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Lactobacillus rhamnosus lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism

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Probiotic treatment reduces appetite and glucose level in the zebrafish model

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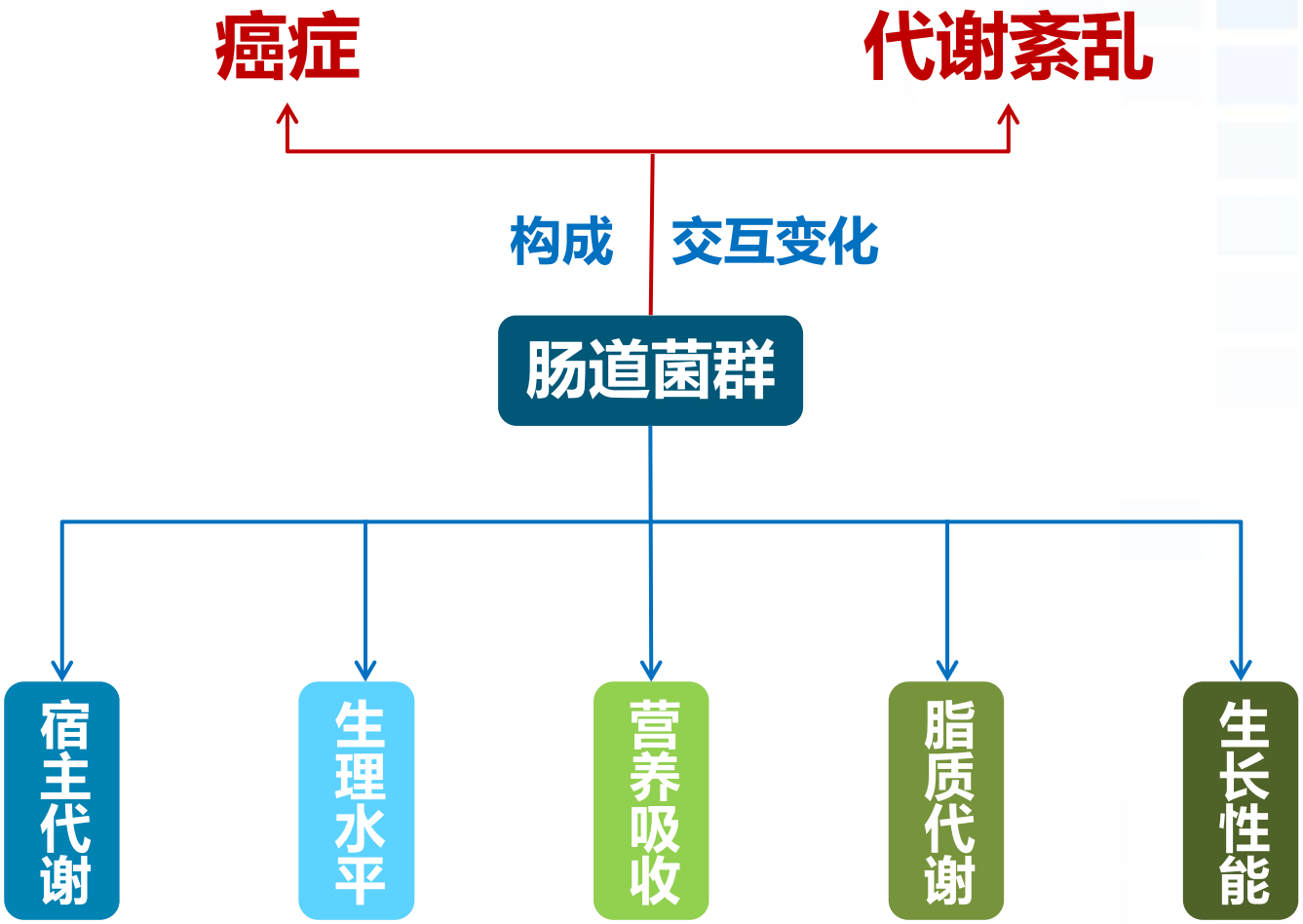
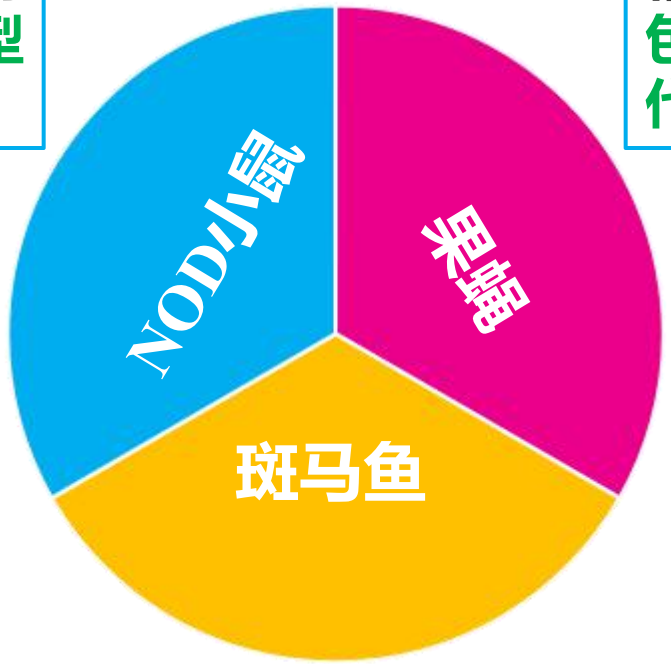
将斑马鱼幼鱼暴露在鼠李糖乳杆菌内 8 天后，通过高通量测序证明益生菌有调节胃肠道微环境的能力。而肠道微环境的改变分别会导致葡萄糖水平降低相关基因的上调，降低食欲和身体的葡萄糖水平。



