

Dietary *Clostridium butyricum* Induces a Phased Shift in Fecal Microbiota Structure and Increases the Acetic Acid-Producing Bacteria in a Weaned Piglet Model

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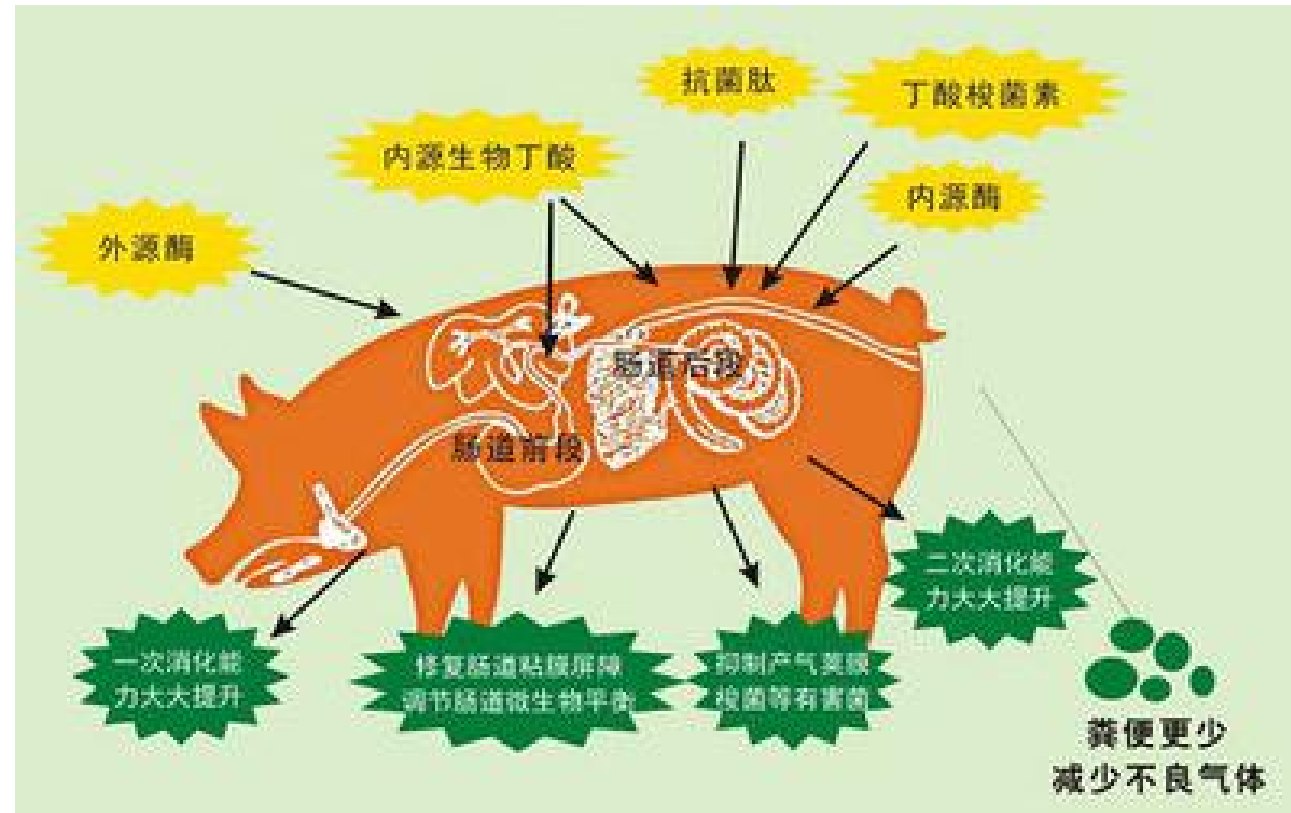
PART 4 DISCUSSION

PART 1 INTRODUCTION

Weaned stress is often accompanied by intestinal dysbiosis with some abrupt changes in the gut microbiota composition of young animals (including infants), resulting in diarrhea, growth retardation, and even mortality.

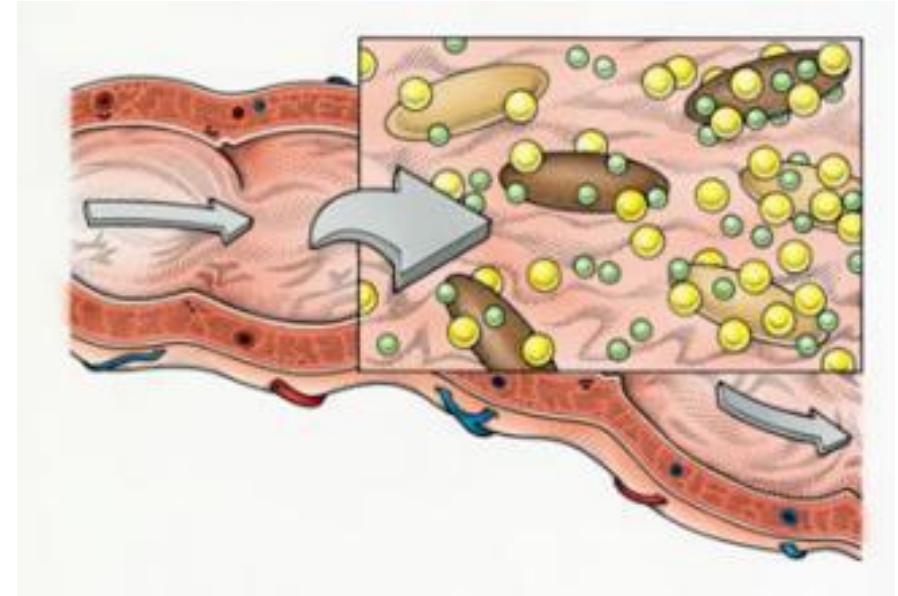
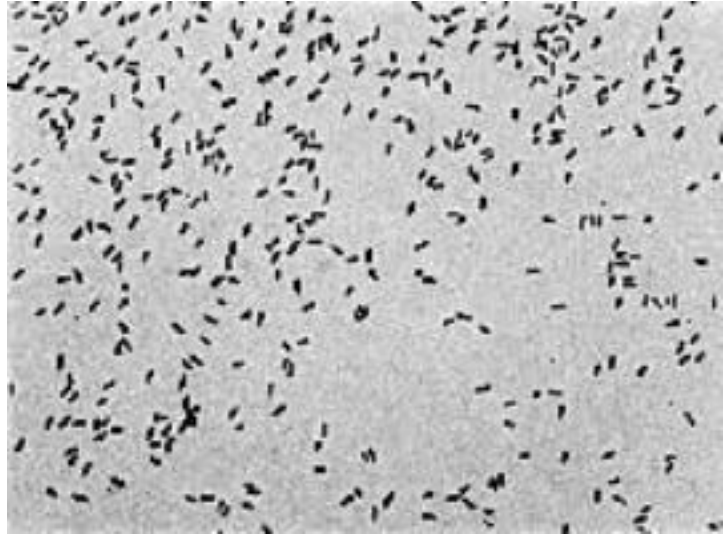


