

Supplementary Results

Table S1. Cell viability was assessed by the MTT assay. The results were expressed as % of control (media only) and represent the means of three independent experiments.

Treatment	Cell Viability (% of Control) ^a
Inducer only (LPS)	96.0 ± 4.8
WPI ^b 1000	91.9 ± 4.8
WPI 100	95.0 ± 5.4
WPI 10	98.1 ± 4.1
WPID ^c 1000	97.2 ± 3.6
WPID 100	97.2 ± 2.8
WPID 10	92.6 ± 4.1
GMP ^d 1000	94.1 ± 9.6
GMP 100	96.6 ± 2.3
GMP 10	96.0 ± 5.6
GMPD ^e 1000	92.9 ± 7.3
GMPD 100	93.6 ± 3.2
GMPD 10	90.0 ± 3.3
WPIINT ^f 1000	94.7 ± 3.5
WPIINT100	94.1 ± 4.9
WPIINT10	100.5 ± 5.8

^a One-sided t-tests were conducted to test if there are significant differences between each treatment from the LPS-only control ($P < 0.05$). No significant different was found between each treatment and LPS-only control. $n = 6$ technical replicates (3 days of cell experiment replication, duplicate wells on each day).

^b Whey protein isolate (WPI)

^c In vitro-digested whey protein isolate (WPID)

^d Glycomacropeptide (GMP)

^e In vitro-digested glycomacropeptide (GMPD)

^f Intestinal-digested whey protein isolate (WPIINT)

Table S2. Concentrations of cytokines in cells based on treatment.

Treatment	TNF- α (pg/mL) ^a	IL-1 β (pg/mL)
Blank cell	33.6±11.9	67.4±10.7
Inducer only (LPS)	216.3 ± 19.3	313.3±47.7
WPI ^b 1000	233.9±18.1	132.2±16.2
WPI 100	234.5±22.5	146.0±17.4
WPI 10	218.4±11.8	178.8±26.3
WPID ^c 1000	115.5±17.3	152.6±14.6
WPID 100	149.4±20.9	157.5±22.8
WPID 10	178.1±22.2	171.9±22.5
GMP ^d 1000	276.3±39.2	492.2±83.8
GMP 100	262.6±19.2	430.2±63.1

GMP 10	89.5±21.6	310.8±46.1
GMPDe 1000	109.0±11.3	151.2±14.6
GMPD 100	157.1±14.4	114.7±17.7
GMPD 10	58.6±26.9	212.5±30.2
WPIINT ^f 1000	58.6±26.9	421.7±57.6
WPIINT 100	60.6±28.3	327.0±50.5
WPIINT 10	63.5±2.9	160.8±14.9

^a n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).

^b Whey protein isolate (WPI)

^c In vitro-digested whey protein isolate (WPID)

^d Glycomacropeptide (GMP)

^e In vitro-digested glycomacropeptide (GMPD)

^f Intestinal-digested whey protein isolate (WPIINT)

Table S3. Two-sided t-tests were performed to determine whether each cytokine concentration (TNF- α and IL-1 β) in each treatment (WPI, GMP, WPID, GMPD, WPIINT at each dose (10, 100 and 1,000 μ g/mL)) were significantly different from the LPS-only control using GraphPad Prism software (version 8.2).

	P value t-test compared to control	
	TNF- α ^a	IL-1 β
WPI 1000 ^b	0.1332	<0.0001
WPI 100	0.1639	<0.0001
WPI 10	0.8233	0.0001
WPID 1000 ^c	<0.0001	<0.0001
WPID 100	0.0002	<0.0001
WPID 10	0.0098	<0.0001
GMP 1000 ^d	0.0071	0.0011
GMP 100	0.0335	0.0047
GMP 10	0.8162	0.9293

GMPD 1000 ^c	<0.0001	<0.0001
GMPD 100	<0.0001	<0.0001
GMPD 10	0.0001	0.0014
WPIINT 1000 ^f	<0.0001	0.0053
WPIINT 100	<0.0001	0.6412
WPIINT 10	<0.0001	<0.0001

^a n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).