研究集中于治疗和恢复肠道菌群的多样性后发现，通过益生菌来调节微生物成分后还可以改善宿主的营养代谢和能量平衡。

改变肠道微生物群落和代谢会改变宿主体内的**葡萄糖代谢**，并导致**1型糖尿病**的发生

宿主基因型和菌群紧密相关，因为**菌群能调节**包括甘油三酯和葡萄糖代谢在内的信号通路



益生菌调控肠道菌群可以通过参与胆固醇和甘油三酯代谢（**FIT2，agpat4，DGAT2，mgll，HNF4A，SCAP和CCK**）基因的下调来调节宿主的**脂质代谢**

糖代谢

食欲

- 胃肠（GI）菌群
- 基因转录水平

糖代谢相关基因

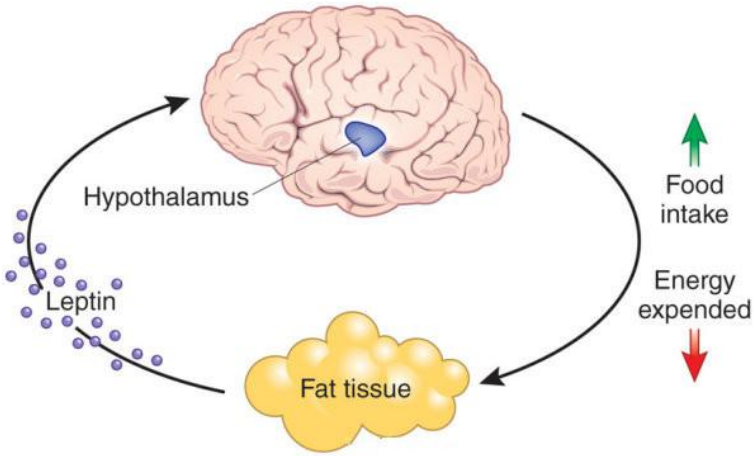
nucb2a → 抑制进食、抑制npy转录、增加胰岛素的释放

Glp-1 → 促进胰岛素释放、抑制胰高血糖素释放、提升饱腹感、减少食物摄取、减缓胃排空

insulin

goat → 启动摄食、增进食欲、增加营养物质尤其是脂类的吸收、促进脂肪形成等多方面的作用，分泌过多可导致代谢紊乱和肥胖

食欲相关基因



黑素皮质素受体4，在控制食欲和体重稳态中起关键作用。

