·学术前沿：肠道菌群与屏障功能专题·

不同剂量生玉米淀粉对小鼠肠道黏膜屏障功能的损伤及机制研究

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摘　要：【目的】探讨不同剂量的二型抗性淀粉生玉米淀粉（UCCS）对小鼠肠道黏膜屏障功能的影响及潜在机制。【方法】将 20 只 C57BL/6 小鼠随机分为 4 组，对照组（普通饲料）及 UCCS 低（188.8 g/kg）、中（314.8 g/kg）、高（440.6 g/kg）剂量组。实验期间动态监测小鼠体质量及疾病活动指数（DAI）。8 周后测量结肠长度；HE 染色观察病理变化并计算炎症评分；免疫荧光法检测紧密连接蛋白 ZO-1、E-钙黏蛋白及 Villin 的表达与共定位；ELISA 法检测血清炎症因子（IL-6、TNF-α、IL-1β、MIP-1α/CCL3）及粪便钙卫蛋白含量；16S rRNA 测序分析肠道菌群结构及功能预测。【结果】与对照组相比，各 UCCS 组小鼠结肠长度显著缩短（P=0.000 5），炎症评分显著增高（P=0.013 3）。免疫荧光显示，UCCS 组结肠黏膜 ZO-1 与 Villin 的表达及共定位阳性细胞数均显著下降（均 P<0.05）。血清 IL-6（P=0.006 4）、TNF-α（P=0.000 1）、IL-1β（P=0.001 4）、MIP-1α/CCL3（P<0.000 1）水平显著升高，粪便钙卫蛋白在干预早期（1~2 周）显著升高（P<0.05）。16S rRNA 测序表明，UCCS 干预显著改变了肠道菌群的 β 多样性（P<0.05）。其中，UCCS 组产丁酸的梭菌纲 UCG-014（*Clostridia*_UCG-014）的相对丰度显著富集，条件致病菌脱硫弧菌（*Desulfovibrio*）的丰度也呈现不同程度的富集。与免疫调节相关的阿克曼氏菌属（*Akkermansia*）和杜博西菌属（*Dubosiella*）丰度降低。PICRUSt2 功能预测分析显示，UCCS 干预影响了肠道菌群的能量代谢、抗氧化及硫循环等多种功能。【结论】UCCS 可能通过干扰肠道菌群稳态及代谢平衡，诱发肠道炎症反应并损伤肠黏膜屏障功能。本研究提示，在应用 UCCS 时应关注其剂量相关的肠道损伤风险。

关键词：生玉米淀粉；肠道菌群；肠道炎症；肠黏膜屏障；抗性淀粉
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Dose-effect and Mechanism of Uncooked Corn Starch on the Intestinal Mucosal Barrier Function in Mice

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Abstract:【Objective】To investigate the effects of different doses of uncooked corn starch (UCCS), type 2 resistant starch, on intestinal mucosal barrier function in mice and to explore the potentional underlying mechanisms.【Methods】

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Twenty C57BL/6 mice were randomly assigned to four groups and fed either a standard diet (control group) or diets supplemented with low (188.8 g/kg), medium (314.8 g/kg), or high (440.6 g/kg) doses of UCCS for 8 weeks. Body weight and disease activity index (DAI) were monitored. At the endpoint, colon length was measured; histopathological changes in were assessed via HE staining. The expression of tight junction proteins (ZO-1, E-cadherin, and Villin) in the colonic mucosa were detected using immunofluorescence. Serum inflammatory cytokines (IL-6, TNF-α, IL-1β, MIP-1α/CCL3) and fecal calprotectin levels were measured by ELISA. Gut microbiota alterations were analyzed via 16S rRNA gene sequencing and PICRUSt2 functional prediction.【Results】Compared with the control group, UCCS intervention across all doses significantly shortened colon length (P=0.000 5) and increased colonic inflammation scores (P=0.013 3). Immunofluorescence revealed a significantly reduction in the expression and co-localization of ZO-1 and Villin in the colonic mucosa ($P<0.05$). Systemic inflammation was evidenced by significantly elevated serum levels of IL-6 (P=0.006 4), TNF-α (P=0.000 1), IL-1β (P=0.001 4), and MIP-1α/CCL3 ($P<0.000\ 1$). Fecal calprotectin levels were also significantly increased during the early phase (weeks 1-2, $P<0.05$). 16S rRNA sequencing showed that UCCS significantly altered the β-diversity of the gut microbiota ($P<0.05$), characterized by the enrichment of butyrate-producing *Clostridia*_UCG-014, and the opportunistic pathogen *Desulfovibrio*, alongside a decreased abundance of immunomodulatory genera *Akkermansia* and *Dubosiella*. PICRUSt2 analysis suggested that UCCS modulated metabolic pathways related to energy metabolism, glutathione metabolism, and sulfur cycling.【Conclusion】Long-term or high-dose intake of UCCS may disrupt gut microbiota homeostasis and trigger inflammation response, thereby damaging the intestinal mucosal barrier. These findings suggest that the clinical or dietary application of UCCS should be cautioned regarding dose-dependent risks of intestinal injury.

