读书报告

Research Seminar

李帅

2017/05/14

01

燕麦-肠道菌群-肥胖

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Oat products modulate the gut microbiota and produce anti-obesity effects in obese rats

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研究背景

1. **肥胖是许多慢性疾病的主要成因，比如说血脂异常、高血压和II型糖尿病等。**
(Brown, Higgins, & Donato, 2000; Cani, Bibiloni, & Knauf, 2008).

2. **越来越多的证据表明，失调的肠道微生物群在肥胖的发展中扮演着重要角色。**

（Firmicutes↑，Bacteroidetes↓） (Turnbaugh, Ley, & Mahowald, 2006)

3. **饮食习惯能够对肠道菌群的组成和代谢产生极大的影响。** (Scott, Duncan, & Flint, 2008).

4. **高脂饮食会引起肠道菌群失调，我们应将肠道菌群作为一个重要作用靶点，寻找慢性疾病的治疗方法。** (Wang, Tang, & Zhang, 2015)

材料与方法

3种燕麦产品

Oat meal — OM

Oat flour — OF

Oat bran — OB

total dietary fibre (AOAC) method 985.29
total β-glucan content (AOAC) method 995.16
crude protein (Kjeldahl Method)
crude fat (Soxhlet abstracting method)
crude ash (Combustion method)
total starch(AOAC) method 991.43
moisture content (Constant weight method)
resistant starch content (AOAC) method 2002.02

制作实验饲料时，将各添加剂组β-葡聚糖含量调为一致 [0.70 g/(kg•BW•d)]

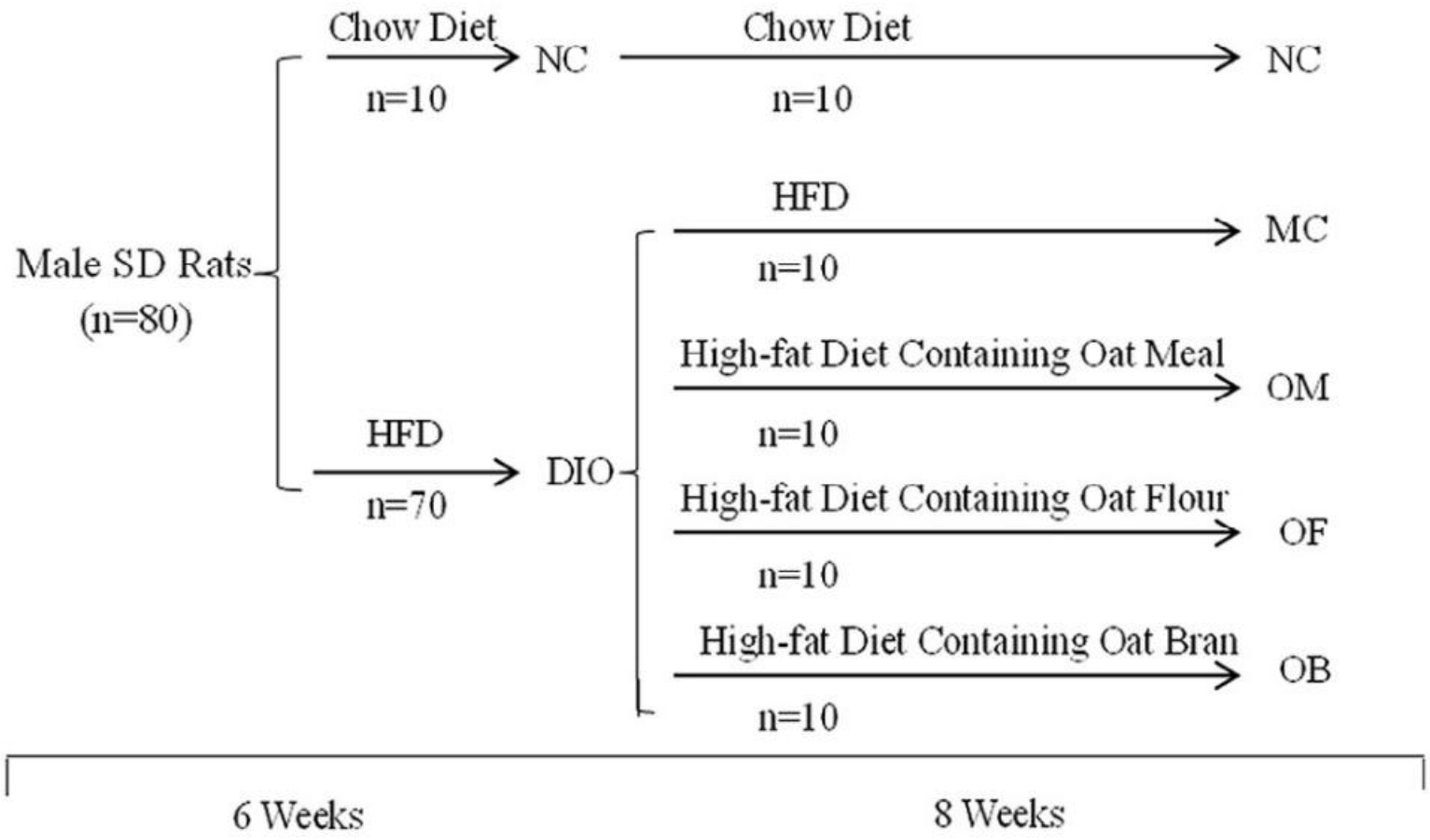
养殖期间，各组小鼠自由饮水和摄食

膨胀能力

表面黏度

材料与方法

分组情况


Male SD Rats
(n=80)
Chow Diet
n=10
NC
Chow Diet
n=10
NC
HFD
n=70
DIO
HFD
n=10
MC
High-fat Diet Containing Oat Meal
n=10
OM
High-fat Diet Containing Oat Flour
n=10
OF
High-fat Diet Containing Oat Bran
n=10
OB
6 Weeks
8 Weeks

材料与方法

取血时间：0w、 3w、 6w、 8w

取材前禁食 12h

血清与粪便：-20℃保存

附睾脂肪组织：小心切开，冷生理盐水冲洗，称重，-70℃保存

材料与方法

血清生化检测和ELISA分析

Triacylglycerol (**TG**)

Total cholesterol (**TC**)

High-density lipoprotein cholesterol (**HDL-C**)

Endotoxin (**ET**)

Tumour cell necrosis factor-α (**TNF-α**)

粪便脂肪含量的测定

干燥后，准确称取1g，加入氯仿：甲醇（1：1，v/v），80℃水浴1.5h，然后3000rpm离心15min，取上清，干燥。

材料与方法

附睾脂肪组织形态观察

10% 中性缓冲福尔马林溶液

放置48 h

福尔马林为40%甲醛溶液（市售商品），取10毫升福尔马林加90毫升水，即成10%福尔马林溶液。同样按比例加入福尔马林及水，可配制其他浓度溶液。

10%中性福尔马林：含10%福尔马林的饱和碳酸钙溶液

10%中性缓冲福尔马林：10毫升甲醛液+90毫升0.01mol/L PBS(PH=7.4)

结肠SCFAs测定

结肠内容物（0.1~0.2g）→ 稀释（2mL 冷生理盐水 + 1mL 50% H_2SO_4）→ 涡旋混匀 → 2mL 乙醚萃取SCFAs

气相色谱分析

结果

1. 燕麦产品成分分析结果

Table 1 – Nutritional component and physicochemical characteristics of three oat products.

	OM	OF	OB
Nutritional component			
Total dietary fibre (%)	20.38 ± 0.05^{b}	14.15 ± 0.11^{c}	29.90 ± 0.12^{a}
Crude protein (%)	13.12 ± 0.07^{a}	13.60 ± 0.08^{a}	11.62 ± 0.30^{b}
Crude fat (%)	7.10 ± 0.11^{a}	4.58 ± 0.08^{b}	6.98 ± 0.17^{a}
Crude ash (%)	2.02 ± 0.04^{b}	1.45 ± 0.06^{c}	2.98 ± 0.10^{a}
Total starch (%)	50.34 ± 0.52^{b}	57.15 ± 1.57^{a}	38.90 ± 0.76^{c}
Moisture (%)	6.16 ± 0.05^{c}	8.53 ± 0.14^{a}	7.81 ± 0.04^{b}
β-Glucan (%)	4.15 ± 0.12^{b}	1.78 ± 0.21^{c}	8.10 ± 0.66^{a}
Physicochemical characteristics			
Apparent viscosity (mPa•s) 25 °C, 5%M/M, 200 rpm	10.33 ± 0.58^{c}	37.33 ± 1.34^{b}	50.00 ± 2.00^{a}
Water-retaining capacity (g/g)	3.14 ± 0.06^{c}	4.28 ± 0.11^{b}	5.02 ± 0.09^{a}
Swelling capacity (mL/g)	0.4 ± 0.03^{b}	2.3 ± 0.11^{a}	2.4 ± 0.08^{a}

Data are mean ± SD (n = 3). Differences among groups were evaluated for significance by the Tukey post hoc test. Values in the same row that do not share the same lowercase letter are significantly different ($p < 0.05$).
Abbreviations: OM, oat meal group; OF, oat flour group; OB, high fibre oat bran group.

Total dietary fibre

β-glucan

Resistant starch

抗肥胖

改善肠道菌群

饲料成分分析结果

Table 2 – Formula, nutritional components, and energy density of different diets.

	Diet (g/100g dried feed)				
	Basic diet*	High-fat diet	Experimental diet I	Experimental diet II	Experimental diet III
Barley flour	20.0	11.6	7.30	1.60	9.40
Soybean flour	20.0	11.6	7.30	1.60	9.40
Corn flour	32.0	18.6	11.7	2.60	15.0
Dehydrated vegetable	10.0	5.80	3.70	0.80	4.70
Fishmeal	10.0	5.80	3.70	0.80	4.70
Bonemeal	5.00	2.90	1.80	0.40	2.40
Salt	2.00	1.20	0.70	0.20	0.90
Yeast	1.00	0.60	0.40	0.10	0.50
Egg yolk powder	——	10.0	10.0	10.0	10.0
Sugar	——	5.00	5.00	5.00	5.00
Peanuts	——	4.00	4.00	4.00	4.00
Lard	——	13.0	13.0	13.0	13.0
Milk powder	——	10.0	10.0	10.0	10.0
OM	——	——	21.4	——	——
OF	——	——	——	49.8	——
OB	——	——	——	——	11.0
Nutritional component (%)					
Total dietary fibre	4.72 ± 0.07^{c}	2.81 ± 0.10^{d}	6.60 ± 0.13^{b}	7.11 ± 0.18^{a}	6.48 ± 0.09^{b}
Crude protein	24.0 ± 1.77^{a}	17.1 ± 1.05^{b}	14.7 ± 1.45^{b}	12.0 ± 0.60^{c}	15.7 ± 0.98^{b}
Crude fat	4.85 ± 0.22^{b}	20.9 ± 1.45^{a}	20.8 ± 0.91^{a}	20.5 ± 1.09^{a}	21.2 ± 1.01^{a}
Crude starch	53.6 ± 1.76^{a}	45.0 ± 2.01^{b}	44.1 ± 1.67^{b}	46.7 ± 2.23^{b}	43.3 ± 2.11^{b}
Crude ash	6.80 ± 0.23^{b}	7.34 ± 0.12^{a}	7.32 ± 0.26^{a}	5.72 ± 0.11^{c}	6.92 ± 0.18^{b}
Moisture	6.55 ± 0.21^{e}	6.90 ± 0.18^{d}	7.55 ± 0.11^{a}	7.33 ± 0.08^{b}	7.11 ± 0.13^{c}
β-Glucan	0.39 ± 0.07^{b}	0.24 ± 0.03^{c}	1.05 ± 0.07^{a}	0.96 ± 0.06^{a}	0.98 ± 0.06^{a}
Resistant starch	4.22 ± 0.11^{a}	2.31 ± 0.16^{d}	2.68 ± 0.09^{c}	1.16 ± 0.10^{e}	3.39 ± 0.21^{b}
kJ/100g feed (×10)					
Energy density	159	192	192	192	192

Data are mean ± SD (n = 3). Differences among groups were evaluated for significance by the Tukey post hoc test. Values in the same row that do not share the same lowercase letter are significantly different ($p < 0.05$).

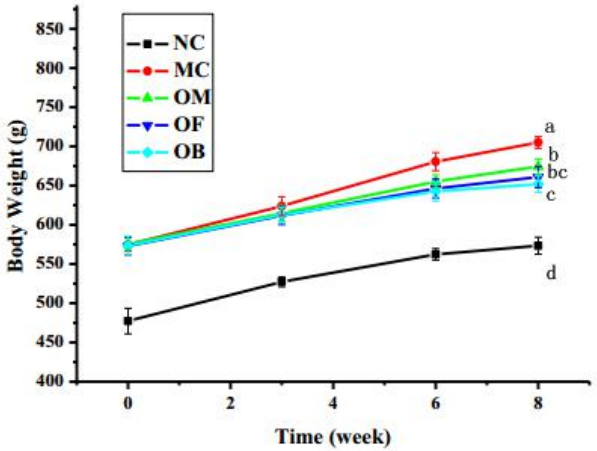
* The basic diet was supplied by Laboratory Animal Centre of Henan Province.

