Approach to Authorization of novel technologies on alternatives to antibiotic in China

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President
General Director

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National Feed Industry Economy Committee
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1. Animal husbandry and feed industry of China
Feed production (MT) in China (1990-2011)
Compound feed product pass rate are steadily increasing.

- Feed quality improved
Today's Challenges

- Feed Safety
  - Feed Row Material
  - Animals
  - Excreta Pollution
  - Animal Product Safety
The balance of protein resources

2010 imported 540 million T.
The balance of energy resources

- Requirement
- Available

Year: 2000, 2010, 2020

Units: 100 MT
New technology adoption

- Government now encourage the feed industry enterprises adopt new high technology to go along with a professional, standard and the industrial model, the animal farm scale will grow bigger and bigger. Integrator farm play an important role in our country and will keep growing.

- New technology such as producing biofeed, nonpolluting feed, no residue feed, and functional feed will be developed to meet overcoming the environment pollution and the animal product quality improving.
2. General situation on alternatives to antibiotic
Fear of the antibiotics use

- Over 90 percent of antibiotics consumption are used for feed all over world.
- 50 percent of the world feed antibiotics were consumed in China.
- The increased use of antibiotics has given rise to a fear of the development of resistant pathogenic bacterial strains and residual contamination of the food chain with antibiotics.
### Growth promoting effect of antibiotics

<table>
<thead>
<tr>
<th>Animal</th>
<th>Daily gain</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>piglet</td>
<td>+4.2~136%</td>
<td>+1.7~42.7%</td>
</tr>
<tr>
<td></td>
<td>(15%)</td>
<td>(6.5%)</td>
</tr>
<tr>
<td>Growing pig</td>
<td>+0~8.9%</td>
<td>-1.8~3.8%</td>
</tr>
<tr>
<td></td>
<td>(3.6%)</td>
<td>(2.4%)</td>
</tr>
</tbody>
</table>

Source: Thaler & Miller, 2000
## Growth promoting effect of antibiotics in China

(1994—2008 trials)

<table>
<thead>
<tr>
<th>Animals</th>
<th>Daily gain</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-20kg piglet (62trials)</td>
<td>11.12±1.19%</td>
<td>8.15±0.82%</td>
</tr>
<tr>
<td>20-60kg growing pig (73trials)</td>
<td>9.80±0.80%</td>
<td>8.02±0.70%</td>
</tr>
<tr>
<td>60-90kg finish pig (39trials)</td>
<td>6.24±1.68%</td>
<td>8.15±1.36%</td>
</tr>
<tr>
<td>chicken (73trials)</td>
<td>4.36±0.44%</td>
<td>3.88±0.47%</td>
</tr>
<tr>
<td>Broiler medium (61trials)</td>
<td>3.81±0.44%</td>
<td>3.93±0.44%</td>
</tr>
<tr>
<td>Broiler large (35trials)</td>
<td>4.21±1.01%</td>
<td>4.12±0.87%</td>
</tr>
<tr>
<td>Duck (41trials)</td>
<td>5.31±0.84%</td>
<td>3.88±0.61%</td>
</tr>
</tbody>
</table>
Many countries have a comprehensive ban of antibiotics in feed.

The feeding environment in China is different.

Feed antibiotics is still a meaningful option to prevention of animal diseases.

China will have to face the antibiotic disabling problem.

Intensive research has focused on the development of alternative strategies with the aim of maintenance of animal health and performance.
3. Management on alternatives to antibiotic
The organization of feed additives and animal medicine in China

- The management for feed ingredient and feed additive are under the animal husbandry department, MOA.
- Any new feed additives have to be approved by the new feed evaluation committee, the Ministry of Agriculture of China (MOA).
- Presently China has published a feed additive list, a guide for feed additive use, and a feed ingredient list.
Organization of feed additives and animal medicine in China

- **The ministry of agriculture of China (MOA).**
- **Department of veterinary**
- **Alternatives to antibiotic**
- **New feed evaluation committee**
- **Feed additive list**
- **Feed resources list guide for feed additive**
- **Producers**
- **Animal new medicine evaluation committee**
- **Antimicrobial peptides (AMP).**
- **Lysozyme (溶菌酶).**
In order to control the feed quality, China has established the Feed quality supervision & detecting system.

