

Article

Investigation of potential gut health biomarkers in broiler chicks challenged by *Campylobacter jejuni* and submitted to a continuous water disinfection program

Tilemachos Mantzios ^{1,2*}, Despoina E. Kiousi ², Georgia D. Brellou ^{3*}, Georgios A. Papadopoulos ⁴, Vangelis Economou ⁵, Marili Vasilogianni ⁶, Elisavet Kanari ², Evangelia Petridou ⁷, Ilias Giannenas ⁸, Guillermo Tellez-Isaias ⁹, Aglaia Pappa ², Alex Galanis ² and Vasilios Tsioris ¹.

Table S1. Analysis of the starter, grower, and finisher diets.

Calculated analysis (g/kg)	Starter diet (1 st -13 th day)	Grower diet (14 th -23 rd day)	Finisher (24 th -36 th day)
Moisture	11.11	10.87	10.95
Crude protein	21.84	20.62	17.83
Crude fat	5.52	6.58	6.96
Crude fiber	2.64	2.86	2.96
Starch	38.13	36.79	38.76
Crude Ash	9.44	9.80	8.98
Sugars	4.16	4.31	4.45
Calcium	1.31	1.57	1.68
Phosphorus	0.57	0.51	0.46
Energy (Mj/kg)	3.000	3.075	3.150

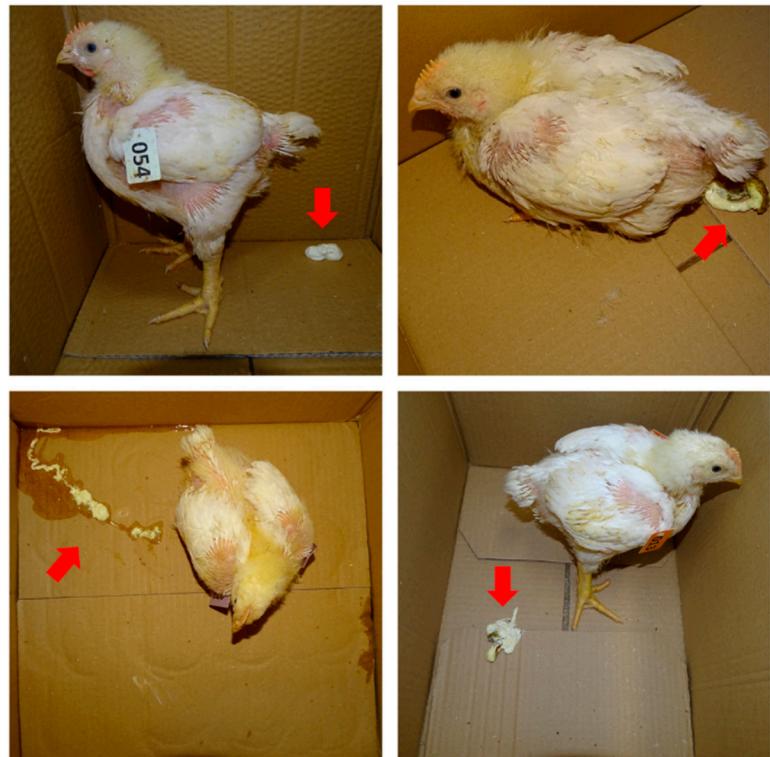


Figure S1. Discharge of white-stained fecal droplets (indicated by the red arrows) following the barium sulfate administration for the determination of the WITT in broiler chicks.

Table S2. Effect of the drinking water disinfection on IL-10 (pg/ml), cortisol (ng/ml) and FITC-d (ng/g) levels in the serum of *C. jejuni* experimentally challenged broiler chicks ($\bar{x} \pm \text{SEM}$).

