

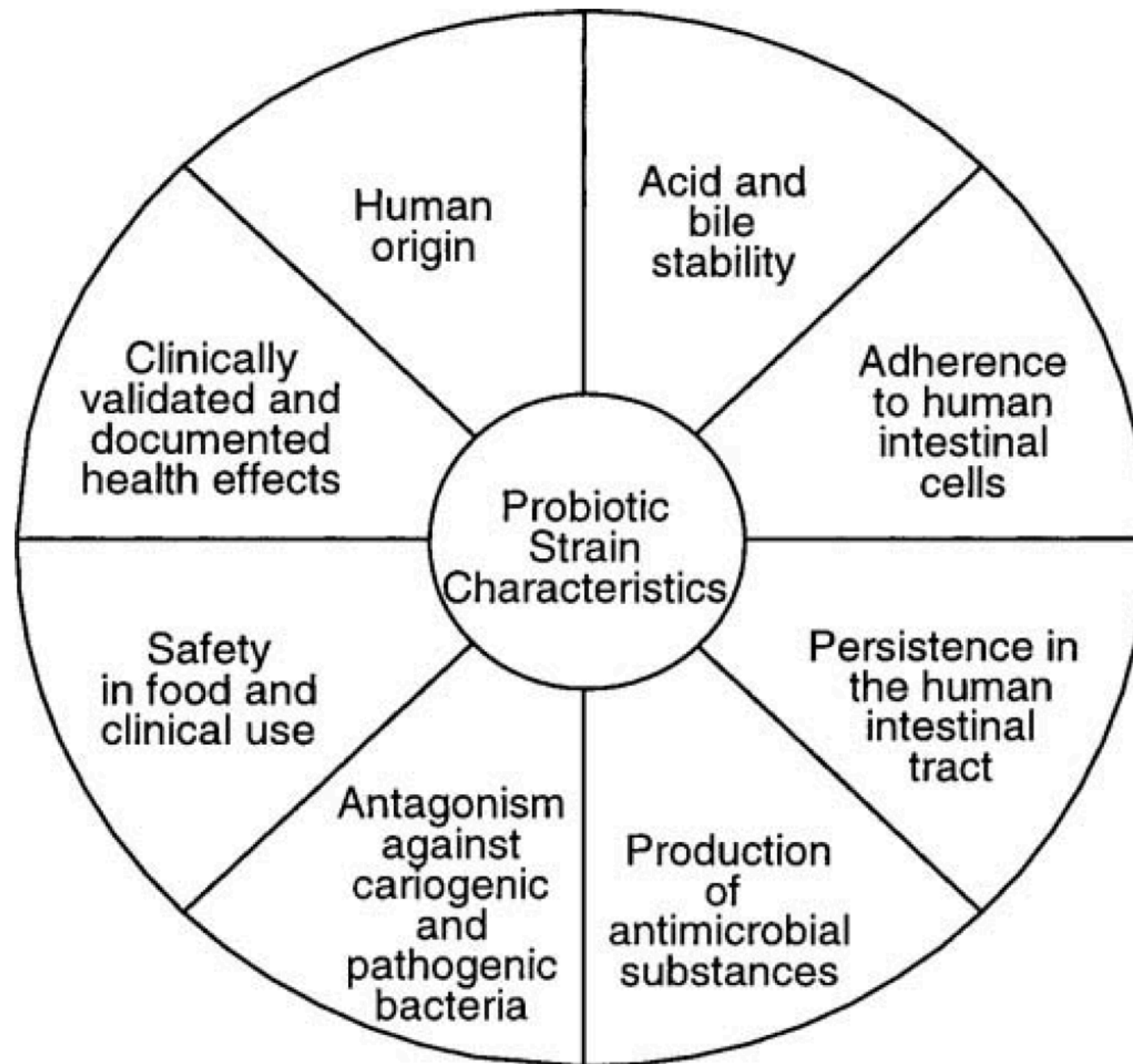
小兒腸胃益生菌 臨床運用與經驗

黃師堯醫師
禾馨民權婦幼中心
2019.4.15

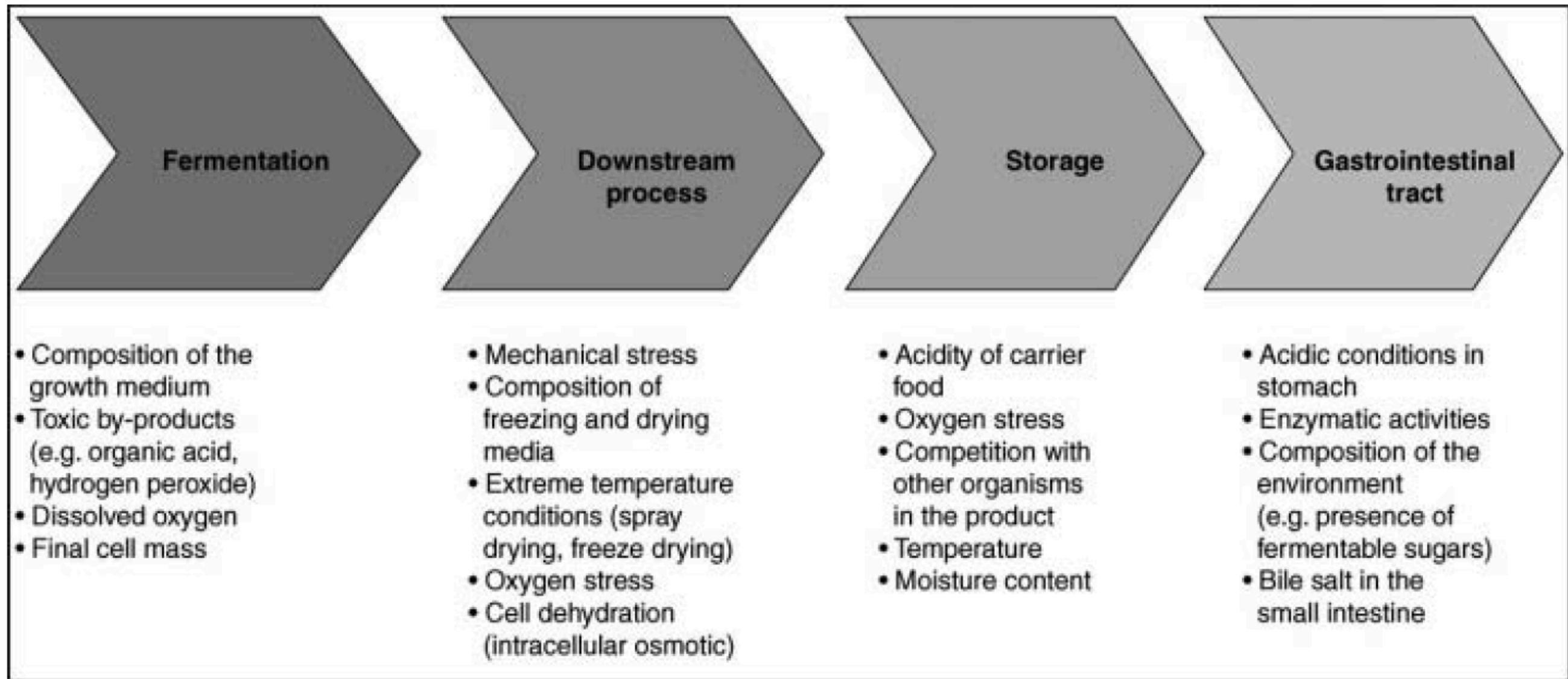
你的 Microbiome

The Hygiene Hypothesis

理想的益生菌



如何維持效果：從發酵到腸胃道



Improving viability

- Thermoprotectants
 - trehalose
 - nonfat milk solids
 - adonitol
- growth-promoting factors
 - probiotic/ prebiotic combinations
 - granular starch

抗發炎の機制是什麼？

SCID/NOD mice model for 5-FU induced intestinal mucositis: safety and effects of probiotics as therapy

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Chuen-Bin Jiang, Jen-Shiu Chiang Chiau, Mei-Lien
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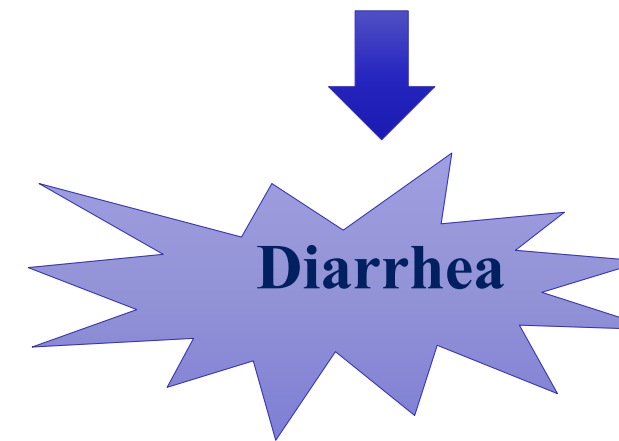
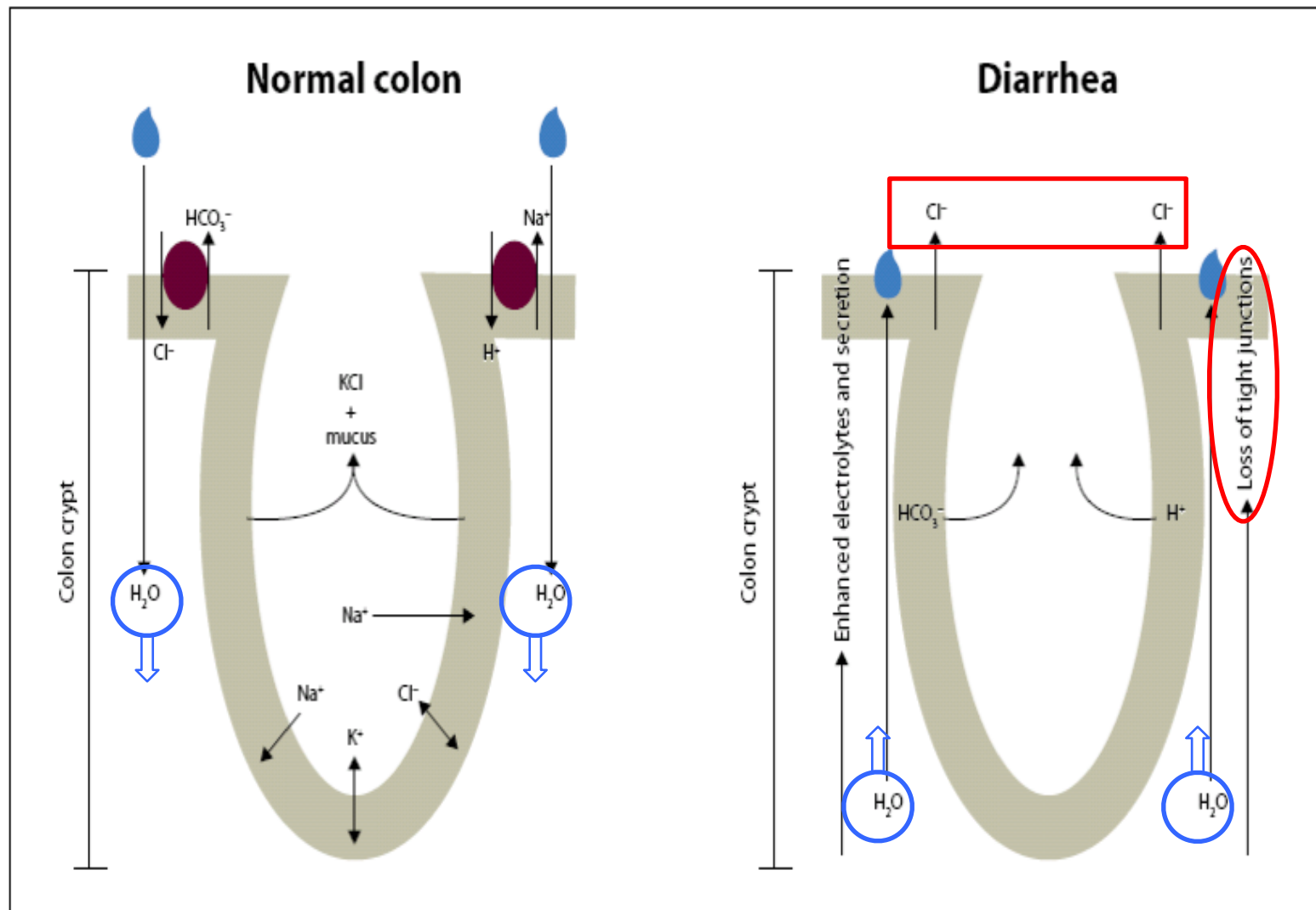
Introduction

- Chemotherapy-induced GI mucositis can occur in about **50-80% of patients**, and it has significant implications on the quality of life for cancer patients.
- The most cytotoxic agents associated with mucositis are the **5-FU, capecitabine and irinotecan**.
- Despite new agents for management such as keratinocyte growth factor and epidermal growth factor in murine models, supportive care with pain relief, oral and IV hydration, and maintaining oral hygiene are still the mainstay of management.