- National Center (1)
- MOA Centers (7)
- Provincial Agencies (30)
Feed quality supervision & detecting items by MOA

- General feed safety.
- Detecting of nutrients and hygiene criteria.
- Detecting of clenbuterol and ractopamine.
- Detecting of illegal additives: e.g. melamine etc.
- Detecting of cattle, sheep & goat derived ingredients in ruminant feed.
Feed Additives Supervision items by MOA

- Routine supervision and management
- Administrative law enforcement
- Reporting complaints
- Information feedback and the Urgent events.
4. Novel technologies on alternatives to antibiotic
Current situation and future development

- **Antibiotics**
  - **Residue** 残留
  - **Resistance** 耐药性

Harmful to animal and human health 影响动物和人类的健康

Reduce the use of antibiotics 减少抗生素的使用

- EU 欧盟
- China 中国
Alternatives to antibiotics in China

- Various natural materials have been investigated as efficient alternatives to antibiotic growth promoters. Many of which are commercially available.
Alternatives to Antibiotics in China

- Prebiotics
- Probiotics
- Antimicrobial Peptides
- Acidifying agents
- Enzymes
- Herbal extracts
1. Probiotics

- Probiotics are one of the most important alternatives to antibiotic in China, which was widely used in feed mills and animal farms.
- Its output is about 50,000 tons a year.
- Focused on three categories: *Bacillus*, Lactic acid bacteria & Yeasts.
Direct-fed microorganisms by MOA

1. *Lactobacillus casei* (干酪乳杆菌)
2. *Streptococcus faecalis* (粪链球菌)
3. *Pediococcus acidilacticii* (乳酸片球菌)
4. *Bacillus natto* (纳豆芽孢杆菌)
5. *Streptococcus lactis* (乳链球菌)
6. *Candida utilis* (产朊假丝酵母)
7. *Lactobacillus Plantarum* (植物乳杆菌)
8. *Streptococcus faeciun* (屎链球菌)
9. *Bacillus subtilis* (枯草芽孢杆菌)
10. *Lactobacillus acidophilus* (嗜酸乳杆菌)
11. *Saccharomyces cerevisiae* (啤酒酵母)
12. *Rhodop seudanonas palustris* (沼泽红假单胞菌)
**Functional synbiotics**

**Synbiotics**

- *Bacillus subtilis*
  - 枯草芽孢杆菌

- Antibacterial substance
  - 抑菌物质

- Organic acid
  - 有机酸

- *Astragalus*, aloe polysaccharide, etc.
  - 黄芪多糖，芦荟多糖等

- Immunity
  - 免疫力

- Animal Health
  - 动物健康
Challenge of the probiotics use

Unstable effects

- Stomach acid and salt
- Zn, Cu in the feed
- Microbial strains Gene stability
- Low live microbes
- Lower resistance
- Bad environment of trans. and store
- Higher cost
- High temp. pressure In feed processing
New processing: pre-fermentation-encapsulation

Pre-fermentation-encapsulation

Realized the integration of fermentation and micro-capsules

① High live microbes
② Higher Micro encapsulation of efficiency
③ Higher stability
④ Lower cost

Make the probiotics industrialization possible
Microscopy photos for preparation of micro-capsule

1 h

8 h

10 h
## Comparison of Encapsulation techniques

<table>
<thead>
<tr>
<th>Encapsulation technique</th>
<th>Processing characteristic</th>
</tr>
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</table>
| **Pre-fermentation encapsulation** | 1. High efficiency of Micro encapsulation  
2. Effective living bacteria content in the product of up to $10^{11}$ cfu/g.  
3. Simplify post-processing and easier to industrialization  
4. Low cost and versatile device  
5. Well product uniformity |
| **Post-fermentation-coating** (fluidized layer coating and spray coating) | 1. Easier to industrialization  
2. Low rate of embedding  
3. Effective living bacteria content in the product of up to $10^9 \sim 10^{10}$ cfu/g  
4. Large energy-consuming, high cost |

Under the same conditions, consumes the same fermentation raw materials, but 10–100 times live bacteria available.
2. Prebiotics

- In China, most of the studied on prebiotic oligosaccharides are focused:
  - Fructo oligosaccharides (FOS)
  - Mannan oligosaccharides (MOS)
  - Arabinoxylol-oligosaccharides
  - Xylo-oligosaccharides.

