



读书报告

READING REPORT

朱振祥

2018.5.12

河南师范大学水产学院

Topic：

British Journal of Nutrition (2014), **112**, 15–29
doi:10.1017/S0007114514000610

Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil

IF=3.706

Wenshu Liu[1]†, Yanou Yang[2]†, Jianli Zhang[2], Delbert M. Gatlin[3]*, Einar Ringø[4] and Zhigang Zhou[1]*

[1]*Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12 Zhongguancun South Street, Beijing 100081, People's Republic of China*
[2]*Science of Animal and Technology College, Anhui Agricultural University, Hefei 230036, People's Republic of China*
[3]*Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX 77843-2258, USA*
[4]*Norwegian College of Fishery Science, Faculty of Biosciences, Fisheries and Economics, University of Tromsø,*

Contents

河南师范大学水产学院

01

Background

2018&读书报告

2018 and reading report

antibiotics

既有有利的一面，又有不好的一面。最终欧盟规定禁止抗生素作为促生长物质用于动物饲料中。

t.163.com/inandu

2018&读书报告

2018 and reading report

lipids oxidise

01

由于脂质含量高，即使添加抗氧化剂，也会有很多在储存中被氧化。

02

脂质氧化过程中的产物会降低饲料的营养价值。

03

脂质氧化产物会对机体细胞膜产生破坏作用。

2018&读书报告

2018 and reading report

Short chain fatty acids

短链脂肪酸（SCFAs）包括乙酸、丙酸、丁酸等。丁酸已被证明可以为肠道上皮细胞的生长提供能量并能够强化肠道防御屏障和减少部分氧化应激反应。基于这些发现，丁酸和丁酸盐可能会对肠道产生积极的影响。

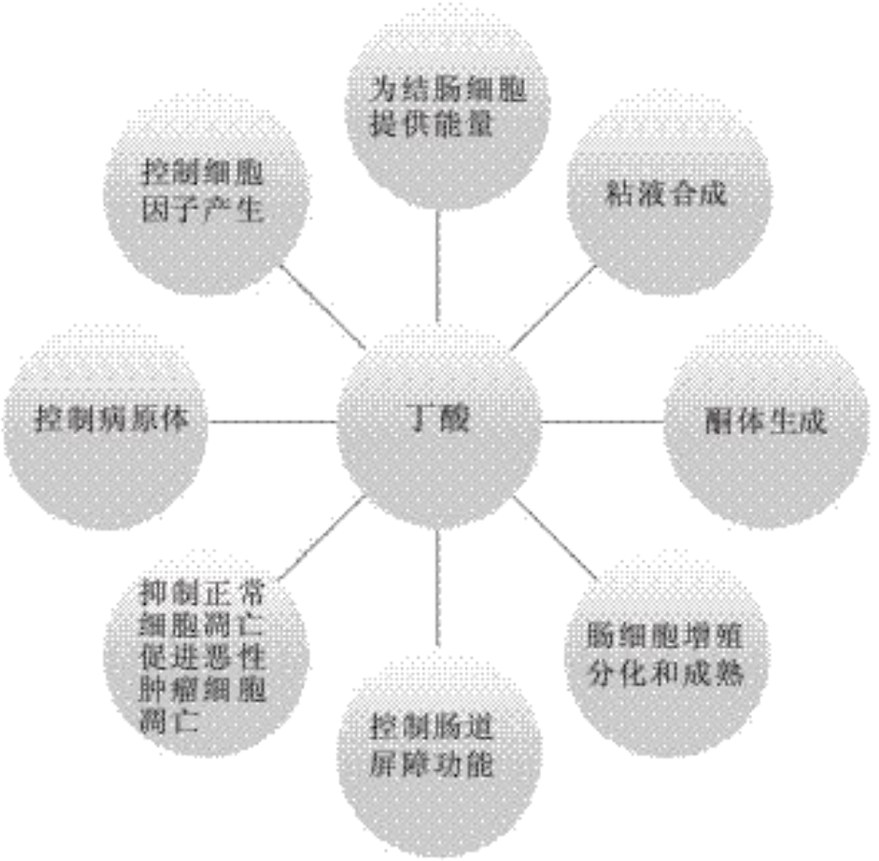
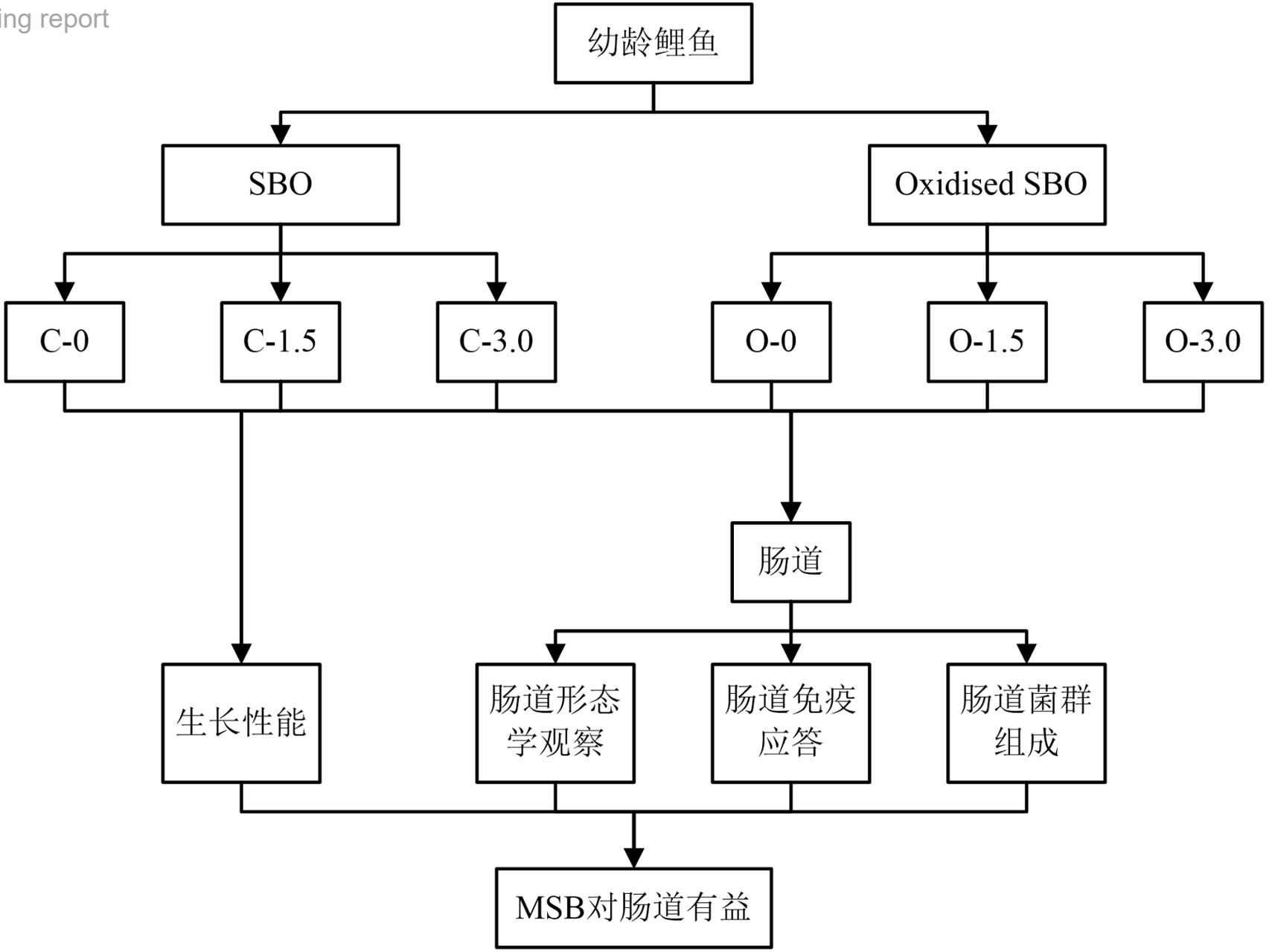