INTRODUCTION



C. butyricum resides in the gastrointestinal tract and has a protective role against pathogenic bacteria and intestinal injury by modulating gut microbial metabolites, such as short-chain fatty acids (SCFAs).

INTRODUCTION



The oral approach of *C. butyricum* to affect intestinal microbial composition and butyrate production in weanling animals remains unclear and whether it acts like butyrate to modulate the intestinal dysbacteriosis in vivo is unknown.

PART 2

**MATERIALS
AND
METHODS**

MATERIALS AND METHODS

Table 1. Nutrient Components of Experimental Diets (Dry Matter Basis)^a

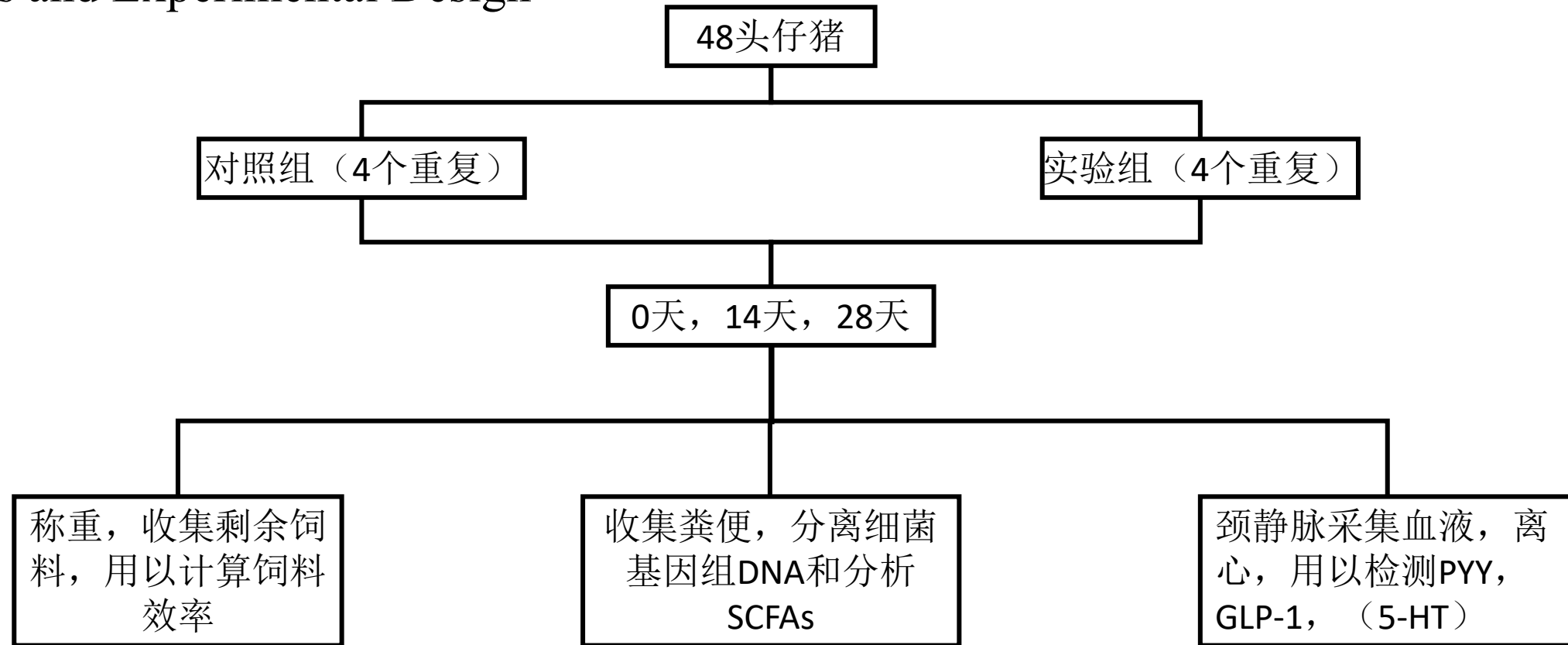
nutrient type	control (%)	<i>C. butyricum</i> (%)	nutrient level	control	<i>C. butyricum</i>
corn	55.02	55.02	digestive energy, MJ/kg	14.50	14.50
DSM	15.70	15.70	crude protein, %	18.50	18.50
EFFSB	5.00	5.00	neutral detergent fiber, %	11.75	11.75
soybean protein concentrate	4.00	4.00	acid detergent fiber, %	4.25	4.25
fish meal	4.00	4.00	Ca, %	0.80	0.80
whey powder	8.00	8.00	P, %	0.60	0.60
sucrose	3.00	3.00	SID Lys, %	1.30	1.30
zinc oxide	0.28	0.28	SID Met + Cystine, %	0.80	0.80
soybean oil	1.30	1.30	SID Thr, %	0.90	0.90
calcium hydrophosphate	1.20	1.20	SID Trp, %	0.30	0.30
limestone	0.50	0.50			
salt	0.30	0.30			
L-lysine (Lys)	0.30	0.30			
DL-methionine (Met)	0.20	0.20			
threonine (Thr)	0.15	0.15			
tryptophan (Trp)	0.10	0.10			
valine (Val)	0.20	0.20			
chromic oxide	0.25	0.25			
premix ^b	0.50	0.50			
<i>C. butyricum</i>	0.00	1.00			
total	100	101			

