Electronic Supplementary Material (ESI) for Food & Function.

Casein-fed mice showed faster recovery from DSS-induced colitis than chicken-protein-fed mice

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Table S1 Crude composition of protein powders (%)

	Casein	Chicken
protein	88.65	86.88
water	9.90	10.34
ash	1.09	1.80
fat	0.36	0.98

Table S2 Mineral composition of protein powders (mg/Kg)

	Casein	Chicken
Ca	305.9	240.0
Cu	4.0	3.0
Fe	41.9	12.1
K	69.7	3171.5
Mg	178.4	493.3
P	5291.0	2982.1
Zn	55.2	13.9
Na	64.2	367.2

Table S3 Ingredient composition of mineral mixes of the two diets (g/Kg diet)

	Casein	Chicken
calcium carbonate, anhydrous, Ca 40.04%	12.33500	12.36500
potassium dihydrogen phosphate, P 22.76% K 28.73%	7.86200	10.02200
sodium dihydrogen phosphate, P 22.76% Na 28.73%	0.69800	0.50700
potassium Citrate, K 36.16%	3.46700	0.00000
sodium chloride, Na 39.34%	2.03400	2.03400
potassium sulphate, S 18.39% K 44.87%	1.63100	1.63100
magnesium oxide, Mg 60.32%	0.79100	0.68400
iron citrate, Fe 16.5%	0.22200	0.25800
zinc carbonate, Zn 52.14%	0.05200	0.06700
curpic carbonate, Cu 57.47%	0.01050	0.01050
manganous carbonate, Mn 47.79%	0.02205	0.02205
potassium iodate, I 59.3%	0.00035	0.00035

sodium selenate, Se 41.79%	0.00036	0.00036
ammonium paramolybdate, 4 hydrate, Mo 54.34%	0.00028	0.00028
sodium meta-silicate, 9 hydrate, Si 9.88%	0.05080	0.05080
chromium potassium sulfate, 12 hydrate, Cr 10.42%	0.00960	0.00960
boric acid, B 17.5%	0.00290	0.00290
sodium fluoride, F 45.24%	0.00220	0.00220
nickel carbonate, Ni 45%	0.00111	0.00111
lithium chloride, Li 16.38%	0.00061	0.00061
ammonium vanadate, V 43.55%	0.00023	0.00023
Total	29.1927	27.6688

Table S4 Ingredient composition and nutritional content of diets (g/Kg diet)

	Casein	Chicken
Protein powder	200.00	204.00
Cornstarch	400.83	400.83
Dextrinised cornstarch	132.00	132.00
Sucrose	100.00	100.00
Soybean oil	70.00	70.00
Fiber	50.00	50.00
Mineral mix[2]	29.19	27.67
Vitamin mix[3]	10.00	10.00
L-cystine	3.00	3.00
Choline chloride	2.50	2.50
Water	2.48	0.00
Total	1000.00	1000.00

Protein[1], the amount of protein powder was adjusted and balanced according to the protein content in casein and chicken protein powder. Mineral mix[2], the formulation of mineral mixes for the two diets is listed in Table S3. Vitamin mix[3]: the formulation of vitamin mix as described [1].

[1] P. G. Reeves, F. H. Nielsen, G. C. Fahey, AIN-93 purified diets for laboratory

rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet, *J Nutr.*, 1993. **123**,1939-1951.

Table S5 Scoring of disease activity index (DAI).

Score	Loss of weight	Visible fecal blood	Stool consistency
0	None	None	Normal
1	0%-5%	Slightly bloody	Slightly loose feces
2	5%-10%	Moderately bloody	Loose feces
3	10%-20%	Bloody	Diarrhea
4	>20%	Severely blood	Severe diarrhea

Table S6 Scoring of colonic histology.

Score	Severity of inflammatory infiltration	Crypt injury	Disappearance of goblet cells
0	Normal	None	None
1	Occasionally, cell limited to submucosa	Some crypt damage	Rarely disappeared
2	Significant presence of inflammatory cells in submucosa	Large spaces between crypts, some shortening of crypts	Slightly disappeared
3	Infiltrate present in both submucosa and lamina propria	Large areas without crypts	Moderately disappeared
4	Large amount of infiltrate in submucosa, lamina propria and surrounding blood vessels	No crypts	Depletion of goblet cells

Table S7 Primer sequences used in RT-qPCR assays in colonic tissue.

