

Supplementary Information

A Proteomic Approach to Investigating the Antioxidant Properties of Passion Fruit Seed Oil on Broiler Liver under Cyclic Heat Stress

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Table S1. Percentage and nutritional composition calculated from pre-initial (1 to 7 days of age), initial (8 to 21 days of age), growth (22 to 33 days of age) and final (34 to 42 days of age) experimental diets.

Ingredient	Pre-initial					Initial				
	0% PFSO	0.3% PFSO	0.5% PFSO	0.7% PFSO	0.9% PFSO	0% PFSO	0.3% PFSO	0.5% PFSO	0.7% PFSO	0.9% PFSO
Corn	54.52	54.55	54.58	54.60	54.63	56.26	56.30	56.32	56.34	56.37
Soybean meal, 46%	36.00	36.00	36.00	36.00	36.00	34.00	34.00	34.00	34.00	34.00
Soy oil	1.057	0.725	0.503	0.282	0.060	1.899	1.567	1.346	1.124	0.903
Offal meal	4.766	4.761	4.757	4.754	4.751	4.487	4.482	4.479	4.476	4.472
Dicalcium phosphate	1.263	1.263	1.264	1.264	1.265	1.080	1.081	1.081	1.082	1.082
Limestone	0.843	0.843	0.843	0.844	0.844	0.765	0.765	0.765	0.765	0.765
Sodium bicarbonate	0.056	0.056	0.056	0.056	0.056	0.035	0.035	0.035	0.035	0.035
PFSO	0.000	0.300	0.500	0.700	0.900	0.000	0.300	0.500	0.700	0.900
DL-Methionine 99%	0.367	0.367	0.367	0.367	0.366	0.350	0.350	0.350	0.350	0.350
L-Lysine HCL 99%	0.227	0.227	0.227	0.227	0.227	0.233	0.233	0.233	0.233	0.233
L-Threonine 98.5%	0.094	0.094	0.094	0.094	0.094	0.092	0.092	0.092	0.092	0.092
Anticoccidial ^a	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055
Hill	0.105	0.105	0.105	0.105	0.105	0.095	0.095	0.095	0.095	0.095
Premix vitamin ^b	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Premix mineral ^c	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Common salt	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450	0.450
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional composition										
ME / (kcal kg ⁻¹)	2975	2975	2975	2975	2975	3050	3050	3050	3050	3050
Crude protein / %	24.27	24.27	24.27	24.27	24.27	23.31	23.31	23.31	23.31	23.31
Calcium / %	0.971	0.971	0.971	0.971	0.971	0.878	0.878	0.878	0.878	0.878
Phosphorus available / %	0.463	0.463	0.463	0.463	0.463	0.419	0.419	0.419	0.419	0.419
Potassium / %	0.597	0.597	0.597	0.597	0.597	0.598	0.598	0.598	0.598	0.598
Sodium / %	0.225	0.225	0.225	0.225	0.225	0.218	0.218	0.218	0.218	0.218
Chlorine / %	0.202	0.202	0.202	0.202	0.202	0.194	0.194	0.194	0.194	0.194
Lysine dig. / %	1.441	1.441	1.441	1.441	1.441	1.384	1.384	1.384	1.384	1.384

Methionine / %	0.591	0.591	0.591	0.591	0.591	0.567	0.567	0.567	0.567	0.567
Met + Cys dig. / %	1.066	1.066	1.066	1.066	1.066	1.024	1.024	1.024	1.024	1.024
Tryptophan / %	0.259	0.259	0.259	0.259	0.259	0.249	0.249	0.249	0.249	0.249
Threonine / %	0.994	0.994	0.994	0.994	0.994	0.955	0.955	0.955	0.955	0.955

