Alternative strategies to control multi-resistant enteric pathogens and improve performance in swine: focus on enzymes, essential oils and 1-monoglycerides

控制耐药性肠道病原菌、提高猪生长性能的可行方案：酶制剂、精油和1-单甘油酯

G. L. Alborali, G. Tosi, M. Parini, V. Bontempo

F. Foresti, A. Paoli

Antimicrobial resistance (AMR): the issue
耐药性：问题

New resistance mechanisms will likely to emerge in future: the discovery of *mcr-1* and *mcr-2* genes
未来可能会有更多新的耐药机理涌现：MCR-1和MCR-2基因的发现

The gene *mcr-1* cause an important and rapid antimicrobial resistance. It can be transferred between different type of bacteria
MCR-1基因引起快速的严重的抗生素耐药性。可以在不同种细菌间传递

The gene was first identified in bacteria (Enterobacteriaceae) in South China
这个基因在中国南方的肠杆菌中第一次鉴定出来

Micro-organism may became resistant to:
- Antibiotics chemically related (Colistin, Neomycin and Kanamycin)
- Antibiotics chemically unrelated (Erythromycin, Lincomycin)

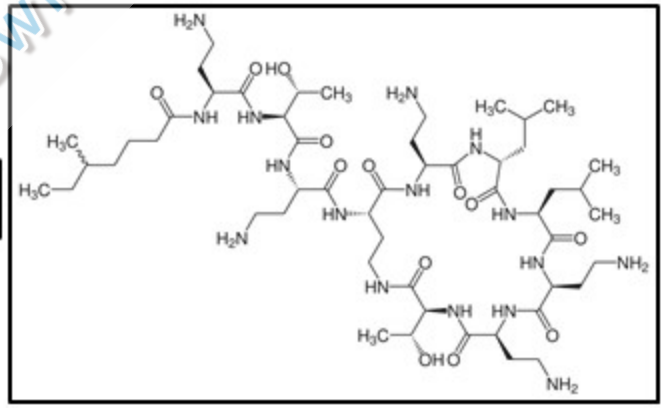
微生物耐药性来源：
- 抗生素化学相关（粘杆菌素、新霉素和卡那霉素）
- 抗生素非化学相关（红霉素、林肯霉素）

Antimicrobial resistance (AMR): the focus
耐药性：焦点

Colistin and Lincomycin
粘杆菌素和林肯霉素

Largerly used in post-weaning and fattening period to control *E.Coli*, *Salmonella spp.* and *Brachyspira hyodysenteriae*
在育肥猪和断奶仔猪中大量使用，以控制大肠杆菌、沙门氏菌和猪痢疾密螺旋体

Colistin



Colistin is actually the last option of treatment against deadly bacteria in human
事实上，粘杆菌素是人医中治疗致死细菌的最后一个手段

Antimicrobial resistance (AMR): the focus
耐药性：焦点

Colistin
粘杆菌素

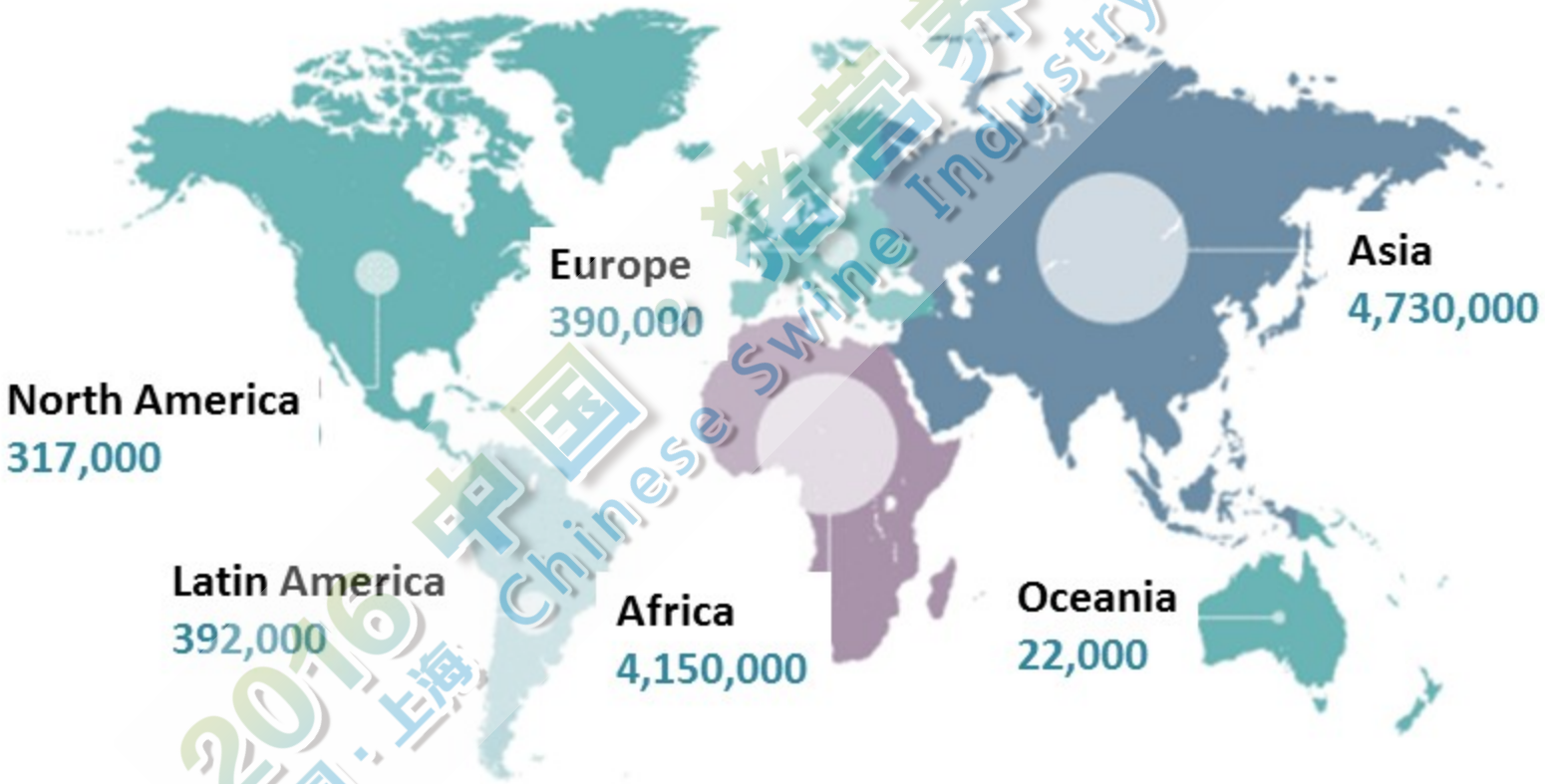
The European Medicines Agency (EMA) has recommended that colistine-containing medicines should only be used as a second line treatment in animals
欧盟药监局建议含粘杆菌素的药物只能当第二类药物在动物中治疗使用

