Supplementary Material

Table S1 Dietary composition and nutrient levels of experimental diets of laying hens.

Ingredient	SM diet	RM diet
Corn (%)	63.00	58.43
Soybean meal (%)	24.85	6.95
Rapeseed meal (%)[1]	0	21.00
Soybean oil (%)	0	2.38
Dicalcium phosphate (%)	1.25	1.06
Limestone (%)	9.45	9.36
Salt (%)	0.30	0.30
DL-Methionine (%)	0.08	0.04
L-Lysine hydrochloride (%)	0	0.11
Vitamin and mineral premix (%)[2]	0.37	0.37
Zeolite powder (%)	0.70	0
Total (%)	100.00	100.00
Nutrient level		
Gross energy (kcal/kg)[3]	3573.9	3657.6
Apparent metabolizable energy (kcal/kg)[4]	2601.3	2601.3
Crude protein (%)[3]	16.02	16.02
Calcium (%)[3]	3.82	3.81
Nonphytate phosphorus (%)[4]	0.32	0.32
Lysine (%)[4]	0.81	0.84
Methionine (%)[4]	0.33	0.33
Choline (%)[3]	0.11	0.12
Sinapine (mg/g)[3]	n.d.[5]	1.33

[1]The rapeseed meal used in this study contained 6.6 mg/g sinapine and 0.34 mg/g vinyloxazolidine thiones.

[2]Provided per kilogram of diet: Vitamin A ,12500 IU; Vitamin D_3, 4125 IU; Vitamin E, 5 IU; Vitamin K, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; calcium pantothenate, 50 mg; niacin, 32.5 mg; pyridoxine, 8 mg; biotin, 2 mg; folic acid, 5 mg; Vitamin B_{12}, 5 mg; Mn, 65 mg; I, 1 mg; Fe, 60 mg; Cu, 8 mg; Zn, 66 mg; Se, 0.3 mg; choline chloride, 500 mg.

[3]Determined values. Gross energy were determined using an Automatic Adiabatic Oxygen Bomb Calorimeter (Parr 6300 Calorimeter, Moline, IL). Crude protein (No 954.01) and calcium (No 927.02) were determined according to the Association of Official Analytical Chemists (AOAC, 2005). Choline content in the diets was determined by the ion chromatography method. Sinapine content in the diets was determined by high-performance liquid chromatography.

[4]Calculated values.

[5]n.d. not detectable. Sinapine contents in SM diets is below the detection limit (0.15 mg/g).

Table S2 Effect of *FMO3* genotype and dietary factors on TMA metabolism in laying hens.

Means of main effects	egg yolk TMA (μg/g)	Cecal chyme TMA (μg/g)	Hepatic FMO3 mRNA	FMO3 activity (nmol/mg/min)	plasma TMAO (μg/mL)	plasma TMA (μg/mL)
Genotype						
AA	2.33	26.69	1.32	2.07	3.57	171.93
TT	3.83	28.80	1.61	1.51	3.84	167.80
Diets						
SM	2.23	14.31	1.44	2.02	3.38	173.04
RM	3.94	41.18	1.49	1.56	4.03	166.7
Source of variation				*P*-value		
Genotype	< 0.001	0.52	0.135	0.004	0.327	0.667
Diets	< 0.001	< 0.001	0.814	0.015	0.021	0.51
Genotype × Diets	< 0.001	0.401	0.009	0.256	0.007	0.001

TMA, trimethylamine; TMAO, trimethylamine-*N*-oxide.

Table S3 Significantly changed plasma metabolites associated with the *FMO3* genetic variant T329S.