Serum biomarker	Group A (Negative control)	Group B (Cid 2000 TM)	Group C (<i>C. jejuni</i>)	Group D (Cid 2000 TM & <i>C. jejuni</i>)	P value
IL-10 (pg/ml)	129.63 \pm 41.99	32.55 \pm 11.33	96.93 \pm 50.33	88.18 \pm 49.93	0.278
Cortisol (ng/ml)	0.38 \pm 0.14 ^a	0.11 \pm 0.04 ^a	0.90 \pm 0.12 ^b	0.27 \pm 0.13 ^a	<0.001
FITC-d (ng/g)	0.02 \pm 0.01 ^a	0.04 \pm 0.01 ^a	0.05 \pm 0.01 ^a	0.09 \pm 0.02 ^b	0.004

^{a,b} Means in the same row with a different superscript differ significantly ($p \leq 0.05$). IL-10: Interleukin-10; FITC-d: Fluorescein isothiocyanate dextran

Table S3. Effect of the drinking water disinfection on ovotransferrin concentration (ng/g) in the feces of *C. jejuni* experimentally challenged broiler chicks ($\bar{x} \pm \text{SEM}$).

Faecal biomarker	Group A (Negative con- trol)	Group B (Cid 2000 TM)	Group C (<i>C. jejuni</i>)	Group D (Cid 2000 TM & <i>C. jejuni</i>)	P value
Ovotransferrin (ng/g)	5.75 \pm 0.30 ^a	13.95 \pm 0.57 ^b	5.72 \pm 0.17 ^a	16.25 \pm 0.54 ^c	<0.001

^{a,b,c} Means in the same row with a different superscript differ significantly ($p \leq 0.05$).

Table S4. Effect of the drinking water disinfection on the whole intestinal transit time (WITT) of barium sulfate in *C. jejuni* experimentally challenged broiler chicks ($\bar{x} \pm \text{SEM}$).

Faecal biomarker	Group A (Negative con- trol)	Group B (Cid 2000 TM)	Group C (<i>C. jejuni</i>)	Group D (Cid 2000 TM & <i>C. je- juni</i>)	P value
WITT (in minutes)	91.92 \pm 11.65 ^{a,b}	130.33 \pm 27.64 ^b	61.55 \pm 6.51 ^a	77.17 \pm 15.76 ^a	0.050

^{a,b} Means in the same row with a different superscript differ significantly ($p \leq 0.05$).

Table S5. Effect of the drinking water disinfection on the gut (duodenum, jejunum, ileum) histomorphometry of *C. jejuni* experimentally challenged broiler chicks ($\bar{x} \pm \text{SEM}$).

Parameter	Group A (Negative con- trol)	Group B (Cid 2000 TM)	Group C (<i>C. jejuni</i>)	Group D (Cid 2000 TM & <i>C. je- juni</i>)	P value
<i>Duodenum</i>					
VH	1011.30±92.92	1283.16±173.63	1240.98±142.28	1175.71±87.37	0.532
VW	93.94±7.96	112.30±7.92	99.10±5.71	130.07±11.48	0.125
CD	147.51±0.49	148.37±8.15	139.70±2.82	144.83±0.95	0.550
VH/CD	6.96±0.62	8.80±1.65	8.95±0.90	8.19±0.59	0.568
<i>Jejunum</i>					
VH	982.48±19.25 ^a	1237.77±94.65 ^b	1208.90±60.14 ^{a,b}	1401.03±31.84 ^b	0.033
VW	90.86±6.31	143.62±25.52	139.19±1.63	122.62±11.78	0.177
CD	139.27±5.26	170.73±3.18	159.67±6.29	153.26±9.63	0.105
VH/CD	7.11±0.42	7.41±0.70	7.72±0.71	9.26±0.77	0.248
<i>Ileum</i>					
VH	807.99±59.18 ^{a,b#}	866.18±67.73 ^{b#}	622.48±44.04 ^{a#}	724.29±8.51 ^{a,b#}	0.092
VW	90.71±2.29 ^{a#}	132.26±10.62 ^{b#}	112.39±6.97 ^{a,b#}	108.02±6.63 ^{a,b#}	0.066
CD	113.40±5.18 ^a	145.41±2.37 ^b	139.81±8.71 ^b	114.35±4.58 ^a	0.032
VH/CD	7.28±0.12 ^b	6.12±0.64 ^b	4.50±0.04 ^a	6.47±0.27 ^b	0.021

^{a,b} Means in the same row with a different superscript differ significantly ($P \leq 0.05$). [#] Means in the same row with a different superscript tend to differ significantly ($P \leq 0.10$). VH: Villus Height (μm); VW: Villus Width (μm); CD: Crypt Depth (μm); VH/CD: Villus Height to Crypt depth ratio.