mc4r

食欲减退

促进食欲

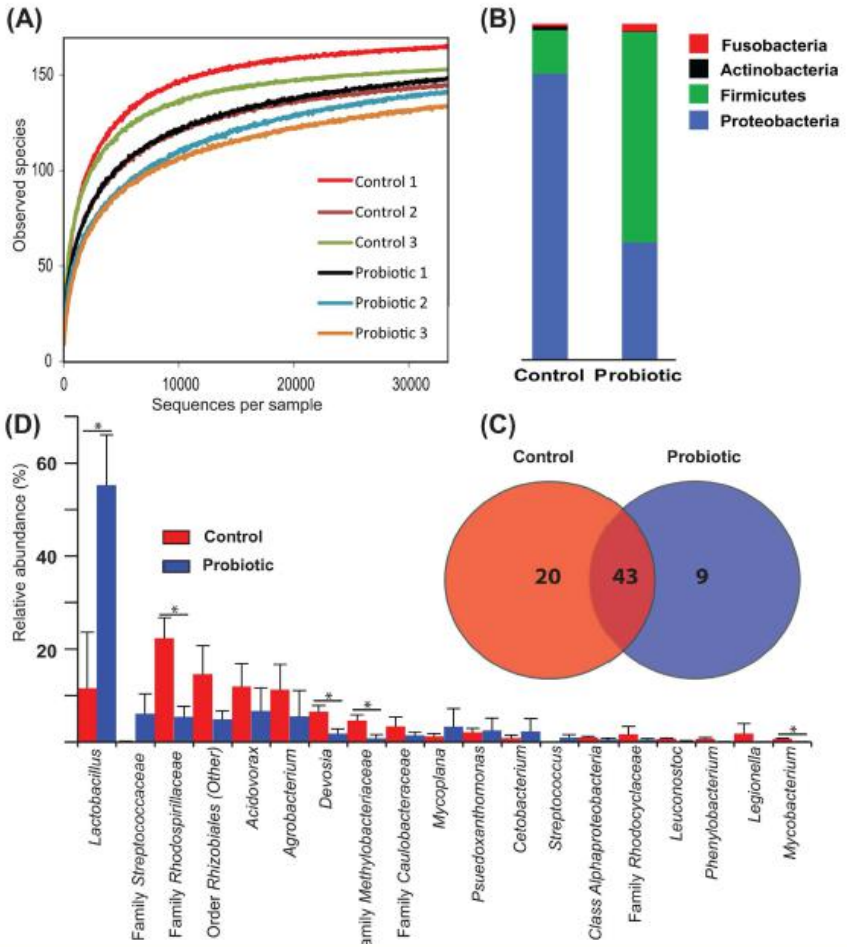
cb1

npy

大麻素受体1，
食物摄取-体重增加

NPY最主要的作用是增加食物的摄入，降低饱食动物的产热效应。

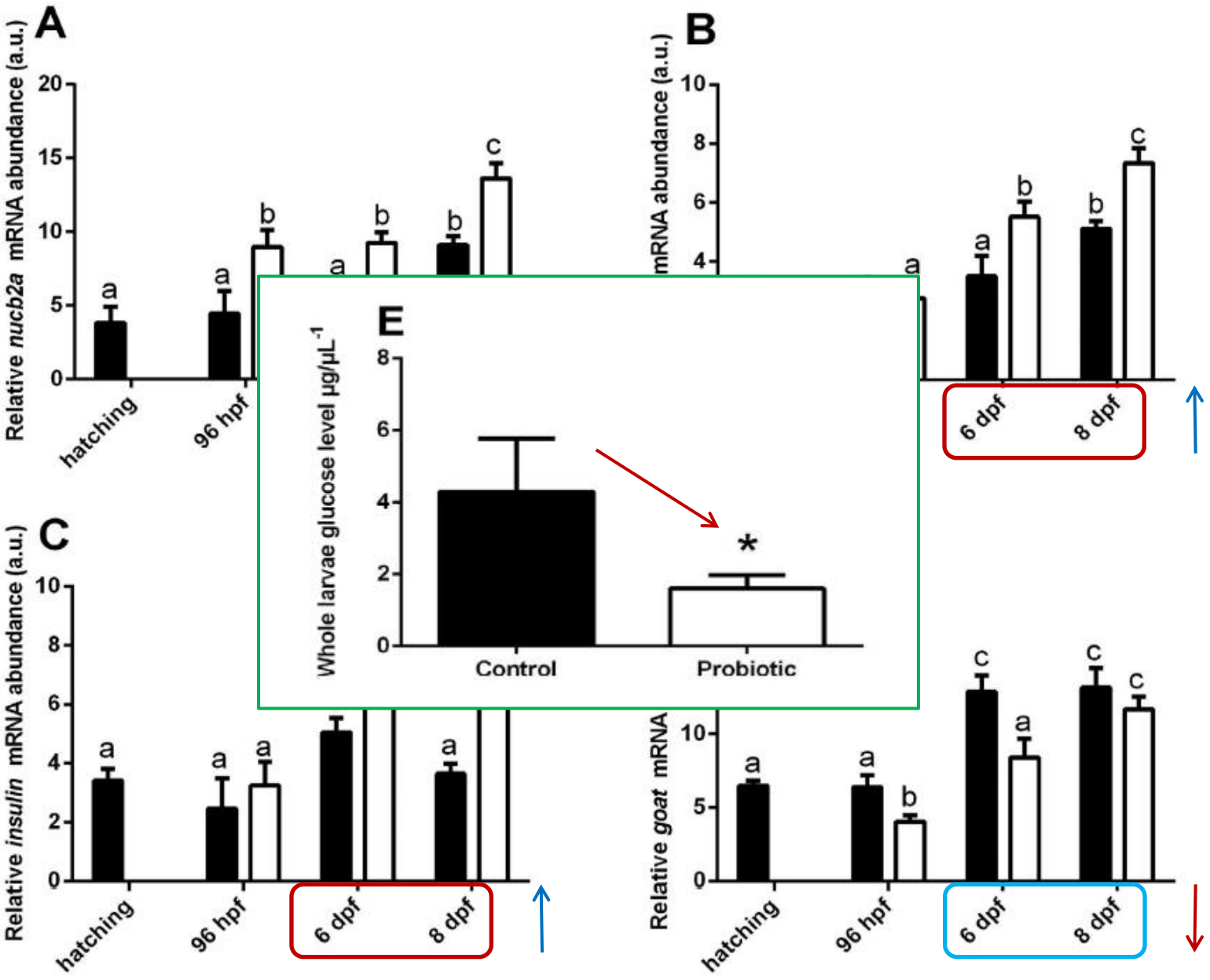
L. rhamnosus modulates the GI microbiome.



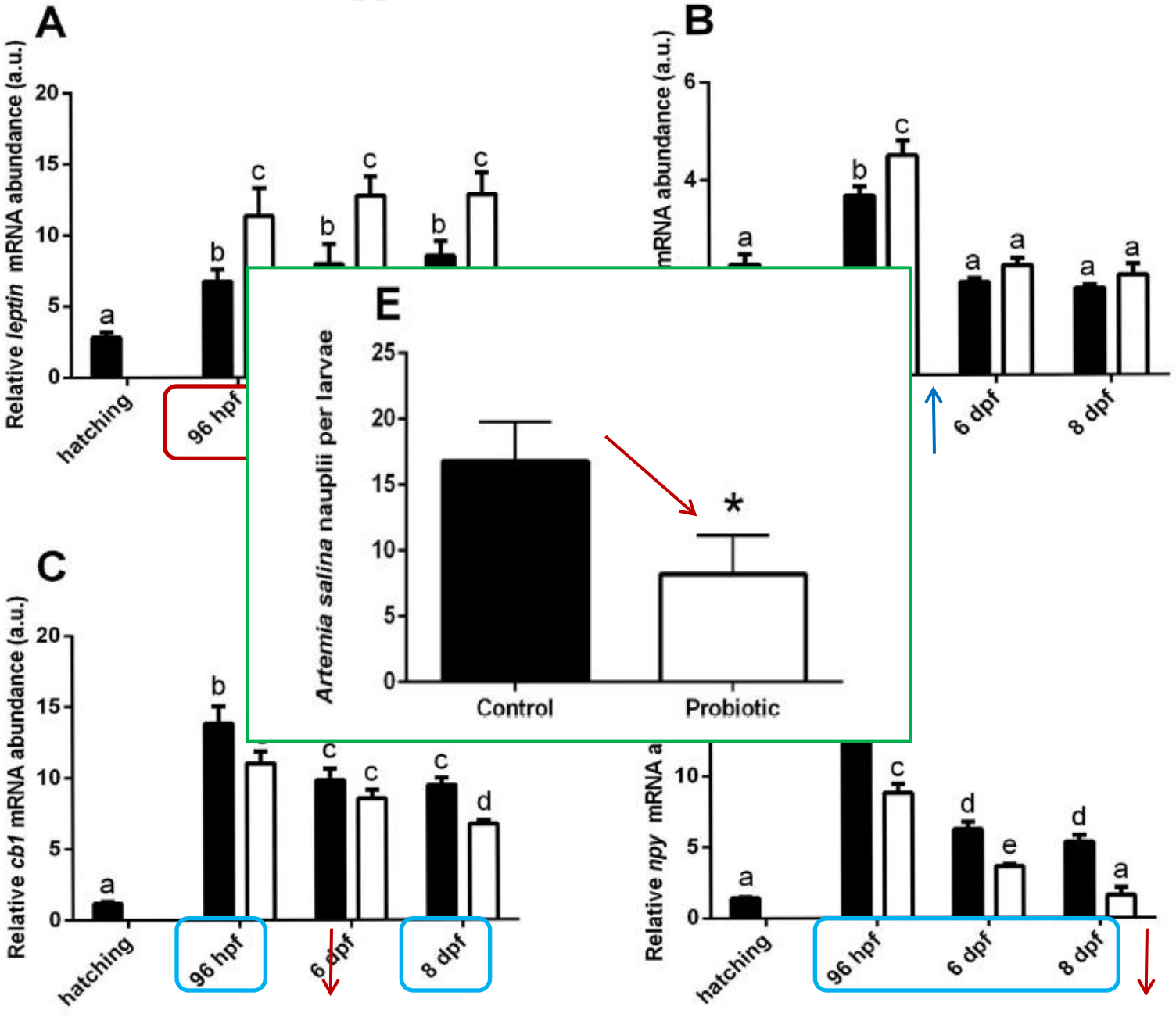
	Observed species	Chao1	Shannon	Phylogenetic diversity	Good's coverage
Control	164.25 ± 7.69	174.14 ± 6.37	4.68 ± 0.21[b]	4.52 ± 0.15	0.9997 ± 0.0000[b]
Probiotic	155.09 ± 5.81	167.23 ± 3.52	3.23 ± 0.65[a]	4.30 ± 0.22	0.9995 ± 0.0001[a]