Abbreviations: OM, oat meal group; OF, oat flour group; OB, high fibre oat bran group.

2. 燕麦产品能够减轻高脂诱导的肥胖

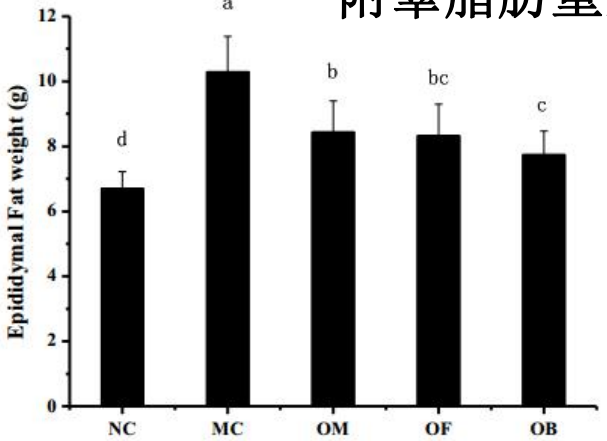
(A)

体重



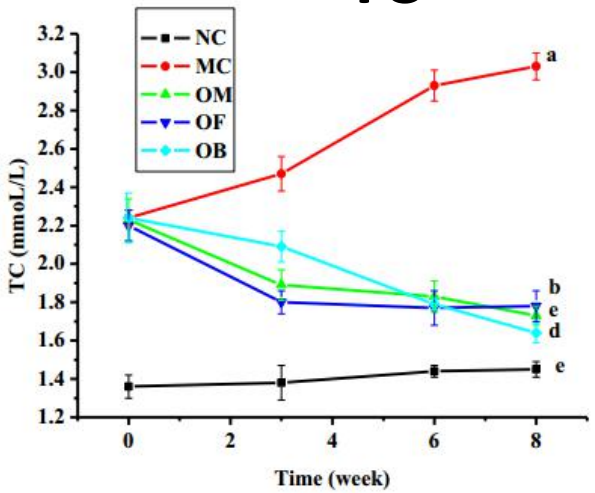
(B)

附睾脂肪重量



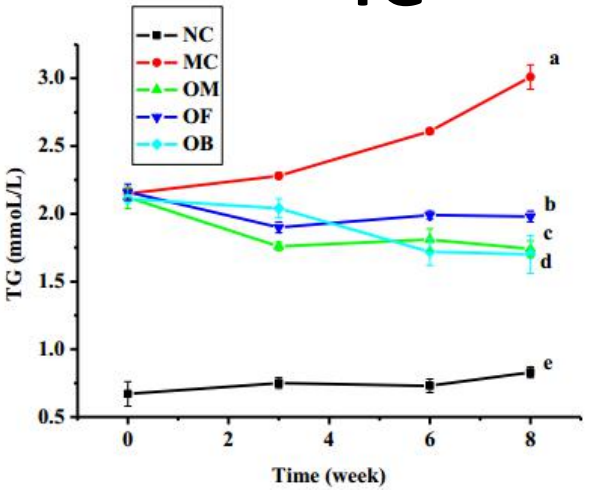
(C)

TC



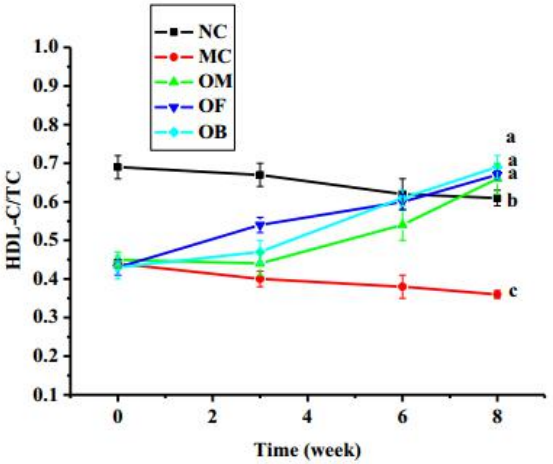
(D)

TG



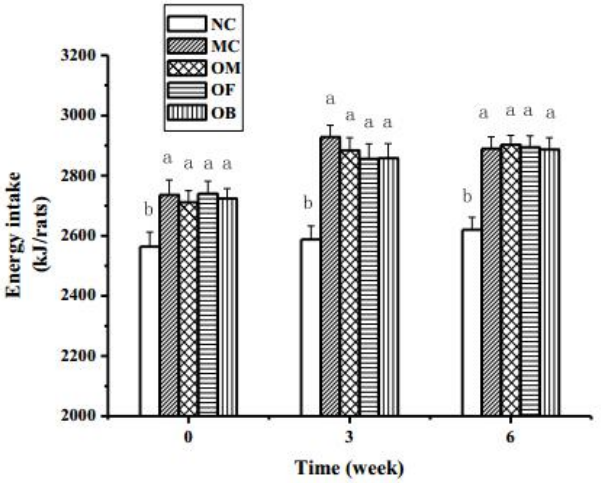
(E)

HDL-C

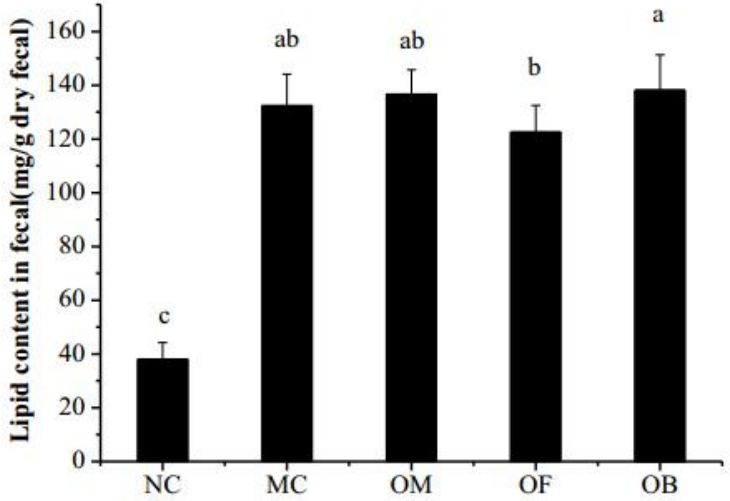


(F)

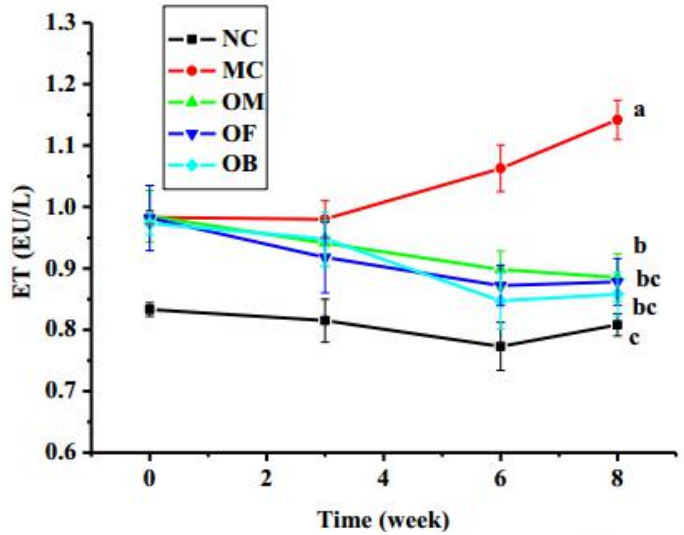
能量获取



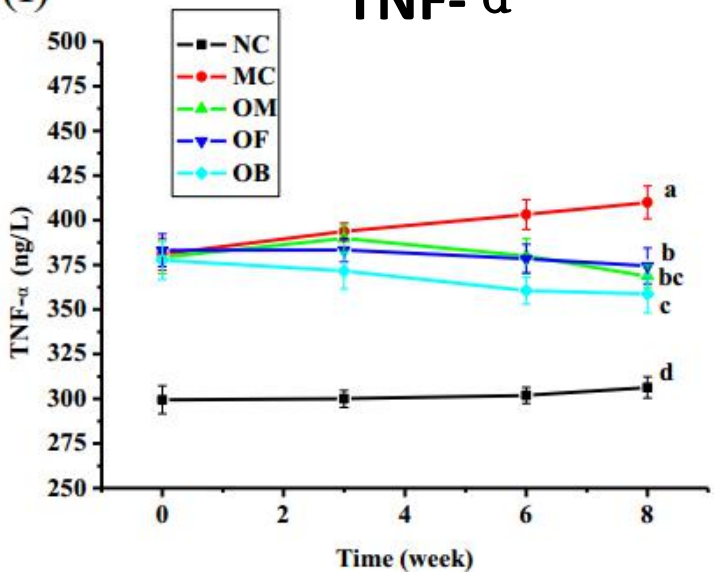
(G) 粪便脂肪含量



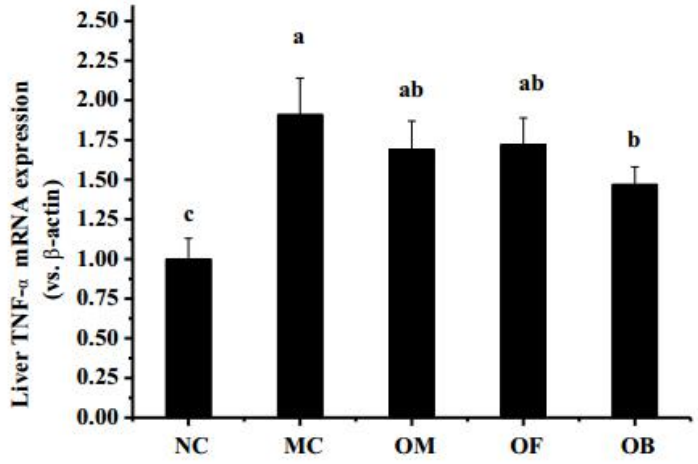
(H) 血清细菌内毒素



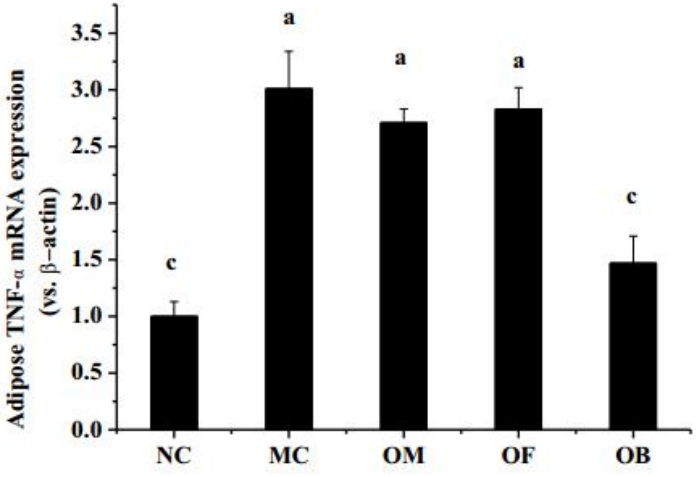
(I) TNF- α



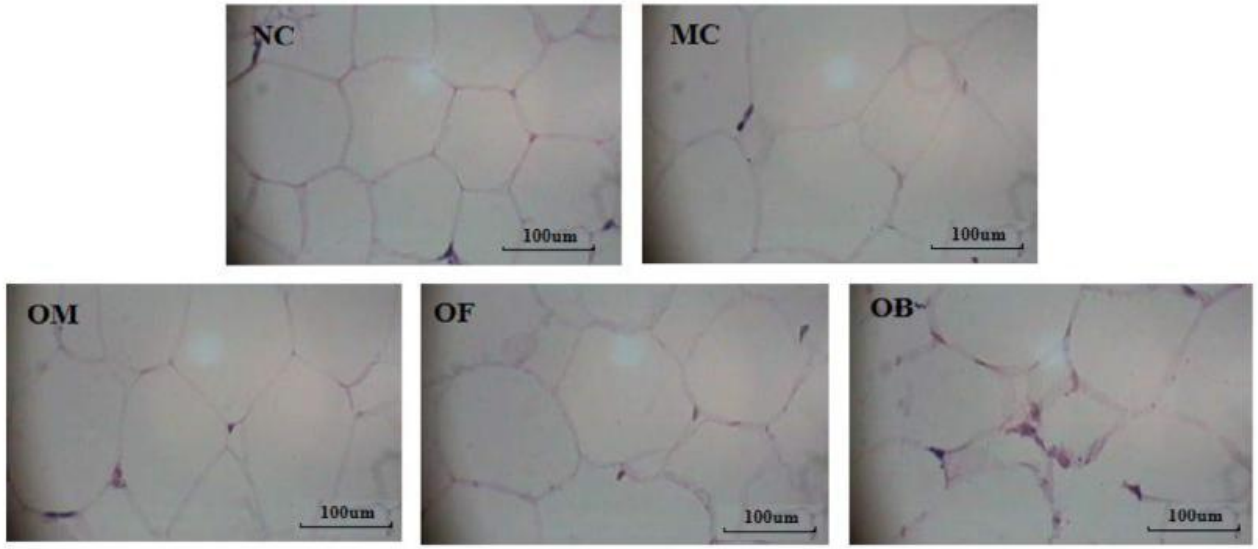
(L)