- Oligosaccharides can be available on the market. Some of the feed mills start to use.
Study on preparative technology of pectic oligosaccharides (果胶寡糖) and chito-oligosaccharide (壳寡糖)

- On the basis of structure-activity relationships study to pectin and oligosaccharides, Endopectate lyase and commercialization were selected respectively for Apple pectin and Chitosan papain degradation, and through targeted monitoring of polymerization degree of degradation products, access to the desired target saccharides.
Fructo oligosaccharides (FOS)

- FOS can be found naturally in some cereal crops and onions.
- Widely used in swine, poultry and aquatic feed in China.
**Effects of FOS**

- Increase *Bifidobacterium*, *Lactobacillus*
- Increase the content of Lactic acid & Short chain fatty acids
- Intestinal fermentation
- Increase digestibility
- Reduce burden on the kidneys
- Inhibit pathogenic bacterial growth
- Promote absorption of Ca, Mg, Fe
- Improve immune function
- Increased excretion of cholesterol
- Decreased cholesterol synthesis
- Promote absorption of isoflavones
Mannan oligosaccharides (MOS)

- MOS is obtained from the cell wall of yeast (Saccharomyces cerevisiae).
- In recent years, with the development of hemicellulose resources in feed industry and aquaculture, the application and research of β – mannanase enter a new stage.
Effects of MOS

1. Regulation of immune defense
   a. Activated macrophages
   b. Improve the antibody response ability, strengthening the protection of vaccine efficacy
   c. Stimulation of hepatic secretion of mannose binding protein, affects the immune system.

2. Regulation of non-specific immune defense
   Interference with intestinal pathogen colonization, prompted the bacteria become intestinal predominant flora.

3. Adsorption of mycotoxin
   Through physical adsorption or direct binding of mycotoxin, eliminate toxin on the body of the harmful effects
A new oligosaccharides, has been highlighted oligosaccharide on the basis of current state at home and domestic.

Considered as food ingredients, arabinoxylo-oligosaccharides have favorable technological properties and cause prebiotic effects derived from their ability to modulate the intestinal function.
Xylo-oligosaccharide

- Xylo-oligosaccharide, a kind of prebiological substance, which can't be digested but can selectively activate bacterial reproduction within intestines.

- It can obviously improve intestinal micro ecological balance, proliferate bifid bacteria and gastric function.
3. Enzyme preparations

酶制剂
3. Enzyme preparation

- The feed enzymes developed in recent 10 years in China, there are a lot of reports about their application.
- Enzymes can degrade the anti-nutrient factors in feedstuff, increase nutrient digestibility, and reduce pollution to the environment, and have been widely supplemented in animal feed.
Presently, 12 kinds feed enzyme preparations are used in China: amylase, cellulase, $\beta$-glucan, glucose oxidase, lipase, maltase, mannanase, pectinase, protease, amylase, amylopectin, phytase xylanase.

Although the enzyme preparation on the microbial itself does not have significant impact, but it can improve the feed digestibility, reduced nutrient residues in digestive tract weight, shorten the residual time, indirect reduction of pathogen growth opportunities, and realize the effect of disease prevention.

The use of feed enzymes as antimicrobial growth promoters will need to be studied further before this strategy finds widespread use within the feed industry.
Phytase is now most important enzyme because of its relatively easy manufactured and be used in all kind feed of animals.

There are 4 companies have set up a international business with 40 countries.

Granular, powder, Instant Soluble.

