



读书报告

胡文攀

2019/1/13

Fermented *Yupingfeng* polysaccharides enhance immunity by improving the foregut microflora and intestinal barrier in weaning rex rabbits

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IF=3.81

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背景介绍

“玉屏风”为中药名方，亦为中药名。出自元代医家[危亦林](#)所著的《世医得效方》一书，由**防风**、**黄芪**、**白术(炒)**三味中药组成。可敛汗固表，也是体质虚弱者预防感冒等感染性疾病的良方。研究还表明，玉屏风散具有调节人体免疫力的之功效，有中成药中的“丙种球蛋白”美称，现代临床在内、外、妇、儿等各科疾病中得到广泛的应用。



背景介绍

玉屏风(YPF)，具有良好的免疫调节活性。然而，其作用机制尚不清楚。现代药理学研究表明YPF多糖是该配方的主要活性成分之一，具有双向免疫调节作用(Du et al. 2013)。此外，益生菌发酵可显著提高生物的活性潜力，具有更大的免疫效应(Bose et al. 2012)。

本实验以兔为实验对象，来探索（寡孢根霉菌）SH发酵玉屏风多糖 (FYP)和未发酵玉屏风多糖(UYP) 的活性。结果表明，UYP和FYP均具有明显的促生长、增强肠道功能、改善肠道菌群稳态、维持肠道屏障完整性和功能的作用。



材料与amp;方法



Preparation of FYP

Determination of serum levels of cytokines

DNA extraction

Quantitative PCR quantification of microbial

Determination of mRNA expression levels by Q-PCR



材料与amp;方法



免疫

免疫：IL-2、TNF- α 、IFN- γ 、IL-4、IL-10

通路：TLR2、TLR4

肠道屏障：ZO-1、Claudin、Occludin
IgA、PIGR、 β -defensin
TFF、EGF

脾脏

肠道

血清：IL-1、IL-2、IL-6、IL-12
TNF- α 、IFN- γ 、IL-4、IL-10



微生物

PCR-DGGE

UPMAG聚类分析

PCA分析

空肠、回肠、盲肠



结果与讨论



Fig. 1

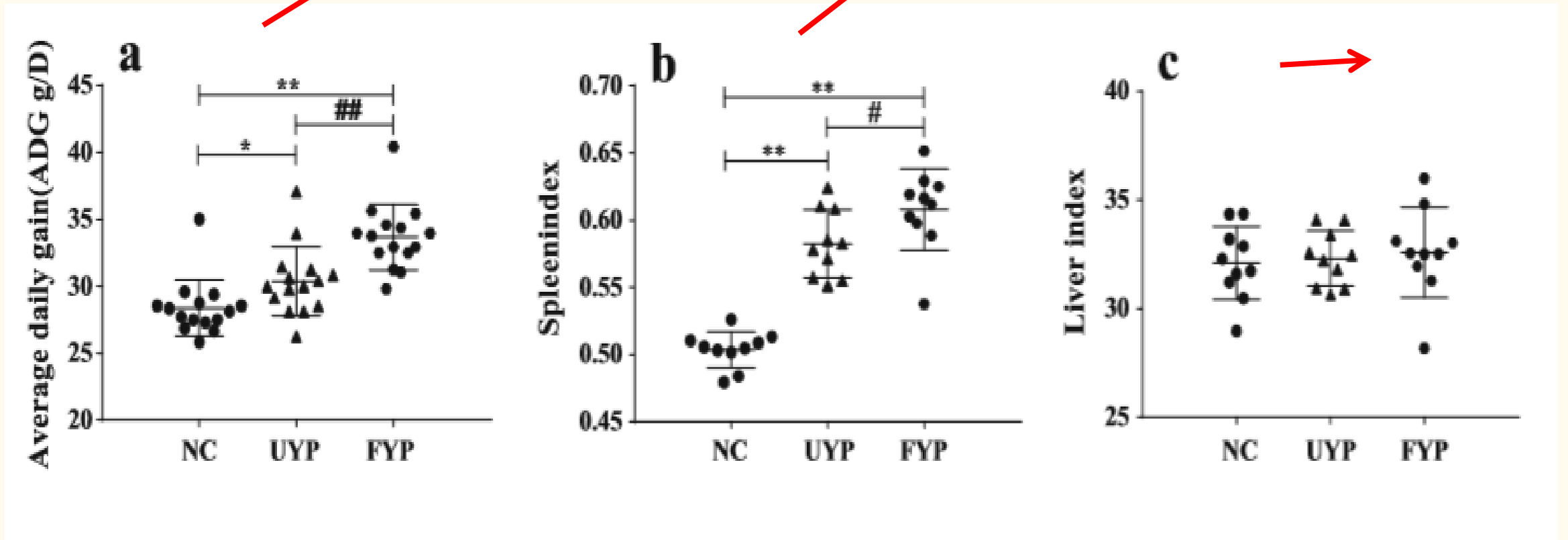


Fig. 1 The ADG and organ indices of rex rabbits in each group. a ADG (n = 15). b Spleen index. c Liver index (n = 10). compared with NC group and UYP group, respectively.

结果与讨论



Fig. 2-脾脏

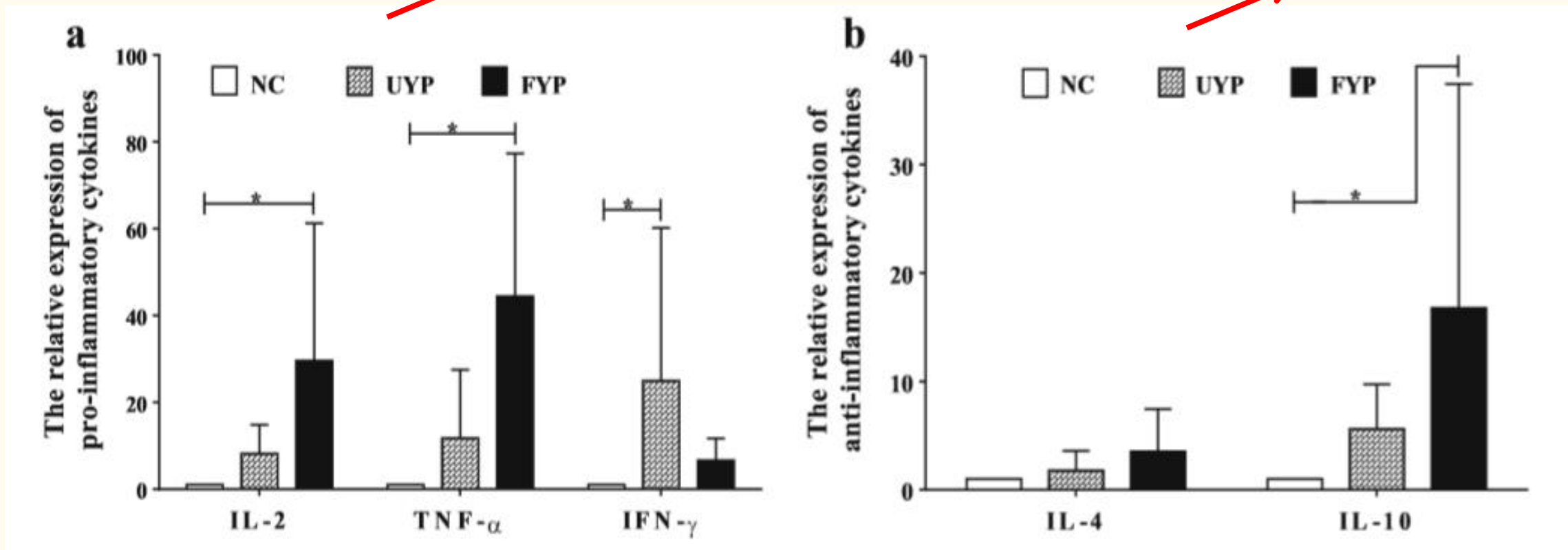


Fig. 2 The mRNA relative expressions of cytokines in the spleen.