图 1　丁酸在肠道中的多种功效(局部)

2018&读书报告

2018 and reading report



河南师范大学水产学院

02

Materials and methods

饲料制备：

油脂氧化：在大豆油中加入铁离子（30mg / kg），铜离子（15mg / kg）和过氧化氢（600mg / kg），37℃下搅拌48小时。然后测定过氧化值。

前2周，其他成分均过200目筛网后与大豆油混合，加水制粒。室内风干24小时，收集储存于-20℃备用。

在随后的8周中，MSB作为饲料添加剂以300mg / kg的剂量进行饲料制备。

2018&读书报告

2018 and reading report

饲料配方

Ingredients	Basal diet	Oxidised SBO diet	C-0	C-1·5	C-3·0	Oxi-0	Oxi-1·5	Oxi-3·0
Fishmeal	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0
Soyabean meal	32·0	32·0	32·0	32·0	32·0	32·0	32·0	32·0
Rapeseed meal	7·25	7·25	7·25	7·25	7·25	7·25	7·25	7·25
Maize gluten meal	2·92	2·92	2·92	2·92	2·92	2·92	2·92	2·92
Cottonseed meal	8·00	8·00	8·00	8·00	8·00	8·00	8·00	8·00
Coated lysine	0·87	0·87	0·87	0·87	0·87	0·87	0·87	0·87
Coated methionine	0·26	0·26	0·26	0·26	0·26	0·26	0·26	0·26
Wheat flour	24·9	24·9	24·9	24·9	24·9	24·9	24·9	24·9
Soyabean oil	6·00	4·00	4·00	4·00	4·00	4·00	4·00	4·00
Oxidised SBO	0·00	2·00	0·00	0·00	0·00	0·00	0·00	0·00
Vitamin C phosphate ester	0·10	0·10	0·10	0·10	0·10	0·10	0·10	0·10
Vitamin premix 1	0·50	0·50	0·50	0·50	0·50	0·50	0·50	0·50
Mineral premix 2	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
$Ca(H_2PO_4)_2$	2·20	2·20	2·20	2·20	2·20	2·20	2·20	2·20
Zeolite powder	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00
Starch	2·00	2·00	2·00	2·00	2·00	2·00	2·00	2·00
Moisture	10·2	10·2	10·2	10·2	10·3	10·2	10·1	10·3
Chemical composition (DM basis)								
Crude	35·6	35·5	35·6	35·7	35·4	35·4	35·7	35·8
Crude lipid	6·07	6·09	6·07	6·12	6·01	6·10	5·96	5·92
Ash	10·2	10·2	10·2	10·2	10·1	10·1	10·2	10·3
Crude fibre	3·50	3·47	3·50	3·60	3·53	3·61	3·45	3·68
N-free extract	34·4	34·5	34·4	34·2	34·7	34·6	34·6	34·0

SBO, soyabean oil.

* See Table 2 for details of the treatments C-0, C-1·5, C-3·0, Oxi-0, Oxi-1·5 and Oxi-3·0.