Key words: uncooked corn starch; gut microbiota; intestinal inflammation; intestinal mucosal barrier; resistant starch

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抗性淀粉(resistant starch, RS)指一类耐受小肠消化而直达结肠、被肠道菌群发酵的碳水化合物,根据其来源与结构,主要可分为4类(RS1~RS4)[1-3]。其中,RS2作为一种天然存在的益生元,在调控“肠道菌群-短链脂肪酸”轴、改善葡萄糖稳态与脂质代谢,以及增强饱腹感等方面展现出广泛的代谢调节潜力[4-7]。生玉米淀粉(uncooked corn starch, UCCS)是RS2的典型代表[8],常被用于特殊膳食管理,例如作为Ⅰ型糖原累积病(glycogen storage disease, GSD)患者维持血糖稳定的基础饮食[9]。在特定的病理状态或长期大量使用UCCS的情况下,其对肠道的调节作用可能发生显著逆转甚至产生不良影响。现有研究指出,高剂量RS的摄入可因结肠菌群的过度发酵而显著增加产气,引发腹胀、腹痛等胃肠道症状[10];更有证据表明,在已存在肠道屏障损伤或微生态失调的背景下,RS的介入非但不能发挥抗炎保护作用,反而可能通过改变菌群构成或代谢产物谱,加剧肠黏膜的炎症反应与屏障功能障碍[11]。这一现象与UCCS在GSD患者中频繁引发消化道不耐受的临床现象高度契合[12],提示UCCS与肠黏膜屏障功能之间存在依赖于机体状态和剂量的复杂关系。

为此,本研究制备含不同剂量的UCCS饲料,通过C57BL/6小鼠实验,结合肠道组织病理学评估、上皮紧密连接蛋白表达检测、血清炎症因子水平分析等多维度技术手段,系统性评估不同剂量UCCS对肠黏膜屏障结构与功能的综合影响,旨在明确其潜在的不良反应与量效规律,为临床UCCS的合理应用提供关键的实验依据。

1 材料与方法

1.1 实验动物

20只35~42日龄的SPF级C57BL/6 WT雄性小鼠购自于广东省医学实验中心(实验动物生产许可证号:SCXK(粤)2022-0002,体质量约15~17 g,小鼠在12 h光照和黑暗循环及恒温恒湿的SPF环境中饲养,小鼠可自由获得食物和水。动物实验方案经广东省人民医院实验动物管理与使用委员会审核通过(伦理批号:KY-N-2022-053-07)。

1.2　玉米淀粉饲料制备及小鼠实验

本研究中，实验饲料委托广东省人民医院实验动物中心配制。依据UCCS在饲料碳水化合物中所供能量的比例（30%、50%和70%），设置低、中、高这3个剂量实验组，并确保除碳水化合物来源外，饲料其他成分与普通饲料维持一致。普通饲料（对照组）中等热量添加熟玉米淀粉。最终，各实验组饲料中UCCS含量分别为188.8 g/kg、314.8 g/kg和440.6 g/kg，对照组中熟玉米淀粉含量为397.15 g/kg。所有配制饲料均于-20℃条件下保存备用。

20只小鼠购入后适应性饲养7 d，随后采用随机数字法将其随机分为4组。分组后比较各组基线体质量，差异无统计学意义（$P>0.05$），根据饲料中UCCS占碳水化合物热量的百分比，由低至高分为3个试验组，其中30%为低剂量组、50%为中剂量组、70%为高剂量组，普通饲料饲养为对照组。隔日记录小鼠进食量，体质量，粪便次数、性状及便血程度，持续8周。记录疾病活动度评分（DAI评分，包括腹泻及便血程度）。实验结束前禁食8 h，小鼠麻醉后（10 g/L戊巴比妥，40 mg/kg腹腔注射），采用眼眶采血法收集外周血，室温静置2 h后以3000 ×*g*离心10 min，取上层血清，于-80℃保存备用。处死动物后采集结肠，测量结肠长度，取1 cm结肠组织于40 g/L多聚甲醛中固定，剩余结肠于-80℃冷冻保存备用。

1.3　H&E染色

结肠组织脱水后用石蜡包埋并切成3 μm厚的组织切片，脱蜡至水后滴加苏木素染色5 min，自来水冲洗返蓝，返蓝液浸泡30 s，95%酒精浸泡30 s，伊红染色20 s后自来水冲洗，梯度酒精脱水，二甲苯透明，中性树脂封片后于徕卡正置荧光显微镜拍摄。评估炎症程度及范围、溃疡和增生情况，进行组织学评分[13]。

1.4　免疫荧光染色

石蜡切片脱蜡至水后置于枸橼酸抗原修复液中进行抗原修复，用含90%PBS、100 mL/L山羊血清及体积分数0.5%Tritonx-100的封闭液室温孵育1 h，随后滴加一抗，4℃下孵育过夜（ZO-1，抗体稀释度1∶100，1151720201，ABclonal；E-cadherin，抗体稀释度1∶100，20874-1-AP，proteintech；Villin，1∶100，1648-1-AP，proteintech）。荧光二抗室温避光孵育1 h（mouse IgG1 Alexa Fluor-488，抗体稀释度1∶200，abcam，ab150113和rabbit IgG Alexa-Fluor 647，抗体稀释度1∶200，ab150079，abcam）。最后用含DAPI的防荧光淬灭封片剂染核并封片。通过徕卡正置荧光显微镜（Leica DM2000）采集图像。