Control group: Basic diet
 Experimental group: Basic diet+10 g/kg *C. butyricum* (1×10^8 CFU/g)

^aValues of nutrient level in this table are calculated values. Abbreviations: *C. butyricum*, *Clostridium butyricum*; SID, standard ileal digestibility; DSM, dehulled soybean meal; EFFSB, extruded full fat soybean. ^bPremix contained the following per kg: vitamin A, 12,000 IU; vitamin D₃, 2,500 IU; vitamin E, 30 IU; vitamin K₃, 3 mg; vitamin B₁₂, 0.012 mg; riboflavin (VB₂), 4 mg; niacinamide (VB₃), 40 mg; D-calcium pantothenate (VB₅), 15 mg; choline chloride, 400 mg; folacin, 0.7 mg; vitamin B₁, 1.5 mg; vitamin B₆, 3 mg; biotin, 0.1 mg; Zn, 100 mg; Mn, 40 mg; Fe, 90 mg; Cu, 200 mg; I, 0.35 mg; Se, 0.3 mg.

MATERIALS AND METHODS

Animals and Experimental Design



MATERIALS AND METHODS

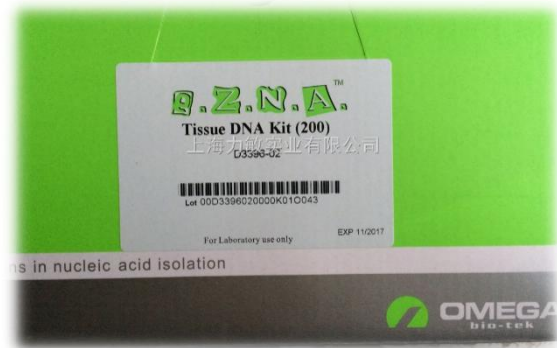
Flora analysis

Extraction of Fecal DNA

PCR Amplification

Illumina MiSeq Sequencing

Bioinformatics Analysis of Sequencing Data



MATERIALS AND METHODS

Detect ion chromatography (IC) is a method for the continuous separation, identification, and quantification of multiple anions or cations. For cation analysis, the separation column is filled with low-capacity cation exchange resin, and hydrochloric acid solution is used as the eluent. Ion chromatography can determine the following types of ions: organic anions, alkali metals, alkaline earth metals, heavy metals, rare earth ions, and organic acids, as well as amines and ammonium salts.

~ 0.5 ml of the sample is diluted with water (1:10) and filtered through a 0.22 µm filter, and finally injected into a high-performance ion chromatograph (ICS 3000 Dionex, USA)

Detection of Plasma Hormones

The concentrations of hormones (PYY, GLP-1, and 5-HT) in porcine plasma samples were detected using the porcine PYY, GLP-1, and 5-HT ELISA assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocols

PART 3

RESULTS

RESULTS

Dietary *C. butyricum* Improved Feed Efficiency

ADFI: 平均每日采食量

ADG: 平均日增重

F / G: 饲料转化率

Dietary supplementation with *C. butyricum* for 28 days improved the feed efficiency by dramatically reducing the ratio of F/G ($P < 0.05$) with no significant effect on ADG or ADFI.

Table 2. Effect of Dietary *C. butyricum* on Growth Performance in Weaned Piglets^a

variable	growth performance		P-value
	control	<i>C. butyricum</i>	
BW, kg			
1 d	8.38 ± 0.21	7.98 ± 0.27	>0.05
14 d	12.76 ± 0.43	12.18 ± 0.50	>0.05
28 d	17.87 ± 0.60	17.60 ± 0.56	>0.05
0–14 d			
ADFI, g/d	518.75 ± 27.55	461.31 ± 47.49	>0.05
ADG, g/d	312.50 ± 22.48	307.25 ± 37.79	>0.05
F/G	1.67 ± 0.08	1.51 ± 0.05	>0.05
14–28 d			
ADFI, g/d	714.79 ± 39.09	664.79 ± 31.19	>0.05
ADG, g/d	426.25 ± 16.95	451.25 ± 13.06	>0.05
F/G	1.68 ± 0.05	1.47 ± 0.06	<0.05
0–28 d			
ADFI, g/d	609.23 ± 31.85	555.22 ± 39.91	>0.05
ADG, g/d	369.38 ± 16.61	379.25 ± 20.69	>0.05
F/G	1.65 ± 0.04	1.46 ± 0.03	<0.05

^aValues are means ± SEM ($n = 24$). Abbreviations: ADFI, average daily feed intake; ADG, average daily weight gain; *C. butyricum*, *Clostridium butyricum*; F/G, feed conversion ratio, the ratio of ADFI to ADG.