Chemotherapy-induced intestinal mucositis

- Diarrhea, malabsorption, abnormal inflammatory processes
- Mechanism: **unbalance gut microflora?**

mucositis \longrightarrow loss of tight junction, reduced absorption of Cl^- \longrightarrow change fluid transport \longrightarrow released fluid



Amelioration of Chemotherapy-Induced Intestinal Mucositis by Orally Administered Probiotics in a Mouse Model

Chun-Yan Yeung^{1,2,3,4}✉, Wai-Tao Chan¹✉, Chun-Bin Jiang¹, Mei-Lien Cheng⁴, Chia-Yuan Liu^{4,5}, Szu-Wen Chang¹, Jen-Shiu Chiang Chiau⁴, Hung-Chang Lee^{6,7}*



5FU-treated
mice



Lactobacillus
rhamnosus (Lcr35)



L. acidophilus
+ *B. bifidum* (LaBi)

72 mice were injected saline or 5-Fluorouracil (5-FU) IP daily. Mice were either orally administered probiotic suspension of *Lactobacillus casei* variety *rhamnosus* (Lcr35) or *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (LaBi).

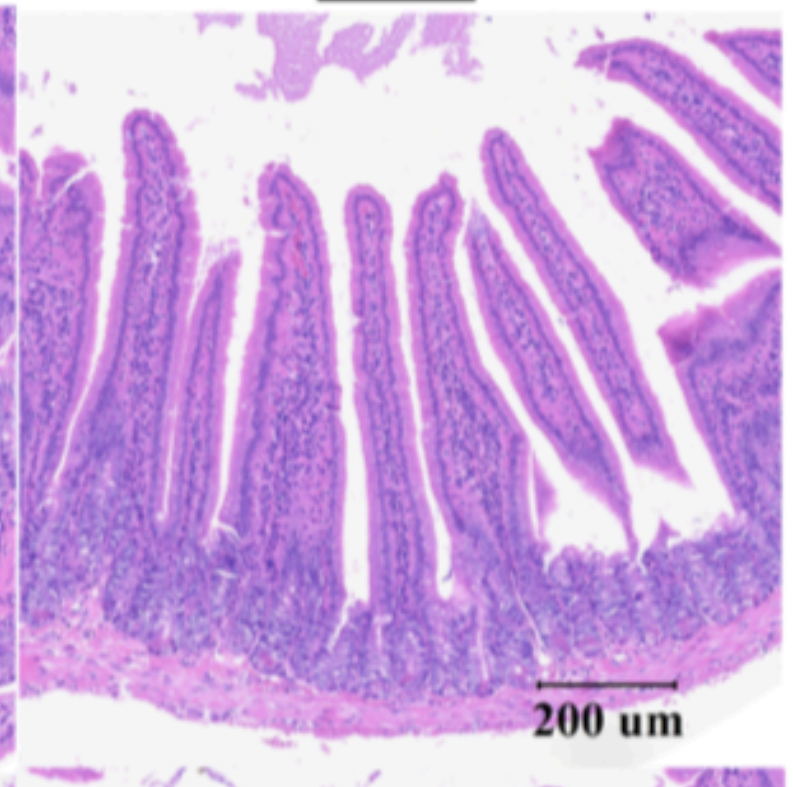
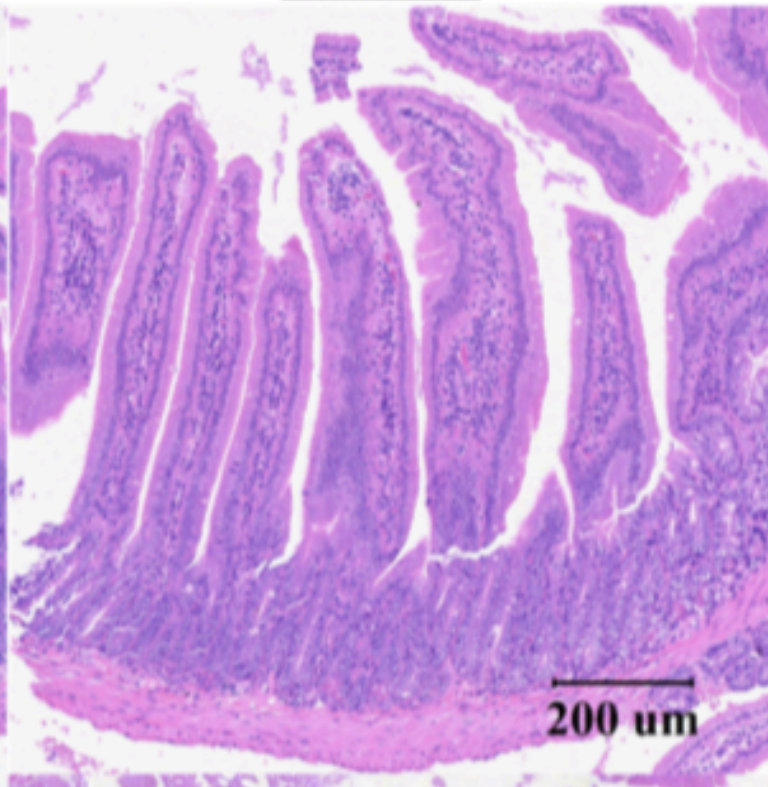
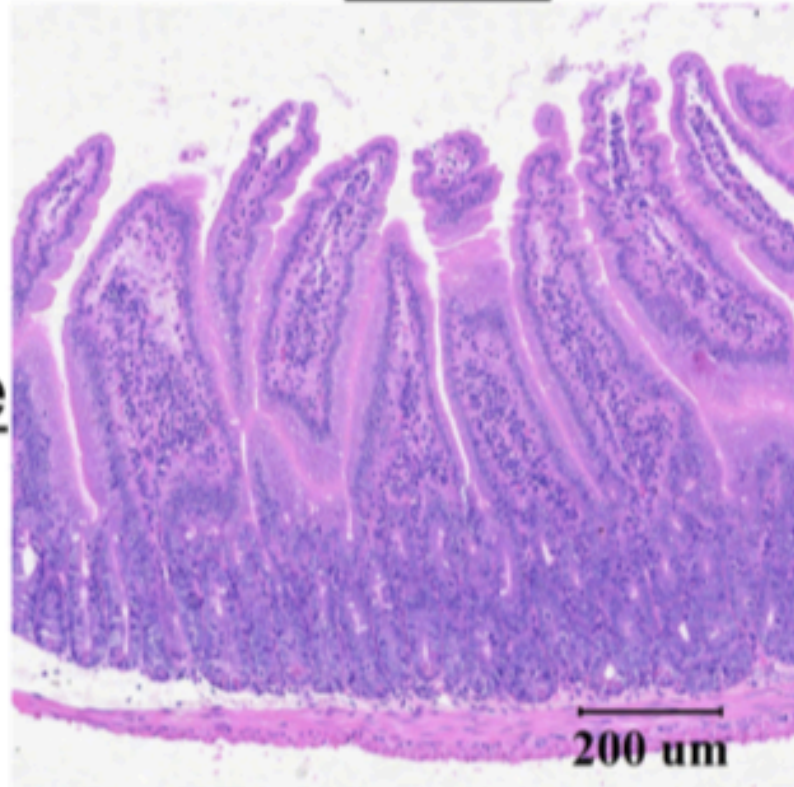
A

saline

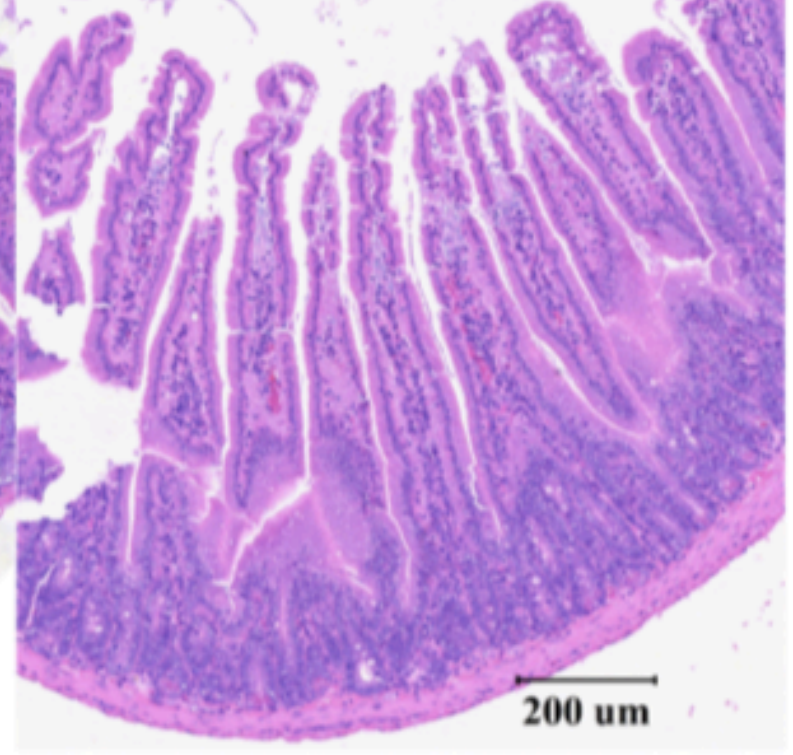
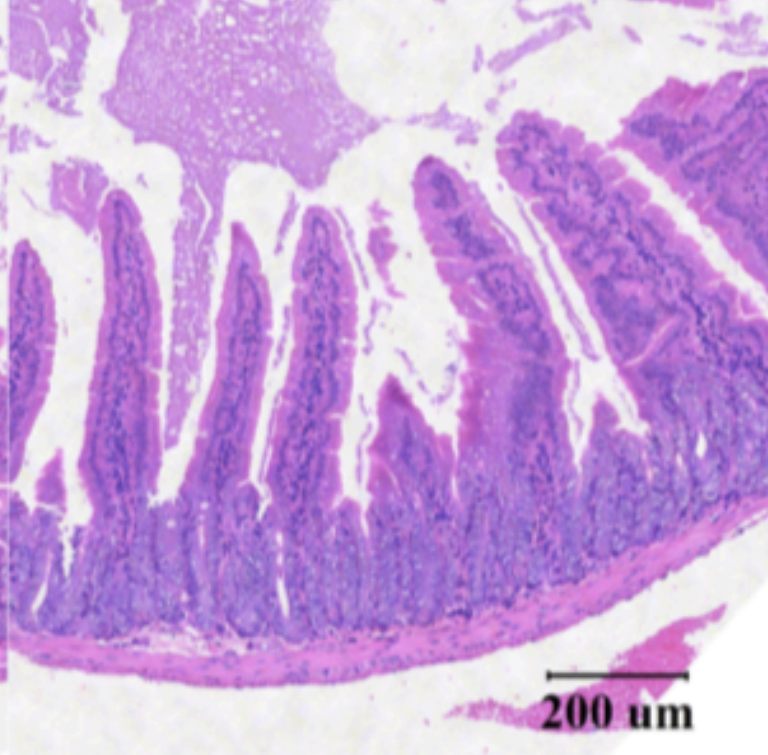
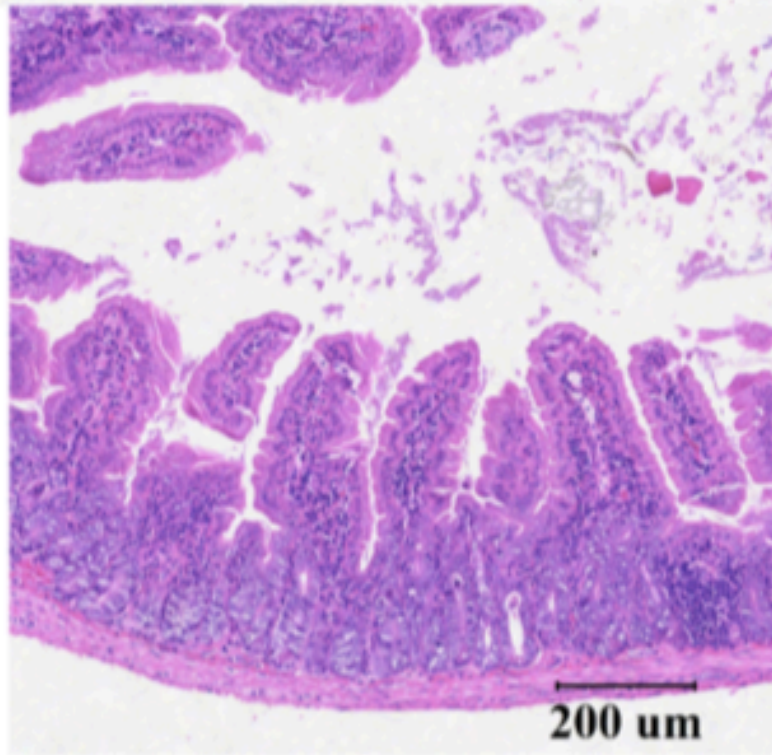
Lcr35