(M)

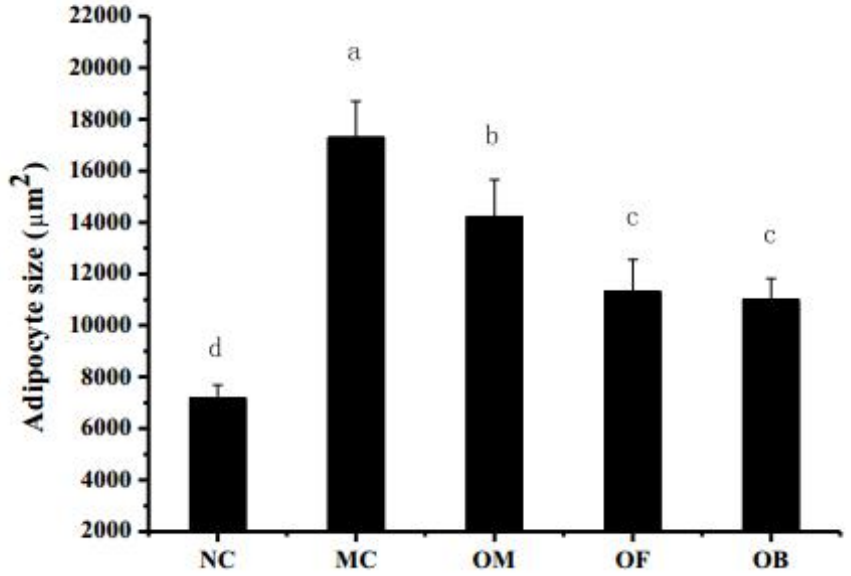


(K)

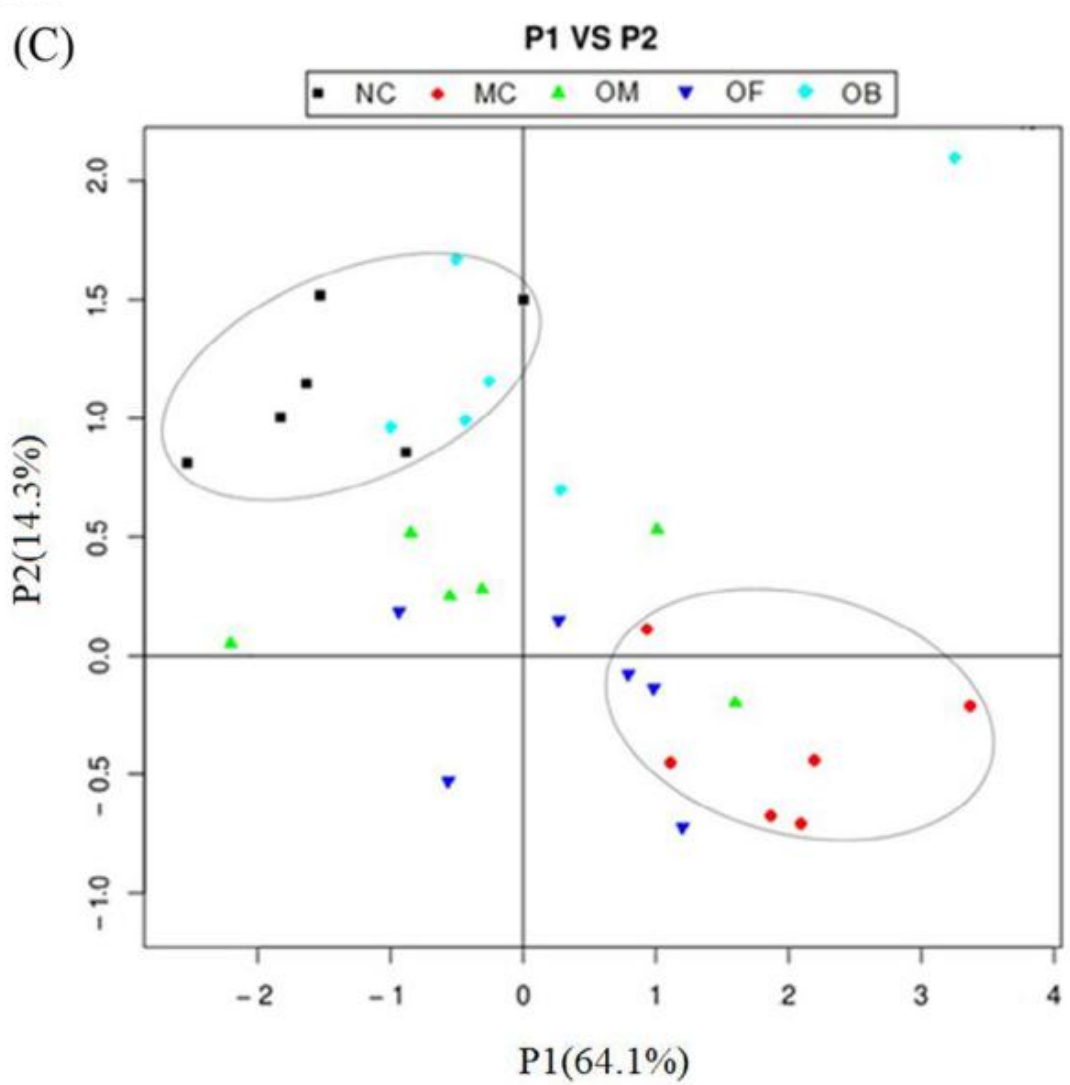


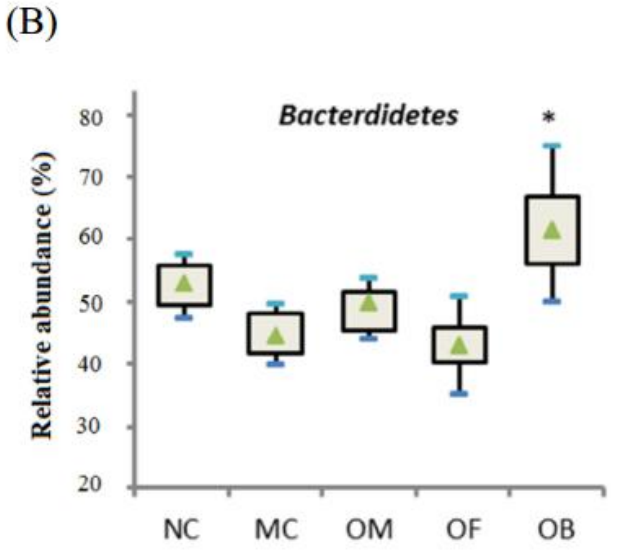
附睾脂肪组织切片

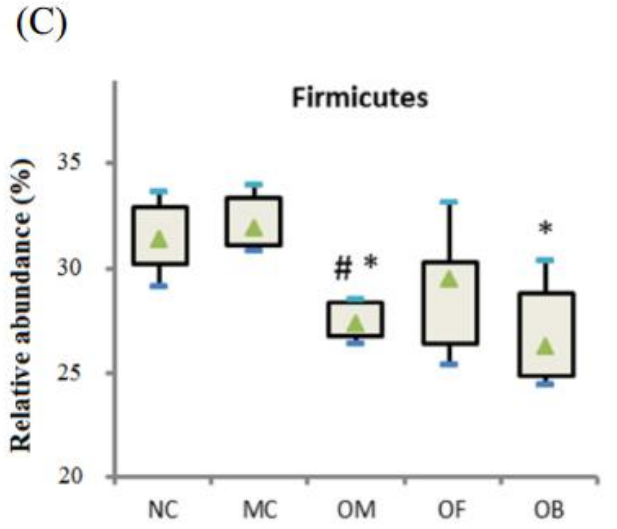
(J)

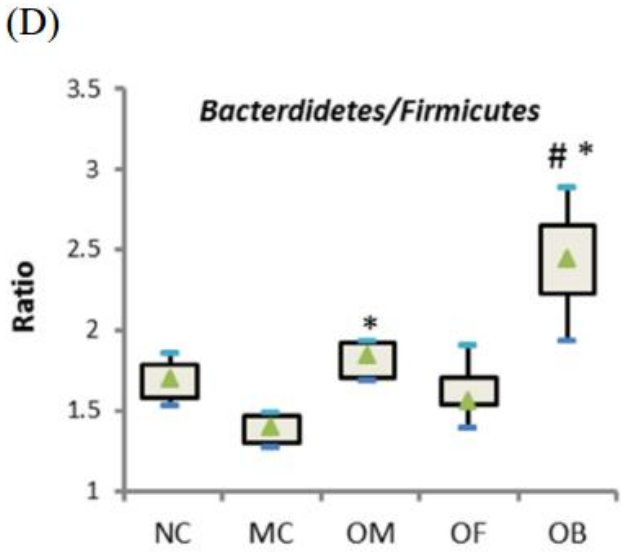


脂肪细胞尺寸


(C)
P1 VS P2
NC
MC
OM
OF
OB
P2(14.3%)
P1(64.1%)


(B)
Bacterdidetes
Relative abundance (%)
NC
MC
OM
OF
OB


(C)
Firmicutes
Relative abundance (%)
NC
MC
OM
OF
OB


(D)
Bacterdidetes/Firmicutes
Ratio
NC
MC
OM
OF
OB

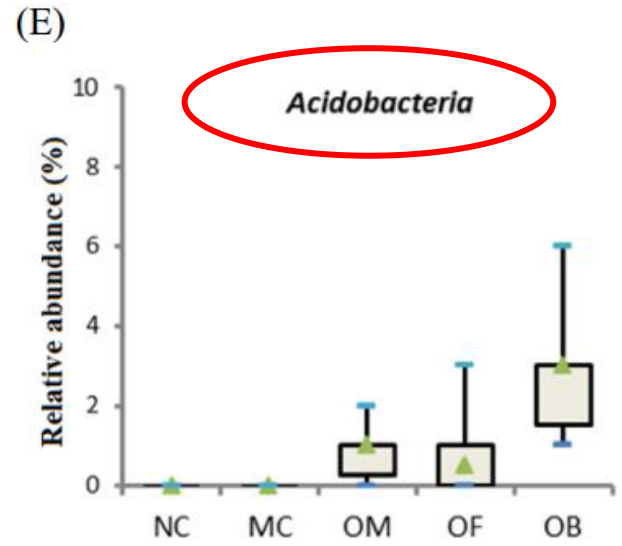

(E)
Acidobacteria
Relative abundance (%)
NC
MC
OM
OF
OB

Table 3 – Spearman's correlation between gut microbiota compositions represented by the first two coordinates of weighted UniFrac PCA and HFD induced obesity and hyperlipaemia parameters.

	P1		P2	
	r	p	r	p
Body weight	0.60	0.285	-0.80	0.104
Epididymal fat weight	0.60	0.285	-0.80	0.104
TC*	0.70	0.188	-0.90	0.037
TG*	0.70	0.188	-0.90	0.037
HDL-C/TC	0.00	1.000	0.60	0.285
ET*	0.60	0.285	-0.80	0.037
TNF-α*	0.70	0.188	-0.90	0.037
Liver TNF-α mRNA*	0.70	0.188	-0.90	0.037
Adipocyte TNF-α mRNA*	0.70	0.188	-0.90	0.037

Correlations were identified using Spearman's correlation. Correlations were considered significant when $p < 0.05$.

* Present a significant correlation with P2.

Abbreviations: NC, normal control group; MC, model control group; OM, oat meal group; OF, oat flour group; OB, high fibre oat bran group; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; ET, endotoxin; TNF-α, tumour necrosis factor α.

斯皮尔曼相关分析

“等级差数法”。

斯皮尔曼等级相关是根据等级资料研究两个变量间相关关系的方法。

它是依据两列成对等级的各对等级数之差来进行计算的，所以又称为“等级差数法”。只要两个变量的观测值是成对的等级评定资料，或者是由连续变量观测资料转化得到的等级资料，不论两个变量的总体分布形态、样本容量的大小如何，都可以用斯皮尔曼等级相关来进行研究。

rs=1-[6*∑di^2/(n*n^2-1)]

等级相关系数记为rs
di为两变量每一对样本的等级之差
n为样本容量。

某厂工人工作周工作时数与月工资水平原始数据如下:

表 1 某厂工人工作时数与月工 资水平

工作时数	原始数据 /h	37	38	39	40	41	42	43
	排队等级 X	1	2	3	4	5	6	7
工资水平	原始数据 /元	800	900	900	900	900	900	1000
	排队等级 Y	1	4	4	4	4	4	7

燕麦改变了结肠内短链脂肪酸的组成和含量

Table 4 – Changes in colonic SCFAs of rats in each at week 8 (mmol/g colonic digesta).

