

Supplementary Information

Evaluation of in-field efficacy of dietary ferric tyrosine on performance, intestinal health and meat quality of broiler chickens exposed to natural Campylobacter jejuni challenge

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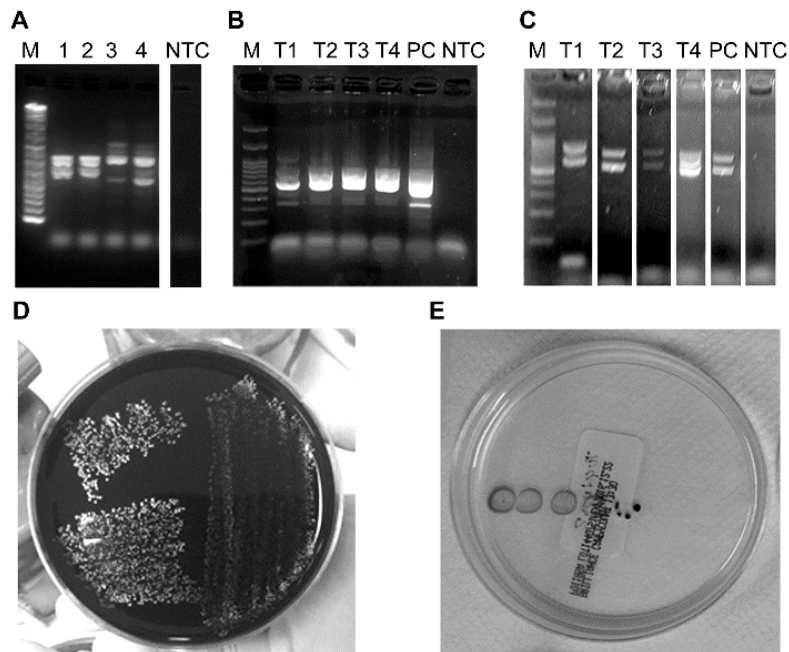
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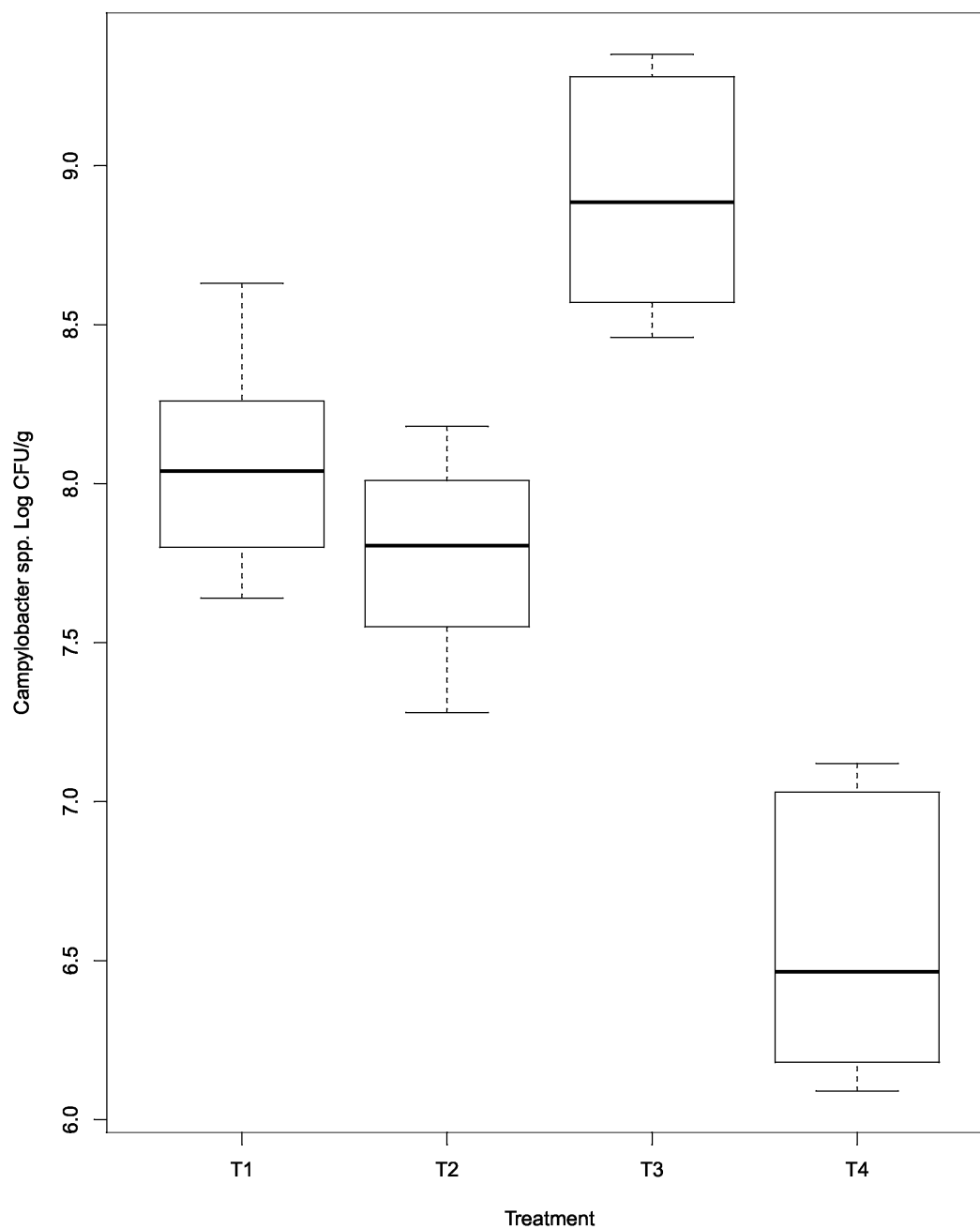
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Suppl. Fig. 1.



