Supplementary Materials for

Potential of Queen Bee Larvae as a Dietary Supplement for Obesity Management: Modulating Gut Microbiota and Promoting Liver Lipid Metabolism

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Table. S1 The composition of D12450J and D12492

Product#	D12450J	D12492
Protein	20% kcal	20% kcal
Carbohydrate	70% kcal	20% kcal
Fat	10% kcal	60% kcal
Total	100%	100%
kcal/g	3.82	5.21

Table. S2. Primer sequences used in the RT-qPCR experiment

Target gene	names	Related pathways	Forward	Reverse
G6pc	Glucose-6-phosphatase catalytic subunit	AMPK signaling pathway	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
Cyp4a14	Cytochrome P450 family 4 subfamily A member 10	PPAR signaling pathway	TTTAGCCCTACAAGGTACTTGGA	GCAGCCACTGCCTTCGTAA
PPARg	Peroxisome proliferator-activated receptor gamma	PPAR signaling pathway	GCTGAACGTGAAGCCCATCG	GGCGAACAGCTGAGAGGACT
CD36	Cluster of differentiation 36	PPAR signaling pathway	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
Plin4	Perilipin 4	PPAR signaling pathway	GTGTCCACCAACTCACAGATG	GGACCATTCCTTTTGCAGCAT
Mapk15	Mitogen-activated protein kinase 15	TNF signaling pathway	CCTATGGCATTGTGTGGAAGG	TCCTCTGAGCATCTATCTGGTC
Traf1	TNF receptor-associated factor 1	TNF signaling pathway	AGGGTGGTGGAATTACAGCAA	GCAGTGTAGAAAGCTGGAGAG
Cxcl10	C-X-C motif chemokine ligand 10	IL-17 signaling pathway	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Creb3l1	CAMP responsive element binding protein 3-like 1	TNF signaling pathway	GCCCTGGGAAACAAGCTGT	AGCTGAGTCATTTCTCCTGGG
Ccl2	C-C motif chemokine ligand 2	TNF signaling pathway	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT

Table. S3 Comparative analysis of amino acid composition between QBL and other meats in previously reported studies (mg/100g)

Amino acids	QBL	Mutton leg	Veal leg	Pork shoulder	Beef sirloin	Duck carcass	Chicken breast	Chicken drumstick
Aspartate	1594.28 ± 11.12	1362	1514	1540	1862	752	2157	1694
Threonine	557.29 ± 0.78	727	688	966	951	370	911	715
Serine	631.82± 0.31	650	688	620	835	372	886	697
Glutamate	1532.22 ± 3.13	2289	2311	2542	3165	1445	3505	2756
Glycine	523.39 ± 0.13	919	881	921	1007	656	1334	1049
Alanine	560.76 ± 0.87	1026	991	1064	1210	589	1441	1134
Cystine	65.32 ± 1.54	198	137	207	265	104	279	220
Valine*	739.32 ± 2.80	785	853	927	1038	479	1345	1057
Methionine*	335 .31± 4.03	381	413	487	560	214	631	497
Isoleucine*	615.94± 3.75	773	826	821	997	391	1251	982
Leucine*	914.63 ± 3.13	1195	1293	1432	1680	611	1579	1240
Tyrosine	470.27 ± 0.89	512	578	622	746	280	735	578
Phenylalanine*	617.93 ± 10.34	621	660	699	911	329	772	606
Lysine*	1130.60 ± 2.12	1267	1349	1483	1844	686	2022	1590
Histidine	341.85 ± 1.85	425	551	584	706	250	941	739
Arginine	698.72 ± 0.27	1068	1074	1085	1309	580	321	1154

* indicated the EAA.

Note: The amino acid content in QBL was measured in this study, whereas the concentrations for the other groups were sourced from reference.[1]

Table. S4 Comparative analysis of minerals content between QBL and other meats in previously reported studies (mg/100g)

Variety	QBL	Mutton leg	Veal leg	Pork shoulder	Beef sirloin	Duck carcass	Chicken breast	Chicken drumstick
K	370.06±1.74	319.20	194.30	293.60	382.00	154.90	385.00	334.00
Na	14.35±0.38	65.50	101.10	50.00	52.00	42.20	55.00	91.00
Ca	14.04±0.17	8.40	7.90	4.10	4.00	5.10	5.00	8.00
Mg	39.20±0.08	19.30	12.60	15.60	26.00	9.00	33.00	26.00
Cu	0.55±0.01	0.10	0.20	0.10	0.10	0.10	0.01	0.10
Zn	2.24±0.03	2.70	1.60	2.20	2.90	0.90	0.50	1.40
Fe	2.82±0.23	2.30	1.90	0.90	3.10	1.30	0.40	0.70
Mn	0.10±0.00	0.03	0.02	0.01	0.04	0.02	0.01	0.01
P	158.86±1.72	178.90	126.40	130.40	212.00	95.40	240.00	215.00