Ingredient	Growth					Final				
	0% PFSO	0.3% PFSO	0.5% PFSO	0.7% PFSO	0.9% PFSO	0% PFSO	0.3% PFSO	0.5% PFSO	0.7% PFSO	0,9% PFSO
Corn	61.43	61.47	61.50	61.52	61.54	66.89	66.93	66.96	66.99	67.01
Soybean meal, 46%	30.00	30.00	30.00	30.00	30.00	25.80	25.79	25.78	25.78	25.78
Soy oil	2.973	2.640	2.419	2.198	1.976	2.916	2.583	2.360	2.138	1.916
Offal meal	2.273	2.268	2.265	2.261	2.258	1.462	1.461	1.461	1.460	1.460
Dicalcium phosphate	1.170	1.170	1.171	1.171	1.171	0.890	0.890	0.890	0.890	0,890
Limestone	0.678	0.678	0.678	0.678	0.678	0.660	0.660	0.661	0.661	0.661
Sodium bicarbonate	0.031	0.031	0.031	0.031	0.031	0.150	0.150	0.150	0.150	0.150
PFSO	0.000	0.300	0.500	0.700	0.900	0.000	0.300	0.500	0.700	0.900
DL-Methionine 99%	0.314	0.314	0.314	0.314	0.314	0.275	0.275	0.275	0.275	0.275
L-Lysine HCL 99%	0.257	0.257	0.257	0.257	0.257	0.271	0.271	0.271	0.272	0.272
L-Threonine 98.5%	0.091	0.091	0.091	0.091	0.091	0.082	0.082	0.082	0.082	0.082
Anticoccidial ^a	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055
Hill	0.075	0.075	0.075	0.075	0.075	—	—	—	—	—
Premix vitamin ^b	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0,100
Premix mineral ^c	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Common salt	0.450	0.450	0.450	0.450	0.450	0.350	0.350	0.350	0.350	0.350
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Calculated nutritional composition										
ME / (kcal kg ⁻¹)	3150	3150	3150	3150	3150	3200	3200	3200	3200	3200
Crude protein / %	20.58	20.58	20.58	20.58	20.58	18.57	18.57	18.57	18.57	18.57
Calcium / %	0.758	0.758	0.758	0.758	0.758	0.634	0.634	0.634	0.634	0.634
Phosphorus available / %	0.374	0.374	0.374	0.374	0.374	0.296	0.296	0.296	0.296	0.296

Potassium / %	0.599	0.599	0.599	0.599	0.599	0.593	0.593	0.593	0.593	0.593
Sodium / %	0.208	0.208	0.208	0.208	0.208	0.197	0.197	0.197	0.197	0.197
Chlorine / %	0.183	0.183	0.183	0.183	0.183	0.172	0.172	0.172	0.172	0.172
Lysine dig. / %	1.239	1.239	1.239	1.239	1.239	1.118	1.118	1.118	1.118	1.118
Methionine / %	0.508	0.508	0.508	0.508	0.508	0.458	0.458	0.458	0.458	0.458
Met + Cys dig. / %	0.917	0.917	0.917	0.917	0.917	0.827	0.827	0.827	0.827	0,827
Tryptophan / %	0.223	0.223	0.223	0.223	0.223	0.201	0.201	0.201	0.201	0.201
Threonine / %	0.855	0.855	0.855	0.855	0.855	0.771	0.771	0.771	0.771	0.771

PFSO: passion fruit seed oil; dig: digestible; ME: metabolizable energy; ^asalinomycin (66 ppm); ^bvitamin premix (guaranteed levels *per* kg of feed): vitamin A (min) 10.000 I.U.; vitamin D3 (min); 2.500 I.U.; vitamin E (min) 17.5 I.U.; vitamin K3 (min) 2 mg; vitamin B1 (min) 1.5 mg; vitamin B2 (min) 7.5 mg; niacin (min) 25 mg; pantathenic acid 15 mg; vitamin B6 (min) 2 mg; folic acid (min) 0.5 mg; biotin (min) 60 mcg; vitamin B12 (min) 15 mcg; ^cmineral premix (guaranteed levels *per* kg of feed): iron (min) 30 mg; copper (min) 6 mg; manganese (min) 70 mg; zinc (min) 50 mg; iodine (min) 1 mg; selenium (min) 0.35 mg; cobalt (min) 0.2 mg.

