读书报告

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Full length article

Screening of intestinal probiotics and the effects of feeding probiotics on the growth, immune, digestive enzyme activity and intestinal flora of *Litopenaeus vannamei*

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肠道益生菌的筛选及饲喂益生菌对凡纳滨对虾

生长、免疫、消化酶活性和肠道菌群的影响

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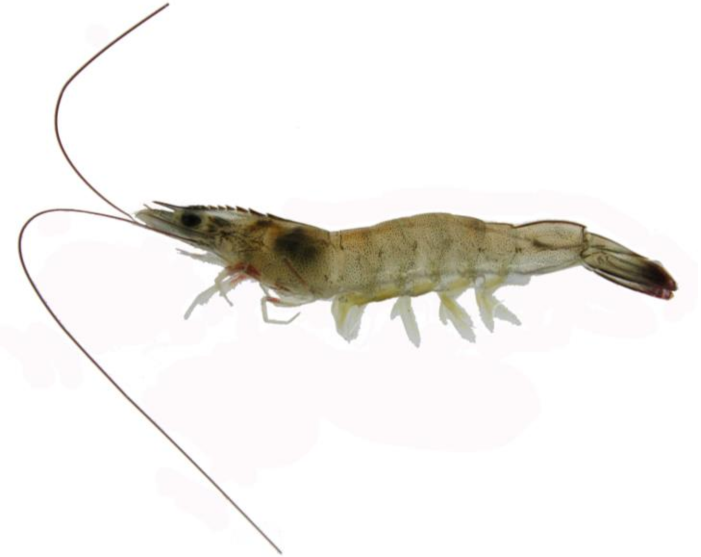
01

Introduction

Introduction



随着凡纳滨对虾养殖的迅速发展，对虾养殖的病害日益突出，对虾的危害越来越大。研究表明，抗生素的滥用不仅扰乱了虾肠道的正常菌群，使病原体对药物产生耐药性，而且还使其残留。由于益生菌在动物体内不产生残留或耐药性，作为抗生素替代品的益生菌已成为研究的热点。

Introduction

The isolation of strains with various digestive enzyme activities from the intestinal tract of healthy animals has become one of the effective ways to screen probiotics [9,10]. It has been shown that the selected beneficial bacteria should be used in the shrimp body, which can not only improve the utilization rate of the shrimp feed and promote the growth of the shrimp, some probiotics can but also promote the immunity of shrimp immune system to virus and pathogenic bacteria and reduce the outbreak of shrimp disease [11].

In this experiment, the candidate probiotics were screened from the intestinal tract of healthy shrimp and feeding experiment was conducted to attempt to evaluate the biological function of the intestinal endogenous probiotics.

补充益生菌不仅可以提高饲料利用率和生长速度。同时也通过改善鱼类和虾的免疫系统来减少疾病的爆发

从健康虾肠道中分离出426株菌，其中11株有产酶和抗菌活性，溶血试验，药敏实验是选出2株益生菌，鉴为*Enterobacter hominis* （E3），*lactobacillus*（L3）

