

SUPPORTING INFORMATION

Inactivation of *Ascaris* eggs in human fecal material through *in-situ* production of carboxylic acids

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Table S1. Treatments for Experiment 2.

pH	[HA] ^a <i>n</i> -Butyric Acid (mM)	[HA] ^a <i>n</i> -Caproic Acid (mM)	[HA+A ⁻] ^b <i>n</i> -Butyric Acid (mM)	[HA+A ⁻] ^b <i>n</i> -Caproic Acid (mM)
2	100	10.0	100	10.0
2	135	13.5	135	13.5
2	170	17.0	170	17.0
2	205	20.5	205	20.5
2	240	24.0	240	24.0
4	100	10.0	115	11.3
4	135	13.5	155	15.3
4	170	17.0	196	19.2
4	205	20.5	236	23.2
4	240	24.0	276	27.2
5	100	10.0	251	23.2
5	135	13.5	339	31.3
5	170	17.0	427	39.4
5	205	20.5	515	47.5
5	240	24.0	603	55.6

^a [HA] represents the concentration of the uncharged species only. The same five concentrations of [HA] were used at each pH level.

^b [HA + A⁻] represents the total concentration of uncharged acid *plus* charged conjugate base

Table S2. Treatments for Experiment 3.

Acid	Exposure Time (d)	Concentrations Tested (mM)
<i>n</i> -Butyric	2	250, 275, 300*, 325, 350, 370*, 400, 450
	6	50, 100, 150*, 200, 250, 300*, 350, 400
	12	10, 25, 50*, 100, 150, 200*, 250, 300
	20	5, 10, 25*, 50, 100, 150*, 200, 250
<i>n</i> -Caproic	2	16, 20, 22*, 24, 26, 30*, 32, 36
	6	4, 6, 8*, 12, 16, 22*, 26, 30
	12	1, 2, 4*, 6, 8, 12*, 16, 22
	20	1, 2, 4*, 6, 8, 12*, 16, 20

*Duplicates were tested at these concentrations

Table S3. Treatments for Experiment 4 and measured initial conditions.

Treatment Number	pH	<i>n</i> -Butyric Acid (uncharged acid <i>plus</i> conjugate base)	<i>n</i> -Caproic Acid (uncharged acid <i>plus</i> conjugate base)	Autoclaved? (Y/N)
1	Low (pH=4.72)	Spiked (269 mM)	Spiked (22.5 mM)	N
2	Low (pH=5.03)	No amendment (27.5 mM)	No amendment (3.1 mM)	N
3	Raised pH after adding carboxylic acids to equal pH of Treatment 4 (pH=6.30)	Spiked (243 mM)	Spiked (30.1 mM)	N
4	No adjustment (pH=6.40)	No amendment (36.1 mM)	No amendment (4.2 mM)	N
5	No adjustment (pH=6.50)	No amendment (16.7 mM)	No amendment (1.7 mM)	Y

