Supplementary File – for Online Publication Only

Structural changes in the small intestine of female turkeys receiving a probiotic preparation are dose and region dependent.

P. Dobrowolski1\*, E. Tomaszewska2\*, R. Klebaniuk3, A. Tomczyk-Warunek2, S. Szymańczyk2, J. Donaldson4, I. Świetlicka5, M. Mielnik-Błaszczak6, D. Kuc6, S. Muszyński5

*Animal* journal

\*Corresponding authors: Piotr Dobrowolski, Email: [piotr.dobrowolski@umcs.lublin.pl](mailto:piotr.dobrowolski@umcs.lublin.pl);

**Materials and methods**

Small intestinal absorptive surface was calculated according to the Kisielinski equation (Kisielinski *et al.*, 2002)

Where:

M – absorptive surface of the small intestine

a – villous width

b – villous length

c – crypt width

*Statistical analysis*

The following statistical model was used:

xi = μ+αi +εi

Where:

xi - an observation (small intestine parameter value)

µ - the fixed effect, population mean

αi - factor level (presence and/or concentration of the probiotic: 0, 107 cfu•g-1, 108 cfu•g-1 and 109•cfu g-1, in 500g•1000 kg-1) (cfu – a colony-forming unit)

εi - random error

High power of statistical tests (87–99%) was reached for all the parameters assessed.

**Table S1.** *Composition and nutritive value of the basal diet*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ingredient (%) | Age of birds (weeks) | | | | |
|  | 1-3 | 4-6 | 7-9 | 10-12 | 13-16 |
| Corn | 15.10 | 15.10 | 25.00 | 25.00 | 25.00 |
| Wheat | 34.70 | 37.50 | 32.16 | 38.32 | 44.98 |
| Soybean meal1 | 42.00 | 39.20 | 32.70 | 26.80 | 20.30 |
| Soybean oil | 1.50 | 1.50 | 3.80 | 4.00 | 4.50 |
| Phosphate 1-calcium | 1.66 | 1.66 | 1.46 | 1.16 | 0.85 |
| Limestone | 1.59 | 1.59 | 1.40 | 1.26 | 1.10 |
| Sodium bicarbonate | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Sodium chloride | 0.31 | 0.31 | 0.31 | 0.31 | 0.31 |
| Premix vita-min | 0.502 | 0.502 | 0.503 | 0.504 | 0.505 |
| Concentrate protein-fat6 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| DL-methionine 99% | 0.34 | 0.34 | 0.32 | 0.26 | 0.25 |
| L-lysine-HCl 78% | 0.26 | 0.26 | 0.26 | 0.25 | 0.29 |
| L-threonine 99% | - | - | 0.05 | 0.10 | 0.10 |
| The nutritional value of 1 kg diet: | | | |  |  |
| \*\*Metabolic Energy, kcal⋅kg−1 | 2784 | 2744 | 3016 | 3070 | 3161 |
| \*Total protein | 27.2 | 24.81 | 21.98 | 20.57 | 18.50 |
| \*Crude fiber | 2.80 | 3.24 | 3.11 | 3.26 | 3.26 |
| \*Crude fat | 4.62 | 5.13 | 5.78 | 6.91 | 7.52 |
| \*Lysine | 1.76 | 1.53 | 1.32 | 1.22 | 1.12 |
| \*Methionine + Cystine | 1.12 | 1.10 | 0.98 | 0.91 | 0.86 |
| \*Total calcium | 1.31 | 1.15 | 1.03 | 0.95 | 0.83 |
| \*Total phosphorus | 0.92 | 0.80 | 0.73 | 0.67 | 0.60 |
| \*\*Available phosphorus | 0.61 | 0.53 | 0.47 | 0.41 | 0.34 |
| \*\*Total calcium/available phosphorus | 2.15 | 2.17 | 2.19 | 2.34 | 2.48 |