Member States should reduce the colistin level below 1 mg colistin/PCU as desiderable level (EMA 2016)
理想状况下成员国应当将粘杆菌素用量降低到1mg/PCU的水平以下（EMA 2016）

Over the course of the next 3 years, all Member States should reduce the use of colistin in animals to to a target level of 5 mg colistin/population correction unit (PCU)
在未来3年内，所有成员国的目标应该是降低粘杆菌素用量至5mg/PCU

Antimicrobial resistance (AMR): the future
耐药性：未来

Deaths attributable to antimicrobial resistance every year by 2050
2050年每年由于耐药性而死亡的人数



Source: Review on Antimicrobial Resistance – 2014

Antimicrobial resistance (AMR): the alternatives
耐药性：可行方案

During last years several products were identified as possible alternatives to antimicrobials:
最近几年替代抗生素的可行方案

- Acidifiers for water and feed 水和饲料酸化剂
- Probiotics and prebiotics 益生素和益生元
- Enzymes酶
- Plant extracts and essential oils植物提取物和精油
- 1-monoglycerides of short and medium chain fatty acids 中短链脂肪酸1-单甘油酯

Enzymes and essential oils: trial
酶和精油：试验

Jiang X.R., et al. (2015) investigated the hypotesis of an improved gut enviroment of post-weaning piglets when administered a blend of essential oils (EO; thymol and cinnamaldehyde) and an enzyme combination (XB; xylanase and β-glucanase)

Jiang等（2015）研究了断奶仔猪日粮中添加精油（麝香草酚和肉桂醛）和酶制剂（木聚糖酶和β-葡聚糖）是否能改善肠道环境

To asses the effect of dietary treatments some parameters have been evaluated:

为了确定处理的效果，检测以下指标：

- 营养物质消化率 Faecal nutrient digestibility
- 微生物菌落数 Microbial counts
- 回肠肠道形态 Ileum hystology
- 炎症调节因子基因表达 Inflammatory mediators gene expression

Enzymes and essential oils: experimental protocol
酶和精油：试验设计

- 192头断奶仔猪
  Number of animal: 192 weaned piglets
- 试验处理（42天）Treatments (42 days):
  - 对照组 Control Group (basal diet)
  - 精油组 EO Group (basal diet + 0.05 g/kg essential oils)
  - 酶制剂组 XB Group (basal diet + 0.1 g/kg enzymes)
  - 精油+酶制剂组 EO + XB Group (basal diet + 0.05 g/kg essential oils + 0.1 g/kg enzymes)

- 消化率试验：2期 Digestibility experiment: 2 periods
  第一期（第15-21天）；第二期（第29-35天）
  (period 1: days 15 to 21; period 2: 29 to 35)
- 在第20、21、34、35天收集粪便
  Feaces were collected on days 20, 21, 34 and 35

Enzymes and essential oils: results
试验结果

On day 42, six piglets form each test group were slaughtered
第42天，每个处理组屠宰6头仔猪

	Treatment 处理				SEM	P-value
	CTRL 对照	EO 精油	XB 酶制剂	EO+XB 精油+酶制剂		
Day 0-42 第0-42天						
ADG 日增重	426	430	415	381	15.2	0.1
ADFI 日采食量	751	732	743	650	27.9	0.052
G:F 肉料比	0.57	0.59	0.56	0.59	0.012	0.2

ADG = average daily gain; ADFI = average daily feed intake; G=gain; F=feed.

[1]n = 4 8(12pens/treatment).