对这两株菌进行生物学特性研究，进行纯培养，将培养的益生菌（10^7CFU /g）加入饲料饲喂分成三组，250只体长4厘米的虾

血液相关免疫酶和肠道消化酶活性的测定

每7天采样一次，每组取20只虾

WSSV感染，观察累积死亡率

每组选取120只虾，三个重复，每只虾注射10ul,每隔12小时记录一次死亡率

生长指标

每组随机抽取10只虾，每周测一次增重率

中肠的电镜观察

样品经过戊二醛清洗，酒精梯度脱水，在电镜下观察

检测对虾肠道微生物群落

经过28天的喂养试验。在1，5，10天随机抽取实验组和对照组20只对虾进行肠道微生物群落测试

从健康虾的肠道中得到的两株土著益生菌可用于解决虾病的问题或促进虾的生长

02

Materials and methods

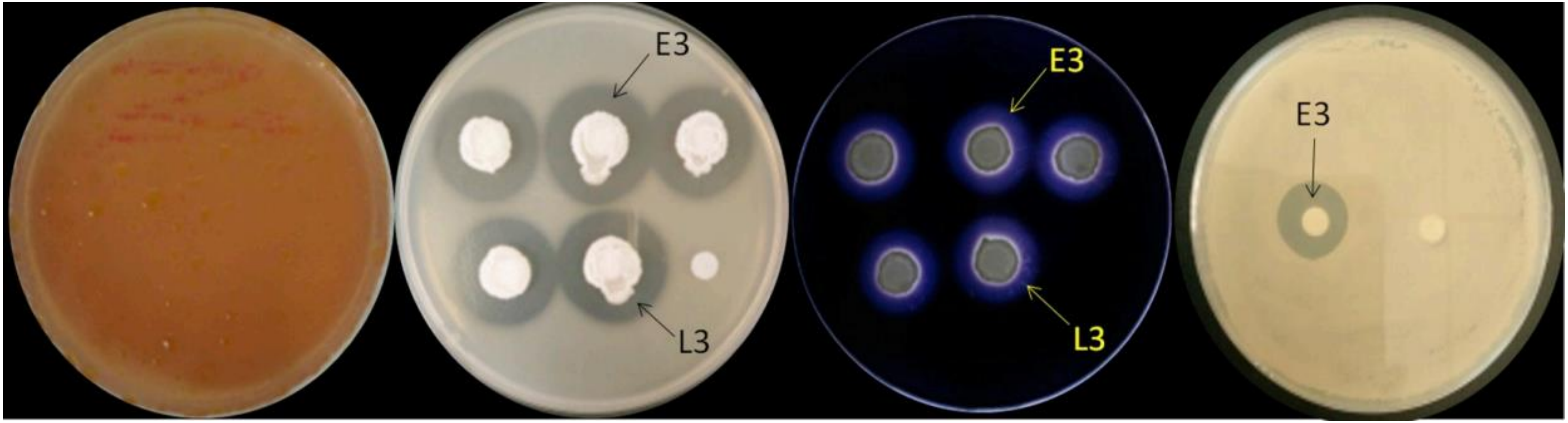
Materials and methods

- 在MRS、YPD、TSB、LBS和2216E培养基上分离菌株
- 对纯化菌株进行，溶血实验和药敏实验
- 16SDNA 测序为*Enterobacter hominis* ，*lactobacillus*
- Biolog-System
- 研究了该菌株的最佳生长条件：温度、pH值、NaCl浓度和牛胆盐浓度。
- 血液相关免疫酶（SOD, PPO, ACP, POD, CAT, LZM）和肠道消化酶活性(NP、AL、LPS)的测定
- 攻毒实验WSSV肌肉注射，
- 扫描电镜和透射电镜对肠道进行观察
- 肠道菌群多样性检测（Simpson指数和McIntosh指数）

03

Results

Results



脂肪酶阳性　　蛋白酶阳性　　淀粉酶阳性　　E3的拮抗点。

Results

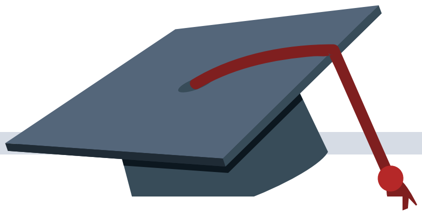
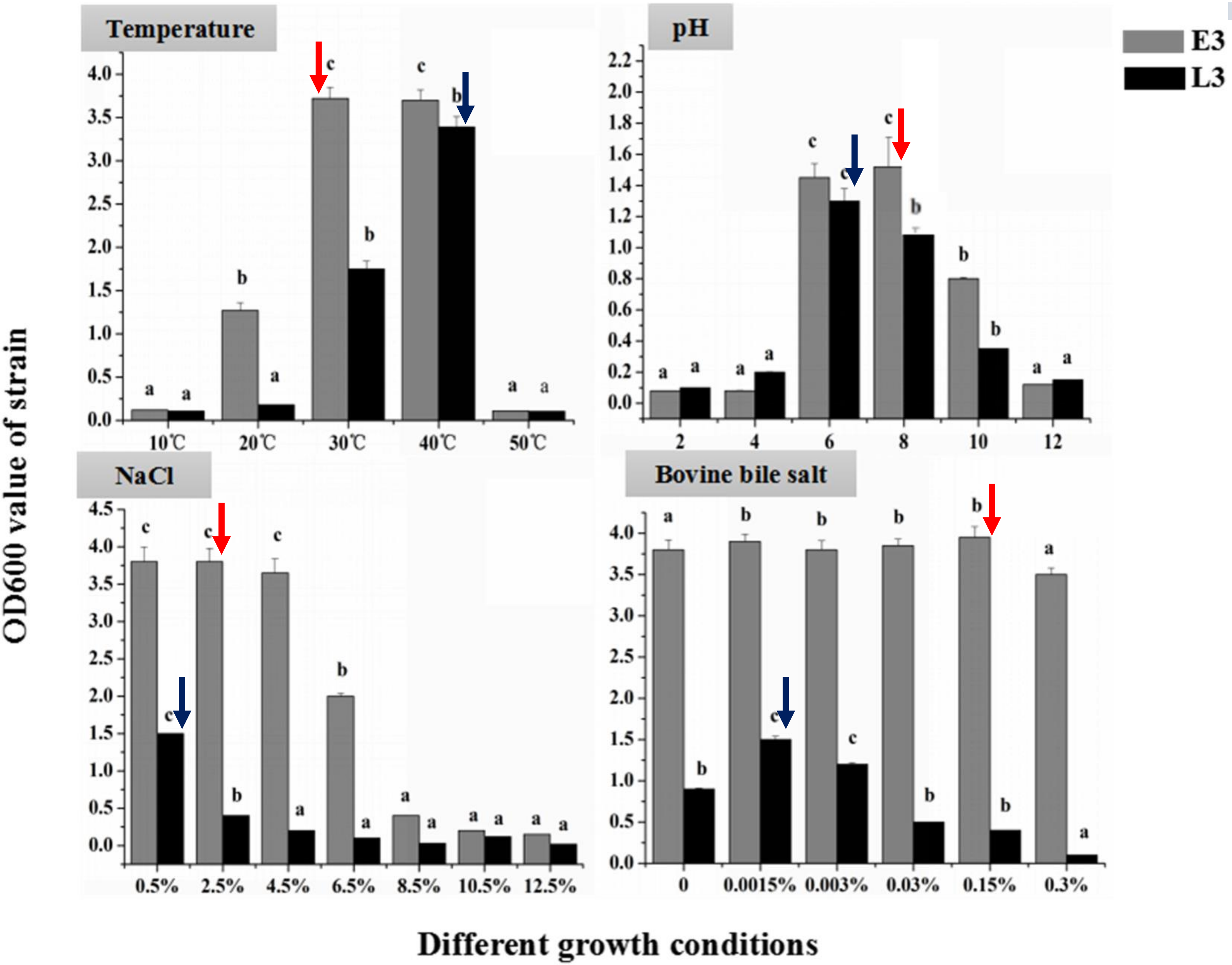


