不同品种猪肠道菌群结构和肠道发育模式在无菌小鼠上的传递性

Intestinal microbiota can transfer host gut characteristics from pigs to mice

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主要内容
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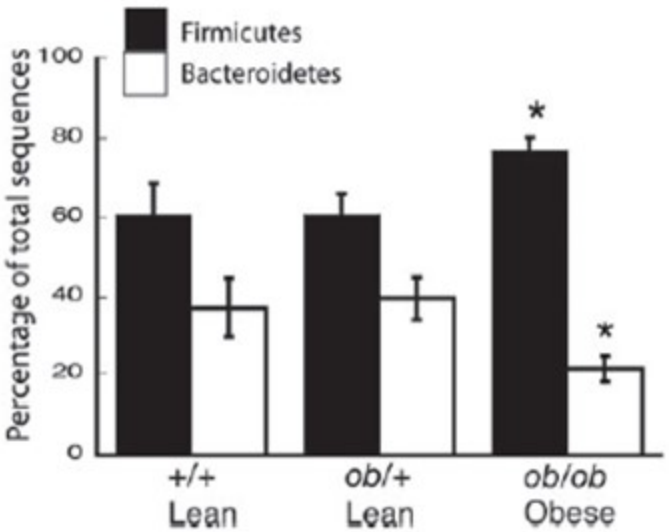
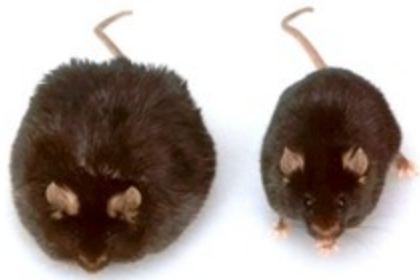
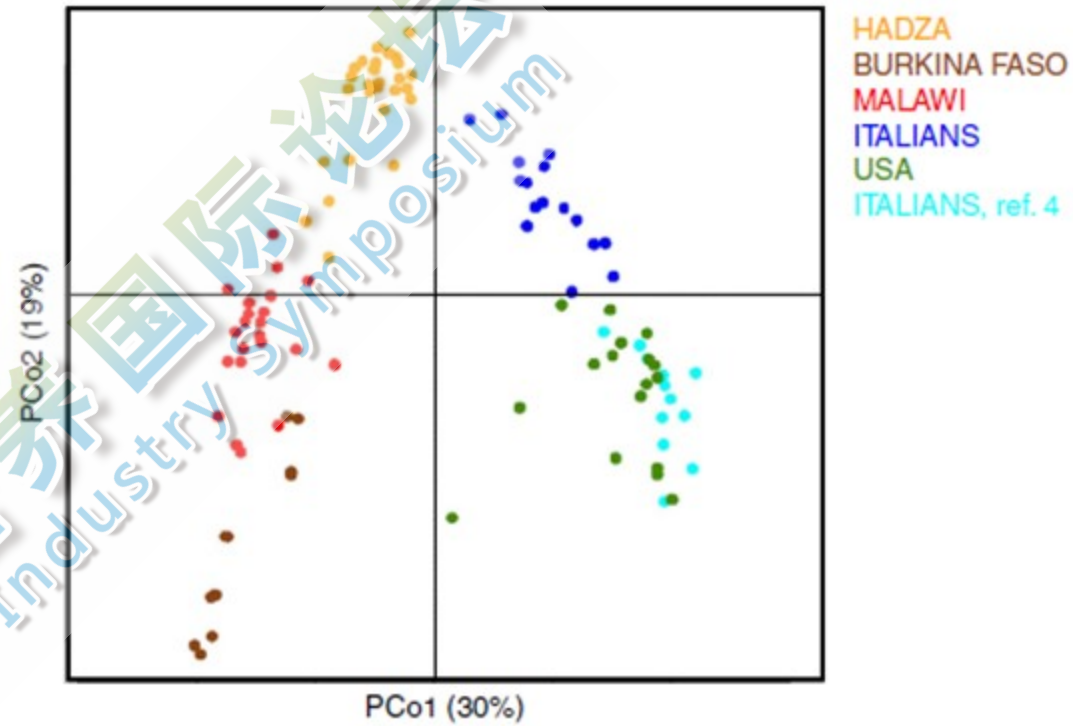
前言 Introduction



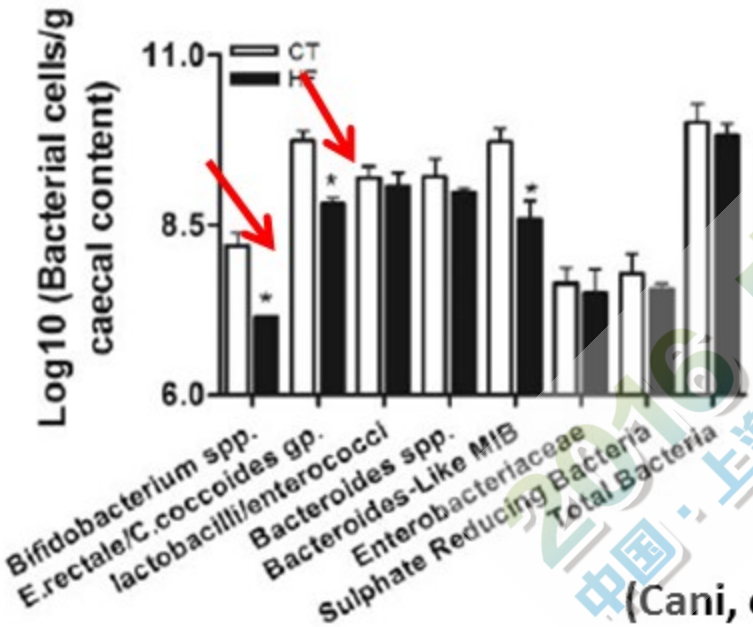





(Gordon, et al. 2005)



（Schnorr, et al. 2014）



(Cani, et al. 2007)

表型不同---微生物不同

Different phenotype---different microbiota

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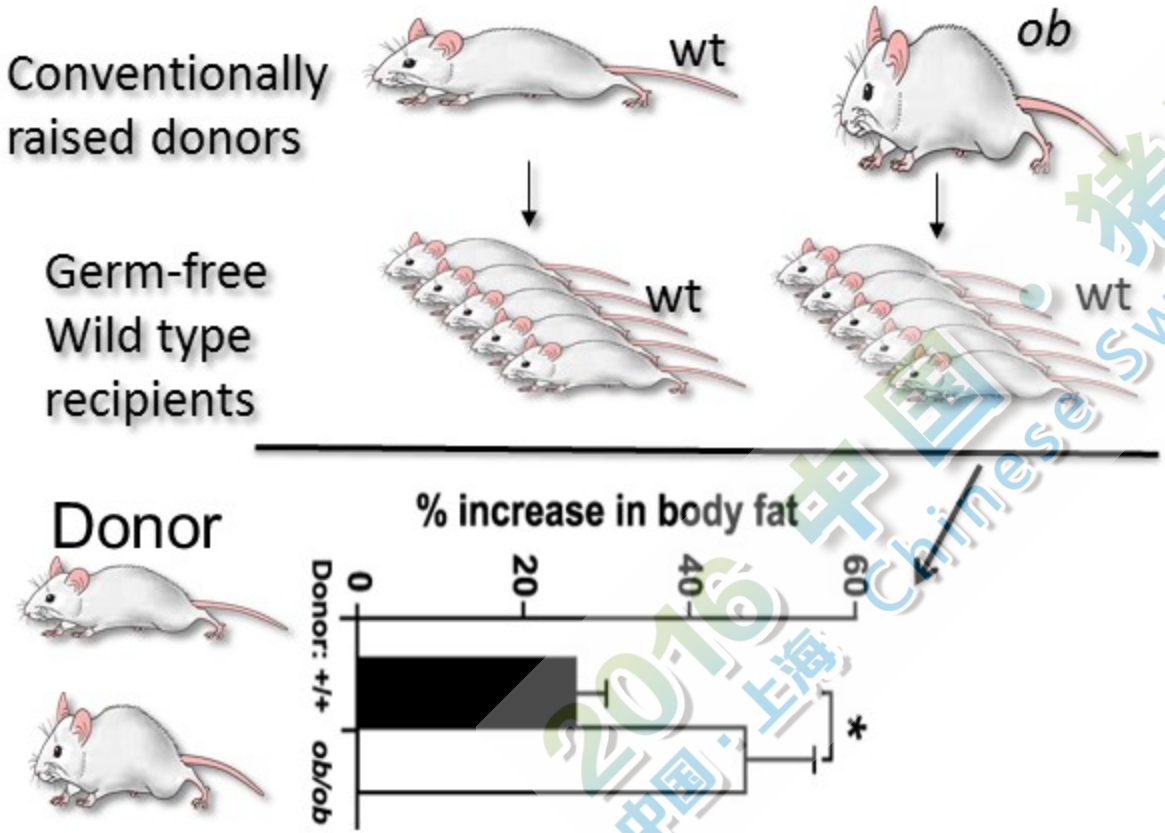
Probiotic pioneer: Lawrence Brandt is one of the leading proponents of fecal transplantation.