结果与讨论

Fig. 3-血清

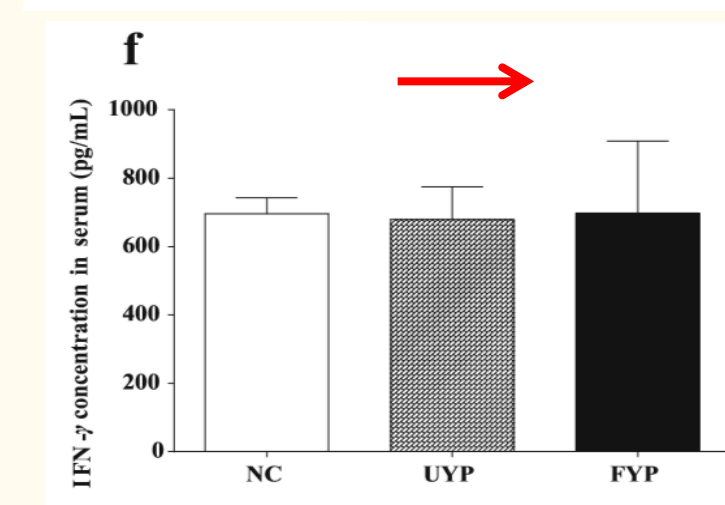
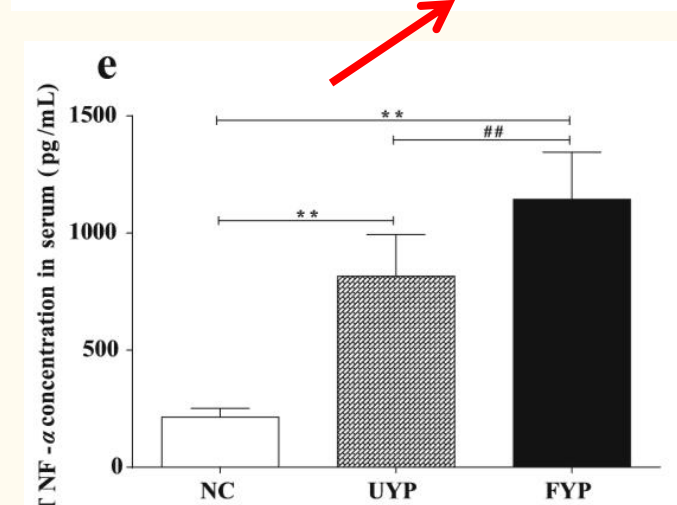
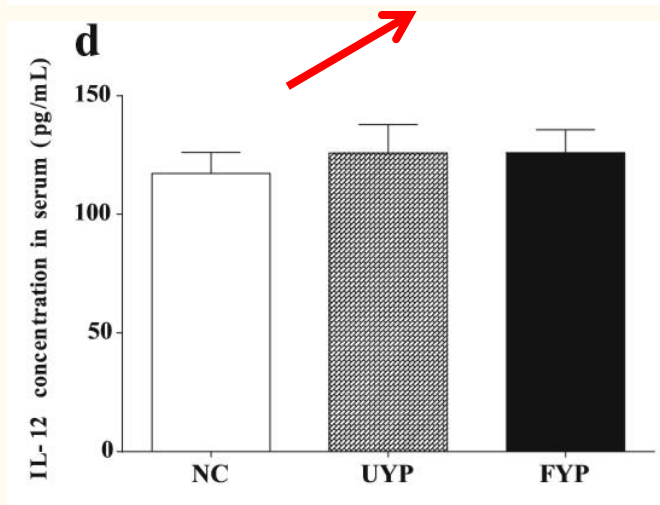
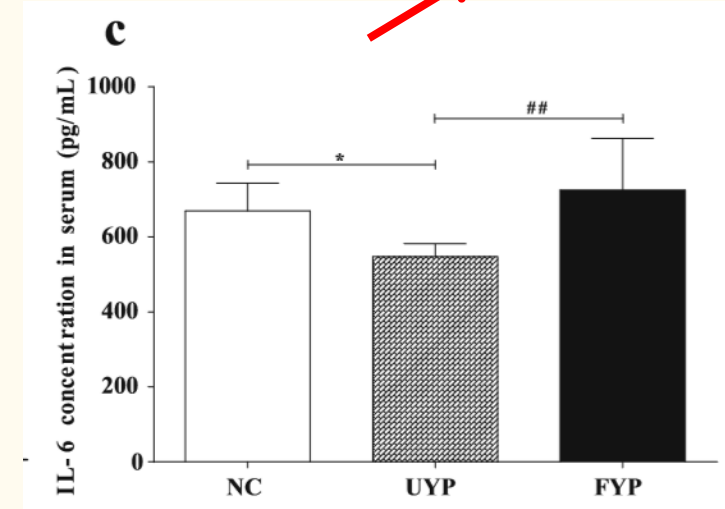
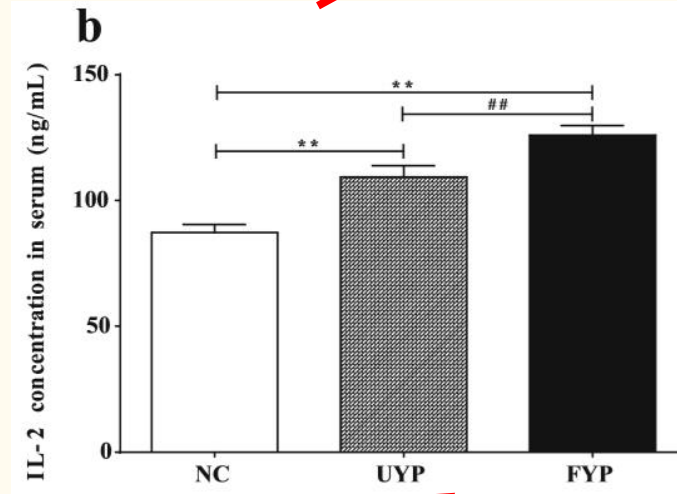
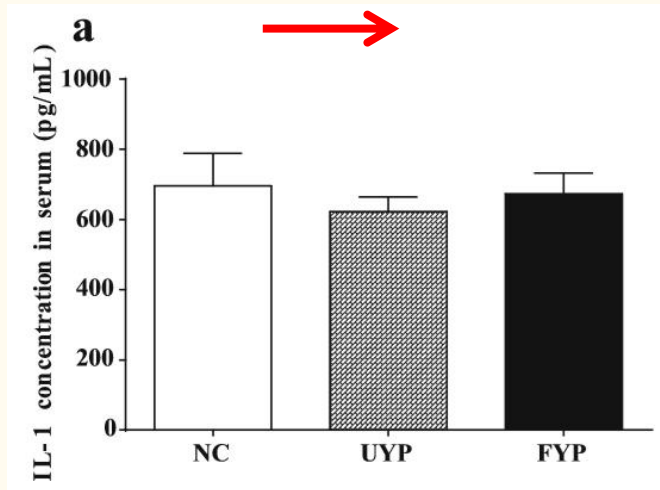


Fig. 3 Effects of UYP and FYP on cytokine concentration in serum. a–h Concentration of IL-1, IL-2, IL-6, IL-12, TNF- α , IFN- γ , and IL-4 and IL10, respectively.