RESULTS

Dietary *C. butyricum* Had No Effects on Three Kinds of Intestinal Hormones in Plasma

Table 3. Effect of Dietary *C. butyricum* on Intestinal Hormone Concentrations in the Plasma of Weaned Piglets^a

hormone level	control	<i>C. butyricum</i>	<i>P</i> -value
PYY, ng/mL	1.58 ± 0.16	1.20 ± 0.05	>0.05
GLP-1, ng/L	432.68 ± 20.46	450.68 ± 20.14	>0.05
5-HT, ng/mL	518.41 ± 55.79	443.85 ± 27.17	>0.05

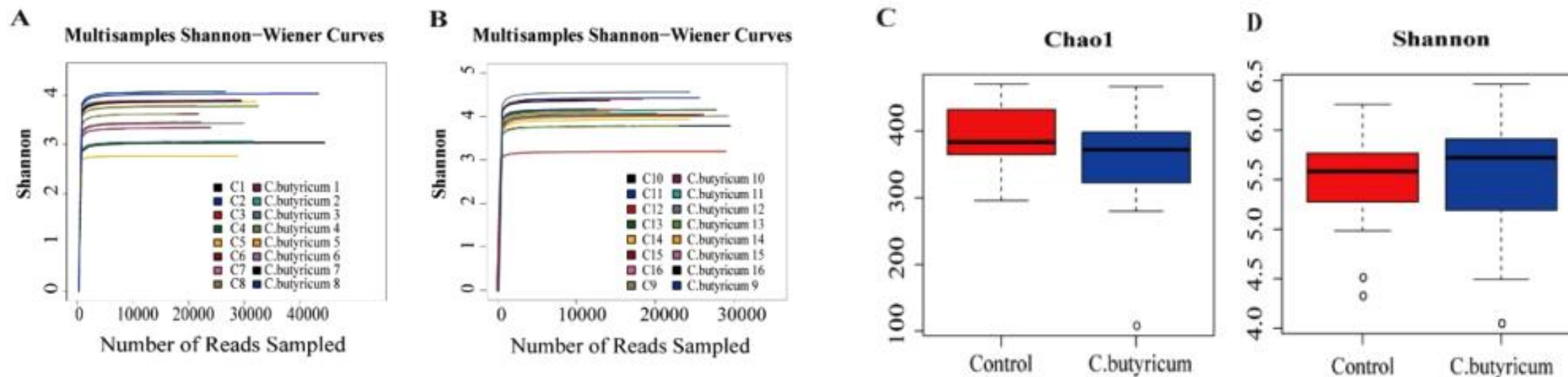
Dietary addition of *C. butyricum* had no effects on any of these hormones.

PYY :一种对多种组织及肿瘤细胞有抑制功能的胃肠激素

GLP-1:作为一种神经递质，抑制食欲和摄食

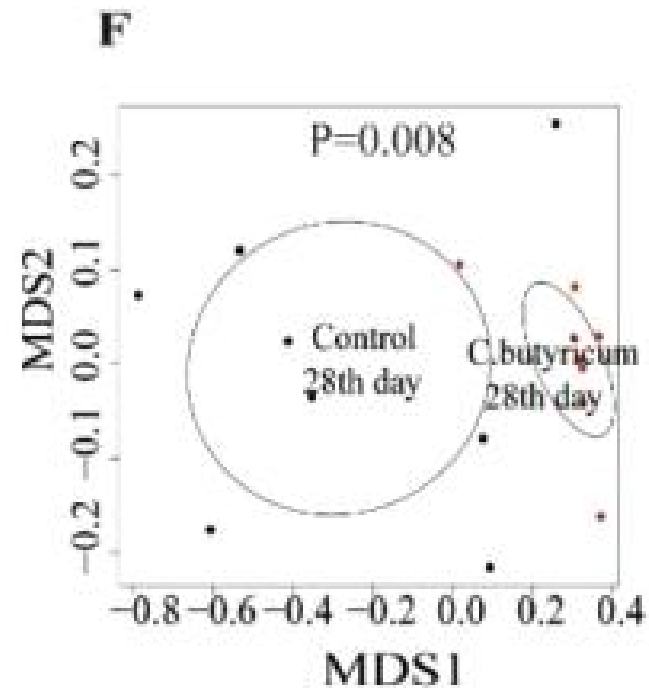
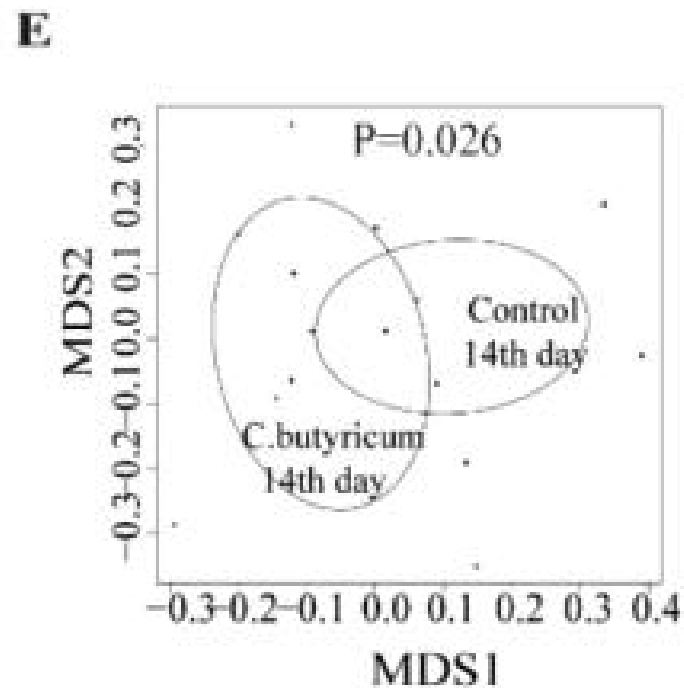
5-HT :一种测量肠应激的指标