"You're transfusing bodily fluid from one person to another."

(Smith, et al. 2013)

粪便移植 FMT: Fecal microbiota transplantation



(Turnbaugh, et al. 2006)

Gut microbiota

菌群移植可以使表型在个体间和物种间传递。

Gut microbiota can transfer different phenotypes in individuals and species.

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FMT　动物生产---起步阶段 Animal production----starting stage

肠道疾患
Intestinal diseases

应用 Application

- 难辨梭菌 Clostridium difficile infection
- 肠炎型疾病 Inflammatory bowel diseases
- 过敏性肠综合征 Irritable bowel syndrome

FMT

肠道发育
Intestinal development

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不同品种猪

Different breeds of pigs

肠道表型

Gut characteristics

传递性 **Transitivity**

无菌鼠

Germ-free Mice

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材料与方法 Materials and Methods




试验设计 Experiment design

1) 大白猪源菌群小鼠

Yorkshire flora-associated mice (YFM)

2) 藏猪源菌群小鼠

Tibetan pig flora-associated mice (TFM)

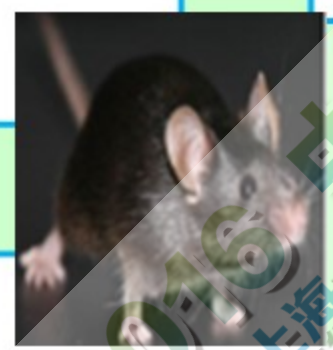
3) 荣昌猪源菌群小鼠

Rongchang pig flora-associated mice (RFM)

大白猪 Yorkshire（YP）

供体猪 Donors:

5 pigs each　56 d



1d无菌小鼠
1 d GF mice

每个处理8只小鼠

8 mice each

28 d

藏猪 Tibetan pig（TP）

荣昌猪 Rongchang pig（RP）

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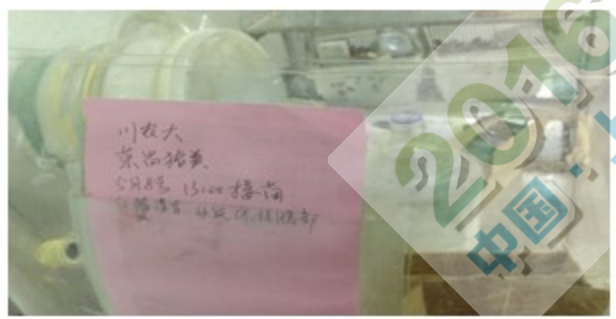
饲养管理 Animals and housing

所有猪只单笼饲养于温度可控的环境中，自由采食及饮水。
All pigs were were housed in individual metabolic cages in an environmentally controlled room that allowed ad libitum access to water and diet.



无菌BALB/C小鼠及日粮由解放军第三军医大学实验动物科学中心提供。
Germ-free BALB/C mice and the diet of mice used in this study were provided by the Department of Laboratory Animal Science of the Third Military Medical University.



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粪便移植
Fecal microbiota transplantation

对1日龄的BALB/C乳鼠灌胃接种0.05 mL（1.4 CFU×10^7）粪便悬液，并将2 mL粪便悬液涂抹于代乳鼠皮肤和毛发上，接种后的小鼠按照无菌鼠的饲喂方式进行饲养。

Germ-free mice were inoculated by intragastric gavage with 0.05 mL feces suspension, and 2-mL aliquots were spread on the fur of each germ-free foster mouse. These mice were maintained in the same manner as germ-free mice.

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无菌小鼠存活率
Survival rate of GF mice

初生GF小鼠接种菌群后存活率较高。

The new born germ-free mice inoculated by feces suspension is an ideal model with good Survival to our study.

通过预先置于隔离器中的培养基是否变色污染来判断菌群的转入情况。

Judge whether the microbes were transplanted successfully by observing the medium in isolators.

成功！
Success!

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样品采集 Samples collection

- 粪便样品 Fecal samples
- 十二指肠、空肠和回肠 Duodenum, jejunum and ileum

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测定指标 Measurements

- ✓肠道微生物 Microbiota analysis (16S)
- ✓肠道指数 intestinal index (Relative length, density and weight of intestine)
- ✓肠道形态 Intestinal morphology (Villus height and crypt depth)
- ✓酶活 Enzyme activity (amylase, lipase,, trypsin, lactase, sucrase, maltase, AKP, Na^+, K^+-ATPase, Ca^+, Mg^+-ATPase and γ-GT)
- ✓空肠抗氧化能力 Jejunal antioxidant capacity (MDA, SOD and T-AOC)
- ✓基因表达 Gene expression: CDX2, EGF, GLP-2, ANG-4, IGF-1, IGF-1R, SGLT-1, GLUT-2, SLC7A1, DMT1, ZnT1, MUC1, MUC2, ZO-1, Occludin

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数据统计与分析 Statistical analysis

基因表达 Gene expression: 2^{-DDCT}

数据分析 Data analysis: SAS version 8.2, GLM & Duncan's test for groups difference ($P<0.05$)

数据呈现方式 Data present: means with SEM

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试验结果Results




微生物分析Microbiota analysis （16S）

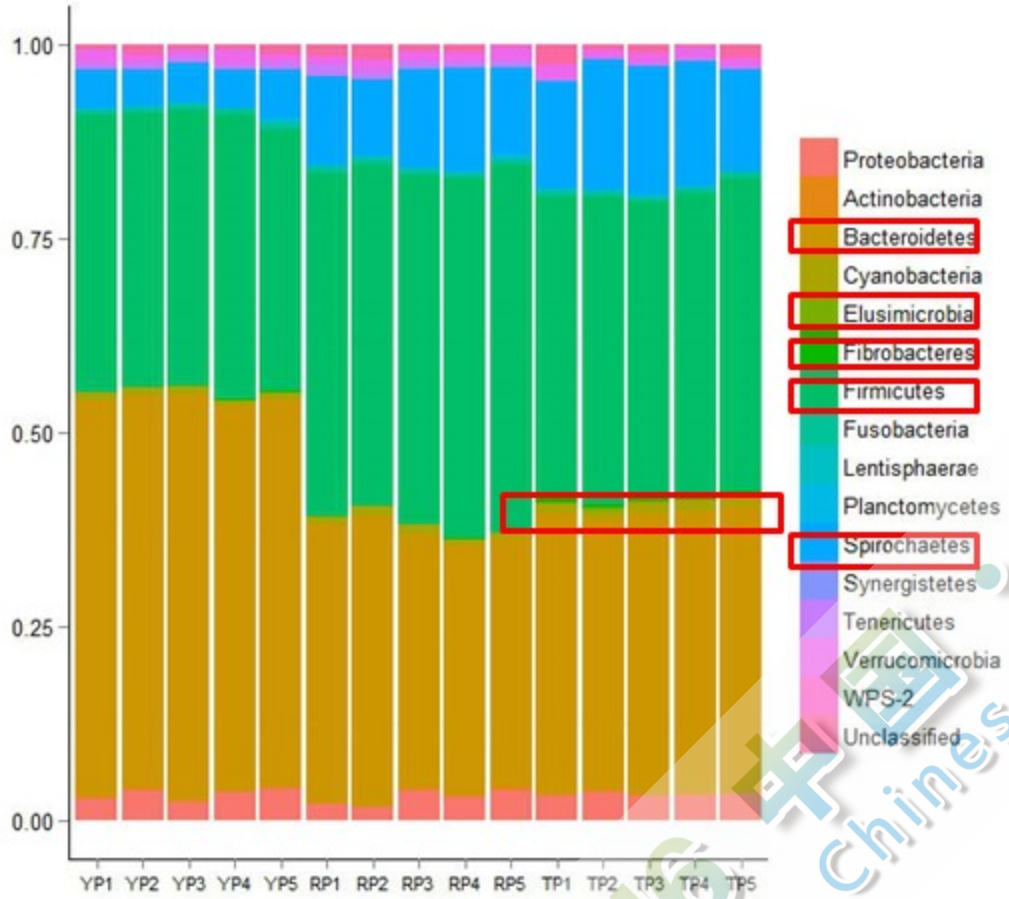

图1 16S rRNA分析藏猪、大白猪和荣昌猪粪便微生物在门水平上的差异

Fig.1 16S rRNA gene analysis reveal phyla level differences in microbiota of TP, YP and RP

