读书报告

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Dietary lipid levels could improve growth and intestinal microbiota of juvenile swimming crab, *Portunus trituberculatus*

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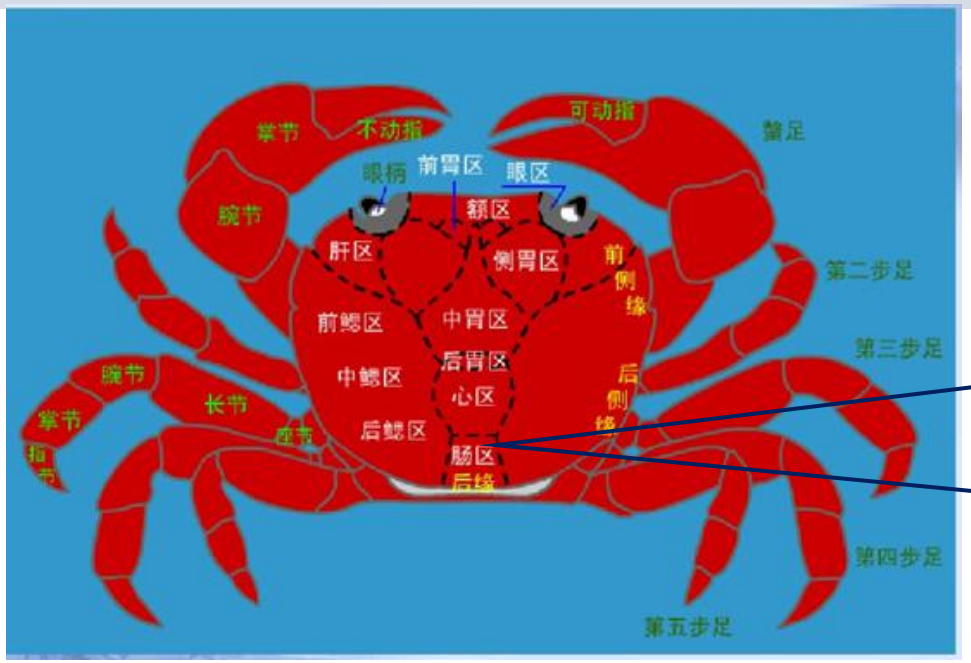
目　　录

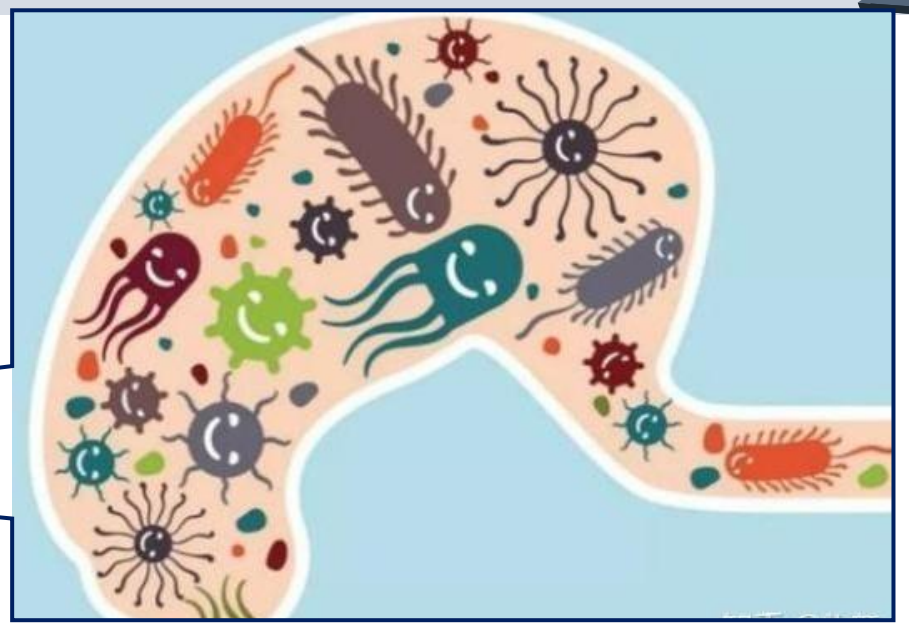
背景意义

三疣梭子蟹，属于甲壳纲、十足目、梭子蟹科，是中国沿海的重要经济蟹类。三疣梭子蟹具有肉质鲜美、营养丰富、是海味品中的上品。并且生长迅速、产量高、成本低，易管理、经济效益好。2015年该蟹的养殖产量约达117772公吨。