1.5　酶联免疫吸附实验

按照小鼠酶联免疫吸附测定（ELISA）试剂盒（IL-6：MU30044，TNF-α：MU30030，IL-1β：MU30369，MIP-1α/CCL3：SMU30805，贝莱茵生物，中国武汉）说明书，对小鼠血清中相应炎症因子进行检测。按照小鼠钙卫蛋白酶联免疫吸附测定试剂盒（MU30827，贝莱茵生物，中国武汉）说明书，对小鼠粪便钙卫蛋白的水平进行检测。小鼠粪便ELISA样品制备：0.10 g粪便加入0.60 mL PBS研磨匀浆后于4 ℃以12 000 ×*g*离心20 min，取上清用待测。

1.6　16S rRNA检测

收集粪便样本，由杭州谷禾信息技术有限公司完成肠道菌群检测及相关数据分析。

基于PICRUSt预测肠道菌群功能，使用Metagenomic Profiles（STAMP）软件包v2.1.3对输出文件做进一步分析。FAPROTAX可根据16S序列的分类注释结果对肠道菌群功能进行注释预测。

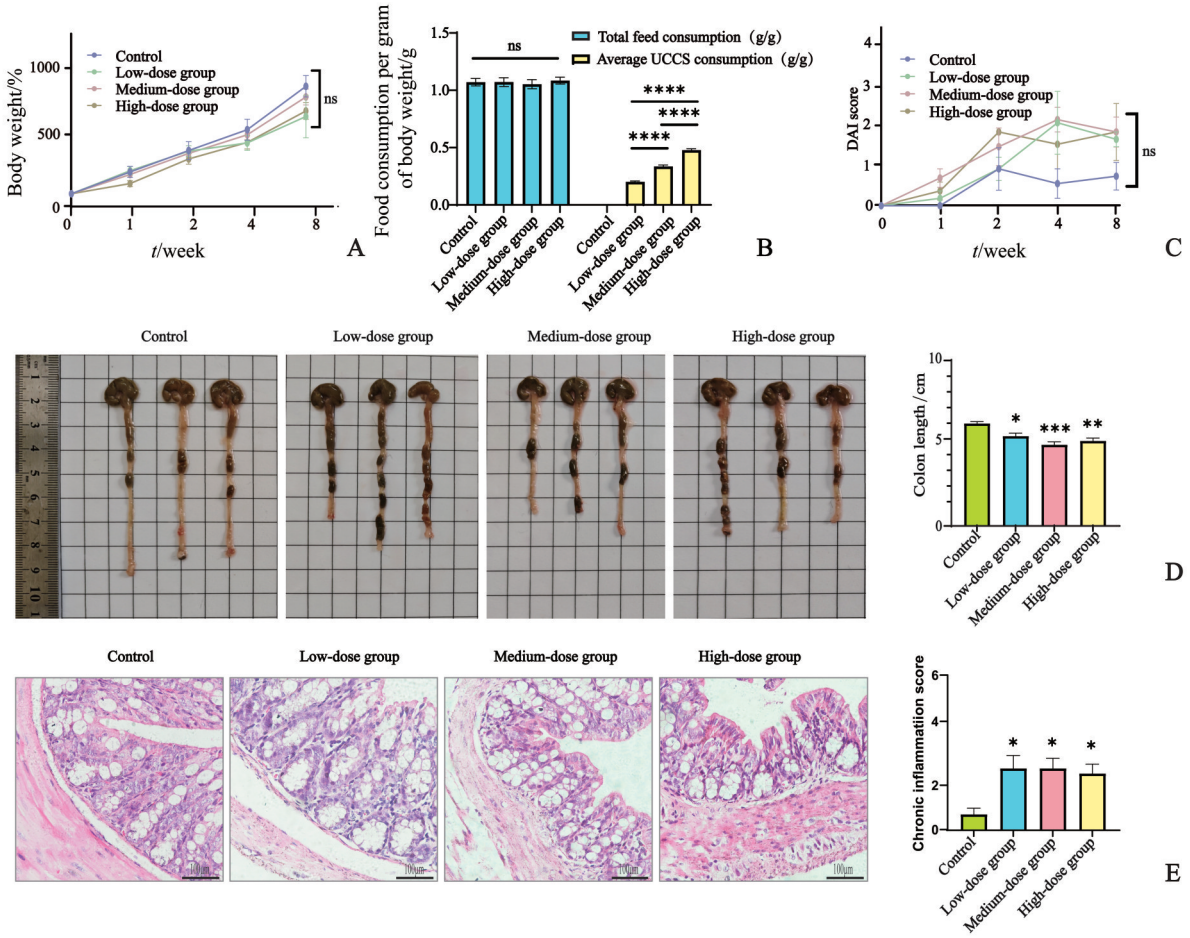
1.7　统计学处理

研究采用GraphPad Prism 10软件进行分析，所有定量实验至少重复3次。数据正态性检验采用Shapiro-Wilk检验，方差齐性采用Brown-Forsythe检验。符合正态分布的计量资料以mean±SEM表示，非正态分布数据以中位数（四分位间距）表示。符合正态分布且方差齐的多组数据，采用单因素方差分析（One-way ANOVA）进行比较，所有组间两两比较采用Tukey's多重比较检验，实验组与对照组间差异采用Dunnett检验。不符合参数检验条件则采用Kruskal-Wallis检验，如果差异具有统计学意义，则进一步使用Dunn's多重比较检验。体质量及DAI评分随时间的变化采用重复测量方差分析。以$P<0.05$为差异有统计学意义。

