State of Genetically Modified Pigs for Agriculture in China

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2021-08-31
Pork is the main meat food in China (65%) 

China consumes more than 50% pork of the World 
World: about 1.4 billion pigs; China: about 0.7 billion pigs

The number of slaughtered pig in the world
The number of slaughtered pig in China
China has no native breeds of pigs with high productivity

Import 20 thousand elite boars from other countries each year
The Chinese government attaches importance to generation of genetically modified pig strains with favorable traits for agriculture

1. Setting up National special funding for transgenic livestock

2. Setting up big pig facilities for large scale characterization of GM pigs

3. More than 15 research teams working on GM pigs
The genetical modification of pigs can be made to:

✓ Promote growth: GH, IGF
✓ Improve meat quality: FAT1, FAT2, MSTN
✓ Reduce environmental emissions: NSP-degrading and phytatea-degrading enzymes
✓ Gain resistance to pathogens or other environmental stress
1. Genetical Modification of pigs for promoting growth
Over expression of growth hormone

Significant improvements in both daily weight gain and feed efficiency

<table>
<thead>
<tr>
<th>Group</th>
<th>Average daily weight gain (g)</th>
<th>Feed efficiency (kg feed/kg gain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Founder animals*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>743 ± 32 (6)</td>
<td>3.12 ± 0.15</td>
</tr>
<tr>
<td>Transgenic</td>
<td>690 ± 65 (6)</td>
<td>2.62 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>*P = 0.480</td>
<td>*P = 0.026</td>
</tr>
<tr>
<td>37-06 G2 progeny†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>760 ± 24 (8)</td>
<td>2.99 ± 0.12 (8)</td>
</tr>
<tr>
<td>Transgenic</td>
<td>874 ± 30 (5)</td>
<td>2.46 ± 0.16 (5)</td>
</tr>
<tr>
<td></td>
<td>*P = 0.016</td>
<td>*P = 0.026</td>
</tr>
<tr>
<td>37-06 G3 progeny‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>867 ± 21 (15)</td>
<td>ND</td>
</tr>
<tr>
<td>Transgenic</td>
<td>933 ± 31 (8)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>*P = 0.098</td>
<td></td>
</tr>
<tr>
<td>31-04 G2 progeny§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>869 ± 44 (7)</td>
<td>ND</td>
</tr>
<tr>
<td>Transgenic</td>
<td>988 ± 62 (7)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>*P = 0.15</td>
<td></td>
</tr>
<tr>
<td>Combined progeny</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>815 ± 17 (30)</td>
<td>ND</td>
</tr>
<tr>
<td>Transgenic</td>
<td>905 ± 21 (20)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>*P = 0.001</td>
<td></td>
</tr>
</tbody>
</table>

a marked reduction in subcutaneous fat

pathological changes

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transgenic</td>
</tr>
<tr>
<td>Gastric ulcers</td>
<td>5/5</td>
</tr>
<tr>
<td>Synovitis</td>
<td>4/5</td>
</tr>
<tr>
<td>Cardiac myocyte nuclear hypertrophy</td>
<td>4/5</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>4/5</td>
</tr>
<tr>
<td>Nephritis</td>
<td>3/5</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3/5</td>
</tr>
</tbody>
</table>

Science, 1989
The transgenic pig with controllable expression of growth hormone

the pCAGGS-rtTA-TRE-GH12 (pTTGH) vector

Growth rates and feed efficiencies

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*Scientific Reports, 2015*
Editing porcine *IGF2* regulatory element to improve meat production in Chinese Bama pigs

**IGF2**, an important growth factor which affects skeletal muscle and fat deposition

*IGF2*-intron 3–nucleotide 3072:

5′-GCTC[G]C-3′, the G allele, recognized by repressor ZBED6, negatively regulates *IGF2* expression

**Experiment Design**
Growth performance of founder and F1 gene-edited pigs

Meat quality and other phenotypes

Cellular and Molecular Life Sciences, 2018
2. Improvement of the pig meat quality
Expression of FAD2 in transgenic pigs promotes synthesis of polyunsaturated fatty acids

