Biotechnologies for Improving Animal Growth

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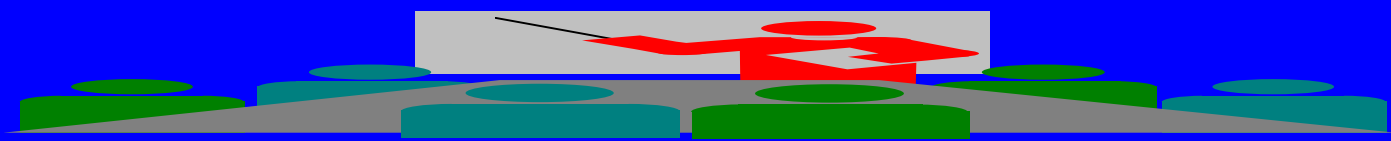

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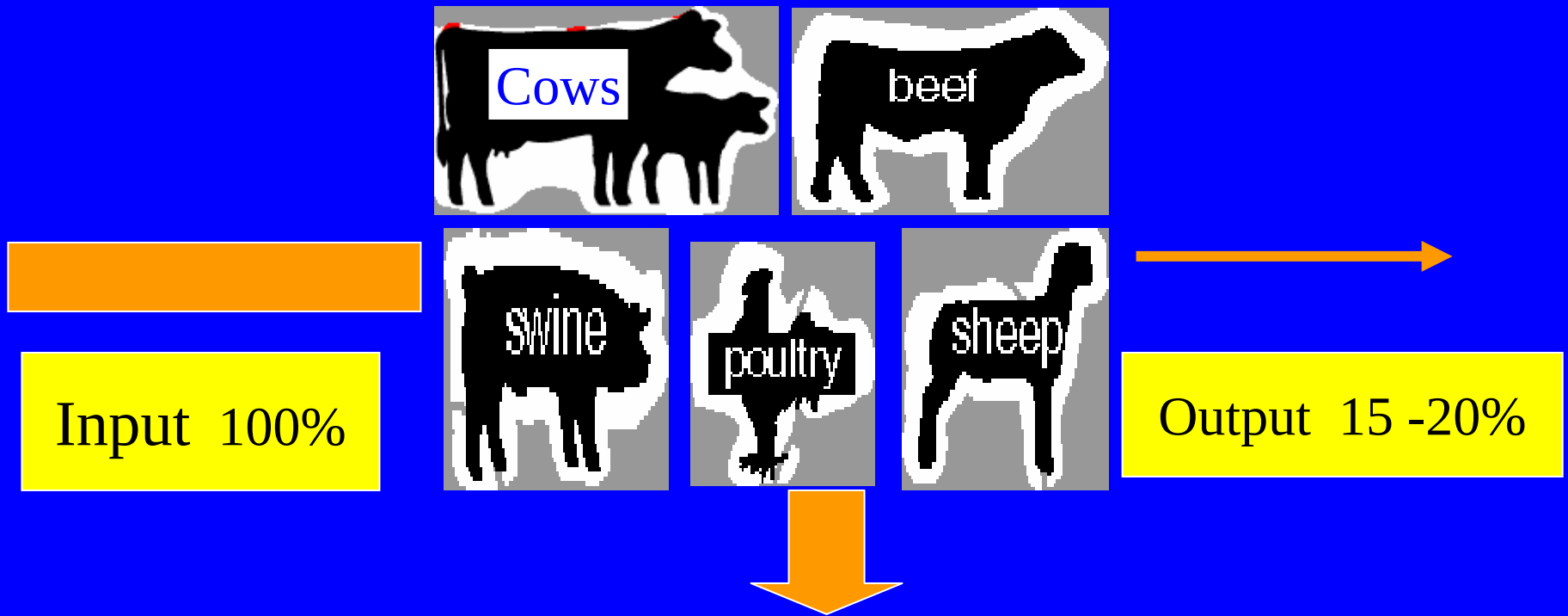
14(12):1794-1802,2001

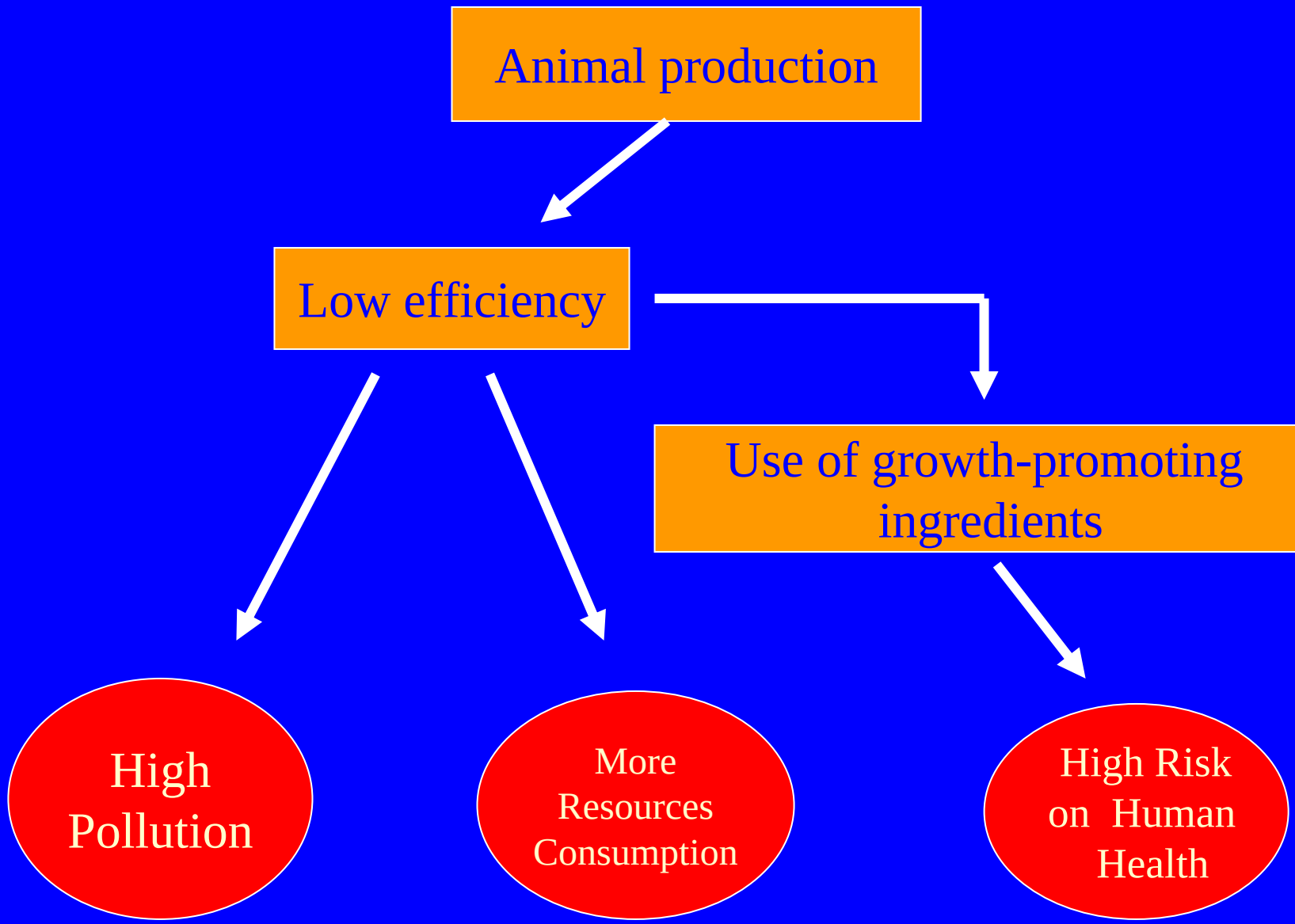
Why is biotechnology needed for animal production?