No.	Metabolite	Assignments	Chemical shift (ppm)[1]	TT hens vs. AA hens				RM diet vs. SM diet			
				SM diet		RM diet		AA hens		TT hens	
				log_2 (FC)	*P*	log_2 (FC)	*P*	log_2 (FC)	*P*	log_2 (FC)	*P*
1	Lipid (high-density lipoprotein)	CH_3	0.86 (t)	0.446	0.031	–0.195	0.260	0.514	0.022	–0.127	0.422
2	Lipid (low-density lipoprotein)	CH_3	0.87 (t)	0.543	0.039	–0.345	0.126	0.539	0.045	–0.349	0.115
3	Lipid (very low-density lipoprotein)	CH_3, CH_2CH_2CO	0.88 (m), 1.57 (m)	0.760	0.023	–0.523	0.128	0.609	0.081	–0.675	0.044
4	3-Hydroxybutyrate	γCH_3, βCH, αCH_2	1.19 (d), 2.30 (m), 2.40 (m), 4.17 (m)	–0.600	0.022	0.047	0.904	–0.932	0.009	–0.285	0.338
5	Lipid (saturated fatty acid)	$(CH_2)_n$	1.25 (m)	0.487	0.019	–0.107	0.526	0.539	0.017	–0.054	0.724
6	Lactate	CH_3, CH	1.33 (d), 4.11 (q)	–0.016	0.896	0.166	0.23	–0.138	0.235	0.045	0.752
7	Lipid (unsaturated lipids)	$CH_2CH_2CH=CH$	2.01 (m)	0.642	0.017	–0.394	0.11	0.549	0.043	–0.487	0.038
8	Acetone	CH_3	2.22 (s)	0.650	0.012	–0.779	0.013	0.913	0.007	–0.516	0.029
9	Glutamate	γCH_2	2.35 (m)	–0.171	0.487	0.110	0.579	0.044	0.842	0.325	0.159
10	Lipid (PUFA)	$CH=CHCH_2CH=CH$	2.74 (m)	0.770	0.01	–0.675	0.043	1.142	0.009	–0.303	0.136
11	Trimethylamine *N*-oxide	$(CH_3)_3$	3.26 (s)	–0.322	0.029	0.224	0.118	–0.384	0.003	0.162	0.291
12	β-Glucose	1-CH	3.24 (dd), 3.40 (t), 3.46 (m), 3.89 (d), 4.64 (d)	–0.424	0.015	0.317	0.065	–0.370	0.024	0.371	0.031
13	α-Glucose	1-CH	3.53 (d), 3.71 (t), 3.82 (m), 5.23 (d)	–0.384	0.022	0.323	0.061	–0.335	0.041	0.372	0.026

[1]s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet.
FC, Fold change.

Table S4 Primer pairs for qRT-PCR analysis.

Gene	Forward primer (5′–3′)	Reverse primer (3′–5′)	NCBI accession number	Amplication lengths (bp)
GAPDH	CCAGAACATCATCCCAGCGT	TTGGCTGGTTTCTCCAGACG	NM_204305.1	153
THRSP	CGGCAGCTTTATTCCTGTGC	ATTTCTGCCCTGCCTACTGC	NM_213577.2	172
ABCG5	TGTGGACACTCGAAGCAAGG	AACGGTATGGGTGGAAGCTC	XM_419457.4	150
ABCG8	ACTCTTGCCAGCTTGTTCCA	GCTTTNMGCTTGCTGGTAGTGG	XM_419458.4	102
TRC8	ATGGAATGACAGACCCCGTG	GGCAGGATGAAAAGGCAAGC	NM_001030955.1	106
MC5R	AAGATGCCTTCGTCCGTCAT	TTGTGGTAACGCAGGGCATA	NM_001031015.1	136
ApoVLDL-II	AGTGTCCTCTTTCCCCTTGC	TCTTTGATGCTGGGTTCCCTC	NM_205483.2	101
FMO3	TCAGGAGCATGAACCAGCAG	ATAACCGCATCGAGGTCGTC	NM_20457.9	208

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; *THRSP*, thyroid hormone responsive spot 14 protein; *ABCG5*, ATP binding cassette transporter G5; *ABCG8*, ATP binding cassette transporter G8; *TRC8*, translocation in renal carcinoma on chromosome 8 protein; *MC5R*, melanocortin 5 receptor; *ApoVLDL-II*, apovitellenin 1; *FMO3*, flavin-containing monooxygenase 3.