The subjects of the study were female turkeys. 1 46% total protein in dry matter; 2 content of vitamins and minerals per 1 kg: Mn 60 mg, I 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 15 000 UI, vitamin D3 3 166 UI, vitamin E 60 UI, vitamin K3 3.5 mg, vitamin B1 2.3 mg, vitamin B2 6.5 mg, vitamin B6 4.2 mg, vitamin B12 10.01 mg, biotin 0.13 mg, folic acid 1.2 mg, nicotinic acid 30 mg, pantothenic acid 17 mg, choline 40.30 mg; 3,4 content of vitamins and minerals per 1 kg: Mn 60 mg, J 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 14 100 UI, vitamin D3 3 325 UI, vitamin E 40 UI, vitamin K3 2.75 mg, vitamin B1 1.9 mg, vitamin B2 5.5 mg, vitamin B6 3.6 mg, vitamin B12 15.01 μg, biotin 0.11 mg, folic acid 1.00 mg, nicotinic acid 25 mg, pantothenic acid 14.5 mg, choline 20.00 mg; 5 content of vitamins and minerals per 1 kg: Mn 60 mg, J 0.80 mg, Fe 50 mg, Cu 10 mg, Se 0.20 mg, vitamin A 12 460 UI, vitamin D3 2 995 UI, vitamin E 32 UI, vitamin K3 2.45 mg, vitamin B1 1.74 mg, vitamin B2 5.1 mg, vitamin B6 3.36 mg, vitamin B12 15.00 μg, biotin 0.11 mg, folic acid 0.92 mg, nicotinic acid 23 mg, pantothenic acid 13.5 mg, choline 12.30 mg; 6 concentrate protein-fat: protein – 65%, fat – 15% **\***analysed values; \*\*calculated values

**Results**

**Table S2.** *The effect size (Cohen’s f for one way ANOVA) of differences in histomorphometric parameters of the small intestine of female turkeys, affected by dietary probiotic dose.*

|  |  |  |
| --- | --- | --- |
| Parameter | Duodenum | Jejunum |
| Thickness of the outer muscle layer [µm] | 0.50 | 1.22 |
| Thickness of the inner muscle layer [µm] | 0.15 | 1.15 |
| Thickness of the submucosa [µm] | 1.51 | 0.84 |
| Thickness of the mucosa [µm] | 0.75 | 0.28 |
| Crypt depth [µm] | 0.98 | 0.49 |
| Villi height [µm] | 1.11 | 0.59 |
| Crypt width [µm] | 1.23 | 0.10 |
| Villi width [µm] | 0.59 | 1.04 |
| Total number of crypts /mm | 0.45 | 0.21 |
| Number of open crypts /mm | 1.63 | 0.69 |
| Number of closed crypts /mm | 0.77 | 0.44 |
| Number of villi /mm | 0.02 | 1.27 |
| Enterocyte height [µm] | 5.46 | 1.33 |
| Number of enterocytes /100 [µm] | 2.52 | 0.20 |
| Villus/crypt ratio | 0.78 | 0.36 |
| Absorption surface [μm2] | 0.06 | 0.64 |

The results are presented as Cohen’s f: small effect size 0.10 ≥ f < 0.25; medium effect size 0.25 ≥ f < 0.40; large effect size f ≥ 0.40 (Kotrlik et al., 2011). The subjects of the study were female turkeys.

**Table S3.** *The effect size (Cohen’s f for one way ANOVA) of differences in the morphology of intestinal Meissner and Auerbach plexuses in the small intestine of female turkeys, affected by dietary probiotic dose.*

|  |  |  |
| --- | --- | --- |
| Parameter | Duodenum | Jejunum |
| *Meissner plexus* |  |  |
| Mean sectional area of nerve ganglia [µm2] | 0.39 | 0.59 |
| Ganglia perimeter [µm] | 0.04 | 0.46 |
| Shape factor | 0.59 | 0.44 |
| Minimal radius [µm] | 0.68 | 0.15 |
| Minimal diameter [µm] | 0.68 | 0.15 |
| Mean diameter [µm] | 0.31 | 0.33 |
| Sphericity | 0.40 | 0.35 |
| Convexity | 0.34 | 0.14 |
| *Auerbach plexus* |  |  |
| Mean sectional area of nerve ganglia [µm2] | 0.48 | 0.05 |
| Ganglia perimeter [µm] | 0.27 | 0.05 |
| Shape factor | 0.33 | 0.40 |
| Minimal radius [µm] | 0.58 | 0.35 |
| Minimal diameter [µm] | 0.58 | 0.35 |
| Mean diameter [µm] | 0.32 | 0.13 |
| Sphericity | 0.15 | 0.29 |
| Convexity | 0.22 | 0.35 |

The results are presented as Cohen’s f: small effect size 0.10 ≥ f < 0.25; medium effect size 0.25 ≥ f < 0.40; large effect size f ≥ 0.40 (Kotrlik et al., 2011). The subjects of the study were female turkeys.

Kotrlik JW, Williams HA and Jabor MK 2011. Reporting and Interpreting Effect Size in Quantitative Agricultural Education Research. Journal of Agricultural Education 52, 132–142.