

Supplementary information

Exploring metabolic effects of dipeptide feed media on CHO cell cultures by *in silico* model-guided flux analysis

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Table S1 Coefficients and coefficients-sum during the post-feeding phase (days 4-14) from BLM-PLS analysis.

Coefficient	Day 4	Day 5	Day 6	Day 7	Day 8	Day 10	Day 12	Day 14	Coef. sum
Glu	0.009354	0.010637	0.009046	-0.011671	-0.009962	-0.010962	-0.011601	-0.012961	-0.0571
Leu	0.002228	-0.006680	0.004521	-0.011397	-0.013037	-0.008309	-0.009883	-0.013420	-0.0560
Val	0.003094	-0.007485	0.000744	-0.011577	-0.007720	-0.008549	-0.009359	-0.013157	-0.0503
Thr	0.003276	-0.006324	0.001176	-0.012714	-0.006737	-0.007501	-0.008352	-0.012431	-0.0477
Asp	0.012441	0.001422	0.00790	-0.012541	-0.006546	-0.010657	-0.013175	-0.016756	-0.0472
Ile	0.002040	-0.007119	0.003908	-0.009385	-0.006702	-0.008277	-0.009330	-0.012931	-0.0466
Trp	-0.007596	-0.006408	0.001433	-0.011955	-0.011038	-0.006738	-0.006627	-0.009567	-0.0459
Lys	0.006545	-0.007107	0.002341	-0.012738	-0.006151	-0.006797	-0.007841	-0.012379	-0.0459
Orn	0.005827	-0.007293	0.003758	-0.010768	-0.017803	-0.014411	-0.009511	-0.013375	-0.0455
Phe	-0.00606	-0.008396	-0.004011	-0.011329	-0.005672	-0.007838	-0.007815	-0.011579	-0.0442
Ser	0.005437	-0.005430	-0.000733	-0.011886	-0.008511	-0.010789	-0.007999	-0.012785	-0.0439
Met	0.000152	-0.008198	-0.005655	-0.010384	-0.006008	-0.007635	-0.007603	-0.012241	-0.0438
His	-0.001931	-0.006983	-0.005486	-0.009993	-0.005176	-0.007648	-0.008367	-0.012364	-0.0435
Ala	-0.001537	-0.010792	-0.007174	-0.009946	-0.011502	-0.010382	-0.013891	-0.012619	-0.0368
Arg	-0.001330	-0.008577	-0.005695	-0.006205	-0.004988	-0.007355	-0.005897	-0.011215	-0.0356
NH4	0.009966	0.011917	0.012620	-0.008455	-0.005247	-0.005994	-0.009323	-0.006553	-0.0355
Tau	-0.002099	-0.002128	0.006143	-0.000594	-0.006557	-0.001327	-0.010686	-0.016453	-0.0342
Pro	0.000251	-0.009223	-0.003321	-0.007633	-0.005685	-0.006942	-0.005956	-0.011729	-0.0319
Cys	0.014034	0.006436	0.009781	0.009111	0.005964	-0.013371	-0.011315	-0.013460	-0.0241
Gln	0.003793	0.003733	0.015242	-0.009210	-0.011267	-0.005982	-0.004774	-0.006475	-0.0224
Asn	0.008428	-0.001657	0.010767	0.002095	0.005683	0.000166	-0.004767	-0.014241	-0.0190
Lac	-0.002584	-0.005693	-0.003846	0.002147	-0.004641	-0.007501	-0.012015	-0.018766	-0.0187

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Table S2 Metabolites with VIP scores exceeding 1.00 during the post-feeding phase (days 4-14) based on BLM-PLS analysis.