Dietary *C. butyricum* Changed Fecal Microbiota Composition



实验中，在第14天和第28天分别测得469,757和466,487条序列，图A、B表示样品拥有足够的微生物信息，图C、D表示相对于对照组，*C. butyricum*对细菌的 α -多样性没有显著影响。

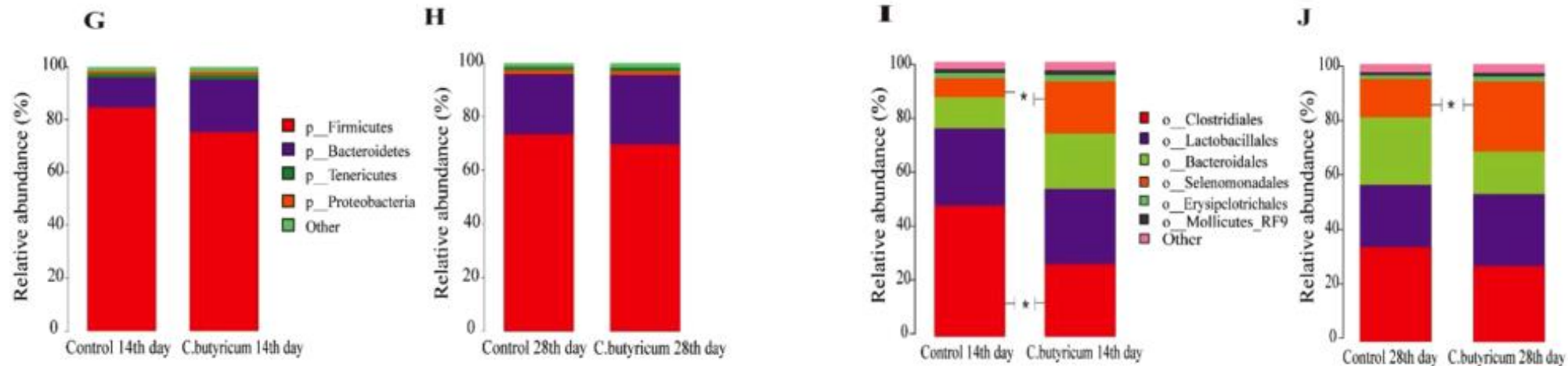
Dietary *C. butyricum* Changed Fecal Microbiota Composition



diff-NMDS plot analysis (one kind of β -diversity analysis) showed that compositions of fecal microbiota were statistically different between two groups on both day 14 ($P < 0.05$) (Figure 1E) and day 28 ($P < 0.01$) (Figure 1F)

RESULTS

Dietary *C. butyricum* Changed Fecal Microbiota Composition



Firmicutes: 厚壁菌门
Clostridiales: 梭菌
Bacteroidales: 拟杆菌属

Bacteroidetes: 拟杆菌门
Lactobacillales: 乳杆菌
Selenomonadales

RESULTS

Dietary *C. butyricum* Increased the Proportion of Common Bacteria and the Number of Dominant Bacteria