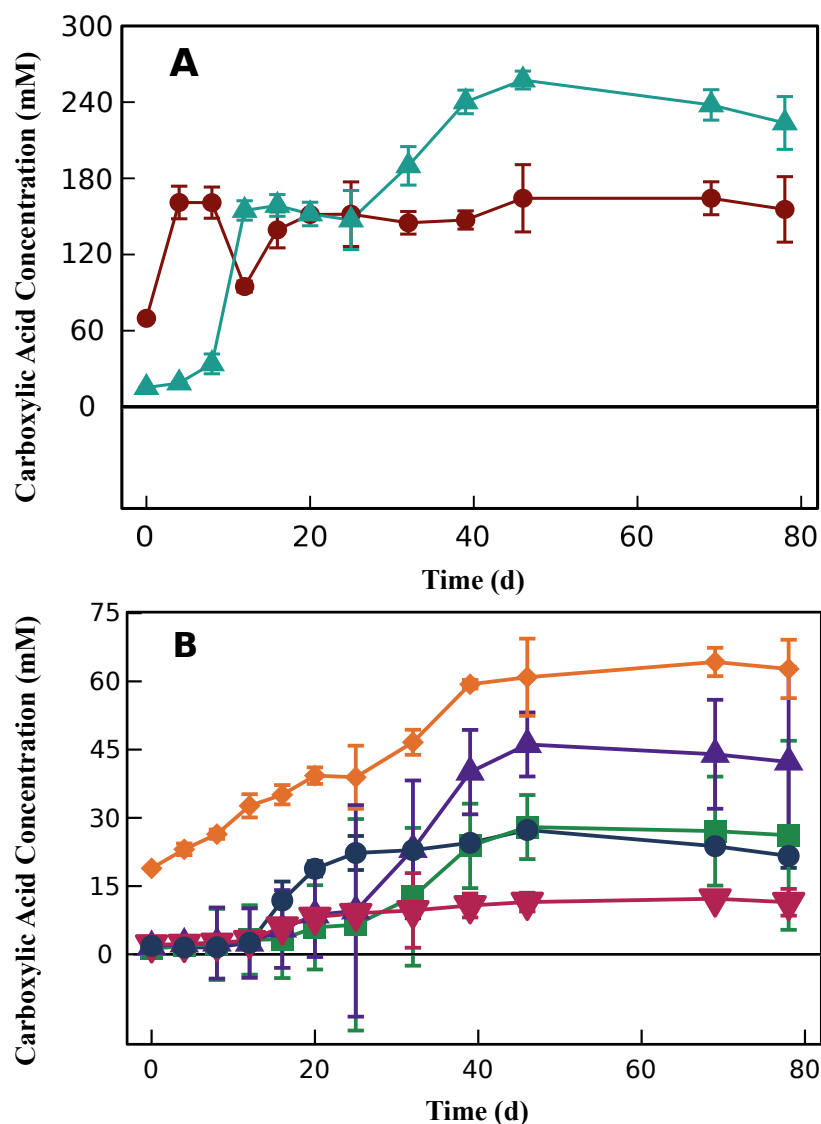


Figure S1. Accumulation of carboxylic acids from batch fermentation of HFM at 30°C. Panel A shows concentrations of acetic acid (●) and *n*-butyric acid (▲). Panel B shows concentrations of propionic acid (◆), *i*-butyric acid (■), *i*-valeric acid (▲), *n*-valeric acid (▼), and *n*-caproic acid (●). Concentrations represent the total concentrations of uncharged acid *plus* conjugate base. Error bars show the standard deviation of three biological replicates. Note that the scale of the y-axis is different in each panel.

Table S4. Individual model parameters and statistics at three different pH levels for inactivation of *Ascaris* eggs when exposed to *n*-butyric acid and *n*-caproic acid for 3 days at 37°C.

pH	Parameter <i>a</i> (SE ^a)	<i>p</i> -value <i>a</i>	Parameter <i>b</i> (SE ^a)	<i>p</i> -value <i>b</i>
2	0.0997 (1.40 x 10 ⁻²)	1.41 x 10 ^{-8*}	146 (1.06)	< 2 x 10 ^{-16*}
4	0.0936 (1.50 x 10 ⁻²)	2.28 x 10 ^{-7*}	149 (0.944)	< 2 x 10 ^{-16*}
5	0.106 (1.80 x 10 ⁻²)	7.49 x 10 ^{-7*}	148 (0.775)	< 2 x 10 ^{-16*}

The model presented in the main text incorporated the data from all three pH levels and was not statistically different from the model shown here with data separated by pH ($p=0.0748$).