^b Whey protein isolate (WPI)

^c In vitro-digested whey protein isolate (WPID)

^d Glycomacropeptide (GMP)

^e In vitro-digested glycomacropeptide (GMPD)

^f Intestinal-digested whey protein isolate (WPIINT)

Digested	Concentration	-Digested	-Concentration	Difference	Std Error	t Ratio	Prob> t	Lower 95%	Upper 95%
No	10	No	100	-0.090000	0.0636479	-1.41	0.8861	-0.298034	0.118034
No	10	No	1000	-0.091667	0.0636479	-1.44	0.8755	-0.299701	0.116367
No	10	Simulated	10	0.226667	0.0636479	3.56	0.0236*	0.018633	0.434701
No	10	Simulated	100	0.381667	0.0636479	6.00	<.0001*	0.173633	0.589701
No	10	Simulated	1000	0.571667	0.0636479	8.98	<.0001*	0.363633	0.779701
No	10	Intestinal	10	0.853333	0.0636479	13.41	<.0001*	0.645299	1.061367
No	10	Intestinal	100	0.875000	0.0636479	13.75	<.0001*	0.666966	1.083034
No	10	Intestinal	1000	0.885000	0.0636479	13.90	<.0001*	0.676966	1.093034
No	100	No	1000	-0.001667	0.0636479	-0.03	1.0000	-0.209701	0.206367
No	100	Simulated	10	0.316667	0.0636479	4.98	0.0004*	0.108633	0.524701
No	100	Simulated	100	0.471667	0.0636479	7.41	<.0001*	0.263633	0.679701
No	100	Simulated	1000	0.661667	0.0636479	10.40	<.0001*	0.453633	0.869701
No	100	Intestinal	10	0.943333	0.0636479	14.82	<.0001*	0.735299	1.151367
No	100	Intestinal	100	0.965000	0.0636479	15.16	<.0001*	0.756966	1.173034
No	100	Intestinal	1000	0.975000	0.0636479	15.32	<.0001*	0.766966	1.183034
No	1000	Simulated	10	0.318333	0.0636479	5.00	0.0003*	0.110299	0.526367
No	1000	Simulated	100	0.473333	0.0636479	7.44	<.0001*	0.265299	0.681367
No	1000	Simulated	1000	0.663333	0.0636479	10.42	<.0001*	0.455299	0.871367
No	1000	Intestinal	10	0.945000	0.0636479	14.85	<.0001*	0.736966	1.153034
No	1000	Intestinal	100	0.966667	0.0636479	15.19	<.0001*	0.758633	1.174701
No	1000	Intestinal	1000	0.976667	0.0636479	15.34	<.0001*	0.768633	1.184701
Simulated	10	Simulated	100	0.155000	0.0636479	2.44	0.2925	-0.053034	0.363034
Simulated	10	Simulated	1000	0.345000	0.0636479	5.42	<.0001*	0.136966	0.553034
Simulated	10	Intestinal	10	0.626667	0.0636479	9.85	<.0001*	0.418633	0.834701
Simulated	10	Intestinal	100	0.648333	0.0636479	10.19	<.0001*	0.440299	0.856367
Simulated	10	Intestinal	1000	0.658333	0.0636479	10.34	<.0001*	0.450299	0.866367
Simulated	100	Simulated	1000	0.190000	0.0636479	2.99	0.0976	-0.018034	0.398034
Simulated	100	Intestinal	10	0.471667	0.0636479	7.41	<.0001*	0.263633	0.679701
Simulated	100	Intestinal	100	0.493333	0.0636479	7.75	<.0001*	0.285299	0.701367
Simulated	100	Intestinal	1000	0.503333	0.0636479	7.91	<.0001*	0.295299	0.711367
Simulated	1000	Intestinal	10	0.281667	0.0636479	4.43	0.0020*	0.073633	0.489701
Simulated	1000	Intestinal	100	0.303333	0.0636479	4.77	0.0007*	0.095299	0.511367
Simulated	1000	Intestinal	1000	0.313333	0.0636479	4.92	0.0004*	0.105299	0.521367
Intestinal	10	Intestinal	100	0.021667	0.0636479	0.34	1.0000	-0.186367	0.229701
Intestinal	10	Intestinal	1000	0.031667	0.0636479	0.50	0.9999	-0.176367	0.239701
Intestinal	100	Intestinal	1000	0.010000	0.0636479	0.16	1.0000	-0.198034	0.218034