Mammalians lack the desaturases required for synthesis of polyunsaturated fatty acids

FAD2: a delta12 fatty acid desaturase from spinach

The coding region of cDNA for a 12 desaturase (FAD2) from Arabidopsis thaliana

fatty acid composition of accumulated lipids in pig adipose cells.
The delta-15 desaturase (fat1) gene pigs synthesize n-3 PUFAs from n-6 PUFAs

Transgenic pigs with hfat-1, humanized Caenorhabditis elegans gene, encoding an n-3 fatty acid desaturase

the polyunsaturated fatty acid profiles of total lipids

<table>
<thead>
<tr>
<th>Table 1</th>
<th>n-3 and n-6 fatty acids concentration and n-6/n-3 ratios in tail samples from hfat-1 transgenic and wild-type piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acids in tails</td>
<td>Transgenic piglets (n = 8)</td>
</tr>
<tr>
<td>ALA (18:3 n-3, %)</td>
<td>0.94 ± 0.10</td>
</tr>
<tr>
<td>EPA (20:5 n-3, %)</td>
<td>4.21 ± 0.60</td>
</tr>
<tr>
<td>DPA (22:5 n-3, %)</td>
<td>1.69 ± 0.19</td>
</tr>
<tr>
<td>DHA (22:6 n-3, %)</td>
<td>1.75 ± 0.23</td>
</tr>
<tr>
<td>Total n-3 FA (%)</td>
<td>8.59 ± 0.84</td>
</tr>
<tr>
<td>Total n-6 FA (%)</td>
<td>14.28 ± 1.31</td>
</tr>
<tr>
<td>n-6/n-3 ratio</td>
<td>1.69 ± 0.30</td>
</tr>
</tbody>
</table>
Co-expression of fat1 and FAD2 in transgenic pigs

Endogenously produced n-6 PUFAs was used as substrates to synthesize n-3 PUFAs
Genes related to muscle mass: **myostatin (MSTN)**, negatively regulates skeletal muscle cell proliferation

*MSTN*-edited pigs to overcome lameness and sustainably improve nutritional meat production

Alternative edit-site-based solution avoids ER stress and overcomes the hindlimb weakness
Similar feed-conversion ratio

No dystocia or significant effects on the maternal reproductive traits

different trends of change for lean and fat rates
3. Reduction of environmental impact

Only 1/3 of feed nitrogen and phosphorus were utilized from feedstuff diets in pig production. Inefficient feed digestion can cause serious nutrient emissions to the environment.

- Phytates, negatively charged saturated cyclic acids, bind to positively charged molecules in the diet such as minerals and protein, thereby reducing nutrient digestibility and increasing discharge of the unabsorbed nutrients to the environment.

- Pigs are inherently incapable of digesting Non-starch polysaccharides that are primarily present in plant cell walls.
Salivary gland-specific expression of phytase

Phytate phosphorus passes undigested, most important manure pollutant

Phytase: allows the pigs to digest the phosphorus in phytate

Phytase transgene constructs

◆ salivary gland-specific expression promoter: PSP

Total phosphorus content of fecal matter

Nature Biotechnology, 2001
Expression of three microbial enzymes, b-glucanase, xylanase, and phytase in the salivary glands

Experiment Design

- salivary gland-specific expression promoter: mPSP
- **NSP-degrading enzymes**: two β-glucanases genes (bg17A and eg1314), a xylanase gene (xynB)
- **Phytate-degrading enzyme**: phytase gene (eappA)
fetal nutrient output

Growth performance

tract nutrient digestibility values
4. Stress resistance pigs

- Overexpressing endogenous resistance genes
- Introducing exogenous resistance genes
- Editing pathogens targeting receptor genes
- Introducing synthetical virus-killing genes

- Cold resistance
- Bacterial resistance
- Broad-spectrum virus resistance
- FMDV resistance
- PCV2 resistance
- CSFV resistance
- PRRSV resistance
- TGEV resistance
Generation of adiponectin-UCP1 KI transgenic pigs: Cold resistance transgene pigs

Pigs lack a functional UCP1 gene, resulting in poor thermoregulation and susceptibility to cold.