^aSE= standard error

* Significant at $\alpha=0.001$

Table S5. Fraction of viable *A. suum* eggs for each treatment in Experiment 2 after exposure to carboxylic acids for 3 days at 37°C.

pH	Uncharged Carboxylic Acid Concentration ^a (mM) (SD [*])	Total Carboxylic Acid Concentration ^a (Uncharged Acid <i>Plus</i> Conjugate Base) (mM) (SD ^c)	Fraction Viable ^b (SD ^c)
2	114 (1.41)	474 (6.70)	0.958 (0.046)
	151 (7.95)	630 (33.4)	0.376 (0.118)
	189 (8.06)	786 (34.2)	0.00377 (0.00144)
	230 (2.85)	958 (12.1)	0.00123 ^d (0)
	267 (2.08)	1111 (8.49)	0.00132 ^d (0)
4	110 (1.94)	526 (10.9)	0.955 (0.0312)
	152 (2.04)	722 (10.3)	0.453 (0.0368)
	188 (1.26)	916 (5.75)	0.00463 (0.00229)
	226 (1.22)	1108 (5.82)	0.00136 ^d (0)
	268 (1.53)	1317 (7.99)	0.00210 ^d (0)
5	106 (1.36)	1199 (15.5)	0.949 (0.0325)
	147 (1.77)	1636 (20.5)	0.532 (0.0364)
	180 (5.06)	2093 (59.3)	0.0143 (0.00783)
	209 (2.54)	2509 (30.1)	0.00151 ^d (0)
	246 (2.07)	2946 (24.6)	0.00161 ^d (0)
Controls			0.856 (0.013)

^a Concentrations shown are combined concentrations of *n*-butyric acid and *n*-caproic acid. Each solution contained 10 mM *n*-butyric acid for every 1 mM *n*-caproic acid.

^b Fraction viable is normalized to the average viability of the controls (*A. suum* eggs suspended in deionized water for 3 days at 37°C).

^c SD= standard deviation of three replicates.