脂肪是饲料中的重要营养素之一，作为主要的能量来源，提供必需的脂肪酸。作为脂溶性维生素的载体，并在甲壳类动物中发挥着重要作用，如甲壳动物中的二十烷类、激素和酶的辅助性因子。

背景意义





肠道微生物与脂质：蟹的肠道中居住着数万亿细菌，它们在宿主健康中发挥着重要作用。如吸收营养、提高能量生产和平衡免疫反应。肠道细菌也参与脂质代谢，包括脂肪储存，能量平衡、胆汁酸合成。甲壳类动物的肠道微生物群落受宿主的营养习惯的影响，它们会代谢部分摄入的食物。

三种等蛋白日粮，含有粗脂肪5.8%、9.9%和15.1%

将(21.1±1.1g)幼蟹分为三组，每组三个重复，每个重复20只，每天饲喂两次，饲养8周

生长性能与生存指标

增重，存活率

取肠道内容物，提取DNA

肠道菌群的16sRNA高通量测序

肠道菌群丰富度，多样性

群落组成

与日粮脂质水平有关的菌属

前100菌属发育关系

三疣梭子蟹特有的肠道微生物和饮食脂肪水平对这些肠道菌群有选择性的压力，肠道微生物群的组成可以作为蟹营养和生长状况的一个指标

材料方法

Table 1
Formulation and proximate composition of experimental diets.

Ingredient (%)	Dietary lipid levels (%)		
	5.8	9.9	15.1
White fish meal	25.0	25.0	25.0
Wheat gluten meal	12.0	12.0	12.0
Soybean protein concentrate	19.0	19.0	19.0
Krill meal	5.0	5.0	5.0
Dextrin	19.7	19.7	19.7
Fish oil	2.0	7.0	12.0
Soy lecithin	1.0	1.0	1.0
Vitamin premix[a]	1.0	1.0	1.0
Mineral premix[b]	1.5	1.5	1.5
Choline chloride	0.3	0.3	0.3
$Ca(H_2PO_4)_2$	1.5	1.5	1.5
Cellulose	10.0	5.0	0.0
Sodium alginate	2.0	2.0	2.0
Proximate composition (dry matter %)			
Dry matter	90.8	91.5	91.6
Crude protein	47.3	47.3	47.6
Crude lipid	5.8	9.9	15.1
Ash	9.5	9.5	9.5

Table 2
Fatty acid composition of the experimental diets (% of total fatty acids).

Fatty acid	Dietary lipid level (%)		
	5.8	9.9	15.1
C14:0	4.6	6.1	7.0
C16:0	23.7	24.6	24.1
C18:0	4.7	5.0	4.6
C20:0	0.3	0.6	0.7
C22:0	0.2	0.2	0.2
ΣSFA[a]	33.6	36.5	36.7
C16:1n-7	5.3	6.7	7.5
C18:1n-9	17.2	16.9	16.0
C20:1n-9	4.4	4.1	3.8
C22:1n-9	0.9	0.9	0.8
ΣMUFA[b]	27.8	28.7	28.1
C18:3n-3	1.6	1.4	1.4
C20:5n-3	7.3	8.2	9.5
C22:6n-3	10.1	10.7	11.5
Σn-3PUFA[e]	19.1	20.3	22.4
C18:2n-6	16.5	10.2	7.9
C20:4n-6	0.6	0.9	0.8
Σn-6PUFA[f]	17.1	11.1	8.7
DHA/EPA	1.4	1.3	1.2
ΣPUFA[c]	18.1	11.6	9.3
ΣHUFA[d]	18.9	20.7	23.0

[a] ΣSFA, saturated fatty acids: C14:0, C16:0, C18:0, C20:0, C22:0.
[b] ΣMUFA, monounsaturated fatty acids: C16:1n-7, C18:1n-9, C20:1n-9.
[c] ΣPUFA, polyunsaturated fatty acids: C18:2n-6, C18:3n-3.
[d] ΣHUFA, highly unsaturated fatty acids: C20:4n-6, C20:5n-3, C22:5n-3, C22:6n-3.
[e] Σn-3PUFA: C18:3n-3, C20:5n-3, C22:6n-3.
[f] Σn-6PUFA: C18:2n-6, C20:4n-6.

结果分析

生长性能研究

Growth performance of swimming crab fed with different dietary lipid levels for 8 weeks.