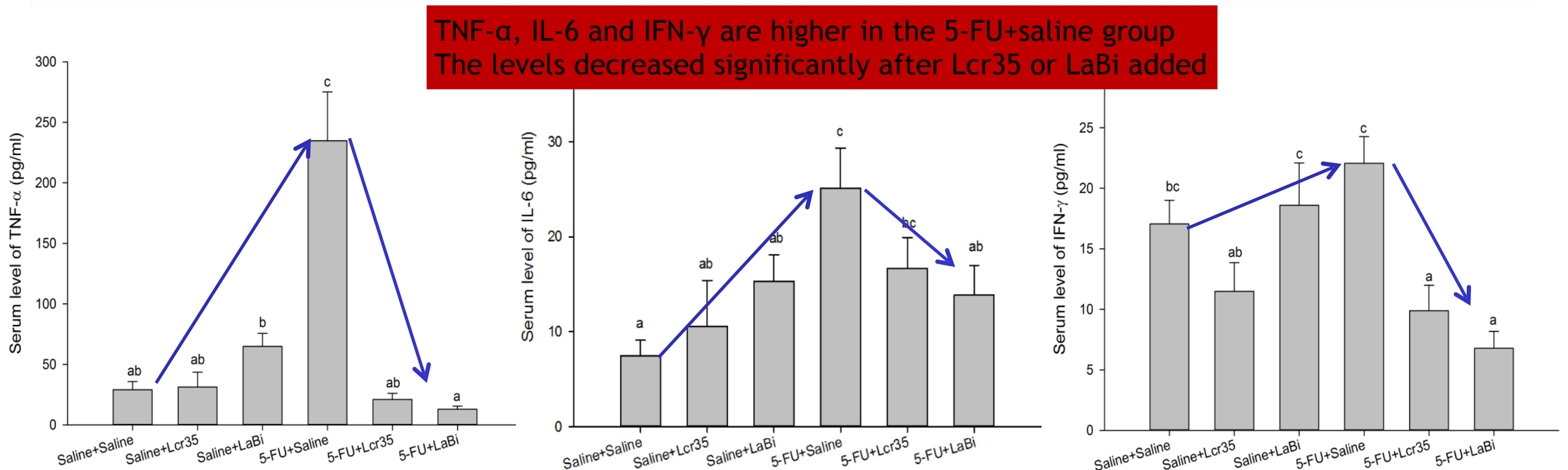
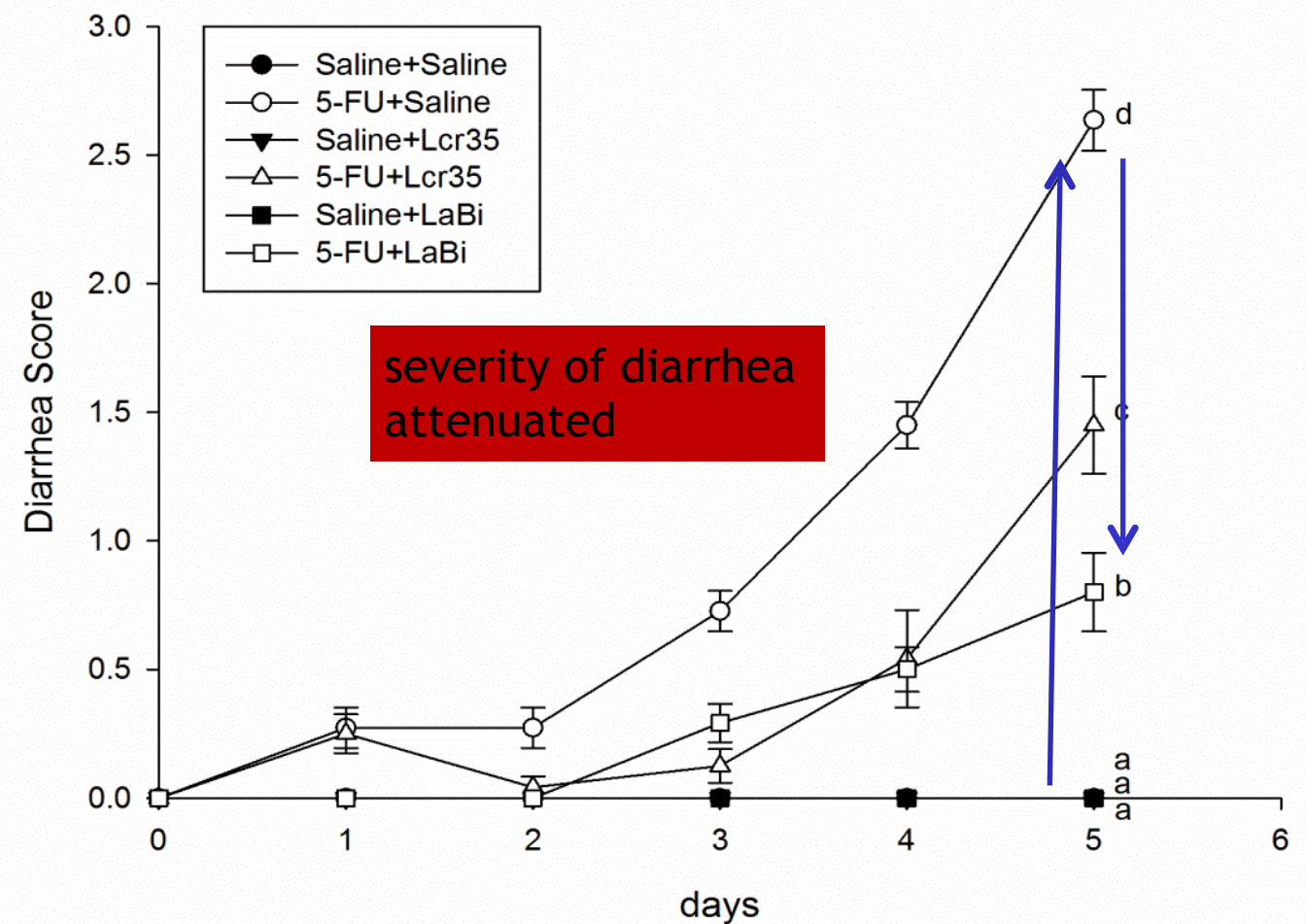
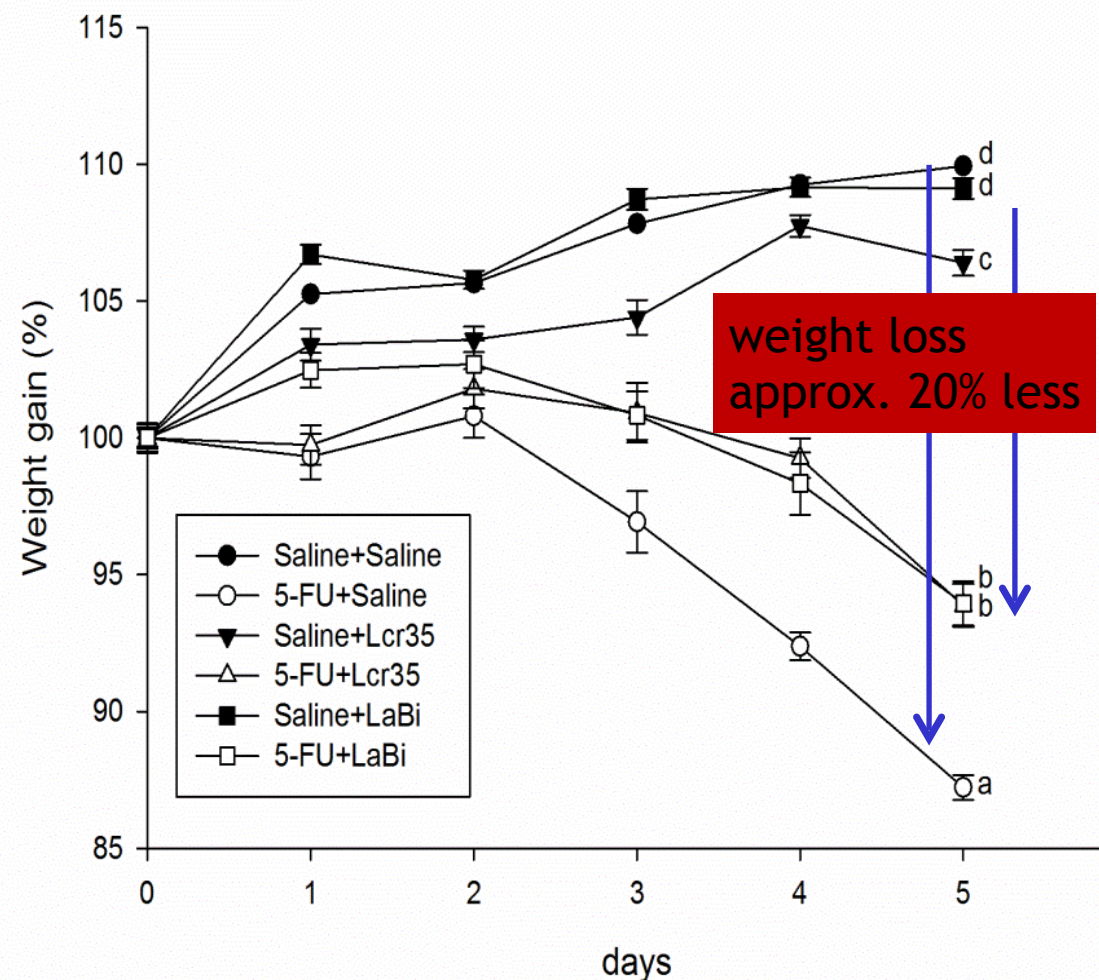
LaBi