Target genes	Primer Sequence (5′-3′)	Annealing T (ºC)
TNF-α	FW: CTCATGCACCACCATCAAGG	59
	RV: ACCTGACCACTCTCCCTTTG	
ZO-1	FW: TGTTTATGCGGACGGTGGCG	64
	RV: TCCATTGCTGTGCTCTTAGCGG	
MUC1	FW: TGCCCTTCCAAGTGAGGAAA	59
	RV: CTGGAGTGGTAGTCGATGCT	
COX-2	FW: CCCATTAGCAGCCAGTTGTC	59
	RV: CAGGATGCAGTGCTGAGTTC	
MUC2	FW: TTTGGGTCCTGTGGGACTTT	59
	RV: ACTGGTCTTCTCCTCCTTGC	
TLR-4	FW: GCCACCAGTTACAGATCGTC	58
	RV: GAGGCATCATCCTGGCATTT	
IL-15	FW: AGAGTTCTGGATGGATGGCA	58
	RV: TGCCCAGGTAAGAGCTTCAA	
GAPDH	FW: GGACTTACAGAGGTCCGCTT	59
	RV: CTATAGGGCCTGGGTCAGTG	

Table S8. The degree of hydrolysis (DH) of samples during gastrointestinal digestion.

	DH (%)			
	G_{15}	G_{120}	I_{15}	I_{120}
Casein	3.86 ± 0.54	13.15 ± 0.25	47.20 ± 3.86**	60.71 ± 2.45**
Chicken Protein	3.67 ± 0.55	14.21 ± 1.97	29.22 ± 4.83	37.83 ± 8.13

G15 and G120 indicate digestibility after 15 or 120 min of gastric digestion; I15 and I120 indicate digestibility after 120 min of gastric digestion and 15 or 120 min of intestinal digestions.

Degree of hydrolysis (DH)

Method

Chicken protein and casein were digested by gastric and intestinal digestive enzymes using the INFOGEST method [1]. The protein digestibility was calculated by the change of primary amino group content before and after enzyme treatments, which was quantified by a fluorescamine method as described previously [2]. The degree of hydrolysis (DH) was determined as follows:

$$\mathrm{DH} = \frac{[-\mathrm{NH2\ (h)}] - [-\mathrm{NH2\ (0)}]}{[-\mathrm{NH2\ (\infty)}] - [-\mathrm{NH2\ (0)}]}$$

[-NH2] indicates the concentration of primary amines in the hydrolyzed (h) or unhydrolyzed (0) samples and [-NH2(∞)] indicates the maximum primary amine concentration assuming total digestion to free amino acids (AAs). The [-NH2(∞)]

value was determined the fluorescence of each sample (before digestion) that was totally hydrolyzed by 6 M hydrochloric acid at 100 °C for 48 h.

References

[1] A. Brodkorb, L. Egger, M. Alminger, P. Alvito, R. Assunção, S. Ballance, T. Bohn, C. Bourlieu-Lacanal, R. Boutrou, F. Carrière, A. Clemente, M. Corredig, INFOGEST static in vitro simulation of gastrointestinal food digestion, *Nat Protocols*., 2019, **14**, 991-1014.

[2] D.Zhao, Y. Xu, T. Gu, H. Wang, Y. Yin, B. Sheng, Y. Li, Y. Nian, C. Wang, C. Li, X. Xu, G. Zhou, Peptidomic investigation of the interplay between enzymatic tenderization and the digestibility of beef semimembranosus proteins, *J Agr. Food Chem.,* 2020, **68**, 1136.

Figure S1. The mRNA levels of TNF-α (A), IL-15 (B), TLR-4 (C), ZO-1 (D) and COX-2 (E) in the colon, respectively.