2 结 果

2.1 生玉米淀粉诱发肠道炎症及结构重塑

为整体评估UCCS对肠道的影响,给予动态监测不同剂量的UCCS饲养下小鼠的体质量增长、腹泻及血便情况。结果发现:各组小鼠体质量随时间增长无显著差异($P>0.05$;图1A)。各组小鼠的饲料消耗量差异无统计学意义,但UCCS消耗量差异显著($F=150.600$, $P<0.000\ 1$;Tukey's 检验:UCCS干预的组间比较$P<0.000\ 1$;图1B)。疾病活动指数(disease activity index, DAI)评分随时间变化无显著差异($P>0.05$;图1C)。与对照组相比,低、中、高剂量组小鼠结肠长度均显著缩短($F=10.320$, $P=0.000\ 5$;Dunnett检验:低剂量$P=0.018\ 4$,中剂量$P=0.000\ 3$,高剂量$P=0.001\ 6$;图1D)。HE染色显示,各UCCS组结肠黏膜炎症细胞浸润增多,炎症评分显著增高($F=4.903$, $P=0.013\ 3$;Dunnett检验:低剂量$P=0.013\ 8$,中剂量$P=0.013\ 8$,高剂量$P=0.028\ 7$;图1E)。以上结果表明,UCCS摄入虽不影响整体的能量摄入,但可引发肠道慢性炎症。



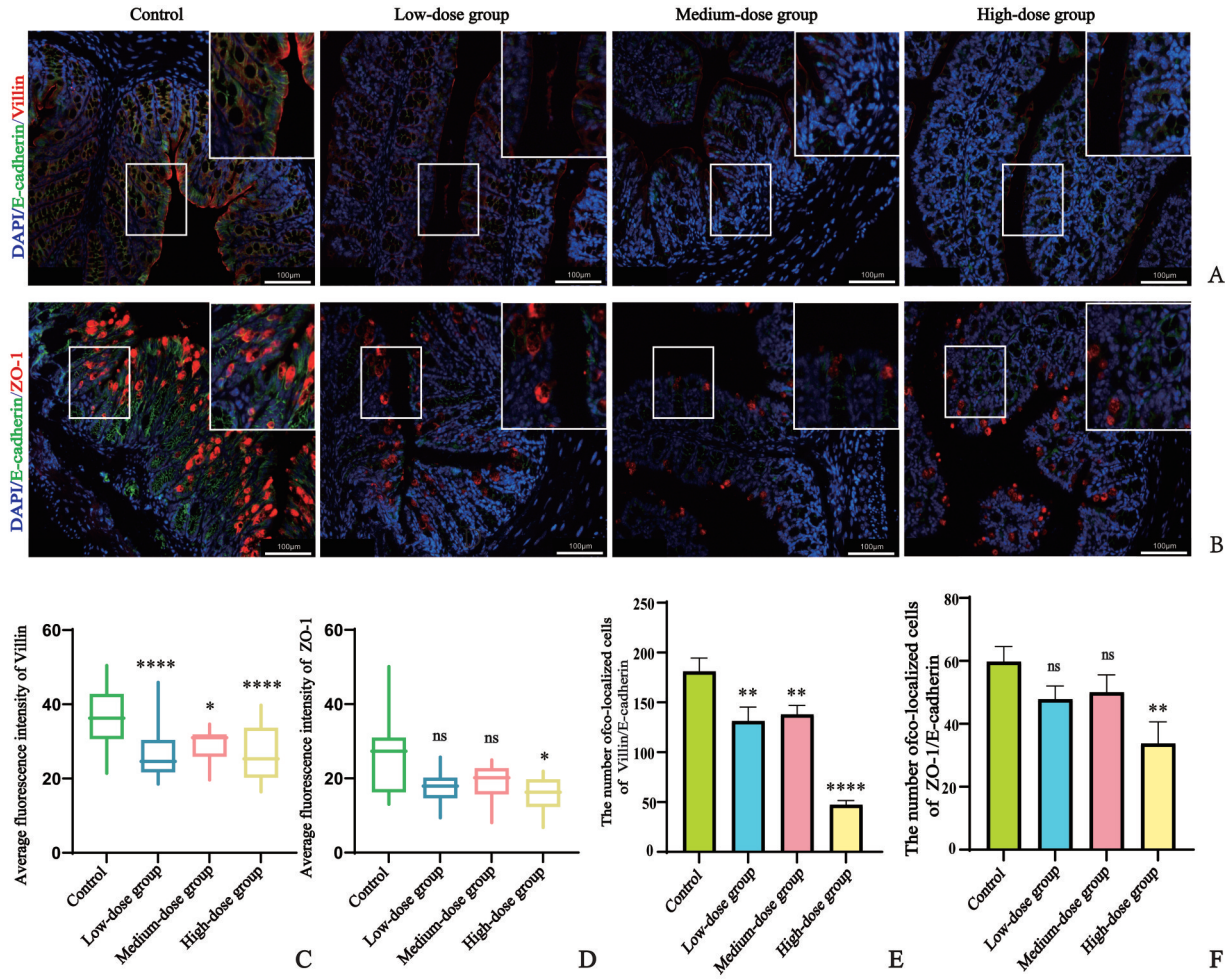
A: Body weight changes in mice over the 8-week experimental period (n=5). B: Total feed intake and average UCCS consumption (g/g body weight). The average UCCS consumption in the low-, medium-, and high-dose groups was 0.20±0.01, 0.34±0.01, and 0.48±0.01 (g/g body weight), respectively. C: Dynamic changes in DAI scores throughout the experiment. D: Colon length measured after 8 weeks of UCCS treatment. Colon lengths in the control, low-dose, medium-dose, and high-dose groups were 6.62±0.15 cm, 5.72±0.22 cm, 5.10±0.23 cm, and 5.38±0.22 cm, respectively. E: Representative HE staining images of mouse colon tissue (20×magnification), Scale bar=100 μm. Inflammation scores were 0.60±0.25 (control), 2.40±0.51 (low-dose), 2.40±0.40 (medium-dose), and 2.20±0.37 (high-dose). All data are presented as mean±SEM. Statistical analysis was performed using ordinary one-way ANOVA. ns: not significant, *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.000\ 1$.