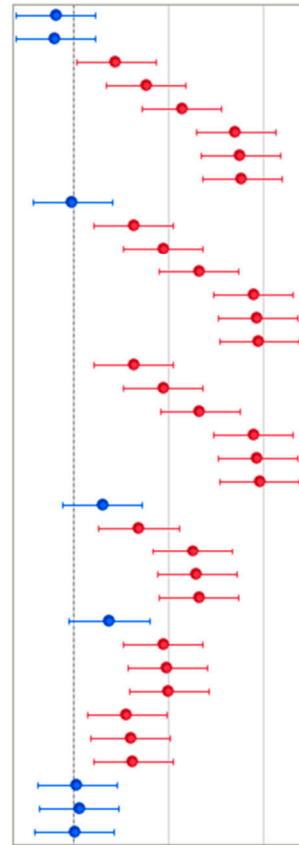


Figure S1. The significance of the differences in percent change of TNF- α from the LPS-only control among WPI, WPID and WPIINT at each concentration were assessed using multi-factor ANOVA followed by Tukey's HSD tests, using JMP software (version 17). n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).

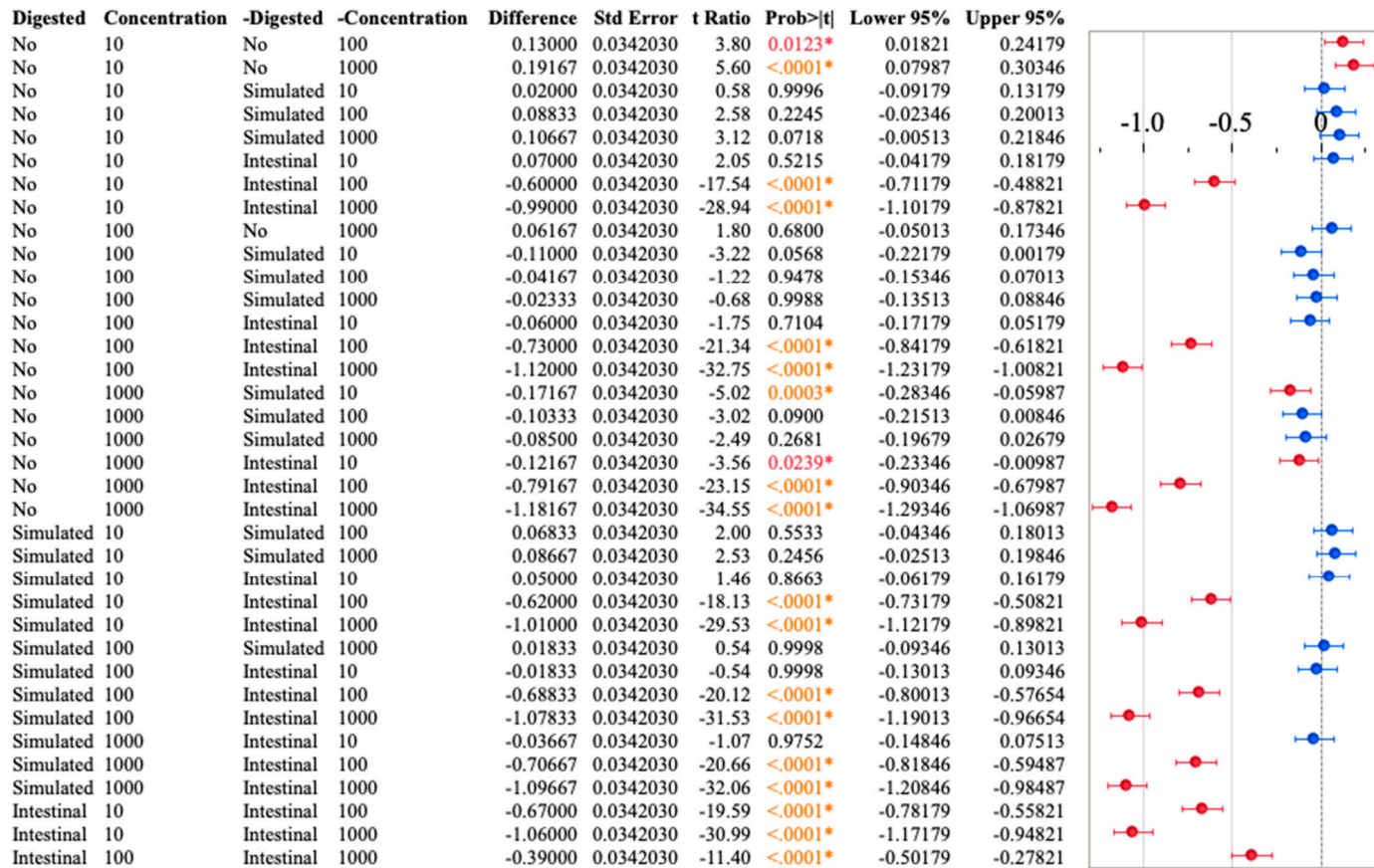


Figure S2. The significance of the differences in percent change of IL-1 β from the LPS-only control among WPI, WPID and WPIINT at each concentration were assessed using multi-factor ANOVA followed by Tukey's HSD tests, using JMP software (version 17). n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).

