

Supplementary materials:

Table S1. Information regarding the selected broilers for the preparation of the cecal inoculum used in this study.

Broiler	Cecal inoculum		
	Live Body weight (kg)	Cecal content (g)	Representation in the inoculum (%)
1	2.04	3.25	3.80
2	1.84	3.45	4.03
3	1.70	3.67	4.29
4	1.68	3.62	4.23
5	2.06	8.21	9.59
6	2.088	2.61	3.05
7	2.187	3.50	4.09
8	2.33	10.3	12.04
9	1.81	4.10	4.79
10	1.85	4.26	4.98
11	1.67	4.23	4.94
12	1.76	6.03	7.05
13	2.01	6.50	7.60
14	1.69	6.35	7.42
15	2.10	2.58	3.01
16	1.88	1.66	1.94
17	1.70	7.8	9.11
18	1.78	3.46	4.04
Mean	1.91 ± 0.19	4.83 ± 2.22	5.56 ± 2.63

Table S2. Bacterial viable cell counts (log (CFU/mL), mean ± SD) of the cecal inoculums in different culture media. Different letters mark statically significant ($p < 0.05$) differences between conditions at each culture media.

Storage time (days)	Preservation condition	Culture media	Bacterial viable cell counts (log CFU/ mL)
0	Inoculum A (None)	CBA with 5 % (v/v) DSB	9.12 ± 0.06 ^d
		MCA	7.47 ± 0.06 ^d
		MRSA with 0.1% (w/v) cysteine	7.53 ± 0.08 ^b
15	Inoculum B (5% DMSO, -80 °C)	CBA with 5 % (v/v) DSB	8.37 ± 0.09 ^c
		MCA	7.40 ± 0.02 ^d
		MRSA with 0.1% (w/v) cysteine	7.50 ± 0.06 ^b
	Inoculum C (30% Glycerol, -20 °C)	CBA with 5 % (v/v) DSB	7.97 ± 0.07 ^a
		MCA	7.04 ± 0.02 ^b
		MRSA with 0.1% (w/v) cysteine	6.59 ± 0.05 ^a
90	Inoculum B (5% DMSO, -80 °C)	CBA with 5 % (v/v) DSB	8.24 ± 0.02 ^b
		MCA	7.15 ± 0.03 ^c
		MRSA with 0.1% (w/v) cysteine	7.41 ± 0.05 ^b
	Inoculum C (30% Glycerol, -20 °C)	CBA with 5 % (v/v) DSB	7.96 ± 0.03 ^a
		MCA	6.79 ± 0.04 ^a
		MRSA with 0.1% (w/v) cysteine	6.66 ± 0.04 ^a

Legend: CBA- Columbia base agar; DSB- defibrinated sheep blood; MCA- MacConkey agar; MRSA- de man, de rogosa and sharpe agar.

Table S3. DMSO concentration (mM, mean \pm SD) in the frozen cecal inoculum during the two washing-out cycles.

Sample	DMSO concentration (mM)
No washing cycle	595.80 \pm 1.74
First washing step	40.77 \pm 6.17
Second washing step	5.58 \pm 0.96

Table S4. Bacterial quantification (log (CFU/mL), means \pm SD) of the different bacterial populations in cecal fermentation for different conditions with fresh (A) and frozen (B) inoculum. Different letters mark statically significant ($p < 0.05$) differences between the same conditions at each sampling time, in each inoculum.