VIP score	Day 4	Day 5	Day 6	Day 7	Day 8	Day 10	Day 12	Day 14
Glu	0.7161	0.7641	0.6380	1.0540	1.3001	1.3862	1.4109	1.4615
Leu	0.5640	0.4778	0.3755	1.3467	1.3490	1.2708	1.3476	1.4686
Val	0.6335	0.5423	0.3132	1.3174	1.2484	1.2853	1.3067	1.4416
Thr	0.7120	0.4844	0.4956	1.4140	1.2080	1.2408	1.2734	1.4202
Asp	1.0009	0.1022	0.5848	1.1445	1.1888	1.3739	1.4134	1.5271
Ile	0.5649	0.5073	0.3221	1.2511	1.2069	1.2733	1.3222	1.4512
Trp	0.6589	0.4754	0.3982	1.3883	1.3052	1.1898	1.2220	1.3022
Lys	0.9030	0.5461	0.6279	1.3909	1.1761	1.2222	1.2398	1.4158
Orn	0.9007	0.5565	0.6232	0.8060	1.4448	1.2685	0.7663	1.1655
Phe	0.4420	0.5972	0.4260	1.3314	1.1662	1.2346	1.2622	1.3693
Ser	0.6369	0.3856	0.2035	1.2428	1.1895	1.2852	0.9002	1.2568
Met	0.4095	0.5821	0.4000	1.2800	1.1743	1.2314	1.1985	1.3448
His	0.3731	0.4991	0.4077	1.2203	1.1471	1.2248	1.2524	1.3733
Ala	0.6409	0.7917	0.7105	0.7062	0.8324	1.1591	1.380	1.3422
Arg	0.5020	0.6081	0.4082	1.0587	1.1229	1.1968	1.0555	1.2685
NH4	0.7028	0.8565	0.8979	1.1672	1.1376	1.1752	1.3104	1.1003
Tau	0.2683	0.2738	0.5363	1.0279	1.1831	0.9496	1.1615	1.4508
Pro	0.4914	0.6523	0.2638	0.9966	1.1369	1.1824	0.9847	1.2548
Cys	1.0868	0.5302	0.9863	0.9775	0.7051	1.2706	1.4020	1.4659
Gln	0.8203	0.7097	1.0972	1.3004	1.3802	1.1742	1.1059	1.1861
Asn	0.6413	0.2391	0.9282	0.5788	0.751	0.9524	1.0263	1.5039
Lac	0.4912	0.5080	0.3917	0.8997	0.7097	0.7844	0.8883	1.3323