^d Viability below detection limit

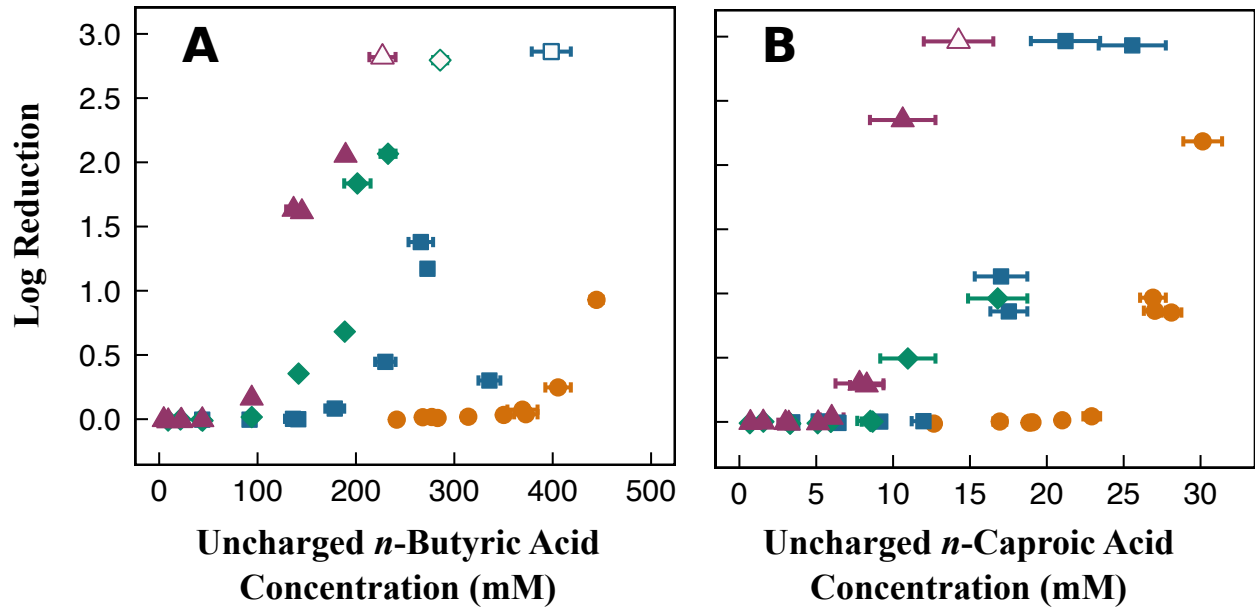


Figure S2. Log Reduction of *Ascaris* eggs due to exposure to *n*-butyric acid (A) and *n*-caproic acid (B). *Ascaris* eggs were exposed to carboxylic acids at 30°C for four different exposure times: 2 days (●), 6 days (■), 12 days (◆), and 20 days (▲). Open symbols indicate viability below the detection limit. The carboxylic acid concentration shown is the average of measurements made before and after the exposure period for each treatment, and the x-error bar shows the range between the two measurements. Replicate treatments are shown as individual points.

Table S6. Fraction of viable *A. suum* eggs for each treatment in Experiment 3.

<i>n</i> -Butyric Acid			<i>n</i> -Caproic Acid		
Exposure Time (d)	Average Uncharged <i>n</i> -Butyric Acid Concentration ^a (mM)	Fraction Viable ^b	Exposure Time (d)	Average Uncharged <i>n</i> -Caproic Acid Concentration ^a (mM)	Fraction Viable ^b
2	241	1.00	2	12.7	1.00
	268	0.966		16.9	0.993
	277	0.962		18.9	1.000
	283	0.979		19.1	1.000
	314	0.957		21.0	0.971
	350	0.927		22.9	0.905
	373	0.915		27.0	0.136
	369	0.843		26.9	0.108
	405	0.566		28.1	0.141
	444	0.118		30.1	0.007
	Controls	0.908 (0.0108 ^d)		Controls	0.908 (0.0108 ^d)
6	43.7	1.00	6	3.44	1.00
	91.9	1.00		5.16	0.991
	141	1.00		6.45	1.00
	136	0.994		6.37	1.00
	178	0.827		9.16	0.994
	230	0.358		12.0	0.987
	266	0.042		17.5	0.138
	273	0.067		17.0	0.074
	336	0.500		21.2	0.00108
	399	0.00126 ^c		25.6	0.00117
	Controls	0.917 (0.0104 ^d)		Controls	0.917 (0.0104 ^d)
12	9.06	1.00	12	0.680	1.00
	21.6	1.00		1.56	0.995
	42.7	1.00		3.31	1.00
	44.0	1.00		3.28	1.00
	94.1	0.963		5.09	1.00
	142	0.442		5.95	0.999
	189	0.208		8.52	0.982
	201	0.015		8.67	0.995
	233	0.009		11.0	0.321
	286	0.00146 ^c		16.8	0.109
	Controls	0.913 (0.0116 ^d)		Controls	0.913 (0.0116 ^d)
20	4.66	1.00	20	0.718	1.00
	9.26	1.00		1.56	1.00
	22.6	1.00		3.00	1.00
	22.2	1.00		3.21	1.00
	43.9	1.00		5.12	1.00
	94.1	0.688		6.01	0.925
	145	0.024		7.81	0.502
	137	0.023		8.28	0.518
	189	0.009		10.6	0.004
	227	0.00137 ^c		14.3	0.00099 ^c
	Controls	0.904 (0.0276 ^d)		Controls	0.904 (0.0276 ^d)

^a Concentration is the average of measurements made before and after the exposure period.

^b Fraction viable is normalized to the average viability of the controls for each exposure period.