Inoculum	Condition	Time (h)	Bacterial concentration (log (CFU/mL))					
			Firmicutes (Firm)	Bacteroidetes (Bdt)	<i>Lactobacillus</i> group (Lac)	<i>Bacteroides</i> (Bac)	<i>Bifidobacterium</i> (Bif)	<i>Enterobacteriaceae</i> family (Enb)
Fresh (A)	IC	0	7.68 \pm 0.03 ^b	6.72 \pm 0.04 ^b	6.47 \pm 0.04 ^a	6.51 \pm 0.02 ^a	2.38 \pm 0.16 ^a	6.89 \pm 0.03 ^c
		24	7.37 \pm 0.04 ^b	6.93 \pm 0.09 ^b	6.13 \pm 0.04 ^a	6.55 \pm 0.14 ^a	2.41 \pm 0.14 ^a	7.70 \pm 0.11 ^a
		48	7.12 \pm 0.08 ^a	6.95 \pm 0.14 ^{a, b}	5.99 \pm 0.05 ^a	6.59 \pm 0.17 ^a	2.27 \pm 0.10 ^a	7.60 \pm 0.12 ^a
	SCF	0	7.59 \pm 0.11 ^{a, b}	6.98 \pm 0.04 ^d	6.48 \pm 0.08 ^a	6.57 \pm 0.04 ^{a, b}	2.47 \pm 0.07 ^a	7.07 \pm 0.05 ^d
		24	6.81 \pm 0.06 ^a	7.45 \pm 0.11 ^d	7.06 \pm 0.11 ^c	7.09 \pm 0.08 ^{c, d}	4.97 \pm 0.10 ^{c, d}	7.69 \pm 0.06 ^a
		48	7.63 \pm 0.11 ^c	7.16 \pm 0.05 ^b	6.81 \pm 0.06 ^c	6.75 \pm 0.06 ^{a, b}	5.54 \pm 0.02 ^d	7.65 \pm 0.02 ^a
	SCF + FOS	0	7.64 \pm 0.09 ^b	6.86 \pm 0.06 ^c	6.58 \pm 0.04 ^a	6.50 \pm 0.04 ^a	2.59 \pm 0.09 ^a	6.99 \pm 0.05 ^d
		24	7.01 \pm 0.10 ^a	7.11 \pm 0.11 ^c	6.96 \pm 0.10 ^c	6.77 \pm 0.06 ^d	4.63 \pm 0.23 ^c	7.86 \pm 0.03 ^{b, c}
		48	7.24 \pm 0.04 ^{a, b}	7.03 \pm 0.13 ^{a, b}	6.91 \pm 0.20 ^c	6.60 \pm 0.14 ^a	4.92 \pm 0.06 ^c	7.75 \pm 0.14 ^a
Frozen (B)	IC	0	7.59 \pm 0.09 ^{a, b}	6.37 \pm 0.05 ^a	6.60 \pm 0.12 ^{a, b}	6.68 \pm 0.06 ^{b, c}	2.42 \pm 0.10 ^a	6.61 \pm 0.03 ^a
		24	7.63 \pm 0.03 ^c	6.62 \pm 0.08 ^a	6.35 \pm 0.10 ^b	6.90 \pm 0.03 ^{b, c}	2.87 \pm 0.12 ^b	7.65 \pm 0.03 ^a
		48	7.19 \pm 0.09 ^{a, b}	6.78 \pm 0.19 ^a	6.36 \pm 0.06 ^b	7.02 \pm 0.06 ^{b, c}	2.69 \pm 0.08 ^b	7.69 \pm 0.08 ^a
	SCF	0	7.55 \pm 0.02 ^{a, b}	6.45 \pm 0.05 ^a	6.86 \pm 0.02 ^c	6.64 \pm 0.11 ^b	2.57 \pm 0.15 ^a	6.80 \pm 0.02 ^b
		24	6.81 \pm 0.23 ^a	7.11 \pm 0.11 ^c	7.29 \pm 0.08 ^d	7.06 \pm 0.09 ^{c, d}	5.23 \pm 0.06 ^d	7.75 \pm 0.11 ^{a, b}
		48	7.52 \pm 0.07 ^c	6.87 \pm 0.07 ^a	6.94 \pm 0.13 ^c	7.03 \pm 0.31 ^{b, c}	5.58 \pm 0.09 ^d	7.69 \pm 0.10 ^a
	SCF + FOS	0	7.48 \pm 0.10 ^a	6.45 \pm 0.07 ^a	6.67 \pm 0.11 ^b	6.77 \pm 0.05 ^c	2.51 \pm 0.11 ^a	6.77 \pm 0.05 ^b
		24	6.93 \pm 0.14 ^a	7.23 \pm 0.06 ^c	7.29 \pm 0.07 ^d	7.41 \pm 0.06 ^e	5.54 \pm 0.31 ^e	7.93 \pm 0.04 ^c
		48	7.31 \pm 0.02 ^b	6.95 \pm 0.12 ^{a, b}	6.73 \pm 0.19 ^c	7.18 \pm 0.12 ^c	5.82 \pm 0.08 ^e	7.76 \pm 0.12 ^a

Legend: FOS- fructo-oligosaccharides; IC- inoculum control; SCF- standard chicken feed.