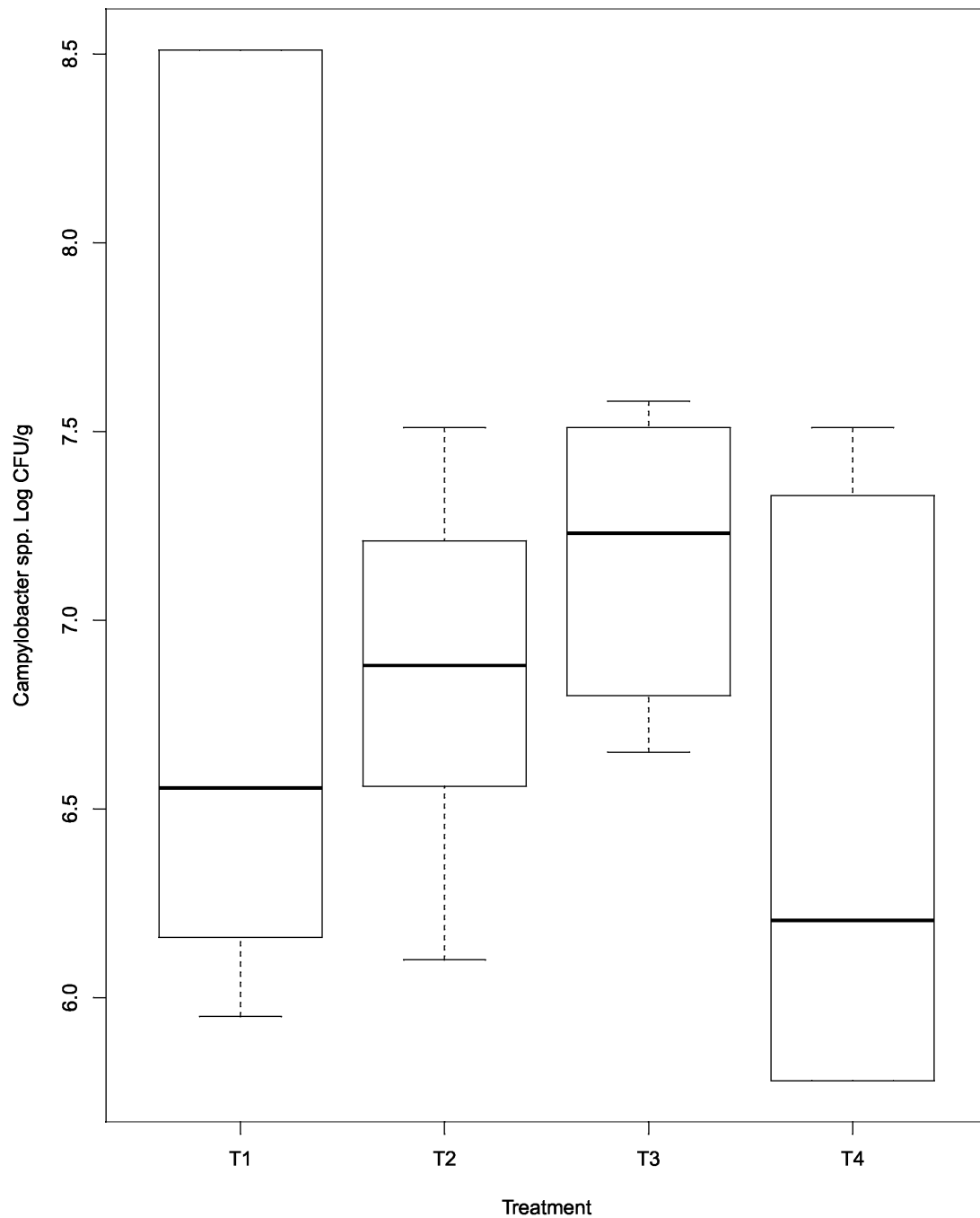
- A.** Farm monitoring of *C. jejuni*. *C. jejuni* detected in the litter of 4 poultry farms, day 40 and *C. coli* detected in the litter of 2 poultry farms, day 42. M) Marker; 1) Farm 1; 2) Farm 2; 3) Farm 3; 4) Farm 4; NTC) Negative control.
- B.** Detection of *C. jejuni* at day 25 in faeces. M) Marker; 1) Typlex 1; 2) Typlex 2; 3) Typlex 3; 4) Typlex 4; PC) Positive Control; NTC) Negative Control.
- C.** Detection of *C. jejuni* at day 42 in caeca. M) Marker; 1) Typlex 1; 2) Typlex 2; 3) Typlex 3; 4) Typlex 4; PC) Positive Control; NTC) Negative Control.
- D.** Isolation of *Campylobacter* spp on Campylobacter Blood – free selective agar (CCDA agar).
- E.** Enumeration of *Campylobacter* spp typical colonies (3 colonies on fourth (10^{-4}) serial decimal dilution) on Brilliance CampyCount agar.