^c Viability below detection limit

^d Standard deviation of three replicates

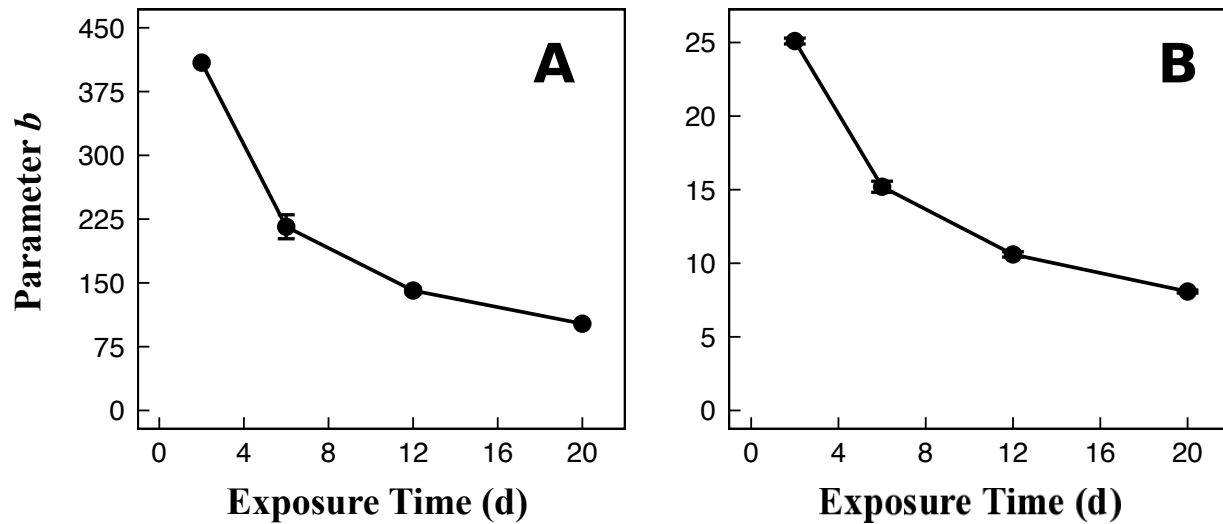


Figure S3. Model parameter b at the four different exposure times tested in Experiment 2 for n -butyric acid (A) and n -caproic acid (B). Error bars represent the standard error of the parameter b .

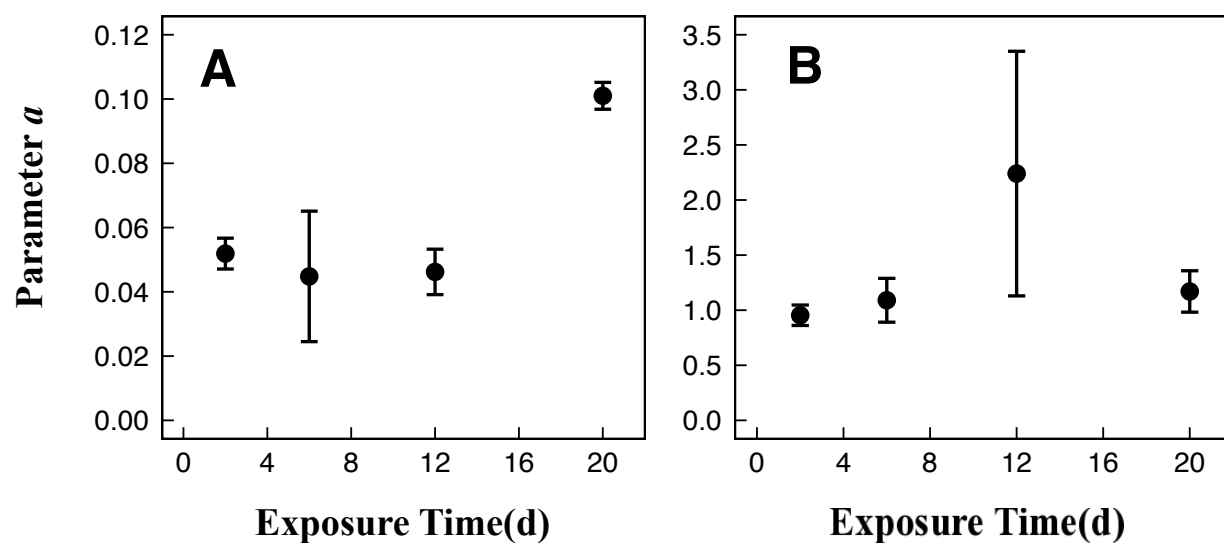


Figure S4. Model parameter a at the four different exposure times tested in Experiment 2 for *n*-butyric acid (A) and *n*-caproic acid (B). Error bars represent the standard error of the parameter a .

