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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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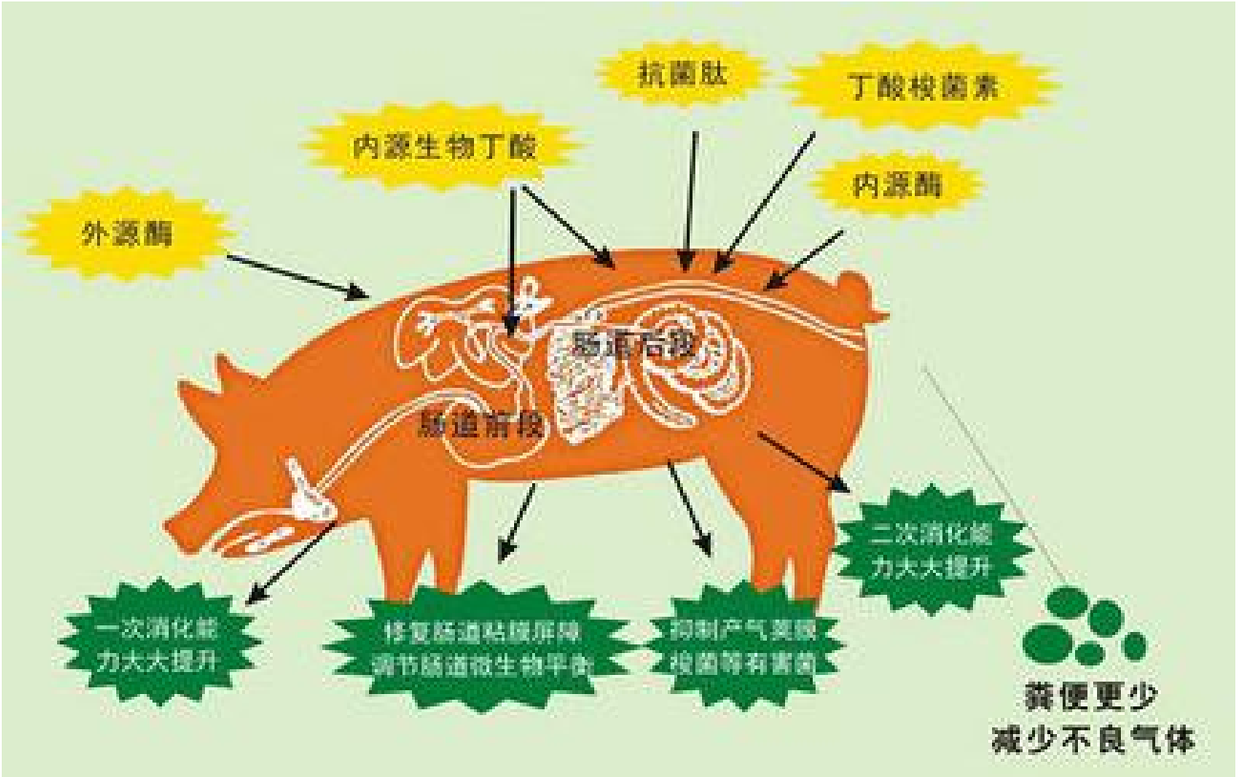
PART 1 INTRODUCTION

INTRODUCTION

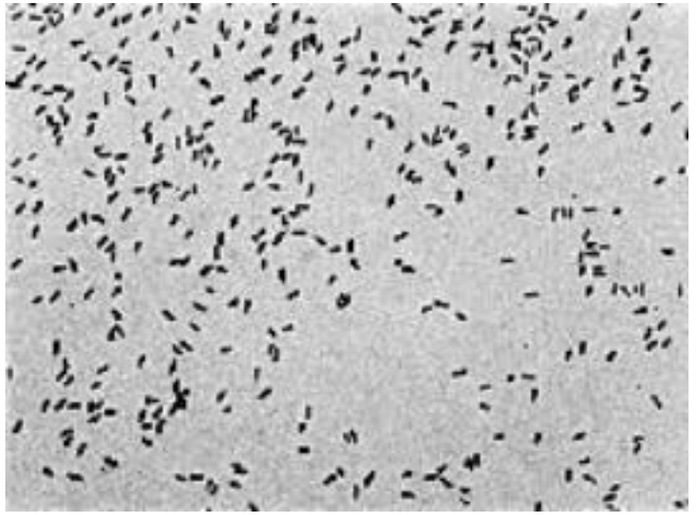
Weaned stress is often accompanied by intestinal dysbiosis with some abrupt changes in the gut microbiota composition of young animals (including infants), resulting in diarrhea, growth retardation, and even mortality.