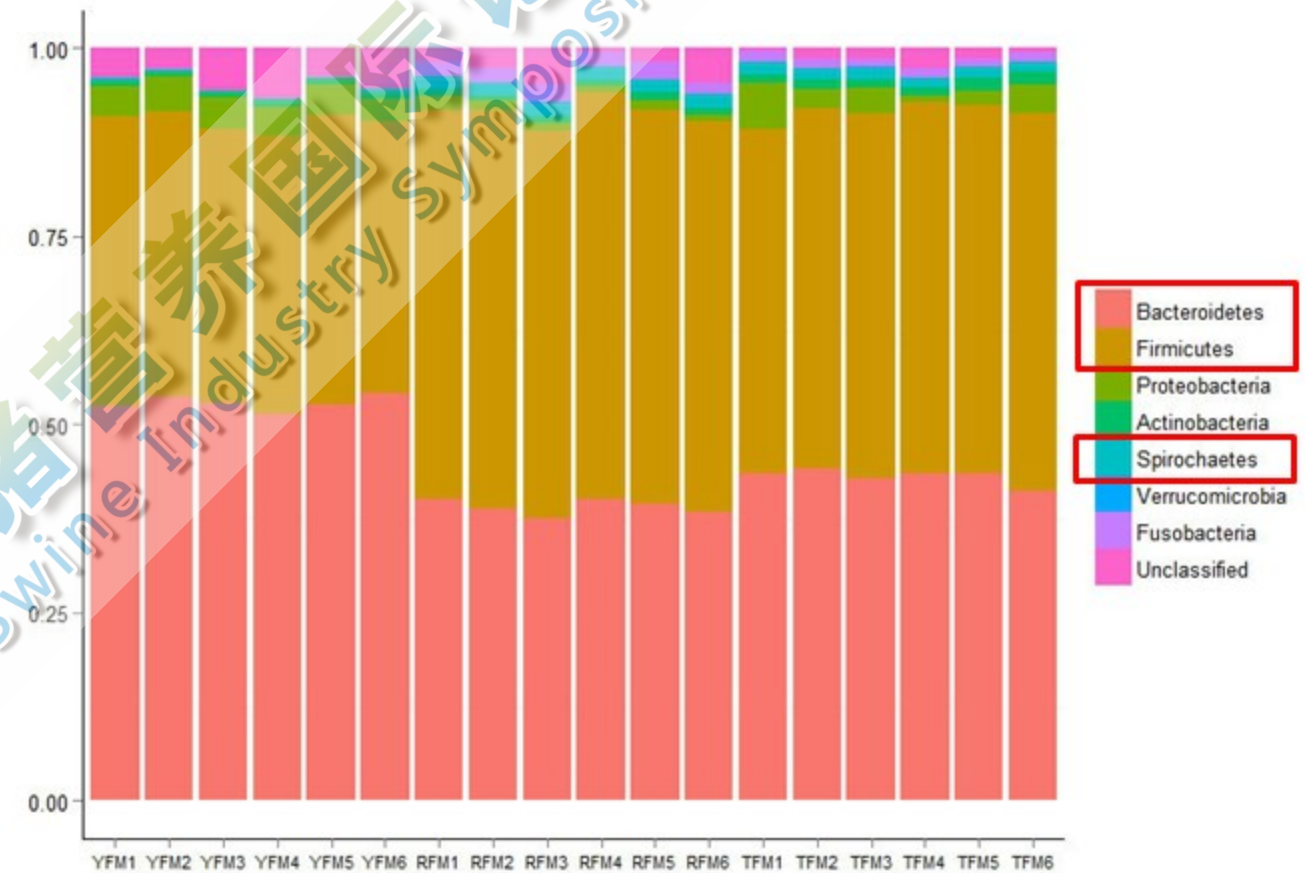

图2 16S rRNA分析藏猪、大白猪和荣昌猪源菌群小鼠粪便微生物在门水平上的差异

Fig.2 16S rRNA gene analysis reveal phyla level differences in microbiota of TFM, YFM and RFM

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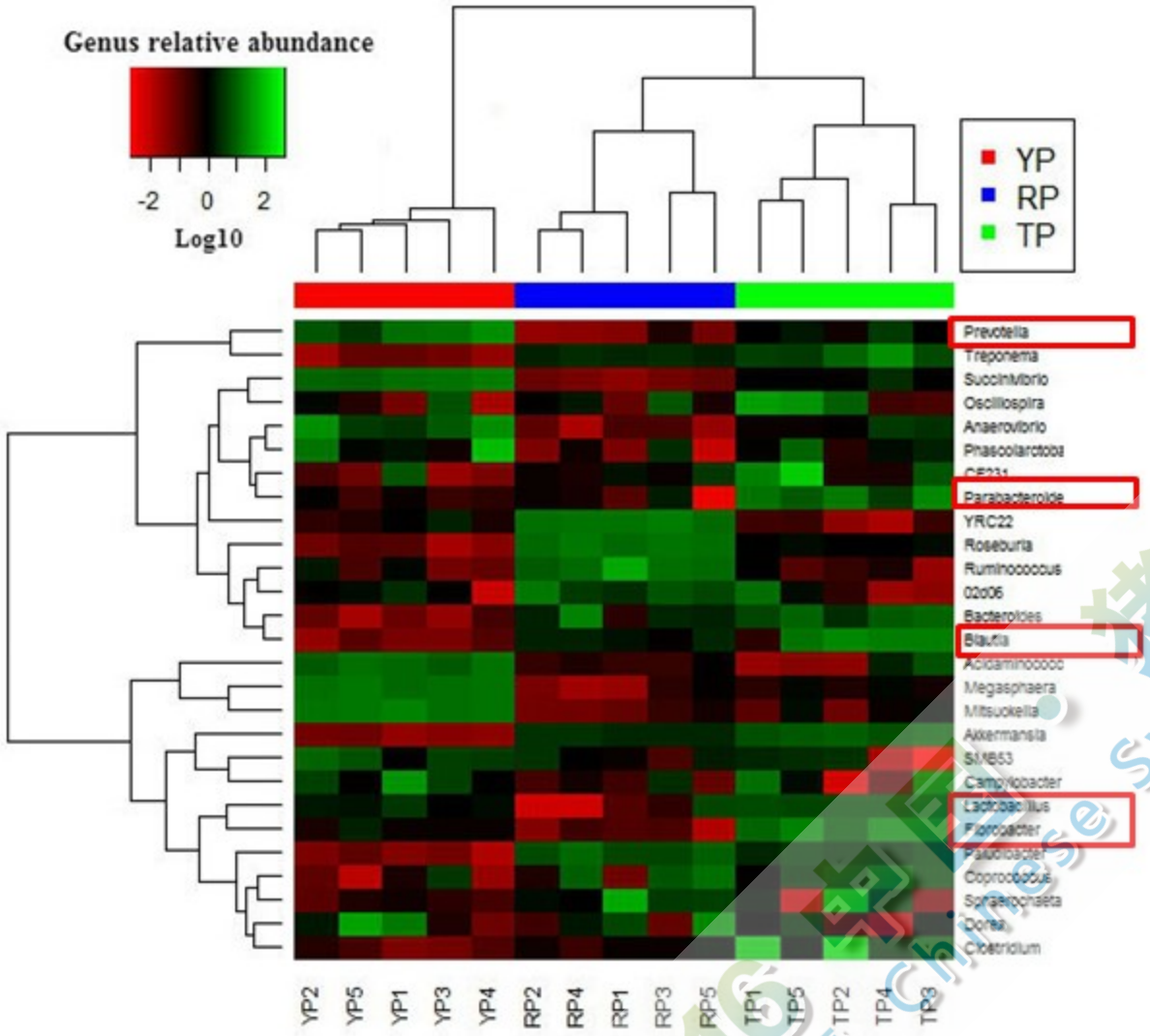