	Acetate	Propionate	Isobutyrate	Butyrate	Total SCFA
NC	4.71 ± 0.22^{d}	1.69 ± 0.43^{bc}	0.28 ± 0.02^{ab}	0.99 ± 0.09^{c}	7.67 ± 0.31^{d}
MC	2.81 ± 0.13^{e}	0.76 ± 0.08^{d}	0.10 ± 0.01^{d}	0.61 ± 005^{d}	4.28 ± 0.67^{e}
OM	9.37 ± 0.56^{b}	2.40 ± 0.21^{b}	0.19 ± 0.04^{bc}	2.01 ± 0.21^{a}	13.99 ± 0.58^{b}
OF	8.01 ± 0.11^{c}	2.21 ± 0.50^{bc}	0.22 ± 0.05^{abc}	1.39 ± 0.33^{b}	11.88 ± 1.01^{c}
OB	11.95 ± 1.01^{a}	3.92 ± 0.33^{a}	0.22 ± 0.01^{bc}	2.90 ± 0.78^{a}	18.99 ± 0.88^{a}

Data are mean ± SD (n = 10). Differences among groups were evaluated for significance by the Tukey post hoc test. Values in the same row that do not share the same lowercase letter are significantly different ($p < 0.05$).

Abbreviations: SCFA, short chain fatty acid; NC, normal control group; MC, model control group; OM, oat meal group; OF, oat flour group; OB, high fibre oat bran group.

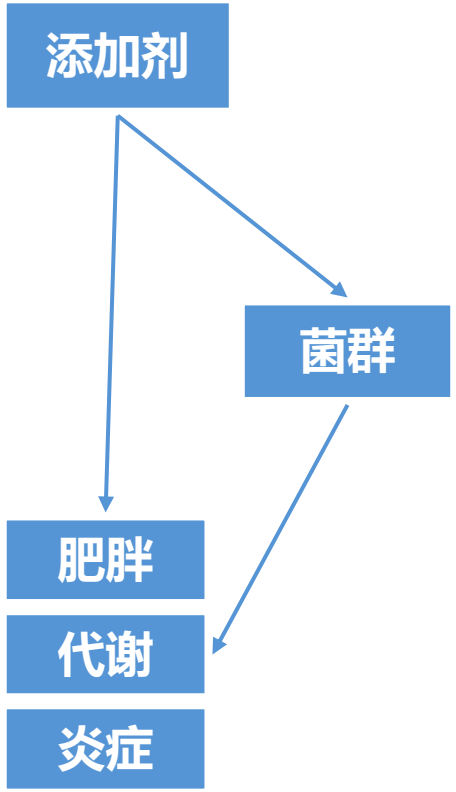
食物纤维、抗性成分

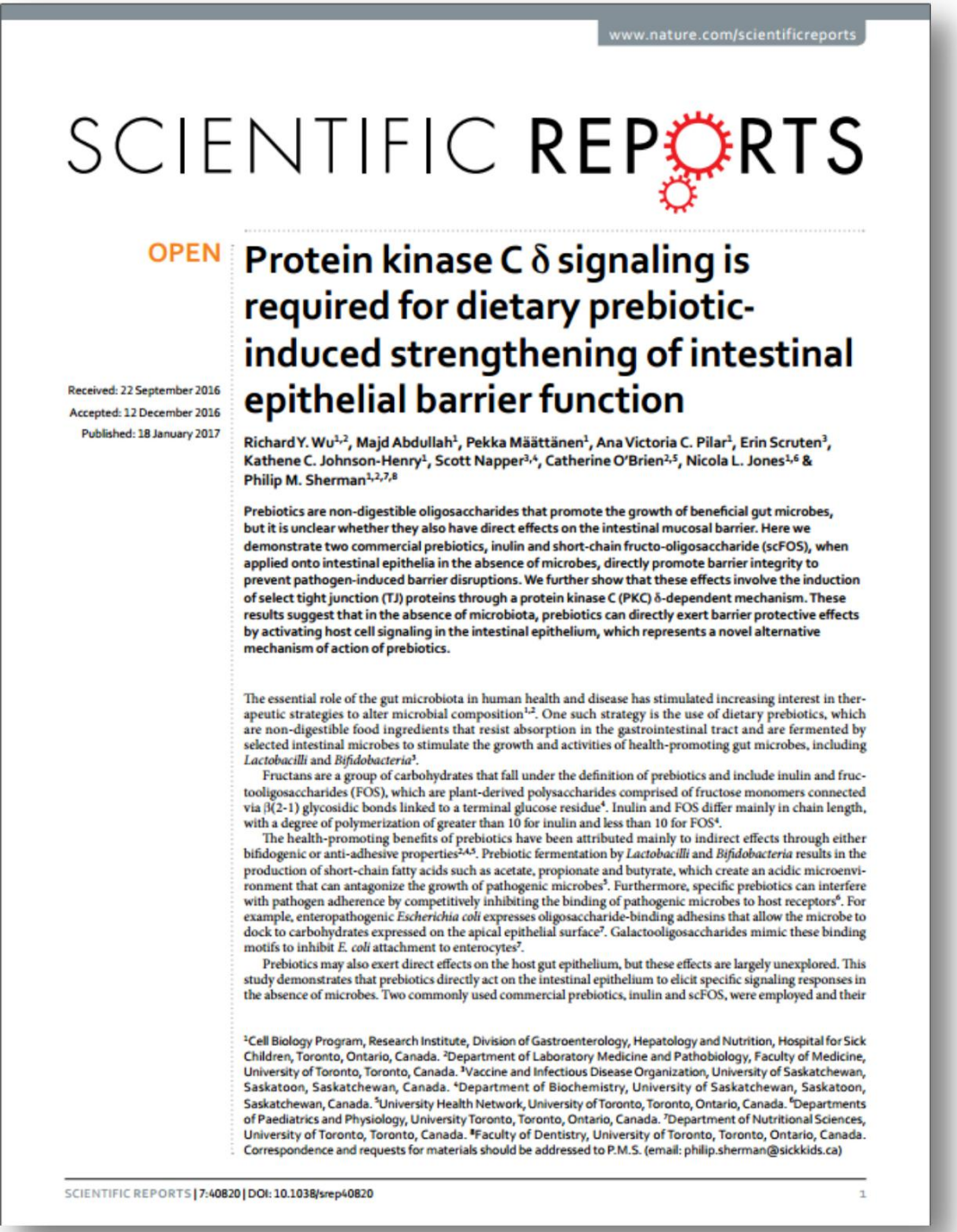
Acidobacteria

写作套路

We firstly found that changes in the gut microbiota induced by oat products were correlated with obesity related metabolic disorders, especially for serum lipid and inflammation levels in DIO rats. Therefore, a speculation that oat products protect DIO rats from some chronic diseases through modifications of gut microbiota structure was proposed. However, to provide direct evidence for this speculation, a further faecal transplant research is needed.

In conclusion, the three oat products individually attenuate the HFD induced obesity and related metabolic disorders in DIO rats. However, the overall gut microbiota structure was altered by these oat products. OB exhibited the most promising effects on inhibiting weight gain and epididymal fat accumulation, improving serum lipid and inflammation levels, modifying gut microbiota composition and increasing SCFAs concentration. This new finding from our study compared with previous studies is that changes in gut microbiota induced by oat products were correlated with obesity-related metabolic disorders (serum lipid and inflammation levels) more than weight gain and fat accumulation. We anticipated that our results will contribute to the development of oat-based diet food for anti-obesity.





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Protein kinase C δ signaling is required for dietary prebiotic-induced strengthening of intestinal epithelial barrier function

Richard Y. Wu[1,2], Majd Abdullah[1], Pekka Määttänen[1], Ana Victoria C. Pilar[1], Erin Scruten[3], Kathene C. Johnson-Henry[1], Scott Napper[3,4], Catherine O'Brien[2,5], Nicola L. Jones[1,6] & Philip M. Sherman[1,2,7,8]

Prebiotics are non-digestible oligosaccharides that promote the growth of beneficial gut microbes, but it is unclear whether they also have direct effects on the intestinal mucosal barrier. Here we demonstrate two commercial prebiotics, inulin and short-chain fructo-oligosaccharide (scFOS), when applied onto intestinal epithelia in the absence of microbes, directly promote barrier integrity to prevent pathogen-induced barrier disruptions. We further show that these effects involve the induction of select tight junction (TJ) proteins through a protein kinase C (PKC) δ-dependent mechanism. These results suggest that in the absence of microbiota, prebiotics can directly exert barrier protective effects by activating host cell signaling in the intestinal epithelium, which represents a novel alternative mechanism of action of prebiotics.

The essential role of the gut microbiota in human health and disease has stimulated increasing interest in therapeutic strategies to alter microbial composition[1,2]. One such strategy is the use of dietary prebiotics, which are non-digestible food ingredients that resist absorption in the gastrointestinal tract and are fermented by selected intestinal microbes to stimulate the growth and activities of health-promoting gut microbes, including *Lactobacilli* and *Bifidobacteria*[3].

Fructans are a group of carbohydrates that fall under the definition of prebiotics and include inulin and fructooligosaccharides (FOS), which are plant-derived polysaccharides comprised of fructose monomers connected via β(2-1) glycosidic bonds linked to a terminal glucose residue[4]. Inulin and FOS differ mainly in chain length, with a degree of polymerization of greater than 10 for inulin and less than 10 for FOS[4].

The health-promoting benefits of prebiotics have been attributed mainly to indirect effects through either bifidogenic or anti-adhesive properties[2,4,5]. Prebiotic fermentation by *Lactobacilli* and *Bifidobacteria* results in the production of short-chain fatty acids such as acetate, propionate and butyrate, which create an acidic microenvironment that can antagonize the growth of pathogenic microbes[3]. Furthermore, specific prebiotics can interfere with pathogen adherence by competitively inhibiting the binding of pathogenic microbes to host receptors[6]. For example, enteropathogenic *Escherichia coli* expresses oligosaccharide-binding adhesins that allow the microbe to dock to carbohydrates expressed on the apical epithelial surface[7]. Galactooligosaccharides mimic these binding motifs to inhibit *E. coli* attachment to enterocytes[7].

Prebiotics may also exert direct effects on the host gut epithelium, but these effects are largely unexplored. This study demonstrates that prebiotics directly act on the intestinal epithelium to elicit specific signaling responses in the absence of microbes. Two commonly used commercial prebiotics, inulin and scFOS, were employed and their

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无需菌群，菊粉和低聚果糖能直接保护肠屏障

① 2种商业化益生元——菊粉及短链低聚果糖，可在无菌群参与的情况下，直接增强肠道的屏障功能，抑制病原体诱导的肠道屏障损伤；

② 益生元增强肠道屏障功能的效应与通过蛋白激酶C δ依赖性机制，诱导选择紧密连接蛋白的表达相关；

③ 这是首次证实菊粉和低聚果糖在促进有益菌增殖外直接对肠屏障功能有保护作用，这充分证明并延展了它们的益生元作用。

02

酒精中毒

大黄提取物

肠道菌群

肠屏障

Mol. Nutr. Food Res. 61, 1, 2017, 1500899 DOI 10.1002/mnfr.201500899 (1 of 12) 1500899

RESEARCH ARTICLE

Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota

Audrey M. Neyrinck[1], Usune Etxeberria[1,2]*, Bernard Taminiau[3], Georges Daube[3], Matthias Van Hul[1,4], Amandine Everard[1,4], Patrice D. Cani[1,4], Laure B. Bindels[1] and Nathalie M. Delzenne[1]*

[1] Louvain Drug Research Institute, Metabolism and Nutrition Research Group, Université catholique de Louvain, Brussels, Belgium
[2] Department of Nutrition, Food Science and Physiology, University of Navarra, Pamplona, Spain
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Revised: February 26, 2016
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Scope: Binge consumption of alcohol is an alarming global health problem. Acute ethanol intoxication is characterized by hepatic inflammation and oxidative stress, which could be promoted by gut barrier function alterations. In this study, we have tested the hypothesis of the hepatoprotective effect of rhubarb extract in a mouse model of binge drinking and we explored the contribution of the gut microbiota in the related metabolic effects.

Methods and results: Mice were fed a control diet supplemented with or without 0.3% rhubarb extract for 17 days and were necropsied 6 h after an alcohol challenge. Supplementation with rhubarb extract changed the microbial ecosystem (assessed by 16S rDNA pyrosequencing) in favor of *Akkermansia muciniphila* and *Parabacteroides goldsteinii*. Furthermore, it improved alcohol-induced hepatic injury, downregulated key markers of both inflammatory and oxidative stresses in the liver tissue, without affecting significantly steatosis. In the gut, rhubarb supplementation increased crypt depth, tissue weight, and the expression of antimicrobial peptides.

Conclusions: These findings suggest that some bacterial genders involved in gut barrier function, are promoted by phytochemicals present in rhubarb extract, and could therefore be involved in the modulation of the susceptibility to hepatic diseases linked to acute alcohol consumption.