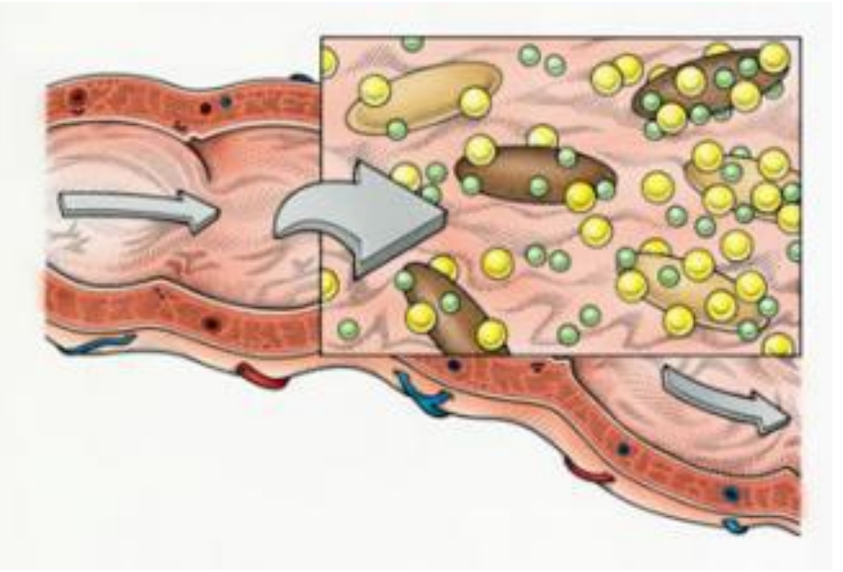
INTRODUCTION



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 (%)	*C. butyricum* (%)	nutrient level	control	*C. butyricum*
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			
C. butyricum	0.00	1.00			
total	100	101			

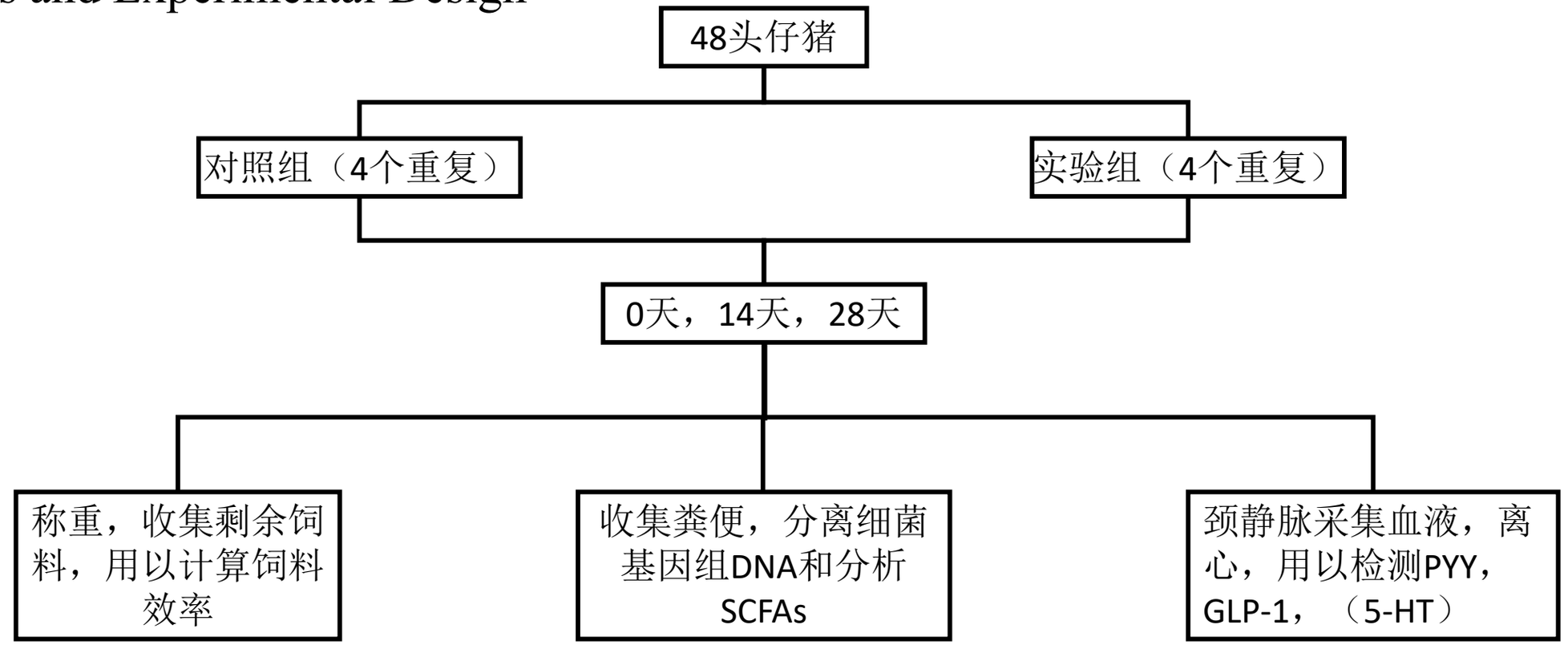
[a]Values of nutrient level in this table are calculated values. Abbreviations: *C. butyricum, Clostridium butyricum*; SID, standard ileal digestibility; DSM, dehulled soybean meal; EFFSB, extruded full fat soybean. [b]Premix contained the following per kg: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; vitamin B_{12}, 0.012 mg; riboflavin (VB_2), 4 mg; niacinamide (VB_3), 40 mg; D-calcium pantothenate (VB_5), 15 mg; choline chloride, 400 mg; folacin, 0.7 mg; vitamin B_1, 1.5 mg; vitamin B_6, 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.