图3 16S rRNA分析藏猪、大白猪和荣昌猪粪便微生物在属水平上的差异

Fig.3 16S rRNA gene analysis reveal genus level differences in microbiota of TP, YP and RP

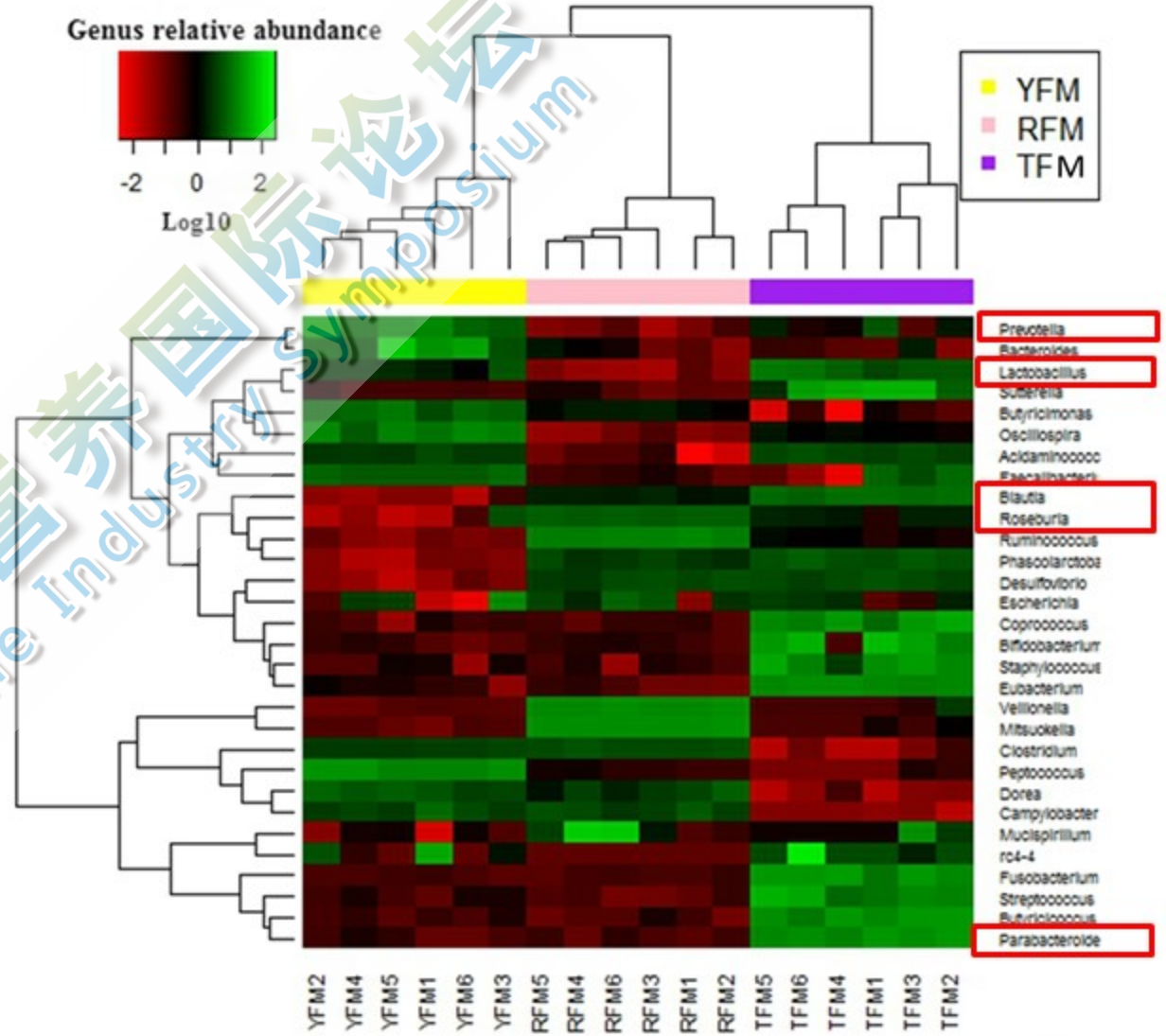

图4 16S rRNA分析藏猪、大白猪和荣昌猪源菌群小鼠粪便微生物在属水平上的差异

Fig.4 16S rRNA gene analysis reveal genus level differences in microbiota of TFM, YFM and RFM

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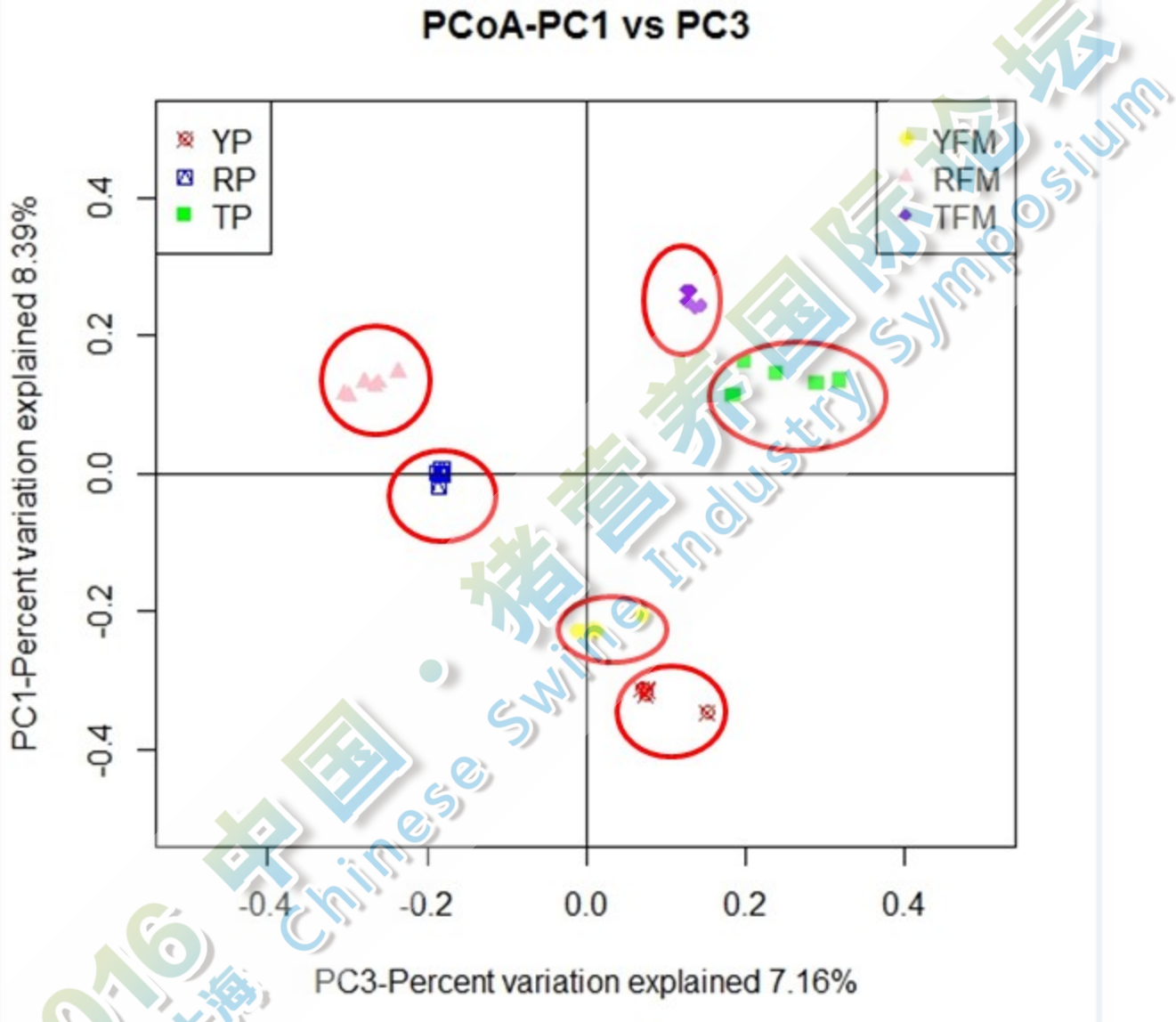