Saline



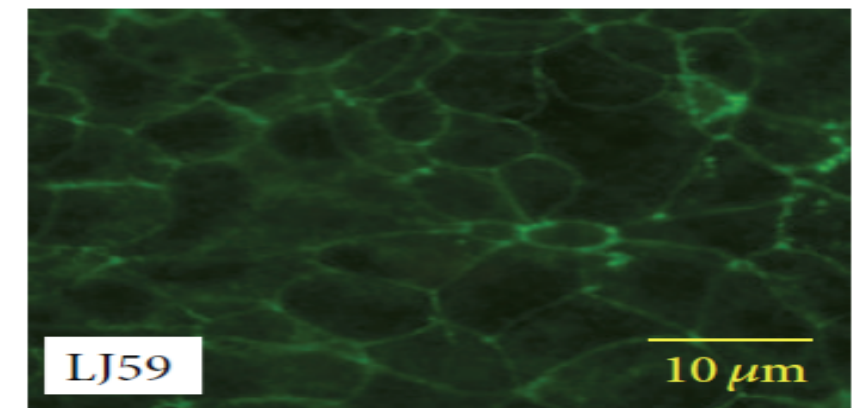
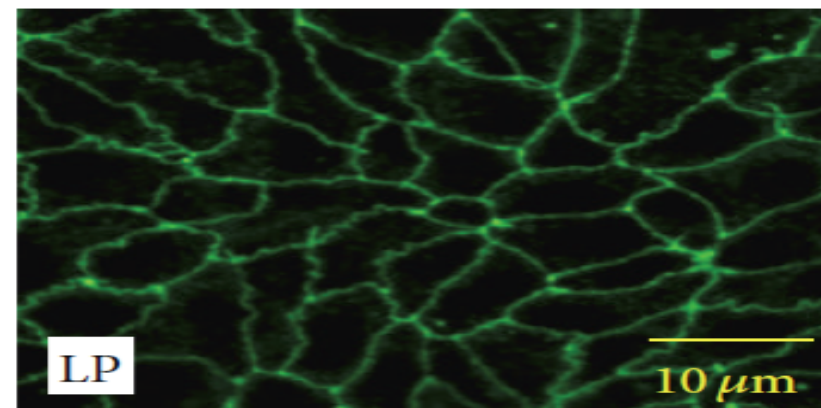
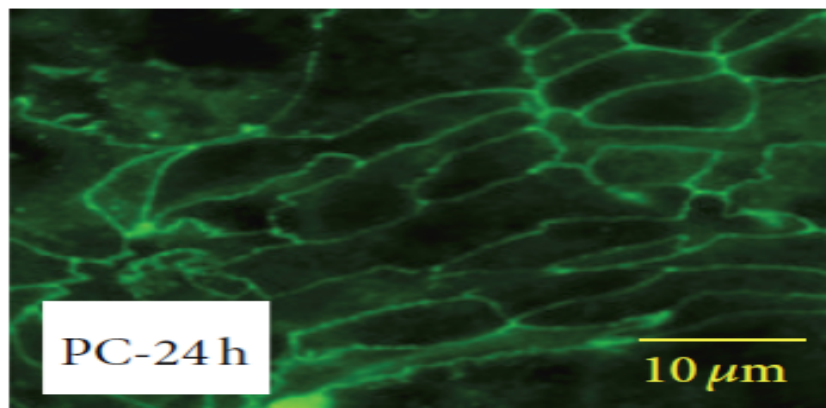
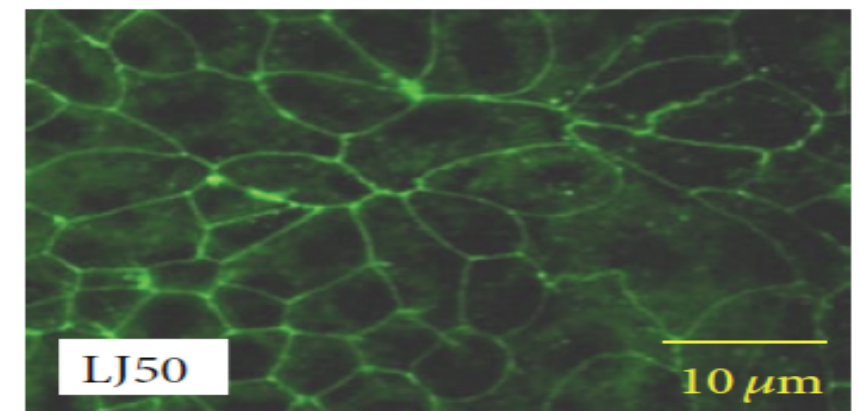
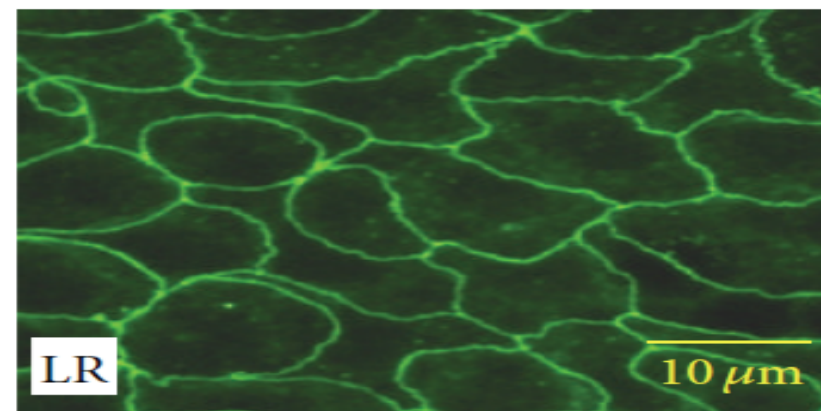
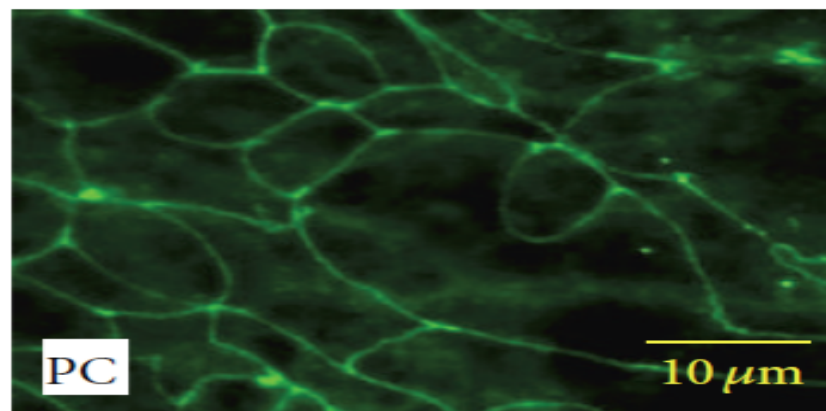
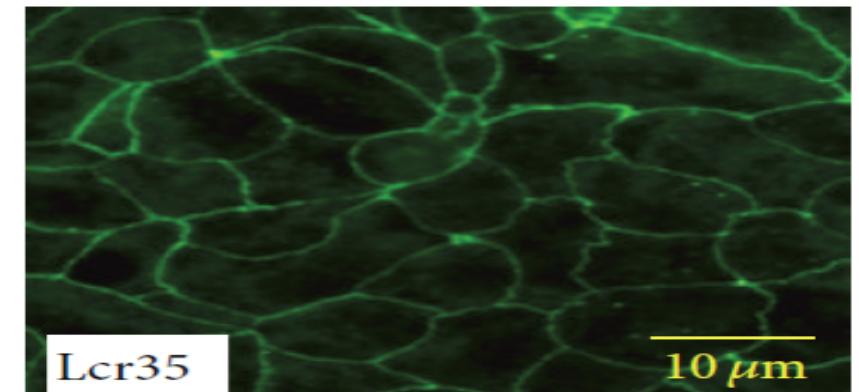
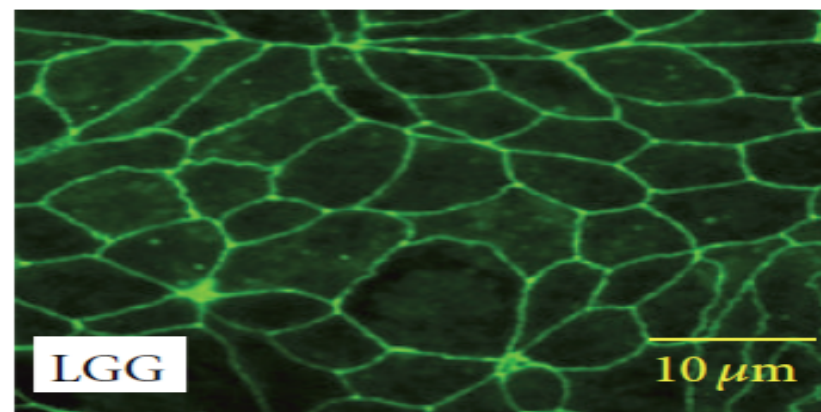
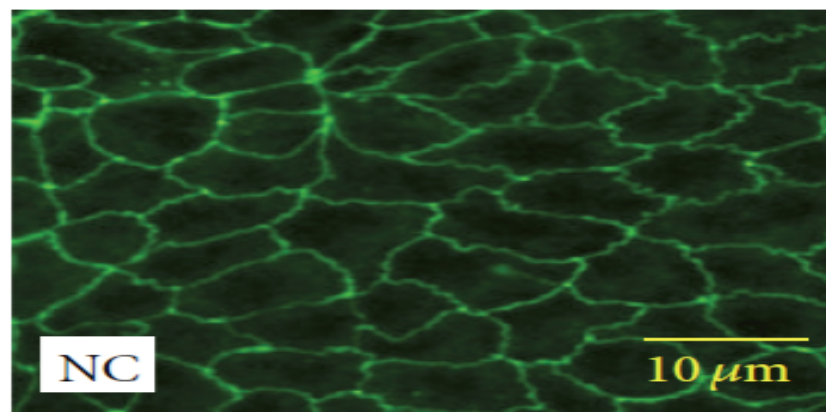
5-FU





***In Vitro* Prevention of *Salmonella* Lipopolysaccharide-Induced Damages in Epithelial Barrier Function by Various *Lactobacillus* Strains**

Chun-Yan Yeung,^{1,2,3,4} Jen-Shiu Chiang Chiau,⁵ Wai-Tao Chan,^{1,2,3} Chun-Bin Jiang,^{1,2} Mei-Lien Cheng,⁵ Hsuan-Liang Liu,³ and Hung-Chang Lee^{6,7}



Aim

To further test for safety of probiotics and more closely simulate the immunodeficiency status for cancer patients, we used the SCID/NOD mice model both to explore effects and test for translocation of oral probiotics given for 5-FU induced mucositis.

Materials and Methods

- 36 SCID/NOD mice were injected with **saline (3 control groups) or 5-FU (3 experimental groups)** i.p. daily for 5 days.
- Mice were given either oral saline daily, probiotic suspension of 2 probiotics.
- Blood, liver, spleen, and lymph node tissue samples were evaluated for probiotic translocation via culture and Q-PCR.
- Weight change, diarrhea score, jejunal villus height and crypt depth and various serum cytokines levels were also assessed.

Probiotics preparation

- *Lactobacillus casei variety rhamnosus* (Lcr 35, Antibiophilus, France) and
- *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (LaBi, Infloran, Italy) were used.
- Probiotics were diluted in sterile saline and administered by oral gavages.
- The mice received 100 uL of saline or probiotic suspension containing 1×10^7 CFU daily for 5 days.

Assessment

- Diarrhea score
- BW loss
- Jejunal mucosa villi and crypt changes
- Effect on serum cytokine levels post oral probiotics
- Probiotic strain organ and blood translocation via Q-PCR and culture

Diarrhea Score

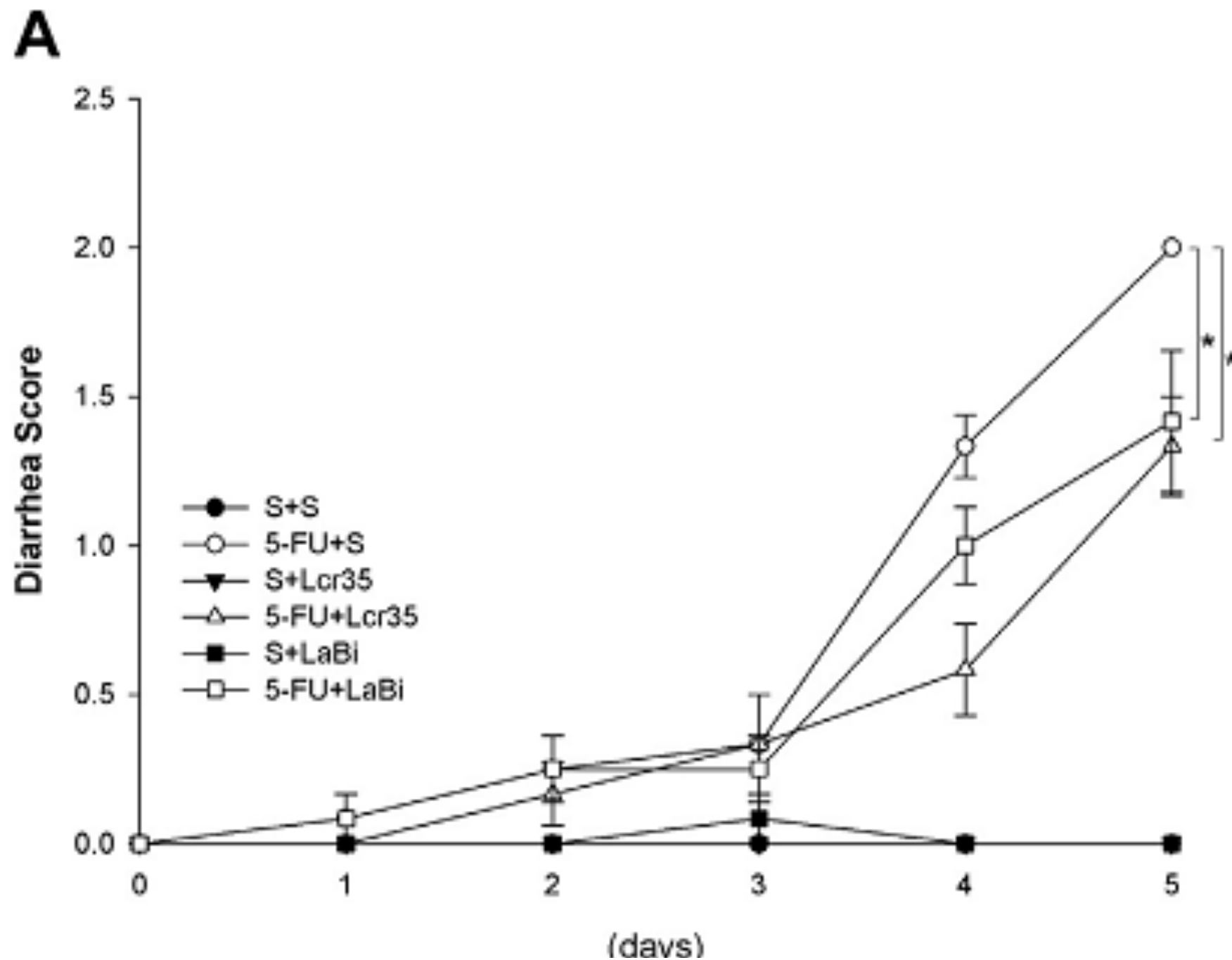
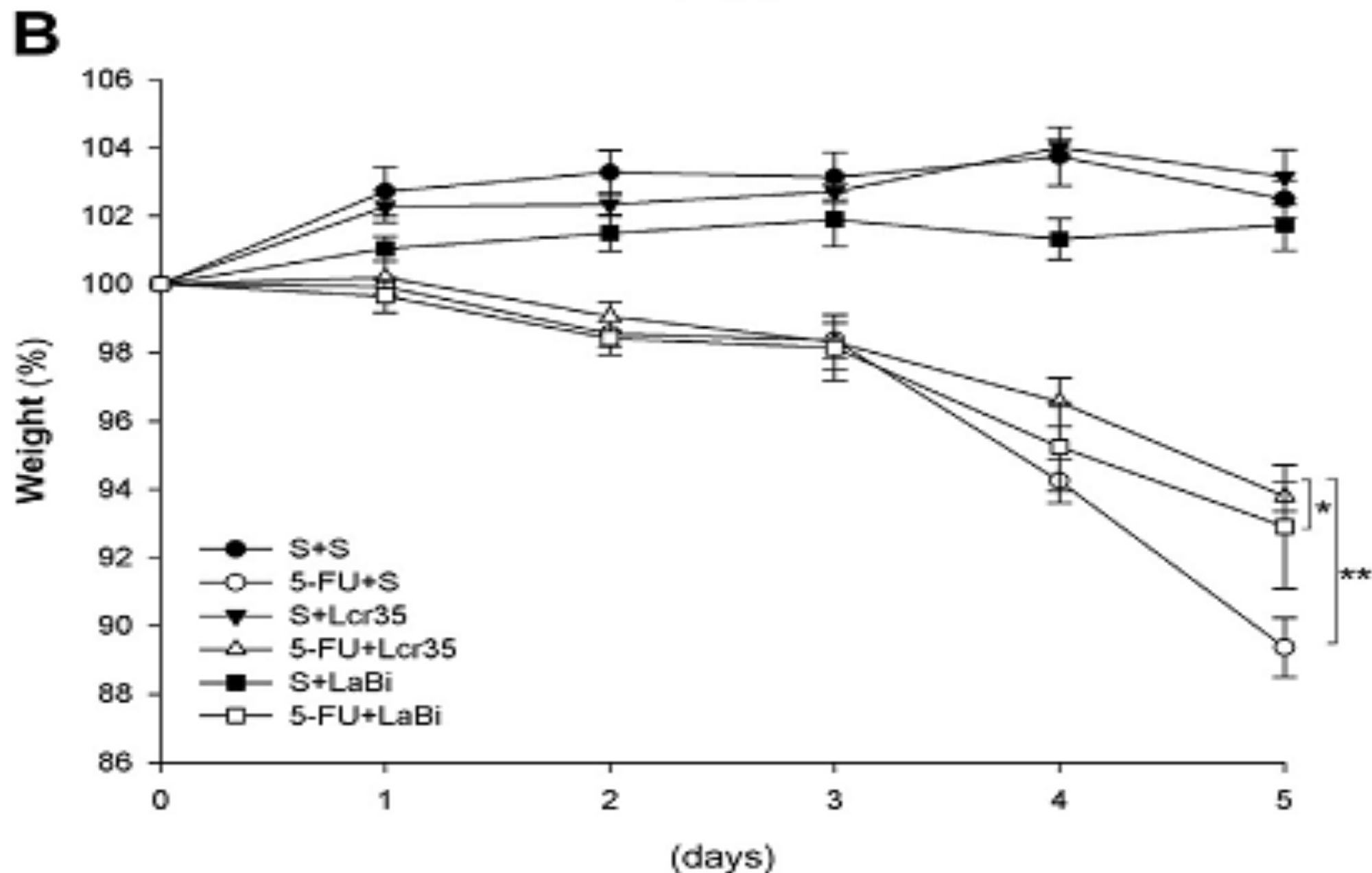