Control group： Basic diet

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

MATERIALS AND METHODS

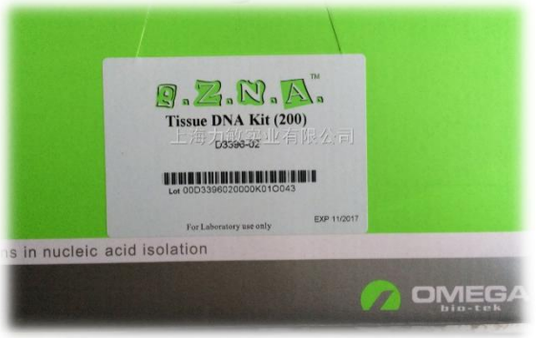
Animals and Experimental Design



MATERIALS AND METHODS

Flora analysis

Extraction of Fecal DNA → PCR Amplification → Illumina MiSeq Sequencing → Bioinformatics Analysis of Sequencing Data



MATERIALS AND METHODS

Detect

~ 0.5
distille
then ce
diluted
high-performance ion chromatograph (ICS 3000 Dionex, USA)

离子色谱法(IC)是利用离子交换原理,连续对共存的多种阴离子或阳离子进行分离、定性和定量的方法。分析阳离子时，分离柱填充低容量的阳离子交换树脂，用盐酸溶液做淋洗液。离子色谱能测定下列类型的离子:有机阴离子、碱金属、碱土金属、重金属、稀土离子和有机酸,以及胺和铵盐等。

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: control	growth performance: *C. butyricum*	*P*-value
	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

[a]Values 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	*C. butyricum*	*P*-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

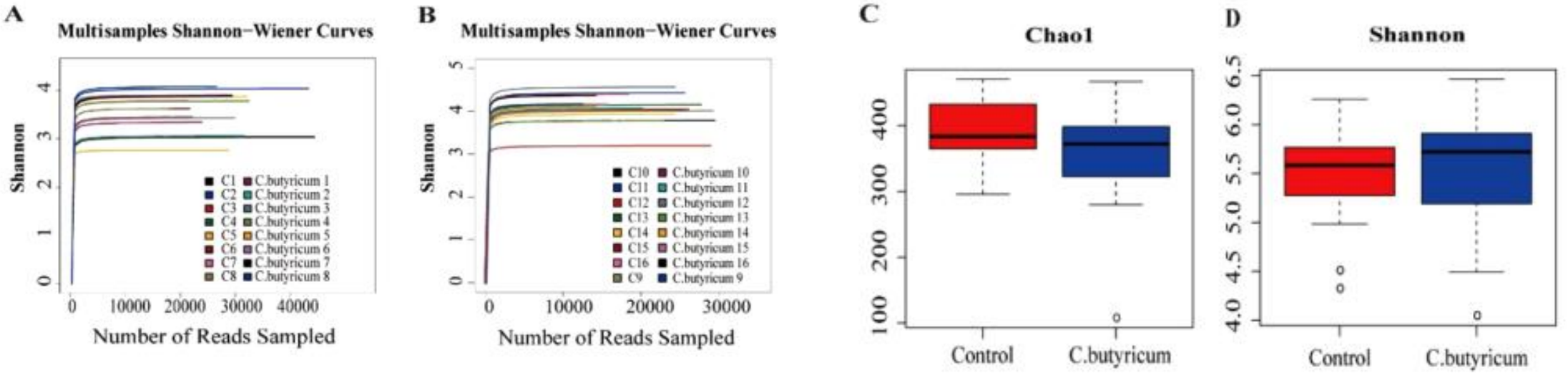
Dietary addition of C. butyricum had no effects on any of these hormones.