结果与讨论



Fig. 3

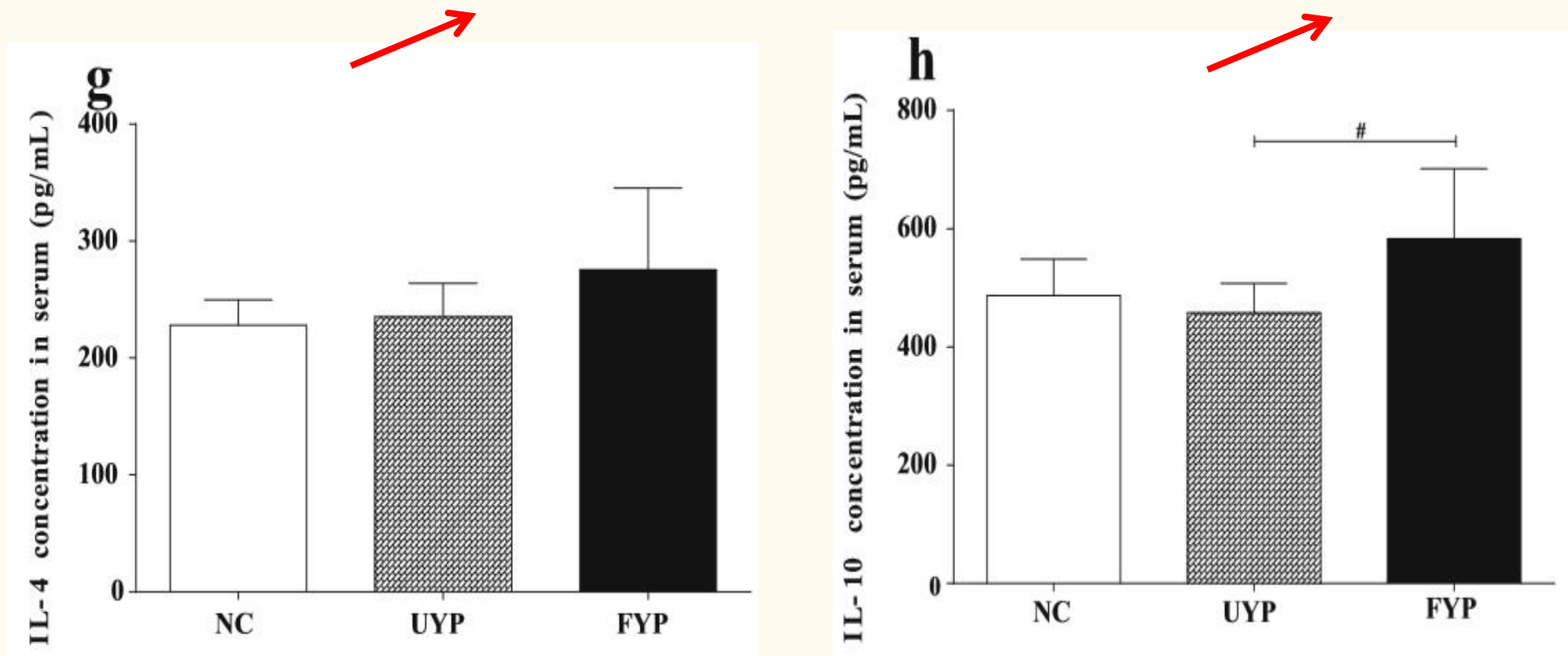


Fig. 3 Effects of UYP and FYP on cytokine concentration in serum. a–h Concentration of IL-1, IL-2, IL-6, IL-12, TNF- α , IFN- γ , and IL-4 and IL10, respectively.

结果与讨论



Fig.1:

- 1、UYP和FYP可提高日增重，促进生长。
- 2、脾脏是生物重要的免疫器官，UYP和FYP可以促进脾脏的发育，且FYP在促进免疫器官指标方面可能比UYP更有效。

Fig.2- Fig.3:

这些结果表明，UYP和FYP均可通过调节细胞因子合成改善免疫应答，且FYP比UYP更有效。此外，血清中细胞因子浓度和脾脏中细胞因子mRNA水平的变化趋势并不完全一致，说明UYP和FYP不仅影响细胞因子的转录，而且调节细胞因子的翻译。

结果与讨论



A number of studies have reported that TCMs induced appropriate immune responses through regulation of mRNA and protein expression of cytokines genes (Burnsetal. 2010). Moreover, the biotransformation of YPF could enhance the immune function by improving the contention of IL-2 in serum(Wangetal.2010). Our results were consistent with these findings.

Ligands induce TLR2 constructing a heterodimer with either TLR1 or TLR6 to regulate cytokine synthesis. TLR2/1 elevates IFN- γ secretion, while TLR2/6 stimulates IL-10 production, which indicated that TLR2 signaling possesses bidirectional immunomodulatory activity (Bryant et al. 2015). Maybe, this explains why both pro- and anti-inflammatory cytokines were improved by UYP or FYP.

结果与讨论



Fig. 4

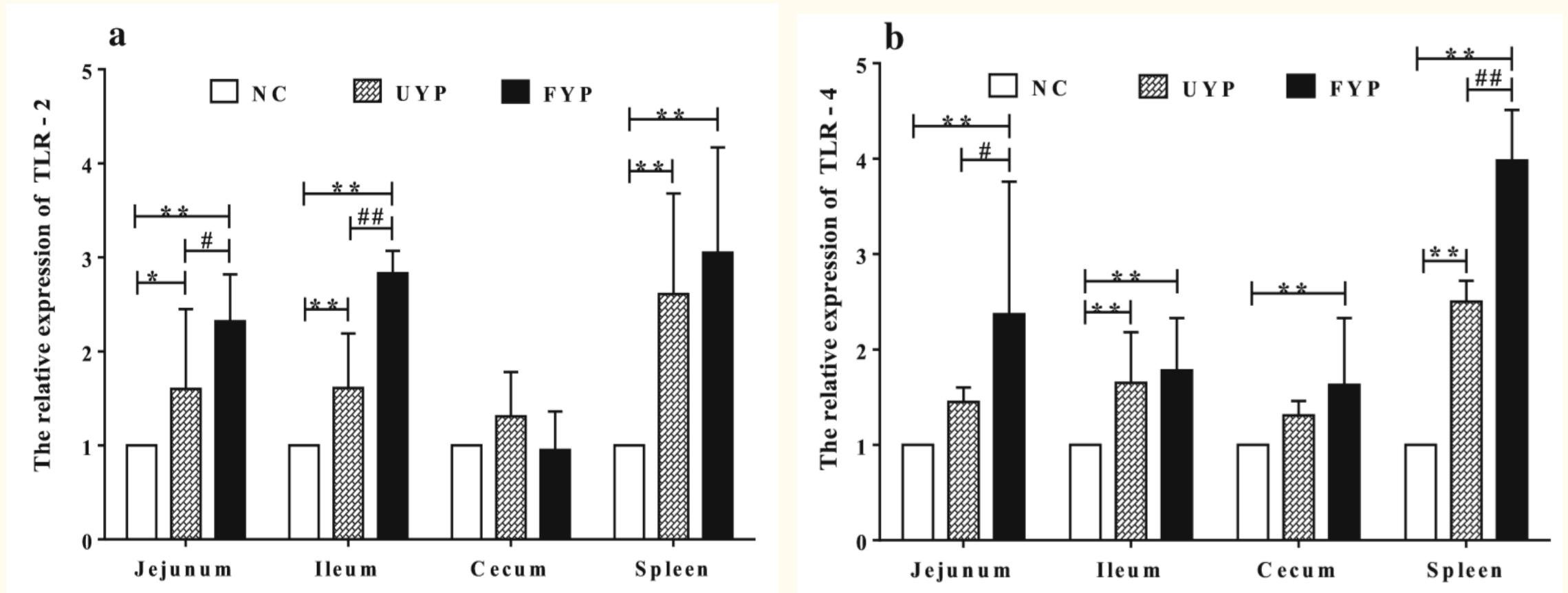


Fig. 4 Effects of UYP and FYP on TLR2 and TLR4 expressions in rex rabbit.a,bTLR2 and TLR4,respectively.