Table 1
Susceptibility of 11 strains to common antibiotics (Notes: R. resistance; I. intermediate; S. sensitive.).

antibiotics	Susceptibility of candidate probiotic strains										
	B1	B2	E3	B3	B4	L3	B5	E4	B6	B7	B8
Erythromycin	R	I	R	S	R	S	R	R	S	R	S
Midecamycin	S	I	R	R	R	S	S	R	S	R	S
Tetracyclie	R	S	S	I	R	S	I	I	R	S	I
Lomefloxacin	S	R	S	R	S	R	R	S	R	R	S
Ciprofloxacin	S	R	S	R	R	S	I	S	R	R	S
Chloramphenicol	R	S	S	S	S	S	S	R	R	S	I
Sulfamethoxazole	I	R	S	S	S	S	R	R	R	R	I
Nitrofurantoin	R	R	R	R	R	R	R	R	R	S	R
Rifampin	R	S	R	R	R	S	S	S	R	R	R
Penicillin	S	R	R	S	R	S	S	R	S	S	R
Mezlocillin	R	R	R	R	R	S	S	R	R	S	R
Ampicillin	S	R	R	R	R	S	R	I	S	I	R
Cefotaxime	R	S	I	I	R	I	I	I	R	R	S
BacitracinR	S	R	R	R	R	R	S	R	R	S	R
GentamicinS	R	R	R	R	R	R	R	S	R	R	R
KanamycinS	I	R	I	R	I	R	R	S	R	S	R
TreptomycinS	S	R	S	S	R	I	S	S	R	S	S