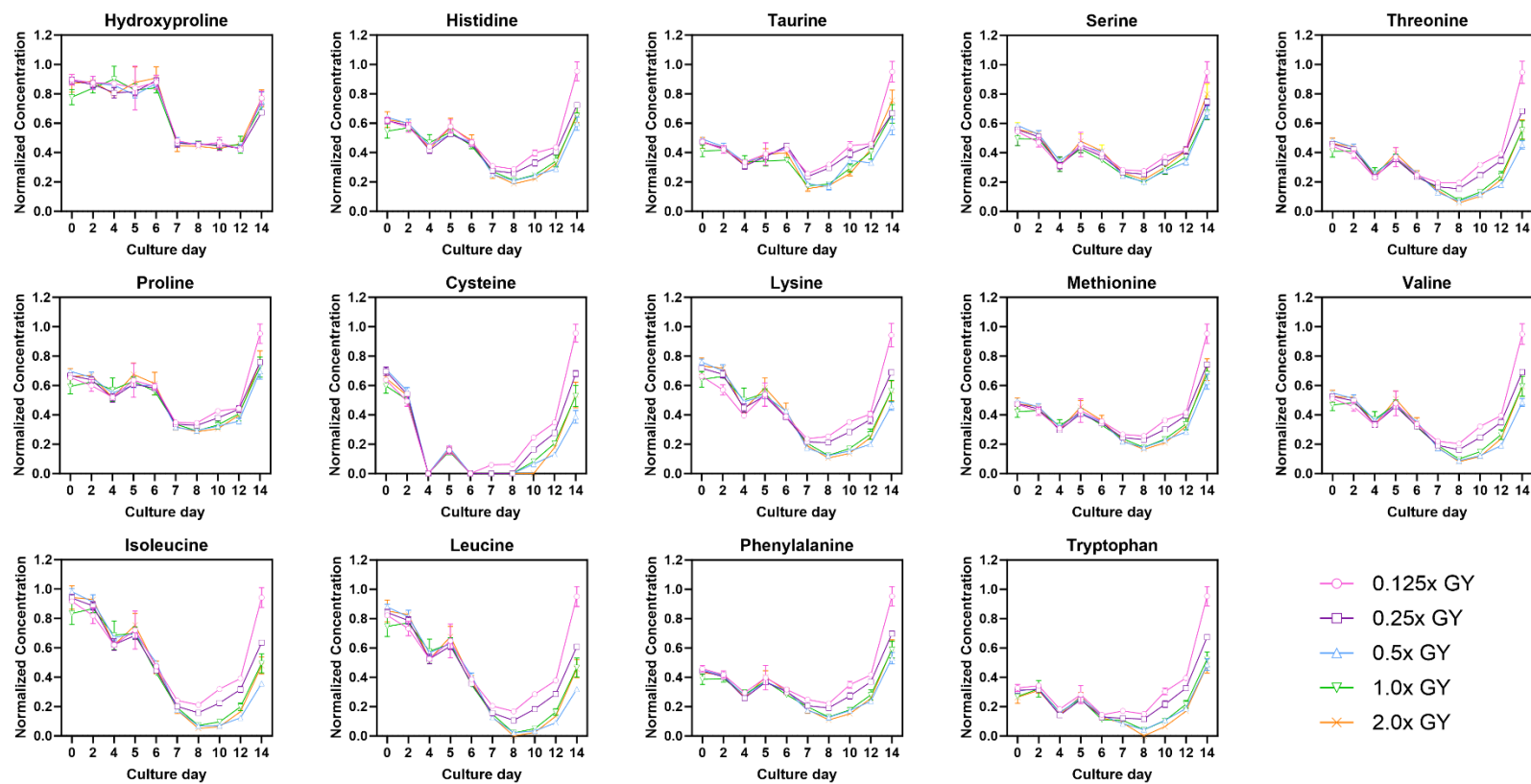


Fig. S1 Profiles of residual concentration of amino acids not shown in Fig. 2.

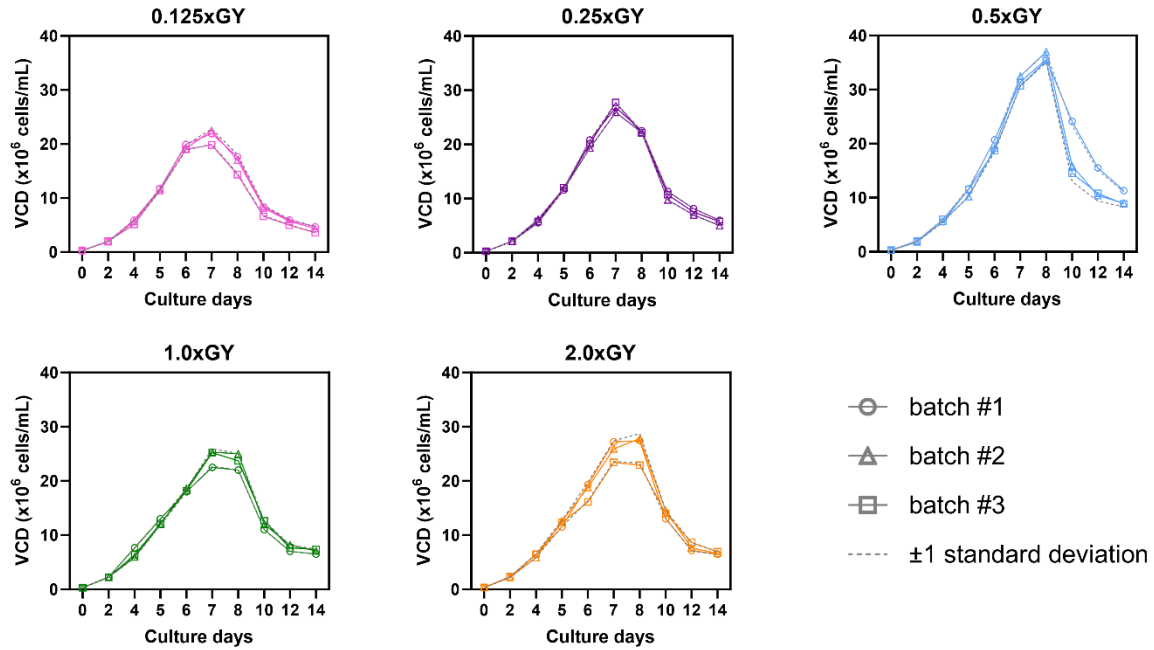


Fig. S2 Identification of outlier batch based on the evaluation of VCD profiles from each GY concentration condition's batch dataset.