Table 1. Alpha diversity metrics of observed species, Chao1, Shannon's diversity, phylogenetic diversityand Good's coverage of zebrafish larvae of 8 dpf (mean ± s.d.; n = 3).

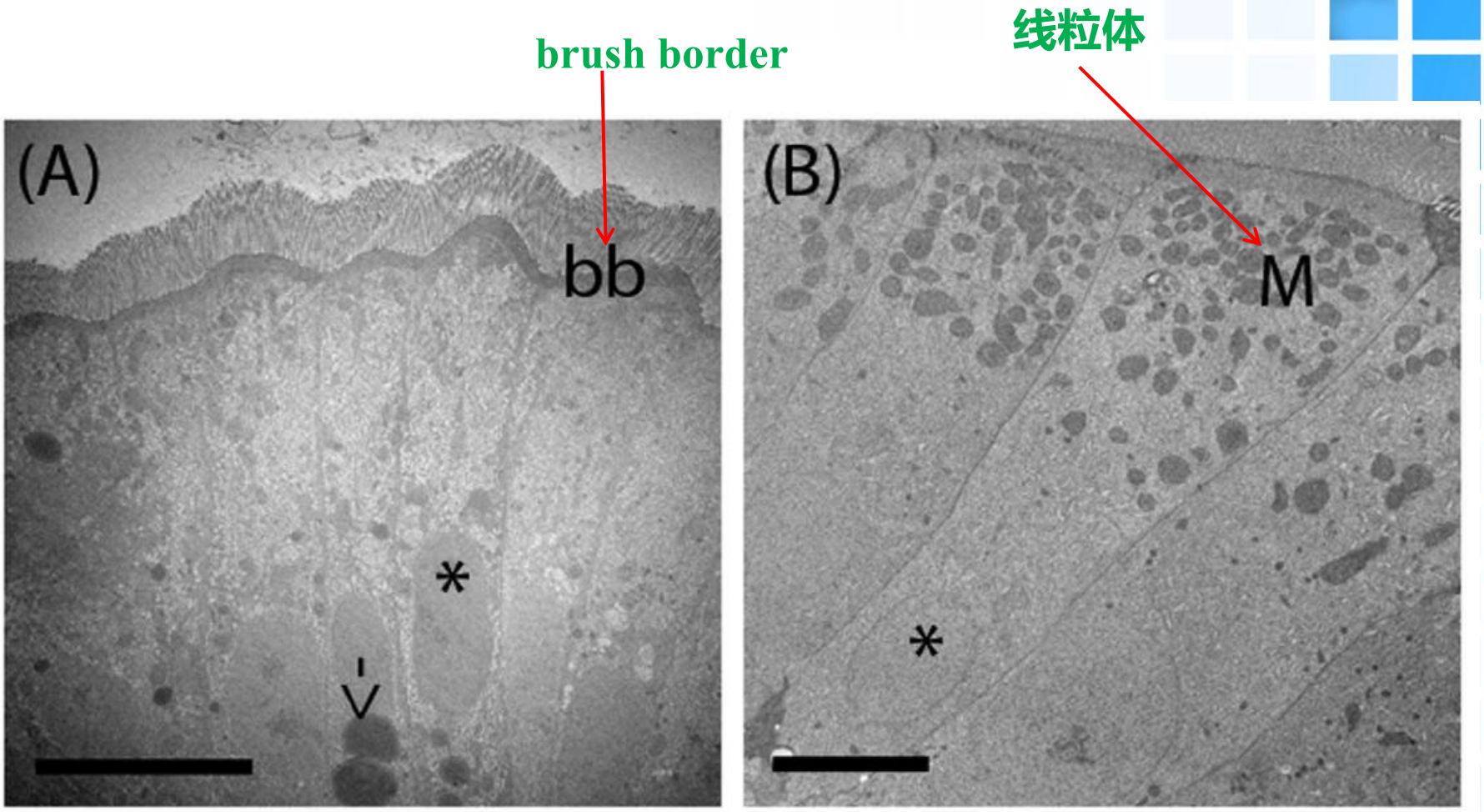
Probiotic treatment modulates the expression of genes involved in glucose metabolism and reduces the total glucose levels in zebrafish larvae.