A

Control 14th day *C. butyricum* 14th day

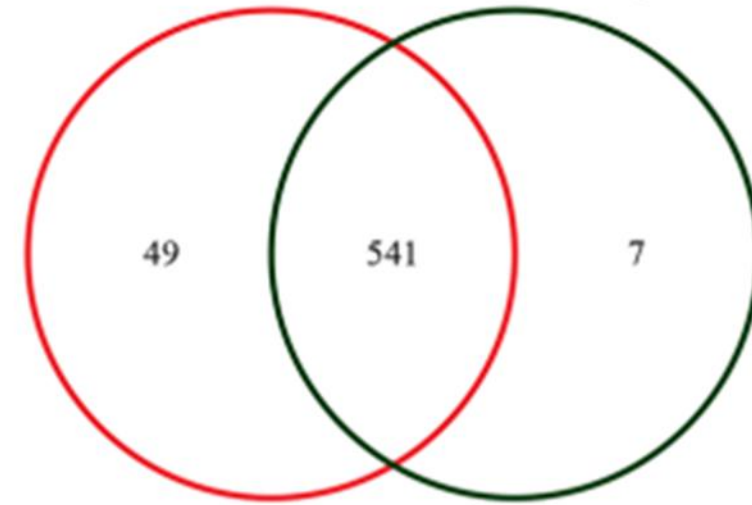


Unique objects: All = 568; S1 = 546; S2 = 534

B

Control 28th day

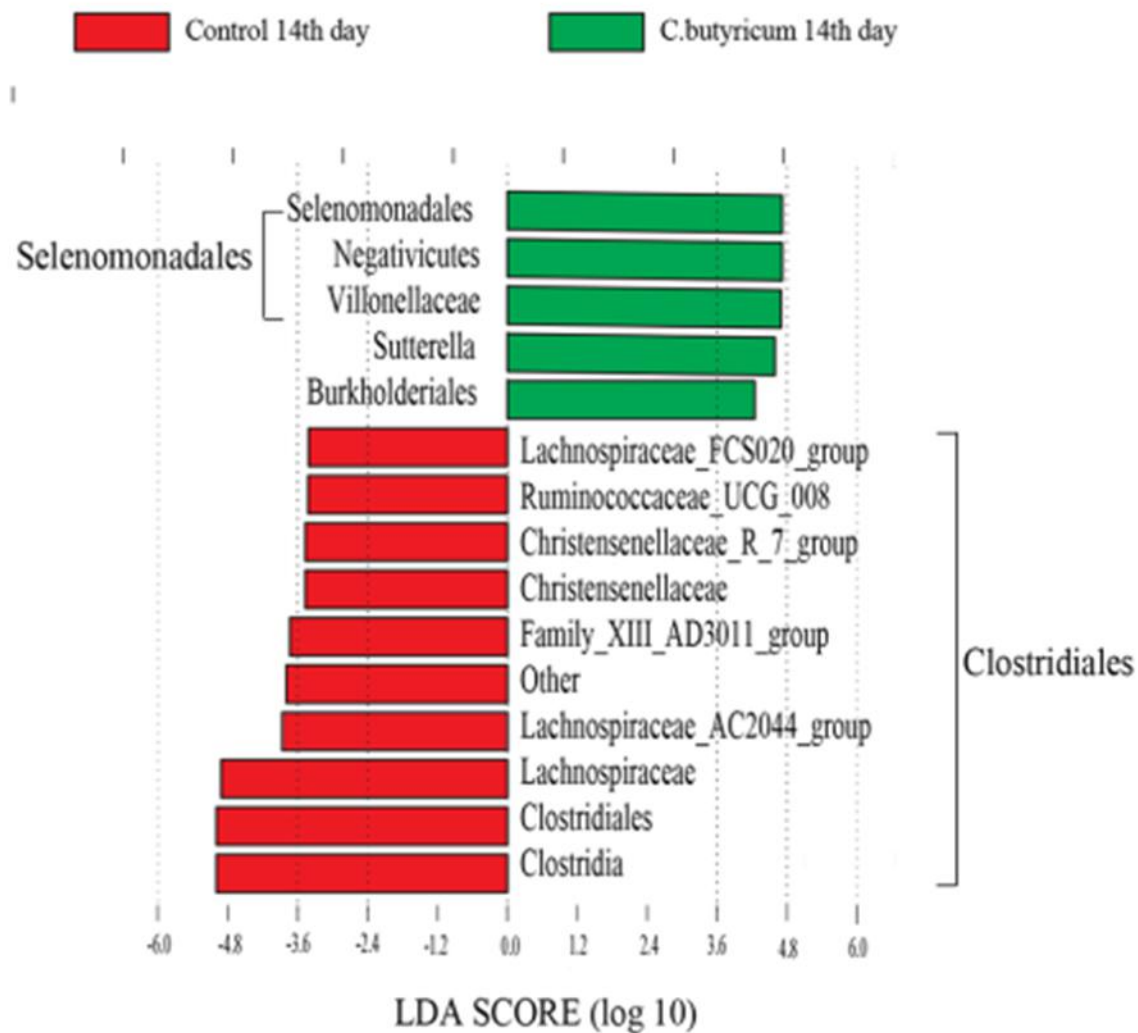
C. butyricum 28th day



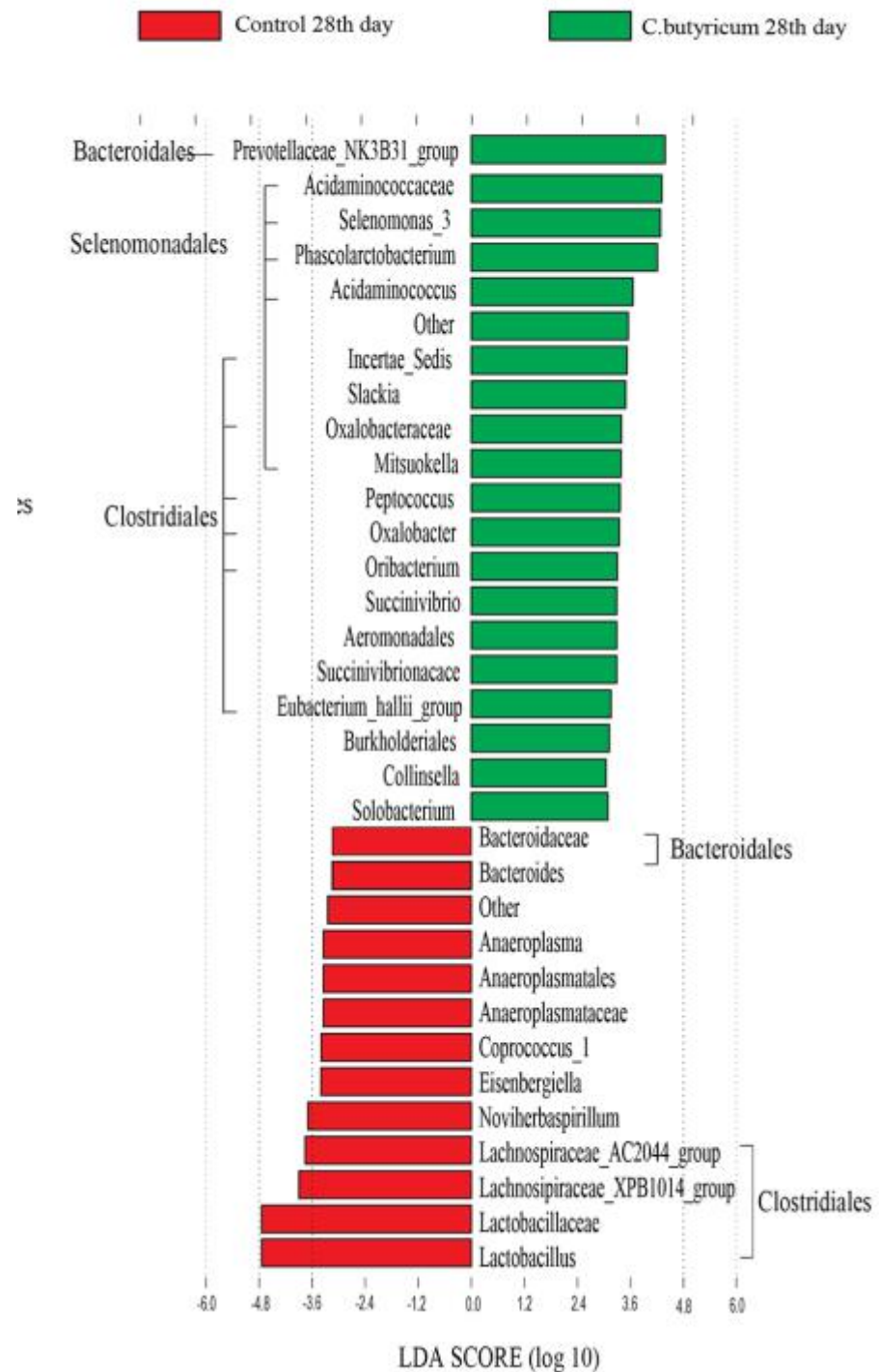
Unique objects: All = 597; S1 = 590; S2 = 548

