# *Supplementary Material*

Effect of supplementing a *Bacillus* multi-strain probiotic to a post-weaning diet on nutrient utilization and nitrogen retention of piglets.

Anne M.S. Huting, L. Vanessa Lagos, Lea H.B. Hansen, and Francesc Molist

**Supplementary Table S1.** Analysed dietary nutrient content of the experimental diets (i.e. control diet = CD; supplemented diet = SD), in g/kg.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Moisture | Ash | CP | CFat | CF | Starch | Sugar | Ti |
| CD | 112 | 55 | 186 | 51 | 37 | 370 | 40 | 2.95 |
| SD | 109 | 56 | 188 | 51 | 37 | 373 | 43 | 3.01 |

**Supplementary Table S2.** CFU recovery analysis of the experimental diets (i.e. control diet = CD; supplemented diet = SD).

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Sample type | Expected,  CFU/g | Result,  CFU/g |
| CD | Mash | <1.00E+05 | 3.10E+04 |
| CD | Pellet | <1.00E+05 | 3.10E+04 |
| SD | Mash | 1.10E+06 | 1.51E+06 |
| SD | Pellet | 1.10E+06 | 1.41E+06 |

**Supplementary Table S3.** The effect of experimental treatment (i.e. control diet = CD; supplemented diet = SD) on piglet performance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter1 | CD | SD | SEM | *P*-value |
| Weaning age, day |  |  |  |  |
| Day 02 | 29.8 | 29.6 | 0.53 | 0.64 |
| Body weight, kg |  |  |  |  |
| Day 02 | 8.48 | 8.48 | 0.013 | 1.00 |
| Day 11 | 11.5 | 11.2 | 0.26 | 0.46 |
| Day 192 | 15.3 | 15.0 | 0.62 | 0.74 |
| ADG, g/piglet |  |  |  |  |
| Day 0-11 | 275 | 246 | 23.5 | 0.46 |
| Day 11-192 | 285 | 263 | 11.2 | 0.27 |
| ADFI, g/piglet |  |  |  |  |
| Day 0-11 | 285 | 263 | 11.2 | 0.27 |
| FCR, g/g |  |  |  |  |
| Day 0-11 | 1.05 | 1.09 | 0.088 | 0.78 |
| Faecal score2 |  |  |  |  |
| Day 0-11 | 6.78 | 6.96 | 0.239 | 0.63 |
| Day 11-192 | 6.69 | 6.72 | 0.105 | 0.86 |

1 The experimental unit was pen (*n* = 4) in case not stated otherwise. Replicate (1 to 4) was used as random effect. The experimental results were analysed using a two-way analysis of variance (ANOVA) by GenStat®.

2 An 8-point scale from severe water thin diarrhoea to hard, dry and lumpy faeces was used for faecal consistency determination. Faecal score 6 was considered the optimal faecal score (see for protocol [13]).

3 The experimental unit was piglet (*n* = 8). Replicate (1 to 8) was used as random effect. The experimental results were analysed using a two-way analysis of variance (ANOVA) by GenStat®.

**Supplementary Table S4.** The effect of experimental treatment (i.e. control diet = CD; supplemented diet = SD) on apparent ileal digestibility of amino acids.1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | CD | SD | SEM | *P*-value |
| Indispensable AA, % |  |  |  |  |
| Arg | 77.6 | 76.9 | 2.47 | 0.87 |
| His | 78.0 | 77.7 | 2.34 | 0.93 |
| Ile | 74.2 | 75.2 | 2.53 | 0.80 |
| Leu | 75.6 | 76.5 | 2.29 | 0.80 |
| Lys | 82.3 | 83.7 | 1.97 | 0.64 |
| Met2 | 88.1  (-93.6 - 93.8) | 88.5  (-93.5 - 93.8) | - | 0.85 |
| Phe | 78.8 | 78.4 | 2.52 | 0.93 |
| Thr2 | 74.9  (-84.9 - 85.7) | 75.5  (-84.8 - 85.8) | - | 0.85 |
| Trp | 71.6 | 72.3 | 2.28 | 0.84 |
| Val | 75.6 | 78.0 | 3.20 | 0.61 |
| Dispensable AA, % |  |  |  |  |
| Ala | 65.4 | 64.9 | 3.48 | 0.92 |
| Asp | 69.7 | 68.2 | 3.53 | 0.78 |
| Cys | 66.0 | 61.6 | 4.39 | 0.51 |
| Glu | 81.0 | 79.2 | 2.73 | 0.65 |
| Gly | 47.6 | 44.6 | 6.95 | 0.77 |
| Pro | 71.4 | 56.6 | 8.43 | 0.27 |
| Ser | 73.1 | 71.6 | 2.48 | 0.69 |
| Tyr | 70.5 | 73.4 | 3.06 | 0.54 |
| All indispensable AA, % | 77.0 | 77.6 | 2.45 | 0.88 |
| All dispensable AA, % | 72.4 | 67.0 | 4.17 | 0.40 |
| All AA, % | 74.5 | 71.7 | 3.34 | 0.60 |

1 The experimental unit was piglet (*n* = 8). Replicate (1 to 8) was used as random effect. The experimental results were analysed using a two-way analysis of variance (ANOVA) by GenStat®. From replicate 2 of treatment SD not enough ileal content was left to perform the AA analysis, therefor this piglet was treated as missing value.

2 This parameter was considered not normally distributed in its original form (i.e. Shapiro Wilk *P*<0.05). Transformation suggestions were made by the “ABOXCOX” procedure in Genstat. The apparent ileal Met and apparent ileal Thr digestibility coefficients were transformed using (X-100)^-4. For presentation purposes the calculated means were back transformed and are presented together with the 95% confidence interval (CI, using Bonferroni inequality) instead of SEM.

**Supplementary Table S5.** The effect of experimental treatment (i.e. control diet = CD; supplemented diet = SD) on pH of the digesta content.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter1 | CD | SD | SEM | *P*-value |
| Jejunum | 6.50 | 6.48 | 0.085 | 0.86 |
| Ileum | 6.70 | 6.63 | 0.095 | 0.64 |
| Colon | 5.75 | 5.84 | 0.037 | 0.15 |

1 The experimental unit was piglet (*n* = 8). Replicate (1 to 8) was used as random effect. The experimental results were analysed using a two-way analysis of variance (ANOVA) by GenStat®. Piglets from replicate 2 (i.e. one piglet from the CD and one piglet from the SD treatment) had a ileum pH content that was > 2.5 times lower than the standard error of the residuals and were therefore considered outlier.