2018&读书报告

2018 and reading report

丁酸钠微囊（MSB）溶解率

将1g的MSB室温下溶解于200ml水中，然后分别15，30，45，60，90，120，150和180分钟时取200微升使用高效液相色谱进行检测。

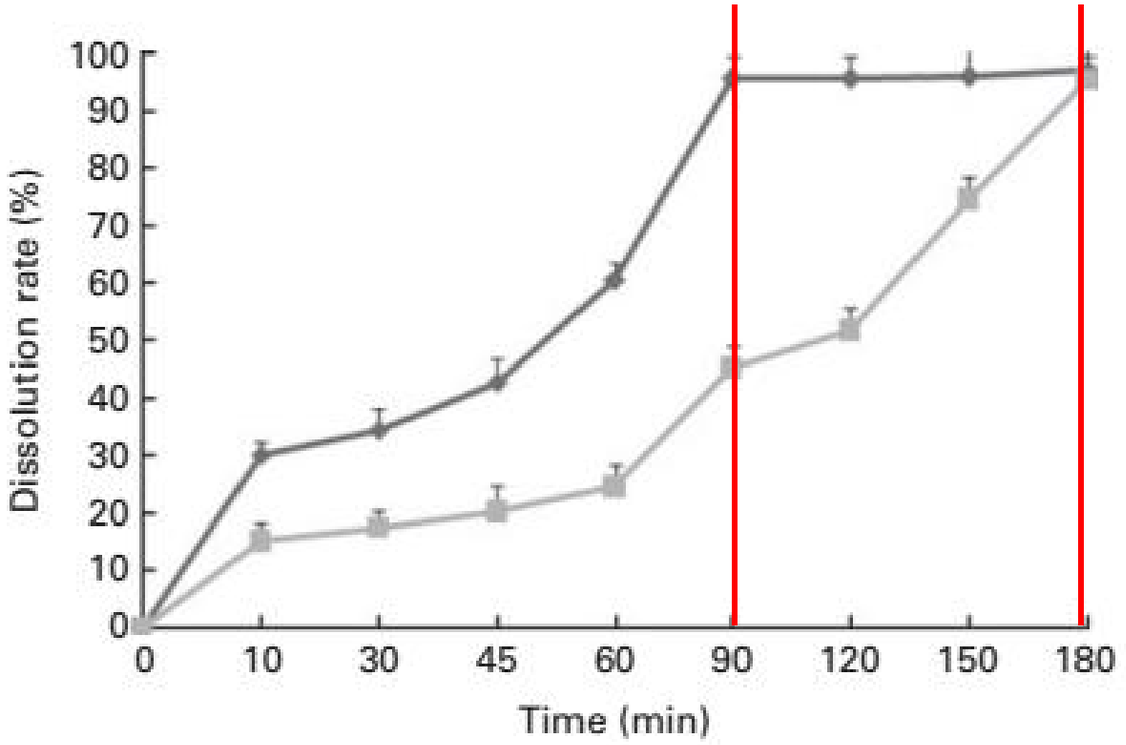


Fig. 1. Dissolution rates of the two types of sustained-release micro-encapsulated sodium butyrate products (1·5 h (◆) and 3·0 h (■) sustained release).

2018&读书报告

2018 and reading report

养殖管理

实验鱼：鲤鱼

（6.22±0.03g）

Table 2. Experimental treatments as well as feeding regimens

	Feeding regimen	
Treatment	Baseline to week 2	Week 3 to week 10
C-0	Basal diet	Basal diet
C-1·5	Basal diet	Basal diet containing MSB1·5 (300 mg/kg)
C-3·0	Basal diet	Basal diet containing MSB3·0 (300 mg/kg)
Oxi-0	Oxidised soyabean oil supplement	Basal diet
Oxi-1·5	Oxidised soyabean oil supplement	Basal diet containing MSB1·5 (300 mg/kg)
Oxi-3·0	Oxidised soyabean oil supplement	Basal diet containing MSB3·0 (300 mg/kg)

MSB, microencapsulated sodium butyrate.

实验分为6组：C-0，C-1.5，C-3.0，Oxi-0，Oxi-1.5，Oxi-3.0，每组4个重复，每个重复20尾鱼。每天两次投喂，投喂量为体重3%，每周调整一次。

2018&读书报告

2018 and reading report

生长性能

养殖试验结束时，每个处理组随机选取14条鱼进行麻醉，称重解剖，取出肠和肝脏用PBS冲洗，将肠道分为前肠、中肠、后肠并单独称重。

$$\mathrm{SR}\,(\%) = (\text{no. of fish counted}/\text{no. of stocked fish}) \times 100,$$

$$\mathrm{WG}\,(\%) = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100,$$

$$\mathrm{SGR}\,(\%/\mathrm{d}) = \frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)}}{\text{days}} \times 100,$$

$$\begin{aligned}\mathrm{FCR} = {} & (\text{total feed consumption (total feed casting} \\ & - \text{total food residue) (g))}/(\text{total final weight (g)} \\ & - \text{total initial weight (g)} + \text{total mortality weight (g)}) \\ & \times 100,\end{aligned}$$

$$\begin{aligned}\mathrm{BI} = {} & (\text{body weight (g)})/(\text{body length (cm)} \times \text{body height (cm)} \\ & \times \text{body thickness (cm)} \times 1000),\end{aligned}$$

$$\mathrm{HSI} = \frac{\text{hepatopancreas weight (g)}}{\text{body weight (g)}} \times 100,$$

$$\mathrm{WII} = \frac{\text{intestinal weight (g)}}{\text{body weight (g)}} \times 100.$$

2018&读书报告

2018 and reading report

肠道形态学检测

每组取3条鱼，取其前、中、后肠，用2.5%戊二醛固定，磷酸盐缓冲液洗涤两次，然后经过一系列酒精脱水步骤后，在扫描电子显微镜下观察肠粘膜的外观及微绒毛形态并测量肠上皮细胞的微绒毛密度。