Table S2. Proteins identified in the liver of broilers supplemented with passion fruit seed oil under cyclic heat stress by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Protein access	Gene symbol	Protein	pI/MW / Da	Score
P07341	ALDOB	fructose-bisphosphate aldolase B	8.81/39295.78	1746.25
P00368	GLUD1	glutamate dehydrogenase 1, mitochondrial	8.48/55712.06	380.1942
P31335	ATIC	bifunctional purine biosynthesis protein ATIC	8.40/64414.82	112.1429
P00356	GAPDH	glyceraldehyde-3-phosphate dehydrogenase	8.70/35703.97	65.1689
Q8JFP1	EIF4A2	eukaryotic initiation factor 4A-II	5.33/46371.31	4602.994
Q5ZMQ2	ACTG1	actin, cytoplasmic 2	5.31/41792.84	1221.098
P53478	N/A	actin, cytoplasmic type 5	5.30/41835.87	1221.098
P60706	ACTB	actin, cytoplasmic 1	5.29/41736.73	1221.098
Q5ZM36	EIF4A3	eukaryotic initiation factor 4A-III	6.30/46815.87	500.7937
P51913	ENO1	alpha-enolase	6.17/47305.01	257.0101
P68139	ACTA1	actin, alpha skeletal muscle	5.23/42051.03	248.5884
P68034	ACTC1	actin, alpha cardiac muscle 1	5.23/42018.97	248.5884
P63270	ACTG2	actin, gamma-enteric smooth muscle	5.31/41876.88	248.5884
P08023	ACTA2	actin, aortic smooth muscle	5.24/41994.92	248.5884
P07322	ENO3	beta-enolase	7.28/47196.08	170.7394
O57391	ENO2	gamma-enolase	4.84/47308.46	87.3216
P19121	ALB	albumin	5.51/69918.19	65.6484
P02552	N/A	tubulin alpha-1 chain	4.96/45900.72	5492.072
Q5ZL72	HSPD1	60 kDa heat shock protein, mitochondrial	5.72/60972.56	3652.898
P09642	N/A	tubulin alpha-3 chain	5.19/36087.83	3589.188
P09644	N/A	tubulin alpha-5 chain	4.94/49948.42	3579.418
P09645	N/A	tubulin alpha-8 chain	4.82/36160.94	3046.951
P08070	N/A	tubulin alpha-2 chain	4.86/49798.11	2571.445
P09643	N/A	tubulin alpha-4 chain	4.76/35935.60	1911.837
P80566	SOD1	superoxide dismutase [Cu-Zn]	6.10/15703.60	2155.948
P00940	TPI1	triosephosphate isomerase	6.71/26620.45	179.4643
Q5ZM98	HSPA9	stress-70 protein, mitochondrial	6.09/73192.32	5425.022
O73885	HSPA8	heat shock cognate 71 kDa protein	5.46/70826.98	430.4864
P08106	N/A	heat shock 70 kDa protein	5.52/69750.82	332.263
Q90593	HSPA5	endoplasmic reticulum chaperone BiP	5.12/72018.51	95.1078
P02789	N/A	ovotransferrin	6.85/77776.53	3700.308
		phosphoenolpyruvate carboxykinase [GTP],		
P21642	PCK2	mitochondrial	7.56/71106.29	491.4611
		trifunctional purine biosynthetic protein		
P21872	GART	adenosine-3	7.51/106544.04	120.8111
Q5ZK03	SEC23A	protein transport protein Sec23A	6.34/86303.91	62.4248