Suppl. Fig. 2.



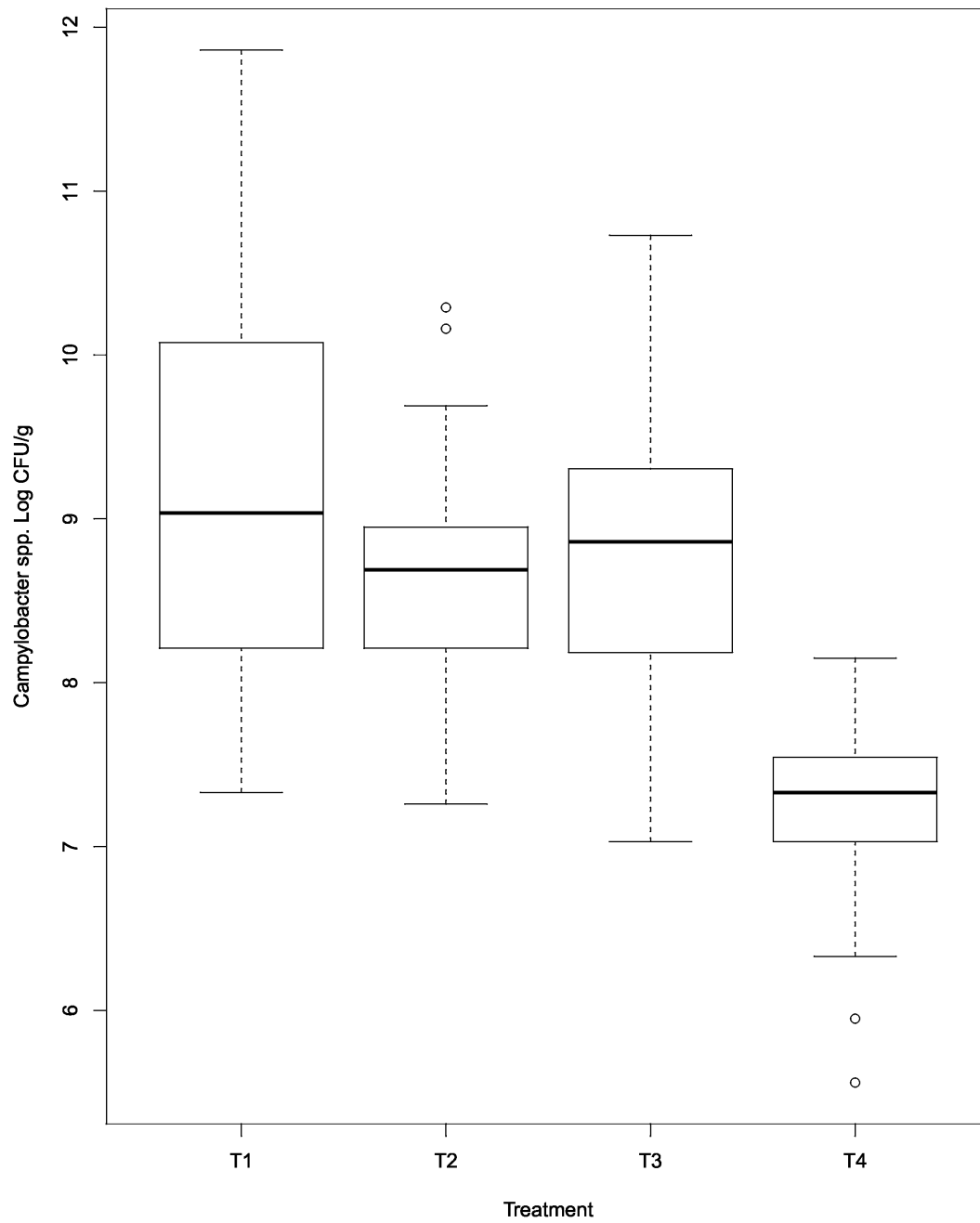
Statistical distribution of *Campylobacter* counts from litter samples taken on day 25.