Keywords:
Akkermansia muciniphila / Alcoholic liver disease / Antimicrobial peptides / Gut barrier / Microbiota / *Parabacteroides goldsteinii* / Steatosis

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

Alcohol abuse represents a risk factor for numerous diseases. In particular, "binge drinking" is on the rise at an alarming rate worldwide. A binge is defined by the National Institute on Alcohol Abuse and Alcoholism as consumption of five and four drinks for men and women, respectively, in 2 h

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E-mail: nathalie.delzenne@uclouvain.be

Abbreviations: **ALAT**, alanine aminotransferase; **ALD**, alcoholic liver disease; **LPS**, lipopolysaccharides; **PIA2g2**, phospholipase A2 group-II; **qPCR**, quantitative PCR; **RegIIIγ**, regenerating islet-derived 3-gamma; **ROS**, reactive oxygen species; **TBARS**, thiobarbituric acid reactive substances; **TLR**, toll-like receptor; **TNF-α**, tumor necrosis factor α

*These authors contributed equally to this work.
Colour online: See article online to view Figs. 1–6 in colour.

IF 4.551

1. 过度消耗酒精带来了全球性的健康问题；
2. 急性酒精中毒常常以肝脏出现炎症和氧化应激来表征，这可能是由于肠道屏障的改变导致。

大黄提取物（Rhubarb extract）

酒精胁迫

Supporting Information Table S1

Composition of the rhubarb extract (% dry matter)

carbohydrate	71
protein (N% x 6.25)	4
fat	<0.5
fiber	27
蒽醌类衍生物 antraquinone derivatives	
大黄酸和大黄酸-8-葡萄糖苷 - rhein and rhein-8-glucoside	7.45
大黄素-甲醚 - physcion	0.43
芦荟大黄素 - aloe-emodin	<0.09
大黄素 - emodin	<0.07
大黄酚 - chrysophanol	<0.04
番泻叶甙 - sennosides	0.89

Data obtained from ORTIS Laboratoires (Elsenborn, Belgium)

Rhein

6.48% (expressed as wet weight)

6.87% (expressed as dry weight)

The diets

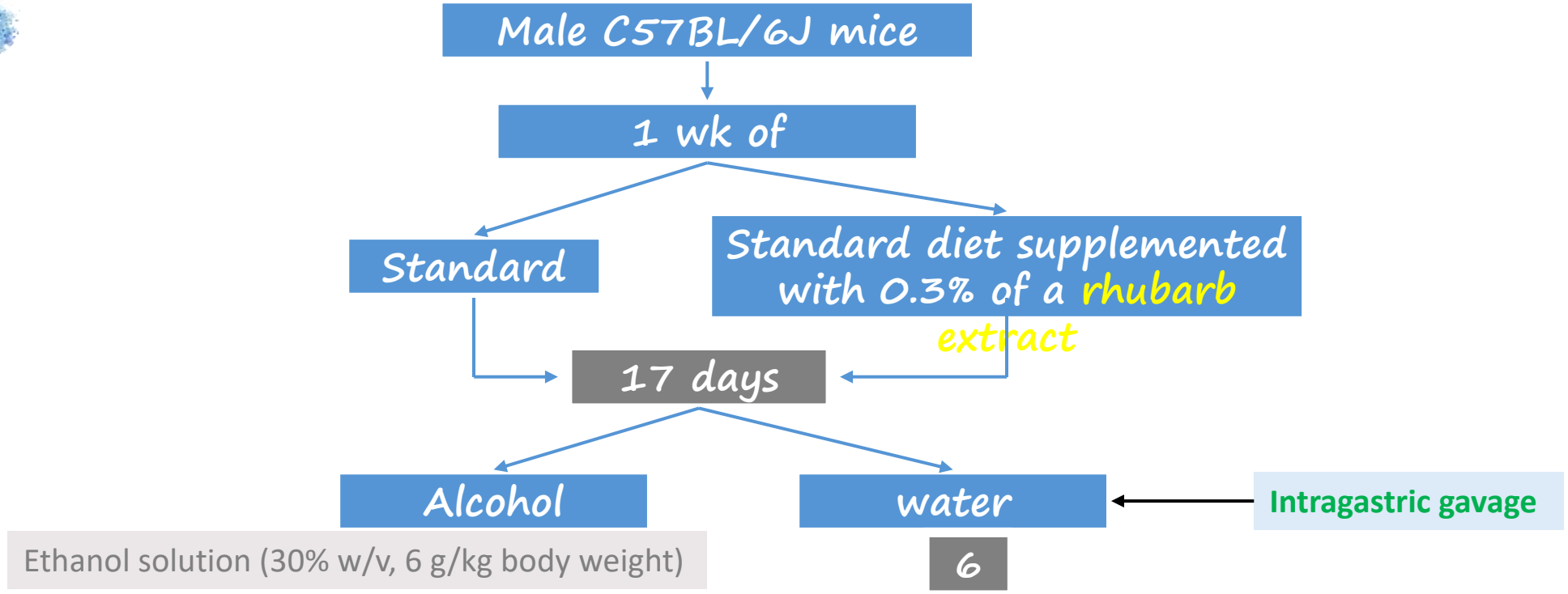
14 g/100 g protein

77 g/100 g carbohydrates

4 g/100 g of lipids

Caloric content of the diet:

3.85 kcal/g



Blood samples

Cecal content

Tissue

Cecal

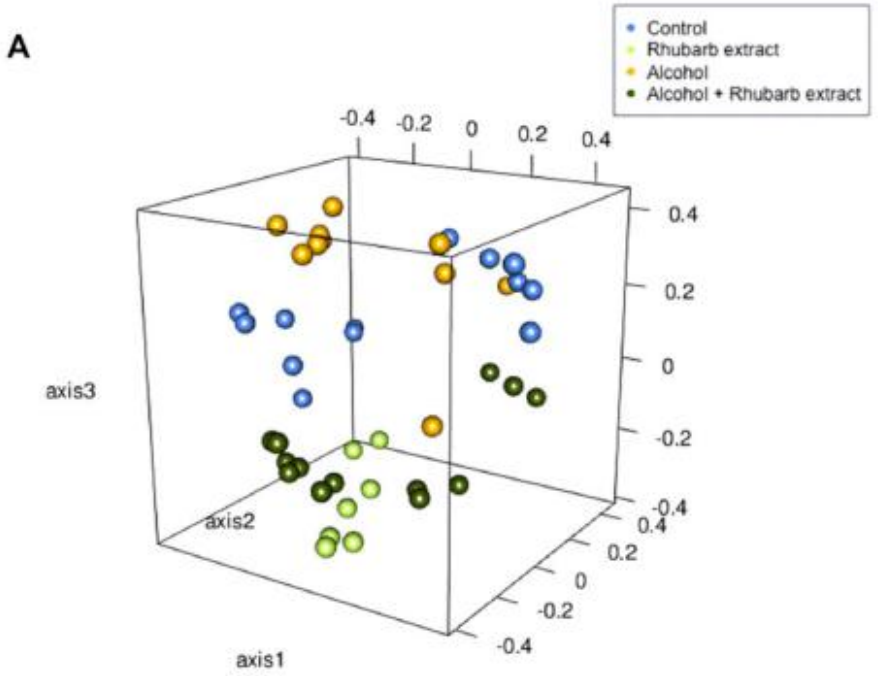
Liver

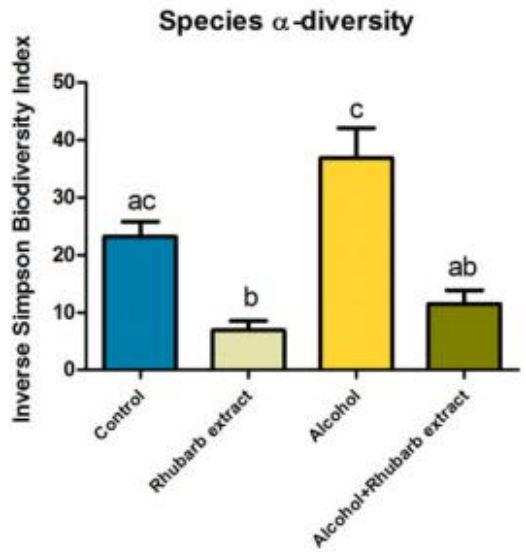
Colon

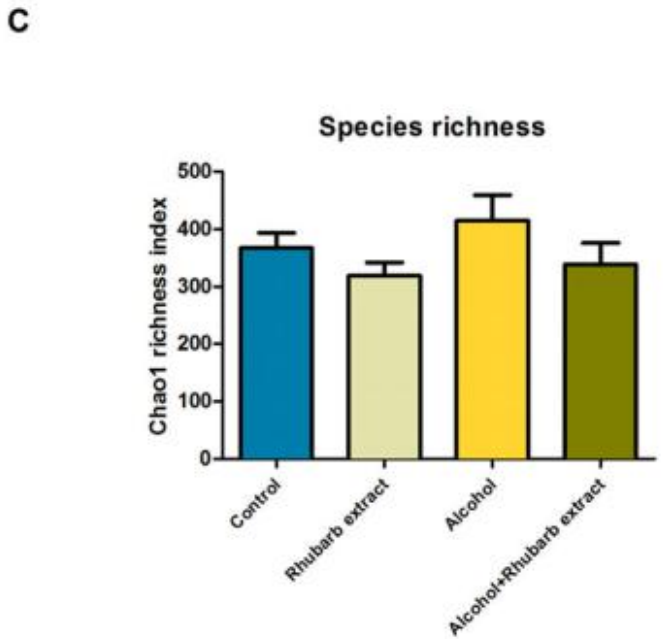
Histological

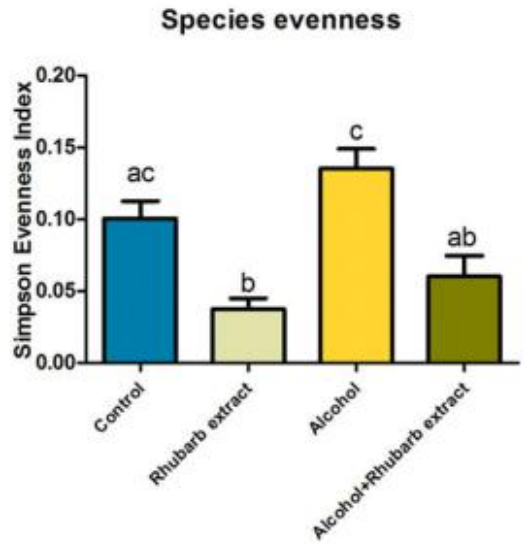
Liver

Colon


A
Control
Rhubarb extract
Alcohol
Alcohol + Rhubarb extract
-0.4
-0.2
0
0.2
0.4
axis3
axis2
axis1


B
Species α-diversity
Inverse Simpson Biodiversity Index
50
40
30
20
10
0
ac
b
c
ab
Control
Rhubarb extract
Alcohol
Alcohol+Rhubarb extract

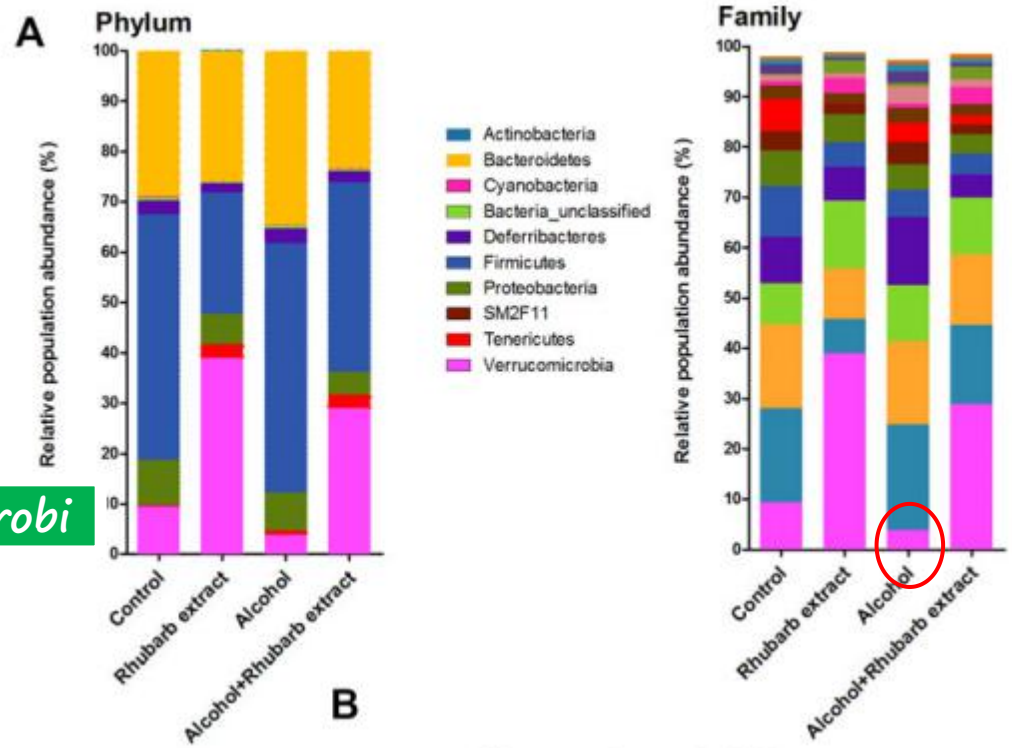

C
Species richness
Chao1 richness index
500
400
300
200
100
0
Control
Rhubarb extract
Alcohol
Alcohol+Rhubarb extract