[2]CTRL = basal diet without supplementation; EO = CTRL + 0.05g/kg EO (thymol and cinnamaldehyde); XB = CTRL + 0.1 g/kg enzymes XB; EO + XB = CTRL +0.05 g/kg EO + 0.1 g/kg enzymes XB.

EO, XB and EO+ XB supplementation did not affect (P>0.05) piglets growth performance
日粮中添加精油、酶制剂或一起添加对仔猪生长性能没有显著影响

Enzymes and essential oils: results
试验结果

Table II – Effect of essential oils (EO), enzymes (xylanase and β-glucanase (XB)) or EO +XB supplementation on faecal microbiological counts (Log10 cfu/g) of weaned

	Treatment (Tr)[2]					P-value		
Items	CTRL	EO	XB	EO + XB	Pooled s.e.m.	Tr	Time (t)	Tr x t
Clostridia								
Day 0	6.02	5.57	5.53	5.72	0.31	0.85	<0.01	0.26
Day 14	5.31	5.46	6.23	5.89				
Day 42	6.38	6.88	6.55	6.58				
Escherichia coli								
Day 0	9.02	8.50	8,64	8,97	0.23	0.22	<0.01	0.16
Day 14	6.94	7.18	7.49	7.44				
Day 42	6.71^{a}	6.44^{ab}	6.00^{b}	6.72^{a}				
Lactobacillus (La)								
Day 0	8,55	8,26	8,54	8,45	0.18	0.03	0.61	0.31
Day 14	8.94^{a}	8.69^{ab}	8.43^{ab}	8.21^{b}				
Day 42	8.86^{a}	8.32^{b}	8.24^{b}	8.44^{ab}				
Coliforms (Co)								
Day 0	10.00^{a*}	9.39^{a*}	10.26^{a*}	9.91^{a*}	0.24	<0.01	<0.01	0.02
Day 14	8.38^{ab†}	7.8^{a†}	8.64^{b†}	8.22^{ab†}				
Day 42	8.20^{a†}	7.29^{b†}	6.91^{b‡}	7.23^{b‡}				
La : Co								
Day 0	0.86^{a*}	0.9^{a*}	0.84^{a*}	0.86^{a*}	0.04	0.19	<0.01	0,03
Day 14	1.07^{ab†}	1.14^{a†}	0.98^{b†}	1.00^{ab†}				
Day 42	1.09^{a†}	1.15^{ab†}	1.20^{b‡}	1.17ab‡				

a,b Means listed in the same row with different superscripts are significantly different (P<0.05).

*,†,‡ Means listed in the same column with different superscripts are significantly different (P<0.05).

Test on the means of diet × time interaction: Means listed in the same row with different letters, or in the same column with different symbols, are significantly different (P<0.05).

[1]n=48 (12pens/treatment).

[2]CTRL = basal diet without supplementation; EO = CTRL + 0.05 g/kg EO (thymol and cinnamaldehyde); XB = CTRL + 0.1 g/kg enzymes XB; EO+XB = CTRL + 0.05 g/kg EO + 0.1 g/kg enzymes XB.

Dietary XB reduced the faecal Latobacillus and E.Coli counts
日粮酶制剂降低粪中乳酸菌和大肠杆菌数量

All the additives supplementations decreased the counts of faecal Coliforms at day 42 (P<0.01)
所有处理均降低第42天粪便中菌落总数

Enzymes and essential oils: results
试验结果

Items		Treatment (Tr)[2] CTRL	EO	XB	EO + XB	s.e.m.	P-value
Villus height (μm)	绒毛高度	397	391	410	372	20	0.60
Crypt depth (μm)	隐窝深度	337	313	296	297	12	0.09
V : C ratio	绒毛:隐窝	1.19[a]	1.26[ab]	1.40[b]	1.26[ab]	0.05	0.048
Total area of lymphatic follicles (Peyer's patches) (mm^2)	淋巴结总面积（mm2）	0.49	0.50	0.43	0.51	0.04	0.39
Lyphatic follicles number (Peyer's patches) (n/mm^2 mucosa)	淋巴结数量	1.23[a]	0.99[b]	1.10[ab]	0.93[b]	0.05	<0.01
Mucosal macrophages number (n/mm^2 mucosa)		283[a]	170[b]	86[b]	110[b]	25	<0.01

a,b Means listed in the same row with different superscripts are significantly different (P<0.05).

[1]n = 4 8(12pens/treatment).

[2]CTRL = basal diet without supplementation; EO = CTRL + 0.05g/kg EO (thymol and cinnamaldehyde); XB = CTRL + 0.1 g/kg enzymes XB; EO + XB = CTRL +0.05 g/kg EO + 0.1 g/kg enzymes XB.