5,000u/g - 200,000u/g are available.
Instant Soluble Phytase

SPECIFICATIONS 99.99% soluble. (see the left solubility test picture) Colour: Buff or white powder Odour: Normal fermentation odour Enzymatic Activity: 100,000u/g Activity Temperature: 20-70°C, favorable at 50°C Carrier Material: Instant soluble Mineral.
Heat-resistant acid Phytase

**Graph 2:** Optimal temperature for Phytase activity.

**Graph 3:** Heat stability of Phytase.

Temperature, °C

Activity, %
Heat-resistant acid Phytase

- High temperature resistant acid Phytase
- pepsin and trypsin-resistant features,
- pH3.5~pH7.0 range
- optimum pH4.0, have a higher stability.
Existing questions and the methods to increase the yield of β-mannanase

✓ Low yield and enzymatic activity, difficult for massive application of β-mannanase

◆ Screen high yielding A. sulphureus mannannase strains by a mutant treatment

◆ Modifying β-mannanase gene sequence according to codon bias
✓ Highest protein expression level (最高蛋白浓度): 3 g/L
✓ Highest enzymatic activity (最高酶活力): 1100 U/mL
✓ Purity (纯度): 96%

Pichia pastoris expression vector 毕赤酵母表达载体
Fermentation device (10 L) 发酵装置（10升）
Aspergillus niger Xylanase gene

Realization of expression in Escherichia coli and Pichia pastoris
Thermostability of the modified recombinant xylanase

Aspergillus sulphureus硫色曲霉

Modified xynA 改造xynA + pGAPZαA 载体

expression in Pichia pastoris

- Good stability: 80°C for 90 min
  
  热稳定性好，80°C保持90

- Enzymatic activity: 120 U/mL
  
  最高酶活力达120 U/mL

- Protein concentration: 3.78 mg/mL
  
  蛋白浓度为3.78 mg/mL
Alpha-Galactose glucoside enzyme could resist the degradation of neutral and alkaline protease. It has high specific activity under the condition of PH value 2-8, and can withstand 80 °C high temperature.

Experimental results show that addition in the broiler diet with different levels of Galactose glucoside enzyme (150 U/kg, 300 U/kg, 1500 U/kg group), it improves FCR, increase energy, protein apparent metabolic rate as compared with the control group.
Established the high level expression of recombinant strain of Alpha-Galactose glucoside enzyme, Cellulase and Pectinase.

The expression of 3 kinds of enzyme in the fermentation tank is up to 10000U/ml, 30000 U/ml and 10000 U/ml respectively.
4. Antimicrobial peptides (AMPs) 抗菌肽
4. Antimicrobial peptides (AMPs)

- AMPs are another major group of promising novel alternatives to antibiotics based on their effectiveness, safety, and enormous diversity.

  ✓ **AMPS**: Important components of the innate immune system
  
  ◆ 先天免疫系统的重要组成部分

  ✓ **Abundant and diverse groups of molecules that are encoded by definite genes**
  
  ◆ 特定编码基因产生的一类小分子多肽
Current situation and existing problems
现状及存在问题

- Research of higher structure and antibacterial mechanism of AMPs
  研究抗菌肽高级结构和抗菌机理
- Short half-life, easy to lose activity
  半衰期短，易失活
- Toxicity toward prokaryotic cell
  抗菌肽对原核细胞的毒性
- Low yield, difficult to purify
  天然抗菌肽纯化难度大，产量低
Key points for product development

- Modify the molecule of AMPs
  - 优化抗菌肽分子

- Select definite strain, and fusion expression
  - 原核系统中选育特定抗性菌株，真核系统中采用融合表达以提高其表达率

- Special induction mode, reduce product cost
  - 以特殊的诱导模式，降低生产成本
Antimicrobial peptides-activity feed products development

① Two Antimicrobial Peptide expression platform establishment of Bacillus subtilis and Yeast.

- Through the *Bacillus subtilis* modified system, antibacterial peptide expression volume reached 1.8mg/ml above.
- The key technology to the low expression volume and low stability solved.
Clear features molecular structure is available.

- **The mass spectrometric analysis for** Antibacterial peptide:
  - cecropin consists of 37 amino acid composition, molecular weight of 3.866KD.
  - *Bacillus subtilis* consists of 37 amino acid, molecular weight: 3.879KD, its amino acid sequence is: GLGKAQCAALWLQCASGGTIGCGGGAVACQNYRQFCR.
Figure 1. Antibacterial peptide cecropin mass spectra
Figure 2. Bacillus subtilis bacteria antimicrobial peptide mass spectra
Three-dimensional structure of the two antibacterial peptides

Figure 3 cecropin three-dimensional structure

Figure 4 Bacillus subtilis three-dimensional structure
③ Complete the antibacterial peptide cecropin and subtilin part antimicrobial **spectrum and stability** test