结果与讨论



TLR4 is other important PRRs associated with immunity and is expressed in immune cells and enterocytes, and it elicits cytokine synthesis with recruitment of innate and adaptive immune cells to improve the body's defense function via activation of NF- κ B (Frosali et al. 2015). TLR2 signaling possesses bidirectional immunomodulatory activity (Bryant et al. 2015).

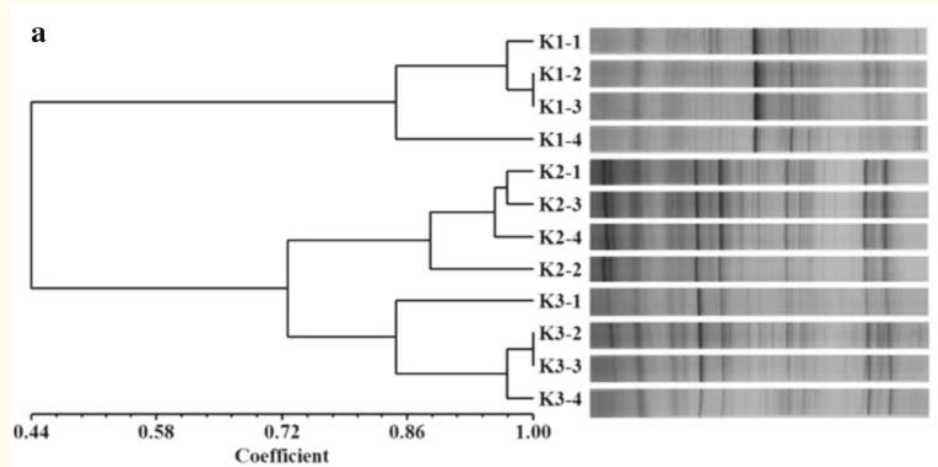
Fig.4:

结果表明, UYP和FYP上调TLR2和TLR4的mRNA表达, FYP能更有效上调TLR2和TLR4的mRNA水平。由此可见, UYP和FYP的免疫增强作用可能是通过激活TLR2和TLR4通路实现的, 且FYP的效果较好。

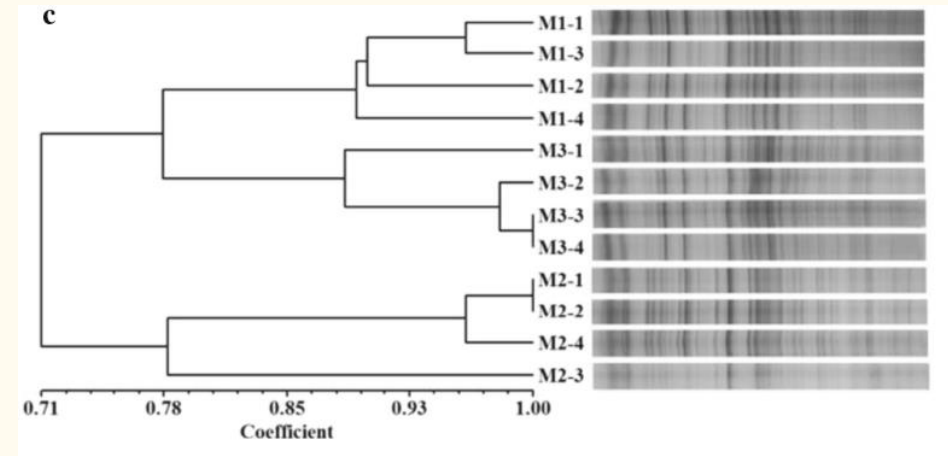
结果与讨论



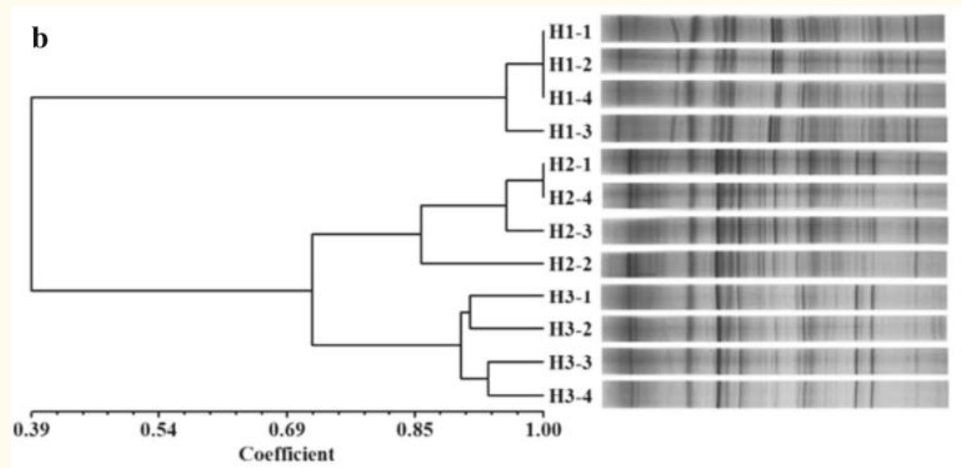
Fig.5 UPGMA dendrogram combined with PCR-DGGE profiles



jejunum



cecum



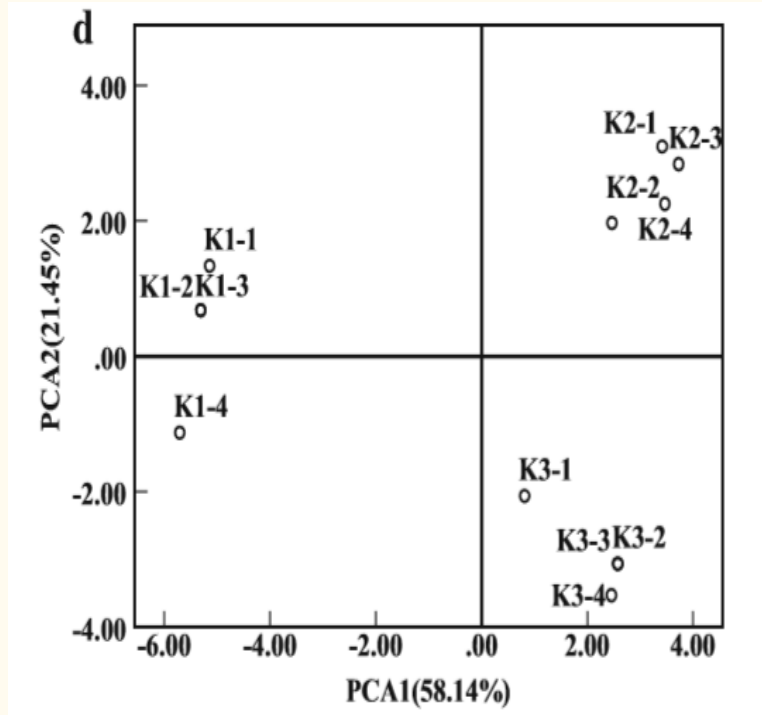
ileum

K jejunum (空肠), H ileum (回肠), M cecum (盲肠)
K1, H1, and M1 NC group;
K2, H2, and M2 UYP group;
K3, H3, and M3 FYP group