XB supplentation reduced the mucosal macrophages number (P<0.01) in the ileum

添加酶制剂降低回肠黏膜巨噬细胞数量

EO and EO+XB supplentation decreased the number of lymphatic follicles and mucosal macrophages

精油或者精油与酶合用降低淋巴结数量和黏膜巨噬细胞数量

Enzymes and essential oils: conclusions
试验结论

- **Despite the positive results obtained over the intestinal histometry in treated animals, no performance improvement, neither enhanced faecal nutrients digestibility were observed**
- 尽管可以改善动物肠道绒毛健康，但是对生长性能和养分消化率没有明显作用效果

- **The pro-inflammatory interleukin (IL-1α) was dowregulated in piglets treated with EO+XB compared with the EO group. This suggest that the synergic combination may have a potential to modulate the expression of proinflammatory cytokines**
- 与精油组相比，日粮中添加精油和酶制剂降低促炎症细胞因子的表达。表明联合使用可以调节促验证因子的表达

1-monoglycerides of fatty acids: trial
1-单脂肪酸甘油酯试验

Aim of the trial:试验目的

- **To asses if 1-monoglycerides of short and medium chain fatty acids may contribute to reduce the impact of ETEC-F4 *E.Coli* strain**

  评估1-单中短链脂肪酸甘油酯是否能降低ETEC-F4大肠杆菌的效果

- **To asses if 1-monoglycerides of short and medium chain fatty acids may contribute to limit injectable antimicrobial treatments in post-weaning period**

  评估1-单中短链脂肪酸甘油酯是否能减少仔猪断奶后抗生素治疗次数

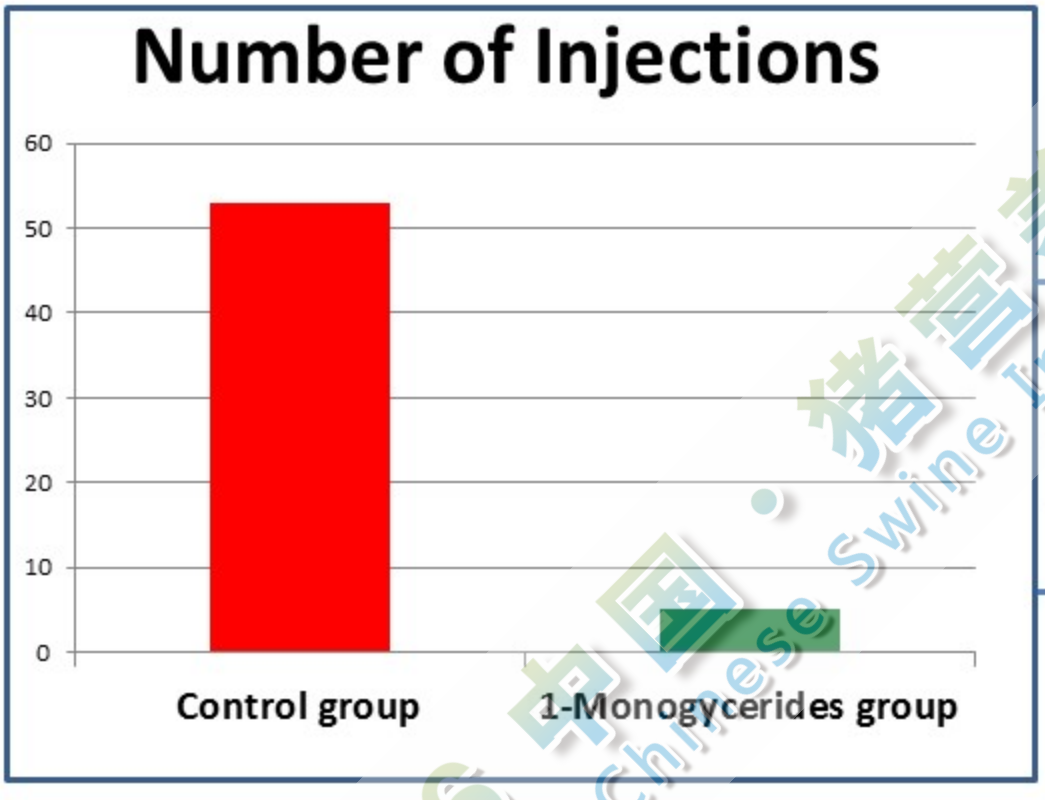
1-monoglycerides of fatty acids: trial
试验方案

- 1343头仔猪 Number of animal: 1343 piglets
- 初始均重：7.7kg Piglets starting weight: 7.7 kg
- 试验期：52天 Trial duration: 52 days
- 试验处理 Treatments :
  - 对照组 – 665头 Control Group – 665 piglets (basal diet)
  - 试验组-678头 （基础日粮+饮水中添加2kg单甘油酯/t）
    Treated Group – 678 piglets (basal diet + 2 kg of 1-monoglycerides /ton of drinking water)

The feed of both groups was supplemented with 3200 ppm of ZnO up to 25 kg of live weight
两组日粮中均添加3.2kg氧化锌直至25kg体重

1-monoglycerides = the product is composed by a mixture with a specific ratio between 1-monopropionin, 1-monobutyrin, 1-monocaprylin, 1-monocapri and 1-monolaurin (produced by SILO S.p.A. – Florence / Italy)
1-单甘油酯由特定比例的丙酸、丁酸、辛酸、癸酸、月桂酸的1-单甘油酯组成，由意大利SILO生产