3株对常用抗生素有耐药，其他对多种抗生素敏感



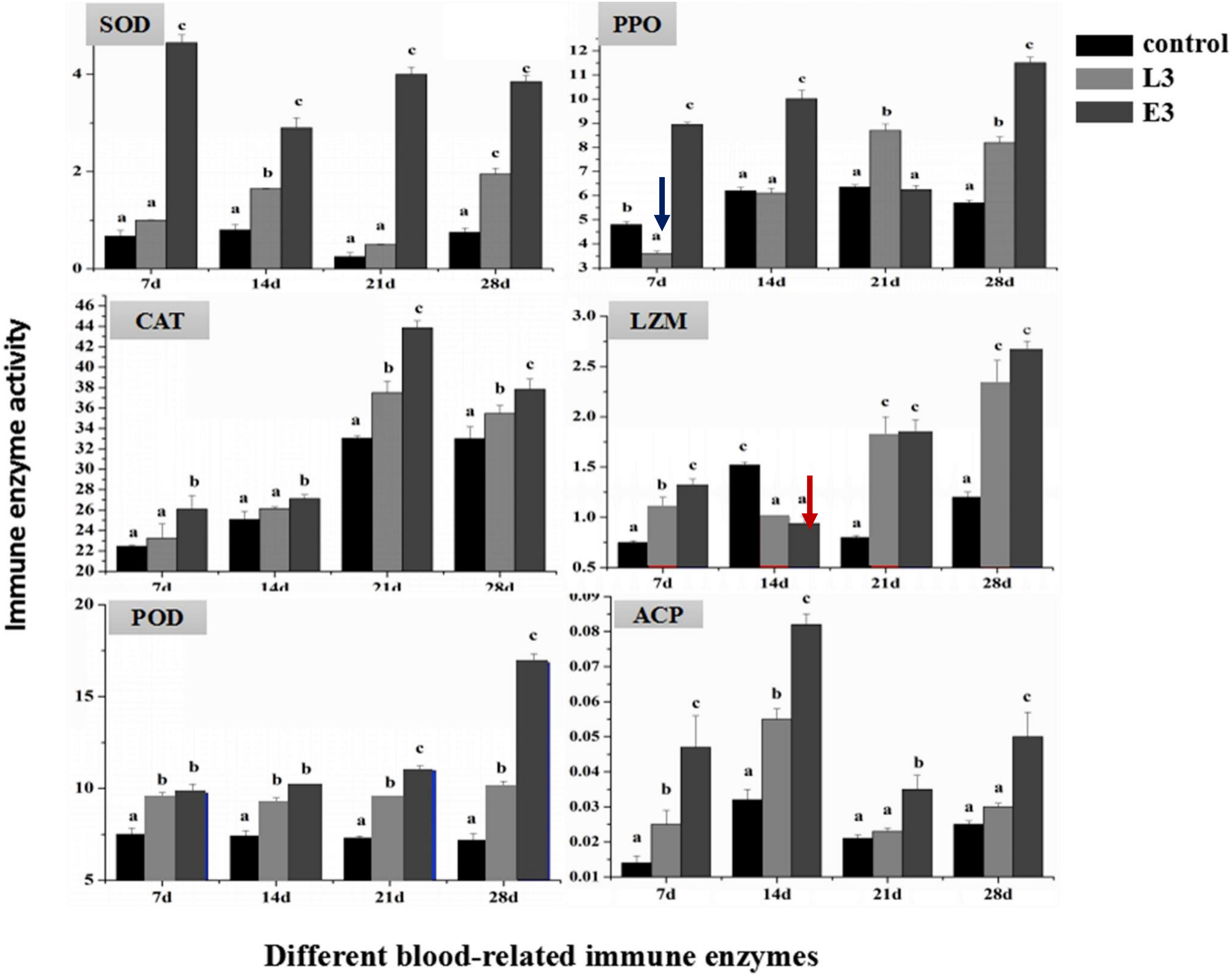
菌株E3的最适生长条件为：温度30°C，pH值8.0，氯化钠浓度2.5%，牛胆盐浓度0.15%