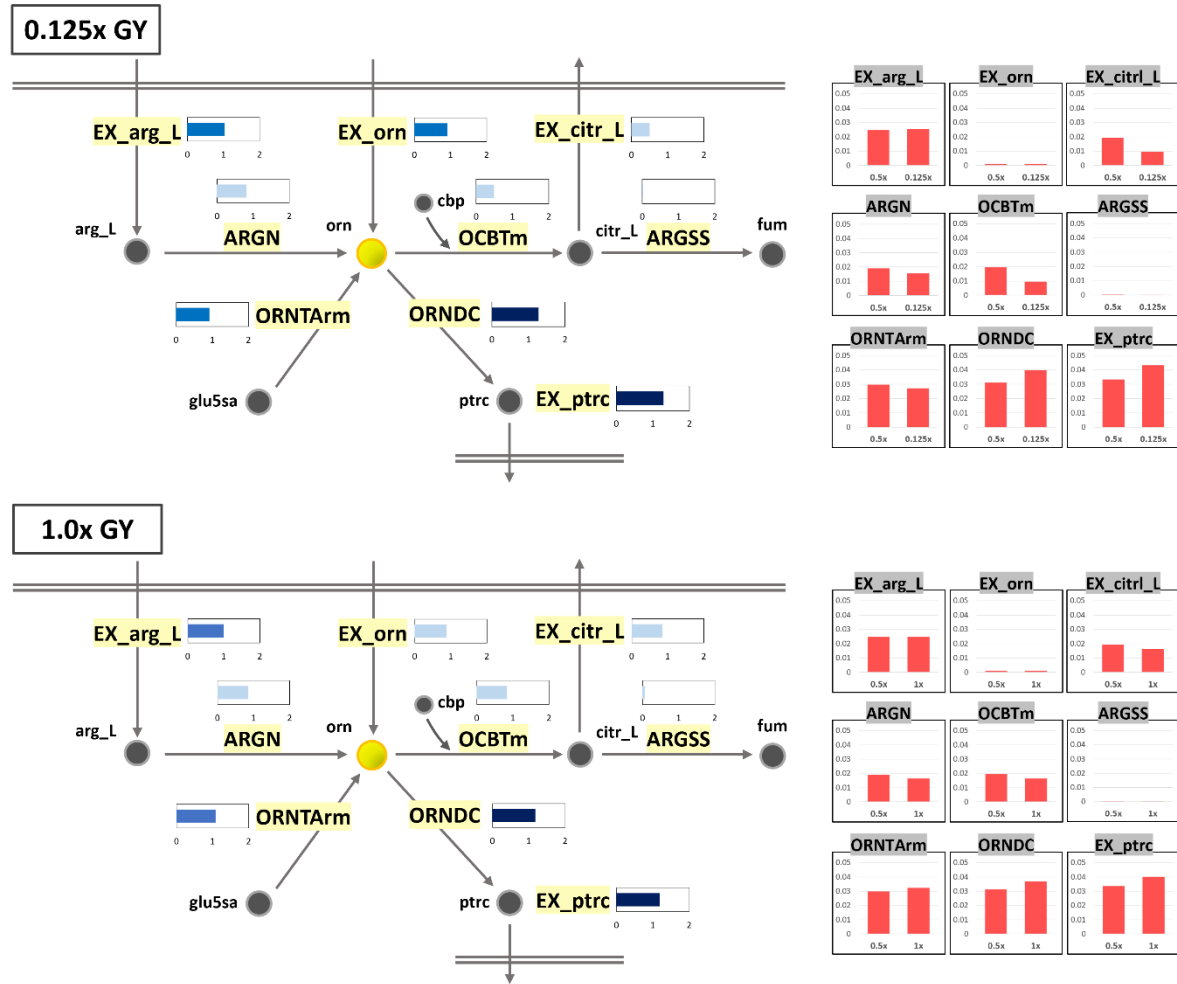


Fig. S3 Metabolic flux comparison of the optimum (0.5×) GY condition with lower (0.125×) and higher (1.0×) GY conditions in ornithine (orn) pathway.

Abbreviation Full name of metabolites and reactions for Fig. 4.

Abbreviation for metabolite listed alphabetically:

2mop, 2-Methyl-3-oxopropanoate; **34hpp**, 3-(4-Hydroxyphenyl)pyruvate; **3aib**, L-3-Amino-isobutanoate; **3aib_D**, D-3-Amino-isobutanoate; **3mob**, 3-Methyl-2-oxobutanoate; **3mop**, (S)-3-Methyl-2-oxopentanoate; **3pg**, 3-Phospho-D-glycerate; **3php**, 3-Phosphohydroxypyruvate; **4abut**, 4-Aminobutanoate; **4fumacac**, 4-Fumarylacetoacetate; **4mop**, 4-Methyl-2-oxopentanoate; **accoa**, Acetyl-CoA; **akg**, 2-Oxoglutarate; **ala_L**, L-Alanine; **asn_L**, L-Asparagine; **asp_L**, L-Aspartate; **cbasp**, N-Carbamoyl-L-aspartate; **cit**, Citrate; **f6p**, D-Fructose 6-phosphate; **fdp**, D-Fructose 1,6-bisphosphate; **fum**, Fumarate; **g3p**, Glyceraldehyde 3-phosphate; **g6p**, D-Glucose 6-phosphate; **glc_D**, D-Glucose; **gln_L**, L-Glutamine; **glu_L**, L-Glutamate; **glu5sa**, L-Glutamate 5-semialdehyde; **ile_L**, L-Isoleucine; **lac_L**, L-Lactate; **leu_L**, L-Leucine; **mal_L**, L-Malate; **malcoa**, Malonyl CoA; **mmcoa_R**, (R)-Methylmalonyl-CoA; **oaa**, Oxaloacetate; **orn**, Ornithine; **pep**, Phosphoenolpyruvate; **phe_L**, L-Phenylalanine; **phpyr**, Phenylpyruvate; **pser_L**, O-Phospho-L-serine; **pyr**, Pyruvate; **succ**, Succinate; **succoa**, Succinyl-CoA; **sucsal**, Succinic semialdehyde; **tyr_L**, L-Tyrosine; **val_L**, L-Valine;

Abbreviation for reactions listed alphabetically:

3AIBTm, L-3-aminoisobutyrate transaminase (mitochondrial); **AAcTA**, Amino acid transaminase; **ACCOAC**, Acetyl-CoA carboxylase; **ACONT**, Aconitate hydratase; **AIBAKGAT_r**, (R)-3-Amino-2-methylpropanoate; **AKGD**, 2-oxoglutarate dehydrogenase; **ALATA**, L-alanine transaminase; **ALDD19y**, Aldehyde dehydrogenase-NADP; **ASNN**, L-asparaginase; **ASPCTr**, Aspartate carbamoyl transferase; **ASPTA**, Aspartate transaminase; **CSm/ACITL**, Citrate synthase (mitochondria)/ATP-Citrate lyase; **DLD**, Dihydrolipoamide dehydrogenase; **EX_4abut(e)**, 4-Aminobutanoate exchange; **EX_glc(e)_r**, D-glucose exchange; **Ex_glu_L(e)_r**, L-glutamate exchange; **EX_pyr(e)**, Pyruvate exchange; **FBA**, Fructose-bisphosphate aldolase; **FUM**, Fumarase; **FUMAC**, Fumarylacetoacetase; **G6PDH**, Glucose 6-phosphate dehydrogenase; **GAPD+PGK_r**, Glyceraldehyde-3-phosphate dehydrogenase, Phosphoglycerate kinase (lumped); **GLNS**, Glutamine synthetase; **GLUD**, Glutamate dehydrogenase; **GLUDC**, Glutamate decarboxylase; **GND**, Phosphogluconate dehydrogenase; **GTHOxm**, Glutathione NAD⁺ oxidoreductase (mitochondrial); **HEX/ADPGK**, Hexokinase/ADP-dependent glucokinase; **ILETA**, Isoleucine transaminase; **LDH_Lm**, Lactate dehydrogenase (mitochondria); **LDH_net**, Lactate dehydrogenase (net reaction); **LEUTA**, Leucine transaminase; **MDH**, Malate dehydrogenase; **MMMm**, Methylmalonyl-CoA mutase (mitochondria); **ORNTArm_r**, Ornithine transaminase reversible (mitochondria); **PCm**, Pyruvate carboxylase (mitochondria); **PDH**, Pyruvate carboxylase (mitochondria); **PEPCK**, Phosphoenolpyruvate carboxykinase; **PFK**, Phosphofructokinase; **PGI**, Glucose-6-phosphate isomerase; **PGM_r+ENO**, Phosphoglycerate mutase, Enolase (lumped); **PHETA**, Phenylalanine transaminase; **PSERT**, Phosphoserine transaminase; **PYK**, Pyruvate kinase; **RADH4**, Retinal dehydrogenase; **SSAL**, Succinate-semialdehyde dehydrogenase reversible (mitochondrial); **SUC_net**, Succinate-CoA ligase (GDP-forming); **SUCD1m**, Succinate dehydrogenase (mitochondria); **TYRTA**, Tyrosine transaminase; **VALTA**, Valine transaminase;