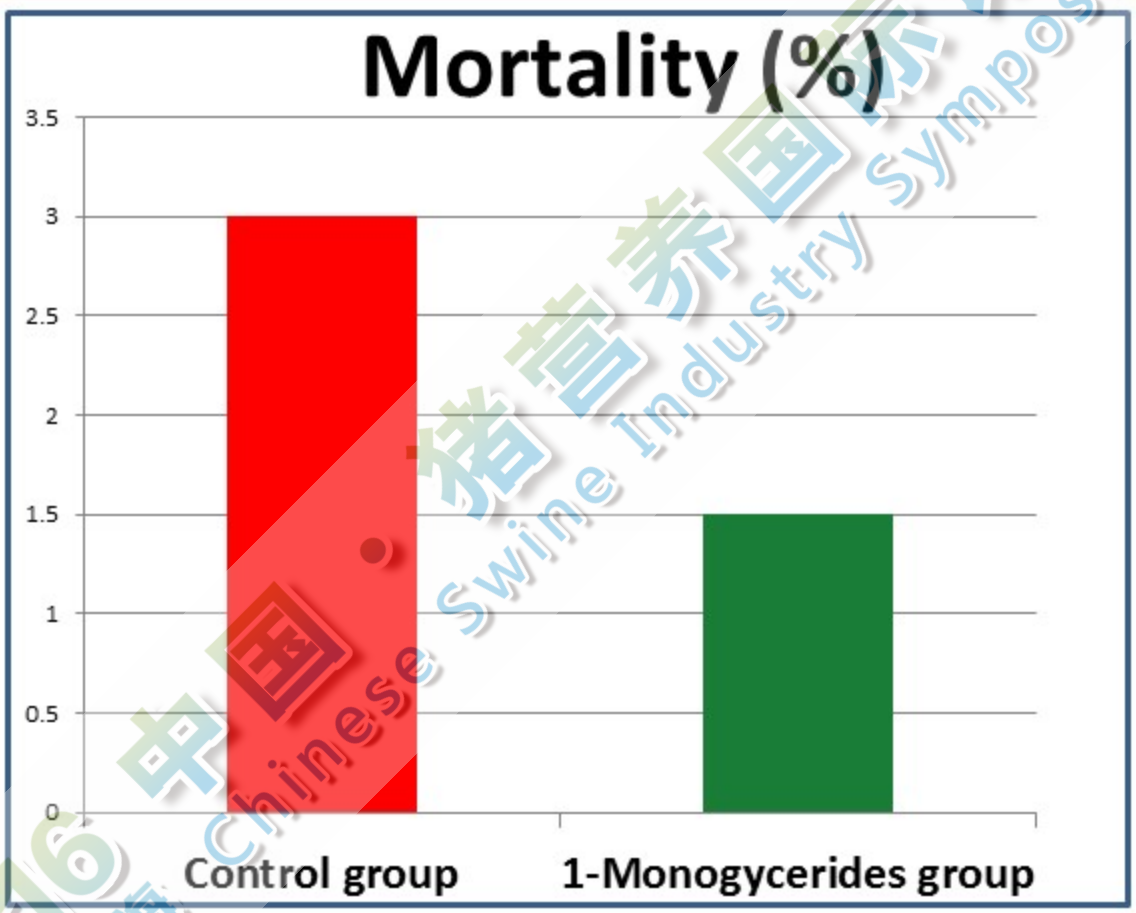
1-monoglycerides of fatty acids: results
试验结果



In control group 53 piglets were injected with enrofloxacin and / or cefquinome to control diarrhea
对照组中有53头次注射恩诺沙星和/或头孢喹肟，以控制腹泻