RESULTS

C

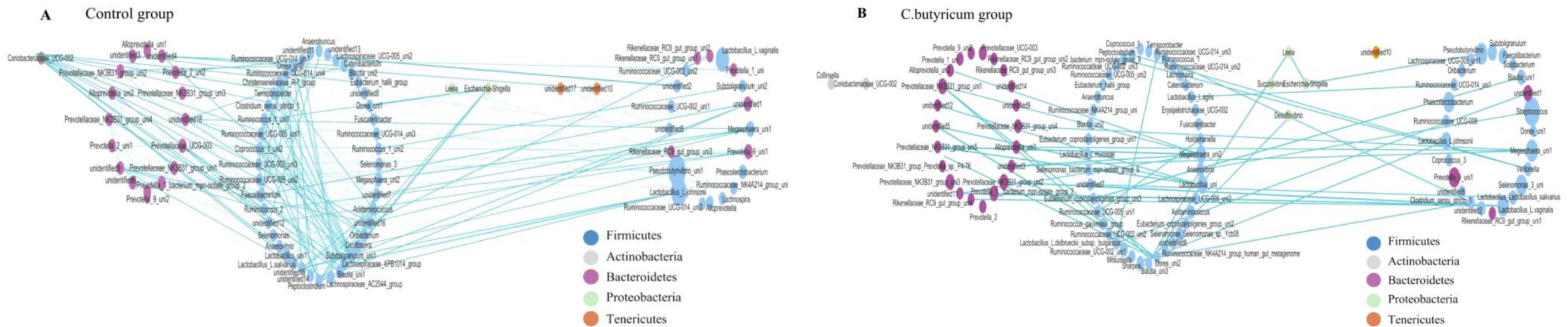


I



RESULTS

Dietary *C. butyricum* Modulated the Correlation between Core Bacteria



Spearman相关矩阵对前100个OUT进行相关网络分析，结果表明丁酸梭菌改变了菌群多样性。

RESULTS

Dietary *C. butyricum* Promoted a More Significant Change of Bacteria Compositional Proportion in the Early Weaned Stage

Table 4. Relative Fecal Microbiota Abundances (%) of Significantly Different Common Bacteria on Days 14 and 28 after *C. butyricum* Supplementation^a

time	classification levels of bacteria				control	<i>C. butyricum</i>	SEM	P-value		
	phylum	order	family	genus						
day 14	Firmicutes	Clostridiales	Lachnospiraceae	<i>Lachnospiraceae_FCS020_group</i>	0.333	0.117	0.001	<0.05		
				<i>Lachnospiraceae_AC2044_group</i>	1.570	0.004	0.008	<0.05		
			Ruminococcaceae	<i>Ruminiclostridium_6</i>	0.204	0.021	0.001	<0.05		
				<i>Ruminococcaceae_UCG-003</i>	0.007	0.033	0.000	<0.05		
				Family_XIII	<i>Eubacterium_nodatum_group</i>	0.109	0.056	0.022	<0.05	
day 28	Firmicutes	Clostridiales	Lachnospiraceae	<i>Christensenellaceae_R-7_group</i>	0.496	0.135	0.009	<0.01		
				<i>Megasphaera</i>	3.790	11.310	0.000	<0.05		
			Selenomonadales	Veillonellaceae	<i>Eubacterium_hallii_group</i>	0.00127	0.00246	0.000	<0.05	
					<i>Incertae_Sedis</i>	0.00005	0.00033	0.000	<0.05	
					<i>Oribacterium</i>	0.00343	0.00658	0.002	<0.05	
Bacteroidetes	Bacteroidales	Lachnospiraceae	<i>Lachnospiraceae_AC2044_group</i>	0.01271	0.00006	0.000	<0.05			
			<i>Eisenbergiella</i>	0.00025	0.00004	0.010	<0.05			
			<i>Coprococcus_1</i>	0.00616	0.00120	0.001	<0.01			
			<i>Selenomonas_3</i>	0.02203	0.06282	0.000	<0.05			
			<i>Mitsuokella</i>	0.00039	0.00345	0.000	<0.05			
		Acidaminococcaceae	<i>Acidaminococcus</i>	0.00391	0.01213	0.000	<0.05			
			Lactobacillales	Lactobacillaceae	<i>Lactobacillus</i>	0.18347	0.07682	0.000	<0.05	
		Erysipelotrichales			Erysipelotrichaceae	<i>Solobacterium</i>	0.00116	0.00263	0.001	<0.01
						Proteobacteria	Burkholderiales	Oxalobacteraceae	<i>Prevotellaceae_NK3B31_group</i>	0.02795
		<i>Prevotella_7</i>	0.00008	0.00218	0.000				<0.05	
Bacteroidaceae	<i>Bacteroides</i>	0.00025	0.00001	0.013	<0.05					
Actinobacteria	Coriobacteriales	Coriobacteriaceae	<i>Slackia</i>	0.00011	0.00050			0.005	<0.05	
			<i>Collinsella</i>	0.00109	0.00200			0.000	<0.05	
<i>Oxalobacter</i>	0.00013	0.00049	0.030	<0.01						

^aOnly those genera that are significantly different between the control and *C. butyricum* group are shown. Values are means ($n = 8$).

