$n_{Treat}$, number of treatments means; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit; *MUC2*, mucin-2; *ZO1*, zonula occludens-1; *OCLN*, occludin; *CLDN1, -3*, claudin-1, -3.

[1]Probiotic genera included for these response variables were *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Paenibacillus*, *Clostridium*, *Enterococcus*, *Pediococcus*, and *Saccharomyces.*

[2]Pathogens included for these response variables were *E. coli, C. perfringens, S. enteritidis, E. maxima, E. tenella, E. acervulina, E. mivati, E. brunetti, E. mitis, E. praecox, F. graminearum, S. pullorum*, and Aflatoxin B1.

[3]Data were calculated as $log_2$fold change between probiotic and control treatments and then expressed in fold-change using a logarithmic scale to base 2.

**Table S6**. Descriptive statistics for predictive and response variables of jejunal, ileal, and caecal gene expression (fold-change) related to immune response in broiler chickens from week 2 to 4 of life with pathogen challenge.

| Variable[1-3] | nTreat | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Jejunum, Week 2 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 14 | 4.8 | 1.15 | 0 | 8.9 | 8.2 |
| Days post-infection | 14 | 7.0 | 0.96 | 3.0 | 12.0 | 6.0 |
| Dietary ME (MJ/kg) | 14 | 12.4 | 0.05 | 12.1 | 12.6 | 12.5 |
| Dietary CP (%) | 14 | 21.9 | 0.21 | 21.0 | 23.0 | 21.8 |
| *IL1B* | 10 | 1.0 | 0.03 | 0.7 | 1.2 | 1.0 |
| *IL10* | 14 | 1.2 | 0.20 | 0.2 | 2.9 | 1.0 |
| *IFNG* | 10 | 0.9 | 0.06 | 0.5 | 1.0 | 0.9 |
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 25 | 5.2 | 0.95 | 0 | 10.7 | 8.5 |
| Days post-infection | 25 | 8.2 | 0.85 | 4.0 | 20.0 | 7.0 |
| Dietary ME (MJ/kg) | 25 | 12.4 | 0.05 | 12.1 | 12.9 | 12.4 |
| Dietary CP (%) | 25 | 21.0 | 0.13 | 20.0 | 22.0 | 21.0 |
| *IL1B* | 17 | 0.8 | 0.07 | 0.2 | 1.3 | 1.0 |
| *IL6* | 12 | 0.9 | 0.08 | 0.4 | 1.1 | 1.0 |
| *IL10* | 13 | 1.7 | 0.33 | 0.5 | 4.6 | 1.0 |
| *IFNG* | 18 | 0.9 | 0.07 | 0.4 | 1.5 | 1.0 |