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

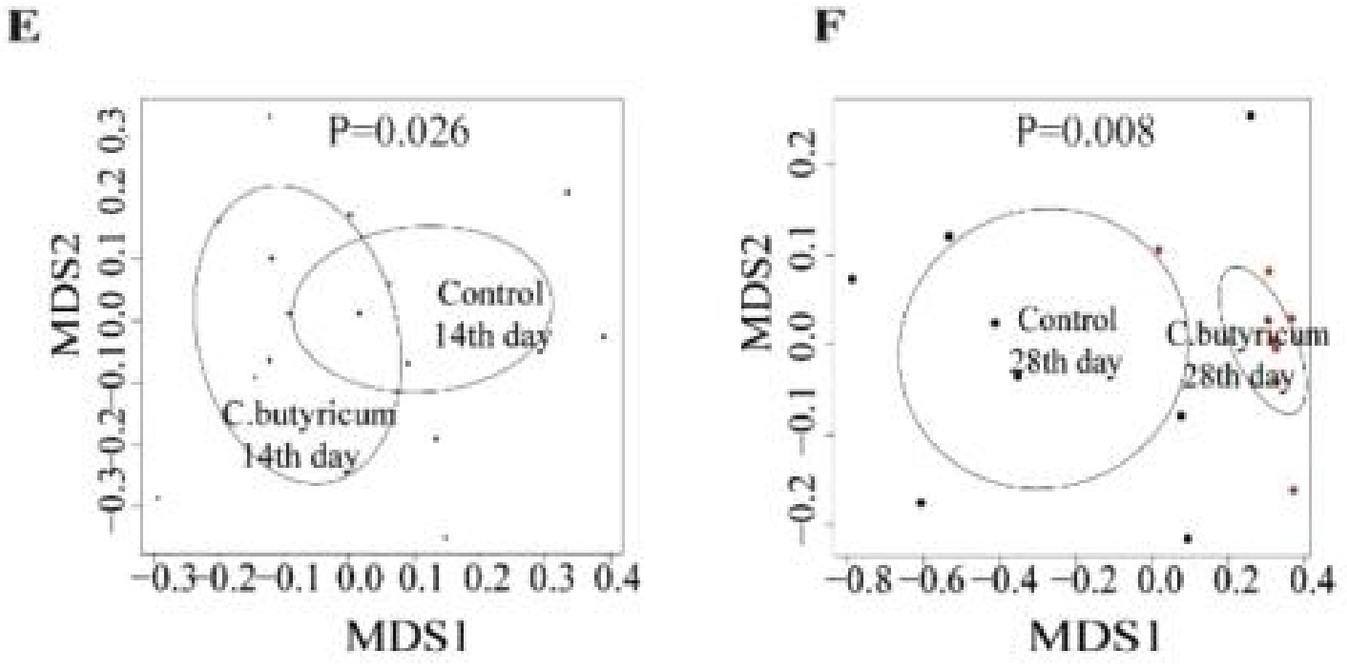
5-HT :一种测量肠应激的指标

Dietary C. butyricum Changed Fecal Microbiota Composition



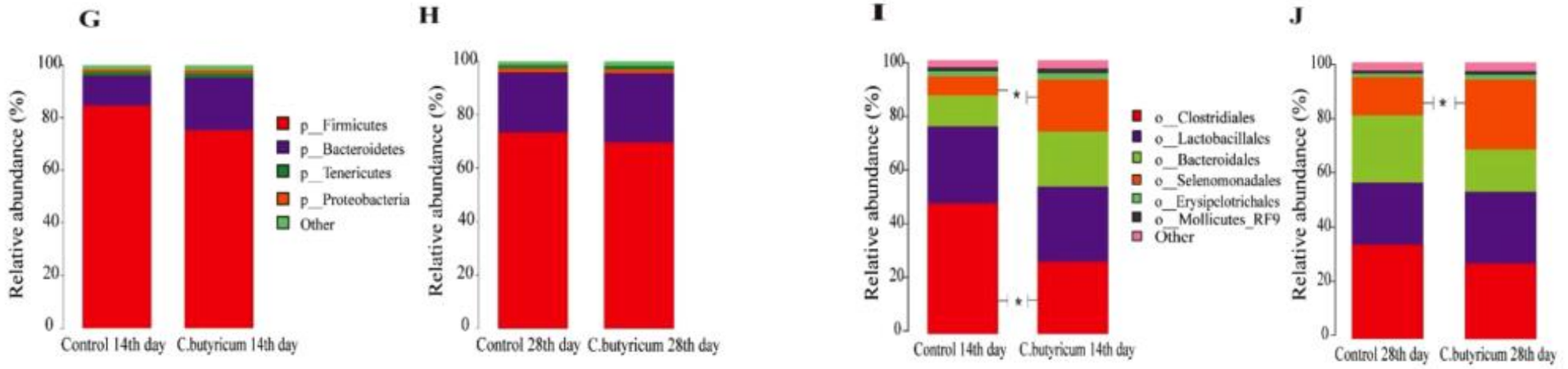
实验中，在第14天和第28天分别测得469,757和466,487条序列，图A、B表示样品拥有足够的微生物信息，图C、D表示相对于对照组，C.butyricum对细菌的α-多样性没有显着影响。

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)

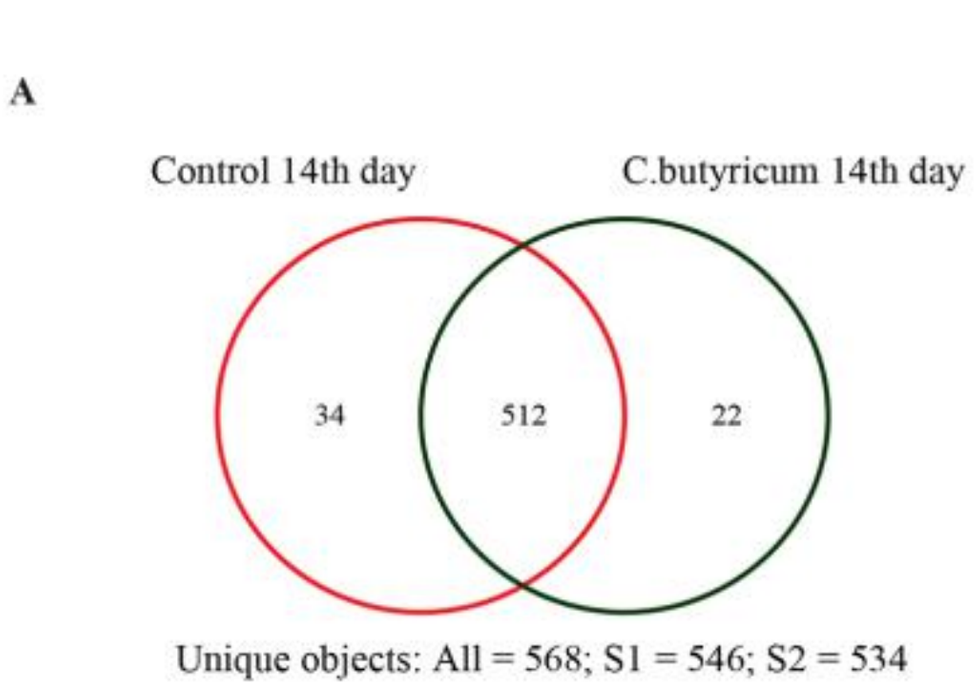
Dietary C. butyricum Changed Fecal Microbiota Composition