Suppl. Fig. 3.



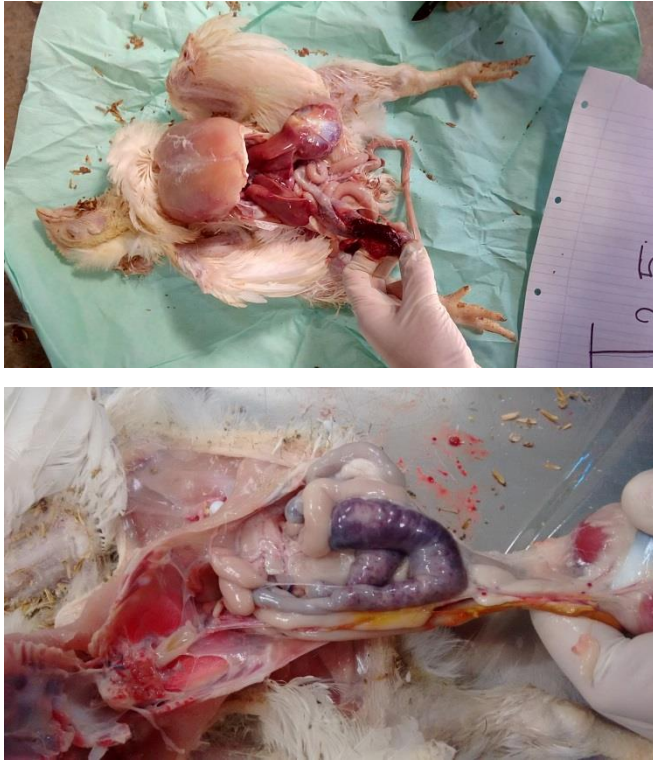
Statistical distribution of *Campylobacter* counts from litter samples taken on day 42.

Suppl. Fig. 4.