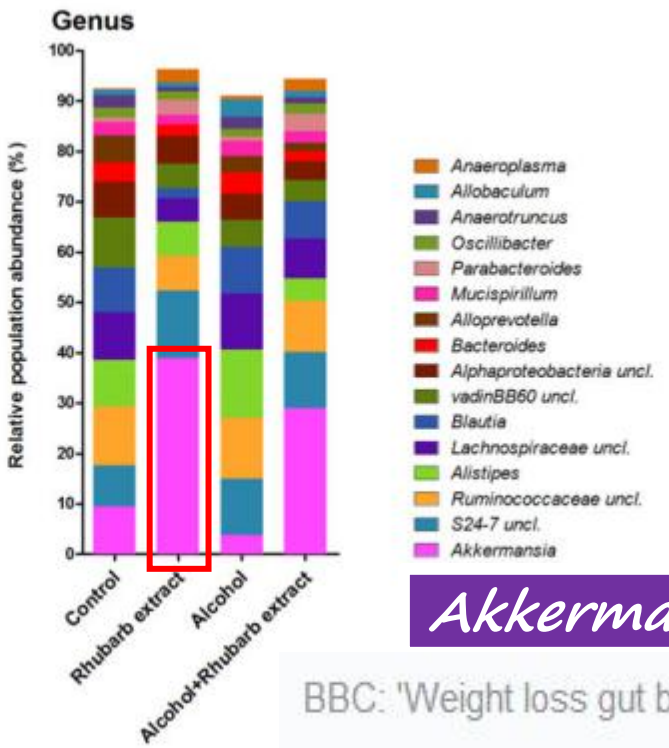

D
Species evenness
Simpson Evenness Index
0.20
0.15
0.10
0.05
0.00
ac
b
c
ab
Control
Rhubarb extract
Alcohol
Alcohol+Rhubarb extract

Firmicute

Verrucomicrobi

疣微菌门

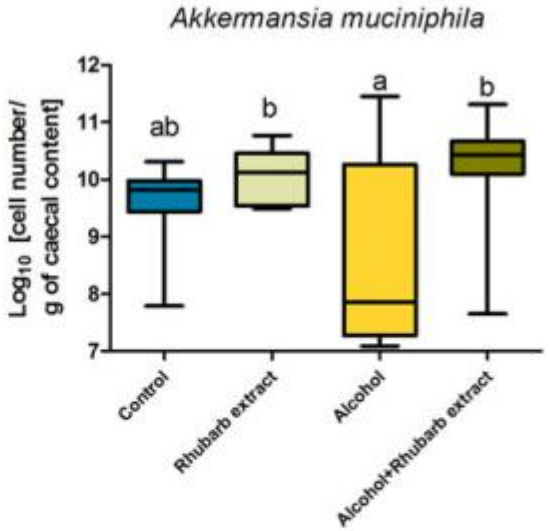


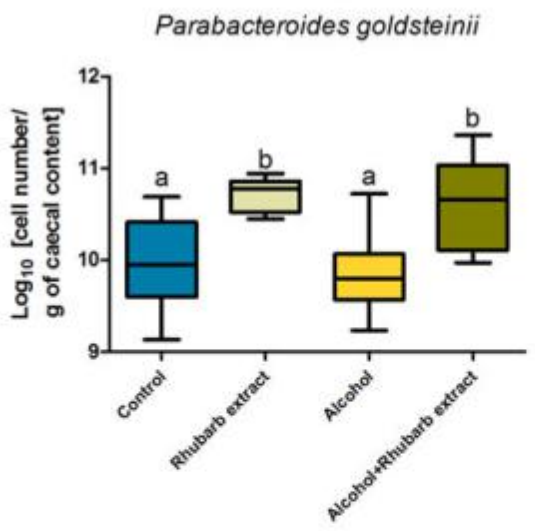


Akkermansia

BBC: 'Weight loss gut bacterium' found

qPCR

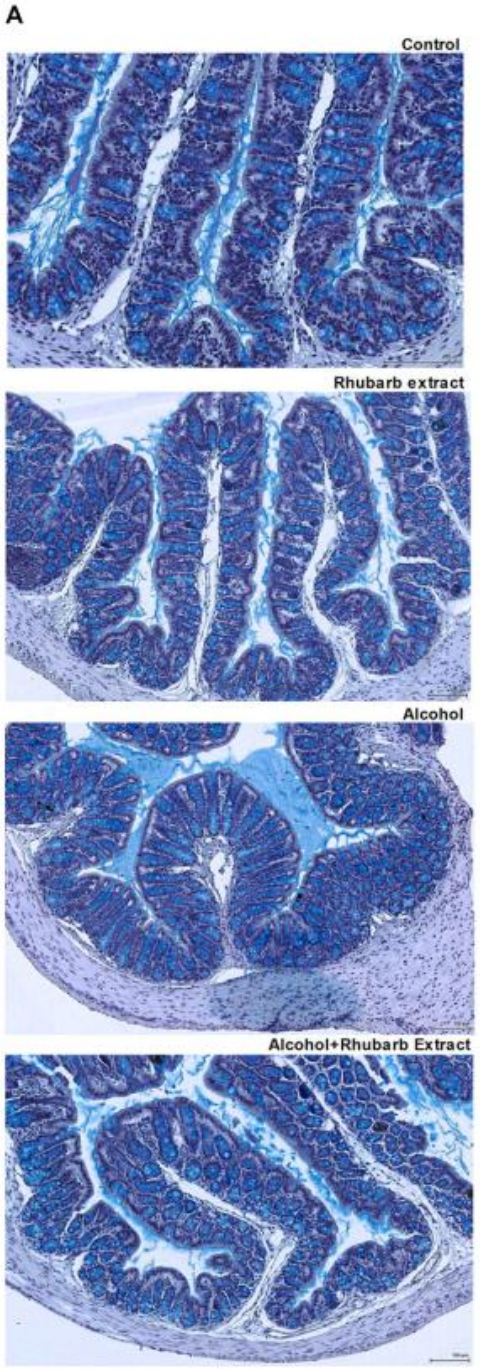




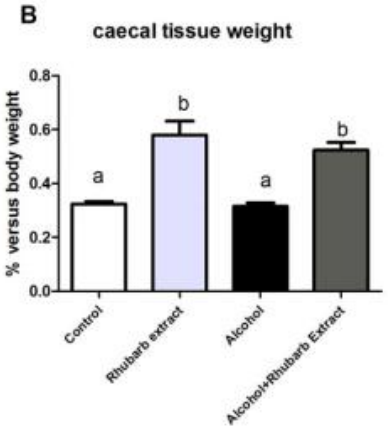
Blautia_HM845948	6.0E-03	0.947	1.078	0.204	0.194	1.739	1.184	0.597	0.39
Allobaculum_EU510831	1.1E-02	1.142	1.045	0.894	0.724	3.390	3.009	1.325	0.93
Alistipes_GQ157664	1.1E-02	0.532	0.584	0.000	0.000	0.646	0.834	0.002	0.00
Bacteroides acidifaciens	1.7E-02	1.949	1.146	1.191	1.199	2.897	1.383	1.393	0.89
Blautia_EF098132	1.7E-02	1.352	1.401	0.278	0.243	2.110	1.489	0.877	0.68
S24-7_EU453189	1.9E-02	0.000	0.000	0.000	0.000	0.568	0.878	0.560	0.56
Anaeroplasma_EF406813	2.8E-02	0.223	0.536	2.642	2.217	0.697	1.642	2.480	3.08
vadinBB60_AB60635									

Species	Referred in Fig.6 as:
Alistipes_JQ084893	*Alistipes_sp_4_s*
Alistipes_JQ085082	*Alistipes_sp_5_s*
Akkermansia muciniphila	*Akkermansia_muciniphila_s*
Bilophila_JQ084163	*Bilophila_sp_s*
S24-7_EU505334	*S24-7_sp_2_s*
Bacteroides acidofaciens	*Bacteroides_acidofaciens_s*
Alistipes_EF603417	*Alistipes_sp_1_s*
Ruminococcaceae_AY991729	*Ruminococcaceae_sp_s*
Alistipes_HQ740259	*Alistipes_sp_3_s*
Parabacteroides goldsteinii	*Parabacteroides_goldsteinii_s*
Anaerotruncus_EU505612	*Anaerotruncus_sp_s*
Blautia_HM845948	*Blautia_sp_2_s*
Allobaculum_EU510831	*Allobaculum_sp_s*
Alistipes_GQ157664	*Alistipes_sp_2_s*
Bacteroides acidifaciens	*Bacteroides_acidifaciens_s*
Blautia_EF098132	*Blautia_sp_1_s*
S24-7_EU453189	*S24-7_sp_1_s*
Anaeroplasma_EF406813	*Anaeroplasma_sp_s*
vadinBB60_AB606358	*vadinBB60_sp_s*

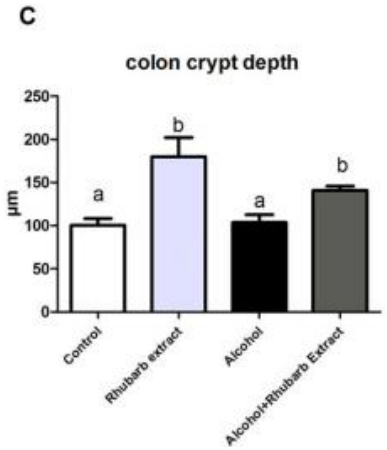
Abundance of bacteria taxa, expressed in percentage, that are impacted by the dietary treatment and/or the alcohol challenge, as determined by pyrosequencing of 16sRNA gene; SD : standard deviation. Data with different superscript letters are significantly different ($p<0.05$) according to 2-way ANOVA followed by Tukey post hoc test; p values were adjusted for multiple testing according to the Tuckey procedure.



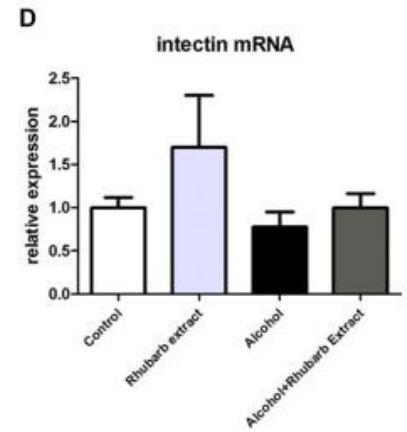
阿尔新蓝染色



盲肠组织重量



结肠隐窝深度

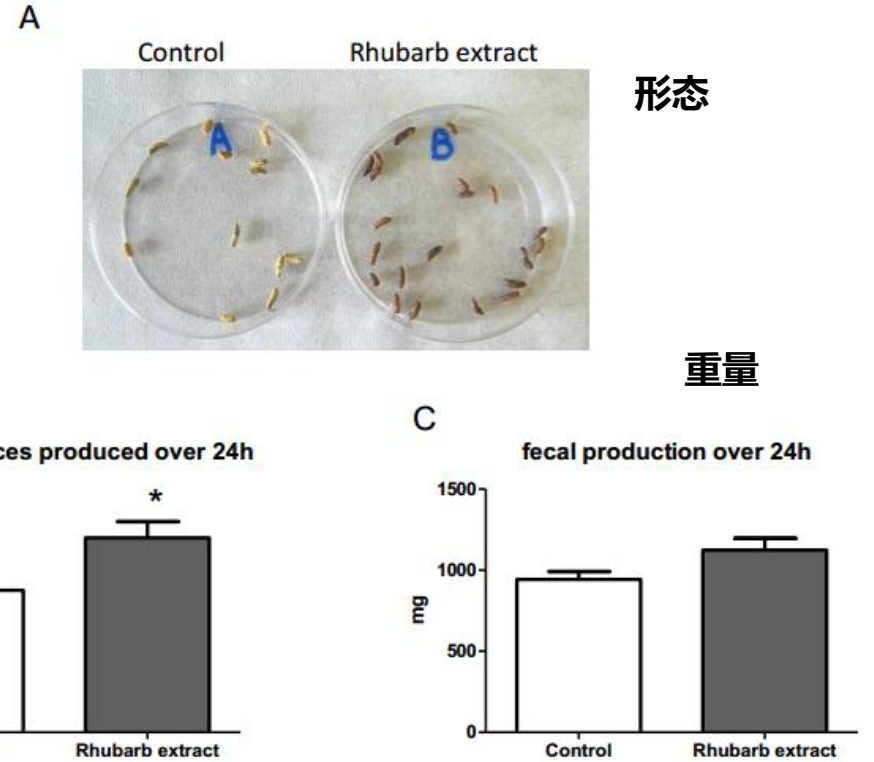


肠上皮细胞凋亡蛋白

Figure 3. Morphologic, morphometric, and molecular changes for the evaluation of cell proliferation in the caecocolon. (A) Representative pictures of the colon after alcian blue staining; (B) cecal tissue weight versus body weight; (C) crypt depth measured by histological analyses after hematoxilin/eosin or blue alcian staining; (D) expression of intectin in the colon. Mice were fed a control diet supplemented with or without rhubarb extract during 17 days before the alcohol challenge. Data are expressed as the mean ± SEM. Data with different superscript letters are significantly different at $p < 0.05$ according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test.