In the 1-monoglycerides group only 5 piglets were injected
而试验组中只有5头次

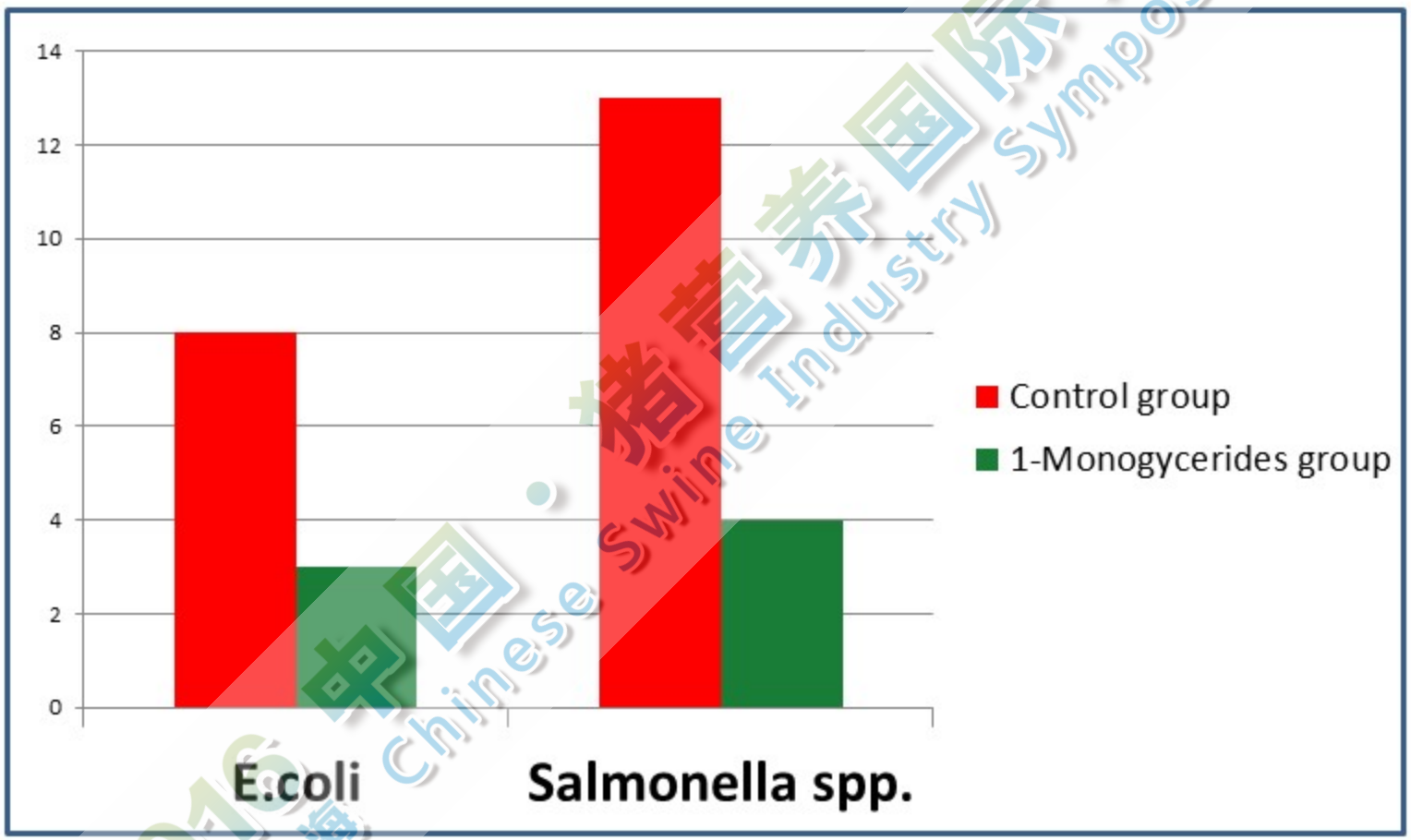
1-monoglycerides of fatty acids: results



Mortality caused by enteric diseases was 3% in the control group and 1.5% in the 1-monoglycerides group

对照组由肠道疾病导致的死亡率为3%，而试验组只有1.5%

1-monoglycerides of fatty acids: results



Bacteriological investigations in intestinal tracts showed a reduction in detection of pathogens in 1-monoglycerides group compared to the control group
肠道细菌检测结果表明1-单甘油酯降低了肠道中病原菌数量

1-monoglycerides of fatty acids: Conclusion
结论

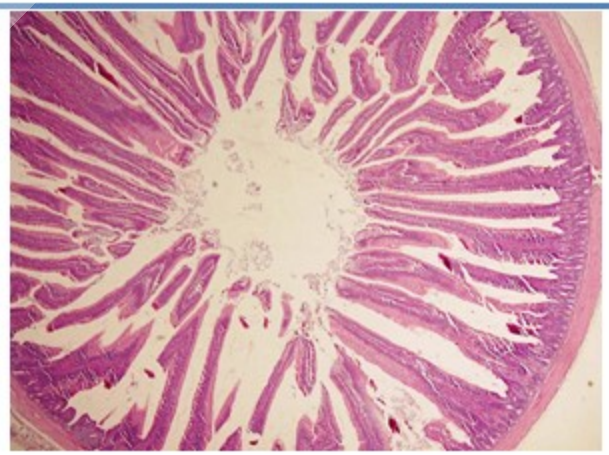
1-monoglycerides resulted to be effective in reducing injectable treatments for controlling mortality rate and the isolation of pathogens in intestinal tracts of death piglets

试验结果表明1-单甘油酯能有效减少抗生素治疗次数，控制死亡率，减少死亡猪肠道内病原菌数量

1-monoglycerides of fatty acids: nutritional aspects
1-单甘油酯：营养作用

The product used in the trial contains 1-monobutyrin which has:
试验中使用的1-单丁酸甘油酯还有以下作用
-促进组织血管增生 A positive angiogenetic effect on tissues
-促进绒毛生长 Positive effect on villus growth
-促进紧密连接蛋白的表达 Positive effect on refosforilation of protein composing tight junctions
-促进有害菌黏膜损伤的修复 Positive effect on reparation of injuries caused by harmful bacteria

Small intestine
Before 1-Monobutyrin



Small intestine
After 1-Monobutyrin

1-monoglycerides of fatty acids: antibacterial aspects
1-单甘油酯：抑菌效果

In Vitro trials conducted by IZSLER
IZSLER进行的体外试验

TABLE IV - MIC of 1-Monoglycerides and organic acids at buffered pH 6-7

	pH	Salmonella Typhimurium	Clostridium p.	E. Coli	Lactobacillus acidophilus / plantarum
1-Monoglycerides	4.5	0.06%	0.01%	0.12%	No inhibition
1-Monoglycerides	7	0.06%	0.01%	0.12%	No inhibition
Formic Acid	6	1.5%	1.8%	1.6%	= =
Propionic Acid	6	2.4%	2.6%	2.5%	= =
Sodium Butyrate	6	2.8%	3%	2.9%	= =

The results of the in vitro test show that concentrations of 1-monoglycerides from 0.01 % to 0.12 % inhibit at pH6 the growth of pathogenic bacteria, whitout inhibiting beneficial bacteria (Lactobacillus)