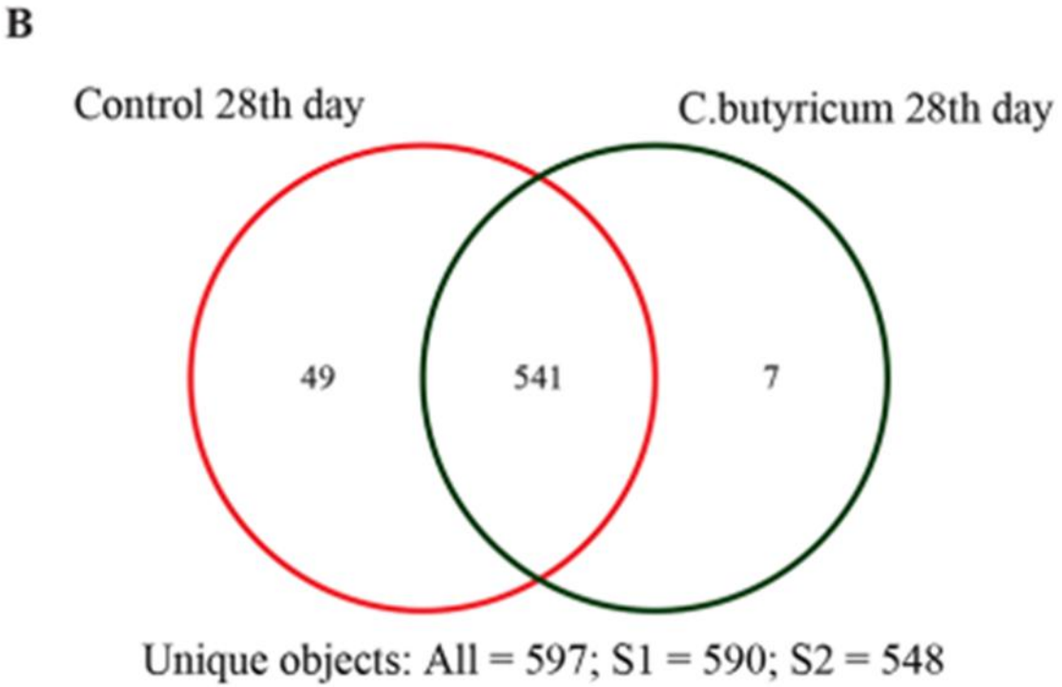


Firmicutes：厚壁菌门　　Bacteroidetes：拟杆菌门
Clostridiales：梭菌　　Lactobacillales：乳杆菌
Bacteroidales：拟杆菌属　　Selenomonadales