Decreased Fat Deposition and Increased Adipose Lipolysis in UCP1 KI Pigs
Anti-bacterial transgenic pigs

Lysozyme has broad-spectrum antibacterial activities

- Bacillus subtilis
- Bacillus cereus
- Staphylococcus aureus
- Escherichia coli
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Streptococcus agalactiae
- Salmonella typhimurium
- …

Human lysozyme gene transgenic pigs

Transgenic pigs milk can inhibit growth of *E. coli*

Transgenic pigs milk can inhibit the growth of *E. coli* in the duodenum of sucking pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>Non-transgenic</th>
<th>Transgenic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>7.62±0.24</td>
<td>6.56±0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Jejunum</td>
<td>7.00±0.39</td>
<td>6.85±0.66</td>
<td>0.707</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.70±0.39</td>
<td>7.58±0.43</td>
<td>0.691</td>
</tr>
<tr>
<td>Colon</td>
<td>7.09±0.35</td>
<td>6.77±0.34</td>
<td>0.236</td>
</tr>
</tbody>
</table>

*PLOS ONE, 2014; PLOS ONE, 2015*
Anti-bacterial transgenic pigs

Porcine beta-defensin 2 (PBD-2) can against *Actinobacillus pleuropneumoniae*

*Actinobacillus pleuropneumoniae* is an important respiratory pathogen causing porcine contagious pleuropneumonia

Overexpression of porcine beta-defensin 2 (PBD-2) enhanced resistance to cohabitation infection by *A. pleuropneumoniae*.

Construct of transgenic pigs overexpressing PBD-2

PBD-2 gene is driven by the CAG promoter

Additional graphs and images showcasing the outcomes of the study are included, such as survival rates and tissue samples.
Broad-spectrum antiviral transgenic pigs

1. The transgenic pigs over-expressing myxovirus resistance gene have broad-spectrum antiviral activities

Transgenic pigs can enhance influenza A viral resistance.

Transgenic pigs can against CSFV infection.
Broad-spectrum antiviral transgenic pigs

2. The transgenic pigs express DRACO gene have broad-spectrum antiviral activities

Transgenic pigs can enhance CSFV and PRV resistance

3. Gene editing pigs enhance NLRP3 expression have broad-spectrum antiviral activities

Transgenic pigs can enhance NLRP3 expression during the stimulation
Specific viral resistance transgenic pigs

1. Knockout virus entry receptors CD163 and porcine aminopeptidase N (pAPN) Gene

pigs are resistant to PRRSV and porcine transmissible gastroenteritis virus (TGEV) and decreased susceptibility to deltacoronavirus (PDCoV)

The strategy of antivirus

CD163 and pAPN DKO pigs are completely resistant to genotype 2 PRRSV and TGEV, and also decreased susceptibility to PDCoV.

DKO pigs maintain normal production performance.
Specific viral resistance transgenic pigs

2. Constructing CSFV resistance pigs through knockin the shRNAs of targeting virus RNA

The strategy of antivirus

- siRNA
- Virus mRNA
- Target virus mRNA cleavage
- No progeny

Transgenic pigs exhibit antiviral response during CSFV infection

Others anti-viral transgenic pigs through introducing RNA interference

<table>
<thead>
<tr>
<th>Type of virus</th>
<th>Journal</th>
<th>Organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot-and-mouth disease virus (FMDV)</td>
<td>eLife, 2015</td>
<td>Shihezi University</td>
</tr>
<tr>
<td>Porcine circovirus type 2 (PCV2)</td>
<td>Chinese Journal of Veterinary Science, 2017</td>
<td>South China Agricultural University</td>
</tr>
</tbody>
</table>
Thanks