P00508	GOT2	aspartate aminotransferase, mitochondrial	9.39/47241.27	566.9792
Q5ZLN1	PGAM1	phosphoglycerate mutase 1	7.02/28898.13	244.7894
P12276	FASN	fatty acid synthase	5.94/274781.57	292.6981
P01994	HBAA	hemoglobin subunit alpha-A	8.54/15428.87	135.4075
P23228	HMGCS1	hydroxymethylglutaryl-CoA synthase, cytoplasmic	5.41/57559.06	95.884
P80226	FABP1	fatty acid-binding protein, liver	8.67/14210.37	465.0665
P16580	GLUL	glutamine synthetase	6.38/42146.46	87.6059
P08629	TXN	thioredoxin	5.10/11700.52	504.1598
P20136	GSTM2	glutathione S-transferase 2	6.84/25892.82	100.2498
P09102	P4HB	protein disulfide-isomerase	4.69/57409.80	78.7594
P02112	HBB	hemoglobin subunit beta	8.84/16466.13	2338.827
P02001	HBAD	hemoglobin subunit alpha-D	7.02/15694.93	473.2623
P02127	N/A	hemoglobin subunit rho	8.78/16589.21	434.5012
P02128	HBE	hemoglobin subunit epsilon	9.07/16603.23	434.5012
P02007	N/A	hemoglobin subunit pi	7.81/15709.04	171.6481
P70081	H4-VIII	histone H4 type VIII	11.20/11439.41	67.8861
P62801	H4-I	histone H4	11.36/11367.34	67.8861
P00337	LDHB	L-lactate dehydrogenase B chain	7.07/36318.09	783.1637
P00378	DHFR	dihydrofolate reductase	7.93/21649.94	148.6893
Q5ZMT0	YWHAЕ	14-3-3 protein epsilon	4.63/29173.90	313.7717
P0DMQ6	SORD	sorbitol dehydrogenase	7.09/38144.20	126.1308
P0CB50	PRDX1	peroxiredoxin-1	8.24/22314.71	121.2862
Q5ZI49	PRPS2	ribose-phosphate pyrophosphokinase 2	6.37/35663.21	1358.28
Q90705	EEF2	elongation factor 2	6.40/95378.24	204.0252
Q5F3X4	EFTUD2	116 kDa U5 small nuclear ribonucleoprotein component	4.85/109477.84	98.9918
Q5ZJF4	PRDX6	peroxiredoxin-6	5.70/24976.79	1684.907
P07630	CA2	carbonic anhydrase 2	6.56/29007.75	90.239
P25324	TST	thiosulfate sulfurtransferase	6.56/32286.51	230.6912
Q5ZKC9	YWHAZ	14-3-3 protein zeta	4.73/27775.13	12351.38
Q5ZLQ6	YWHAB	14-3-3 protein beta/alpha	4.76/27907.15	6195.533
Q5F3W6	YWHAG	14-3-3 protein gamma	4.84/28230.53	5124.856
Q5ZMD1	YWHAQ	14-3-3 protein theta	4.68/27782.28	4262.729
Q9YGGQ1	EEF1B	elongation factor 1-beta	4.54/24761.79	1207.509
P51903	PGK	phosphoglycerate kinase	8.31/44716.87	4811.904
P07850	SUOX	sulfite oxidase	5.80/50204.65	266.6363
Q91348	N/A	6-phosphofructo-2-kinase/fructose-2,6- biphosphatase	6.82/54404.06	215.5867
Q9YH58	FTCD	formimidoyltransferase-cyclodeaminase	5.87/59153.55	8051.222