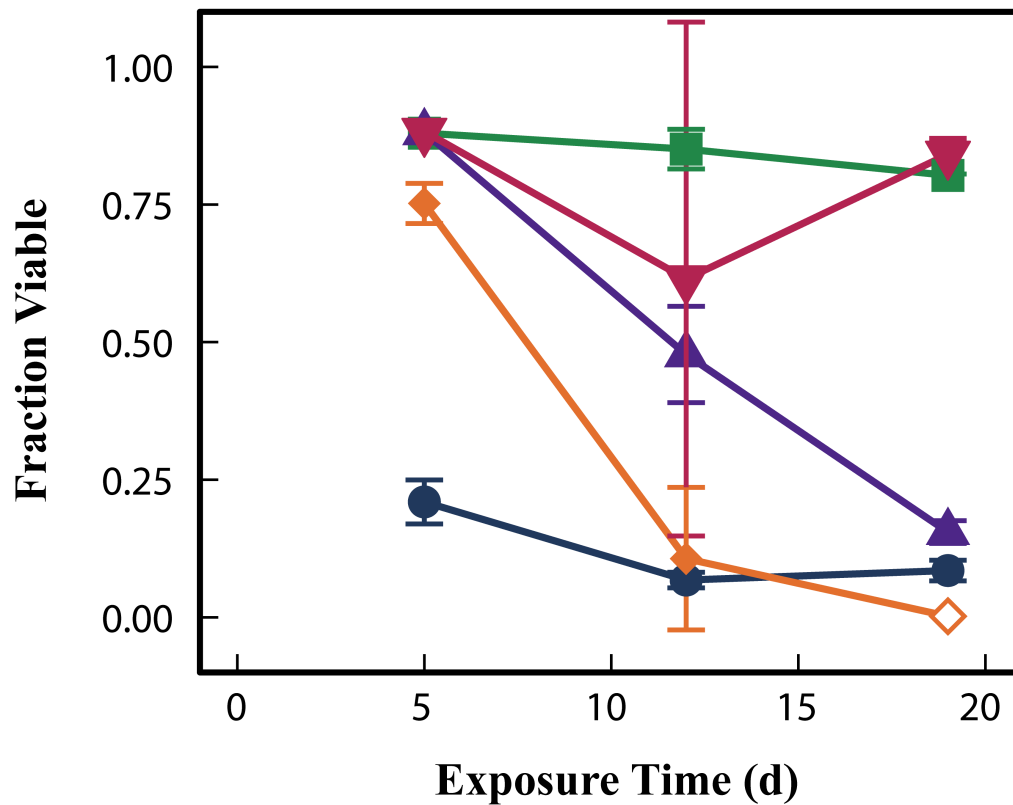


Figure S5. Inactivation of *Ascaris* eggs in HFM after exposure times of 5, 12, and 19 days. *A. suum* eggs were exposed to five treatments at 30°C : Treatment 1 with spiked acids and low pH (●); Treatment 2 with no added acids and low pH (■); Treatment 3 with spiked acids and natural pH (▲); Treatment 4 with no added acids and natural pH (◆); and Treatment 5 with autoclaved HFM, no added acids, and natural pH (▼). Error bars represent standard deviation of viabilities for three replicates. Open symbols indicate that viability was below the detection limit of 0.004.