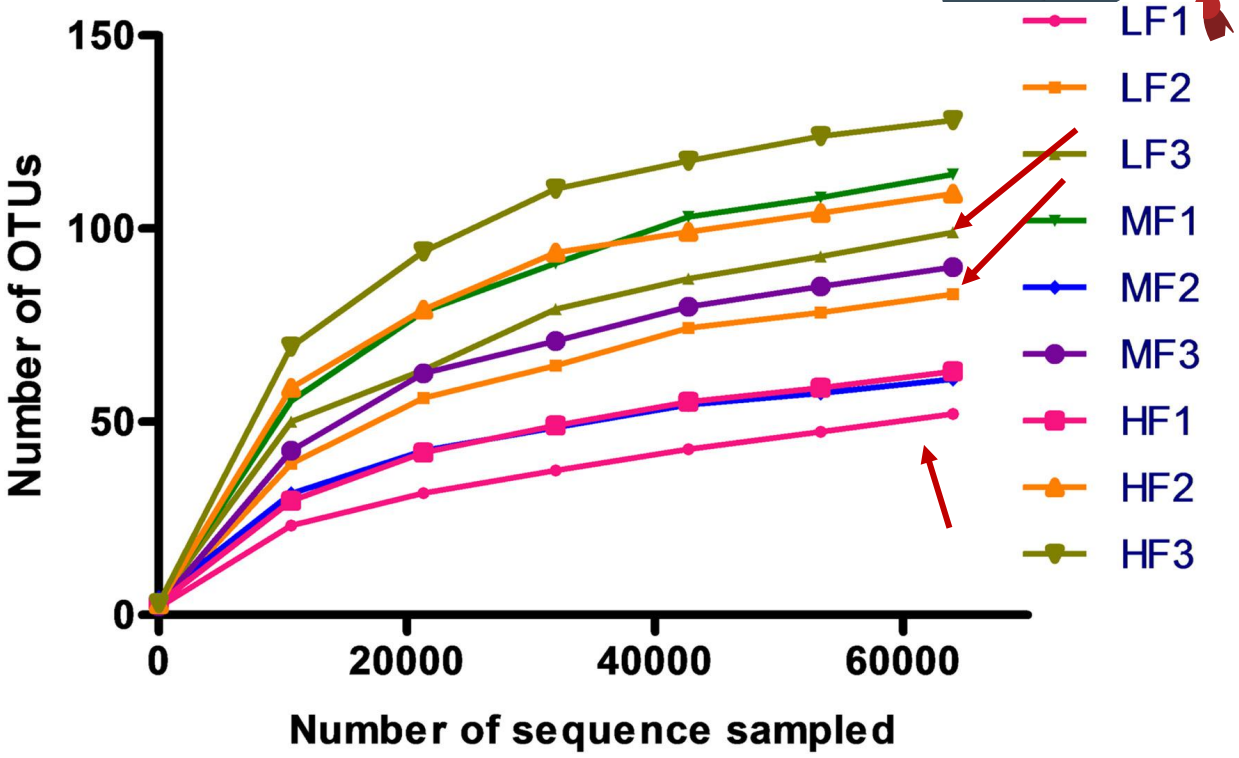
Parameters	Dietary lipid level (%)		
	5.8	9.9	15.1
Initial weight (g)	19.9 ± 1.2	21.4 ± 1.1	22.0 ± 0.3
Final weight (g)	83.9 ± 5.1[a]	84.1 ± 6.5 [a]	76.6 ± 4.7 [b]
Weight gain (%)	322.9 ± 10.6[a]	292.2 ± 14.7[a]	248.0 ± 21.7[b]
Survival (%)	80.0 ± 4.1[a]	83.3 ± 4.9[a]	63.3 ± 1.4[b]

结果分析

Illumina high-throughput data, bacterial diversity richness (OTUs), diversity index (Shannon & Simpson), and estimated OTU richness (Chao & ACE) for intestinal bacterial diversity analysis of *P. trituberculatus* fed with different dietary lipid levels for 8 weeks.