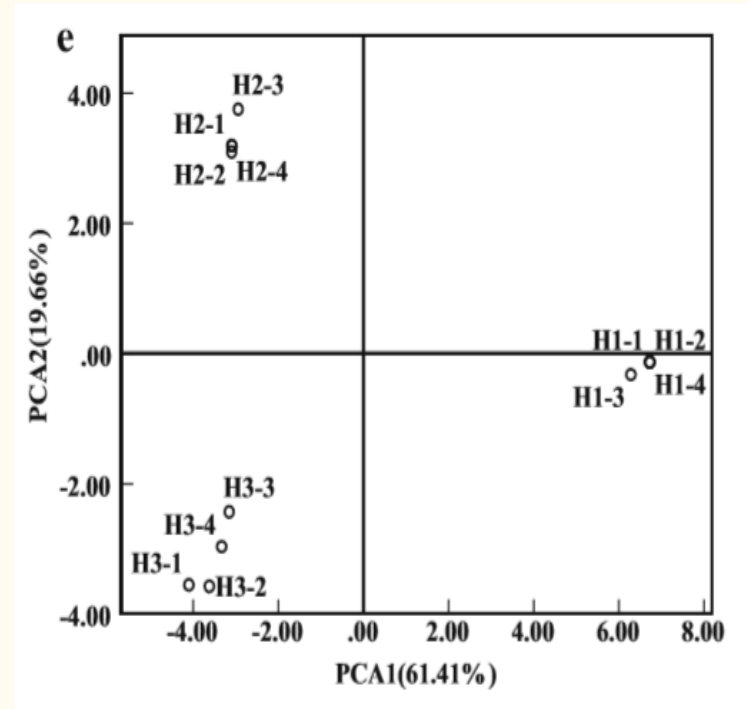
结果与讨论



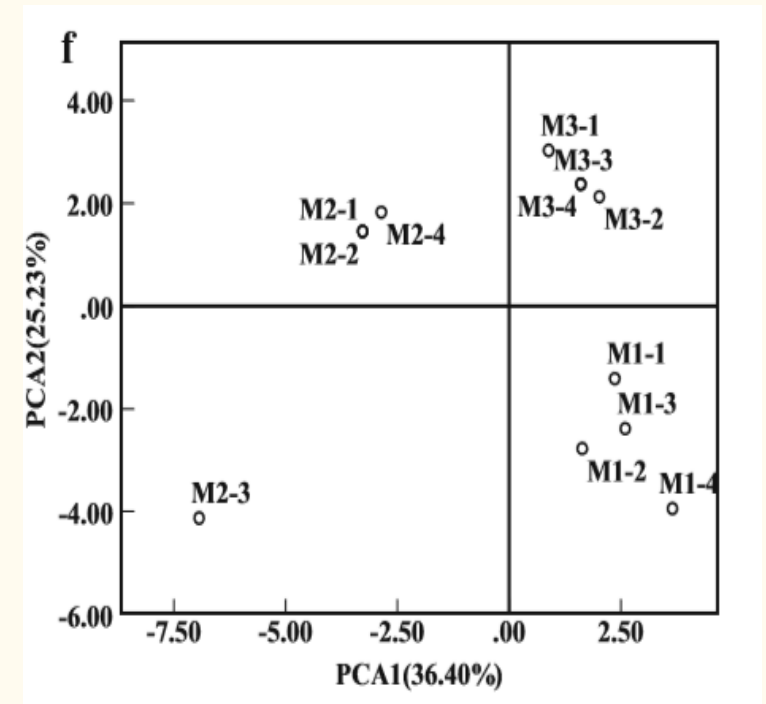
Fig.5 PCA profiles of the jejunum, ileum, and cecum, respectively



jejunum



ileum



cecum

K jejunum (空肠), H ileum (回肠), M cecum (盲肠)

K1, H1, and M1 NC group; K2, H2, and M2 UYP group; K3, H3, and M3 FYP group

结果与讨论



Fig.5

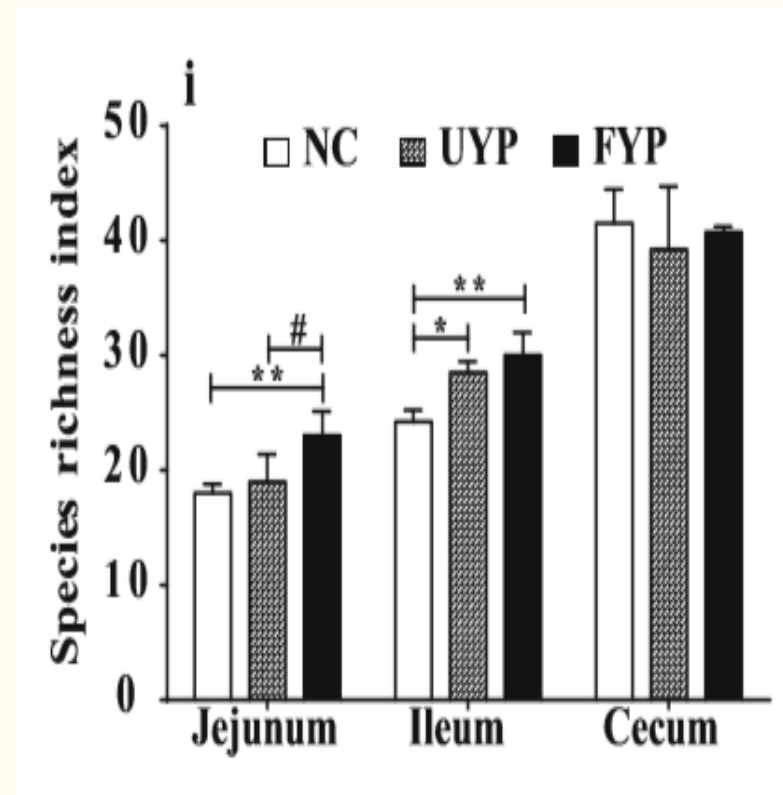
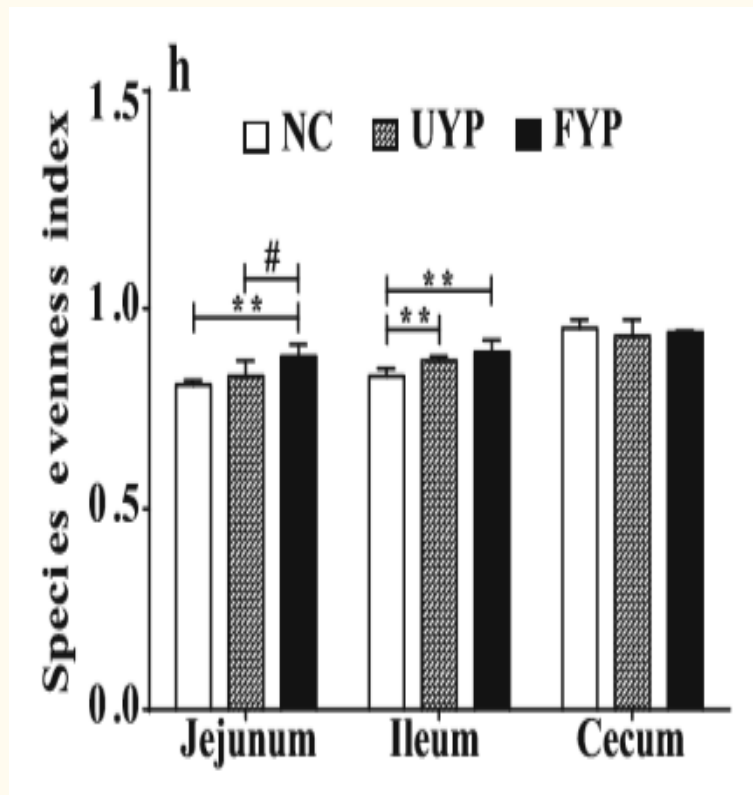
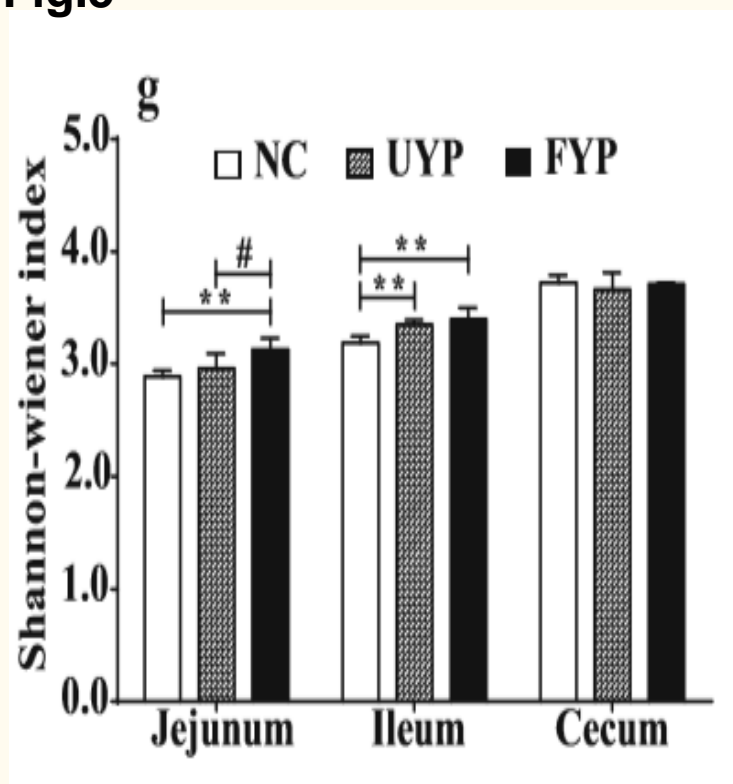