| *TNFA* | 10 | 0.9 | 0.06 | 0.6 | 1.2 | 1.0 |
| Jejunum, Week 4 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 14 | 5.1 | 1.23 | 0 | 10.0 | 7.7 |
| Days post-infection | 14 | 11.8 | 1.04 | 7.0 | 17.0 | 13.5 |
| Dietary ME (MJ/kg) | 14 | 12.7 | 0.09 | 12.4 | 13.2 | 12.6 |
| Dietary CP (%) | 14 | 19.7 | 0.21 | 19.0 | 21.0 | 19.5 |
| *IL1B* | 10 | 1.4 | 0.14 | 1.0 | 2.2 | 1.2 |
| *IFNG* | 14 | 1.2 | 0.13 | 0.6 | 2.4 | 1.0 |
| Ileum, Week 2 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 12 | 4.6 | 1.39 | 0 | 9.7 | 4.1 |
| Days post-infection | 12 | 3.5 | 0.77 | 1.0 | 7.0 | 2.5 |
| Dietary ME (MJ/kg) | 12 | 12.6 | 0.12 | 12.1 | 13.2 | 12.8 |
| Dietary CP (%) | 12 | 21.6 | 0.18 | 20.9 | 22.1 | 21.6 |
| *IFNG* | 10 | 1.0 | 0.16 | 0.4 | 2.2 | 1.0 |
| *TLR4* | 10 | 0.9 | 0.10 | 0.5 | 1.6 | 1.0 |
| Ileum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 12 | 5.1 | 1.32 | 0 | 9.7 | 8.0 |
| Days post-infection | 12 | 13.7 | 2.27 | 4.0 | 20.0 | 20.0 |
| Dietary ME (MJ/kg) | 12 | 12.3 | 0.05 | 12.1 | 12.5 | 12.2 |
| Dietary CP (%) | 12 | 21.4 | 0.17 | 21.0 | 22.4 | 21.1 |
| *IL10* | 10 | 1.1 | 0.11 | 0.7 | 2.0 | 1.0 |
| *IFNG* | 12 | 0.9 | 0.05 | 0.6 | 1.1 | 0.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ileum, Week 4 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 10 | 5.9 | 1.63 | 0 | 11.3 | 9.0 |
| Days post-infection | 10 | 17.3 | 2.60 | 8.0 | 27.0 | 17.0 |
| Dietary ME (MJ/kg) | 10 | 12.4 | 0.11 | 12.1 | 12.8 | 12.1 |
| Dietary CP (%) | 10 | 19.8 | 0.41 | 19.0 | 22.1 | 19.0 |
| *TNFA* | 10 | 0.8 | 0.09 | 0.2 | 1.0 | 1.0 |
| Caeca, Week 2 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 22 | 5.5 | 1.04 | 0 | 12.4 | 8.0 |
| Days post-infection | 22 | 3.4 | 0.60 | 0.2 | 7.0 | 3.0 |
| Dietary ME (MJ/kg) | 22 | 12.0 | 0.10 | 11.4 | 12.8 | 12.1 |
| Dietary CP (%) | 22 | 20.8 | 0.25 | 19.2 | 23.0 | 21.0 |
| *IL6* | 18 | 0.9 | 0.07 | 0.1 | 1.5 | 1.0 |
| *IL8* | 10 | 0.9 | 0.19 | 0.1 | 2.1 | 1.0 |
| *IL10* | 10 | 2.4 | 0.42 | 1.0 | 4.7 | 2.5 |