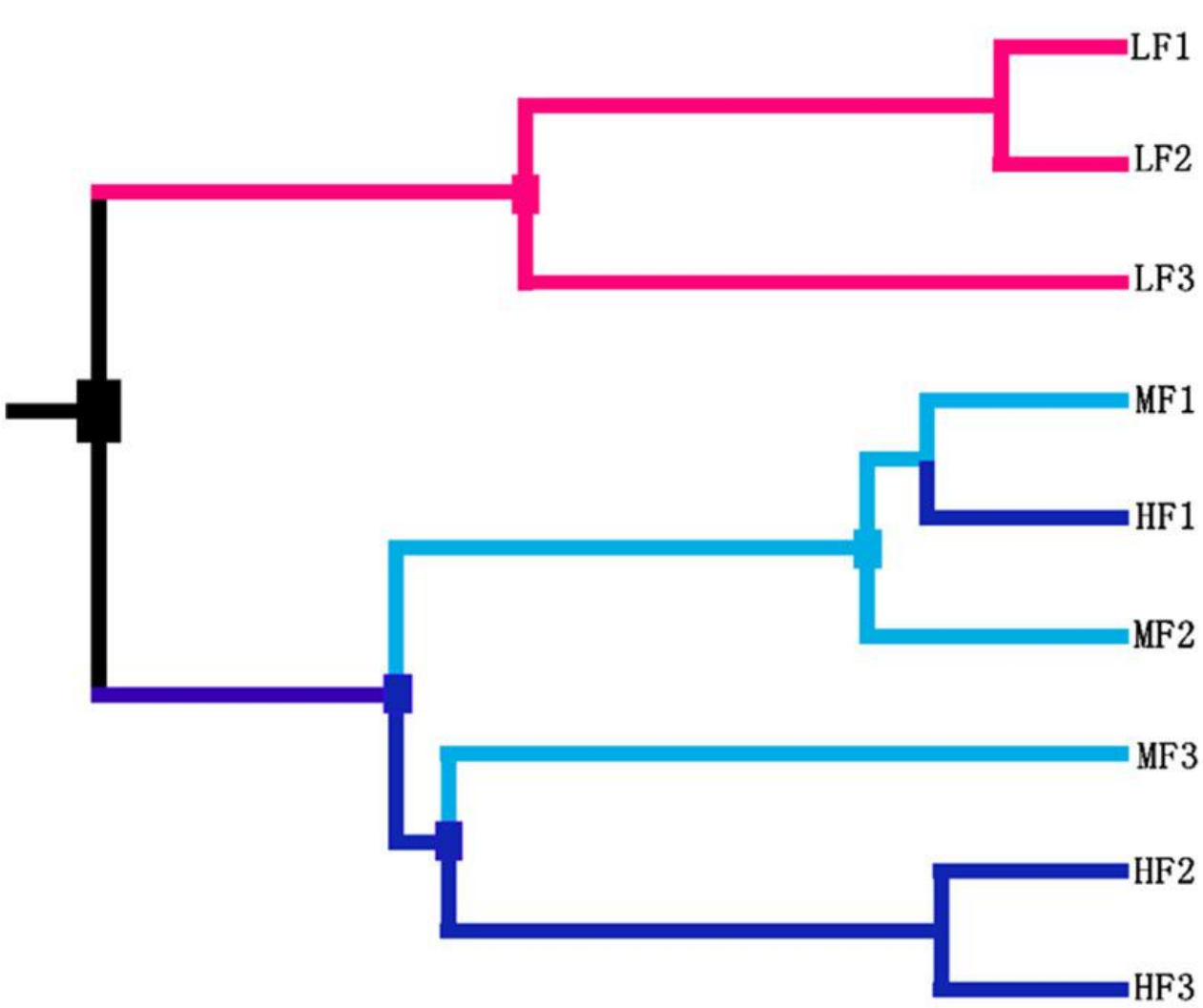
Parameters	Dietary lipid levels (%)		
	5.8	9.9	15.1
Sampling depth			
Mean sequences	74,189	76,025	75,697
Observed OTUs	78.0 ± 13.8	88.3 ± 15.3	100.0 ± 19.3
Richness estimators			
Chao1	97.9 ± 12.0	101.2 ± 14.0	107.6 ± 17.8
ACE	103.0 ± 10.8	103.6 ± 14.1	112.4 ± 17.3
Diversity estimators			
Shannon	1.2 ± 0.3	1.7 ± 0.5	1.7 ± 0.0
Simpson	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.0

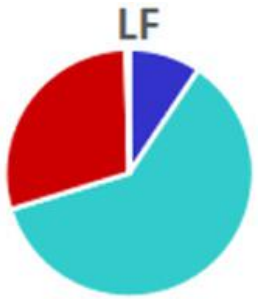
Data represent means ± S.E.M from three repetitions. The values in the same row with different superscripts are different ($P < 0.05$).