Probiotic treatment modulates the expression of genes involved in appetite control and decreases appetite in zebrafish larvae.



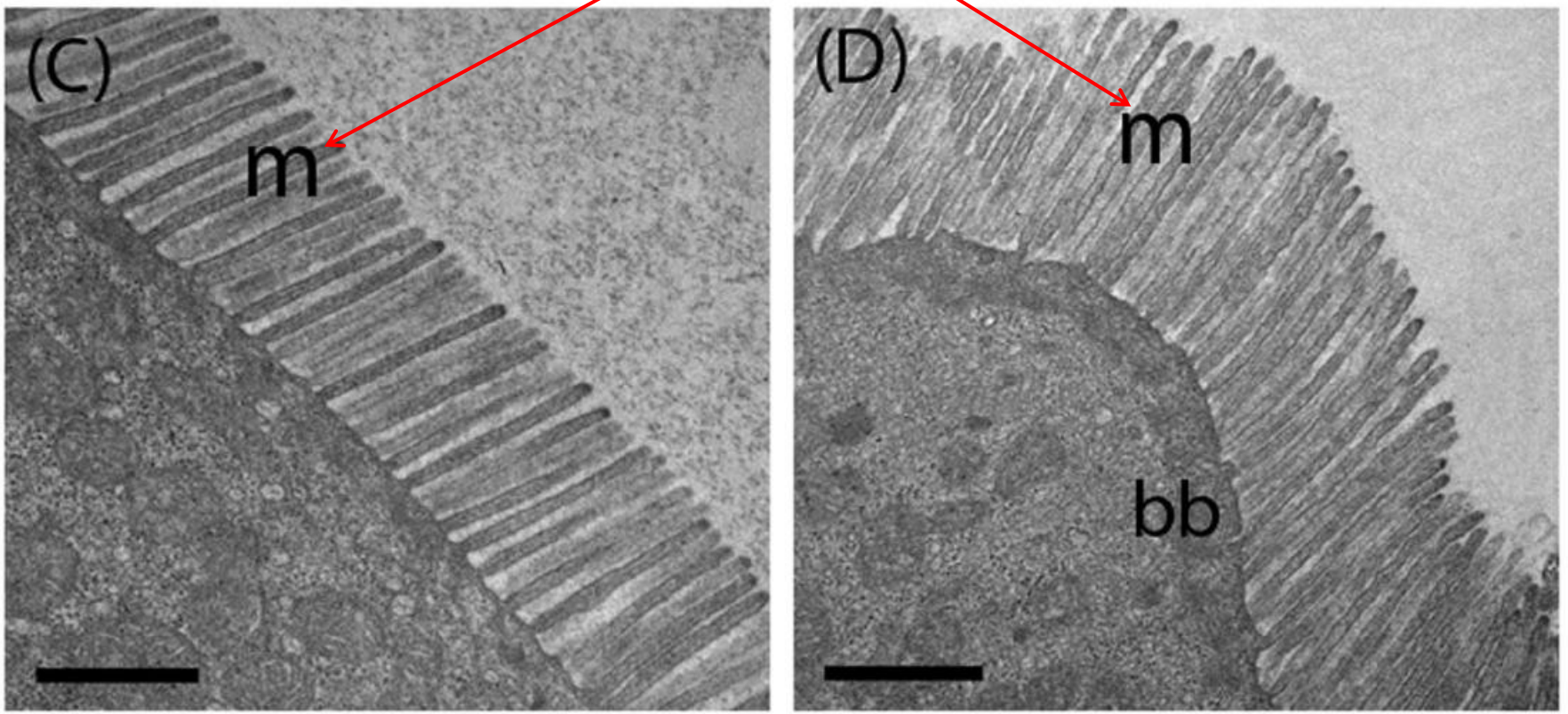
Ultrastructural analysis of zebrafish gut exposed to L. rhamnosus evidenced an increase of absorptive surface area.