2018&读书报告

2018 and reading report

肠道免疫反应

每组三尾鱼，分别测定前中后肠。三步法进行Q-PCR。

Table 3. Sequences of oligonucleotide primers for quantitative RT-PCR

Target genes	Primers	Oligonucleotide (5′–3′)	Reference (NCBI accession no.)
β-Actin	Forward	ATCCGTAAAGACCTGTATGCCA	JQ619775[32]
	Reverse	GGGGAGCAATGATCTTGATCTTCA	
EF-1a	Forward	GTCAAGTCCGTTGAGATGCACC	JQ619777[32]
	Reverse	GGATGATGACCTGAGCATTGAAGC	
18S	Forward	GAGTATGGTTGCAAAGCTGAAAC	JQ619778[32]
	Reverse	AATCTGTCAATCCTTTCCGTGTCC	
IL-1β	Forward	CGACTCTGATGAACTGGACTG	JF957368
	Reverse	CCTCAAGTGTGAAGTTTGTGG	
HSP70	Forward	AAACAGACCCAGACCTTCAC	JF957366
	Reverse	GTTTAGTGATGAGTGGGTTGC	
TGF-β	Forward	GTCACGCTACCTGGAATCAC	JF957371
	Reverse	CCACATAGTAAAAGATGGGCAG	
TNF-α	Forward	TGAAGAAGGAGGATTGCTGC	JF957372
	Reverse	CGAGATAAATCGTGTTGTACCAC	

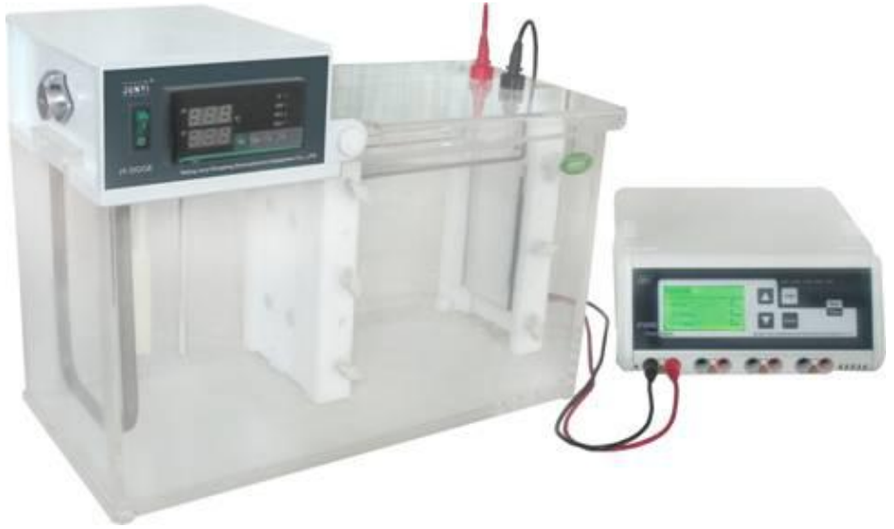
EF-1a, elongation factor 1a; *HSP70*, heat shock protein 70; *TGF-β*, transforming growth factor-β.

2018&读书报告

2018 and reading report

肠道菌落检测

每组3尾鱼，取前中后肠，并将相同组别组织进行混样。以338-GC-f (5’-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3’) 和 519r (5’-ATTACCGCGGCTGCTGG-3’)为引物进行扩增（V3区），随后进行DGGE。



DGGE(变性梯度凝胶电泳)是根据DNA在不同浓度的变性剂中解链行为的不同而导致电泳迁移率发生变化，从而将片段大小相同而碱基组成不同的DNA片段分开。具体而言，就是将特定的双链DNA片段在含有从低到高的线性变性剂梯度的聚丙烯酰胺凝胶中电泳，随着电泳的进行，DNA片段向高浓度变性剂方向迁移，当它到达其变性要求的最低浓度变性剂处，双链DNA形成部分解链状态，这就导致其迁移速率变慢，由于这种变性具有序列特异性，因此DGGE能将同样大小的DNA片段很理想地分开，它是一种很有用分子标记方法。

河南师范大学水产学院

03

Results

2018&读书报告

2018 and reading report

生长性能

Table 4. Effects on weight gain (WG, g), specific growth rate (SGR, %/d), feed conversion ratio (FCR), body index (BI, %), hepatosomatic index (HSI, %) and intestinal index of weight (WII, %) in carp at the end of the different experimental treatments (from week 3 to week 10)

	Control (h of MSB)			Oxidised SBO (h of MSB)					*P* (polynomial contrasts)*						
									Control		Oxidised SBO		h of MSB		
	0	1·5	3·0	0	1·5	3·0	SEM	Interaction	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Diet
IBW	9·40	10·3	9·82	9·63	10·17	9·47	0·13	0·610	–	–	–	–	0·654	0·127	0·794
FBW	22·8	29·1	23·3	18·6	24·5	24·7	1·06	0·367	–	–	–	–	0·169	0·082	0·355
WG	13·4	18·9	13·4	8·96	14·4	15·2	1·03	0·305	–	–	–	–	0·175	0·123	0·358
SGR	1·54	1·86	1·54	1·16	1·57	1·68	0·08	0·303	–	–	–	–	0·175	0·197	0·494
FCR	2·63	2·20	2·46	3·16	2·52	2·45	0·12	0·528	–	–	–	–	0·109	0·061	0·385
BI	0·47	0·49	0·50	0·50	0·49	0·51	0·01	0·672	–	–	–	–	0·300	0·311	0·607
his	1·43	1·21	1·55	1·64	1·49	1·52	0·07	0·507	–	–	–	–	0·908	0·609	0·175
WII	4·29	3·46	4·27	3·43	3·61	3·50	0·15	0·353	–	–	–	–	0·773	0·842	0·088

MSB, microencapsulated sodium butyrate; SBO, soyabean oil; IBW, initial body weight; FBW, final body weight.