图1 不同剂量的生玉米淀粉对小鼠体质量及肠黏膜的影响

Fig. 1 Effects of different doses of uncooked corn starch on body weight and intestinal mucosa of mice

2.2　生玉米淀粉对小鼠肠上皮屏障完整性的影响

我们对小鼠肠黏膜紧密连接蛋白ZO-1、上皮细胞分化相关蛋白Villin和细胞黏附蛋白E-cadherin进行了免疫荧光染色和定量分析，结果表明：UCCS处理可导致小鼠肠上皮屏障损伤，表现为结肠黏膜中关键蛋白表达和共定位的异常。与对照组相比，UCCS组小鼠肠黏膜的Villin蛋白表达均显著降低（Kruskal-Wallis检验：H=26.610，P<0.000 1；Dunn's检验两两比较：低剂量P<0.000 1、中剂量P=0.025 2、高剂量组P<0.000 1）；ZO-1蛋白表达整体下降（H=9.043，P=0.028 7），但仅高剂量组与对照组差异显著（P=0.044 9；图2A-D）。共定位分析显示，E-cadherin与Villin共阳性细胞在各UCCS组均显著减少（方差分析：F=34.820，P<0.000 1；Dunnett检验：低剂量P=0.007 6，中剂量P=0.008，高剂量P<0.000 1；图2E）；而E-cadherin与ZO-1共阳性细胞仅在高剂量组显著减少（F=3.605，P=0.020 4；Dunnett检验：高剂量P=0.006 6；图2F）。综上，中、高剂量UCCS破坏小鼠肠上皮屏障的完整性，损伤肠上皮屏障功能。



A-B: Representative immunofluorescence images of mouse intestinal mucosa. (A) Co-staining of Villin (red) and E-cadherin (green); (B) Co-staining of ZO-1 (red) and E-cadherin (green). Nuclei were counterstained with DAPI (blue). Scale bar=100 μm. C-D: Quantitative analysis of Villin (C) and ZO-1 (D) expression based on immunofluorescence intensity. Data were analyzed using the Kruskal-Wallis test. E-F: Quantification of cell populations co-expressing Villin/E-cadherin (E) and ZO-1/E-cadherin (F). Data were analyzed using ordinary one-way ANOVA (n=3). ns: not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.000 1.

图2　不同剂量的生玉米淀粉对小鼠肠上皮屏障功能的影响

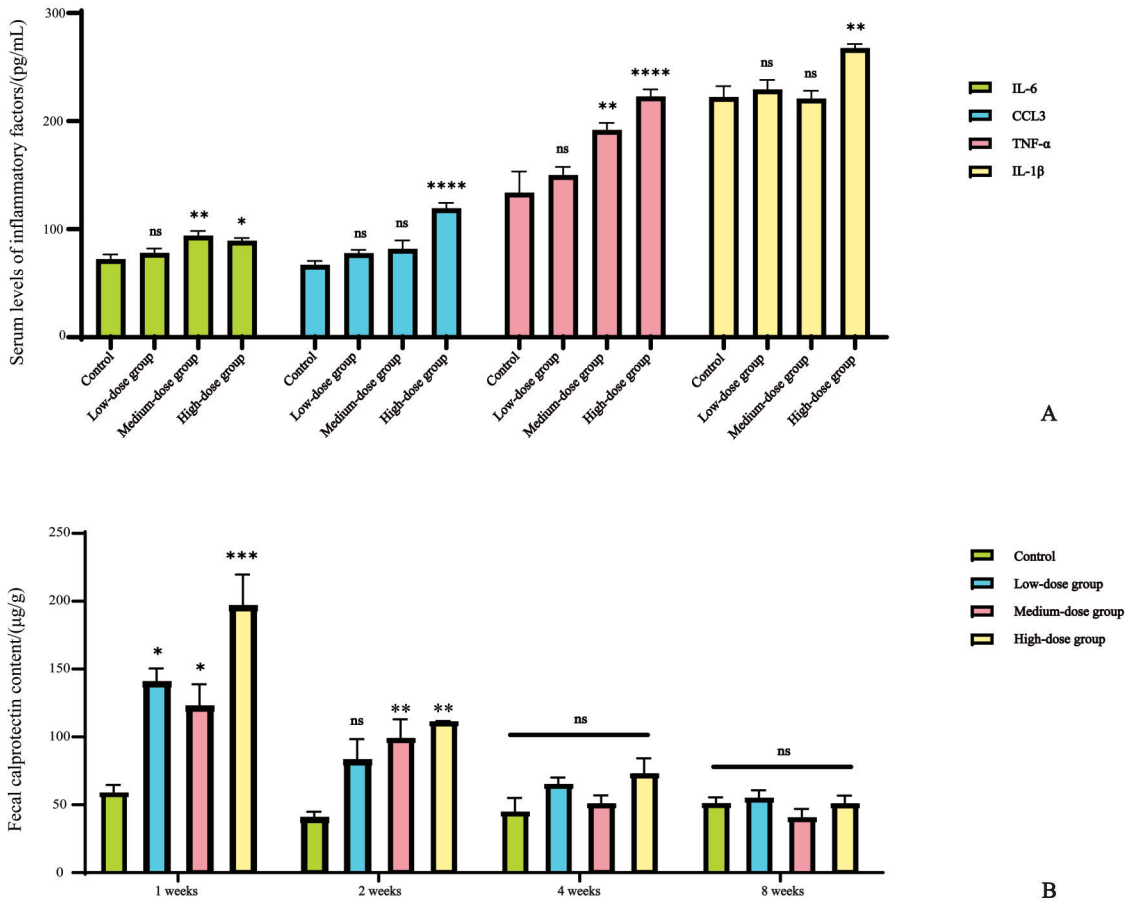
Fig. 2　Effects of different doses of uncooked corn starch on the intestinal epithelial barrier function in mice