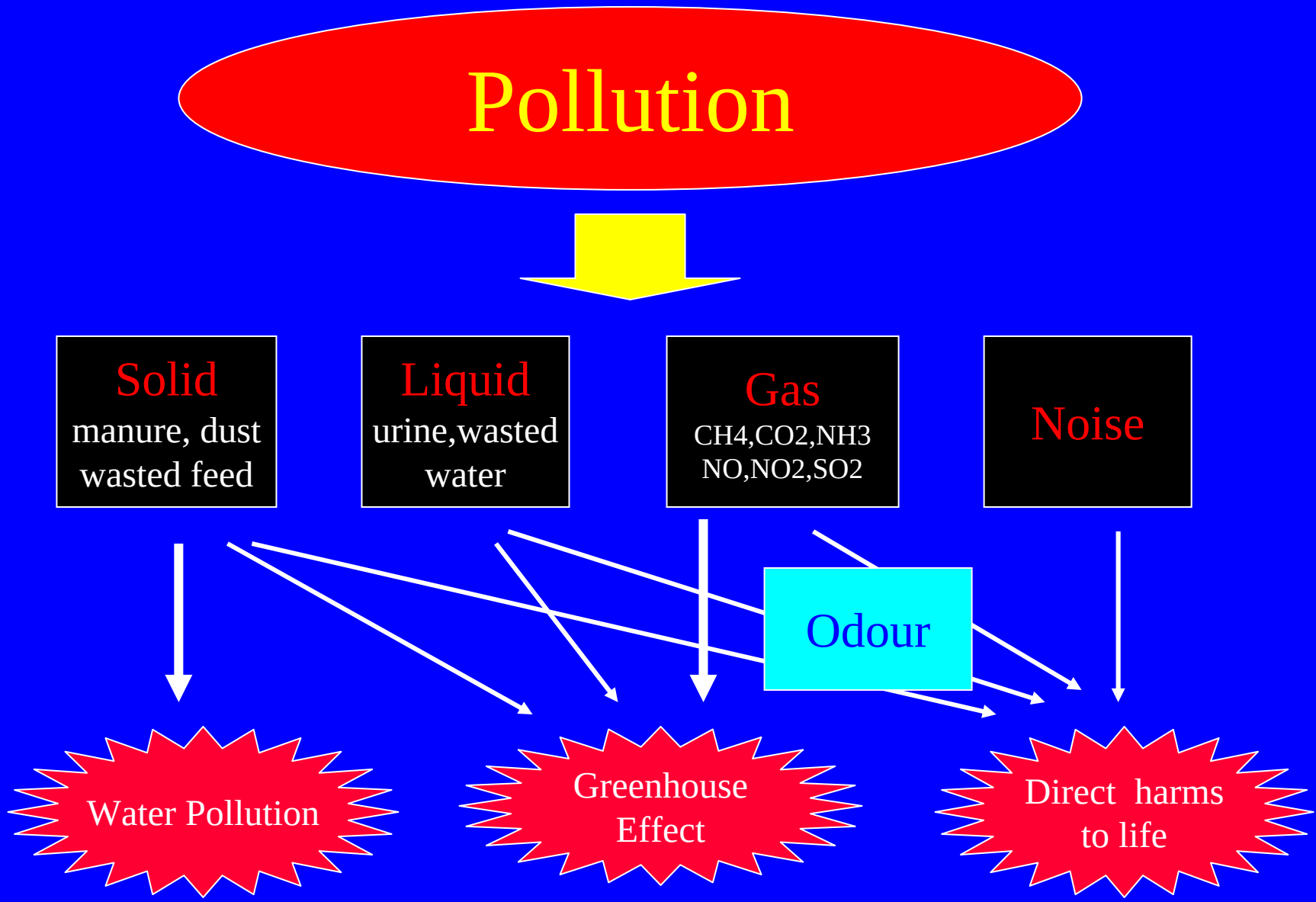
1. Great challenges faced
2. increase of animal protein

Great challenges faced

Flow of nutrients through animals




Animal production
Low efficiency
Use of growth-promoting ingredients
High Pollution
More Resources Consumption
High Risk on Human Health


Pollution
Solid
manure, dust
wasted feed
Liquid
urine,wasted
water
Gas
CH4,CO2,NH3
NO,NO2,SO2
Noise
Odour
Water Pollution
Greenhouse
Effect
Direct harms
to life

Food Safety

- Bovine Spongiform Encephalopathy
- Dioxin contamination of animal feeds
- Drug residues
- Mycotoxins
- E. coli O157:H7
- Salmonella

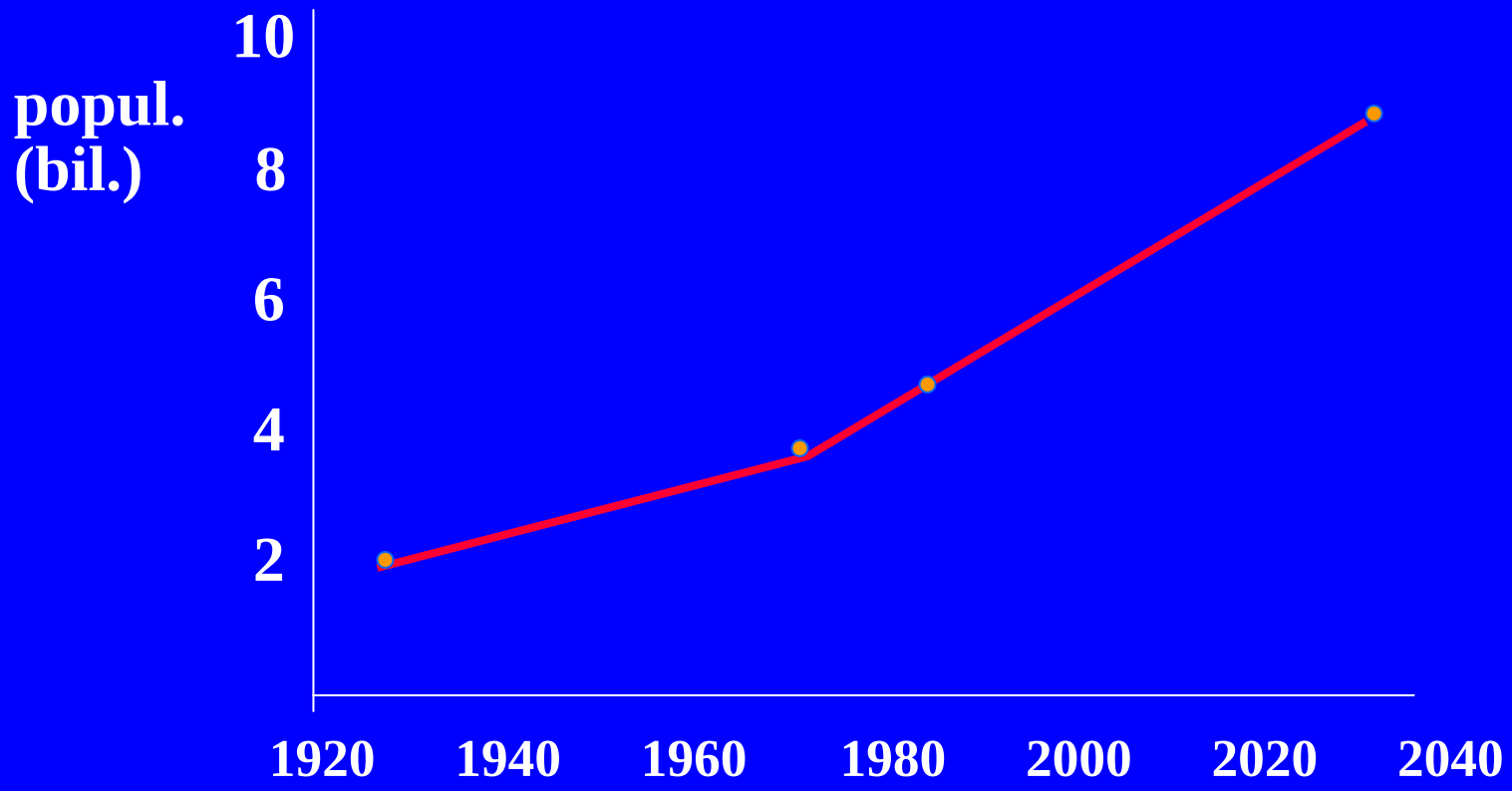
challenges

- Ban of growth promotants (antibiotics)
- Ban of animal feeds(bone and meat meal)