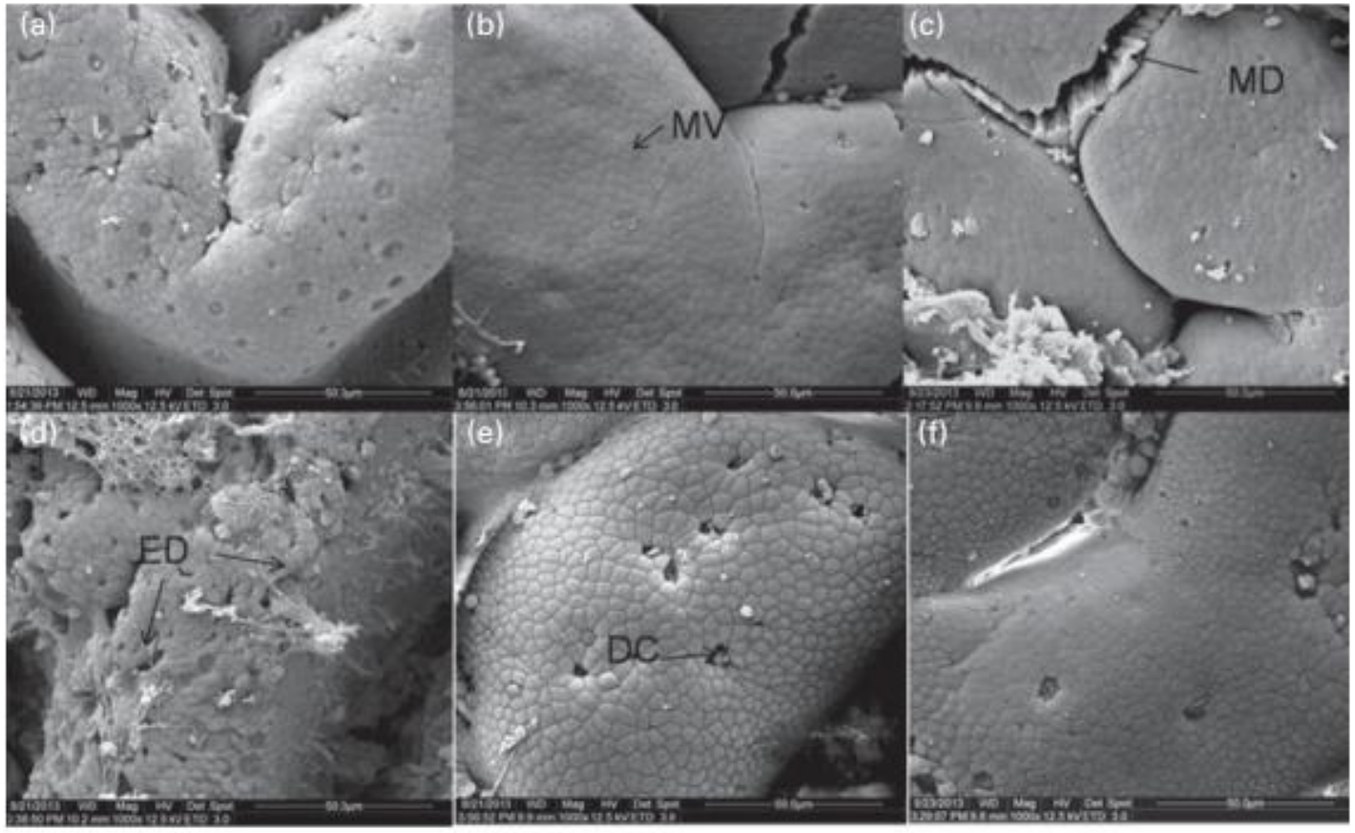
* The interaction term drives the other contrasts as follows: when the interaction is not significant, only the *P* values for linear and quadratic contrasts for pre-fed treatments (main-effect means averaged over the sustained-release time of MSB) and the *P* value for the sustained-release time of MSB are given. When the interaction is significant, only the *P* values for linear and quadratic contrasts for individual pre-fed treatments (control and oxidised SBO) are given.