Note: The mineral content in QBL was measured in this study, whereas the concentrations for the other groups were sourced from reference.[1]

Table. S5 Summary of Sample sequencing data quality

Sample	Raw reads	Clean reads	Q20 (%)	Q30 (%)	GC content (%)
LFD1	80569958	77691164	99.27	97.78	51.75
LFD2	44027838	42749914	99.28	97.85	49.24
LFD3	40211906	39537864	99.15	97.28	47.89
HFD1	69754418	68013058	99.29	97.79	48.89
HFD2	68458080	67104924	99.27	97.75	49.97
HFD3	59357560	57979342	99.26	97.70	50.57
HFD+Q1	47212896	45713484	99.18	97.40	48.06
HFD+Q2	66645060	64718352	99.29	97.81	50.27
HFD+Q3	56971988	55669786	99.30	97.85	50.05

Table. S6 Mapped information of sample

Sample	total_map (%)	unique_map (%)	multi_map (%)
LFD1	88.89	84.07	4.82
LFD2	91.50	84.02	7.48
LFD3	95.63	86.39	9.24
HFD1	89.92	82.21	7.70
HFD2	89.41	83.71	5.70
HFD3	90.50	85.98	4.52
HFD+Q1	94.79	86.01	8.78
HFD+Q2	90.88	85.60	5.28
HFD+Q3	88.91	82.67	6.24

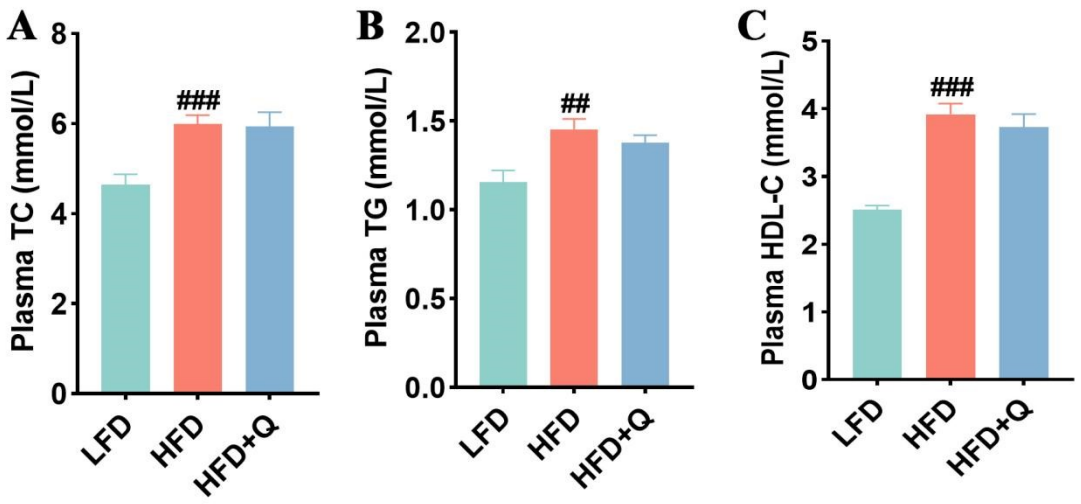

Fig. S1. QBLF showed limited effect on TC (A), TG (B), and HDL-C (C) levels compared to the HFD group. Values are means ± SEM. #P<0.05, ##P<0.01, ###P<0.001 vs. LFD group.

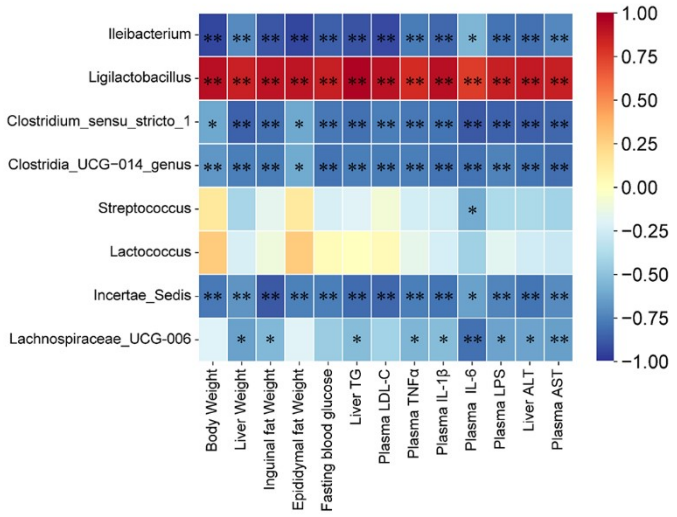

Fig. S2. Heat map of the correlation between gut microbiota and phenotypic index, biochemical markers and inflammation levels. Significant correlation was indicated by $^{*}P<0.05$ and $^{**}P<0.01$.