$n_{Treat}$, number of treatments means; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit; *IL6, -8, -10, -1B*, interleukin-6, -8, -10, -1B; *TLR4*, toll-like receptor -4; *IFNG*, interferon-gamma; *TNFA*, tumor necrosis factor-alpha.

[1] Probiotic genera included for these response variables were *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Paenibacillus*, *Clostridium*, *Enterococcus*, *Pediococcus*, *Streptococcus,* and *Saccharomyces.*

[2] Pathogens included for these response variables were *E. coli, C. perfringens, S. enteritidis, E. maxima, E. tenella, E. acervulina, E. mivati, E. brunetti, F. graminearum, S. minnesota,* and *L. monocytogenes.*

[3] Data were calculated as $log_2$fold change between probiotic and control treatments and then expressed in fold-change using a logarithmic scale to base 2.

**Table S7**. Descriptive statistics for predictive and response variables of duodenal, jejunal and ileal histomorphology (fold-change) in broiler chickens at week 2, 3 and 5 of life with pathogen challenge.

| Variable[1-3] | $n_{Treat}$ | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Duodenum, Week 5 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 15 | 7.2 | 0.97 | 0 | 9.4 | 9.0 |
| Days post-infection | 15 | 32.0 | 1.07 | 24.0 | 34.0 | 34.0 |
| Dietary ME (MJ/kg) | 15 | 12.8 | 0.02 | 12.8 | 13.0 | 12.8 |
| Dietary CP (%) | 15 | 19.2 | 0.11 | 19.0 | 20.0 | 19.0 |
| Villus Height | 15 | 1.1 | 0.02 | 1.0 | 1.3 | 1.1 |
| Crypt Depth | 15 | 1.1 | 0.03 | 0.8 | 1.2 | 1.0 |
| Villus Height/Crypt Depth | 15 | 1.0 | 0.03 | 0.9 | 1.3 | 1.0 |
| Jejunum, Week 2 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 11 | 5.2 | 1.50 | 0 | 10.7 | 8.7 |
| Days post-infection | 11 | 5.2 | 0.48 | 3.0 | 7.0 | 5.0 |
| Dietary ME (MJ/kg) | 11 | 12.4 | 0.09 | 12.1 | 12.8 | 12.4 |
| Dietary CP (%) | 11 | 21.2 | 0.25 | 20.0 | 22.1 | 21.0 |
| Villus Height | 11 | 1.0 | 0.04 | 0.9 | 1.4 | 1.0 |
| Crypt Depth | 11 | 0.9 | 0.03 | 0.7 | 1.0 | 1.0 |
| Villus Height/Crypt Depth | 11 | 1.1 | 0.08 | 1.0 | 1.8 | 1.0 |
| Jejunum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 17 | 5.3 | 1.12 | 0 | 10.7 | 8.2 |
| Days post-infection | 17 | 9.8 | 1.34 | 4.0 | 20.0 | 7.0 |
| Dietary ME (MJ/kg) | 17 | 12.3 | 0.03 | 12.1 | 12.5 | 12.3 |
| Dietary CP (%) | 17 | 21.1 | 0.12 | 20.0 | 22.0 | 21.0 |
| Villus Height | 17 | 1.1 | 0.04 | 0.9 | 1.5 | 1.0 |
| Crypt Depth | 17 | 1.0 | 0.03 | 0.7 | 1.1 | 1.0 |
| Villus Height/Crypt Depth | 17 | 1.2 | 0.07 | 1.0 | 2.2 | 1.1 |
| Jejunum, Week 5 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 17 | 6.9 | 0.96 | 0 | 9.4 | 9.0 |
| Days post-infection | 17 | 30.6 | 1.35 | 20.0 | 34.0 | 34.0 |
| Dietary ME (MJ/kg) | 17 | 12.8 | 0.03 | 12.6 | 13.0 | 12.8 |
| Dietary CP (%) | 17 | 19.2 | 0.09 | 19.0 | 20.0 | 19.0 |
| Villus Height | 17 | 1.0 | 0.02 | 0.9 | 1.2 | 1.0 |
| Crypt Depth | 17 | 1.0 | 0.03 | 0.7 | 1.1 | 1.0 |
| Villus Height/Crypt Depth | 17 | 1.1 | 0.04 | 0.8 | 1.4 | 1.1 |
| Ileum, Week 3 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 13 | 5.5 | 1.26 | 0 | 9.7 | 8.5 |
| Days post-infection | 13 | 10.3 | 1.73 | 4.0 | 20.0 | 7.0 |
| Dietary ME (MJ/kg) | 13 | 12.3 | 0.04 | 12.1 | 12.5 | 12.3 |
| Dietary CP (%) | 13 | 21.2 | 0.10 | 21.0 | 22.0 | 21.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Villus Height | 13 | 1.0 | 0.02 | 1.0 | 1.2 | 1.0 |
| Crypt Depth | 13 | 1.0 | 0.04 | 0.9 | 1.4 | 1.0 |
| Villus Height/Crypt Depth | 13 | 1.0 | 0.02 | 0.9 | 1.2 | 1.0 |
| Ileum, Week 5 | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 15 | 7.2 | 0.97 | 0 | 9.4 | 9.0 |
| Days post-infection | 15 | 32.0 | 1.07 | 24.0 | 34.0 | 34.0 |
| Dietary ME (MJ/kg) | 15 | 12.8 | 0.02 | 12.8 | 13.0 | 12.8 |
| Dietary CP (%) | 15 | 19.2 | 0.11 | 19.0 | 20.0 | 19.0 |
| Villus Height | 15 | 1.0 | 0.03 | 0.8 | 1.2 | 1.0 |
| Crypt Depth | 15 | 1.1 | 0.03 | 0.9 | 1.3 | 1.1 |
| Villus Height/Crypt Depth | 15 | 0.9 | 0.03 | 0.7 | 1.1 | 1.0 |

$n_{Treat}$, number of treatments means; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit.