L3最适生长条件为：温度40°C，pH值6.0，NaCl浓度0.5%，牛胆盐浓度0.0015%

Results

3.4. Growth rate of L. vannamei

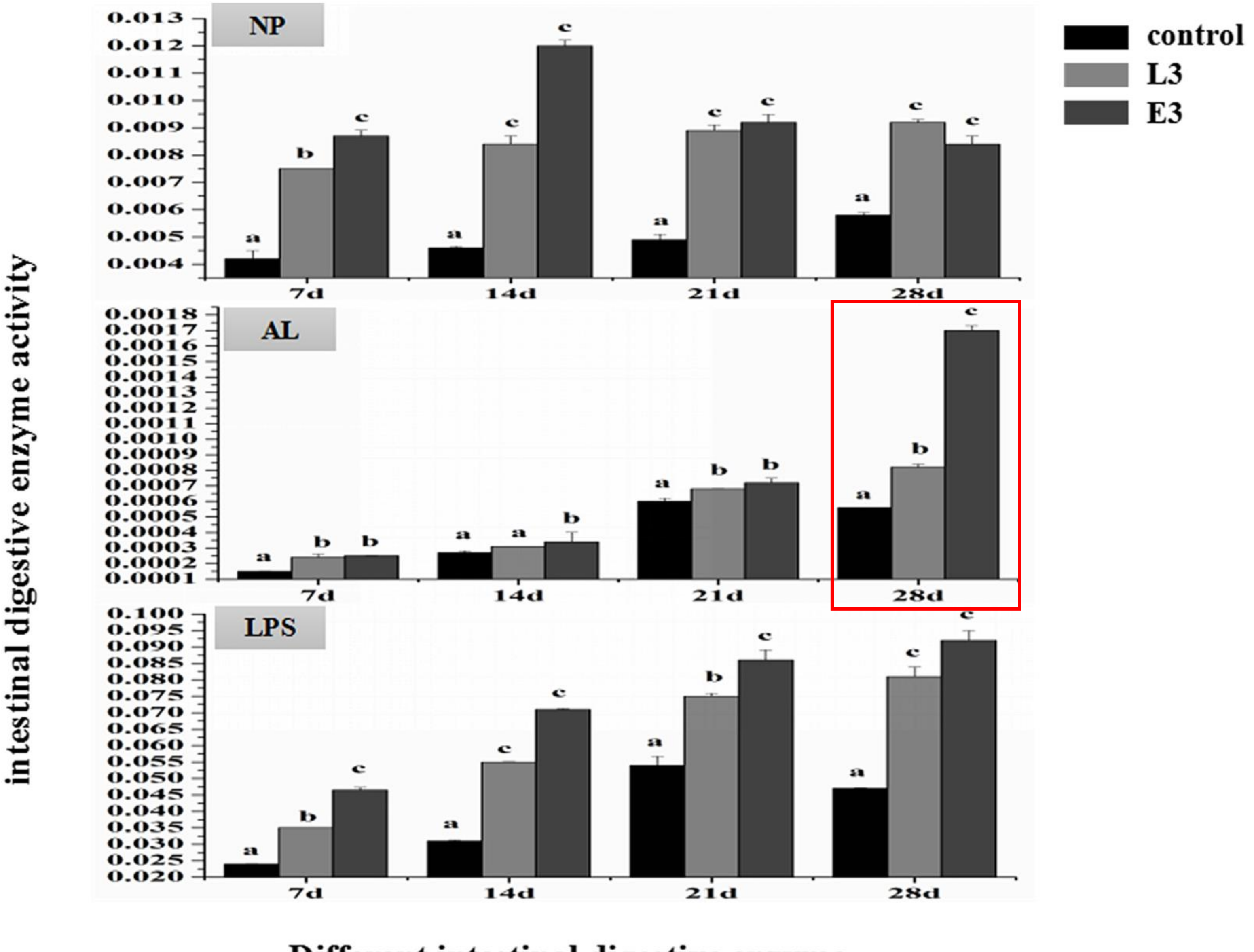
During the period of shrimp culture and feeding, there was no significant ($p < 0.05$) difference in the first week; at the second week, the L3 group (8.95%)was slightly higher than the E3 group (7.05%) and the blank group (6.98%); at the third week, the L3 group (10.94%) and E3 group (10.79%) were significantly higher than the blank group (8.25%). After the end of feeding, the probiotics groups were significantly higher than the control group (10.05%), and the effect of E3 group (13.75%) was more significant.