结果表明：在对照组中，MSB1.5有益于机体的生长。另外，在氧化组，MSB1.5和MSB3.0余对照组相比也有益于生长。

2018&读书报告

2018 and reading report

肠道形态观察



前肠

MV：微绒毛；

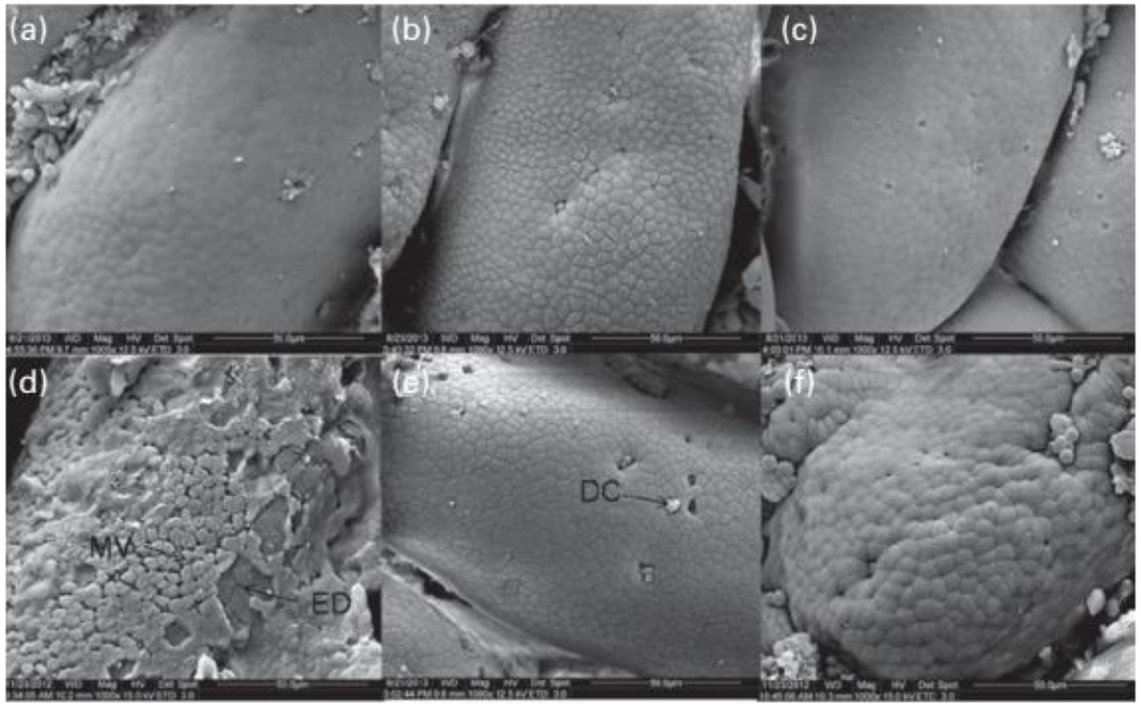
MD：机械损伤；

ED：实验性损伤；

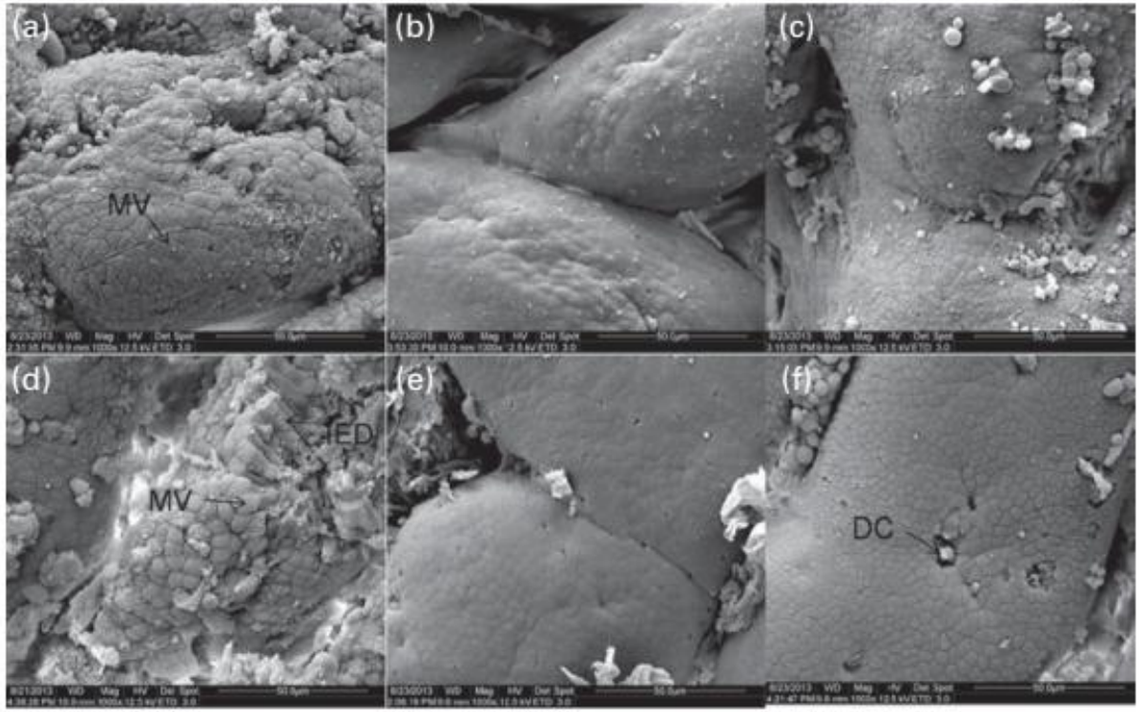
DC：死亡细胞。

2018&读书报告

2018 and reading report



中肠



后肠

与正常的肠上皮对比，MSB1·5和MSB3·0都有预防或修复由氧化油脂引起的肠道损伤的能力。

2018&读书报告

2018 and reading report

肠道绒毛长度

Table 5. Effects on the density of intestinal microvilli of carp at the end of the different experimental treatments

	Control (h of MSB)			Oxidised SBO (h of MSB)					*P* (polynomial contrasts)*						
									Control		Oxidised SBO		h of MSB		
	0	1·5	3·0	0	1·5	3·0	SEM	Interaction	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Diet
FG	97·7	102	95·3	84·3	107	107	2·06	<0·001	0·551	0·215	0·006	0·001	–	–	–
MG	123	121	122	51·3	101	85	6·43	0·423	–	–	–	–	0·314	0·325	<0·001
DG	72·7	78·7	77·3	57	117	69·3	4·59	0·242	–	–	–	–	0·467	0·003	0·618
SEM			3·00												
Interaction			<0·001												

MSB, microencapsulated sodium butyrate; SBO, soyabean oil; FG, foregut; MG, midgut; DG, distal gut.

* The interaction term drives the other contrasts as follows: when the interaction is not significant, only the *P* values for linear and quadratic contrasts for pre-fed treatments (main-effect means averaged over the sustained-release time of MSB) and the *P* value for the sustained-release time of MSB are given. When the interaction is significant, only the *P* values for linear and quadratic contrasts for individual pre-fed treatments (control and oxidised SBO) are given.

2018&读书报告

2018 and reading report

肠道免疫反应

Table 6. Effects on the relative expression levels of intestinal heat shock protein (HSP)-70 and cytokines in carp at the end of the different experimental treatments