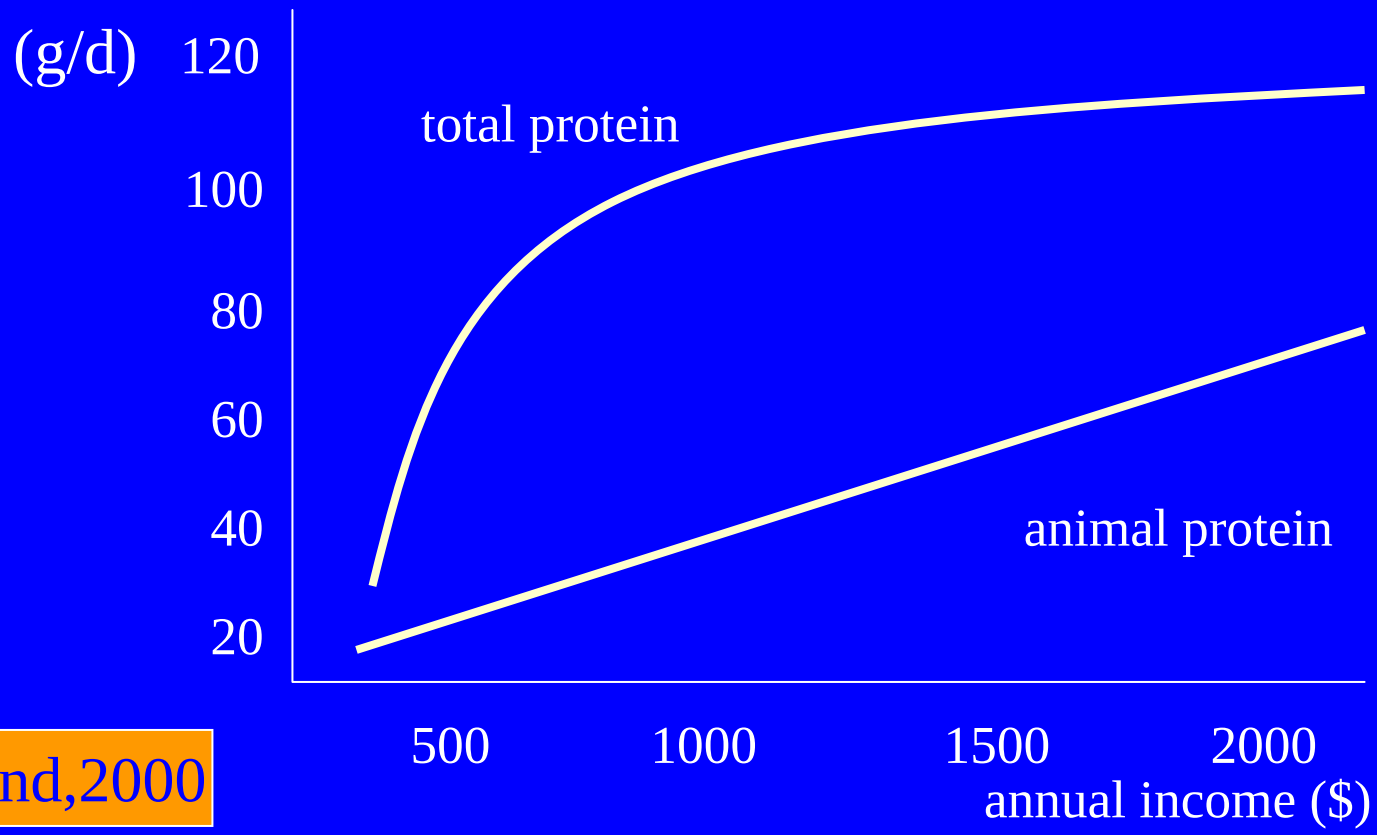
How to maintain productivity ?

1. Great challenges faced
2. increase of animal protein

Increasing World Population



Per capita protein consumption



Pond,2000

Sustainable Animal Agriculture

- Reduce input/ cost.
- Increase output/ productivity.
- Reduce environmental burden/cost.
- Maintain safety.
- Improve quality.
- Increase awareness and confidence
- Maintain competitiveness
- Compatible with other industry

Goal Shift of Animal Agriculture

One goal in the past: **high yield of products**

Multi-goals from now on:

1. **high yield of safe animal products,**
2. **high utilization of natural resources**
3. **minimum environmental pollution**