Figure 1 Assessment of SCID mice Intestinal Mucositis Severity. A. Diarrhea Score of SCID/NOD mice after oral probiotics *Lcr35* or *LaBi* with/without 5-FU treatment. The

Body Weight Change



($P < 0.05$) based on one-way ANOVA. B. Daily body weight change in percentage of saline or 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*. The mice were

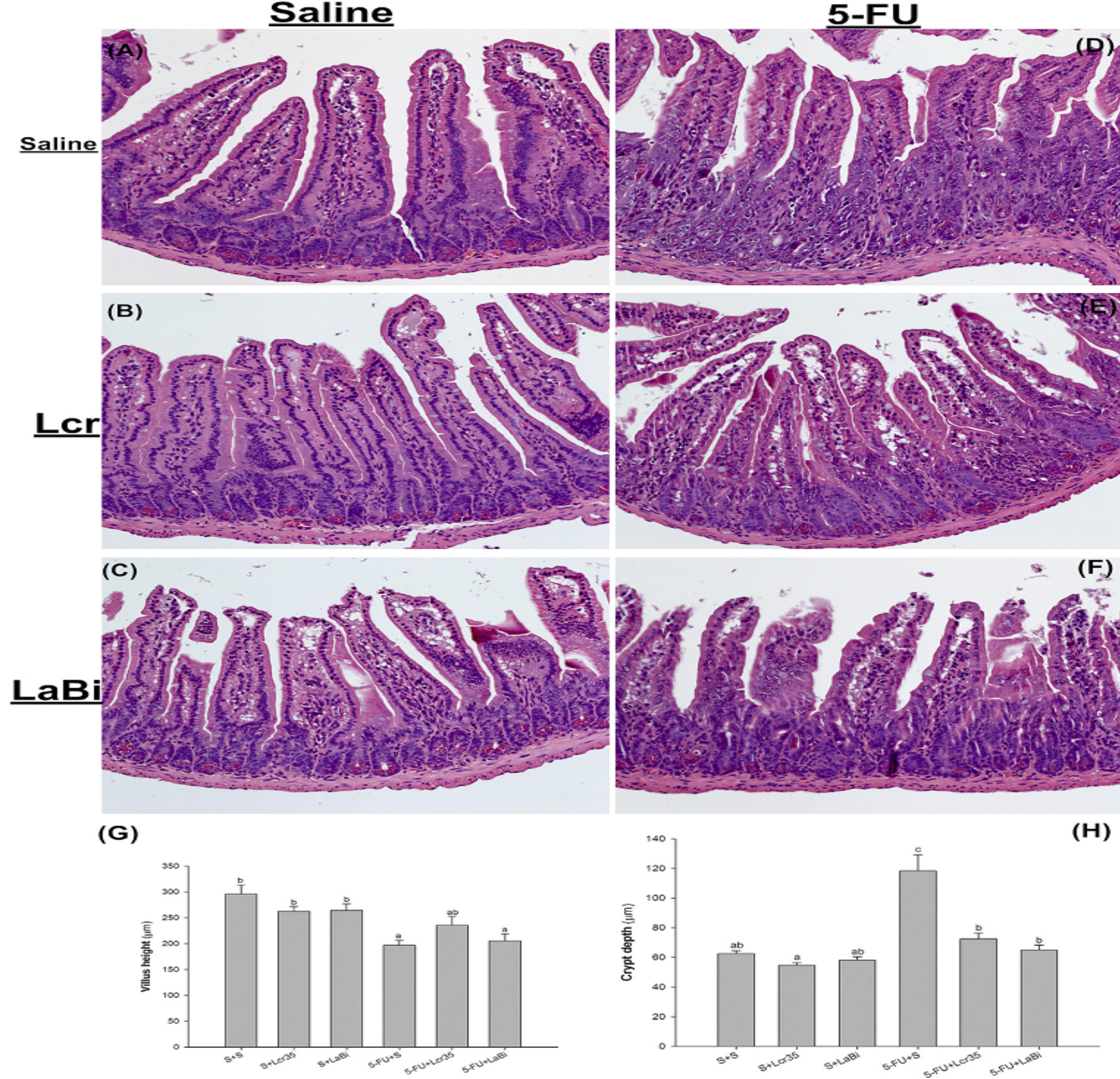


Figure 2 Representative histology of jejunal villus height(VH) and crypt depth(CD) with HE stain in mice on day 5 with saline or 5-FU with/without probiotics(*Lcr35* or *LaBi*). (A, B, C, D, E, F) The image acquisition phase was done with a 20x magnification objective. (G,H) Average jejunal villus height and crypt depth measurements. Values are represented as mean \pm SEM and were analyzed using one-way ANOVA.

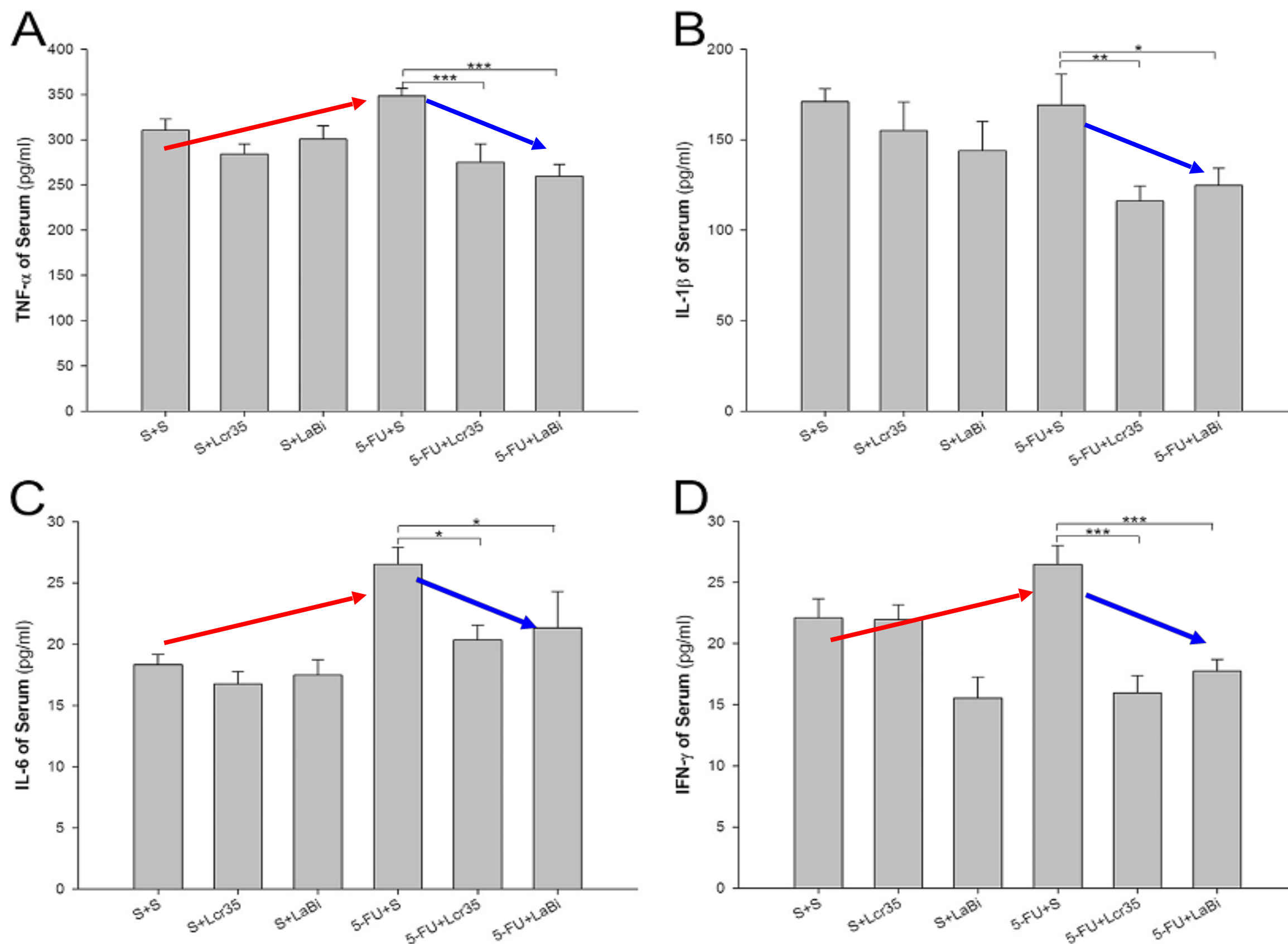


Figure 3 Serum levels of TNF- α , IL-1 β , IL-6, and IFN γ of 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*. The statistical analysis was performed by One-way ANOVA. (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