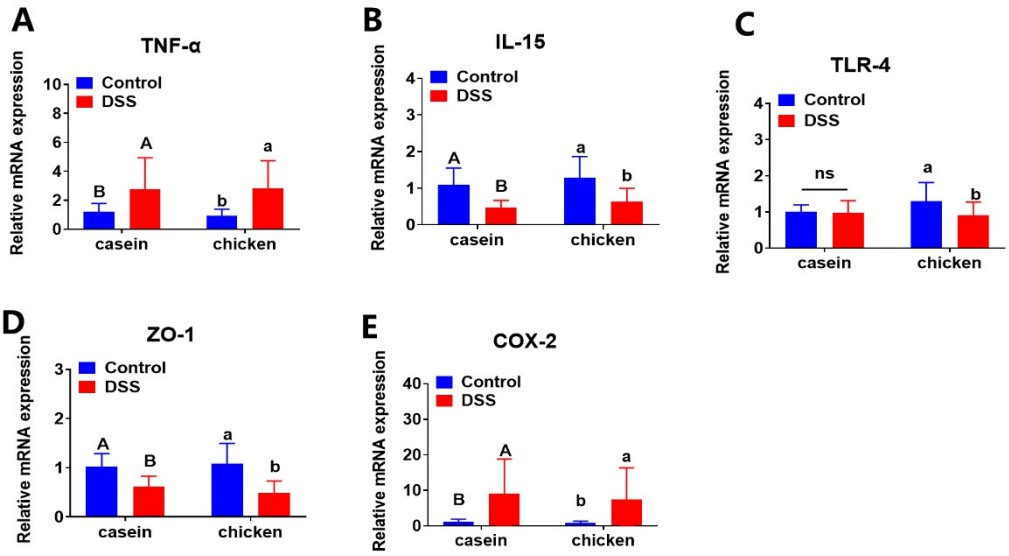

Figure S2. The gut microbiota composition at phylum level on day 21.

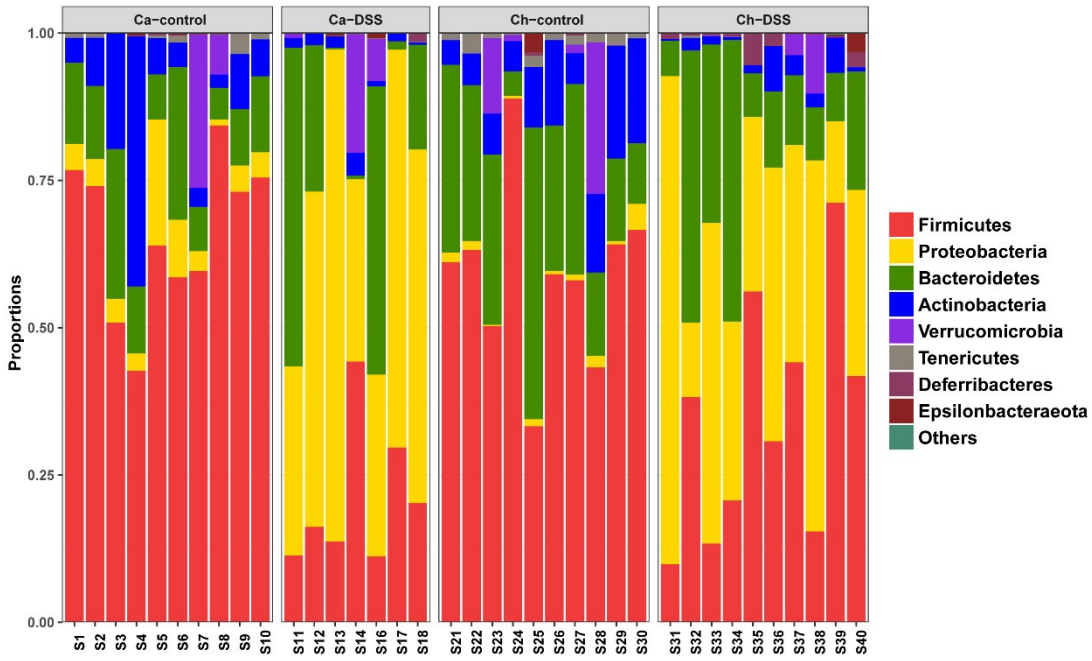

Figure S3. A Venn diagram showing the overlap of the OTUs identified in the gut microbiota among six groups on day 27.