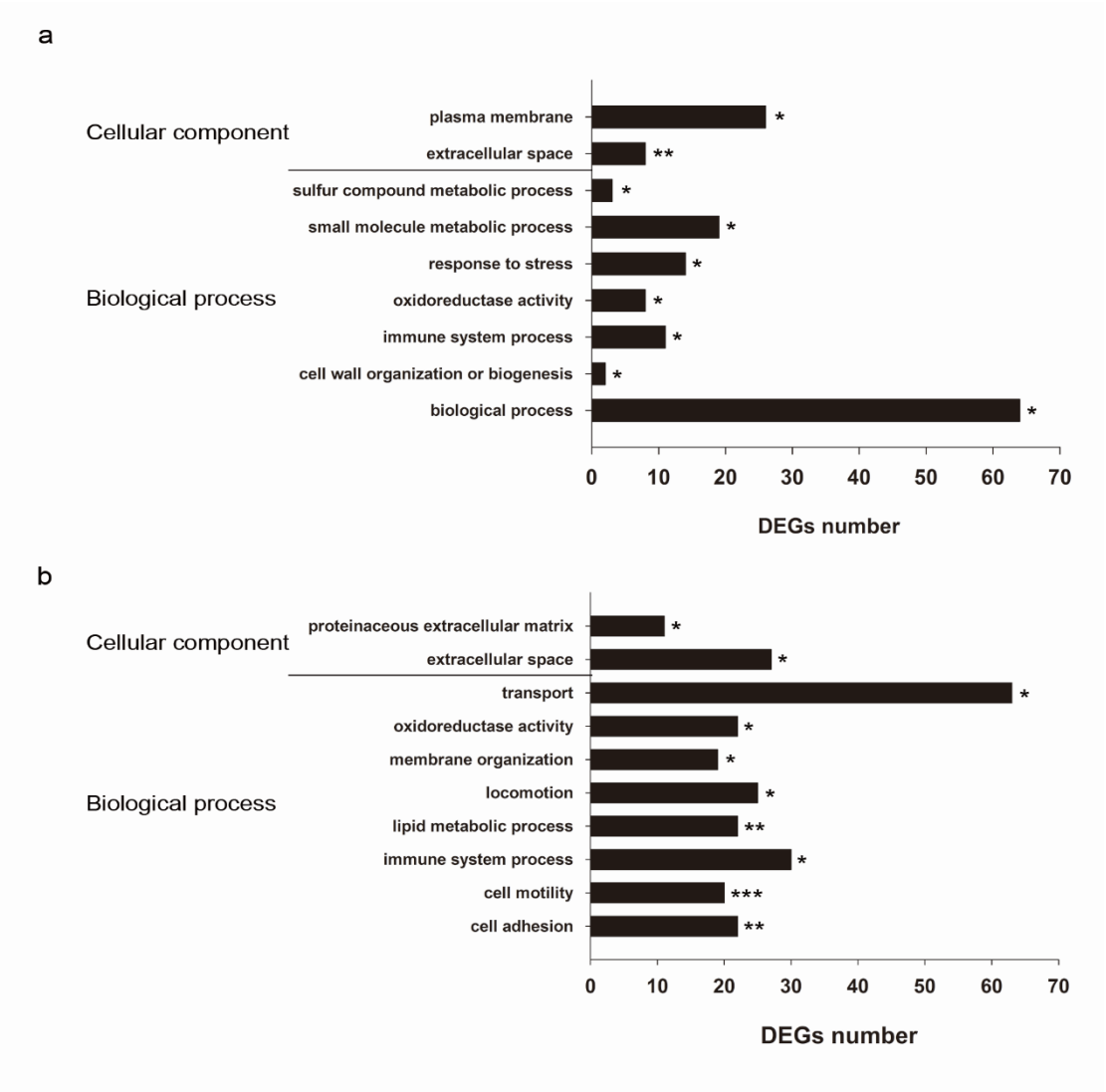

Figure S1 Gene Ontology enrichment analysis of hepatic DEGs**.** Comparisons were made between *FMO3* genotypes (TT vs. AA) under the SM (a) or RM (b) diet condition. For gene ontology analysis, contigs were categorized and statistically analyzed in terms of their annotated molecular function, cellular component or biological process. Only the significantly enriched biological processes are shown. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