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图5 主成分分析藏猪、大白猪、荣昌猪及各粪便移植小鼠肠道微生物组成的差异

Fig.5 Comparison of the gut microbiota composition among 6 groups

肠道生长和发育
Gut growth and development

SI: 小肠 small intestine
LI: 大肠 large intestine
TI: 总肠 total intestine

肠道指数
Intestinal index

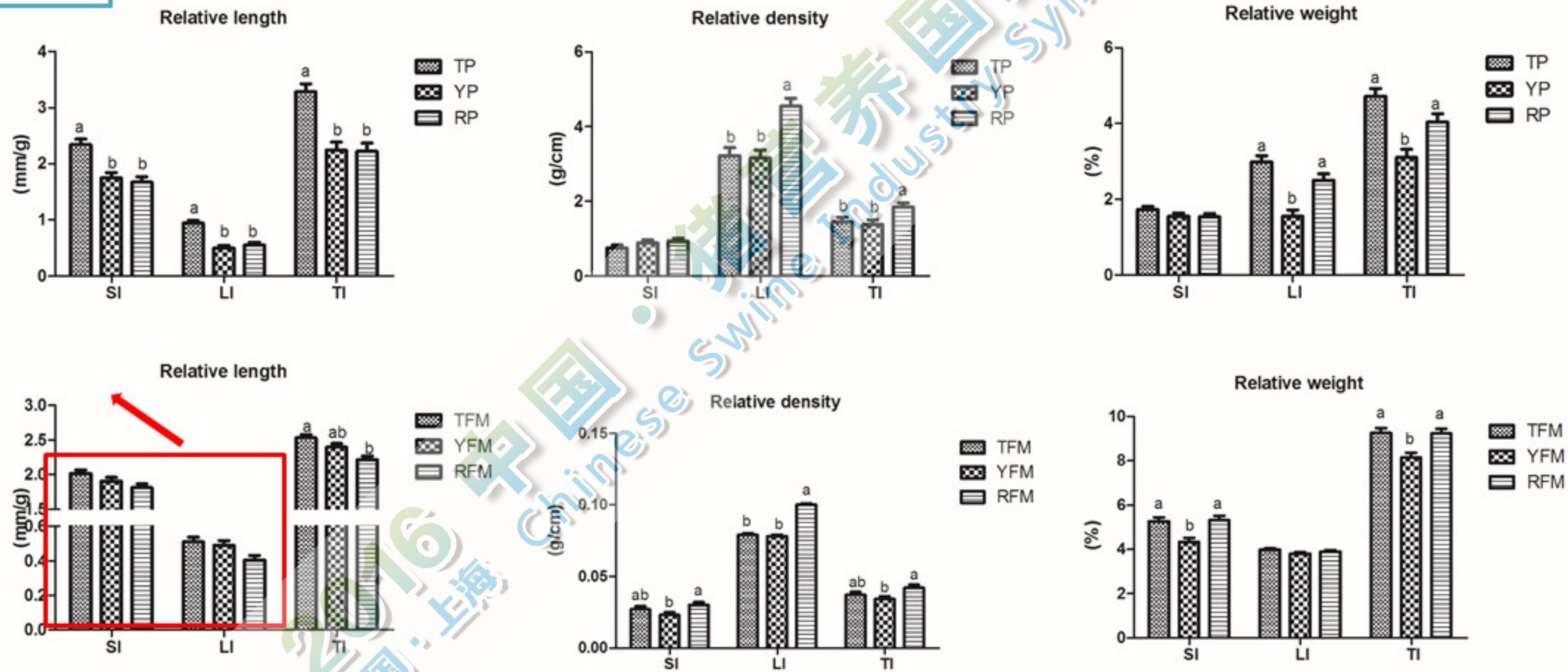

图6 肠道指数（供体猪和受体小鼠）
Fig.6 The intestinal index in pigs and mice

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肠道形态
Intestinal morphology

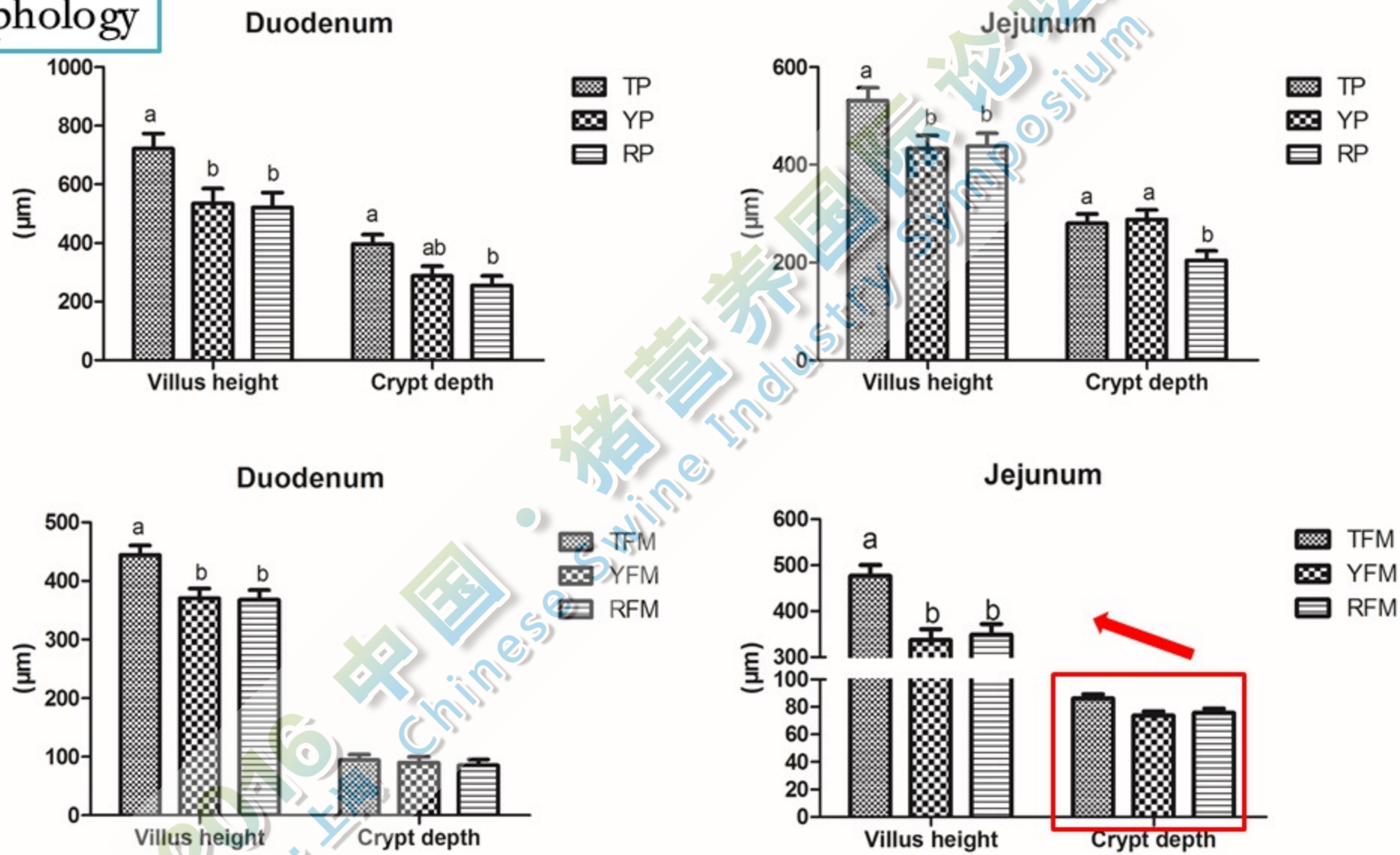

图7 肠道形态（供体猪和受体小鼠）