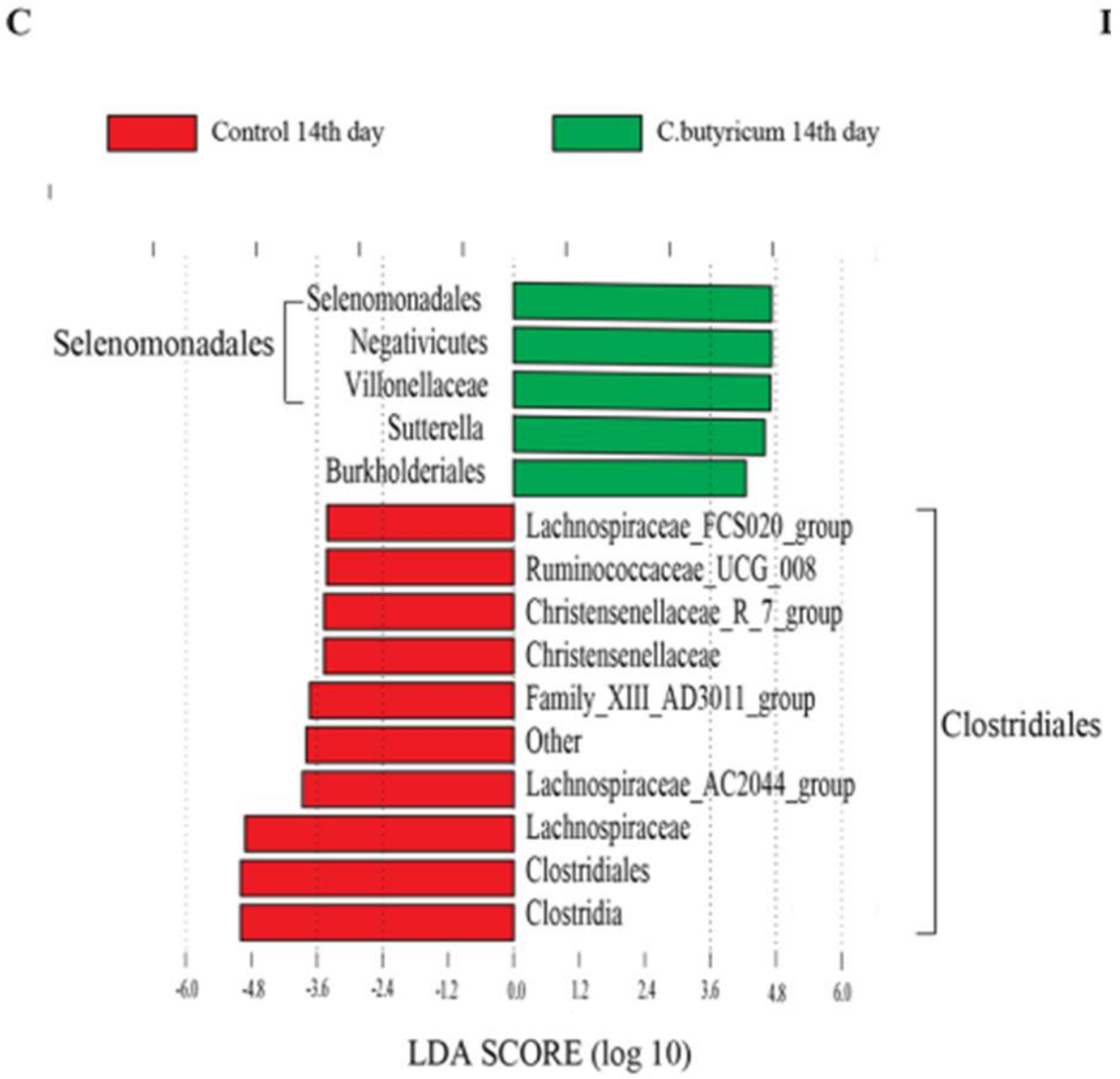
RESULTS

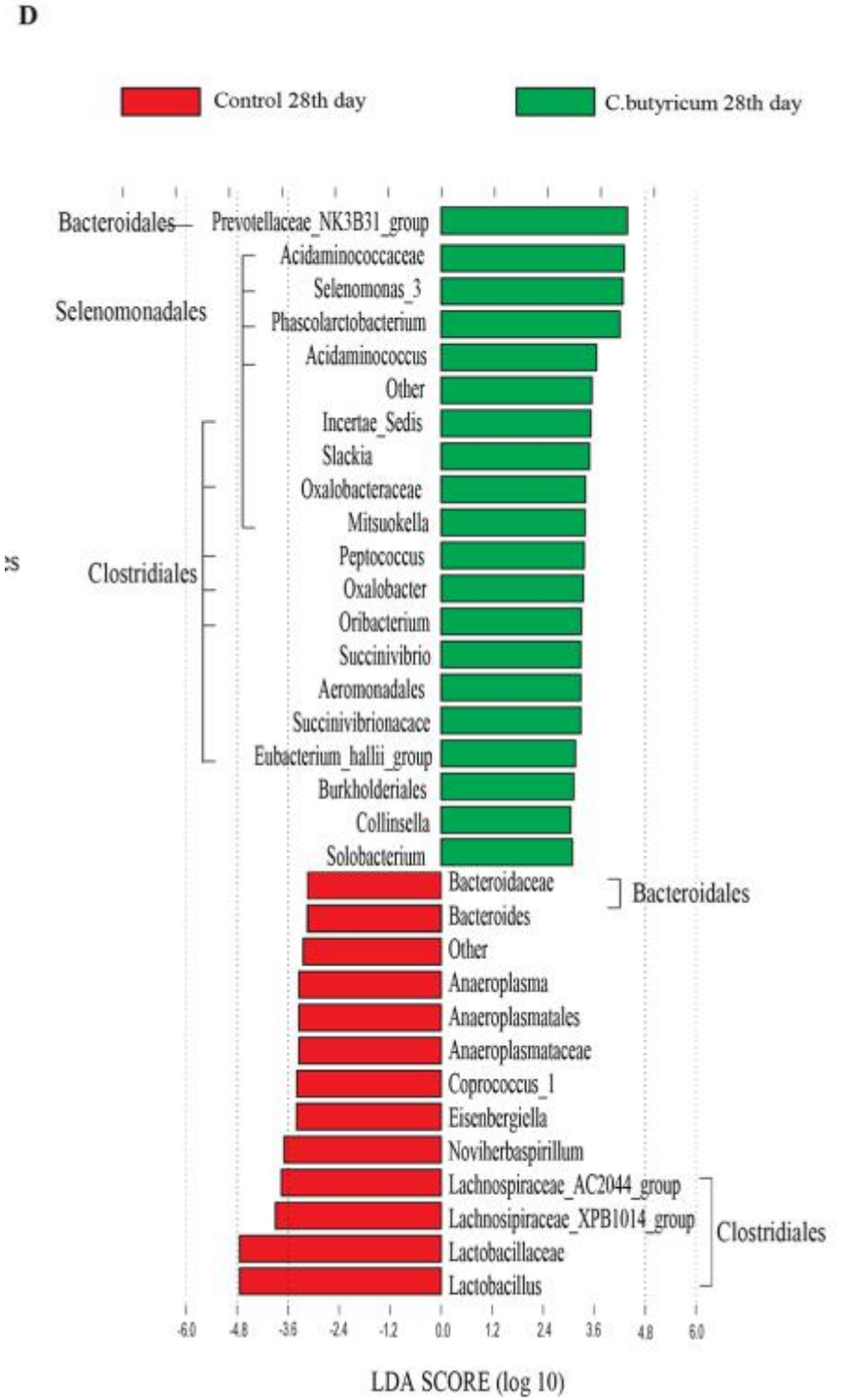
Dietary C. butyricum Increased the Proportion of Common Bacteria and the Number of Dominant Bacteria