		Control (h of MSB)			Oxidised SBO (h of MSB)					*P* (polynomial contrasts)*						
										Control		Oxidised SBO		h of MSB		
		0	1·5	3·0	0	1·5	3·0	SEM	Interaction	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Diet
HSP70	FG	1·01	1·28	6·79	1·80	0·95	10·1	0·87	0·010	0·004	0·001	0·008	<0·001	–	–	–
	MG	0·24	0·14	0·63	0·20	0·22	0·10	0·05	<0·001	0·033	0·002	0·112	0·092	–	–	–
	DG	2·51	1·46	0·63	0·88	0·42	0·45	0·21	0·106	–	–	–	–	0·018	0·062	0·018
	SEM			0·36												
	Interaction			<0·001												
IL-1β	FG	1·04	1·12	11·3	5·29	3·05	18·4	1·48	0·066	–	–	–	–	0·001	<0·001	0·289
	MG	0·88	0·89	1·03	3·21	0·69	7·33	0·68	0·030	0·658	0·054	0·178	0·046	–	–	–
	DG	0·35	0·72	0·15	0·91	0·11	0·39	0·07	<0·001	0·378	0·010	0·085	0·001	–	–	–
	SEM			0·61												
	Interaction			<0·001												
TGF-β	FG	1·12	1·71	7·90	6·42	6·14	1·86	0·73	<0·001	0·011	0·012	0·006	0·003	–	–	–
	MG	0·53	0·87	4·10	1·39	0·44	0·27	0·41	0·009	0·021	0·030	0·132	0·306	–	–	–
	DG	1·32	5·55	1·23	1·19	0·28	0·51	0·52	0·011	0·846	0·009	0·293	0·392	–	–	–
	SEM			0·38												
	Interaction			<0·001												
TNF-α	FG	1·08	0·40	9·04	12·3	1·70	0·30	1·35	0·002	0·063	0·064	0·007	0·007	–	–	–
	MG	3·51	1·90	12·26	3·32	0·41	0·83	1·02	<0·001	0·027	0·003	0·028	0·004	–	–	–
	DG	1·83	8·13	4·18	36·0	0·91	12·9	2·96	<0·001	0·344	<0·001	0·067	<0·001	–	–	–
	SEM			1·20												
	Interaction			<0·001												

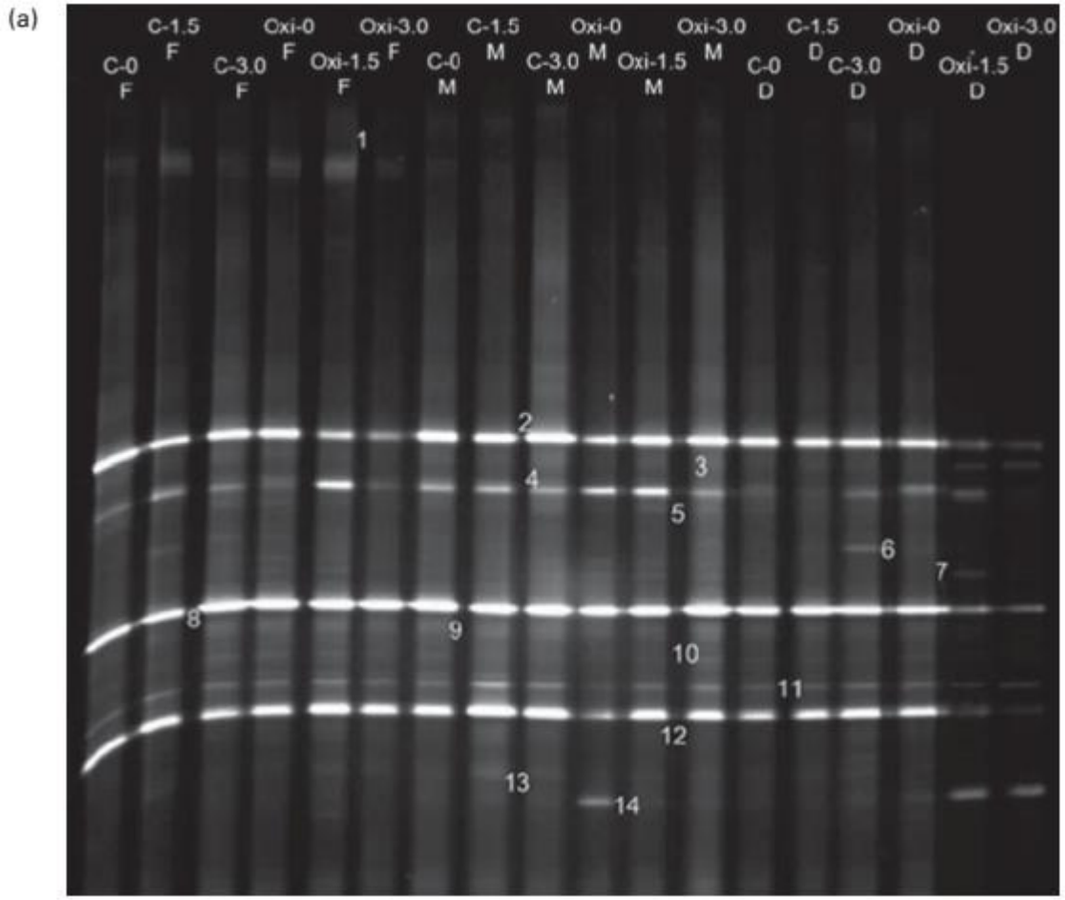
MSB, microencapsulated sodium butyrate; SBO, soyabean; FG, foregut; MG, midgut; DG, distal gut; *TGF-β*, transforming growth factor-β.

* The interaction term drives the other contrasts as follows: when the interaction is not significant, only the *P* values for linear and quadratic contrasts for pre-fed treatments (main-effect means averaged over the sustained-release time of MSB) and the *P* value for the sustained-release time of MSB are given. When the interaction is significant, only the *P* values for linear and quadratic contrasts for individual pre-fed treatments (control and oxidised SBO) are given.