[1]Probiotic genera included for these response variables were *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Saccharomyces*.

[2]Pathogens included for these response variables were *E. coli, C. perfringens, S. enteritidis, E. maxima, E. tenella, E. acervulina, E. mitis, E. praecox*, and *F. graminearum*.

[3]Data were calculated as $log_2$fold change between probiotic and control treatments and then expressed in fold-change using a logarithmic scale to base 2.

**Table S8**. Descriptive statistics for predictors and response variables of growth performance in broiler chickens at starter, finisher, and overall periods with pathogen challenge.

| Variable[1,2] | nTreat | Mean | SEM | Min. | Max. | Median |
|---|---|---|---|---|---|---|
| Starter (1-3 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 22 | 5.5 | 1.00 | 0 | 9.7 | 9.0 |
| Days post-infection | 22 | 10.8 | 1.00 | 5.0 | 20.0 | 10.0 |
| Dietary ME (MJ/kg) | 22 | 12.4 | 0.04 | 12.1 | 12.7 | 12.4 |
| Dietary CP (%) | 22 | 21.1 | 0.05 | 21.0 | 21.8 | 21.0 |
| ADFI (g) | 22 | 52.6 | 1.57 | 40.6 | 63.3 | 52.3 |
| ADG (g) | 22 | 36.2 | 1.46 | 28.4 | 50.2 | 33.2 |
| FCR | 22 | 1.5 | 0.04 | 1.1 | 1.9 | 1.4 |
| Finisher (4-6 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 13 | 5.0 | 1.33 | 0 | 9.6 | 9.0 |
| Days post-infection | 13 | 33.2 | 1.50 | 27.0 | 41.0 | 33.0 |
| Dietary ME (MJ/kg) | 13 | 12.7 | 0.05 | 12.5 | 13.0 | 12.8 |
| Dietary CP (%) | 13 | 19.3 | 0.10 | 19.0 | 19.8 | 19.0 |
| ADFI (g) | 13 | 149.8 | 11.04 | 108.2 | 219.4 | 142.7 |
| ADG (g) | 13 | 72.1 | 7.54 | 31.7 | 114.3 | 80.7 |
| FCR | 13 | 2.0 | 0.09 | 1.7 | 2.8 | 1.9 |
| Overall (1-6 Weeks) | | | | | | |
| Dietary probiotics ($log_{10}$ CFU/kg) | 17 | 4.8 | 1.13 | 0 | 9.6 | 8.1 |
| Days post-infection | 17 | 34.9 | 1.38 | 27.0 | 41.0 | 36.0 |
| Dietary ME (MJ/kg) | 17 | 12.7 | 0.06 | 12.4 | 13.0 | 12.5 |
| Dietary CP (%) | 17 | 20.3 | 0.08 | 19.9 | 20.8 | 20.1 |
| ADFI (g) | 17 | 95.6 | 2.78 | 75.6 | 119.5 | 93.8 |
| ADG (g) | 17 | 57.0 | 3.26 | 31.7 | 75.7 | 55.2 |
| FCR | 17 | 1.7 | 0.06 | 1.4 | 2.3 | 1.7 |

nTreat**,** number of treatments means; SEM, standard error of means; Min, minimum; Max, maximum; ME, metabolizable energy; CP, crude protein; CFU, colony-forming unit; ADFI, average daily feed intake; ADG, average daily weight gain; FCR, feed conversion ratio.

[1]Probiotic genera included for these response variables were *Bacillus, Bifidobacterium, Lactobacillus, Enterococcus,* and *Saccharomyces.*

[2]Pathogens included for these response variables were *E. coli, C. perfringens, S. enteritidis, E. maxima, E. tenella, E. acervulina, E. mivati, E. mitis,* and *E. praecox.*

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