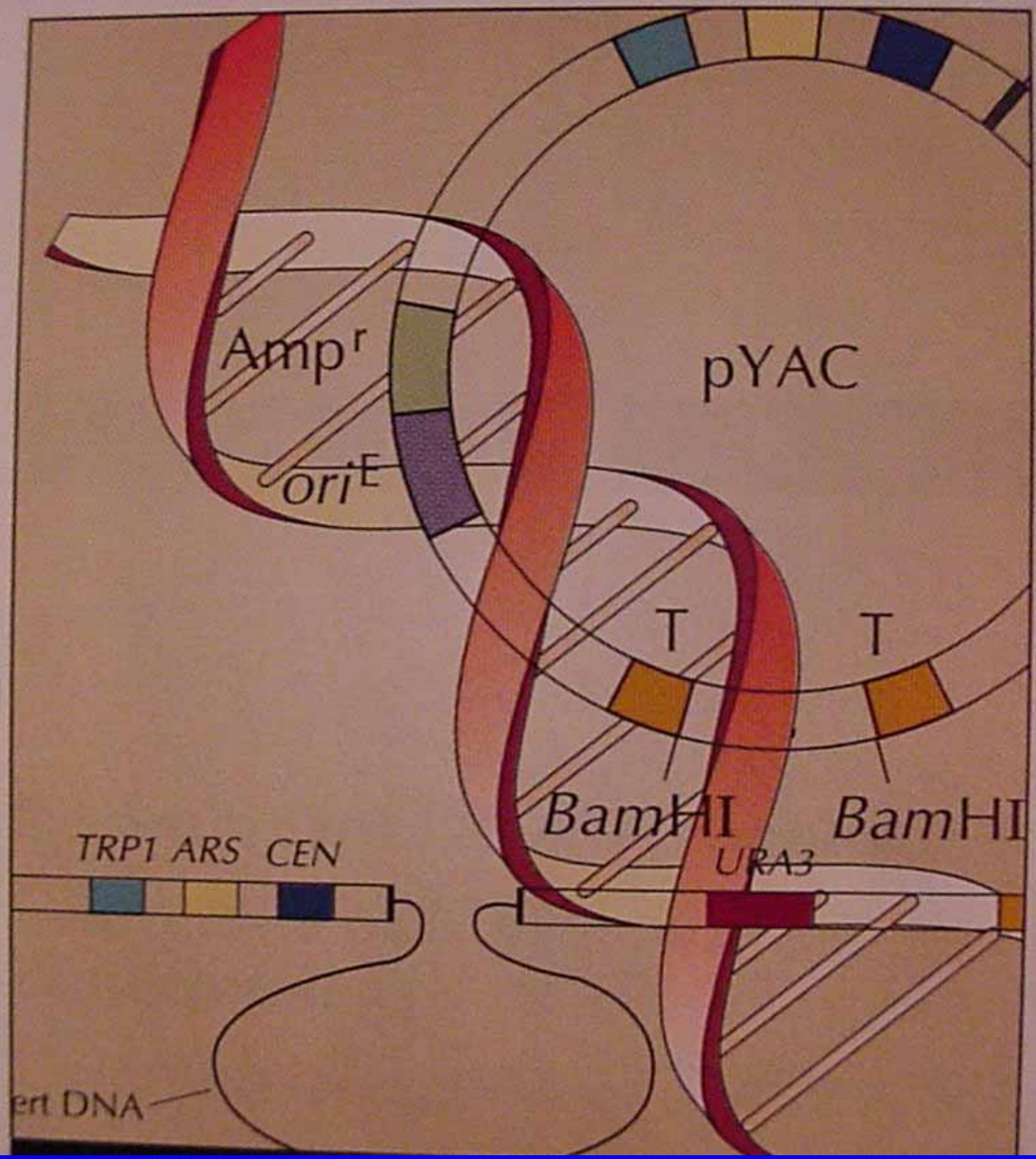
sustainability

Where to go for animal agriculture?

Answer: Biotechnology

biotechnology

biotechnology biotechnology



biotechnology biotechnology

1. Administration of recombinant somatotropin, IGF,and growth hormone-releasing peptides(GHRPs)

Effects of pST in pigs

(Etherton and Bauman,1998)

stage	dose(ug/kg)	ADG (g)	G / F	daily retention (g)	
				Protein	Fat
10-25 kg	0	680	0.61	96	89
	120	680 (0)	0.61 (0)	113 (+17)	61 (-31)
20-50 kg	0	900	0.43	120	207
	150	990 (+10)	0.49 (+13)	150 (+25)	122 (- 41)
50-100 kg	0	1,140	0.33	135	340
	150	1,334 (+17)	0.44 (+33)	235 (+74)	61 (-82)

Injection of exogenous IGF-1

Klindt (1998):

Injection of IGF-1 into Meishan pigs for 28 days

growth rate	22 %
carcass protein accretion	33 %
trimmed lean cuts	5 %

Growth hormone-releasing peptides(GHRPs)

GHRP-6: His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2

GHRP-1: D-Ala-His-D- β Nal-Ala-Trp-D-Phe-Lys-NH_2

GHRP-2: D-Ala-D- β Nal-Ala-Trp-D-Phe-Lys-NH_2

Phung et al.(2000):

pigs of 69 kg

30 μ g GHRP-2 /kg BW s.c.

once daily for 30 days

ADG: 22.35% ($P<0.05$)

G/F: 20.64% ($p<0.01$)

Plasma GH peak concentration :12-15 folds

Administration of ST, IGF and GHRPs

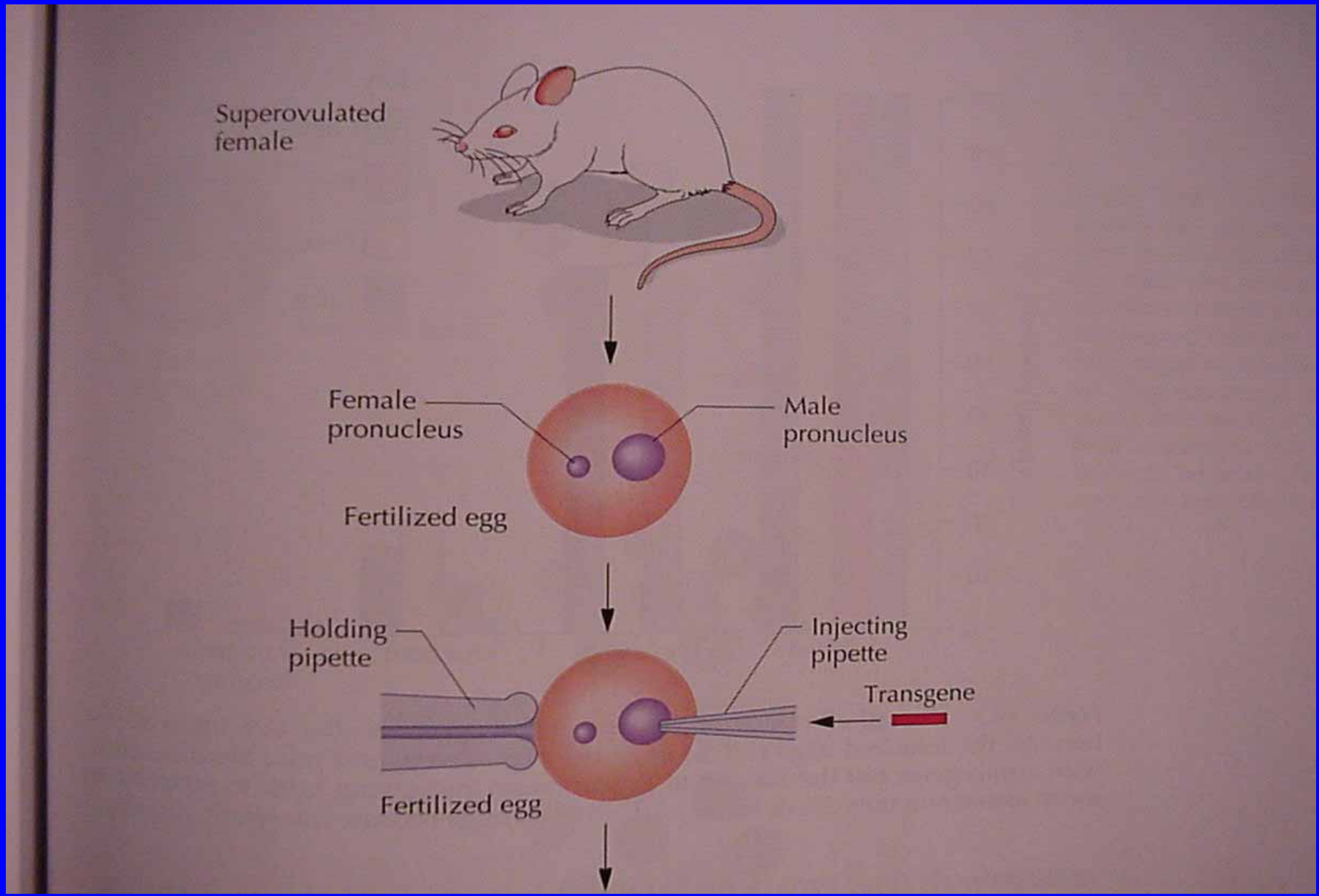
- **Advantage: high efficient**
- **disadvantage:**

  ---intensive labor

  ---high stress on animals

  ---unacceptable by the public

2. Transgenesis


Superovulated female
Female pronucleus
Male pronucleus
Fertilized egg
Holding pipette
Injecting pipette
Transgene
Fertilized egg