柱状上皮细胞

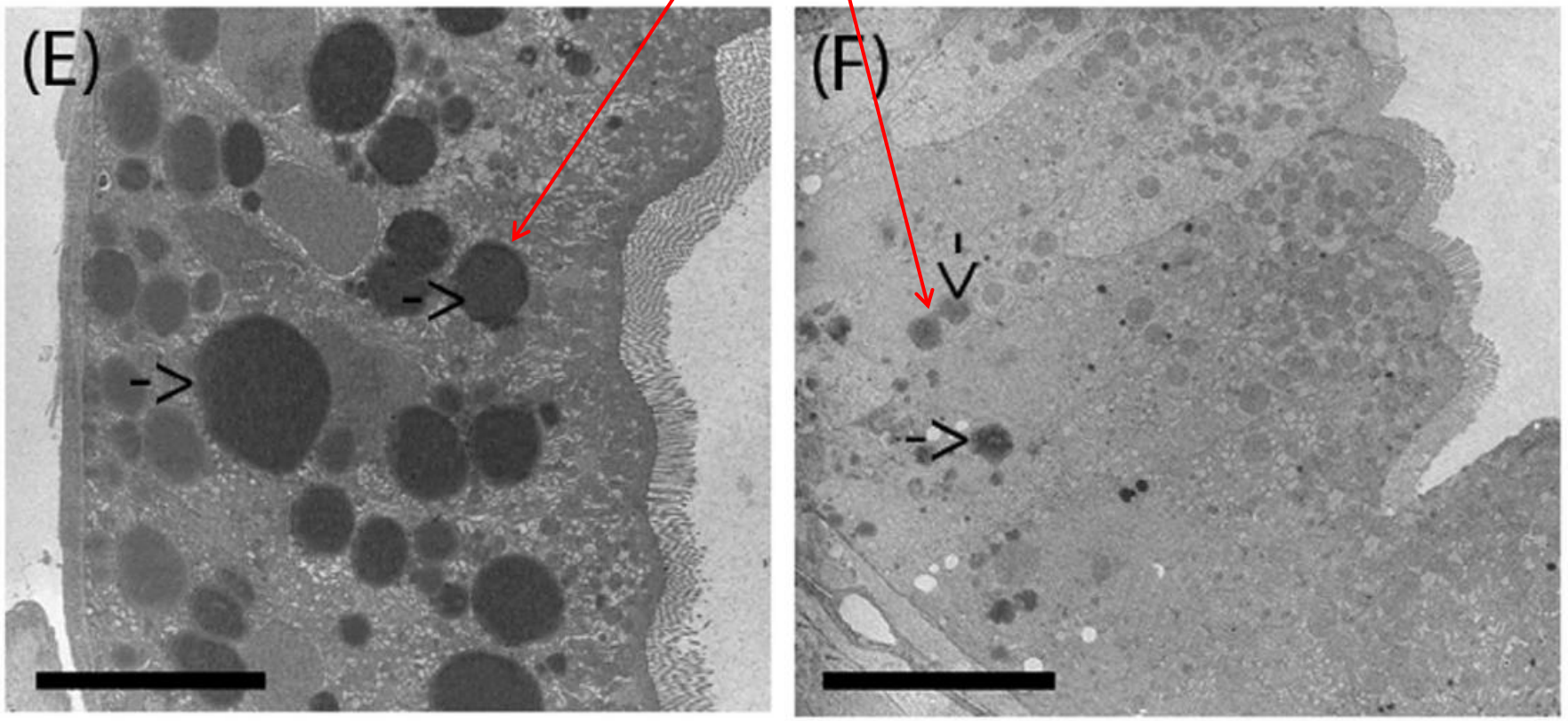
Enterocytes length	34.92 ± 2.34 μm	42.54 ± 1.48 μm	0.0001