2.3　生玉米淀粉对局部及系统性炎症反应的影响

为了评估UCCS对局部和系统性炎症反应的影响，本研究检测了血清炎症因子及粪便钙卫蛋白水平。采用ELISA检测实验小鼠血清IL-6、TNF-

α、IL-1β和CCL3的表达水平。结果显示:中、高剂量UCCS干预显著上调血清IL-6水平(对照组:72.38±4.29 pg/mL;中剂量组:94.15±4.16 pg/mL;高剂量组:89.43±2.39 pg/mL;F=6.258,P=0.006 4);TNF-α水平在中、高剂量组显著升高(对照组:133.80±19.59 pg/mL;中剂量组:191.90±6.49 pg/mL;高剂量组:222.90±6.50 pg/mL;F=18.820,P<0.000 1);IL-1β水平仅在高剂量组显著升高(对照组:222.30±10.05 pg/mL;高剂量组:267.50±3.81 pg/mL;F=9.472,P=0.001 4);CCL3水平仅在高剂量组显著升高(对照组:67.10±3.59 pg/mL;高剂量组:119.30±5.18 pg/mL;F=16.170,P<0.000 1)。低剂量组各炎症因子水平与对照组相比均无显著差异(图3A)。粪便钙卫蛋白水平在UCCS干预早期(1~2周)显著升高,而在后期(4~8周)恢复至基线水平(图3B)。第1周时,各UCCS组钙卫蛋白水平均显著高于对照组(F=12.250,P=0.001 6;低剂量P=0.015 4,中剂量P=0.038 9,高剂量P=0.000 5);第2周时,中、高剂量组仍维持显著升高(F=6.997,P=0.01;中剂量P=0.009,高剂量P=0.006 7),低剂量组与对照组无显著差异(P=0.073 9)。

以上结果表明,UCCS摄入对炎症因子的释放具有剂量和时间依赖性。血清炎症因子的升高提示系统性炎症反应的发生,而粪便钙卫蛋白的早期升高与后续恢复则提示肠道可能存在"早期急性应激-后期适应性免疫"的动态炎症过程。



A: Serum levels of inflammatory cytokines (IL-6, TNF-α, IL-1β) and chemokine (CCL3) measured by ELISA, Control (n=3), UCCS group (n=5). B: Time-course analysis of fecal calprotectin levels in mice over 8 weeks, measured by ELISA, Control (n=3), UCCS group (n=4).All data are presented as mean±SEM. Statistical analysis was performed using ordinary one-way ANOVA. ns: not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.000 1.

图3 不同剂量的生玉米淀粉对炎症因子释放的影响

Fig. 3 The effect of different doses of uncooked corn starch on inflammatory cytokine release in mice

2.4　生玉米淀粉驱动肠道菌群结构重塑

为探索长期摄入UCCS是否影响小鼠菌群结构，收集小鼠粪便进行16S rRNA测序，结果显示：UCCS干预未显著影响肠道菌群的α多样性（Shannon，Simpson和Chao1指数均$P>0.05$）。然而，基于Bray-Curtis距离的Anosim分析显示，菌群β多样性发生显著改变，组间差异大于组内差异（非加权：$R=0.509$，$P=0.001$；加权：$R=0.383$，$P=0.028$），提示UCCS干预显著改变了菌群整体结构（图4A-E）。与对照组比较，丰度排名前10的核心菌属中，多个关键菌属的相对丰度发生改变（图4F）。如阿克曼氏菌属（*Akkermansia*）、毛螺菌科NK4A136群（*Lachnospiraceae_NK4A136_group*）、乳酸菌属（*Lactobacillus*）及粪杆菌属（*Faecalibaculum*）。其中，产丁酸的梭菌纲UCG-014（*Clostridia*_UCG-014）在各UCCS组中丰度升高，以低剂量组最为显著；阿克曼氏菌属（*Akkermansia*）和杜博西菌属（*Dubosiella*）丰度呈不同程度下降。此外，穆尔巴赫菌科（*Muribaculaceae*）、乳酸菌属（*Lactobacillus*）及粪杆菌属（*Faecalibaculum*）丰度呈不同程度升高。值得注意的是，条件致病菌脱硫弧菌属（*Desulfovibrio*）在低、中剂量组中均出现富集。