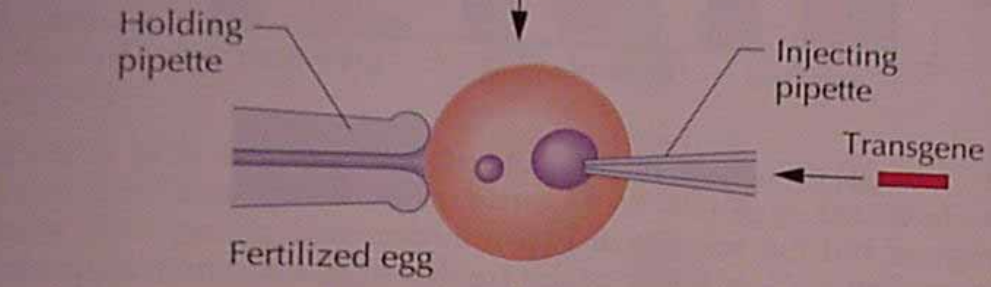


Figure 19.2 Establishing transgenic mice by DNA microinjection. Eggs are ob-

GH-transgenic mouse

Figure 18.40 Transgenic mice. This photograph shows a

GH-transgenic pigs (Pursel and Solomon,1993)：

ADG：13% ↑

G/F： 18%

At 92kg，

total fat in carcass	85%
s- fatty acids	85%
ms-fatty acids	91%
PUFA	66%
IMF in ham	43%
in loin	66%
in shoulder	64%
in belly	69%

↓

Total fat in carcass（%）

BW kg	14	26	48	88
Transgenic	6.19	7.62	7.54	3.27
Control	10.03	12.32	15.50	19.55

Solomon,1992.

- GH-transgenic fish (Chen and Lu,1998) :

  growth rate 30-50%

  G/F: 6-19%

Other transgenes

- GRF、 IGF-1、 cSki
- Lys 、Thr synthases
- cellulase

Problems of gene transfer

low efficiency

health troubles

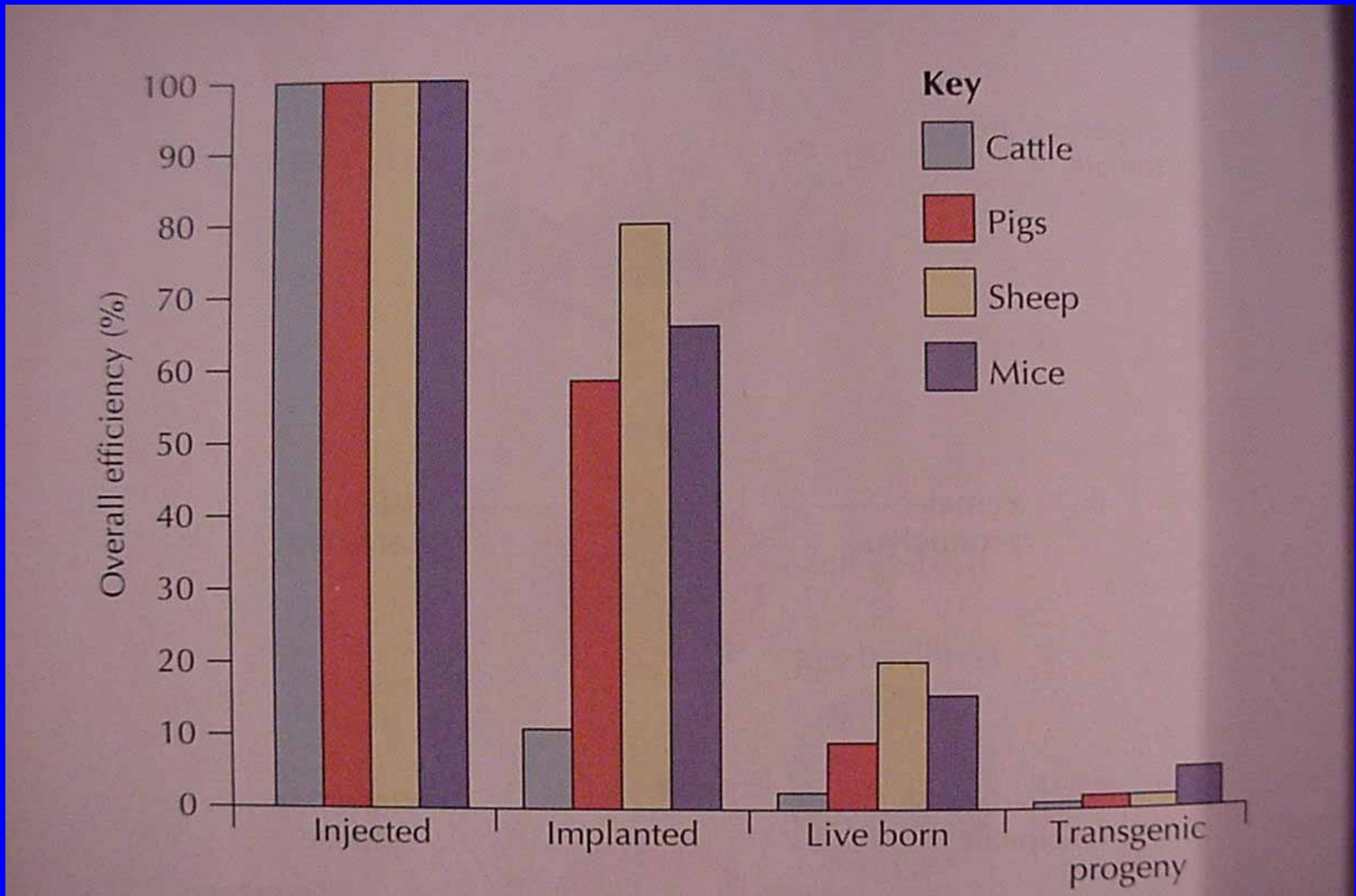


Figure 19.3 Overall efficiency of the transgenesis process after DNA microinjec-

- GH-transgenic pigs had severe health and reproductive problems

3. Gene knockout

GENE KNOCKOUT

Target gene:

general growth-inhibiting gene:

Type Ⅱ IGF receptor gene (Igf2r)

local (tissue-specific) growth-inhibiting gene

myostatin

Gene Knockout

Igf2r knockout mice (Ludwig et al.,1996).