Digested	Concentration	-Digested	-Concentration	Difference	Std Error	t Ratio	Prob> t	Lower 95%	Upper 95%
No	10	No	100	-0.27167	0.0655120	-4.15	0.0037*	-0.472386	-0.07095
No	10	No	1000	-0.35167	0.0655120	-5.37	0.0002*	-0.552386	-0.15095
No	10	Simulated	10	0.31000	0.0655120	4.73	0.0008*	0.109281	0.51072
No	10	Simulated	100	0.57500	0.0655120	8.78	<.0001*	0.374281	0.77572
No	10	Simulated	1000	0.68667	0.0655120	10.48	<.0001*	0.485948	0.88739
No	100	No	1000	-0.08000	0.0655120	-1.22	0.8228	-0.280719	0.12072
No	100	Simulated	10	0.58167	0.0655120	8.88	<.0001*	0.380948	0.78239
No	100	Simulated	100	0.84667	0.0655120	12.92	<.0001*	0.645948	1.04739
No	100	Simulated	1000	0.95833	0.0655120	14.63	<.0001*	0.757614	1.15905
No	1000	Simulated	10	0.66167	0.0655120	10.10	<.0001*	0.460948	0.86239
No	1000	Simulated	100	0.92667	0.0655120	14.14	<.0001*	0.725948	1.12739
No	1000	Simulated	1000	1.03833	0.0655120	15.85	<.0001*	0.837614	1.23905
Simulated	10	Simulated	100	0.26500	0.0655120	4.05	0.0047*	0.064281	0.46572
Simulated	10	Simulated	1000	0.37667	0.0655120	5.75	<.0001*	0.175948	0.57739
Simulated	100	Simulated	1000	0.11167	0.0655120	1.70	0.5407	-0.089052	0.31239

Figure S3. The significance of the differences in percent change of TNF- α from the LPS-only control among GMP and GMPD at each concentration were assessed using multi-factor ANOVA followed by Tukey's HSD tests, using JMP software (version 17). n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).

Digested	Concentration	-Digested	-Concentration	Difference	Std Error	t Ratio	Prob> t	Lower 95%	Upper 95%
No	10	No	100	-0.48167	0.0657943	-7.32	<.0001*	-0.68325	-0.28008
No	10	No	1000	-0.73167	0.0657943	-11.12	<.0001*	-0.93325	-0.53008
No	10	Simulated	10	0.41000	0.0657943	6.23	<.0001*	0.20842	0.61158
No	10	Simulated	100	0.80667	0.0657943	12.26	<.0001*	0.60508	1.00825
No	10	Simulated	1000	0.65667	0.0657943	9.98	<.0001*	0.45508	0.85825
No	100	No	1000	-0.25000	0.0657943	-3.80	0.0088*	-0.45158	-0.04842
No	100	Simulated	10	0.89167	0.0657943	13.55	<.0001*	0.69008	1.09325
No	100	Simulated	100	1.28833	0.0657943	19.58	<.0001*	1.08675	1.48992
No	100	Simulated	1000	1.13833	0.0657943	17.30	<.0001*	0.93675	1.33992
No	1000	Simulated	10	1.14167	0.0657943	17.35	<.0001*	0.94008	1.34325
No	1000	Simulated	100	1.53833	0.0657943	23.38	<.0001*	1.33675	1.73992
No	1000	Simulated	1000	1.38833	0.0657943	21.10	<.0001*	1.18675	1.58992
Simulated	10	Simulated	100	0.39667	0.0657943	6.03	<.0001*	0.19508	0.59825
Simulated	10	Simulated	1000	0.24667	0.0657943	3.75	0.0100*	0.04508	0.44825
Simulated	100	Simulated	1000	-0.15000	0.0657943	-2.28	0.2367	-0.35158	0.05158

Figure S4. The significance of the differences in percent change of IL-1 β from the LPS-only control among GMP and GMPD at each concentration were assessed using multi-factor ANOVA followed by Tukey's HSD tests, using JMP software (version 17). n = 6 technical replicates (3 days of cell experiment replication, duplicate wells on each day).