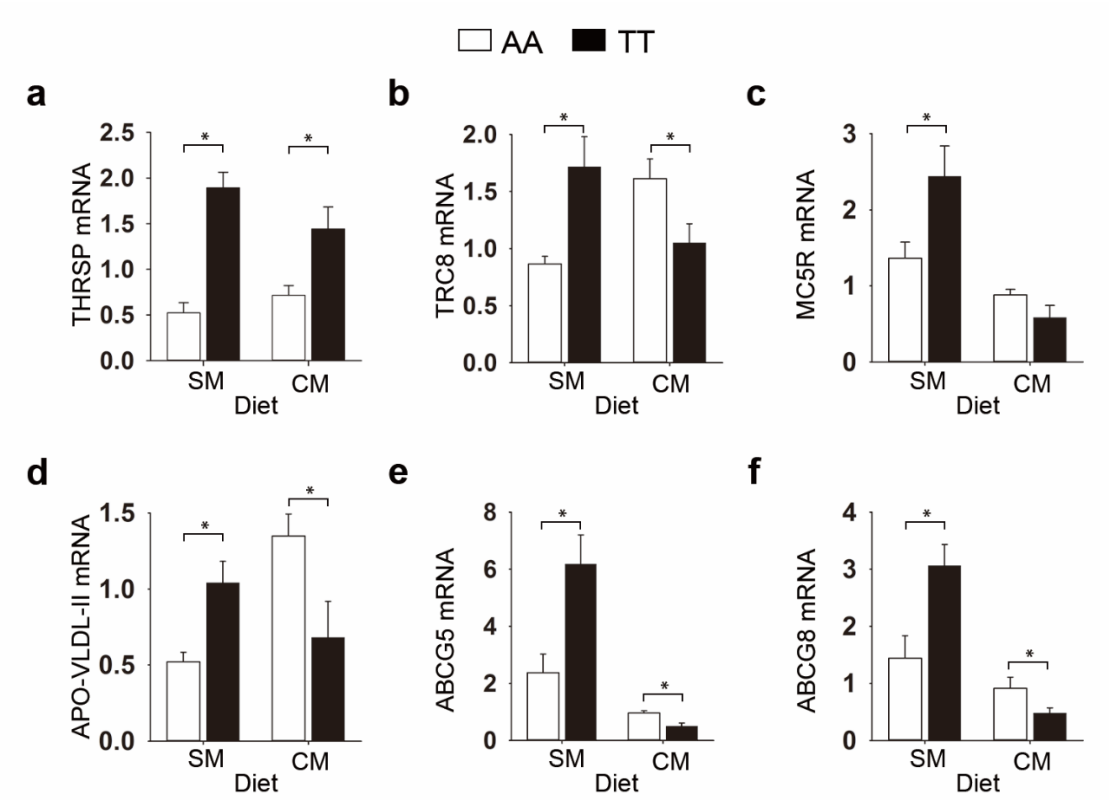

Figure S2 Hepatic expression of the six genes selected for validation of RNA-Seq data by using quantitative real-time polymerase chain reaction. Genotyped hens (AA and TT genotype) were fed the soybean meal (SM) diets or rapeseed meal (RM) diets (n = 6 birds/groups)**.** The six genes include *THRSP* (a), *TRC8* (b), *MC5R* (c), *APO-VLDL-II* (d), *ABCG5* (e) and *ABCG8* (f). The relative gene expression levels were normalized to the endogenous RNA control glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Data are presented as mean ± SEM. Significance was measured with Student's *t*-test. **P* < 0.05.