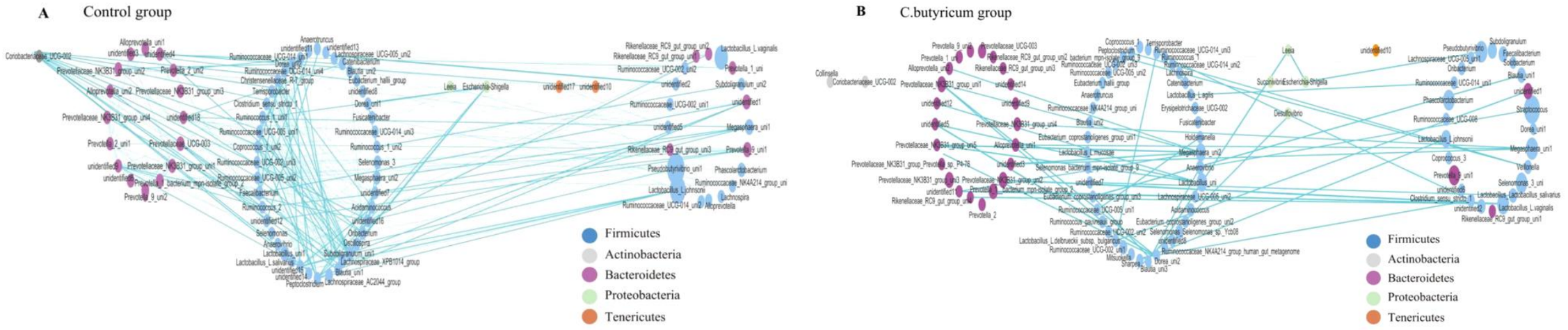
RESULTS


C
Control 14th day
C.butyricum 14th day
Selenomonadales
Selenomonadales
Negativicutes
Villonellaceae
Sutterella
Burkholderiales
Lachnospiraceae_FCS020_group
Ruminococcaceae_UCG_008
Christensenellaceae_R_7_group
Christensenellaceae
Family_XIII_AD3011_group
Other
Lachnospiraceae_AC2044_group
Lachnospiraceae
Clostridiales
Clostridia
Clostridiales
-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0
LDA SCORE (log 10)


D
Control 28th day
C.butyricum 28th day
Bacteroidales
Prevotellaceae_NK3B31_group
Selenomonadales
Acidaminococcaceae
Selenomonas_3
Phascolarctobacterium
Acidaminococcus
Other
Incertae_Sedis
Slackia
Oxalobacteraceae
Mitsuokella
Clostridiales
Peptococcus
Oxalobacter
Oribacterium
Succinivibrio
Aeromonadales
Succinivibrionacace
Eubacterium_hallii_group
Burkholderiales
Collinsella
Solobacterium
Bacteroidaceae
Bacteroidales
Bacteroides
Other
Anaeroplasma
Anaeroplasmatales
Anaeroplasmataceae
Coprococcus_1
Eisenbergiella
Noviherbaspirillum
Lachnospiraceae_AC2044_group
Lachnosipiraceae_XPB1014_group
Clostridiales
Lactobacillaceae
Lactobacillus
-6.0 -4.8 -3.6 -2.4 -1.2 0.0 1.2 2.4 3.6 4.8 6.0
LDA SCORE (log 10)

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]