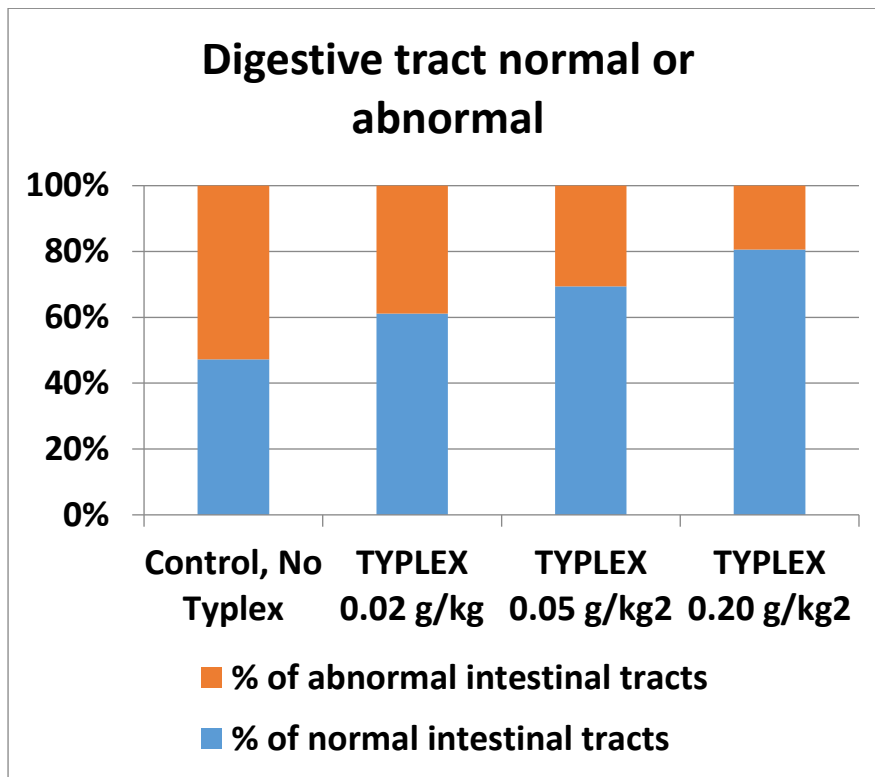
Statistical distribution of *Campylobacter* counts from caecal samples taken on day 42.

Suppl. Fig. 5.



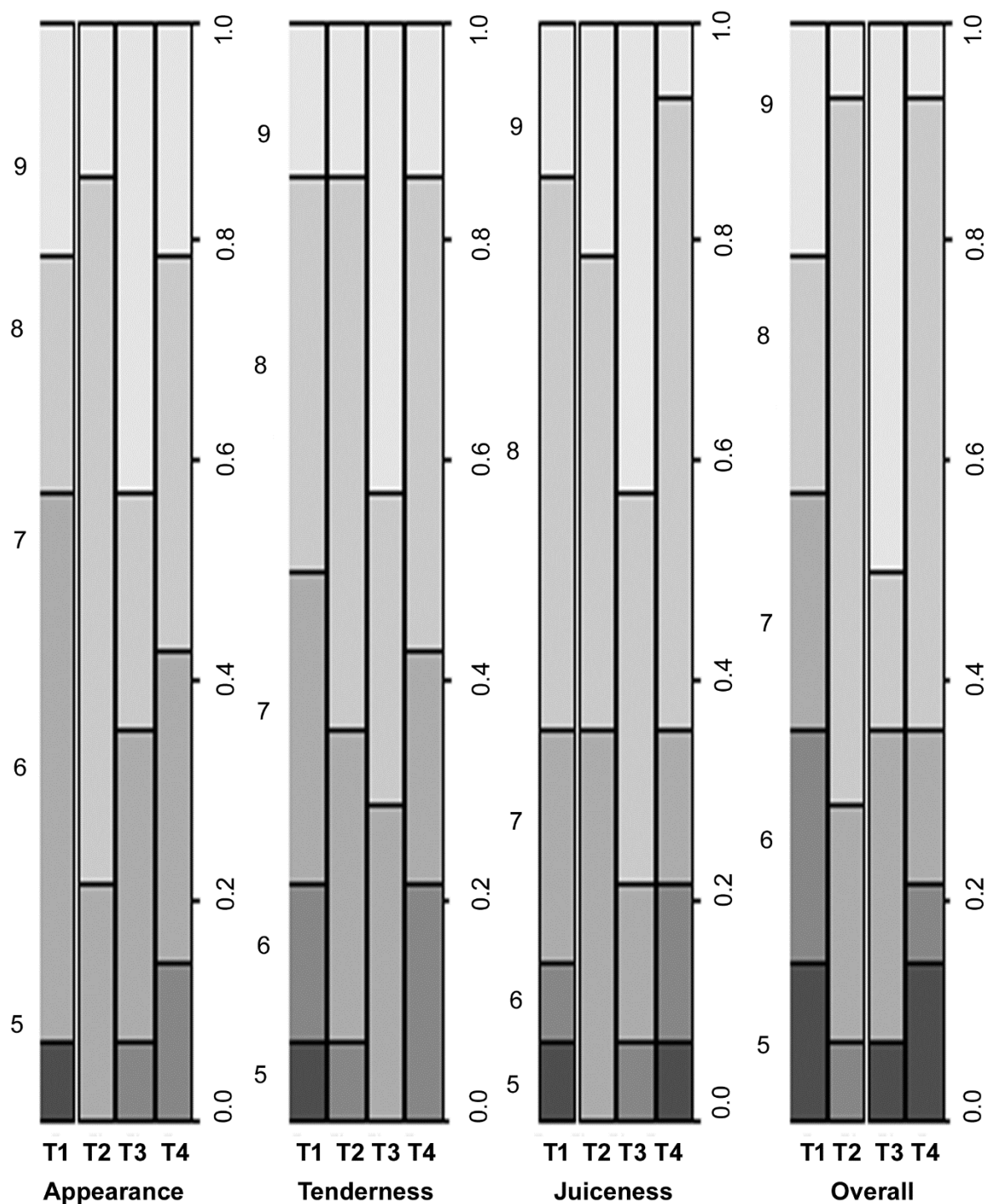
Post-mortem examinations showing typical haemorrhagic lesions in the ceca of broilers infected by *E. tenella*.