Table 1, cecropin antibacterial peptides against pathogenic bacteria of the minimum inhibitory concentration

<table>
<thead>
<tr>
<th>Division bacteria</th>
<th>Minimum inhibitory concentration(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em> K12D31 (大肠杆菌K12D31)</td>
<td>1.8</td>
</tr>
<tr>
<td><em>E.coli</em> K88 (大肠杆菌K88)</td>
<td>2.0</td>
</tr>
<tr>
<td><em>E. coli</em> K99 (大肠杆菌K99)</td>
<td>2.0</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> (鼠伤寒沙门氏菌)</td>
<td>8.0</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em> (肠炎沙门氏菌)</td>
<td>16.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (金黄色葡萄球菌)</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em> (粪链球菌)</td>
<td>24.0</td>
</tr>
</tbody>
</table>
Table 2. Minimum inhibitory concentration of the Bacillus subtilis against pathogenic bacteria

<table>
<thead>
<tr>
<th>Division bacteria</th>
<th>Gram attributes</th>
<th>Minimum inhibitory concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphyloccocus aureus CMCC26003</td>
<td>G+</td>
<td>1.3</td>
</tr>
<tr>
<td>Staphyloccocus aureus CVCC1882</td>
<td>G+</td>
<td>4.69</td>
</tr>
<tr>
<td>Staphyloccocus aureus ATCC29213</td>
<td>G+</td>
<td>23.43</td>
</tr>
<tr>
<td>Staphyloccocus aureus ATCC25923</td>
<td>G+</td>
<td>11.7</td>
</tr>
<tr>
<td>Staphyloccocus aureus ATCC43300</td>
<td>G+</td>
<td>187.5</td>
</tr>
<tr>
<td>Staphyloccocus aureus 野生株（吉林）</td>
<td>G+</td>
<td>23.43</td>
</tr>
<tr>
<td>Staphyloccocus aureus 野生株（华中）</td>
<td>G+</td>
<td>11.7</td>
</tr>
<tr>
<td>A.hydrophila （嗜水气单胞菌）</td>
<td>G−</td>
<td>46.9</td>
</tr>
<tr>
<td>B.cereus （蜡样芽孢杆菌）</td>
<td>G+</td>
<td>0.2</td>
</tr>
<tr>
<td>Vibrio anguillarum （鳗弧菌）</td>
<td>G−</td>
<td>12.8</td>
</tr>
<tr>
<td>C.perfringens （魏氏梭菌）</td>
<td>G+</td>
<td>1.4</td>
</tr>
</tbody>
</table>
pH value effect on the activity of two antibacterial peptide

**Figure 9** pH value effect on the activity of antibacterial peptide cecropin

**Figure 10** pH value on effect of Bacillus subtilis bacteria activity
Antimicrobial peptides-activity feed products development

- Obtained pure purity greater than 99.5% of antibacterial peptide, established two methods for detection of antimicrobial peptides, complete isolation and purification of antibacterial peptide cecropin pilot.

- Completed the feed safety evaluation of antibacterial peptide cecropin, established product quality standards.
Antibacterial assay
抑菌分析

1: control with no insert; 2-6: positive recombinants induced 72 h
1：对照；2-6：重组菌诱导72 h的正对照组（抗菌肽）
Studies on other bioactive peptides and defense peptides

① Recombinant expression of host defense peptides of Palustrin-OG2

Improved OG2 peptide expression in Escherichia coli, purification and activity determination.

② Recombinant expression of Apidaecin (蜜蜂肽) and Spheniscin (企鹅防御肽).