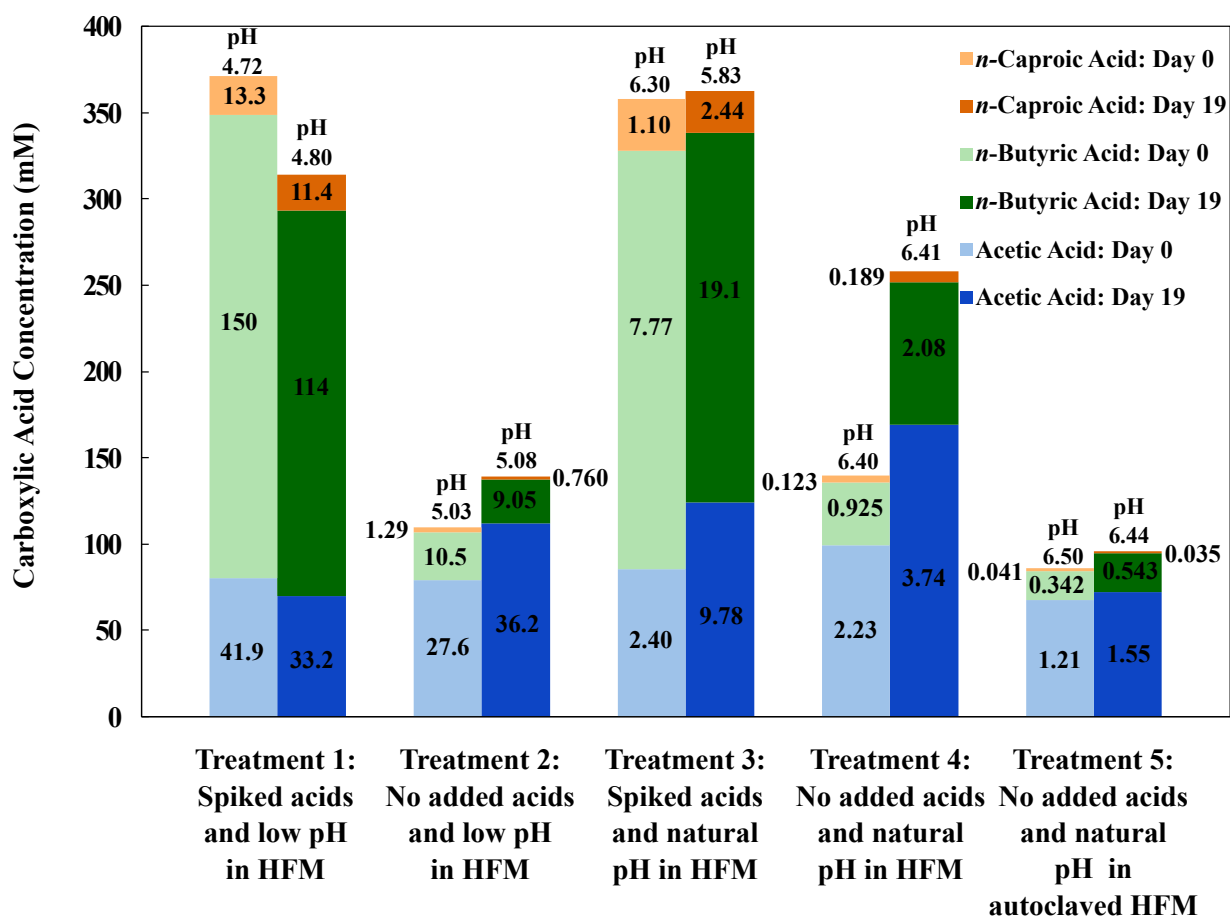


Figure S6. Concentrations of carboxylic acids measured at onset of experiment and after 19-day exposure period for Experiment 4. The height of each bar represents the total carboxylic acid concentration (uncharged acid *plus* conjugate base). The pH is shown on top of each bar, and the calculated uncharged carboxylic acid concentration is written within the bar.