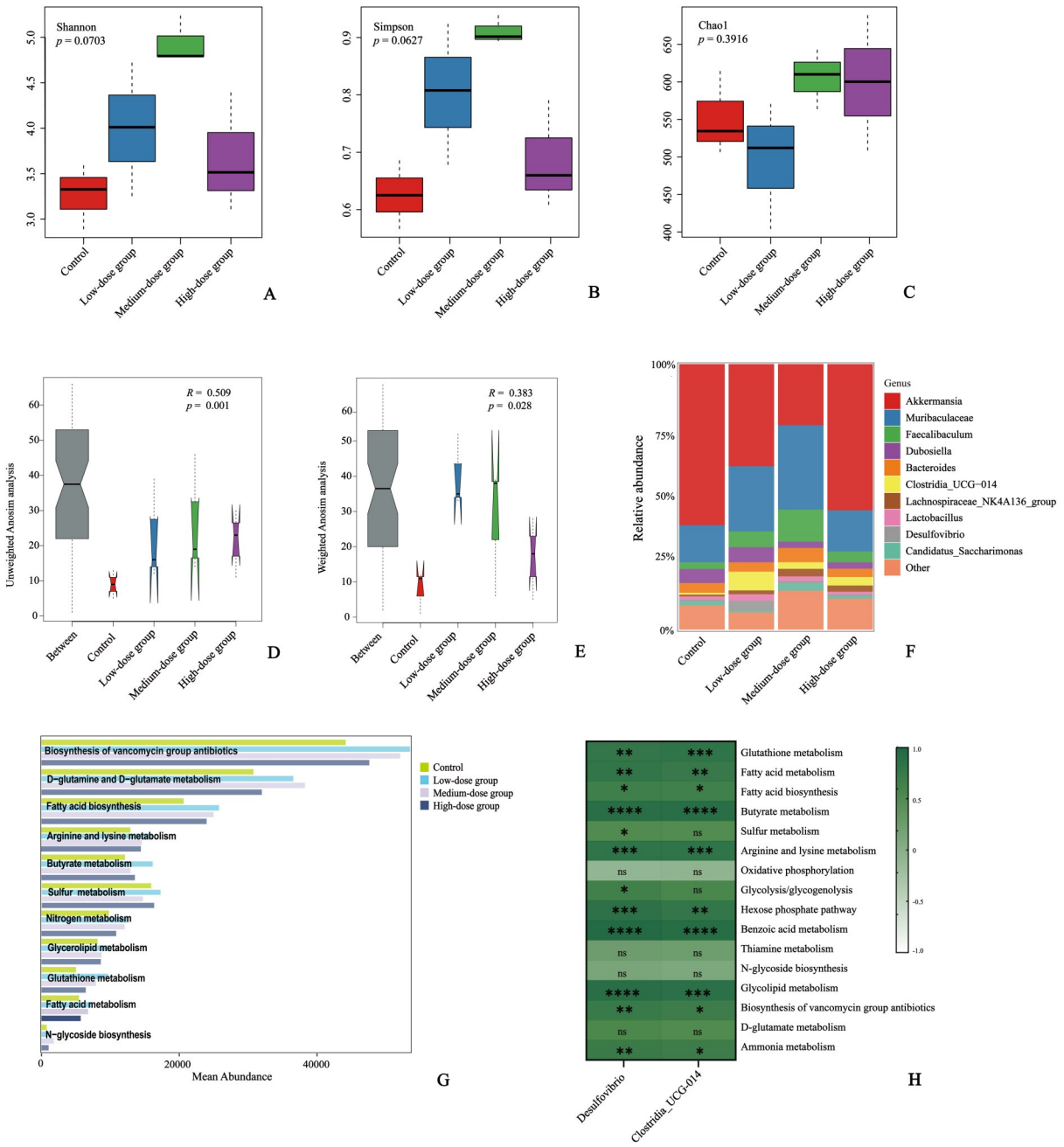
为评估UCCS干预对肠道菌群代谢功能的影响，基于PICRUSt2进行的功能预测分析，结果如图4G所示：UCCS干预重塑了菌群的功能图谱，共激活了11条核心代谢通路，其中所有UCCS组的脂肪酸合成、精氨酸/赖氨酸代谢及谷胱甘肽代谢通路均被不同程度地激活。值得注意的是，低剂量组丁酸代谢的增强最为显著，而硫代谢通路则在低、高剂量组显著升高。UCCS干预改变了肠道菌群的能量代谢、抗氧化能力及硫循环等多种功能。Spearman等级相关分析（图4H）显示，特定菌群相对丰度与上述通路的功能潜力之间存在显著相关性。其中，脱硫弧菌属的相对丰度与谷胱甘肽代谢（$r_s=0.81$，$P=0.001\ 5$）、硫代谢（$r_s=0.68$，$P=0.016$）、精氨酸和赖氨酸代谢（$r_s=0.85$，$P=0.000\ 4$）以及氨代谢（$r_s=0.74$，$P=0.006\ 5$）等通路呈正相关；而梭菌纲UCG-014的相对丰度则与丁酸代谢（$r_s=0.91$，$P<0.000\ 1$）、脂肪酸代谢（$r_s=0.78$，$P=0.003\ 1$）、甘油酯代谢（$r_s=0.88$，$P=0.000\ 1$）、谷胱甘肽代谢（$r_s=0.83$，$P=0.0008$）以及戊糖磷酸途径（$r_s=0.81$，$P=0.001\ 6$）等通路呈正相关。以上结果表明，UCCS可能是驱动肠道微生态失调及代谢失衡的关键因素之一。