Supporting Information Figure S4

Representative picture of feces and fecal production over 24h



A. Representative picture of feces produced during 6h. B. Number of feces produced over 24h. C. Weight of feces produced over 24h. Mice were fed a control diet supplemented with our without rhubarb extract during 14 days.

大黄提取物改善了肠道内稳态

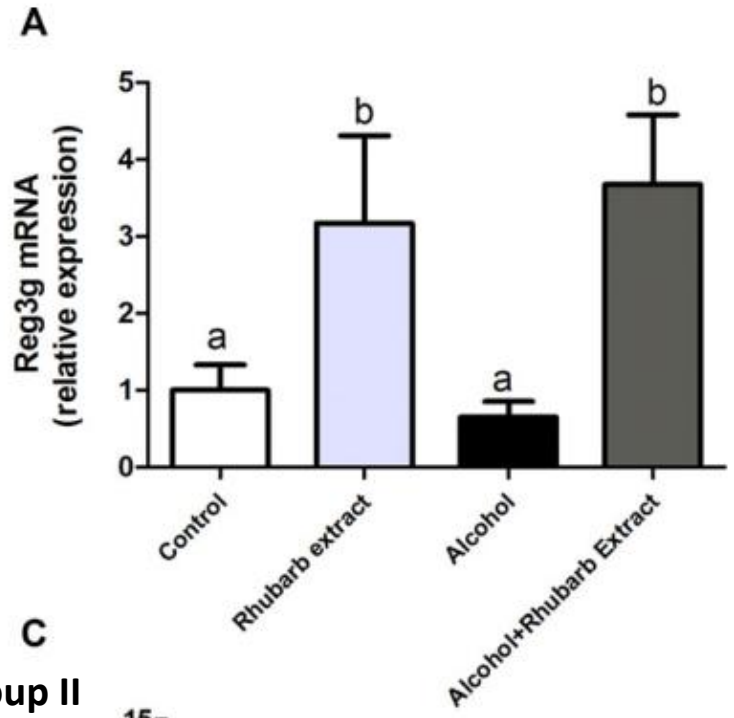
结肠炎症因子

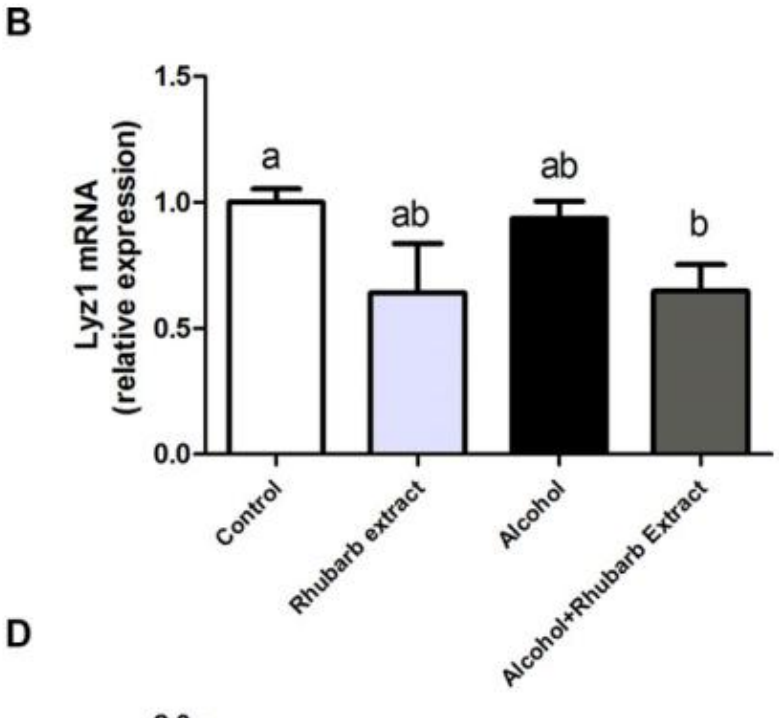
Table 1. Gene expression in the colon

mRNA (relative expression)	Control	Rhubarb extract	Alcohol	Alcohol+ Rhubarb extract
Il-6	1.00 ± 0.08	1.20 ± 0.19	0.94 ± 0.17	0.79 ± 0.23
Il-1β	1.00 ± 0.09[a]	1.10 ± 0.16[a,b]	1.37 ± 0.21[a,b]	1.96 ± 0.37[b]
TNF-α	1.00 ± 0.11	1.17 ± 0.23	1.16 ± 0.39	1.27 ± 0.16
MCP-1	1.00 ± 0.10[a,b]	1.32 ± 0.26[a]	0.57 ± 0.07[b]	1.07 ± 0.20[a,b]

Mice were fed a control diet supplemented with or without rhubarb extract during 17 days before the alcohol challenge. Data are expressed as the mean ± SEM. Data with different superscript letters are significantly different at $p < 0.05$ according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test. MCP-1, monocyte chemotactic protein 1.