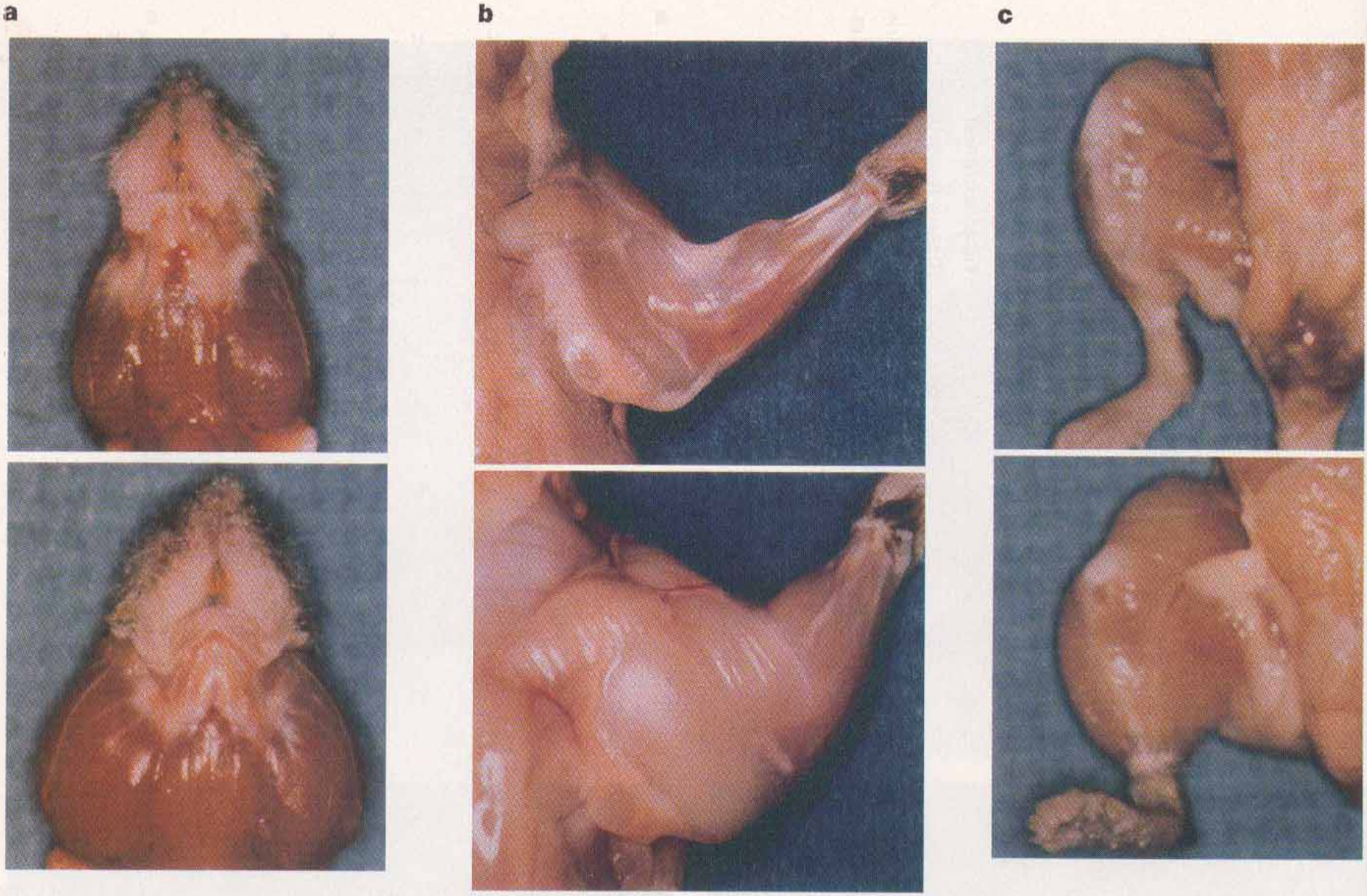
--- IGF-2 levels

--- birth weight being 1.4 times higher

Gene Knockout

myostatin -- knockout mice:

muscle mass : doubled


a
b
c

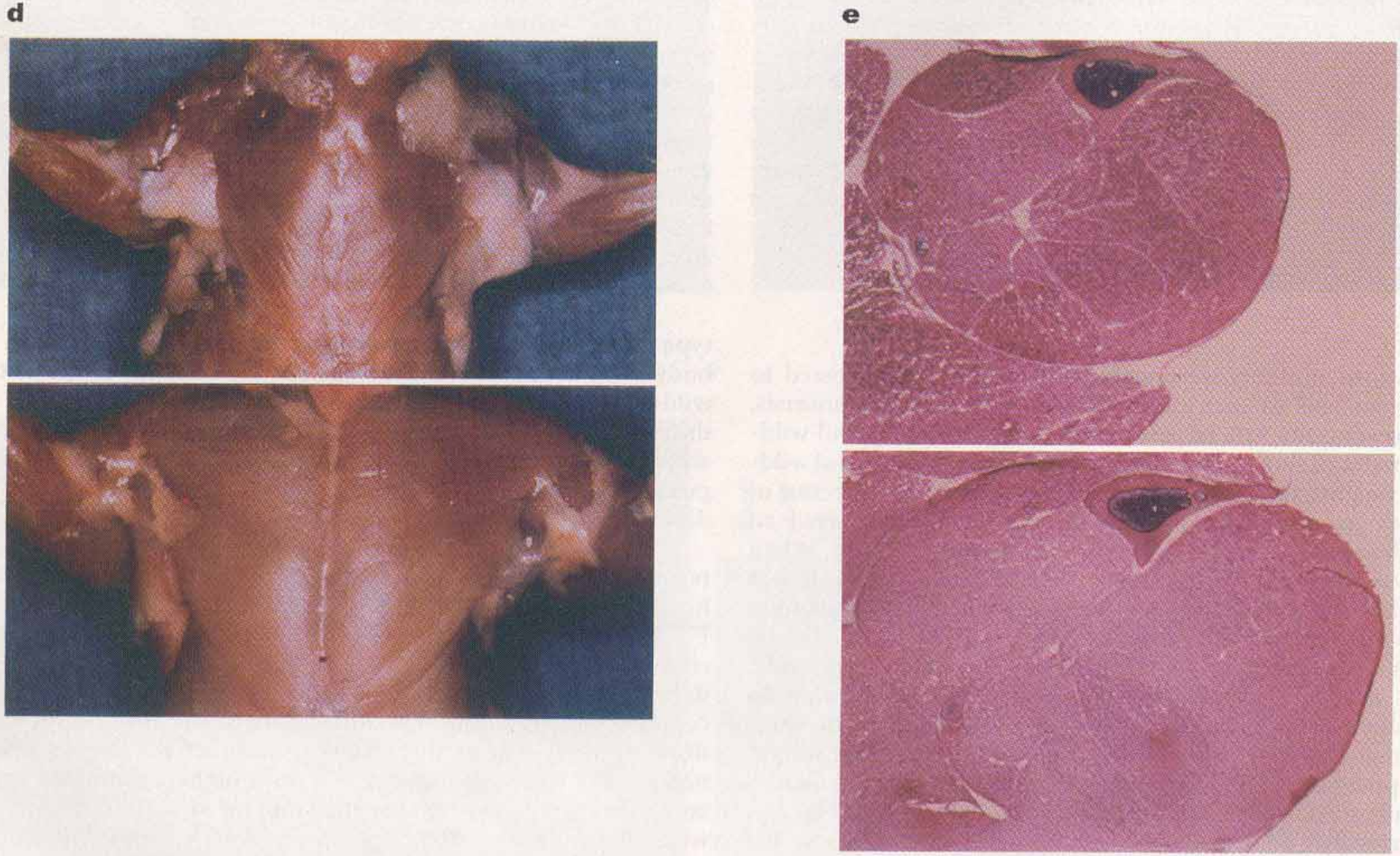


Figure 4 Increased skeletal muscle mass in GDF-8 null mice (bottom panels) compared to wild-type littermates (top panels). **a**–**d**, Facial (**a**), upper limb (**b**), lower limb (**c**) and pectoral (**d**) muscles of skinned animals. **e**, Sections of distal hindlimbs stained with haematoxylin and eosin.