E3组，PPO、SOD、ACP、POD和CAT活性均显著提高，LZM活性在第14天下降

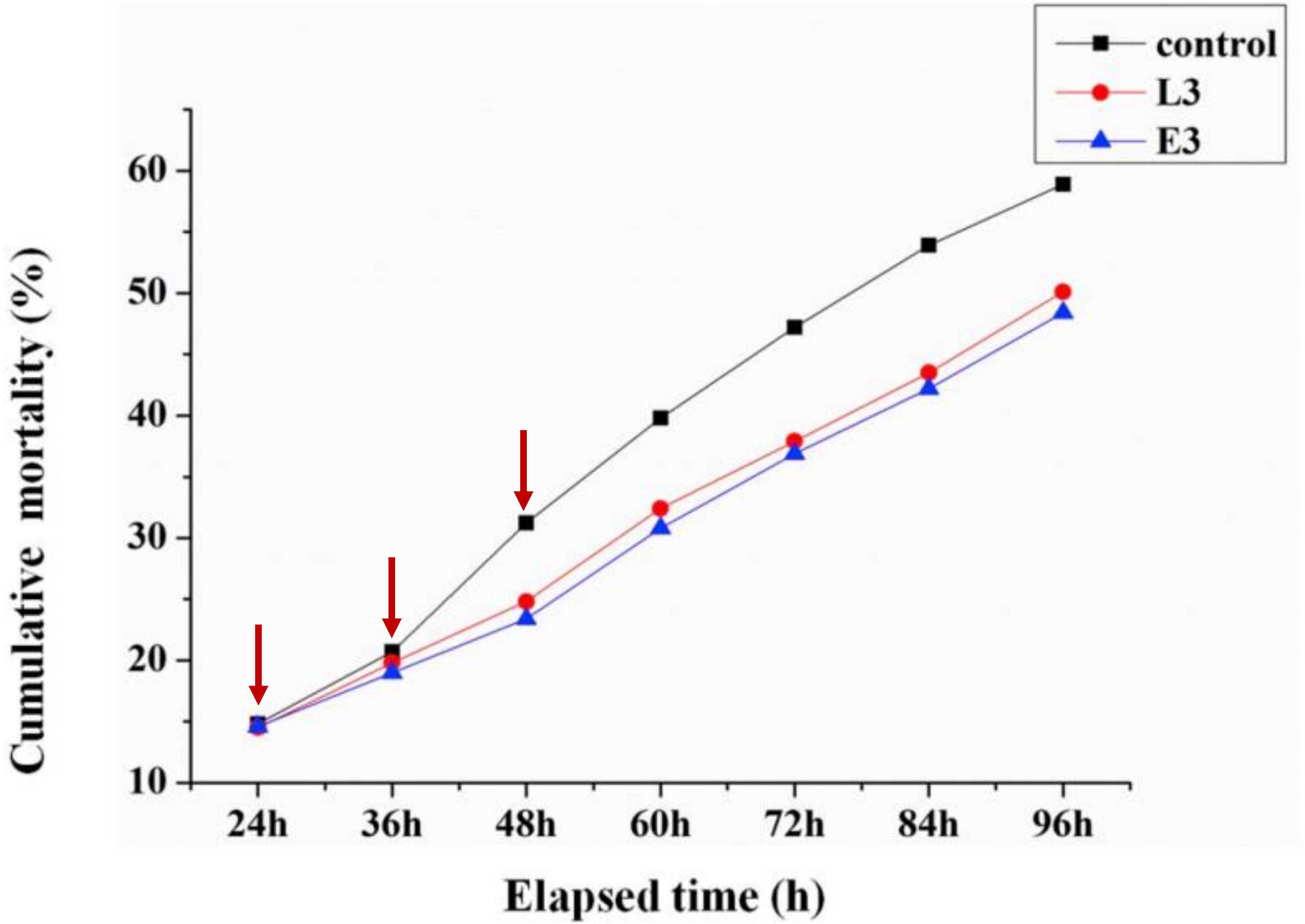
L3组PPO活性在第7天下降。

Results



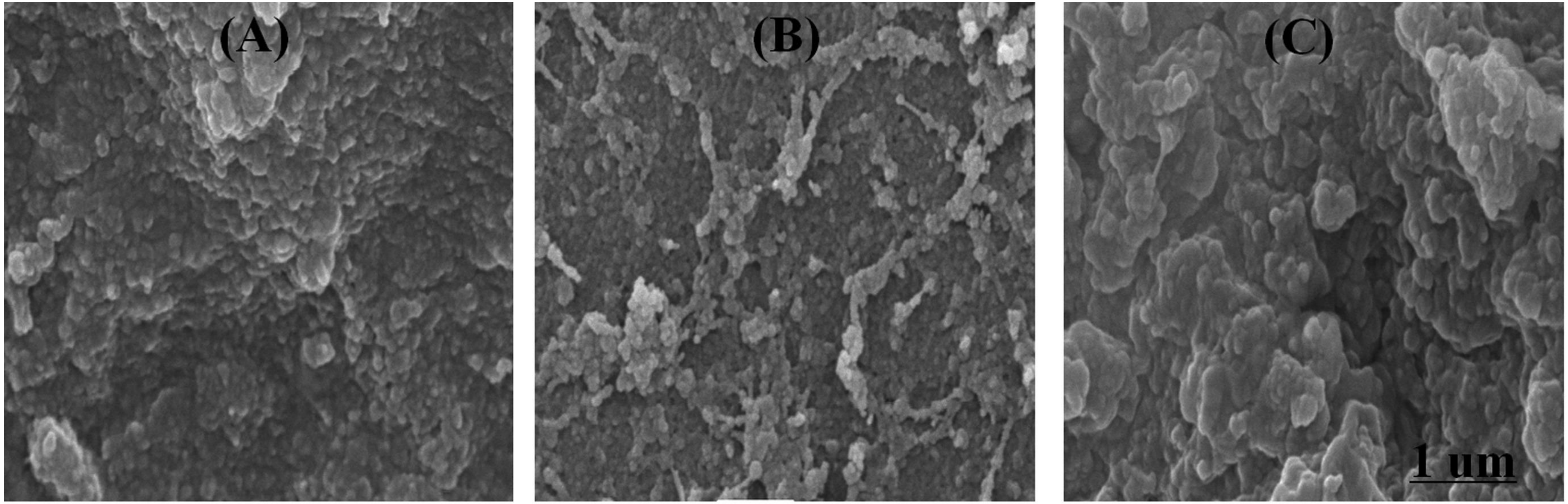