3　讨论

本研究通过为期8周的动物实验证实，长期摄入高剂量UCCS可诱发C57BL/6小鼠的肠道炎症，导致结肠长度显著缩短，紧密连接蛋白ZO-1与分化相关蛋白Villin表达下调以及系统性炎症因子水平上升，该效应呈现明显的剂量依赖性。以上结果提示，当UCCS摄入超过一定阈值时，其作用可能从“代谢改善”转变为“稳态干扰”。

机制上，UCCS对肠黏膜的损伤可能是多重机制共同作用的结果。首先，本研究观察到与肠黏膜屏障维持相关的*Akkermansia*属和与免疫调节密切相关的*Dubosiella*属丰度降低可能导致黏液层更新及免疫稳态维持能力削弱[14-20]。与此同时，与H_2S生成相关的条件致病菌脱硫弧菌属（*Desulfovibrio*）的在低、中剂量组中富集[21-22]。相关性分析进一步表明，脱硫弧菌属（*Desulfovibrio*）的富集与硫代谢通路显著正相关，提示其可能是驱动硫代谢异常的关键菌群。作为典型的硫酸盐还原菌，脱硫弧菌可产生大量H_2S，其异常积累被认为是破坏肠屏障的重要因素之一。过量H_2S可通过多途径损伤肠上皮稳态：一方面，其可抑制线粒体细胞色素c氧化酶活性，导致上皮细胞能量代谢障碍并诱发氧化应激[23]；另一方面，H_2S可破坏黏蛋白中维持凝胶结构的二硫键，削弱黏液层的物理屏障功能，使细菌及其代谢产物更易接触上皮细胞[24-26]。此外，H_2S还可通过激活ERK1/2-NF-κB信号通路上调炎症因子表达，进一步放大局部炎症反应[27]。谷胱甘肽代谢水平升高进一步提示肠道处于活跃的氧化应激状态，这种由氧化应激与硫代谢紊乱共同构成的损伤性微环境。

值得注意的是，本研究中产丁酸的*Clostridia*_UCG-014属呈现显著富集，但并未观察到相应的屏障改善或炎症缓解，这提示功能预测反映的“丁酸代谢潜力增强”可能是代偿性适应，其保护效应在损伤微环境中可能被多重机制抵消。一方面，高



A–C: Alpha diversity analysis of the gut microbiota. (A) Shannon diversity index, (B) Simpson diversity index and (C) Chao1 richness estimator, an increasing trend was observed in the Shannon and Simpson indices of the high-dose group, no statistically significant differences were detected among the groups (P>0.05). D–E: Beta diversity analysis based on Anosim. Both unweighted (D) and weighted (E) Anosim analyses revealed significant separation in microbial community structure among groups, with the high-dose group showing distinct clustering compared to other groups. F: Relative abundance of the top 10 bacterial genera in fecal microbiota composition across groups. G: PICRUSt2-predicted functional potential of core metabolic pathways in the gut microbiota following different doses of UCCS intervention. H: Spearman correlation heatmap between the relative abundances of key differential genera (*Desulfovibrio* and *Clostridia*_UCG-014) and the functional potential of core metabolic pathways predicted by PICRUSt2. Color intensity indicates the strength and direction of correlation (dark green: strong positive correlation, r_s=+1.0; gray: strong negative correlation, r_s=−1.0). The selected pathways encompass functions related to oxidative stress, inflammation, energy metabolism, and substance metabolism.

图4 生玉米淀粉干预后小鼠肠道菌群的组成变化

Fig. 4 Effects of uncooked corn starch on gut microbiota composition in mice

浓度的H_2S可直接抑制丁酸的β-氧化，阻碍其为上皮细胞提供能量[28-29]；另一方面，肠道炎症可能导致结肠上皮细胞丁酸转运蛋白（如MCT1/SMCT1）的表达下调，减少丁酸的细胞内摄取[30]。因此，在高H_2S及高氧化应激的背景下，丁酸的代谢优势难以转化为实际的屏障保护效应。肠黏膜物理屏障的破坏，导致肠道内细菌及内毒素易位，系统性地触发全身炎症反应，促使血清IL-6、TNF-α、IL-1β等炎症因子水平升高。这些炎症因子可以通过NF-κB、MAPK等信号通路放大炎症级联反应[31]，并可以进一步下调ZO-1等紧密连接蛋白的表达[32]，形成“菌群失调-免疫激活-屏障损伤-炎症加剧”的恶性循环。

本研究的局限性包括：①丁酸及H_2S等关键代谢物的水平变化，有待粪便或组织样本的代谢组学分析加以验证；②未能通过干预实验（如使用H_2S抑制剂或特定菌株）直接证实脱硫弧菌与UCCS诱导的肠道炎症的因果关系；③炎症因子如何具体调控紧密连接蛋白表达的分子通路有待阐明；④未能进行更长时间的干预以判断是否会引发肠道适应性变化或导致不可逆损伤；⑤本研究主要聚焦于肠道局部效应，未涉及UCCS对宿主全身代谢的影响。未来我们将构建长期的动物模型，并结合靶向代谢组学（如直接定量H_2S）、基因编辑技术及菌群干预实验，深入揭示其因果机制与信号通路。为全面理解UCCS的生物学效应提供更完整的证据。

综上，本研究表明长期高剂量UCCS可诱导硫代谢相关细菌增强与黏液相关菌减少，并最终导致屏障受损与炎症放大，研究结果为UCCS的长期应用提供了新的实验依据。

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