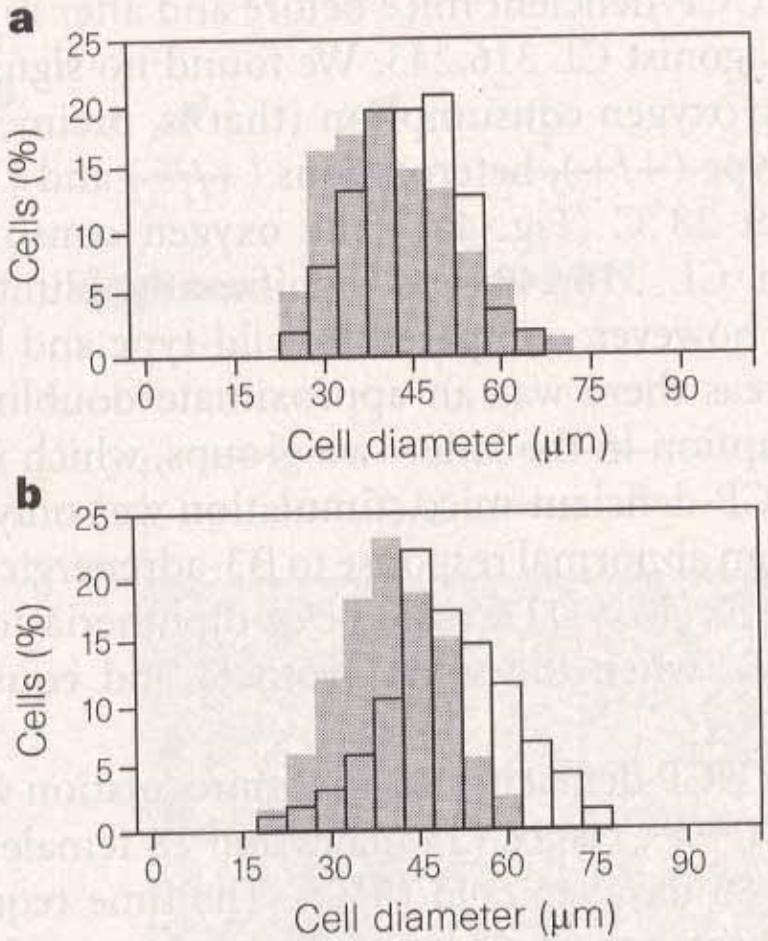


Figure 5 Muscle fibre size distribution in mutant (open bars) and wild-type (shaded bars) animals. Smallest cross-sectional fibre widths were measured for **a**, wild-type ($n = 1{,}761$) and mutant ($n = 1{,}052$) tibialis cranialis or **b**, wild-type ($n = 900$) and mutant ($n = 900$) gastrocnemius muscles, and fibre sizes were plotted as a per cent of total fibre number. Standard deviations were 9 and 10 μm, respectively, for wild-type and mutant tibialis cranialis and 11 and 9 μm, respectively, for wild-type and mutant gastrocneumius muscles.

Gene Knockout

- *in vitro* study(Taylor et al.,2001):

  recombinant MS protein

  inhibited in a dose-dependent manner

  cell proliferation

  DNA synthesis

  protein synthesis

Gene Knockout

- Double-muscled cattle：

  Belgian Blue and Piedmontese

  20-25% more muscle growth

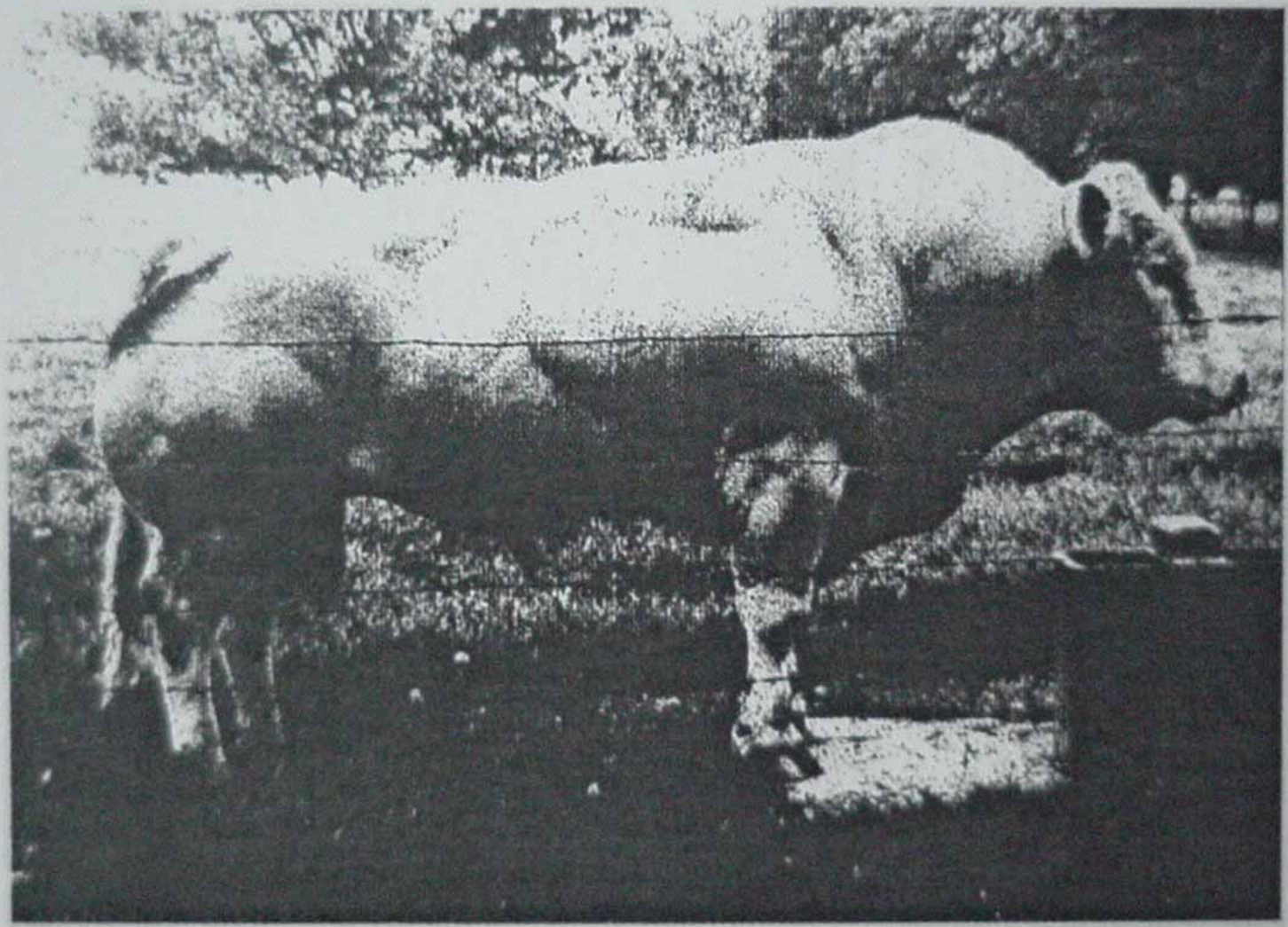


FIG. 2. A fullblood Belgian Blue bull showing the double muscling

Mechanism for double-muscling

- Belgian Blue:

  11 nucleotides in exon 3 deleted

  ⟶ null activity of MS protein.

- Piedmontese:

  guanosine in exon 3 ⟶ adenosine

  ↓

  tyrosine ⟶ cysteine.