Q5ZJK8	CCT7	T-complex protein 1 subunit eta	5.96/60348.58	255.709
P00340	LDHA	L-lactate dehydrogenase A chain	7.75/36514.46	1018.506
O73888	HPGDS	hematopoietic prostaglandin D synthase	6.74/22730.34	126.345
P23007	CS	citrate synthase, mitochondrial	8.31/47378.25	70.1806
P20137	N/A	glutathione S-transferase 5	9.88/3388.92	60.0913
P26697	N/A	glutathione S-transferase 3	9.06/26325.84	113.3562
Q5ZLF0	ST13	Hsc70-interacting protein	5.07/40158.30	79.786
Q08392	N/A	glutathione S-transferase	8.86/25298.52	111.4719
Q90835	EEF1A	elongation factor 1-alpha 1	9.10/50156.86	519.8495
P00504	GOT1	aspartate aminotransferase, cytoplasmic	8.22/45935.27	332.9474
P38024	AIRC	multifunctional protein ADE2	8.18/47240.32	83.3754
P08836	FDPS	farnesyl pyrophosphate synthase	6.16/42153.36	3869.996
P19179	PLS1	plastin-1	5.47/70939.12	455.8107
Q6EE31	CCT8	T-complex protein 1 subunit theta	5.35/59485.49	143.1289
O93277	WDR1	WD repeat-containing protein 1	6.19/66566.90	968.3096
Q5ZJ08	YARS1	tyrosine--tRNA ligase, cytoplasmic	6.24/59284.33	332.9967
P51901	ANXA6	annexin A6	5.41/75218.53	908.1689
P08110	HSP90B1	endoplasmic	4.83/91555.02	4411.261
Q04619	HSP90AB1	heat shock cognate protein HSP 90-beta	4.94/83427.45	209.4907
P11501	HSP90AA1	heat shock protein HSP 90-alpha	5.00/84059.22	92.5225
Q05744	CTSD	cathepsin D	5.90/43298.06	161.7098
P08250	APOA1	apolipoprotein A-I	5.59/30680.16	234.8511
Q8UW59	PARK7	protein/nucleic acid deglycase DJ-1 hydroxyacylglutathione hydrolase,	6.32/19943.16	110.1111
Q5ZI23	HAGH	mitochondrial	6.42/29031.14	219.1373
Q8JG64	PDIA3	protein disulfide-isomerase A3	5.76/56181.64	104.3574
O57601	HMX3	homeobox protein HMX3	8.71/33787.01	79.9531
P81628	ERP29	endoplasmic reticulum resident protein 29	7.66/25373.25	172.6228
P28337	AMT	aminomethyltransferase, mitochondrial	9.12/42058.37	181.8587
Q5ZM25	OLA1	obg-like ATPase 1	8.10/44778.59	82.2543
Q08393	N/A	glutathione S-transferase	8.76/25413.73	3323.991
Q9IAY5	NUDT16L1	tudor-interacting repair regulator protein	5.73/33296.38	85.9432
Q5ZKJ4	FGFR1OP2	FGFR1 oncogene partner 2 homolog	5.96/25197.83	107.5338
Q5ZJL5	ADSS2	adenylosuccinate synthetase isozyme 2	5.93/49454.85	61.3957
Q9I9K9	GATM	glycine amidinotransferase, mitochondrial	8.24/48311.33	372.0159
P27463	ALDH1A1	retinal dehydrogenase 1	7.49/55809.33	322.827
P21265	ADSL	adenylosuccinate lyase	6.69/54652.97	275.7029
O93344	ALDH1A2	retinal dehydrogenase 2	5.87/56731.99	145.75
Q5ZK14	DDA1	DET1- and DDB1-associated protein 1	8.73/11884.31	111.6822

Q9I923	RGN	regucalcin	5.77/33229.57	599.7192
Q5ZME2	MDH1	malate dehydrogenase, cytoplasmic	6.92/36543.40	157.6787
P00565	CKM	creatine kinase M-type	6.50/43328.41	123.1646
Q5ZHZ0	DDX39B	spliceosome RNA helicase DDX39B	5.45/49003.34	151.0137
P23991	ADH1	alcohol dehydrogenase 1	8.69/39807.50	81.5808
Q5ZMA2	PRPF19	pre-mRNA-processing factor 19	6.19/55134.63	69.3062
P09244	N/A	tubulin beta-7 chain	4.78/49670.82	1123.167
P09206	N/A	tubulin beta-3 chain	4.77/49860.99	1011.942
P09652	N/A	tubulin beta-4 chain	4.86/50420.67	948.0264
P09203	N/A	tubulin beta-1 chain	4.78/49909.01	881.7851
P32882	N/A	tubulin beta-2 chain	4.78/49953.06	881.7851
P09653	N/A	tubulin beta-5 chain	4.77/49971.25	788.6357
P09207	N/A	tubulin beta-6 chain	4.96/50153.64	506.2223
O42265	PSMA1	proteasome subunit alpha type-1	6.08/28925.06	2229.996
O73930	PCBD1	pterin-4-alpha-carbinolamine dehydratase	6.04/11997.53	800.0145
P05122	CKB	creatine kinase B-type	5.92/42870.75	231.2479
Q90734	ACTN4	alpha-actinin-4	5.13/104207.29	2364.672
P05094	ACTN1	alpha-actinin-1	5.23/103161.53	699.2343
P20111	ACTN2	alpha-actinin-2	5.26/104275.09	299.6845
Q5ZJ54	CCT6	T-complex protein 1 subunit zeta	6.36/57644.65	204.9525

N/A: not applicable; pI: isoelectric point; MW: molecular weight.