体外试验结果表明0.01%或0.12%的1-单甘油酯可以在PH6下抑制有害菌的生长，而对有益菌无影响

Fatty acids: antibacterial aspects
脂肪酸：抑菌效果

	Chemical Status 化学状态	Antibacteria action 抑菌作用
pH 3 – 4 (stomach 胃)	Un-dissociated 未解离	Yes
pH 6 – 7 (intestinal 肠道)	Dissociated 解离	No

$RCOO^-H^+$

$RCOO^-$

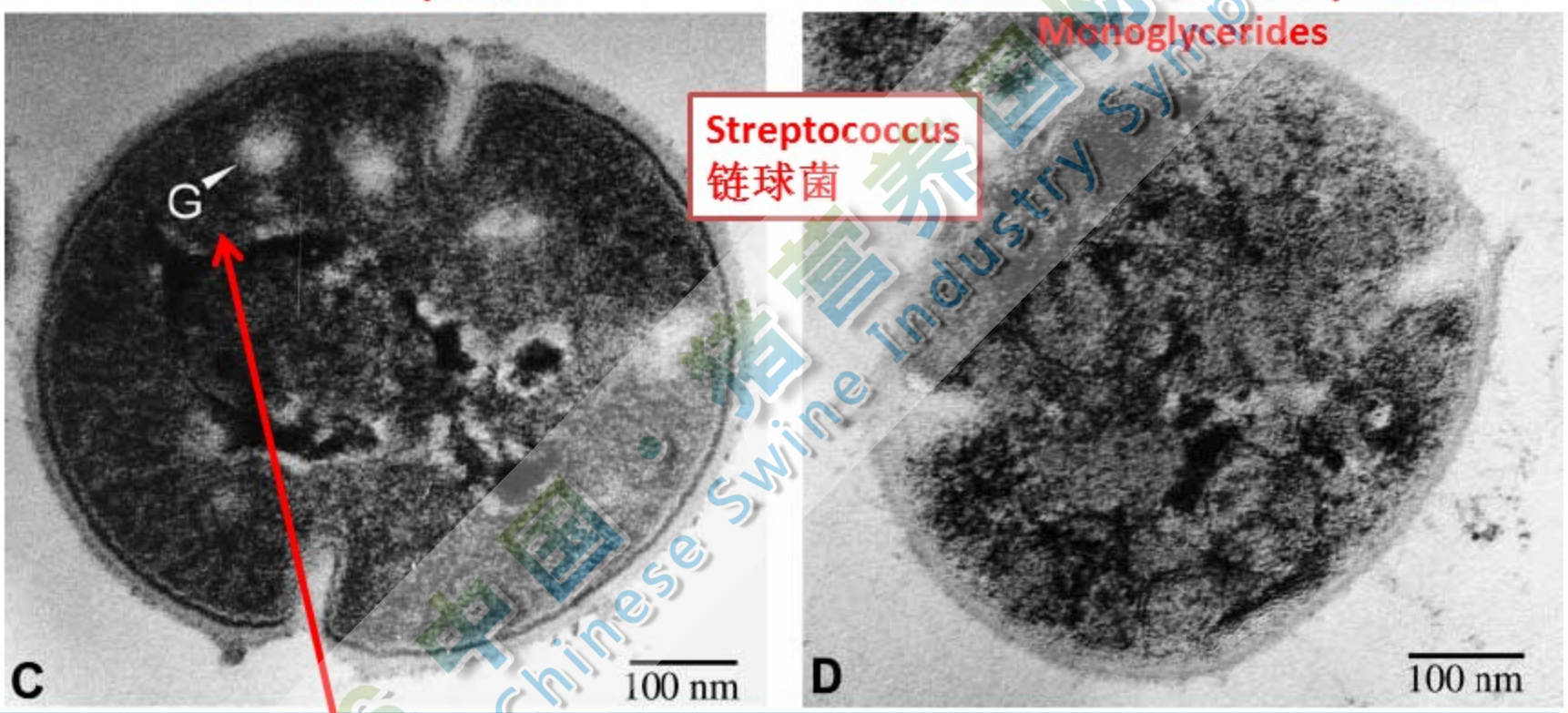
Can penetrate the bacterial membrane
能通过细菌细胞膜

Can not penetrate the bacterial membrane
不能穿过细菌细胞膜

1-monoglycerides of fatty acids: mode of action - Gram positive bacteria
1-单甘油酯：革兰氏阳性菌作用机理