Fig.7 The intestinal morphology in pigs and mice

增殖和分化
Proliferation and differentiation

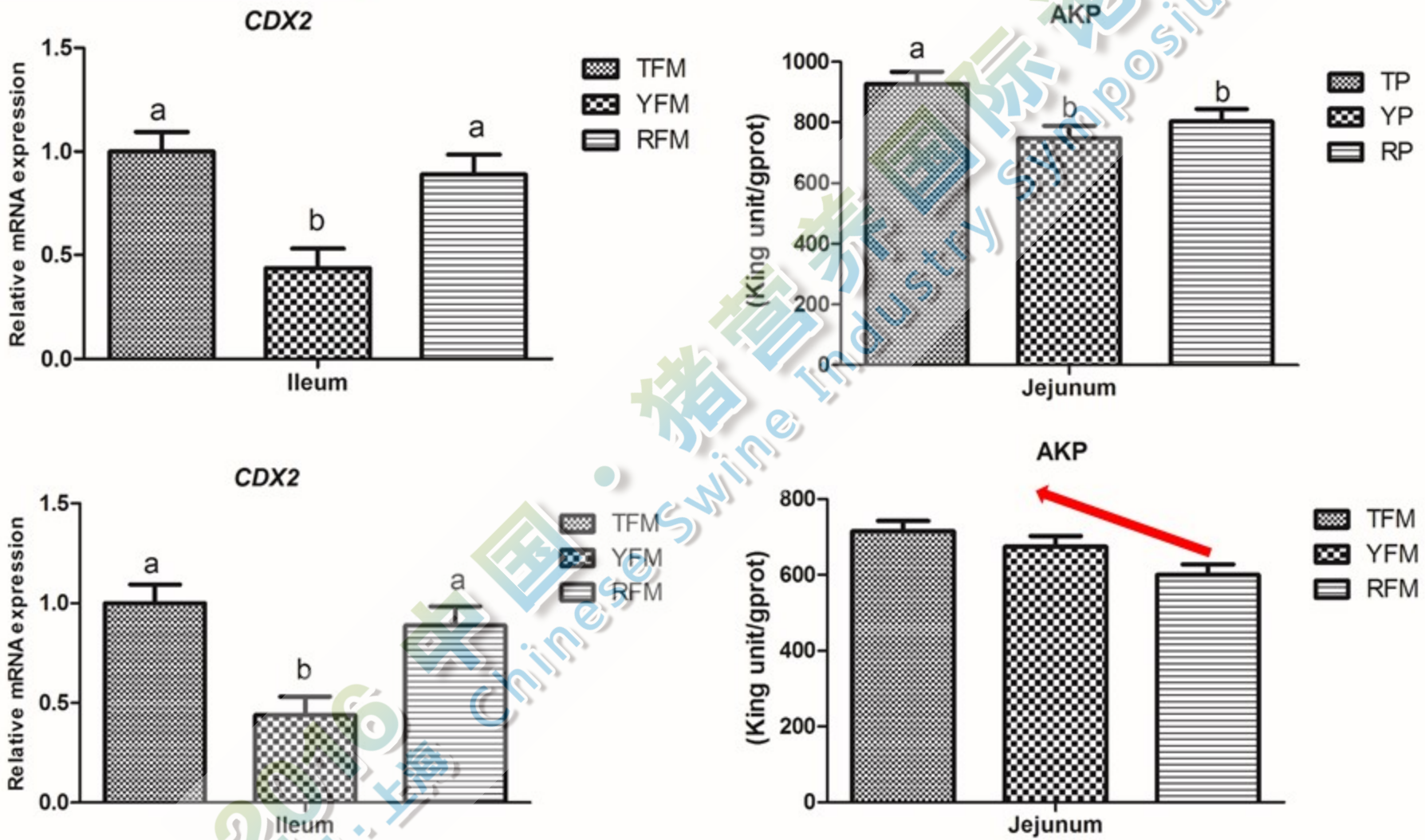

图8 肠道增值与分化（供体猪和受体小鼠）

Fig.8 Intestinal cell proliferation and differentiation in pigs and mice

肠道发育相关基因
Intestinal development-related genes

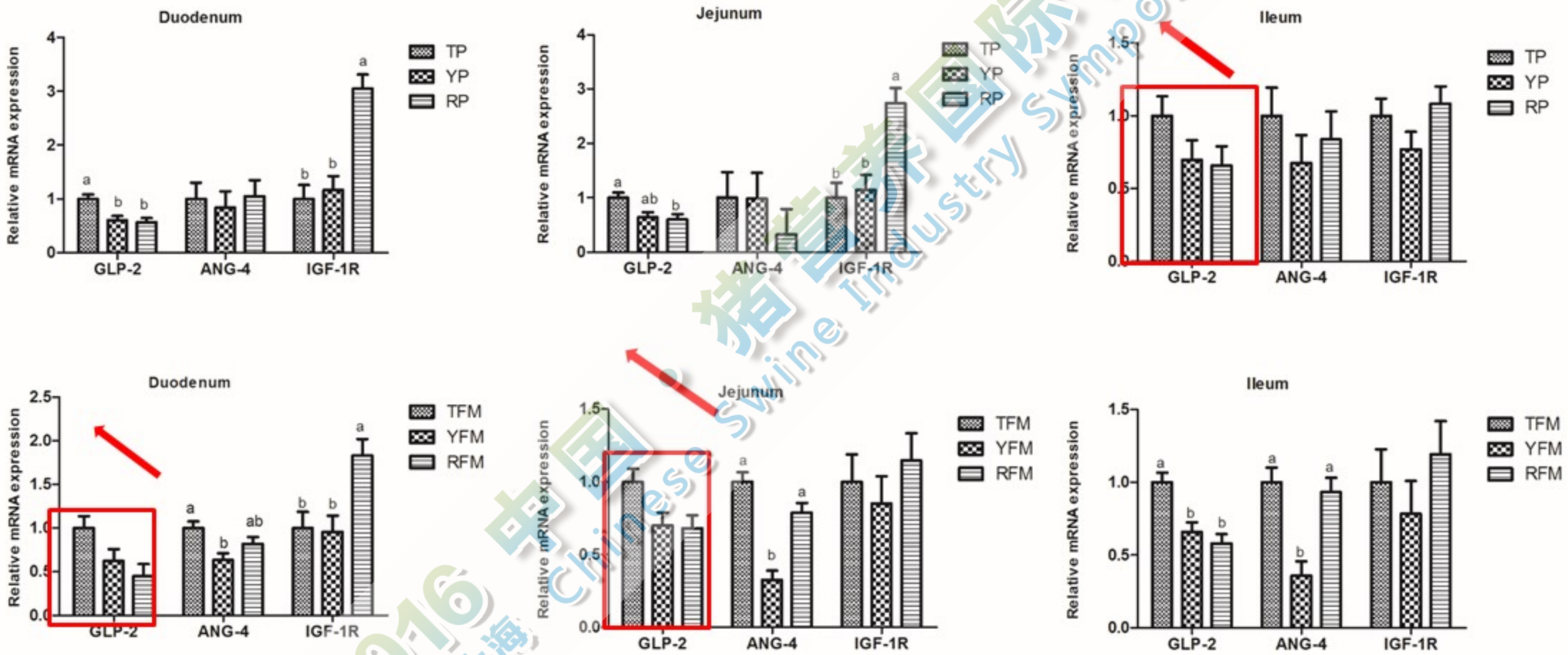

图9 肠道发育相关基因mRNA相对表达量（供体猪和受体小鼠）

Fig.9 The relative mRNA expression of intestinal development-related genes in pigs and mice

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肠道消化和吸收
Gut digestion and absorption