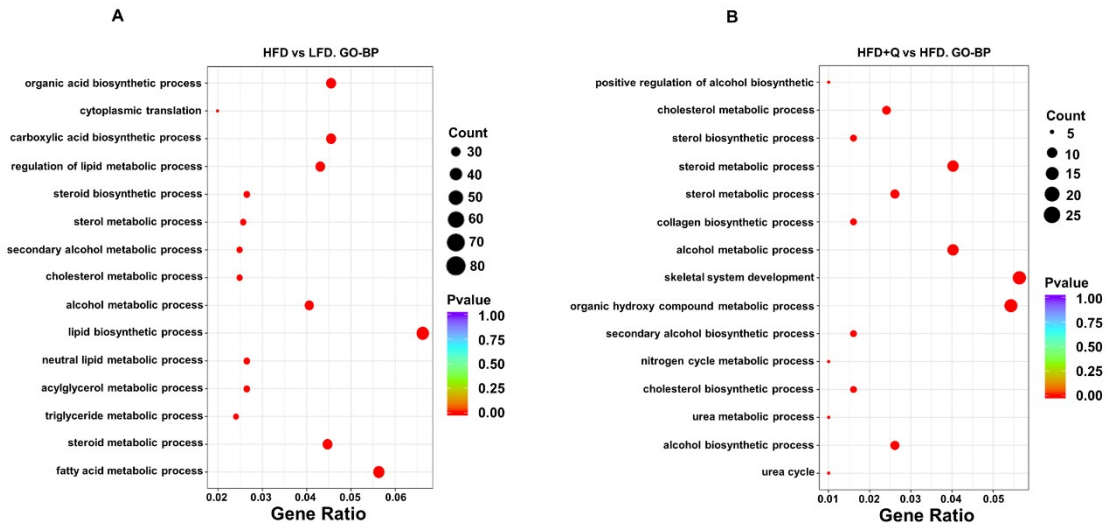

Fig. S3. **GO enrichment analysis of DEGs in mice liver.** (A) GO enrichment analysis of DEGs between HFD and LFD groups; (B) GO enrichment analysis of DEGs between HFD+Q and HFD groups.

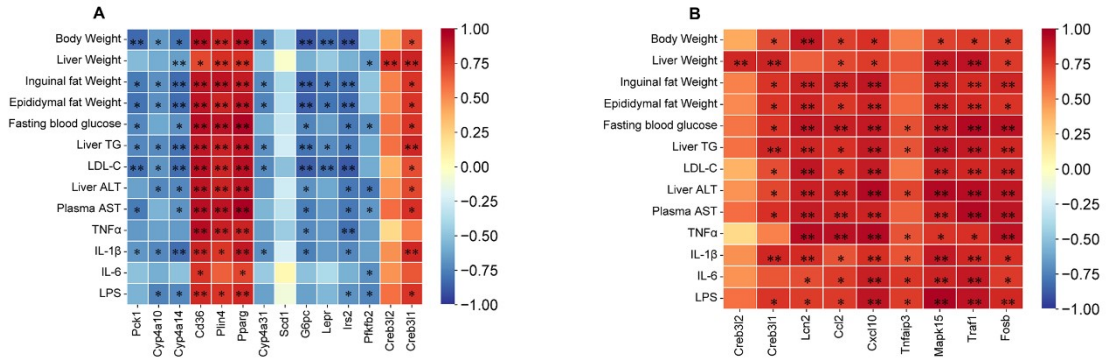

Fig. S4. Heat map of the correlation between transcriptome and phenotypic index, biochemical markers and plasma inflammation levels. (A) Correlation of lipid metabolism genes with phenotypic indices, biochemical indices and plasma inflammation levels; (B) Correlation of inflammation genes with phenotypic indices, biochemical indices and plasma inflammation levels. Significant correlation was indicated by $^{*}P<0.05$ and $^{**}P<0.01$.

References

1 A. Orkusz, Edible Insects versus Meat-Nutritional Comparison: Knowledge of Their Composition Is the Key to Good Health, *Nutrients*, 2021, **13**, 1207.