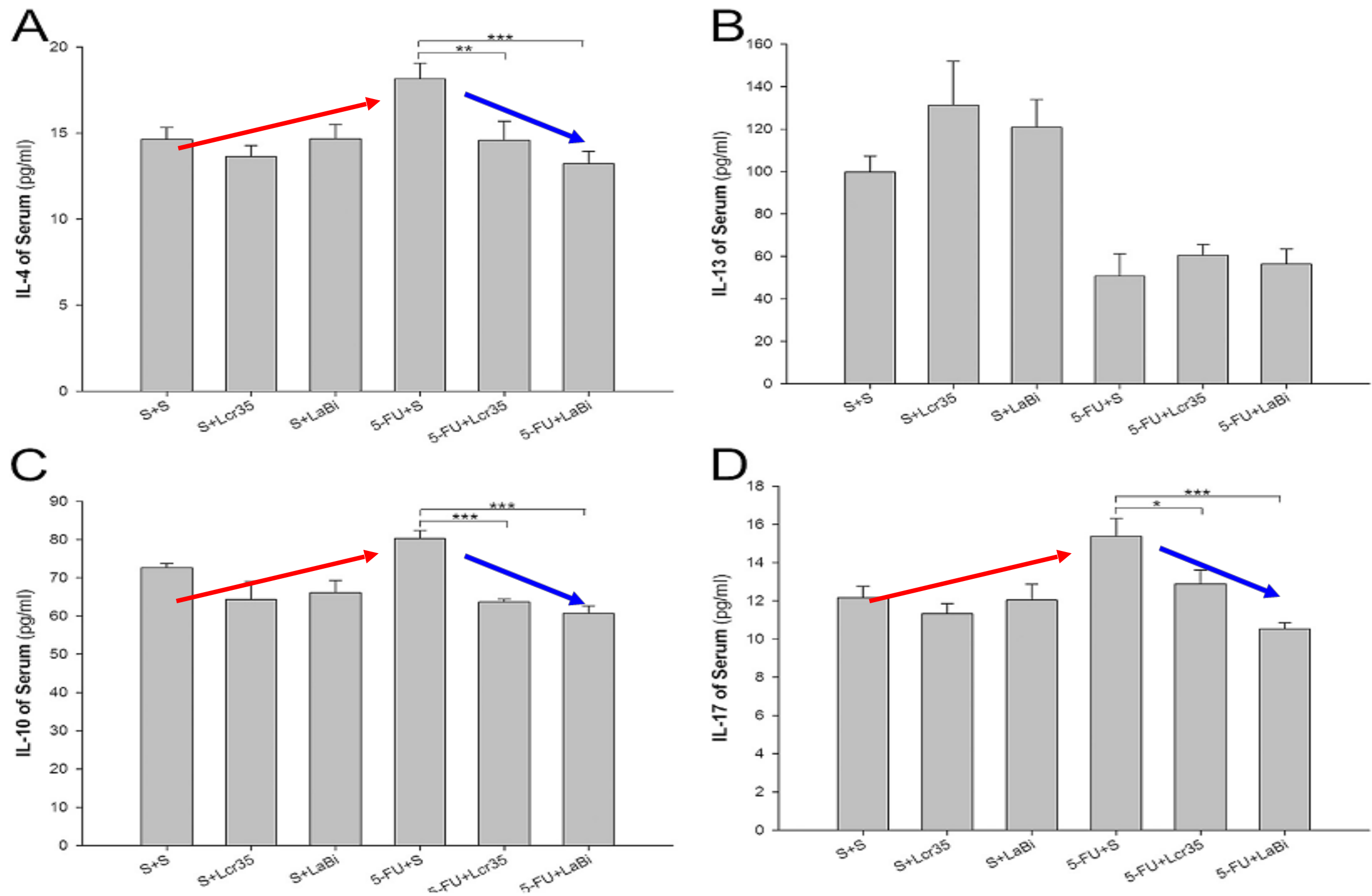
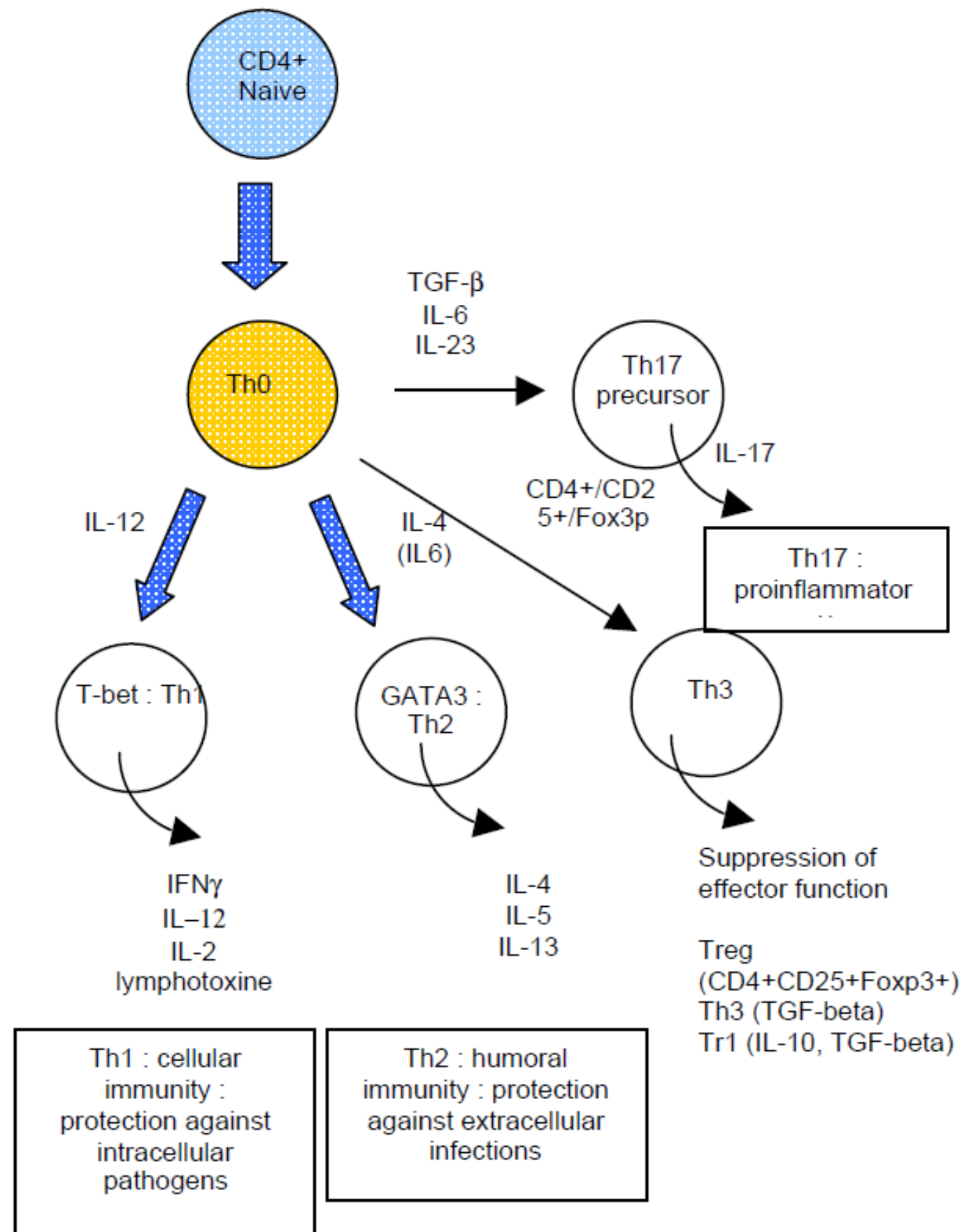


Figure 4 Serum levels of IL-4, IL-10, IL-13, and IL-17 of 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*. The statistical analysis was performed by One-way ANOVA. (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

Table 1 Culture and Q-PCR for individual probiotic strains *Lcr35* and *LaBi* in mesenteric lymph node, spleen, liver, and blood samples.

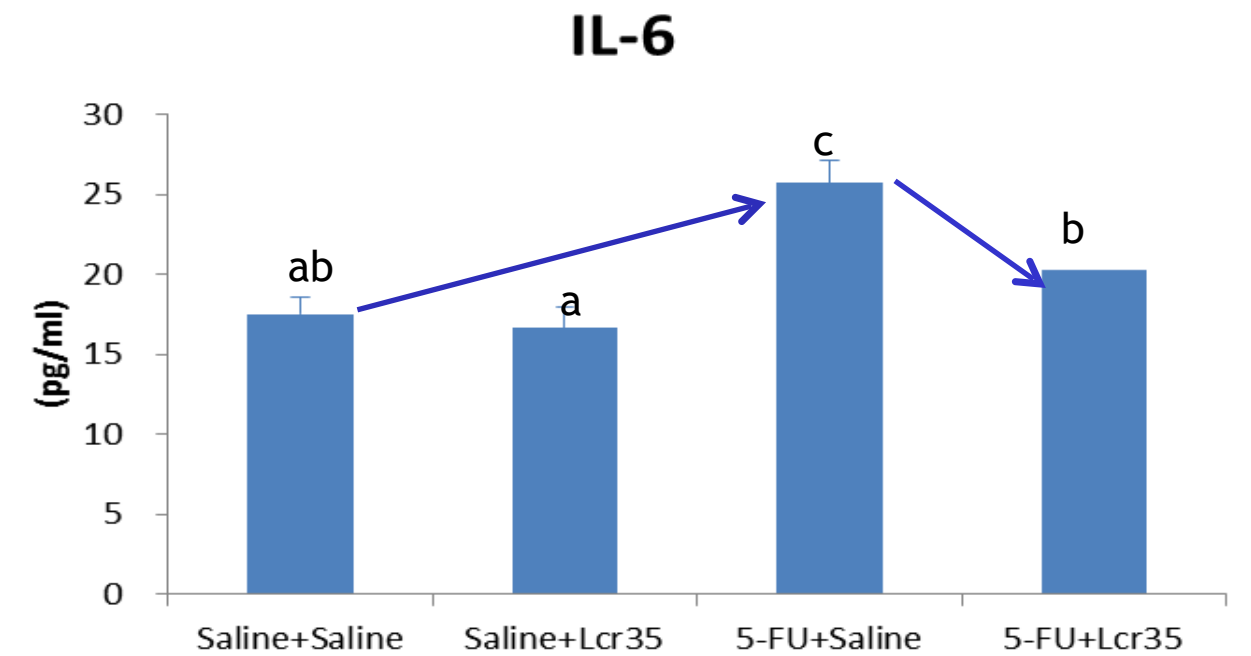
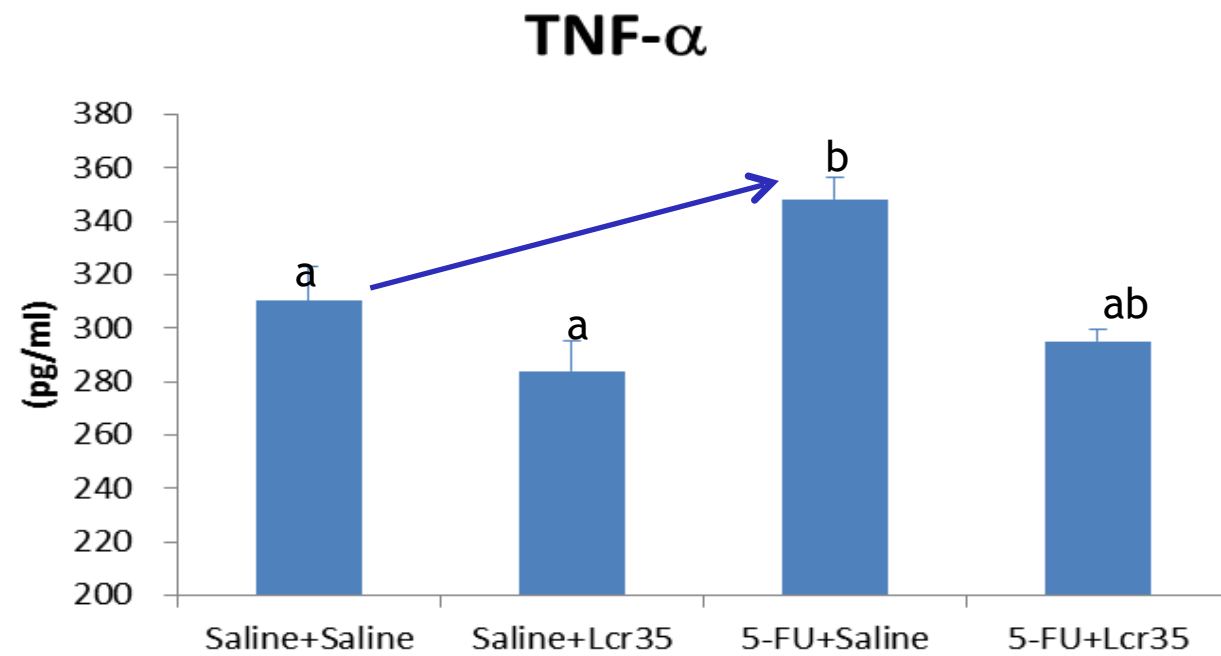
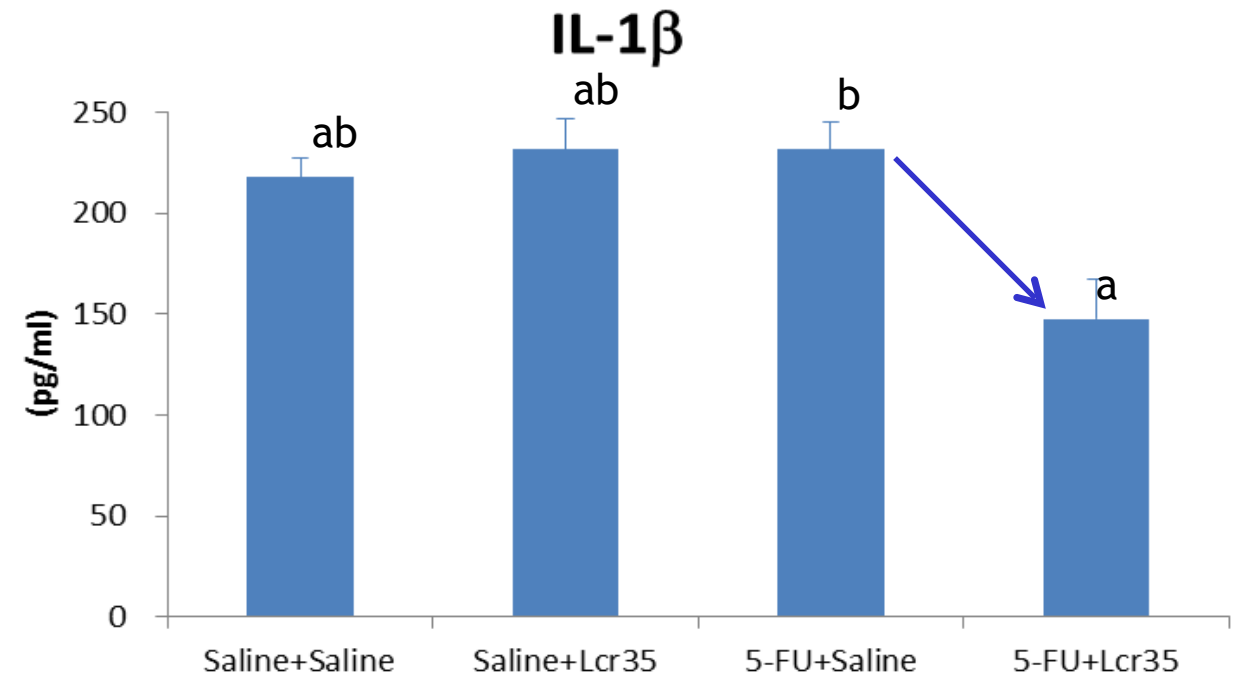
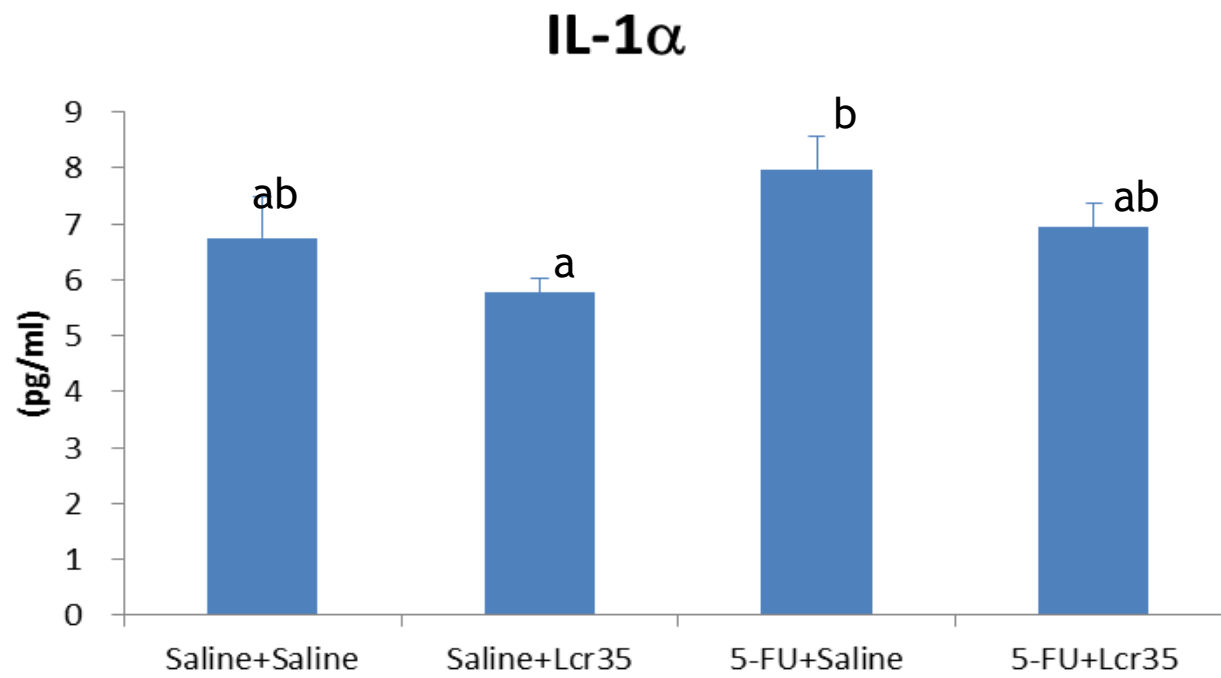
Culture for bacteria	Mesenteric lymph node	Spleen	Liver	Blood
<i>S</i> + <i>S</i>	3/6	0/6	0/6	0/6
<i>S</i> + <i>Lcr35</i>	2/6	1/6	0/6	0/6
<i>S</i> + <i>LaBi</i>	1/6	0/6	0/6	0/6
5-FU + <i>S</i>	3/6	1/6	0/6	0/6
5-FU + <i>Lcr35</i>	2/6	1/6	1/6	0/6
5-FU + <i>LaBi</i>	4/6	1/6	1/6	0/6
PCR for <i>Lcr35</i>	Mesenteric lymph node	Spleen	Liver	Blood
<i>S</i> + <i>S</i>	1/6	0/6	0/6	1/6
<i>S</i> + <i>Lcr35</i>	1/6	0/6	0/6	1/6
5-FU + <i>S</i>	1/6	0/6	0/6	0/6
5-FU + <i>Lcr35</i>	3/6	1/6	0/6	0/6
PCR for <i>LaBi</i>	Mesenteric lymph node	Spleen	Liver	Blood
<i>S</i> + <i>S</i>	4/6	1/6	2/6	0/6
<i>S</i> + <i>LaBi</i>	5/6	2/6	2/6	1/6
5-FU + <i>S</i>	4/6	3/6	2/6	1/6
5-FU + <i>LaBi</i>	6/6	4/6	3/6	1/6