③ Recombinant expression of BuforinII (牛乳铁蛋白素) in the photorhabdus luminescens.
5. Herbal extracts
(Astragalus polysaccharide, APS)
天然提取物（黄芪多糖）
5. Chinese herbal extracts

- Practice has proved it can promote appetite, enhance immunity, prevent and cure diseases etc..
- Traditional Chinese medicine additives is over 50 kinds.
- Slow effect, Much required dose and antibiotics-like effects.
- The main problems: bad palatability and high cost.
- Two important representatives: oregano oil and *Astragalus polysaccharide*. 
APS Hypothesis

Immune suppress (cyclophosphamide)
免疫抑制（环磷酰胺，CY）

Disorder of GH/IGF-I axis
GH / IGF-I轴异常

APS
黄芪多糖

Immune enhancement
免疫增强

Immune type
免疫类型

Cell culture
细胞培养

Inflammation reaction
炎症反应

Inflammation factor secretion
炎性因子分泌

+ + + +
Effects of APS on ratio of CD4⁺/CD8⁺ and Lymphocyte proliferation in piglets

CD4⁺/CD8⁺

(Linear $P < 0.05$)

(Linear $P < 0.05$)

Improve cell immune function, the optimal APS level was 500 mg/kg

APS在断奶仔猪日粮中的最适添加量为500 mg/kg

Effects of dietary supplement of APS on serum cytokines in weaned pigs received cyclophosphamide (CY)

APS（注射环磷酰胺，CY）对断奶仔猪血清细胞因子的影响 (pg/mL)

<table>
<thead>
<tr>
<th></th>
<th>−APS</th>
<th>+APS</th>
<th>−APS</th>
<th>+APS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>55.4</td>
<td>79.8</td>
<td>50.6</td>
<td>77.8</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>133.3</td>
<td>160.1</td>
<td>111.7</td>
<td>138.9</td>
</tr>
</tbody>
</table>

- Reverse CY-induced cell immune suppress and humour immune suppress

Effects of APS on serum index in piglets

APS对仔猪血清指标的影响

- Recover the normal function of GH/IGF-I axis
- APS通过降低炎性细胞因子、GH和皮质醇的含量，增加抗炎性因子的合成，恢复GH/IGF-I轴的正常而发挥其免疫抗炎作用

<table>
<thead>
<tr>
<th></th>
<th>−LPS</th>
<th>+LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>−APS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+APS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>115.3</td>
<td>113.0</td>
</tr>
<tr>
<td>GH</td>
<td>3.82</td>
<td>3.83</td>
</tr>
<tr>
<td>IGF-I</td>
<td>235.6</td>
<td>240.0</td>
</tr>
</tbody>
</table>

Effects of APS on NO and iNOS activity in piglets

- Increase NO and iNOS activity of the peripheral blood lymphocytes in piglets
  - APS显著增加仔猪外周血淋巴细胞NO和iNOS的活性，并呈剂量依赖性

*J. Anim. Sci., X. F. Mao, Defa Li, 2005*
Effects of APS on different cytokines

APS对不同细胞因子的影响

- Increase the IL-2 and IFN-γ secretion
  - APS可在体外促进细胞因子IL-2和IFN-γ的分泌，这可能是APS的免疫调节作用之一

Mechanism of immune function of APS

APS的免疫作用机理

Gene knockout
基因敲除

TLRs Toll 样受体

Signal transduction pathway
信号传导通路

细胞因子表达

IL-2 etc.

IFN-γ

淋巴细胞增殖

改善动物免疫

APS

Receptor

NO

iNOS

细胞因子表达

IL-2 etc.

IFN-γ

淋巴细胞增殖

改善动物免疫
6. Acidifying agent

- Research shows, lactic acid, citric acid or acetic acid and other organic acids can promote the animal appetite, improve weight gain and feed conversion. However, there are some problems of feed supply organic acids must be considered, such as corrosion of equipment and higher cost.

- **Acidification agent application in China feed industry is still in the initial stage, usage and methods have not yet be standardized.**

- Organic acid is added in feed and feed formula to consider the compatibility, high protein and salt minerals will be of the acid buffer action; moreover some special acidification agent will cause the animal reduction of feed intake.
5. Conclusions
1. Wide variety of antibiotic replacements available.

2. Each product or class has distinct characteristics, responses, advantages, and disadvantages.

3. Scientific literature increasing worldwide, and effectiveness of products is continually improving.
Conclusions

Recombinant technique
重组DNA技术

Extraction
提取

Microbiology
微生物学

Probiotics, Antimicrobial peptides, Prebiotics, Herbal extracts, Enzyme preparations, Acidifying agents
酶制剂，天然提取物，益生元，抗菌肽，寡糖，酸化剂

Safety Animal products
绿色畜产品

Antibiotics
抗生素
Thank you for your attention!

The End