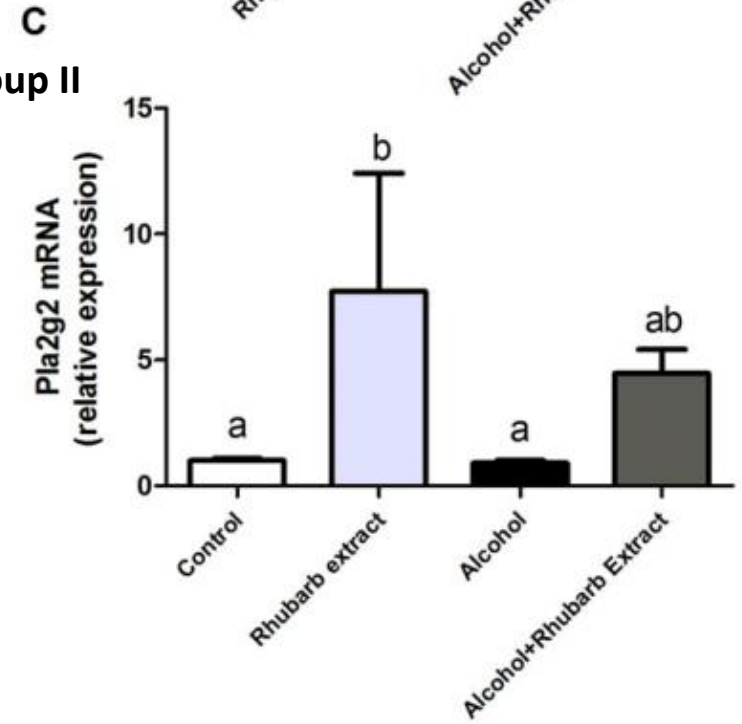
Reg3g
再生胰岛衍生蛋白3

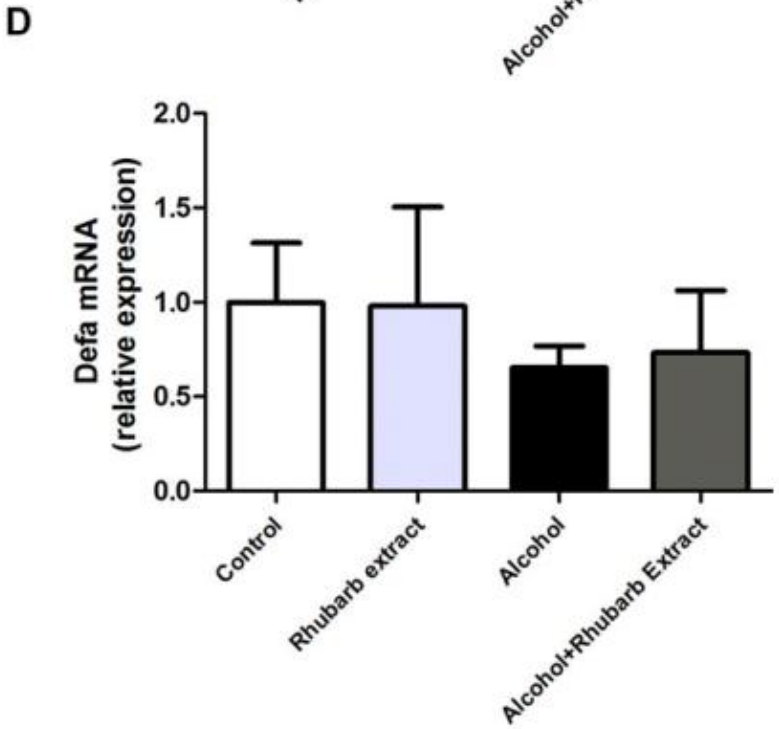




lysozyme C
溶菌酶 C

phospholipase A2 group II
磷脂酶A2





α-defensins
α -防御素

肝脏炎症相关基因

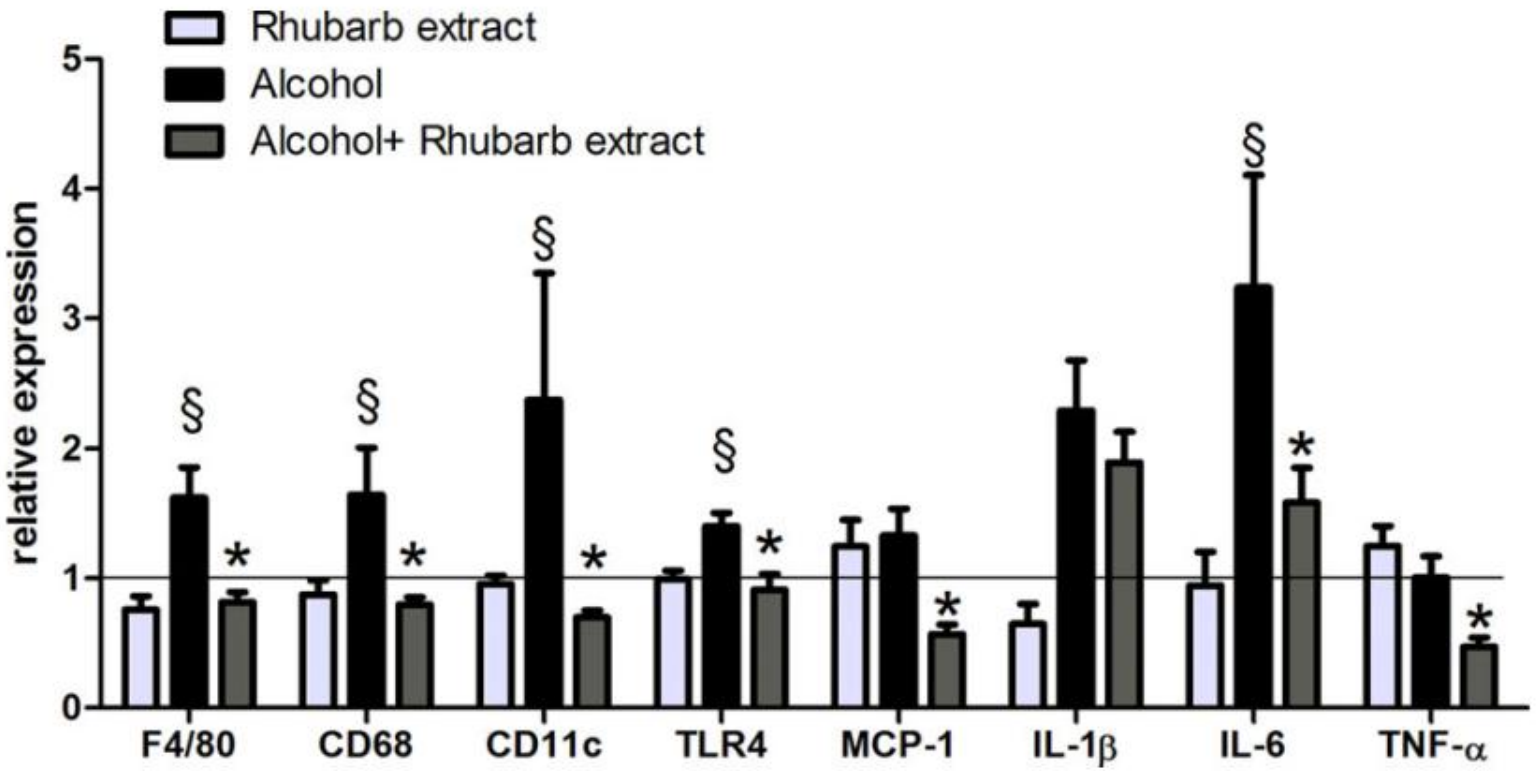
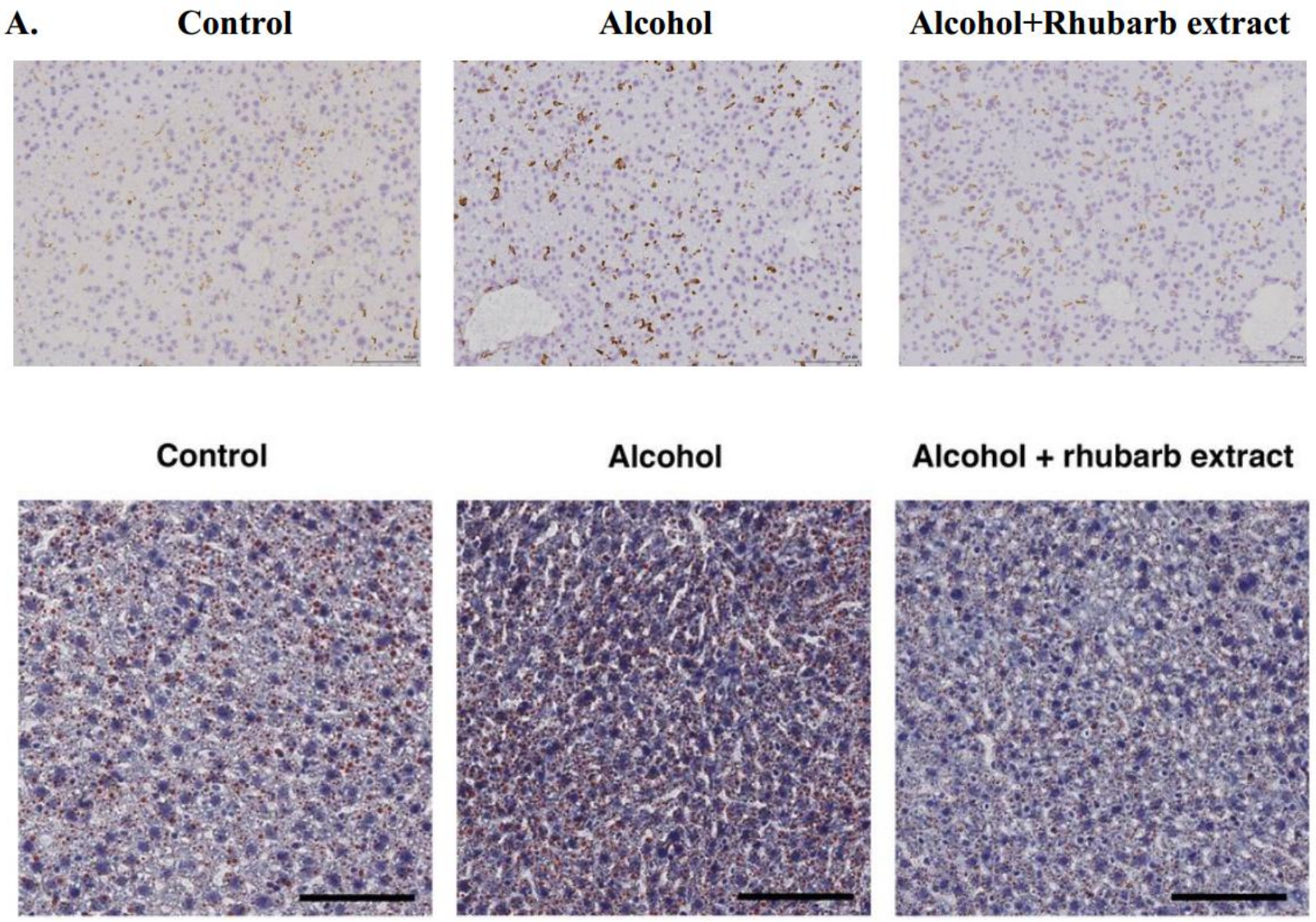


Figure 5. Expression of inflammatory genes in the liver. Data are expressed as the mean ± SEM; values are expressed relative to control group (set at 1). $^{\S}p < 0.05$ versus control group and $^{*}p < 0.05$ versus Alcohol group according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test. Mice were fed a control diet supplemented with or without rhubarb extract during 17 days before the alcohol challenge.

免疫组化

（巨噬细胞-F4/80）

油红O染色



肝脏脂肪变性、氧化应激相关参数

Table 2. Hepatic parameters related to steatosis and oxidative stress

	Control	Rhubarb extract	Alcohol	Alcohol+ Rhubarb extract
Triglycerides content (nmol mg protein)	151 ± 8^{a,b}	118 ± 9^{a}	174 ± 16^{b}	173 ± 6^{b}
Cholesterol content (nmol mg protein)	81 ± 6	83 ± 4	98 ± 9	81 ± 7
TBARS content (mmol MDA/l homogenate H/10)	5.2 ± 0.4	3.7 ± 0.2	5.1 ± 0.7	5.6 ± 0.5
ROS content (% RFU/μg protein)	100 ± 6^{a,b}	84 ± 13^{a,b}	116 ± 10 ^{a}	85 ± 6^{b}
NADPH oxidase mRNA (relative expression)	1.00 ± 0.18^{a,b}	0.87 ± 0.23^{a,b}	1.73 ± 0.56^{a}	0.55 ± 0.06^{b}

Mice were fed a control diet supplemented with or without rhubarb extract during 17 days before the alcohol challenge. Data are expressed as the mean ± SEM. Data with different superscript letters are significantly different at $p < 0.05$ according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test. MDA, relative fluorescence units; RFU, relative fluorescence units; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

硫代巴比妥酸反应物

活性氧类

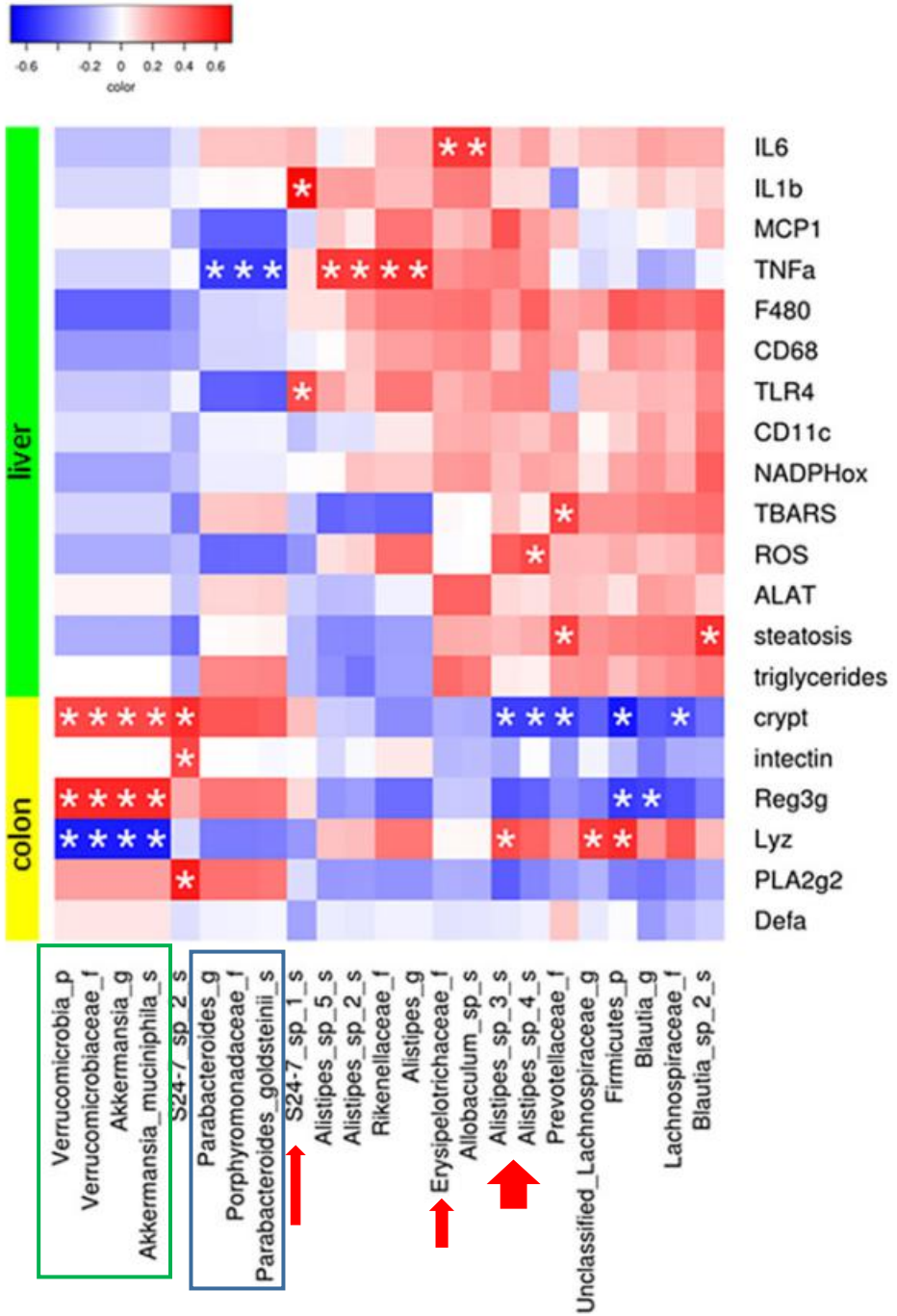


Figure 6. Correlations between bacterial taxa and host parameters. Pearson correlations were computed for all bacterial taxa significantly affected by the treatment (Supporting Information Table 3) and all measured host parameters. *p*-Values were adjusted for multiple testing according to the Bonferroni and Hochberg procedure. The color at each intersection refers to the value of the *r* coefficient; asterisk indicated a significance correlation between these two parameters ($p < 0.05$). Only the bacterial taxa for which at least one significant correlation with a host parameter was detected, are displayed. Bacterial taxonomic level is indicated at the end of the name (p = phylum, f = family, g = genus, s = species).

In summary, this study highlighted that administration of the rhubarb extract at low doses modified host antimicrobial peptide production and gut homeostasis and was associated with profound changes in gut microbial composition. Moreover, the administration of the rhubarb extract had a hepatoprotective effect in binge alcohol induced liver injury acting upon the first step of the disease. This outcome might be the consequence of several pathways related with the down-regulation of expression levels of inflammatory and oxidative markers such as TLR4 and NADPH oxidase, respectively. We hypothesize that the changes in gut bacteria observed upon rhubarb treatment was involved in the higher turnover of epithelial cells contributing to reinforce gut barrier, and thereby, would reduce hepatic damages induced by a binge alcoholic challenge. Future studies should aim at further unraveling the mechanisms by which the gut microbiota impact host physiology related to alcohol abuse, with the hypothesis in mind that the gut microbiota could either be a contribution factor and a therapeutic target in this context.

1. **低剂量的大黄提取物能够改进宿主抗菌肽的产生和肠道内稳态，并与肠道微生物组成的变化相关；**
2. **大黄提取物对大量摄入酒精导致的肝脏损伤具有防护作用；**
3. **研究结果可能与多个代谢路径有关，如炎症（TLR4）和氧化损伤（ NADPH oxidase）；**
4. **推测大黄提取物组肠道菌群的变化可能促进了肠上皮细胞的更新，改善了肠道屏障功能，从而减轻了酒精引起的肝脏损伤；**
5. **机制尚待研究。某些肠道微生物的生理学功能还需进一步验证（假定肠道菌群是一个重要的作用因子，则其可作为治疗靶点来改善肠道内环境和机体健康）。**

实验设计

- 建模、非建模
- 添加剂浓度
- 添加剂—时间节点
- 阴阳性对照
- 添加剂类型—效果比较
- 复合添加剂协同效果
- 不同添加剂效果对比
- ……

营养—肠道菌群—机体代谢研究小结

涉及方面	具体内容
生长指标	增重率、饲料利用率、成活率、肝体比、肝脂含量等
血液生化指标	血清(血浆) GLU/ALT/AST/HDL-C/LDL-C/TG/TC/LPS/胰岛素/免疫相关因子…
常规成分分析	饲料、组织、全动物、粪便成分（水分/粗蛋白/脂肪/灰分）
组织病理学	肝脏、肠道切片（H.E染色、油红O染色、扫描/电子显微镜）
组织抗氧化分析	ROS、SOD、MDA等
肠道消化酶活性	脂肪酶、淀粉酶、胰蛋白酶、纤维素酶等
基因表达	（肝脏、脂肪组织、肠道）脂代谢、免疫或其它相关基因的实时荧光定量（代谢通路）
免疫印迹分析	Western blot analysis
炎症评估	流式细胞术
能量支出	VCO_2/VO_2
耐受试验	葡萄糖耐受/胰岛素耐受
肠道菌群	16S rRNA/宏基因组分析/功能基因预测/不同水平差异挖掘
代谢组	差异代谢产物分析（SCFAs/广筛）

Akkermansia muciniphila

感谢聆听

THANKS VERY MUCH