添加益生菌4周后，E3组淀粉酶活性与L3组比较有显着性差异

WSSV感染



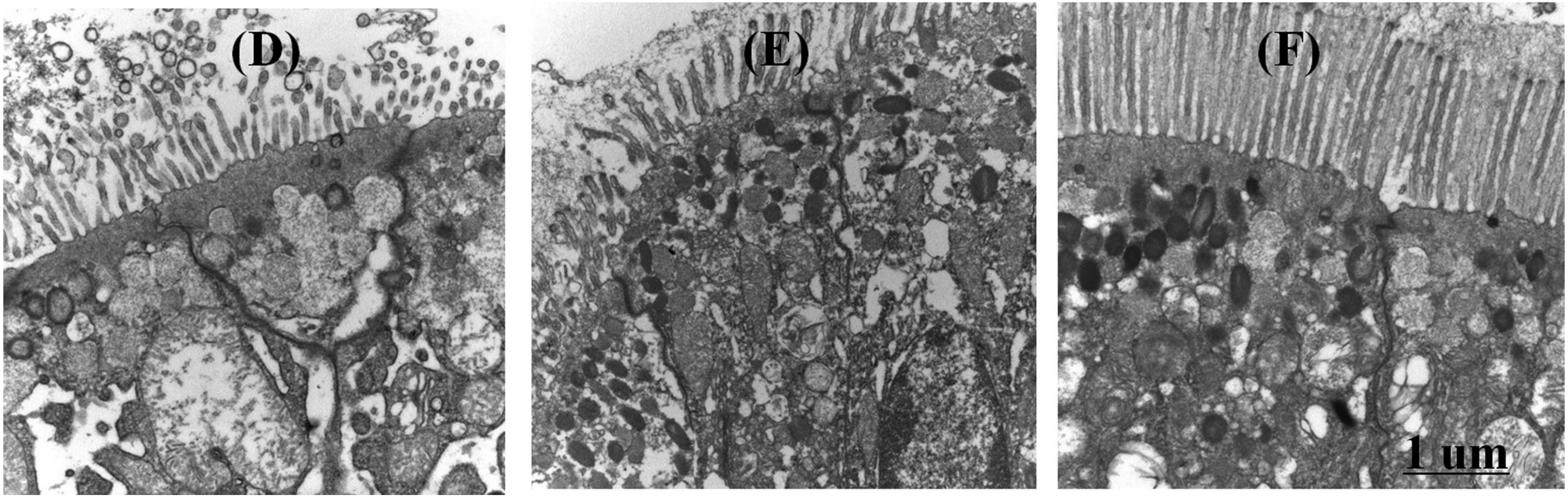
24小时内，对照组和益生菌组的累计死亡率基本相同。在36小时内有一个微小的差别。48 h后不同时间点，空白组虾的累积死亡率显著高于实验组。其中E3组的累积死亡率始终低于L3组。