	classification levels of bacteria							
time	phylum	order	family	genus	control	*C. butyricum*	SEM	*P*-value
day 14	Firmicutes	Clostridiales	Lachnospiraceae	*Lachnospiraceae_FCS020_group*	0.333	0.117	0.001	<0.05
				Lachnospiraceae_AC2044_group	1.570	0.004	0.008	<0.05
			Ruminococcaceae	*Ruminiclostridium_6*	0.204	0.021	0.001	<0.05
				Ruminococcaceae_UCG-003	0.007	0.033	0.000	<0.05
			Family_XIII	*Eubacterium_nodatum_group*	0.109	0.056	0.022	<0.05
			Christensenellaceae	*Christensenellaceae_R-7_group*	0.496	0.135	0.009	<0.01
		Selenomonadales	Veillonellaceae	*Megasphaera*	3.790	11.310	0.000	<0.05
day 28	Firmicutes	Clostridiales	Lachnospiraceae	*Eubacterium_hallii_group*	0.00127	0.00246	0.000	<0.05
				Incertae_Sedis	0.00005	0.00033	0.000	<0.05
				Oribacterium	0.00343	0.00658	0.002	<0.05
				Lachnospiraceae_AC2044_group	0.01271	0.00006	0.000	<0.05
				Eisenbergiella	0.00025	0.00004	0.010	<0.05
				Coprococcus_1	0.00616	0.00120	0.001	<0.01
		Selenomonadales	Veillonellaceae	*Selenomonas_3*	0.02203	0.06282	0.000	<0.05
				Mitsuokella	0.00039	0.00345	0.000	<0.05
			Acidaminococcaceae	*Acidaminococcus*	0.00391	0.01213	0.000	<0.05
		Lactobacillales	Lactobacillaceae	*Lactobacillus*	0.18347	0.07682	0.000	<0.05
		Erysipelotrichales	Erysipelotrichaceae	*Solobacterium*	0.00116	0.00263	0.001	<0.01
	Bacteroidetes	Bacteroidales	Prevotellaceae	*Prevotellaceae_NK3B31_group*	0.02795	0.07623	0.000	<0.05
				Prevotella_7	0.00008	0.00218	0.000	<0.05
			Bacteroidaceae	*Bacteroides*	0.00025	0.00001	0.013	<0.05
	Actinobacteria	Coriobacteriales	Coriobacteriaceae	*Slackia*	0.00011	0.00050	0.005	<0.05
				Collinsella	0.00109	0.00200	0.000	<0.05
	Proteobacteria	Burkholderiales	Oxalobacteraceae	*Oxalobacter*	0.00013	0.00049	0.030	<0.01

[a]Only 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]

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

[a]Only 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

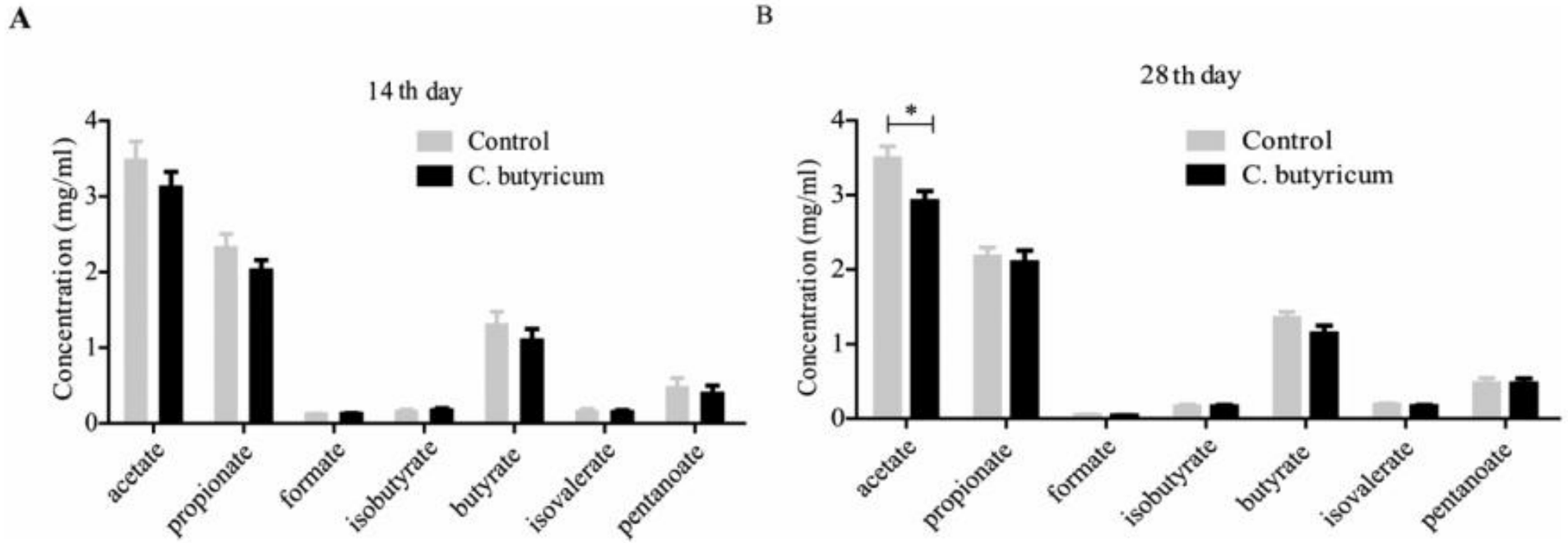


Figure 4. Effect of dietary *C. butyricum* on fecal SCFA concentrations of weaned piglets on days 14 and 28. Each bar represents means ± SEM (n = 8). *P < 0.05. Fecal SCFA concentrations of weaned piglets were analyzed on days 14 (A) and 28 (B).

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

PART 4 DISCUSSION

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着*Clostridiales*，*Selenomonadales*，*Bacteroidales*，*Lactobacillales*和*Coriobacteriales*。

2.有报道称，*Megasphaera*是种重要的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*Anaeroplasma*以便得到更健康的肠道环境。

请各位老师同学批评指正