微绒毛



Microvilli length	0.91 ± 0.07 μm	1.02 ± 0.08 μm	0.008

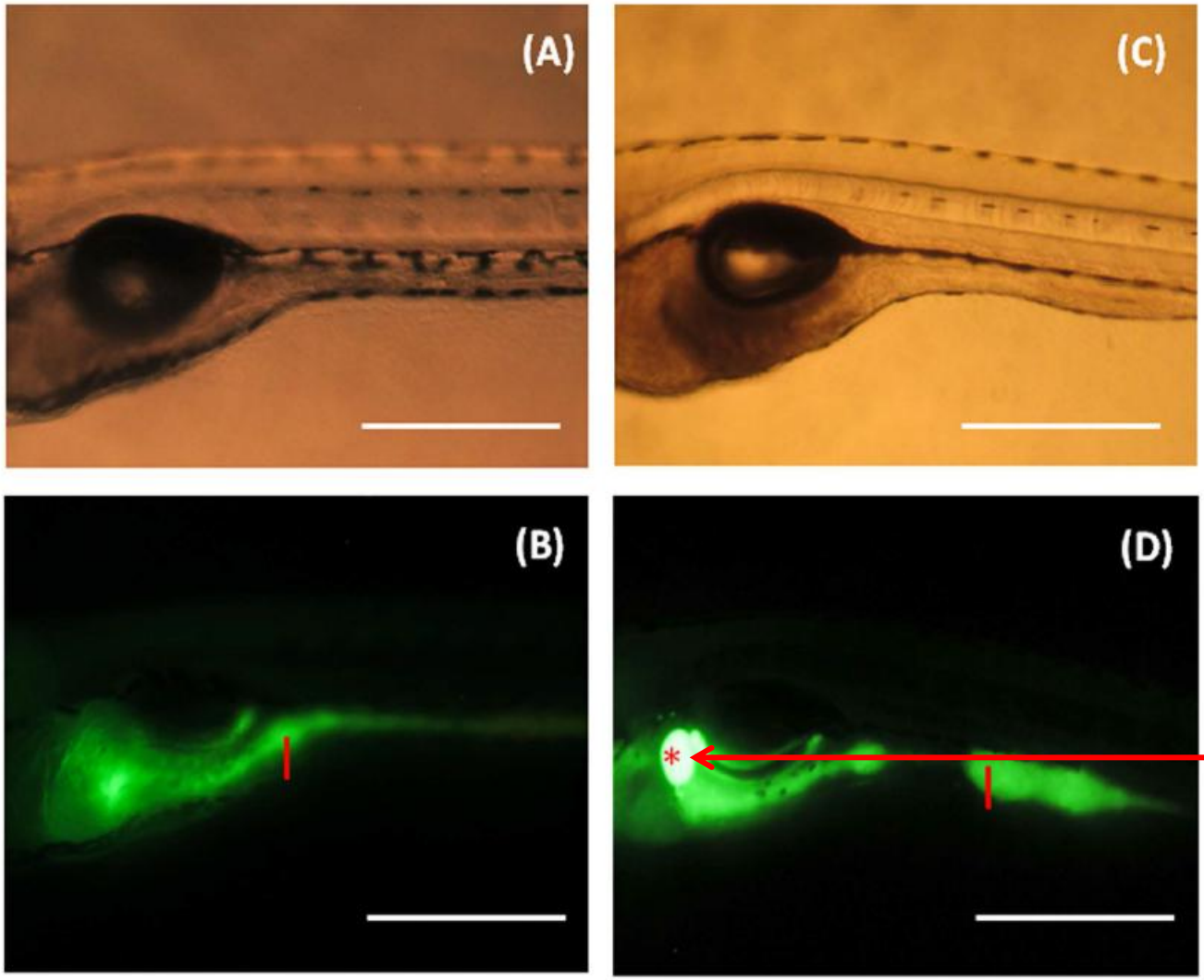
脂滴

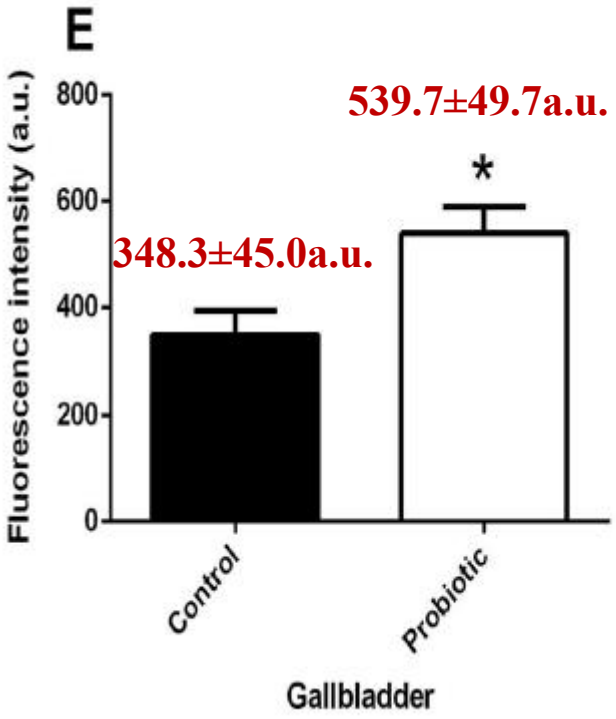


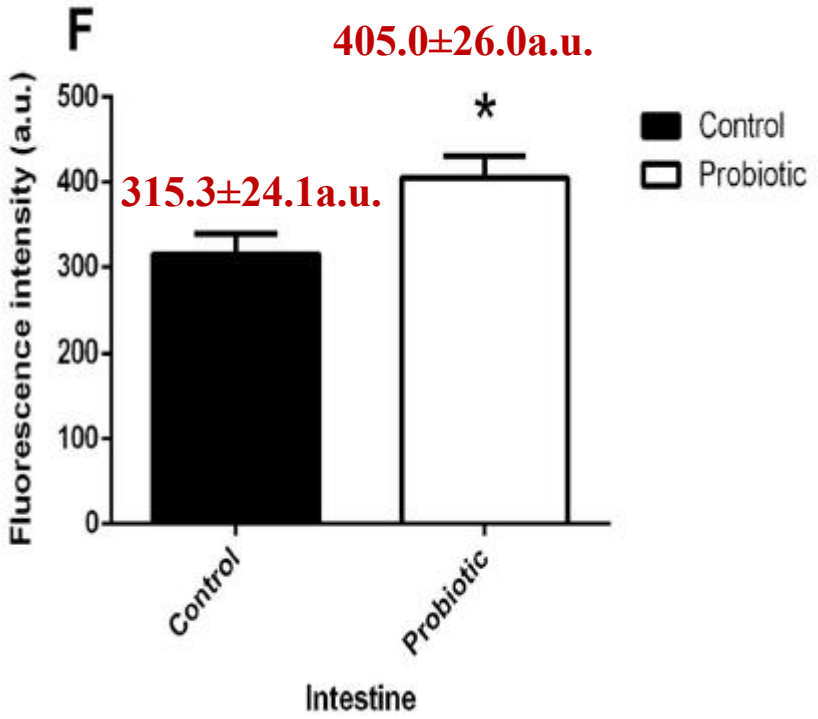
细胞质

Lipid droplets diameter	4.75 ± 1.12 μm	2.02 ± 0.72 μm	0.0001

In vivo short chain fatty acids localization revealed accumulation in the intestine and gallbladder of probiotic treated zebrafish.




E
Fluorescence intensity (a.u.)
800
600
400
200
0
539.7±49.7a.u.
*
348.3±45.0a.u.
Control
Probiotic
Gallbladder


F
Fluorescence intensity (a.u.)
500
400
300
200
100
0
405.0±26.0a.u.
*
315.3±24.1a.u.
Control
Probiotic
Control
Probiotic
Intestine

P=0.04

将斑马鱼幼鱼暴露在鼠李糖乳杆菌内8天后，通过高通量测序证明益生菌有调节胃肠道微环境的能力。

而肠道微环境的改变分别会导致：

葡萄糖水平降低相关基因的上调，

降低食欲和身体的葡萄糖水平。

结果：

1. 强调了益生菌调制肠道微生物群落的能力；
2. 提供洞察益生菌相互作用如何调节参与葡萄糖代谢和食欲控制基因网络的渠道；
3. 表明了鼠李糖乳杆菌作为食物摄入失调和糖耐量降低治疗方式的可能性。

Material and Methods

1. 试验动物、益生菌、养殖取材流程

成年雌雄斑马鱼

产卵

斑马鱼幼体

养殖温度：27.0± 0.5℃

光照周期：12h：12h昼夜循环

对照组

实验组

养殖温度：27.0℃

光照周期：12h：12h昼夜循环

饲料配方：43.00% 粗蛋白，8.30% 粗脂肪，8.10% 灰分，1.9% 粗纤维， 8.00% 水分.