Results

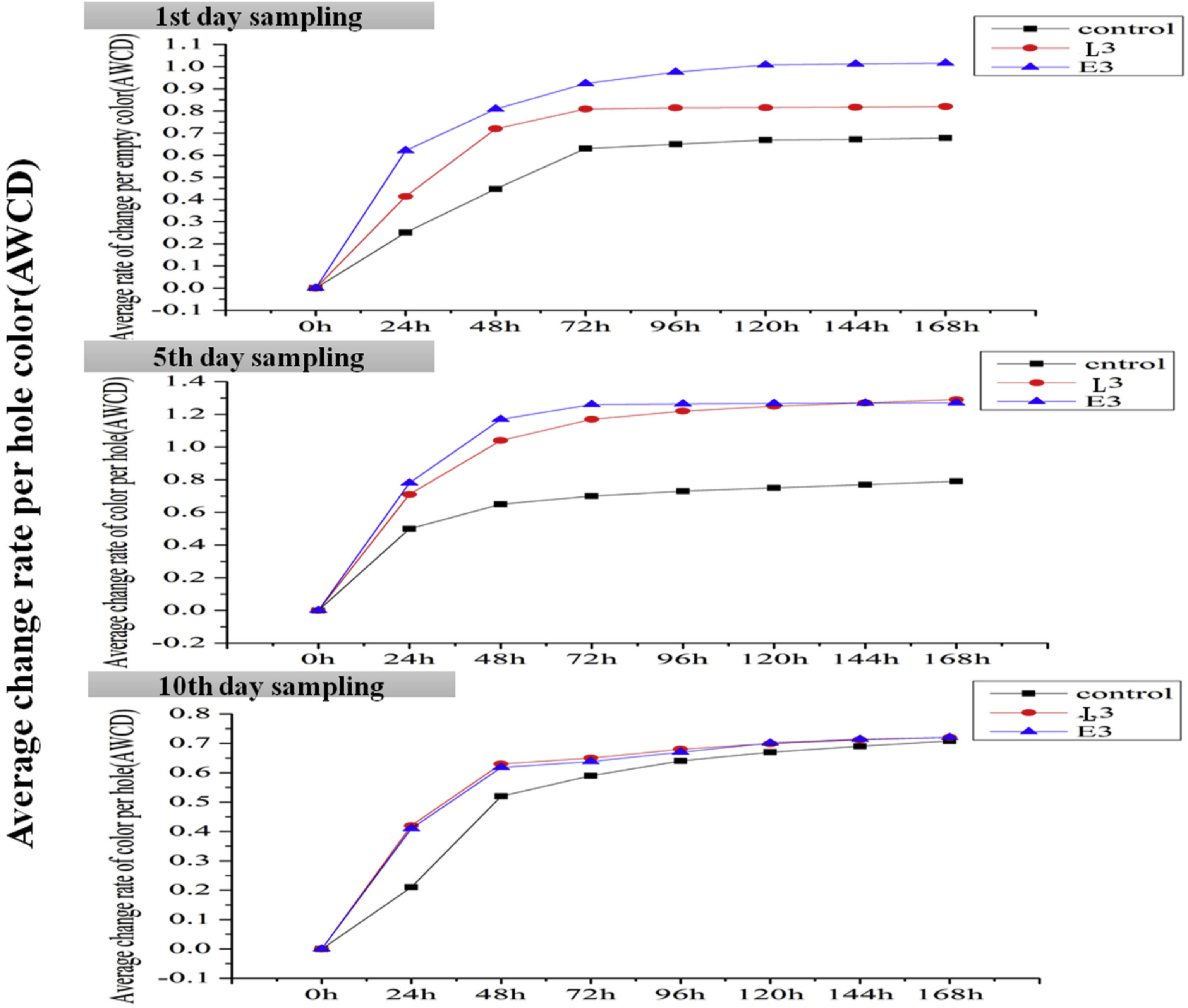


益生菌组的密度和折叠深度均高于对照组；表面有一系列的小凸起。

E3组的皱襞密度高于L3组。



益生菌组粘膜层密度均优于对照组。对照组上皮细胞内颗粒较少，益生菌组胞浆内充满高密度颗粒，呈活跃的分泌状态。其中E3组肠黏膜修复效果优于L3组。



AWCD curves of intestinal microflora in shrimp

E3组和L3组的AWCD(平均每孔颜色变化率)显著高于对照组。E3组明显高于L3组。在第5天，E3组和L3组的趋于一致，但他们仍然显著高于对照组，第10天，L3组、E3组和对照组对虾的微生物基本相同

Sampling time	experimental group	diversity indices		
		shannon index	simpson index	mcIntosh index
1- st day sampling	E3	3.050 ± 0.033*	56.697 ± 0.876*	6.046 ± 0.331*
	L3	3.413 ± 0.231	62.801 ± 0.221*	5.403 ± 0.562*
	control	3.363 ± 0.017	38.393 ± 0.289	4.012 ± 0.389
5- th day sampling	E3	3.155 ± 0.272 *	82.314 ± 0.223*	7.931 ± 0.291**
	L3	3.394 ± 0.013	79.949 ± 0.086*	8.091 ± 0.023**
	control	3.364 ± 0.103	69.328 ± 0.381	3.949 ± 0.048
10- th day sampling	E3	3.293 ± 0.031	91.556 ± 0.852	6.492 ± 0.212
	L3	3.304 ± 0.129	97.015 ± 0.496	6.474 ± 0.539
	control	3.308 ± 0.059	94.050 ± 0.344	6.324 ± 0.542

Note: * the difference between the experimental pool and the control pool was significant ($p < 0.05$), Values are presented as means ± standard deviation (n = 3).

凡纳滨对虾肠道内益生菌的持续时间至少为5d

04

Discussion

Discussion

- E3和L3能提高凡纳滨对虾肠道微生物活性，肠道菌群中碳的整体利用能力显著增强。E3和L3分泌的消化酶增强了对虾肠道的消化吸收能力，促进了虾的快速生长。这一结果进一步证明了消化酶活性测定和对虾生长测定的结果。
- E3和L3组血液中与免疫有关酶的活性提高。
- 用E3和L3饲喂28天后，肠道微生物多样性在前5天与对照组比较(包括Shannon、Simpson和McIntosh指数)有显著差异。
- 在添加益生菌后第10天，多样性差异逐渐减小，且趋于一致。提示益生菌对凡纳滨对虾肠道菌群的影响至少为5天。

Discussion

- 在饲料中添加E3和L3益生菌能提高肠道菌群的竞争力。改变肠道原菌群的数量和结构，促进对虾肠道微生物群落的复杂相互作用，进而在保护或促进对虾体质的正常方面发挥重要作用。
- 添加益生菌对肠道菌群的影响是周期性的，超过这个周期时，菌群的组成将恢复到原来的状态。为益生菌在水产养殖中的应用奠定了基础，饲喂益生菌的间隔时间不能超过其持续时间。

Thanks

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