根据ACE、Chao 1、Shannon、Simpson等指标，不同脂类水平对游泳蟹肠道细菌的丰富度和多样性没有影响。

结果分析

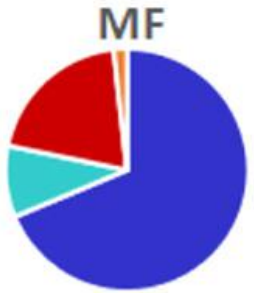




9.4 ± 5.2%

60.9 ± 22.4%

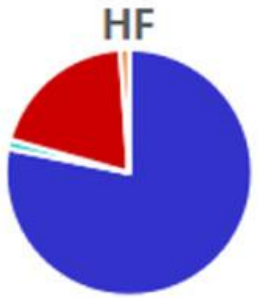
29.3 ± 17.6%



68.7 ± 13.7%

9.5 ± 7.4%

20.0 ± 10.5%



78.1 ± 15.3%

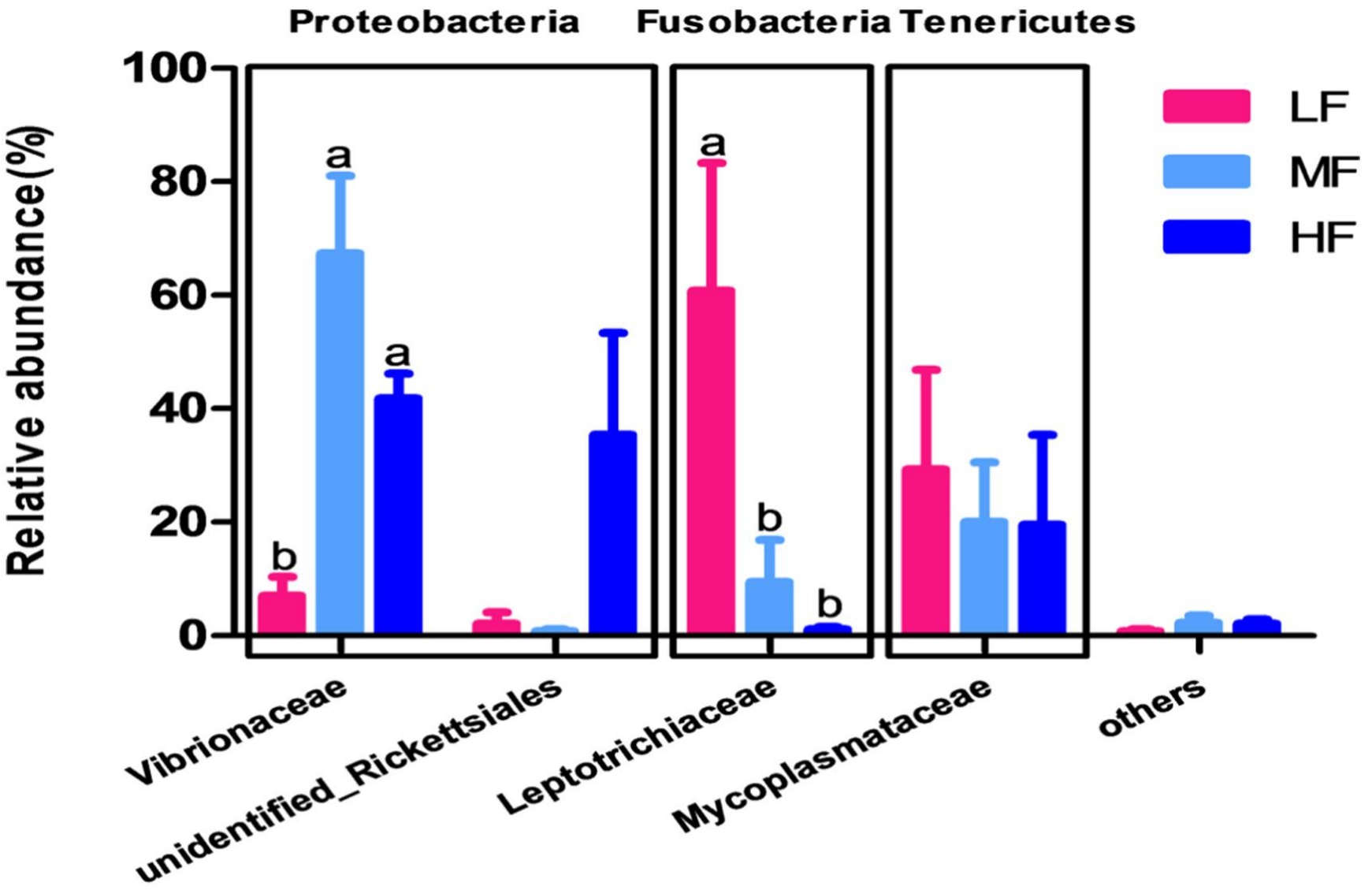
1.2 ± 0.5%

19.6 ± 15.8%

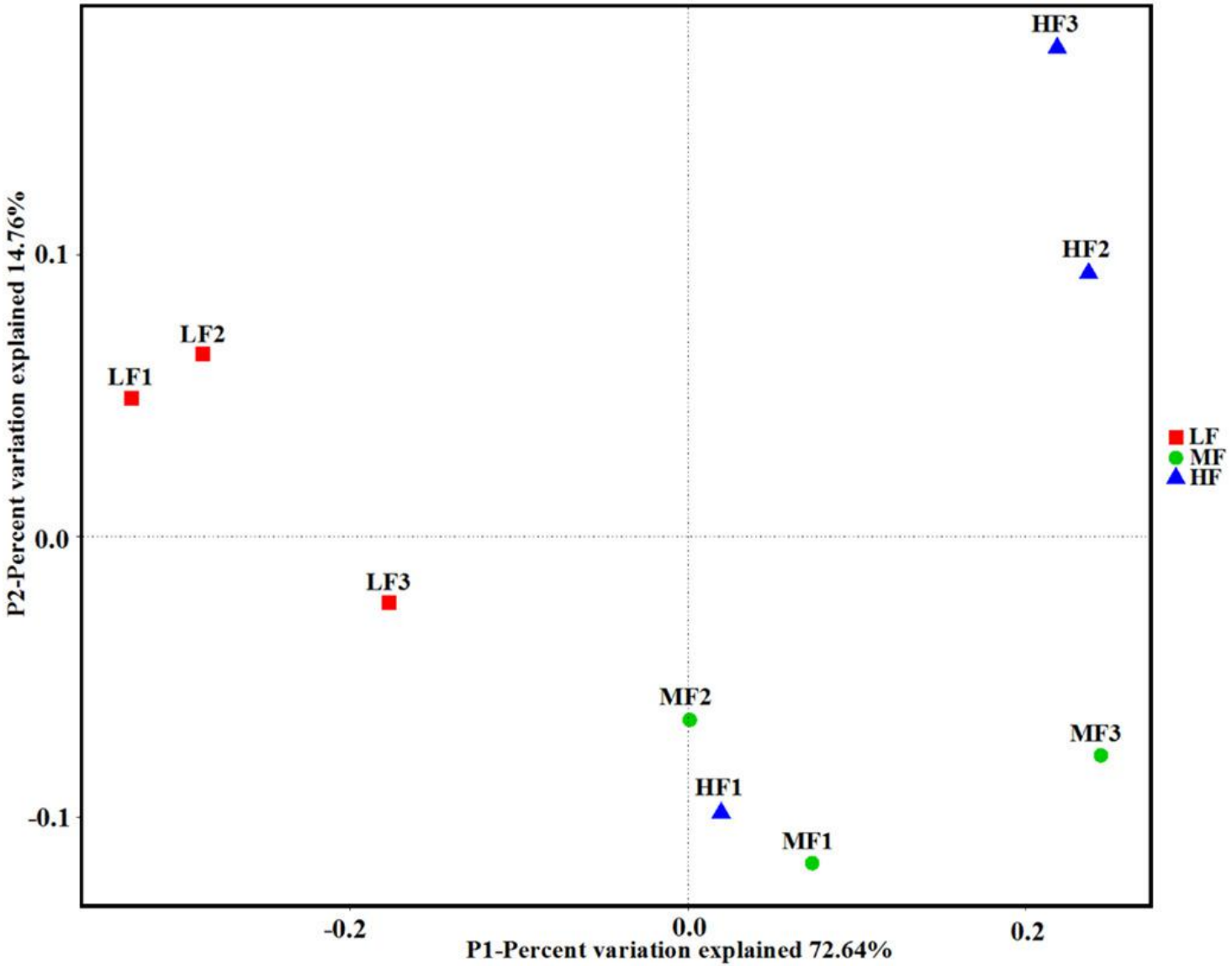
Proteobacteria Fusobacteria Tenericutes other