Suppl. Fig. 6.



Effect of dietary addition of TYPLEXTM on post-mortem examination of intestinal tracts of broiler chickens for pathological abnormality at study end (42 days on trial). N° replicates = 144 (6 or 7 birds sacrificed per pen/treatment).

Suppl. Fig. 7.



Effect of dietary addition of ferric tyrosine on sensory panel scores of cooked thigh meat on appearance, tenderness, juiciness and overall. N° replicates: 48 (2 carcasses per pen/treatment) scored by 14 panelists. Results range: From 1 (negative perception) to 9 (positive perception).

Suppl. Table 1.

Chemical analysis of study diets.

Sample	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)	Fe mg/kg	Microtraced Ferric tyrosine (TYPLEX™ Chelate) inclusion rate, g/kg feed	Microtracer (% recovery)	Calculated ferric tyrosine content (g/kg)*
T ₁ starter	88.15	22.14	4.18	6.93	80	0.000	NA	-
T ₂ starter	88.51	21.87	4.21	6.87	88	0.022	86	0.019
T ₃ starter	88.46	21.97	4.08	7.15	95	0.050	98	0.049
T ₄ starter	88.15	22.14	4.18	6.93	132	0.200	103	0.206
T ₁ grower	88.22	21.18	5.31	6.87	84	0.000	NA	-
T ₂ grower	88.24	21.64	5.12	6.70	93	0.022	78	0.017
T ₃ grower	88.25	21.53	5.23	6.74	106	0.050	99	0.050
T ₄ grower	88.41	21.36	5.24	6.86	140	0.200	91	0.182

NA – Not applicable;

*Calculated ferric tyrosine = % recovery of microtracer x ferric tyrosine dose

Suppl. Table 2.

Primers and probes used in DNA intestinal *Campylobacter* determination.

Gene target	Primers according to EURL	Sequence	Amplicon size
mapA (<i>C. jejuni</i>)	MD mapA1 (3034)	5' CTA TTT TAT TTT TGA GTG CTT GTG 3'	589bp
mapA (<i>C. jejuni</i>)	MD mapA2 (3035)	5' GCT TTA TTT GCC ATT TGT TTT ATT A 3'	
ceuE (<i>C. coli</i>)	COL3 (3036)	5' AAT TGA AAA TTG CTC CAA CTA TG 3'	462bp
ceuE (<i>C. coli</i>)	MDCOL2 (3037)	5' TGA TTT TAT TAT TTG TAG CAG CG 3'	
16S	16S primer 804 RX (442)	5' GAC TAC CNG GGT ATC TAA TCC 3'	800bp
16S	16S primer 10FX (444)	5' AGA GTT TGA TCC TGG CTN AG3'	

Suppl. Table 3

Effect of dietary addition of ferric tyrosine on mortality, culls, and causes of removal of broilers from the trial.