Fig.5 Effects of UYP and FYP on the intestinal microbiota community g–i ShannonWiener index, evenness index, and richness index of the gastrointestinal microbiota on K jejunum, H ileum, M cecum.

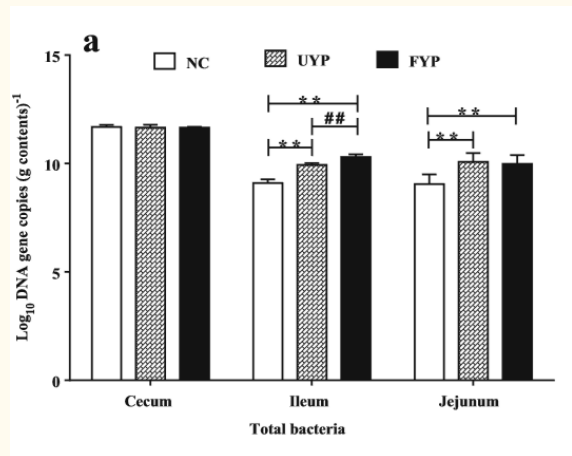
结果与讨论



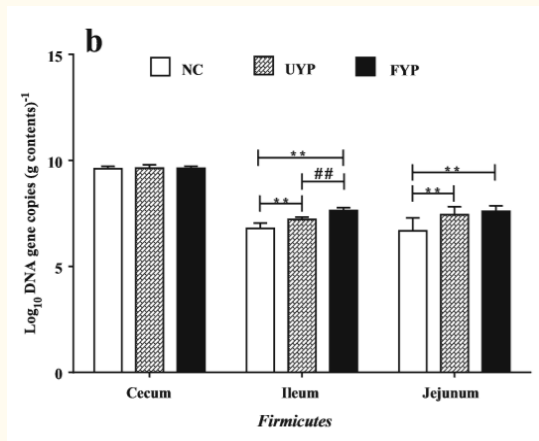
Fig.5:

- 1、PCR-DGGE图谱显示，在空肠和回肠中，UYP组和FYP组肠道菌群多样性明显高于NC组；图谱聚类分析显示，各组均有明显的聚类分离，在空肠和回肠中组间的相似性系数最低。
- 2、PCA分析显示，在空肠和回肠中UYP组和FYP组之间群落相似性较高。
- 3、经UYP或FYP处理后，空肠和回肠的微生物群落的丰度、多样性有明显升高，且FYP的效果更佳。总体而言，这些结果表明，不同的处理导致了不同的微生物群落结构和优势种群。经UYP和FYP处理后，空肠和回肠的肠道菌群结构受影响较大，其中FYP组效果最明显。

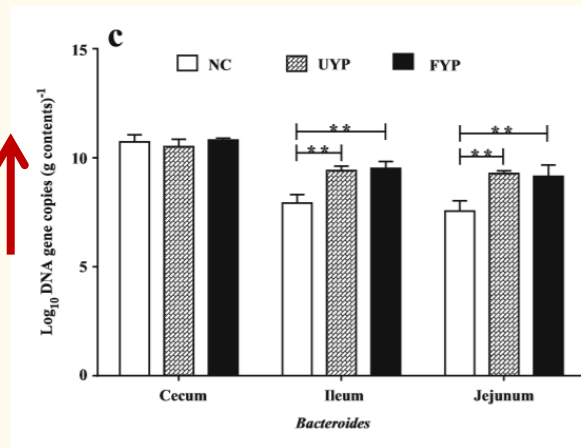
结果与讨论 (cecum盲肠、 ileum回肠、 jejunum空肠)