Control sample对照

处理 Bacteria cell after exposure to Monoglycerides



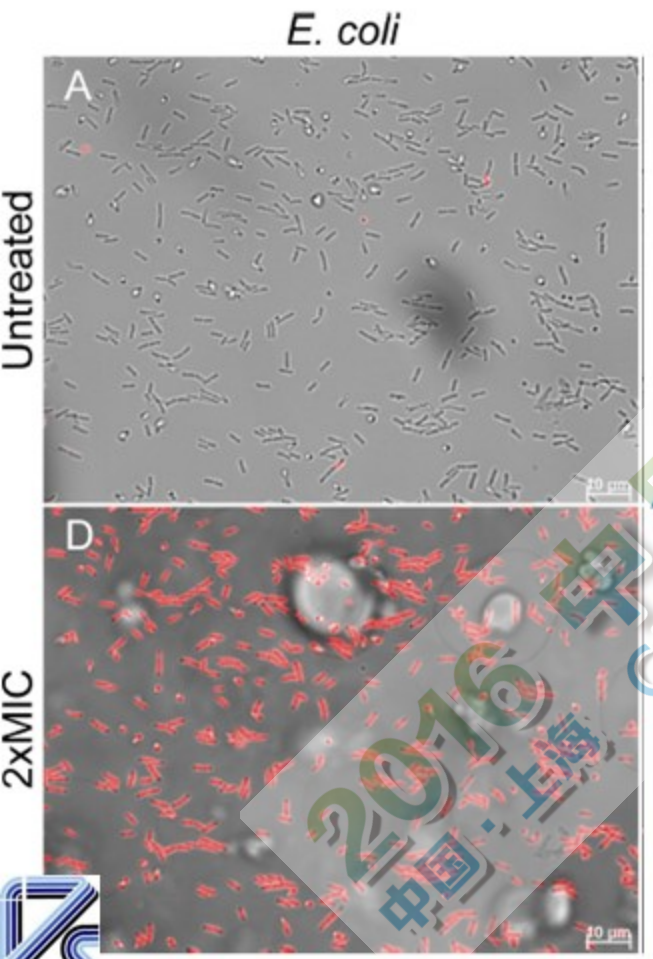
图片C表明来自对照样品的细胞具有完整的细胞核 。细胞质颗粒(G)。图片D表明经过10mM的单甘酯处理30min的细胞，质膜和细胞质颗粒消失。可以看到细胞壁的一些变化。

C picture shows cell from the control samples with intact plasma membrane and intact cytoplasmic granules (G). D shows a cell from samples treated with 10 mM Monoglyceride for 30 min, demonstrating disappearance of plasma membranes and cytoplasm granules. Some changes can be seen in the cell wall as well.

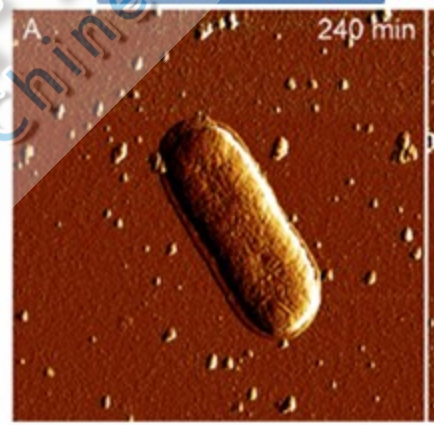
1-monoglycerides of fatty acids: mode of action - Gram negative bacteria
1-单甘油酯：革兰氏阴性菌作用机理

Membrane permeabilization caused by monoglycerides (8 mM) treatment on E.Coli
单甘油酯处理大肠杆菌，导致细胞膜穿透

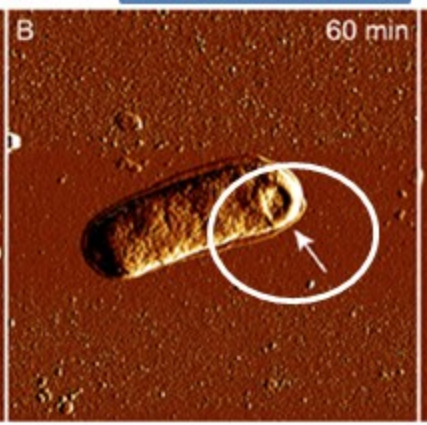
Hyldgaard M., et al 2012



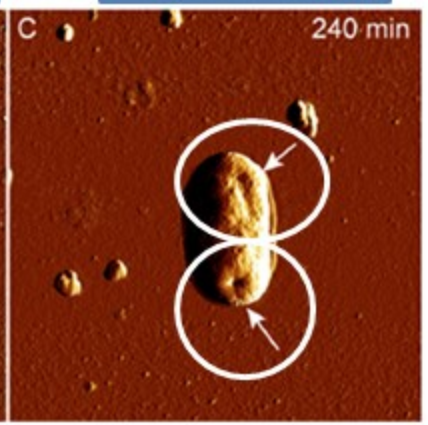
Control对照



Treated处理



Treated处理



Conclusion结论

The administration of EO, XB and their synergic combination, was effective in improving ileum hystology in terms of villus / crypt ratio in post-weaning period
日粮中添加精油和酶制剂可以有效改善断奶仔猪回肠肠道绒毛形态

EO+XB supplementation might benefit the modulation of the expression of inflammatory cytokines 精油和酶联合使用可以有效调节炎症因子的表达

1-monoglycerides of short and medium chain fatty acids proved to be effective in fighting *Clostridium perfringens, Salmonella spp,* ETEC-F4 *E.Coli*
试验证明中短链脂肪酸1-单甘油酯可以有效控制产气荚膜梭菌、沙门氏菌和大肠杆菌

1-monoglycerides do not inhibit beneficial bacteria as Lactobacillus
1-单甘油酯不会抑制有害菌，如乳酸菌

1-monoglycerides supplementation proved to reduce injectable treatments to control diarrhoea in post-weaning piglets
1-单甘油酯可以减少断奶仔猪由于腹泻导致的治疗次数