- The interaction between probiotic and enterocytes is the initiating event in immunomodulation and affects profiles of cytokines secreted by lymphocytes or dendritic cells.
- Pro-inflammatory cytokines: IL-1, TNF- α
- Th1/Th2/Th3 response cytokines
 - Th1: IL-12, IFN- γ
 - Th2: IL-4, IL-5, IL-13
 - Th3-Treg: IL-10 (Tr1)

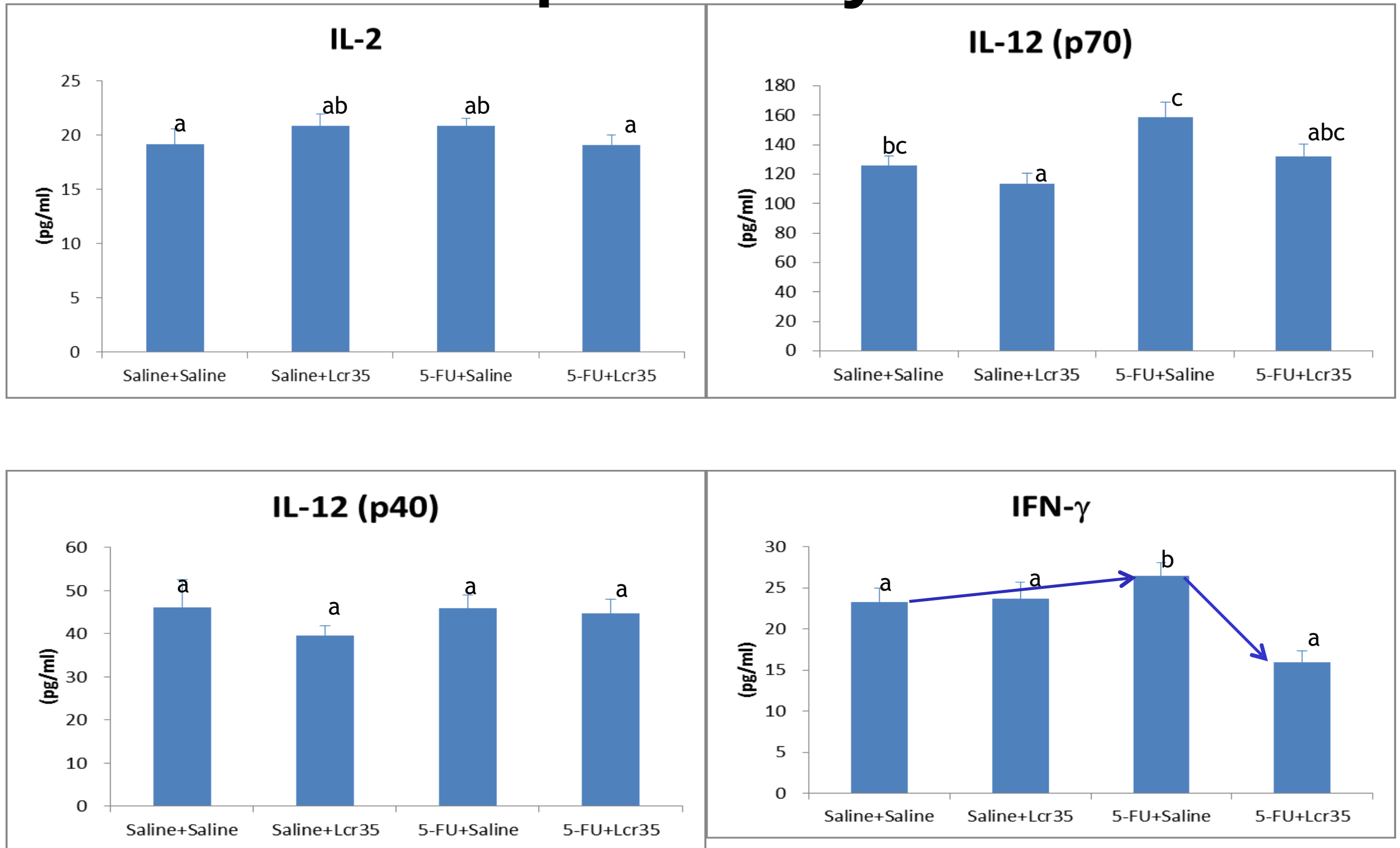


(Deltcenserie et al., (2007) Curr. Issues Mol. Biol. 10:37-54.)