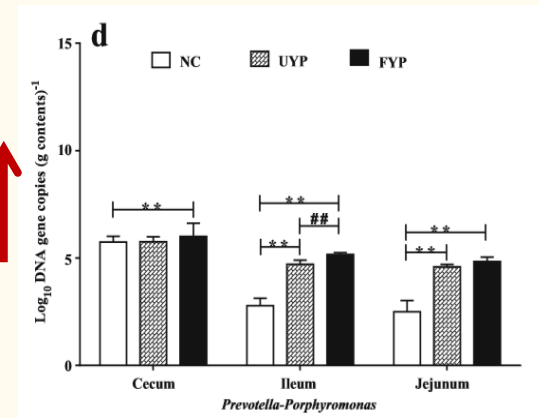
总菌



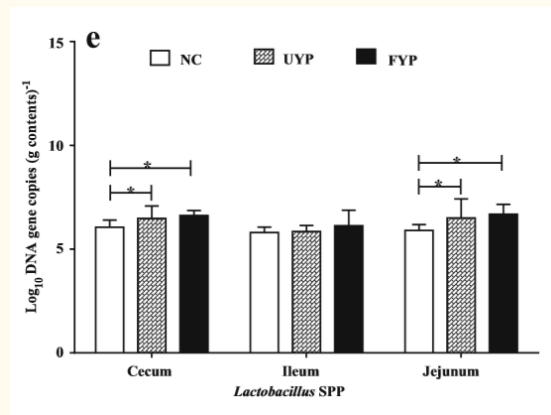
厚壁菌



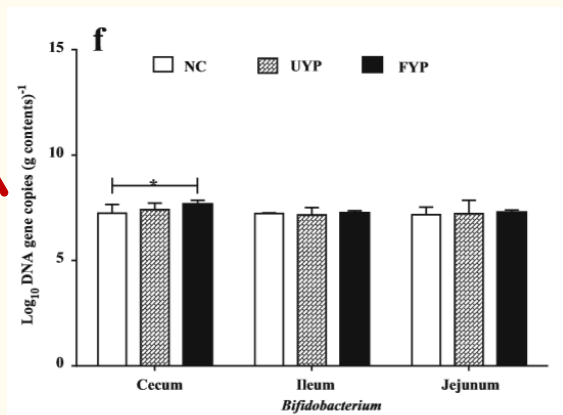
拟杆菌



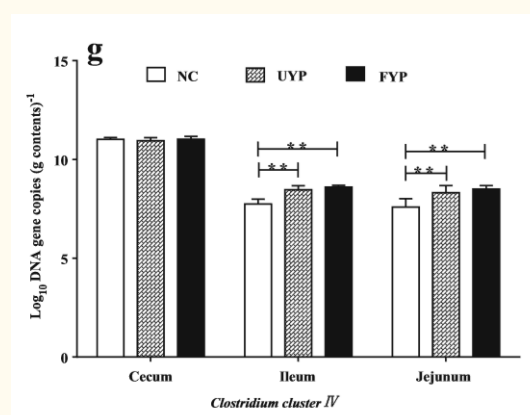
普氏菌属



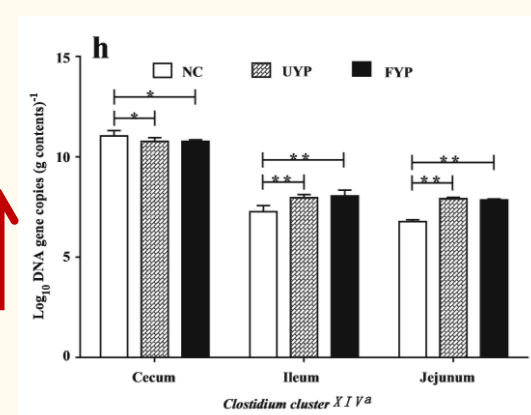
乳杆菌属



双歧杆菌

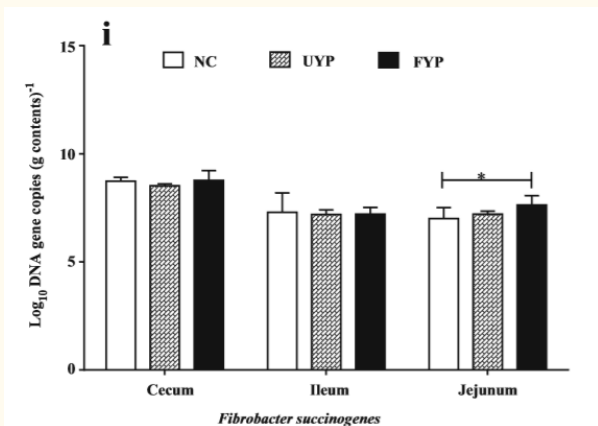


集群IV核状芽胞杆菌

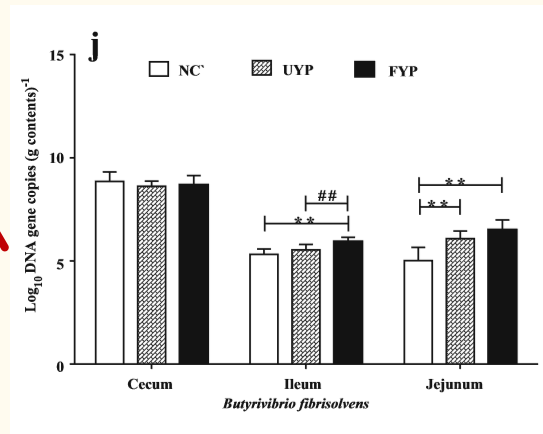


集群XIVa核状芽胞杆菌

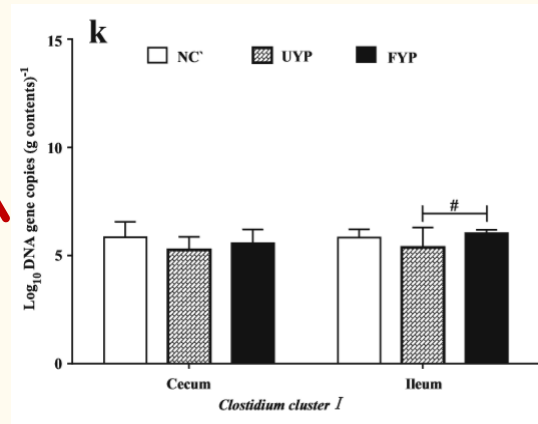
结果与讨论 (cecum盲肠、 ileum回肠、 jejunum空肠)



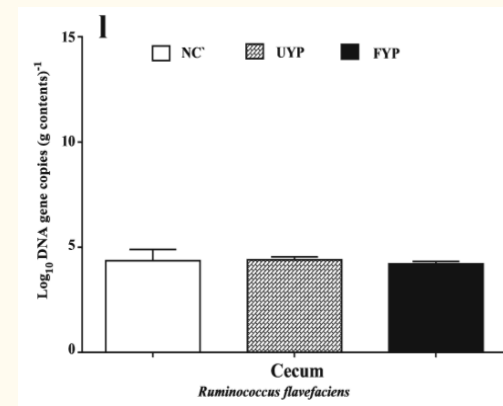
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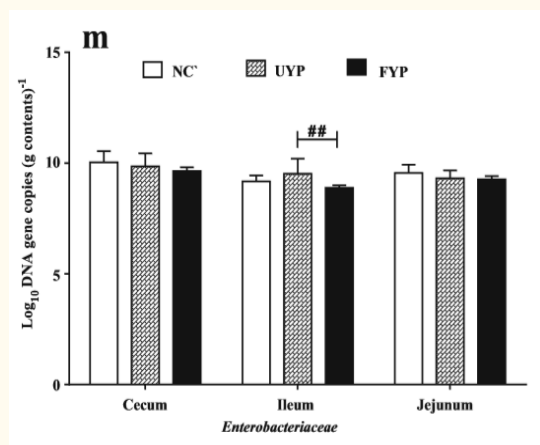
丁酸弧菌属