消化和吸收相关酶活
Digestive and absorptive enzyme activity

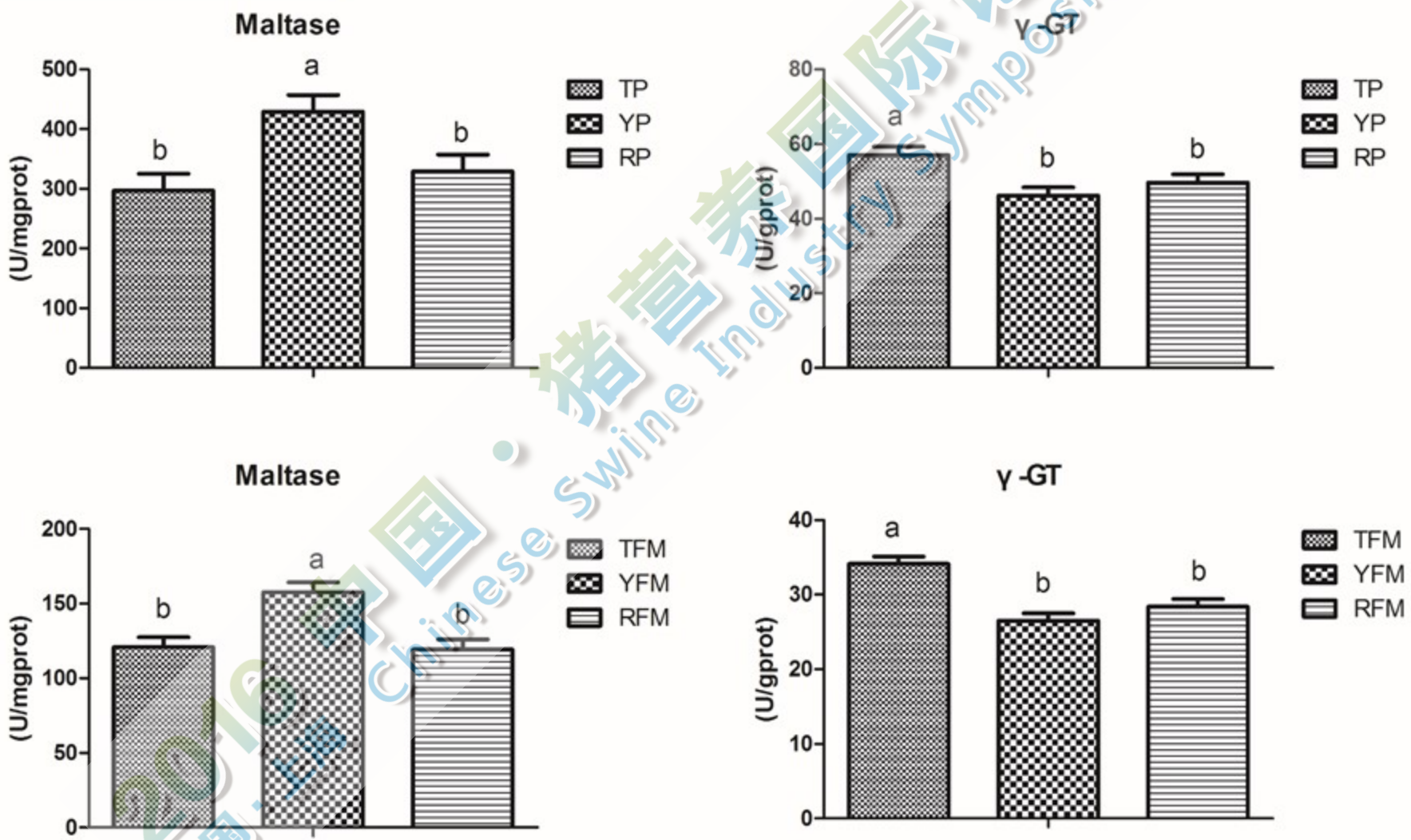

图9 肠道消化和吸收相关酶活（供体猪和受体小鼠）

Fig.9 The digestion and absorption-related enzyme activities in pigs and mice

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肠道消化吸收相关基因
Intestinal digestion and absorption-related genes

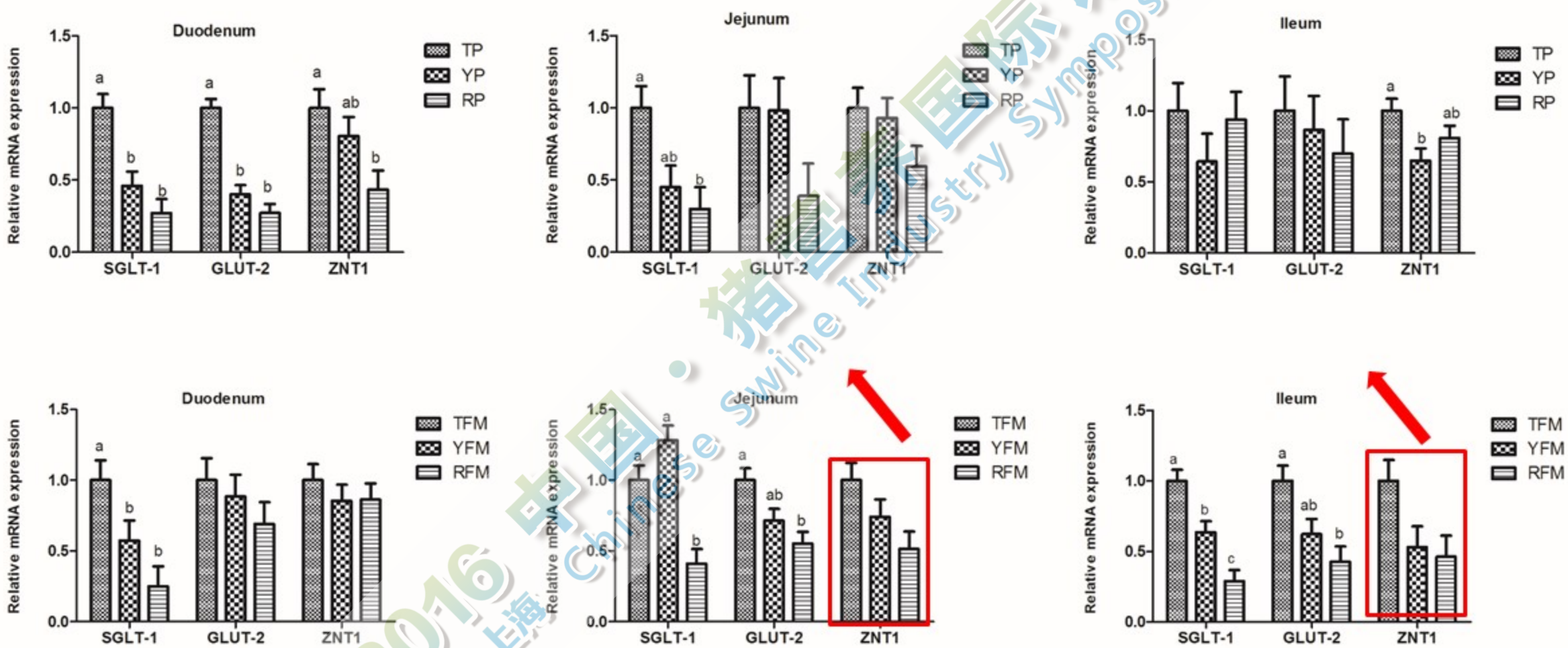

图10 肠道消化吸收相关基因mRNA相对表达量（供体猪和受体小鼠）

Fig.10 The relative mRNA expression of intestinal digestion and absorption-related genes in pigs and mice