2018&读书报告

2018 and reading report

肠道菌落分析



变性梯度凝胶电泳结果显示：共计扩增出14种菌落。

2018&读书报告

2018 and reading report

Table 7. Representatives of intestinal adhesive bacterial communities and their relative abundance (%) obtained from the BLAST (Basic Local Alignment Search Tool) search in denaturing gradient gel electrophoresis fingerprints

Phylogenetic group	Band no.	Closest relative (accession no.)	Identity (%)	Relative abundance (%)																
				C0F	C1-5F	C3-0F	Oxi0F	Oxi1-5F	Oxi3-0F	C0M	C1-5M	C3-0M	Oxi0M	Oxi1-5M	Oxi3-0M	C0D	C1-5D	C3-0D	Oxi0D	Oxi1-5D	Oxi3-0D
Proteobacteria	1	*Acinetobacter* sp. (KC153297·1)	100	5	6	5	4	5	4	3	2										
气单胞菌	2	*Aeromonas* sp. (AY689039·1)	96	20	19	20	21	19	19	21	21	20	21	20	20	21	23	19	22	19	18
	4	*Acinetobacter* sp. (DQ211907·1)	99	11	12	11	8	17	11	11	11	10	10	12	11	10	8	8	10	10	6
	5	Enterobacteriaceae bacterium (GU237035·1)	100	5	4	4	4	3	2	3	3	4	3	4	3	3	2	2	2	2	1
	7	*Acinetobacter* sp. (JX909164·1)	100				2	2				1	2	2	2	2				6	7
假单胞菌属	8	*Acinetobacter* sp. (JN703731·1)	98	21	20	20	20	18	22	21	22	22	21	21	21	21	22	20	21	17	17
	10	*Acinetobacter junii* (JX490076·1)	100	3	2	3	3	3	4	4	3	3	3	3	4	3	3	3	3	2	1
	11	*Sphingobium yanoikuyae* (JX122496·1)	100	10	9	9	9	9	11	11	11	11	10	11	11	11	11	11	11	11	11
鞘氨醇单胞菌属	12	*Sphingomonas* sp. (JX867317·1)	98	20	18	19	20	19	20	20	20	20	18	20	20	21	22	20	20	17	17
Eukaryota	3	*Cyprinus carpio* (JN628435·1)	99	3	2	2	2	1	2	2	2	3	2	2	2	2	2	2	2	8	8
Actinobacteria	6	*Micropruina glycogenica* (JQ899240·1)	99		4	3	2											9	2		
Cyanobacteria	9	*Anabaena circinalis* (EU015860·1)	100	2	2	3	3	3	3	3	3	3	2	3	3	3	3	3	3	4	4
Fusobacteria	13	*Psychrilyobacter* sp. (JF825448·1)	98		2	1	2	1	2	1	2	2	1	1	1	1	2	1	2		
Firmicutes	14	Unidentified anaerobic bacterium (AY756145·2)	95									1	7	1	2	2	2	2	2	11	11

F, foregut; M, midgut; D, distal gut.

2018&读书报告

2018 and reading report

Table 8. Pairwise similarity coefficient matrix for the intestinal adhesive bacterial communities of carp at the end of the different experimental treatments

	C0F	C1·5F	C3·0F	Oxi0F	Oxi1·5F	Oxi3·0F	C0M	C1·5M	C3·0M	Oxi0M	Oxi1·5M	Oxi3·0M	C0D	C1·5D	C3·0D	Oxi0D	Oxi1·5D	Oxi3·0D
C0F	1·00																	
C1·5F	0·86	1·00																
C3·0F	0·93	0·93	1·00															
Oxi0F	0·79	0·93	0·86	1·00														
Oxi1·5F	0·86	0·86	0·79	0·93	1·00													
Oxi3·0F	0·93	0·93	0·86	0·86	0·93	1·00												
C0M	0·93	0·93	0·86	0·86	0·93	1·00	1·00											
C1·5M	0·93	0·93	0·86	0·86	0·93	1·00	1·00	1·00										
C3·0M	0·71	0·71	0·64	0·79	0·86	0·79	0·79	0·79	1·00									
Oxi0M	0·71	0·71	0·64	0·79	0·86	0·79	0·79	0·79	1·00	1·00								
Oxi1·5M	0·71	0·71	0·64	0·79	0·86	0·79	0·79	0·79	1·00	1·00	1·00							
Oxi3·0M	0·71	0·71	0·64	0·79	0·86	0·79	0·79	0·79	1·00	1·00	1·00	1·00						
C0D	0·71	0·71	0·64	0·79	0·86	0·79	0·79	0·79	1·00	1·00	1·00	1·00	1·00					
C1·5D	0·79	0·79	0·71	0·71	0·79	0·86	0·86	0·86	0·93	0·93	0·93	0·93	0·93	1·00				
C3·0D	0·71	0·86	0·79	0·79	0·71	0·79	0·79	0·79	0·86	0·86	0·86	0·86	0·86	0·93	1·00			
Oxi0D	0·71	0·86	0·79	0·79	0·71	0·79	0·79	0·79	0·86	0·86	0·86	0·86	0·86	0·93	1·00	1·00		
Oxi1·5D	0·79	0·64	0·71	0·71	0·79	0·71	0·71	0·71	0·93	0·93	0·93	0·93	0·93	0·86	0·79	0·79	1·00	
Oxi3·0D	0·79	0·64	0·71	0·71	0·79	0·71	0·71	0·71	0·93	0·93	0·93	0·93	0·93	0·86	0·79	0·79	1·00	1·00

F, foregut; M, midgut; D, distal gut.

鲤鱼肠粘附细菌群落的配对相似系数矩阵

04

Discussion

2018&读书报告

2018 and reading report

1.诸多研究发现有机酸对机体有益。实验中虽然MSB作用在统计学上没有显著差异，但在MSB1.5时取得较好效果，可能与缓释剂的剂型与持续释放有关。

2.实验结果表明MSB1.5和MSB3.0似乎可以预防或修复肠粘膜损伤并增加微绒毛的密度。但实际作用机制仍需进一步研究。

3.在目前的研究中，在每个肠道区域内，丁酸钠没有显着影响微生物群落，因为丁酸盐本身是一种由产丁酸菌产生的代谢产物。该微生物群落中的细微差异可能具有是由肠粘膜形态变化和免疫反应引起的。





THANK YOU

请各位老师同学批评指正！