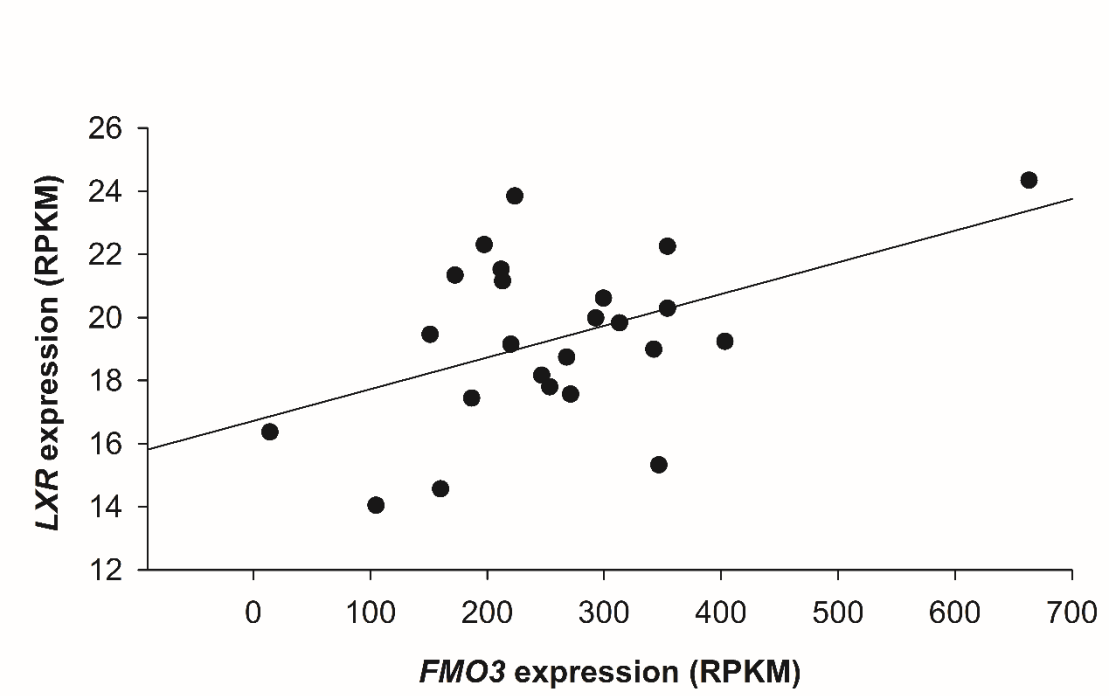

Figure S3 Relationship between hepatic expression levels of *FMO3* and *LXR* in laying hens (n = 24). Laying hens homozygous normal for *FMO3* (AA genotype) and mutant hens genotyped as *FMO3* c.984 A>T (i.e. T239S mutation; TT genotype) were fed either the corn–soybean meal basal diets (SM; < 0.15 mg/g sinapine) or the basal diets supplemented with 21% of rapeseed meal (RM; 1.33 mg/g sinapine) for 8 weeks. Pearson correlation = 0.465, $P = 0.022$. Hepatic expression of *FMO3* and *LXR* were determined by RNA-Seq analysis. RPKM, reads per kilobase per million mapped reads.

Gene Abbreviations

THRSP: thyroid hormone responsive spot 14 protein; TRC8: renal carcinoma on chromosome 8 protein; MC5R: melanocortin 5 receptor; ApoVLDL-II: apovitellenin 1; ABCG5: ATP binding cassette transporters G5; ABCG8: ATP binding cassette transporters G8; MYO 16: Myosin XVI; MOCOS: molybdenum cofactor sulfurtransferase; SULT1C: sulfotransferase family, cytosolic,1C, member 3; PTX3: pentraxin 3; ELOVL2: ELOVL fatty acid elongase 2; ELOVL5: ELOVL fatty acid elongase 5; ELOVL6: ELOVL fatty acid elongase 6; PTPLB: protein tyrosine phosphatase-like, member B; PECR: peroxisomal trans-2-enoyl-CoA reductase; CYP2U1: Cytochrome P450, family 2, subfamily U, polypeptide 1; FASN: fatty acid synthase; SREBP1: sterol regulatory element-binding protein 1; ACACA: acetyl-CoA carboxylase alpha; ACACB: acetyl-CoA carboxylase beta; HMGCR: 3-hydroxy-3-methylglutaryl coenzyme A reductase; INSIG2: insulin induced gene 2; NCEH1: neutral cholesterol ester hydrolase 1; HSD17B4: hydroxysteroid (17-beta) dehydrogenase 4; ACOX2: acyl-CoA oxidase 2; DECR1: 2,4-dienoyl CoA reductase 1; PLTP: phospholipid transfer protein; BAAT: bile acid-CoA:amino acid N-acyltransferase; AMACR: alpha-methylacyl-CoA racemase; HSD3B7: hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7; SCP2: sterol carrier protein 2; SLC51α: solute carrier family 51, alpha subunit; APOB: apolipoprotein B; ACSL5: acyl-CoA synthetase long-chain family member 5; ACSBG1: acyl-CoA synthetase bubblegum family member 1; ACADSB: acyl-CoA dehydrogenase short chain; PECI: delta 3,delta 2-enoyl-CoA isomerases; HSD3B7: bile acid biosynthesis; CYP7A1: cytochrome P450 family 7 subfamily A member 1; MSMO1: methylsterol monooxygenase 1; CYP2R1: cytochrome

P450 family 2 subfamily R member 1; LSS: lanosterol synthase; SQLE: squalene epoxidase; DHCR7: 7-dehydrocholesterol reductase, NSDHL: NAD(P) dependent steroid dehydrogenase-like; CYP51A1: cytochrome P450, family 51, subfamily A, polypeptide 1; CYP24A1: cytochrome P450 family 24 subfamily A member 1; DHCR24: 24-dehydrocholesterol reductase; FDFT1: farnesyl-diphosphate farnesyltransferase 1.