益生菌种类：*L. rhamnosus* IMC 501H (C025396A; Synbiotec, Camerino, Italy)

添加方式：10^6 colony-forming units (CFU)浓度的菌液直接添加到养殖水体中

分组情况：来自同一养殖池中的斑马鱼幼体，随机分到6个养殖单元中，对照组设3个重复，实验组设3个重复。**并且整个养殖实验重复3次。**

取材过程：分别于孵化后96h，6d，8d进行取材；

MS222 (100 mg·L^{-1})作为麻醉剂；样品存于-80℃；

① RT-PCR分析；

② 形态学分析；

③ 8dpf 的样品，用于分子鉴定，分析基因表达的变化情况；

高通量测序、葡萄糖水平、摄食量、TEM、BODIPY 染色等。

2、DNA提取和PCR

3、高通量测序分析

4、RNA提取和cDNA合成

5、Real time PCR

6、Feed intake

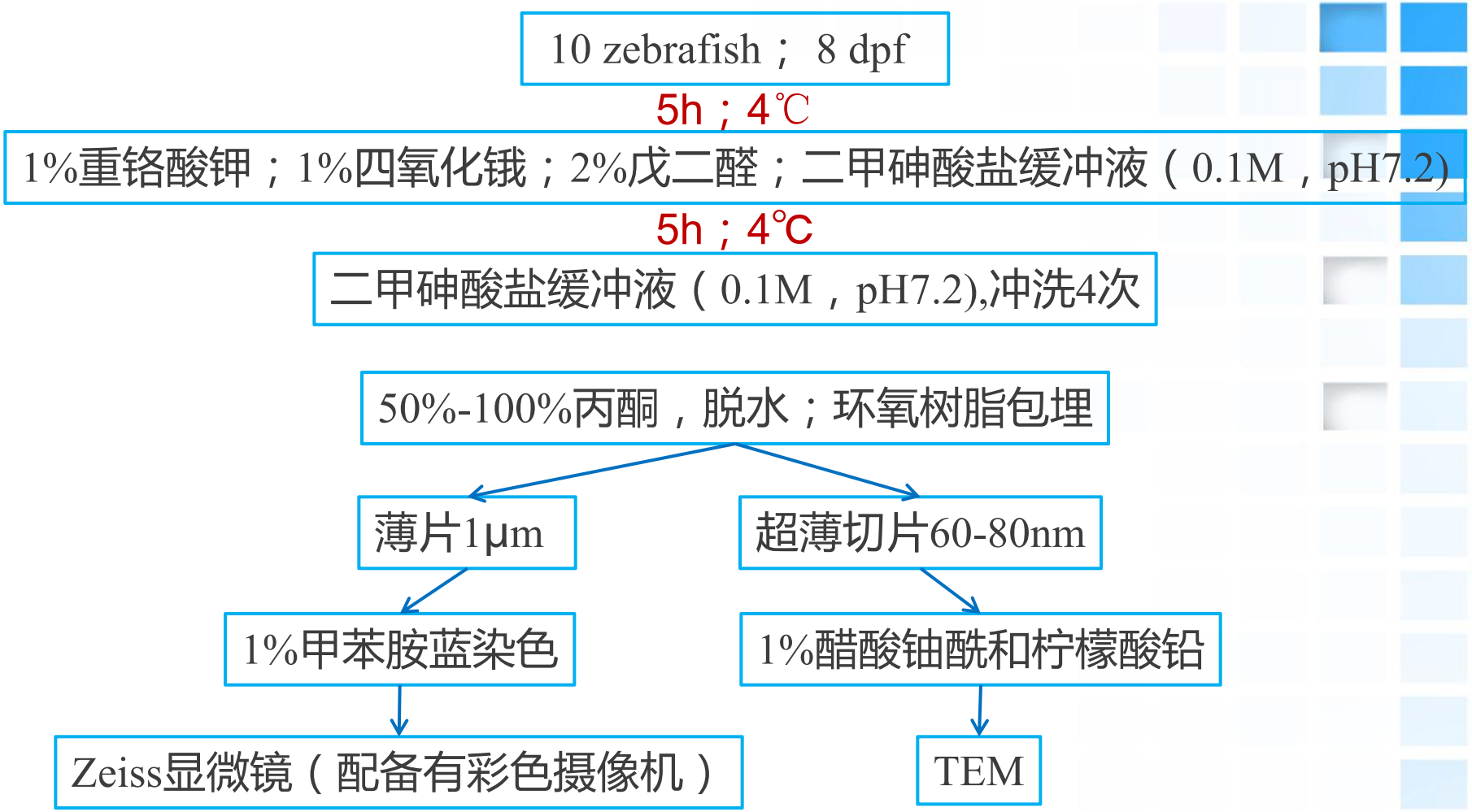
Feed intake. Zebrafish larvae were maintained in tanks at 27.0 ± 0.5 °C under a 12:12 hours light:dark photoperiod. At 8 dpf, 11 zebrafish larvae per group were fed newly-hatched *Artemia salina* nauplii (approximately 400 µm) with the same concentration of *A. salina* (7 *A. salina* per µL). *A. salina* were cultured daily from cysts (*Artemia* Cysts, INVE, Thailand). Zebrafish larvae were fed for 6 min with *A. salina* nauplii, and the food intake activity of each single larvae was monitored with the aid of a Stemi 2000 micrometric Microscope (Zeiss Vision Italia, Castiglione Orona, Italy) and *A. salina* intake per larvae were counted.

7、Larval glucose level

Larval glucose level. Total larvae glucose levels were determined from 4 pools of 15 larvae per treatment at 8 dpf, using an enzymatic kit that detects D-glucose (D-Fructose and D-Glucose, Megazyme, Ireland) following the manufacturer's protocol. The concentration of each sample was determined with a spectrophotometer SHIMADZU UV-1800 (Shimadzu Scientific Instruments, USA).

The final glucose concentration was correlated to the initial pool larvae weight and finally a measurement units conversion from gL^{-1} to $\mu g \mu L^{-1}$ and related to 1 mg of pool larvae.

8、Transmission Electron Microscopy (TEM)



9、BODIPY 505/515 染色实验

10、 统计学分析

思考

糖代谢和控制食欲的相关基因

TEM


THANK