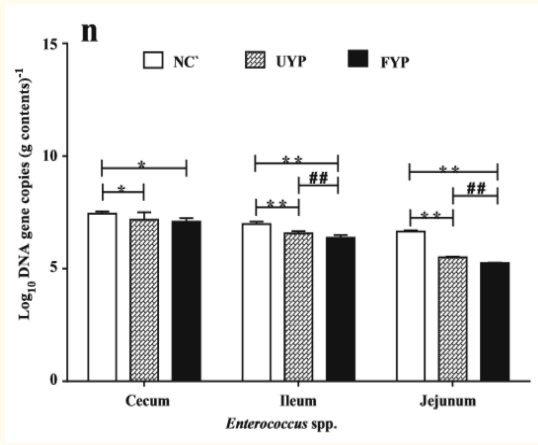
集群I梭状芽胞杆菌



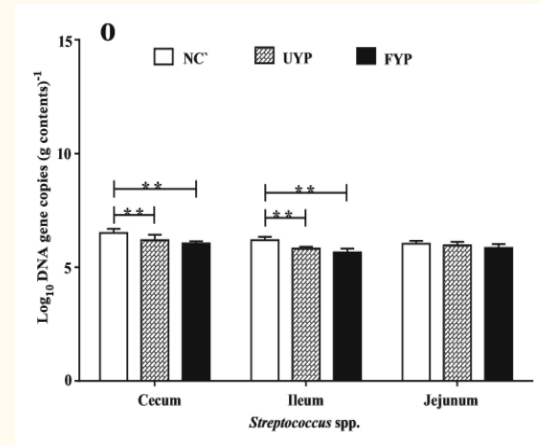
瘤胃球菌属



肠杆菌属



肠球菌属



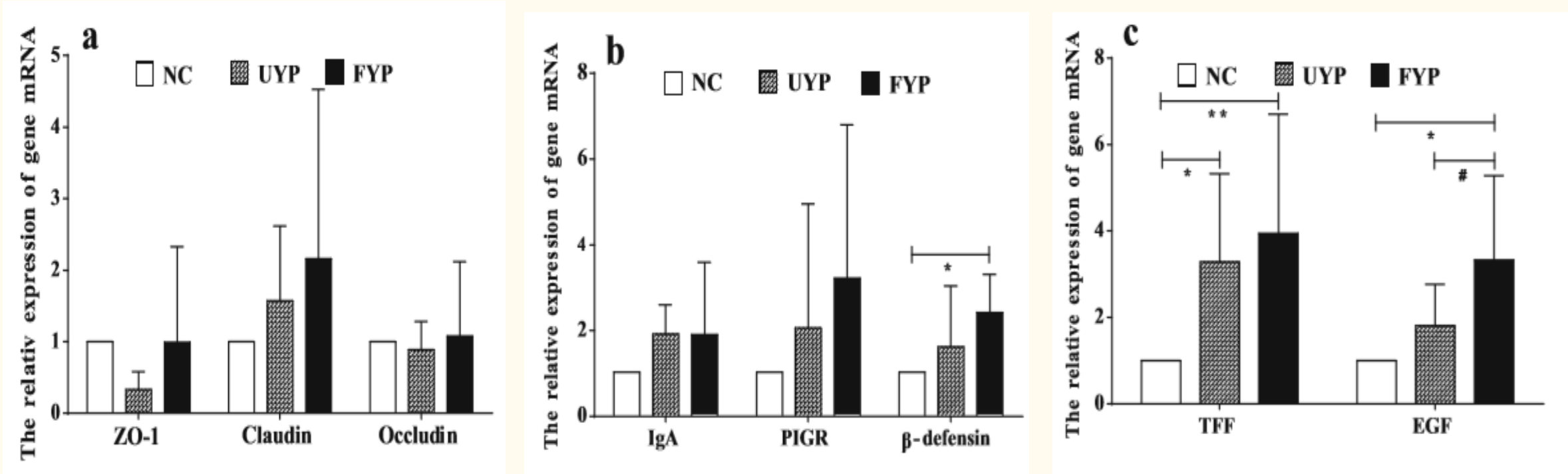
链球菌属

Fig.6 Effects of UYP or FYP on functional bacteria

结果与讨论



Fig.7-空肠



物理屏障

免疫屏障

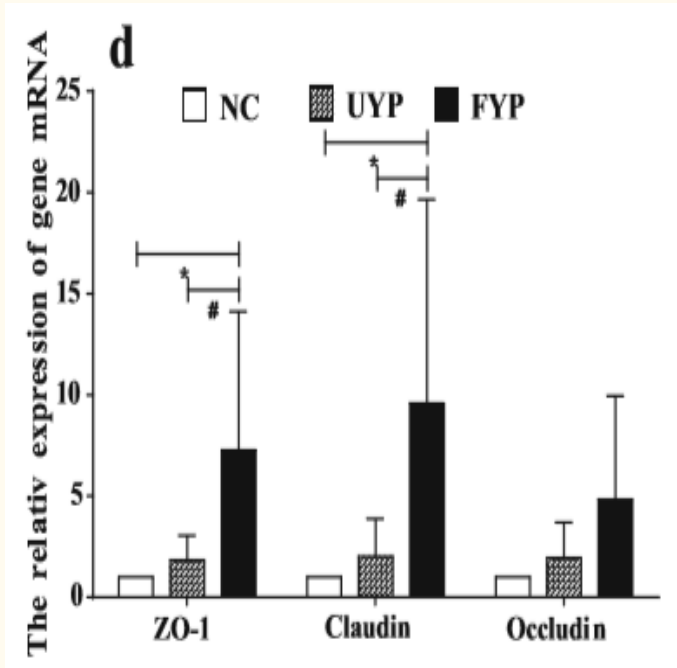
化学屏障

Fig.7 Effects of UYP or FYP on intestinal barrier of rex rabbit. a–c Physical barrier, immunological barrier, and chemical barrier in the jejunum,

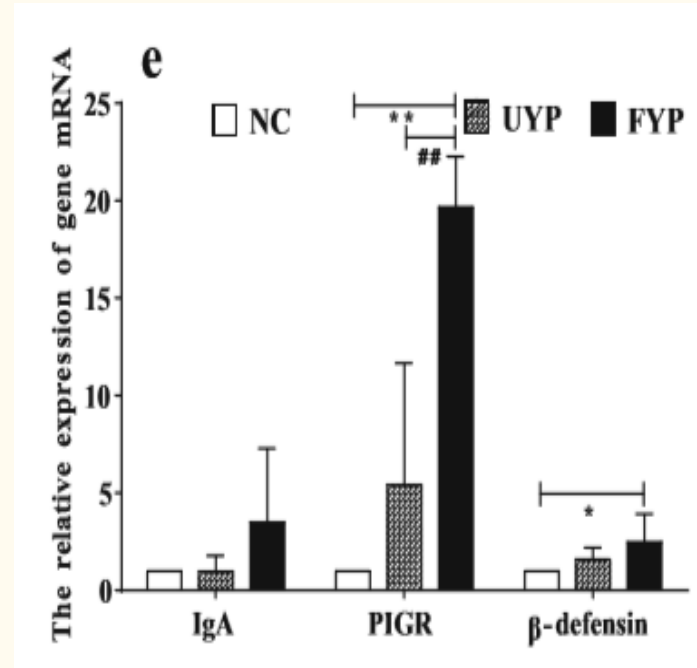
结果与讨论



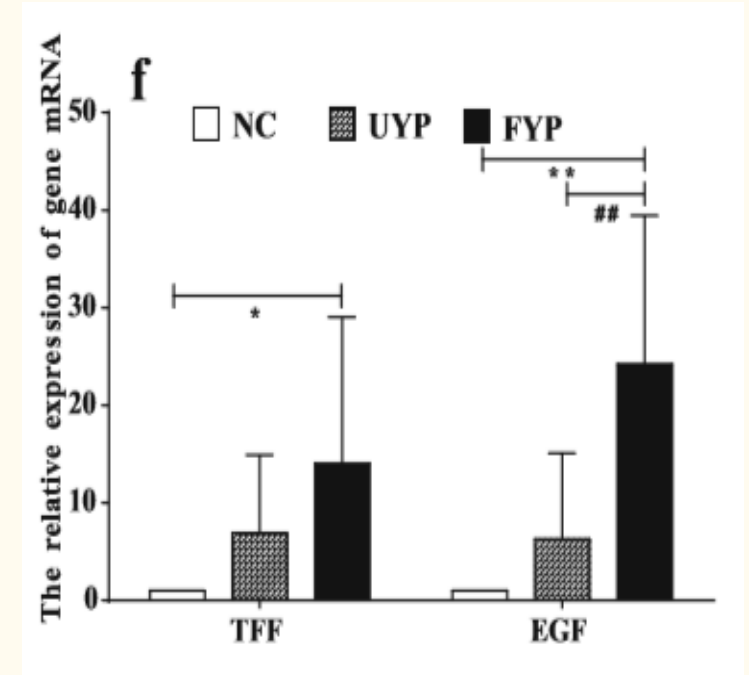
Fig. 7-回肠



物理屏障



免疫屏障



化学屏障

Fig. 7 Effects of UYP or FYP on intestinal barrier of rex rabbit. d–f indicates those in the ileum, respectively. The mRNA quantification data were normalized to GAPDH.

结果与讨论



Fig.6:

UYP和FYP处理后，显著减少肠杆菌、肠球菌和链球菌的含量，增加了乳酸菌、双歧杆菌、普氏菌等的含量。结果表明，UYP和FYP的免疫作用可能是通过增加益生菌的数量来抑制有害细菌实现的。

Fig.7:

结果显示，与对照组相比，UYP和FYP显著提高了物理屏障、免疫屏障、化学屏障相关基因的表达。

总的来说，FYP比UYP更能促进生长和增强免疫。此外，FYP的有益作用可能与TLR2和TLR4通路的激活有关，通过激活TLR2和TLR4通路，促进细胞因子的合成，改善肠道群落结构，改善肠道屏障的完整性和功能。