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空肠抗氧化能力

Jejunal antioxidant capacity

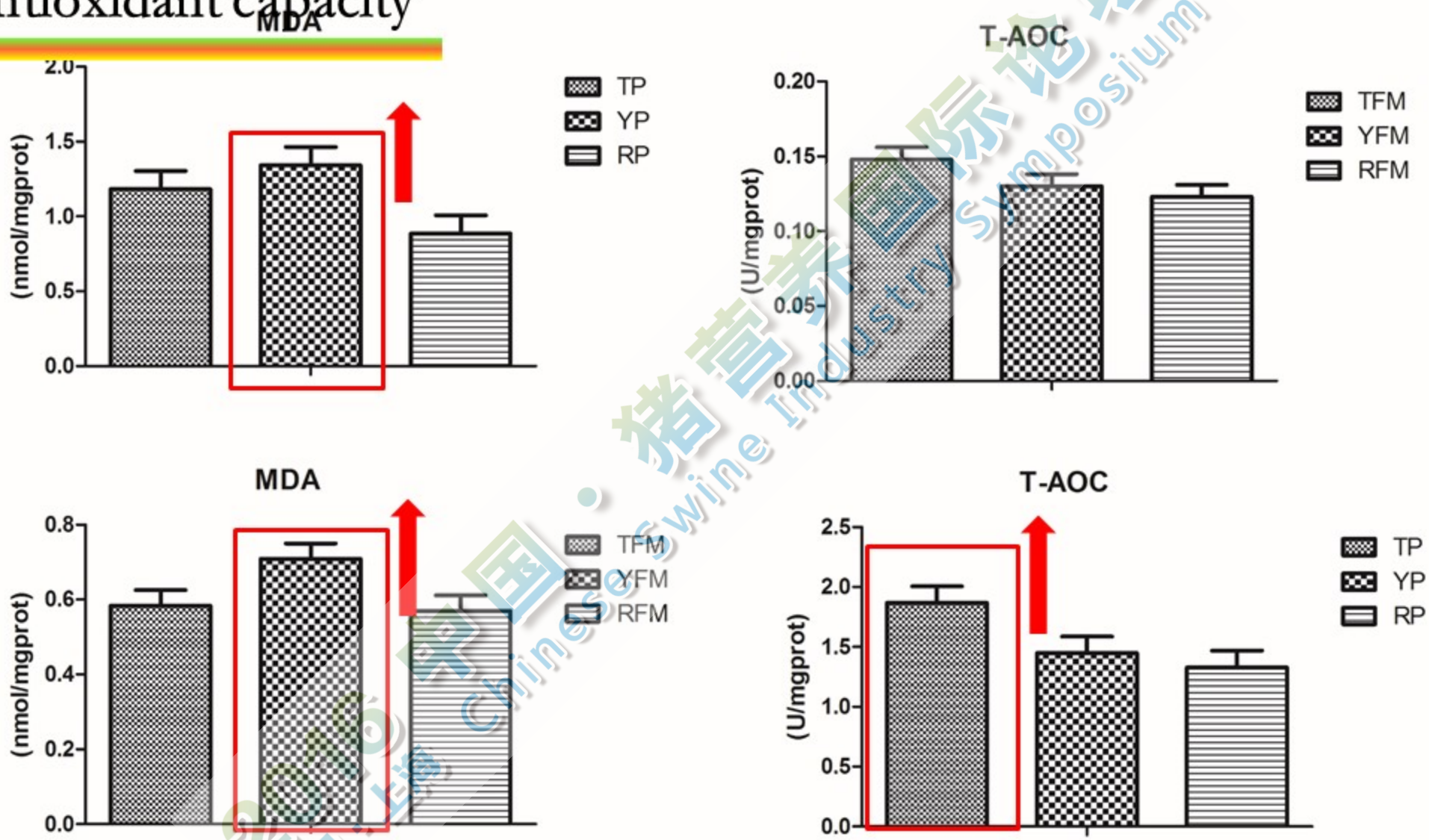

图11 空肠抗氧化能力（供体猪和受体小鼠）

Fig.11 The jejunal antioxidant capacity in pigs and mice

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肠道屏障 Gut barrier

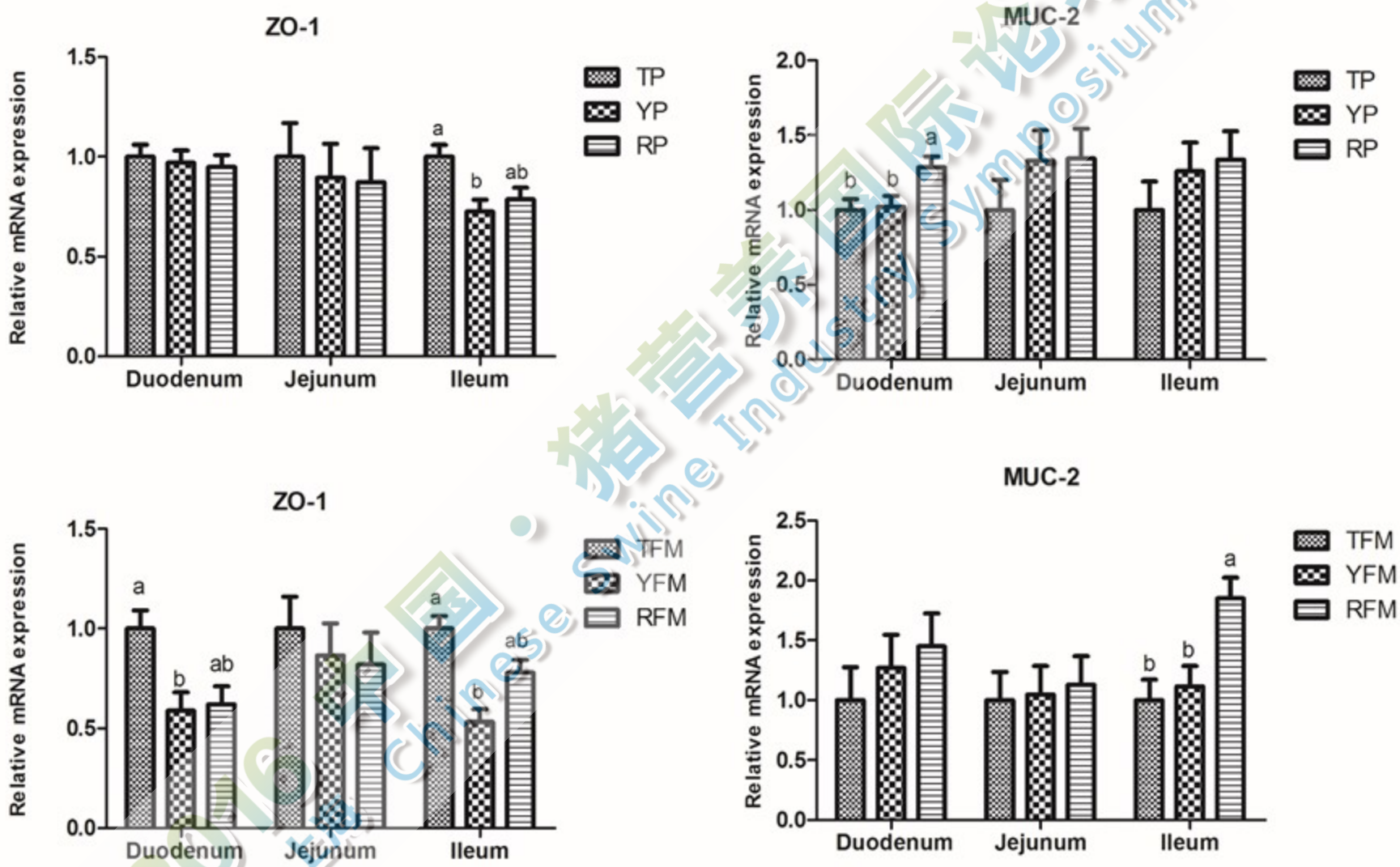

图12 肠道屏障相关基因mRNA相对表达量（供体猪和受体小鼠）

Fig.12 The relative mRNA expression of gut barrier-related genes of small intestine in pigs and mice

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结论与展望 Summary and Prospects




Summary

- 不同品种猪肠道微生物组成和肠道发育模式各不相同，藏猪拥有更好的肠道形态和屏障功能。

There were huge differences in gut microbiota composition and gut characteristics among pig breeds, and Tibetan pig had better intestinal morphology and barrier function than Yorkshire.

- 肠道微生物可以携带供体猪肠道发育的部分特征传递给受体无菌小鼠。

Gut microbiota could partially convey host gut characteristics from pigs to mice.

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Prospects

- 通过粪便移植解决生猪生产中的肠道疾病问题。机制？

Whether we can cure intestinal diseases by FMT in pig production ?

Mechanism ?

- 营养、微生物与宿主如何互作？

The interactions among nutrition, microbiota and host.

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奉献、协作、求实、创新

Thank You for your attention!

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