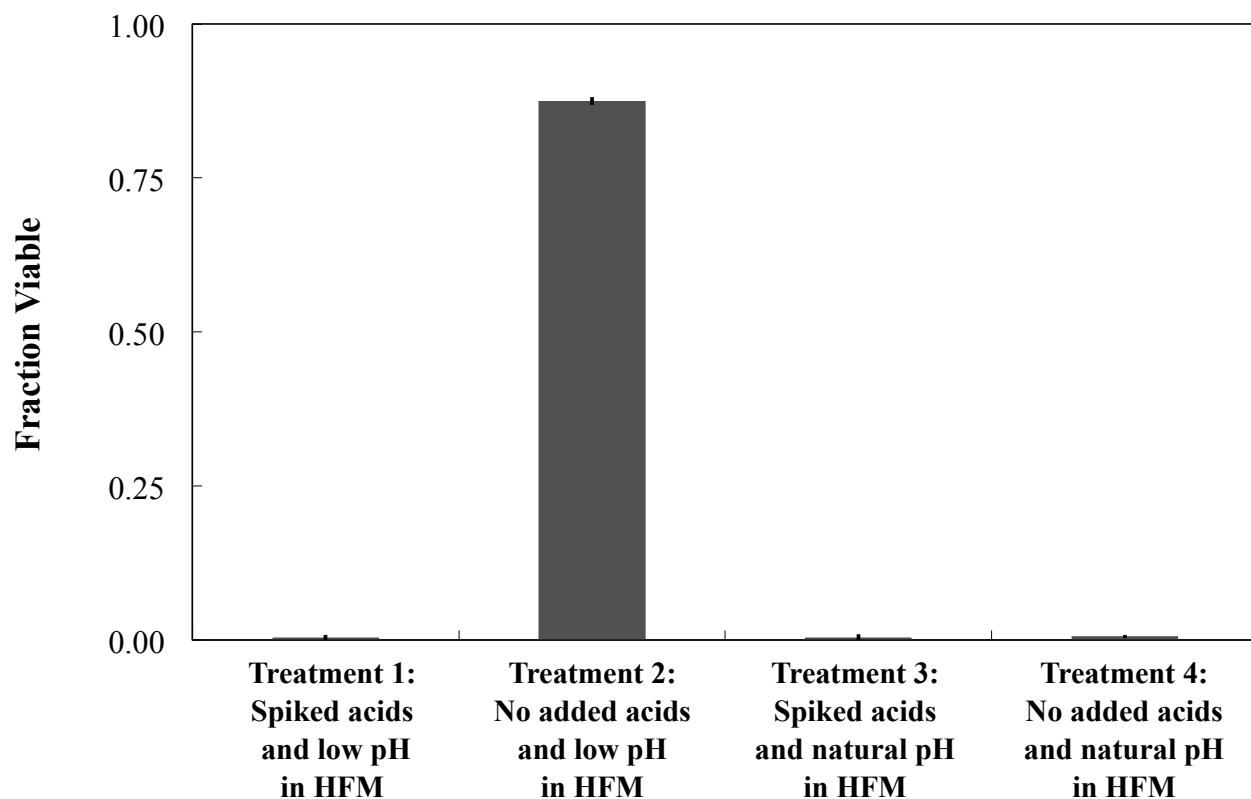


Figure S7. Previous experiment inactivating *A. suum* eggs in HFM. *A. suum* eggs were exposed to treatment conditions in HFM for 14 days at 30°C. For treatments with spiked acids, *n*-butyric acid and *n*-caproic acid were added to achieve final concentrations of 240 mM and 24 mM (uncharged acid *plus* conjugate base), respectively. For treatments with low pH, the pH was adjusted to 5.10 (\pm 0.05) using HCl. All other methods were the same as those for Experiment 4. Error bars represent standard deviation of viabilities for three replicates. Viabilities for Treatments 1, 3, and 4 were 0.004, 0.004, and 0.003, respectively. The detection limit was 0.004.