Table S5. Concentration (mM, means \pm SD) of the different organic acids produced during cecal fermentation with fresh (A) and frozen (B) inoculum. Different letters mark statistically significant ($p < 0.05$) differences between each condition at each sampling time.

Inoculum	Condition	Time (h)	Concentration (mM)					Ratio A: P: B
			L	A	P	B	Sum SCFA (A+P+B)	
Fresh (A)	IC	0	Nd	Nd	Nd	Nd	Nd	Nd
		24	Nd ^a	32.20 \pm 0.53 ^c	5.05 \pm 0.05 ^a	4.76 \pm 0.19 ^d	42	7:1:1
		48	Nd	33.37 \pm 0.47 ^b	5.07 \pm 0.21 ^a	4.79 \pm 0.32 ^c	43	7:1:1
	SCF	0	Nd	Nd	Nd	Nd	Nd	Nd
		24	55.24 \pm 1.11 ^c	22.76 \pm 1.26 ^{a, b}	11.31 \pm 0.46 ^c	3.46 \pm 0.34 ^{b, c}	38	7:3:1
		48	Nd	37.59 \pm 0.82 ^c	28.65 \pm 1.81 ^{b, c}	5.13 \pm 0.34 ^c	71	7:6:1
	SCF + FOS	0	Nd	Nd	Nd	Nd	Nd	Nd
		24	46.16 \pm 2.28 ^b	29.40 \pm 5.96 ^{b, c}	13.25 \pm 0.34 ^d	3.27 \pm 0.10	46	9:4:1
		48	Nd	38.53 \pm 0.90 ^c	32.34 \pm 1.39 ^c	4.49 \pm 0.26	75	9:7:1
Frozen (B)	IC	0	Nd	Nd	Nd	Nd	Nd	Nd
		24	Nd	17.89 \pm 0.80 ^a	5.79 \pm 0.34 ^a	3.84 \pm 0.13 ^c	28	5:2:1
		48	Nd	21.56 \pm 0.06 ^a	6.95 \pm 0.34 ^a	3.44 \pm 0.15 ^b	32	6:2:1

	0	Nd	Nd	Nd	Nd	Nd	Nd
SCF	24	49.98 ± 1.23 ^b	20.19 ± 0.23 ^a	8.95 ± 0.55 ^b	Nd ^a	29	Nd
	48	Nd	42.71 ± 0.82 ^d	26.69 ± 2.27 ^b	2.07 ± 0.68 ^a	71	21:13:1
	0	Nd	Nd	Nd	Nd	Nd	Nd
SCF + FOS	24	57.42 ± 3.34 ^c	21.71 ± 1.59 ^a	8.64 ± 0.33 ^b	Nd ^a	30	Nd
	48	Nd	46.70 ± 0.95 ^e	29.45 ± 2.11 ^{b, c}	1.92 ± 0.09 ^a	78	24:15:1

Legend: A- Acetate; B- Butyrate; FOS- fructo-oligosaccharides; IC- inoculum control; Nd- not detected; P- Propionate; SCFA- Short-chain fatty acids; SCF- standard chicken feed.

Table S6. Concentration (mM, means ± SD) of total ammonia nitrogen produced during cecal fermentation with fresh (A) and frozen (B) inoculum. Different letters mark statistically significant ($p < 0.05$) differences between each condition at the same sampling time.

Inoculum	Condition	Sampling time point (h)	Total ammonia nitrogen (mM)
Fresh (A)	IC	0	3.70 ± 0.03 ^b
		24	33.42 ± 0.47 ^c
		48	41.48 ± 0.29 ^e
	SCF	0	3.79 ± 0.01 ^b
		24	18.17 ± 1.75 ^b
		48	35.93 ± 0.91 ^d
	SCF + FOS	0	3.86 ± 0.02 ^b
		24	19.55 ± 0.61 ^b
		48	32.07 ± 0.29 ^c
Frozen (B)	IC	0	1.42 ± 0.03 ^a
		24	19.26 ± 0.59 ^b
		48	22.85 ± 0.07 ^b
	SCF	0	1.74 ± 0.17 ^a
		24	10.17 ± 0.92 ^a
		48	20.04 ± 0.98 ^a
	SCF + FOS	0	1.63 ± 0.32 ^a
		24	10.25 ± 0.19 ^a
		48	19.41 ± 0.08 ^a

Legend: FOS- fructo-oligosaccharides; IC- inoculum control; SCF- standard chicken feed.