Treatment	Pen N°	Age (d)	Death or Cull	Cause of death/cull	N° birds/treatment	%
T1	T 1.3	26	Death	Coccidiosis	2	
	T 1.3	28	Death	Coccidiosis	1	
	T 1.6	28	Death	Coccidiosis	1	
	T 1.6	30	Death	Coccidiosis	1	
	Total				5	5.2
T2	T 2.4	32	Death	Enteritis	1	
	T 2.5	29	Death	Enteritis	1	
	T 2.6	31	Death	Enteritis	1	
	Total				3	3.1
T3	T 3.3	32	Death	Enteritis	1	
	T 3.4	30	Death	Low body weight due to diarrhea	1	
	T 3.5	28	Death	Coccidiosis	1	
	T 3.5	36	Death	Colibacillosis	1	
	T 3.6	33	Death	Low body weight, due to diarrhea	1	
	Total				5	5.2
T4	T 4.1	31	Death	Enteritis	1	
	T 4.3	30	Death	Low body weight, due to diarrhea	1	
	Total				2	2.1
TOTAL					15	3.9

Replicates = 6 pens of 16 male birds/treatment

T1; Control; 0 g ferric tyrosine/kg feed, T2; 0.02 g ferric tyrosine/kg feed, T3; 0.05 g ferric tyrosine/kg feed, and T4; 0.20 g ferric tyrosine/kg feed

Suppl. Table 4

Effect of dietary addition of ferric tyrosine on the incidence and severity of diarrhoea from day 24 to day 42

Study day	Treatment							
	T1 (Control; 0 g ferric tyrosine /kg feed)		T2 (0.02 g ferric tyrosine /kg feed)		T3 (0.05 g ferric tyrosine /kg feed)		T4 (0.20 g ferric tyrosine /kg feed)	
	Incidence	Average diarrhoea score	Incidence	Average diarrhoea score	Incidence	Average diarrhoea score	Incidence	Average diarrhoea score
Day 24	50% (3/6)	0.5	100% (6/6)	1.0	67% (4/6)	0.7	33% (2/6)	0.3
Day 26	100% (6/6)	2.3	100% (6/6)	2.5	100% (6/6)	2.7	100% (6/6)	2.3
Day 29	100% (6/6)	2.3	100% (6/6)	2.2	100% (6/6)	2.0	100% (6/6)	2.0
Day 31	100% (6/6)	1.2	100% (6/6)	1.2	100% (6/6)	1.2	100% (6/6)	1.3
Day 34	100% (6/6)	1.8	100% (6/6)	1.3	100% (6/6)	1.7	100% (6/6)	1.3
Day 36	100% (6/6)	1.8	100% (6/6)	1.5	100% (6/6)	1.5	100% (6/6)	1.5
Day 39	67% (4/6)	0.7	67% (4/6)	0.7	83% (5/6)	1.0	67% (4/6)	0.7
Day 42	100% (6/6)	1.5	33% (2/6)	0.3	83% (5/6)	1.3	17% (1/6)	0.2

Table shows the percentage incidence and the number of pens affected in parentheses. The severity of diarrhea was scored 0 = no diarrhoea to 4=severe diarrhoea.

Suppl. Table 5

Effect of dietary addition of ferric tyrosine on gut pathology on day 42

Treatment	Birds with normal intestinal tract	Binomial <i>p</i> -value
T1 (Control; 0 g ferric tyrosine /kg feed)	48.6% (18/37)	-
T2 (0.02 g ferric tyrosine /kg feed)	62.2% (23/37)	0.350
T3 (0.05 g ferric tyrosine /kg feed)	62.2% (23/37)	0.350
T4 (0.20 g ferric tyrosine /kg feed)	81.1% (30/37)	0.007

The percentage of birds and the number of birds is presented.

Suppl. Table 6

Effect of dietary addition of ferric tyrosine on the proximate analysis of breast and thigh meat

Meat type	Parameter	Treatment group				SEM	Treatment P (ANOVA)
		T1 (Control; 0 g ferric tyrosine /kg feed)	T2 (0.02 g ferric tyrosine /kg feed)	T3 (0.05 g ferric tyrosine /kg feed)	T4 (0.20 g ferric tyrosine /kg feed)		
Breast	Fat	1.53	1.52	1.66	1.57	0.04	0.575
	Moisture	75.05	75.18	75.01	75.16	0.09	0.882
	Protein	22.53 ^{xy}	22.43 ^{xy}	22.33 ^x	22.78 ^y	0.06	0.087
Thigh	Fat	6.35 ^b	6.27 ^{ab}	5.60 ^a	6.60 ^b	0.09	0.006
	Moisture	73.40 ^a	73.48 ^a	73.34 ^b	73.83 ^a	0.08	0.001
	Protein	19.22 ^b	19.18 ^b	19.05 ^b	18.67 ^a	0.04	0.002

SEM = standard error of mean. N^o replicates: 24 (6 birds per treatment, 1 bird