RESULTS

Table 5. Relative Fecal Microbiota Abundances (%) of Significantly Different Bacteria on Days 14 and 28 after *C. butyricum* Supplementation^a

time	classification levels of bacteria				control	<i>C. butyricum</i>	SEM	P-value	
	phylum	order	family	genus					
day 14	Firmicutes	Selenomonadales	Veillonellaceae	<i>Veillonella</i>	0.000	1.812	0.001	<0.05	
				<i>Dialister</i>	0.153	0.000	0.001	<0.01	
day 28	Proteobacteria	Burkholderiales	Alcaligenaceae	<i>Sutterella</i>	0.000	0.007	0.000	<0.01	
	Firmicutes	Clostridiales	Lachnospiraceae	<i>Tyzzerella</i>	0.00007	0.00000	0.000	<0.01	
				<i>Noviherbaspirillum</i>	0.00005	0.00000	0.000	<0.05	
	Proteobacteria	Burkholderiales	Campylobacterales	Helicobacteraceae	<i>Helicobacter</i>	0.00004	0.00000	0.001	<0.01
				Mycoplasmatales	Mycoplasmataceae	<i>Mycoplasma</i>	0.00000	0.00003	0.001
Tenericutes	Anaeroplasmatales	Anaeroplasmataceae	<i>Anaeroplasma</i>	0.00008	0.00000	0.000	<0.01		

^aOnly those genera that are significantly different between the control and *C. butyricum* group are shown. Values are means ($n = 8$).

Dietary *C. butyricum* Eliminated the Presence of Unbeneficial Bacteria in the Intestine

RESULTS

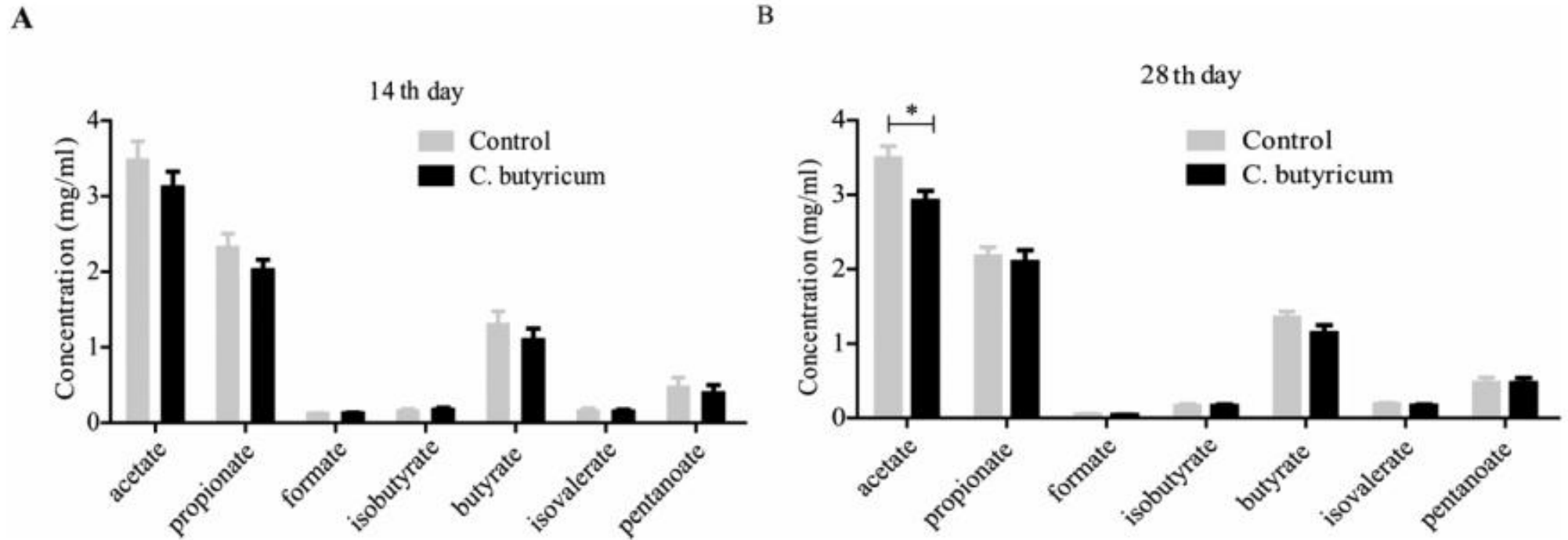


Figure 4. Effect of dietary *C. butyricum* on fecal SCFA concentrations of weaned piglets on days 14 and 28. Each bar represents means \pm SEM ($n = 8$). * $P < 0.05$. Fecal SCFA concentrations of weaned piglets were analyzed on days 14 (A) and 28 (B).

Dietary *C. butyricum* Decreased the Concentrations of Fecal Acetate

PART 4

DISCUSSION

1. 饲料中丁酸梭菌对微生物的影响可能主要集中在特定水平的细菌，如属水平变化最为显著 *Clostridiales*, *Selenomonadales*, *Bacteroidales*, *Lactobacillales* 和 *Coriobacteriales*。

2. 有报道称，*Megasphaera* 是种重要的改善肠胃健康益生菌使用，可以通过将乳酸转化为乙酸盐 *C. butyricum* 处理后 *Megasphaera* 增多，意味着 *C. butyricum* 有助于产生更多的乙酸盐。

3. 丁酸梭菌对猪的有益作用是通过清除无益的细菌，如 *Dialister*，在肠道中发现的 *Helicobacter* 和 *Anaeroplasm* 以便得到更健康的肠道环境。