变形菌门和梭杆菌门的相对丰度受日粮脂类水平的显著影响。聚类分析显示，三个LF饮食相关的肠道群落之间的相似性比MF和HF饮食中的肠道微生物群落更相似

结果分析

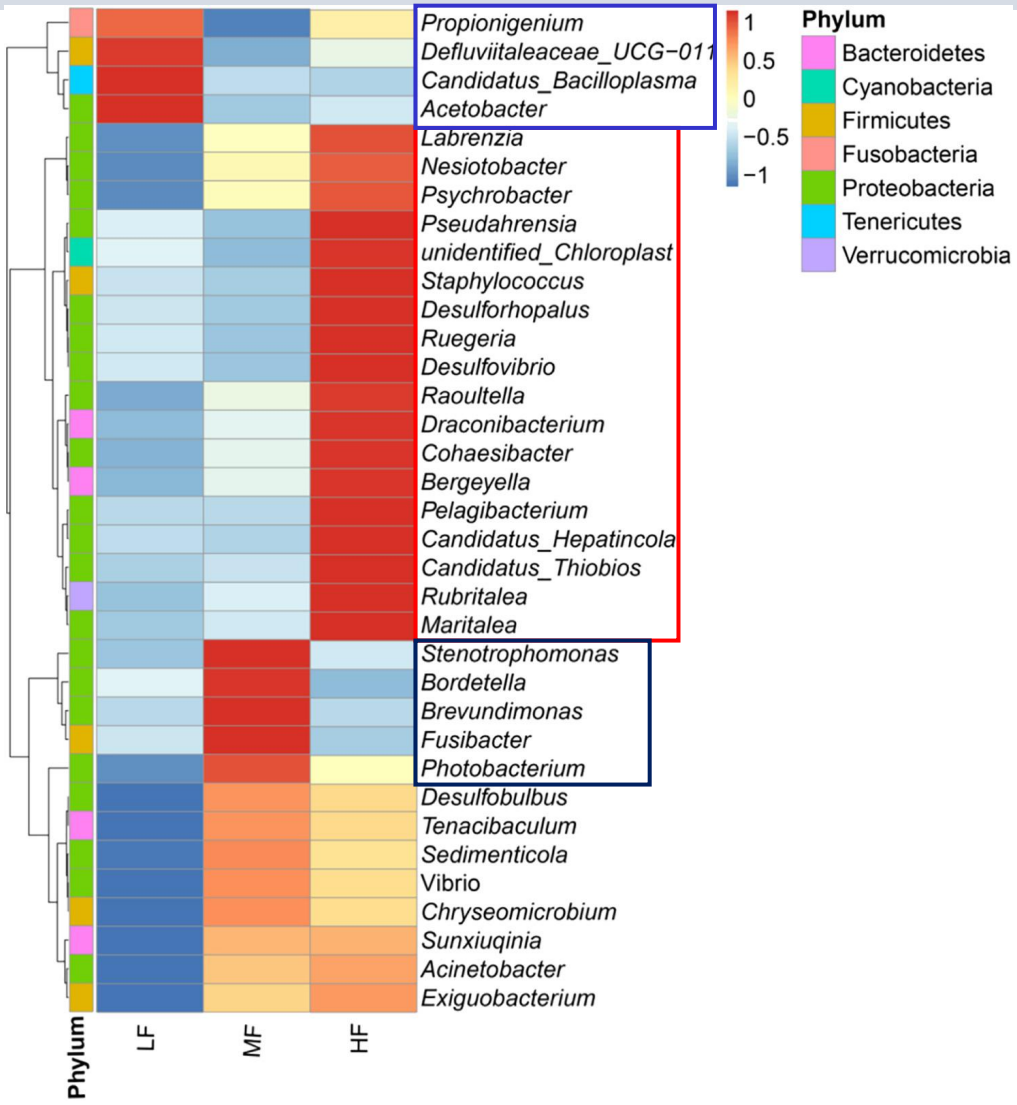

Proteobacteria
Fusobacteria
Tenericutes
Relative abundance(%)
100
80
60
40
20
0
a
a
b
a
b
b
LF
MF
HF
Vibrionaceae
unidentified_Rickettsiales
Leptotrichiaceae
Mycoplasmataceae
others

结果分析



For swimming crab intestines, the LF group was distinctly separated from the MF and HF groups