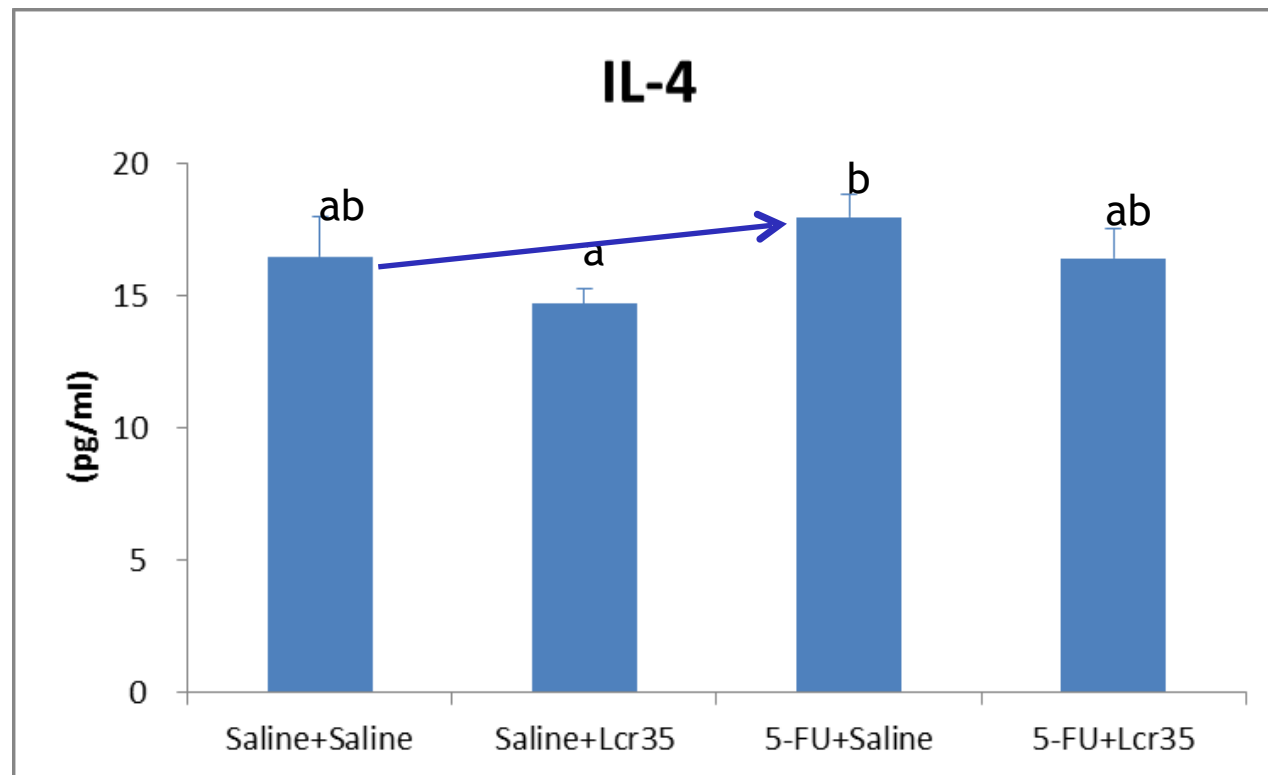
Pro-inflammatory cytokines



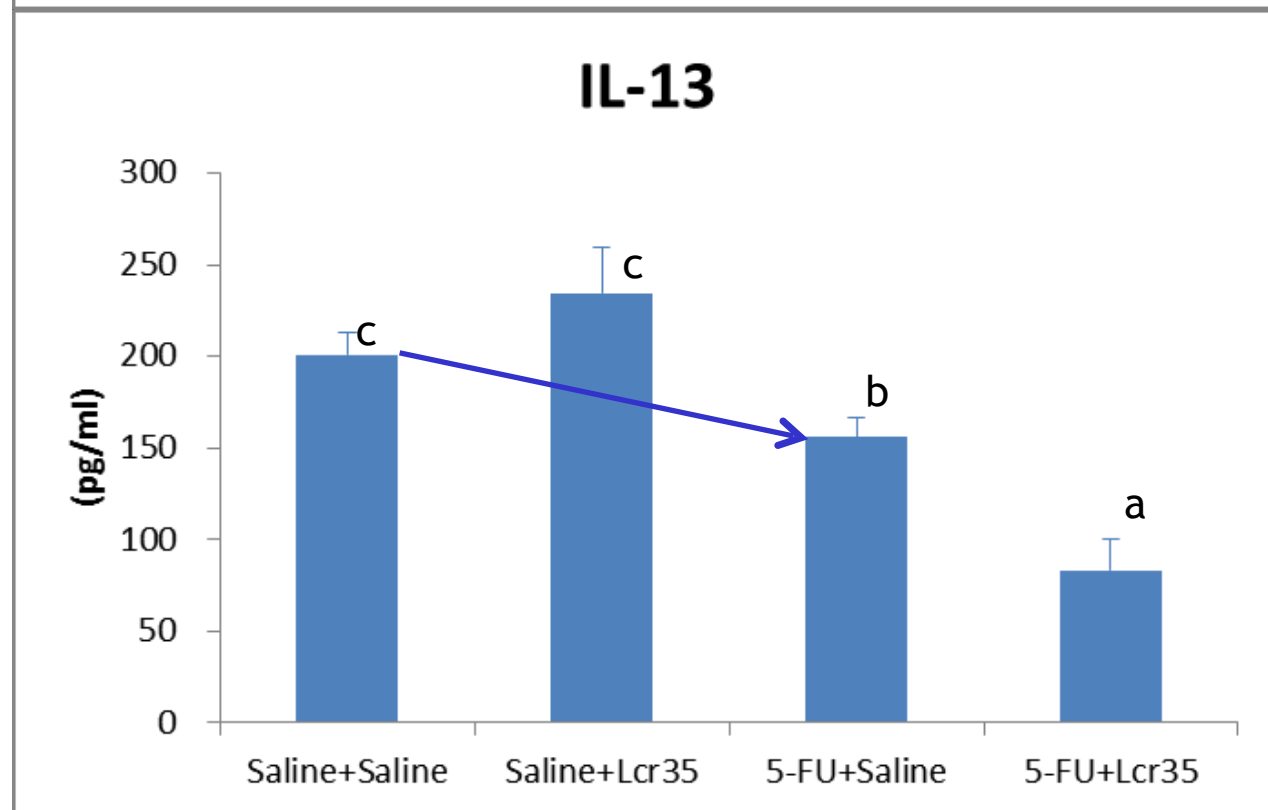
Th1 response cytokines



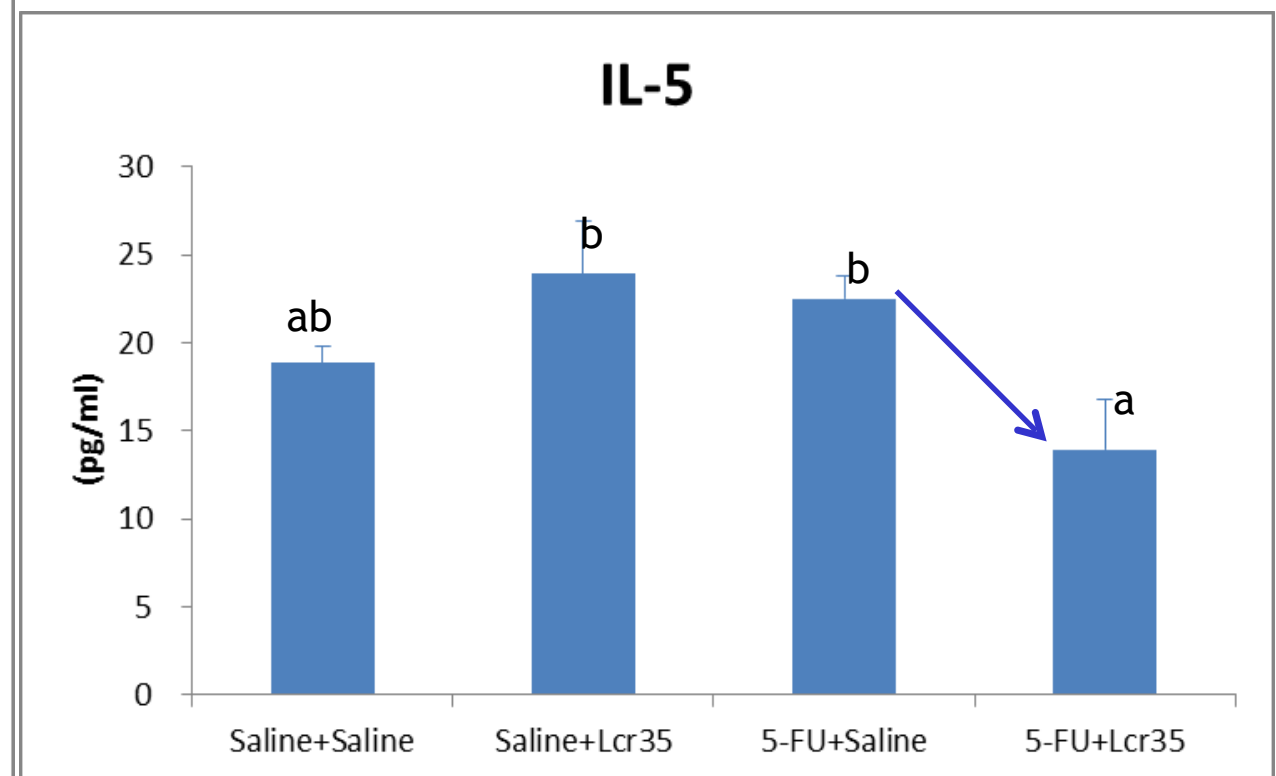
Th2 response cytokines



5-FU increased IL-4 production

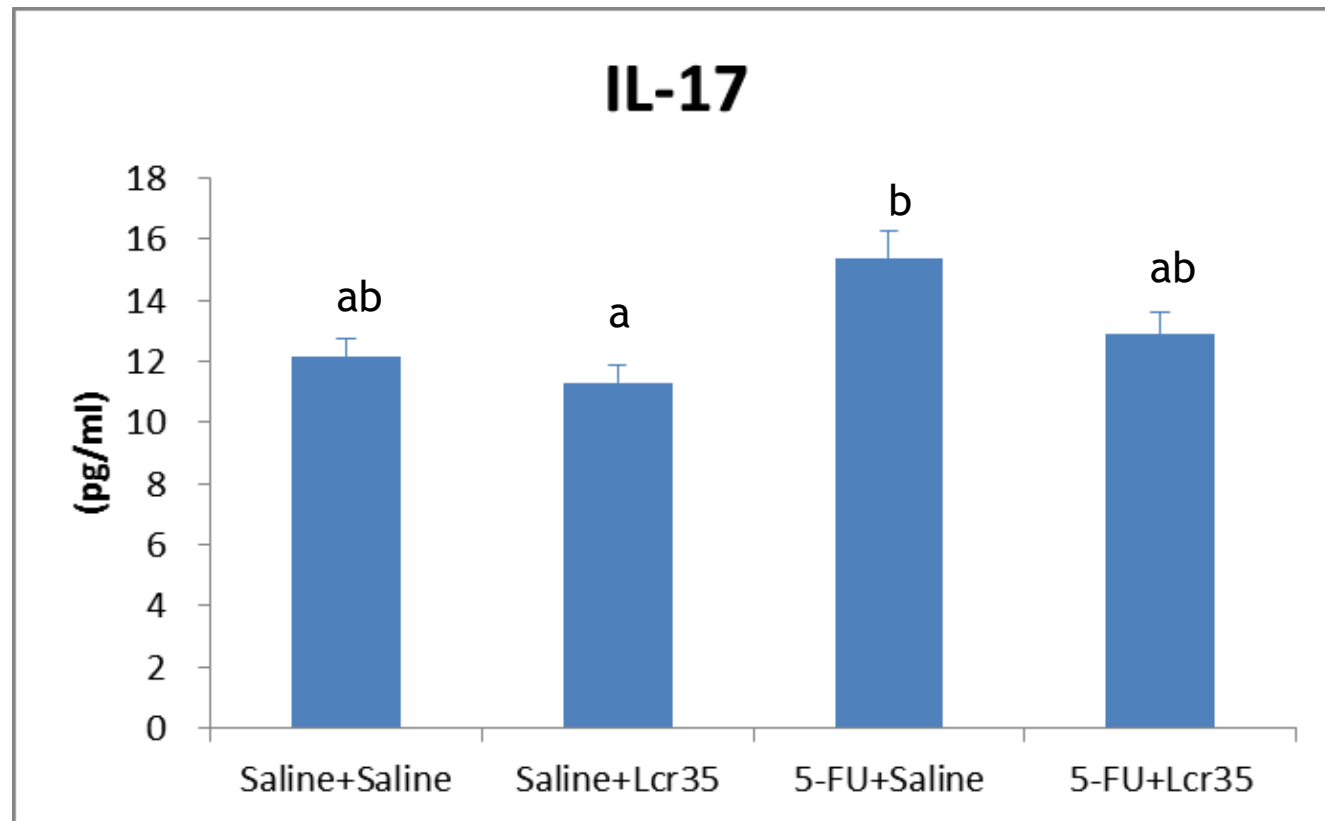
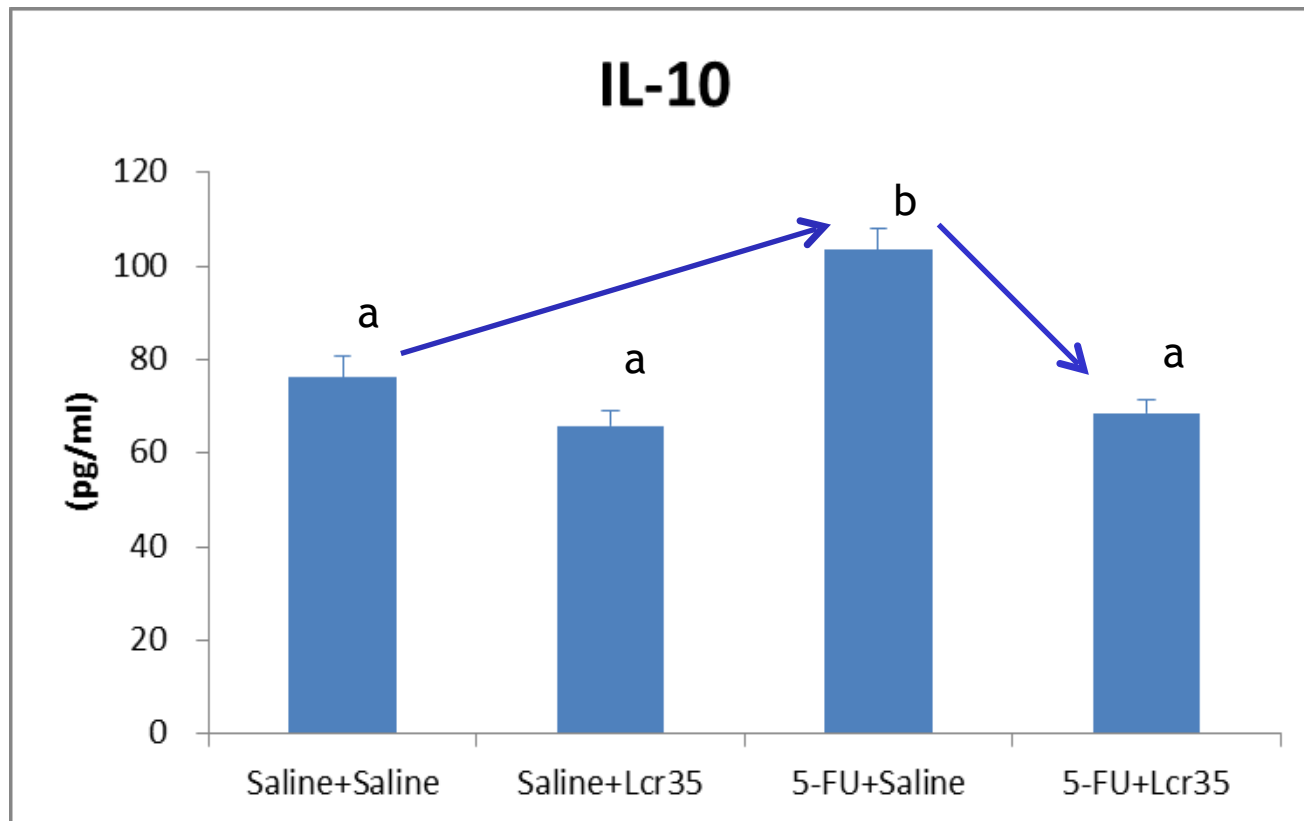


5-FU decreased IL-13 production



Lcr35 reduced IL-5 and production

Th3 response cytokines



Conclusion

- Using SCID/NOD mice as a novel model for 5-FU induced intestinal mucositis, we find that **probiotics *Lcr35* and *LaBi* do not lead to bacteremia, can improve diarrhea and body weight, can restore jejunal crypt depth**, and significantly inhibit cytokines TNF- α , IL-1 β , IFN γ , IL-6, IL-4, IL-10 and IL-17.
- *Lcr35* and *LaBi* are potentially safe therapeutic options.
- To the best of our knowledge, using SCID/NOD mice as model for 5-FU induced intestinal mucositis and probiotic therapy has not been evaluated previously.

Role of probiotics and prebiotics in immunomodulation

N Yahfoufi¹, JF Mallet¹, E Graham², C Matar^{1, 2} 

- Probiotics and prebiotics contribute to balancing the immune response.
- Probiotics modulate the activity of dendritic cells, epithelial cells and natural killers.
- Probiotics increase Treg cells and polarize the immune response towards Th1.
- Probiotics and prebiotics modulate the balance of intestinal microbial population.
- Metabolites of prebiotics' fermentation contribute to changes in cytokines, chemokines and Treg cells.

臨床經驗分享

- Infantile colic 腸絞痛
- Chronic constipation 慢性便秘
- Acute and chronic diarrhea 急性與慢性腹瀉