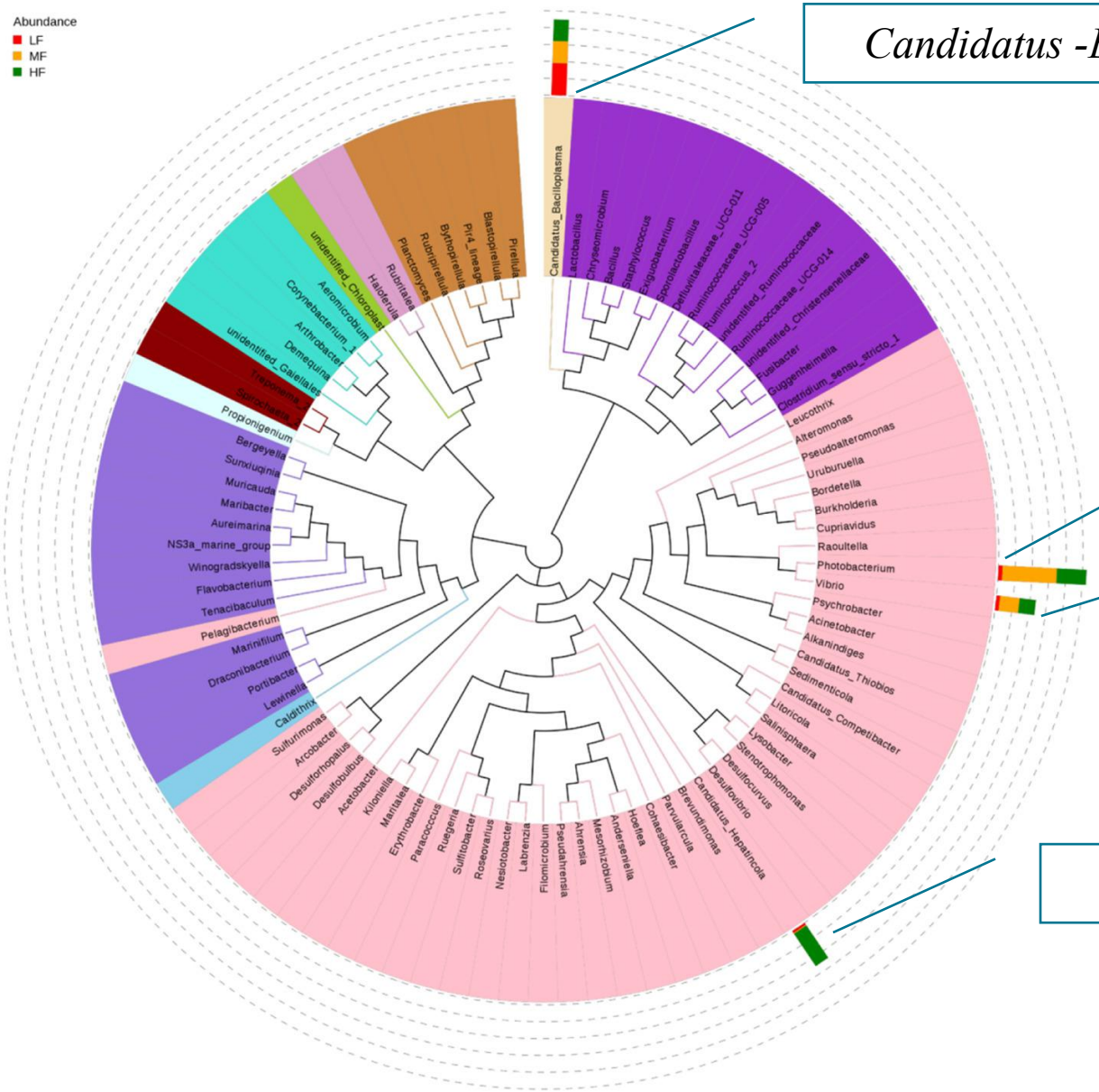
结果分析



The heat map was used to show genera whose abundances were changed by dietary lipid level

These results indicated that the observed microbial assembly is at the genus level

结果分析



Candidatus -Bacilloplasma

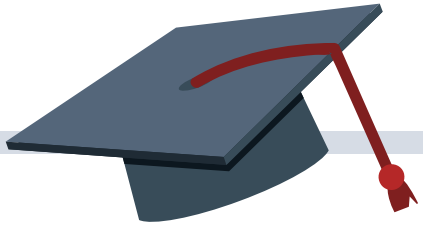
Photobacterium

vibrio

Candidatus-Hepatincola

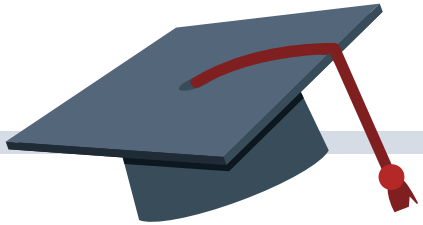
系统发育树的结果表明，MF和HF组集中在同一门中，并与LF组分离

讨论



- The swimming crab in the LF and MF groups showed better growth performance and survival than those in the HF group.
- The intestinal microbiota of swimming crab fed the MF and HF diets were similar to each other, and different from those fed the LF diet. The relative abundance of Fusobacteria decreased, while Proteobacteria increased in the intestine of the crabs fed the LF diet, suggesting that the abundance of these two phyla respond to dietary lipid levels.

讨论



- swimming crab fed the medium- and high-lipid diets had less *Leptotrichiaceae* than those fed the low-lipid diet, but the role of this family in the crustacean intestine remains unclear. Moreover, higher *Vibrionaceae* were detected in the swimming crab fed the medium- and high-lipid diets than in those fed the low-lipid diet.
- Orders of *Rickettsiales* are well-known parasites and pathogens of plants, animals and human .The large number of *Rickettsiales* may explain the low survival rate in swimming crab fed the HF diet.

Thanks