quence. One was a C to A transversion in exon 1, resulting in

Polled Shorthorn, 4 Red Angus, 2 Chianina, and 1 Texas

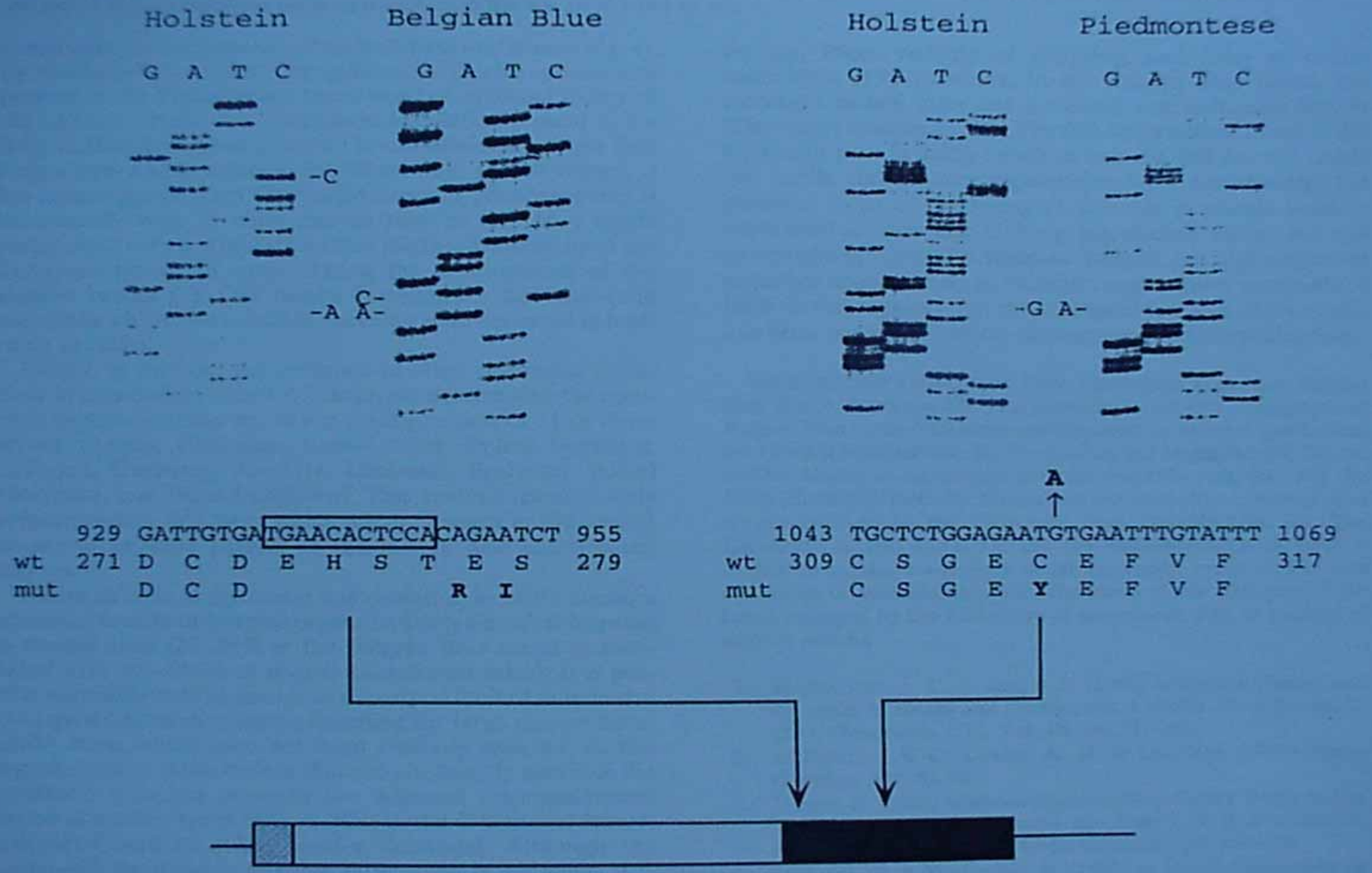


FIG. 3. Myostatin mutations in Belgian Blue (*Left*) and Piedmontese (*Right*) cattle compared with wild-type Holstein cattle. The nucleotides immediately preceding (A936) and following (C948) the Belgian Blue 11-nucleotide deletion are marked. Nucleotide and amino acid sequences

Gene Knockout

In Pigs (Ji et al., 1998):

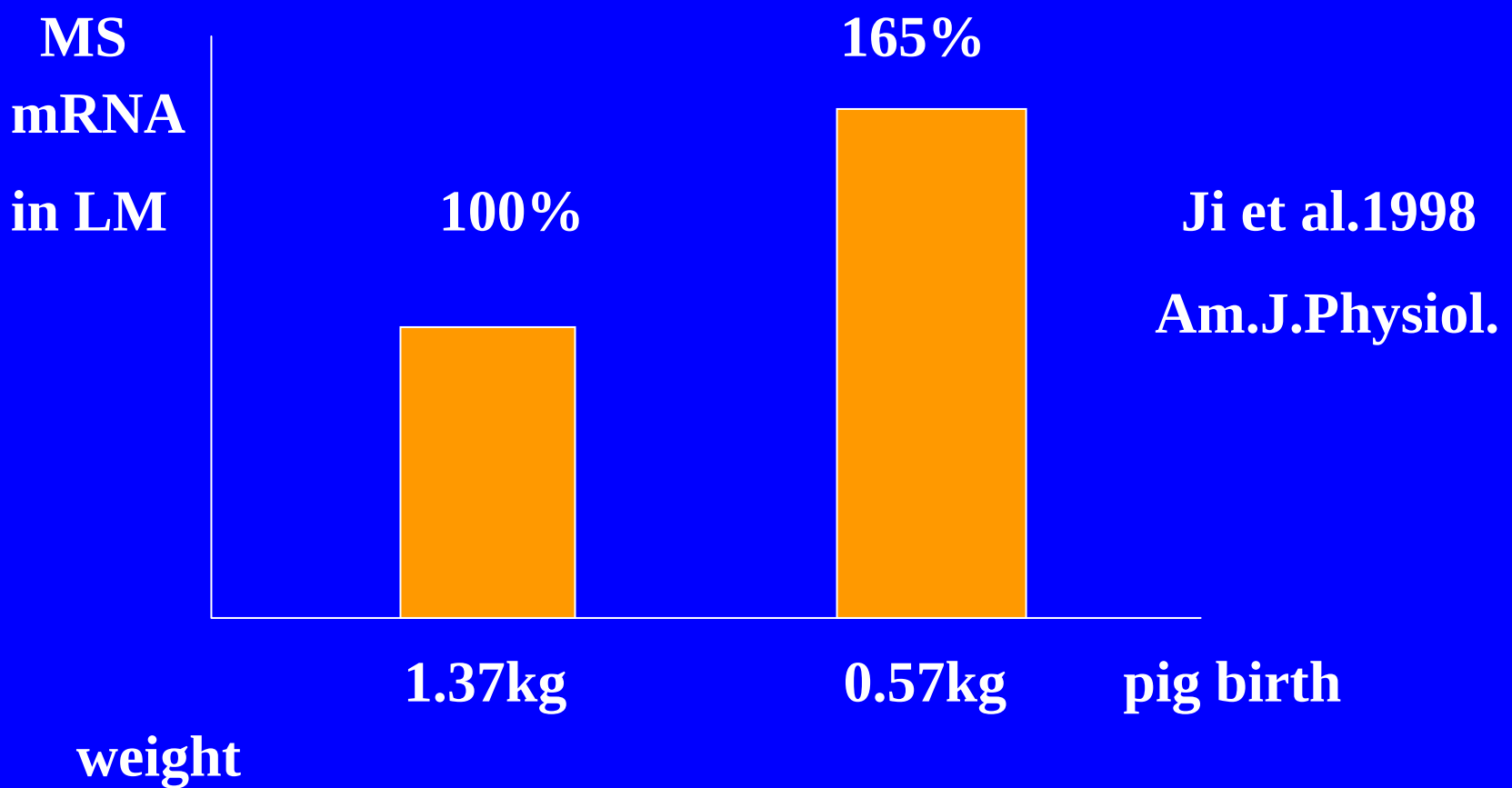
low birth weight (0.57 kg)

vs

high birth weight(1.37kg).

MS expression in LD: 65% higher ($P<0.04$) for low weight

High expression of MS is associated with low birth weight in pigs



4. Immunomodulation

---Immunomodulation of GH

- Active immunization against

  GH，GH receptor、GH binding protein、GHRIH

  → higher GH and anabolic activity

  → higher growth and protein accretion

- pST antibody

  antibody to pST antibody

  synthetic peptide （pGH54-95）

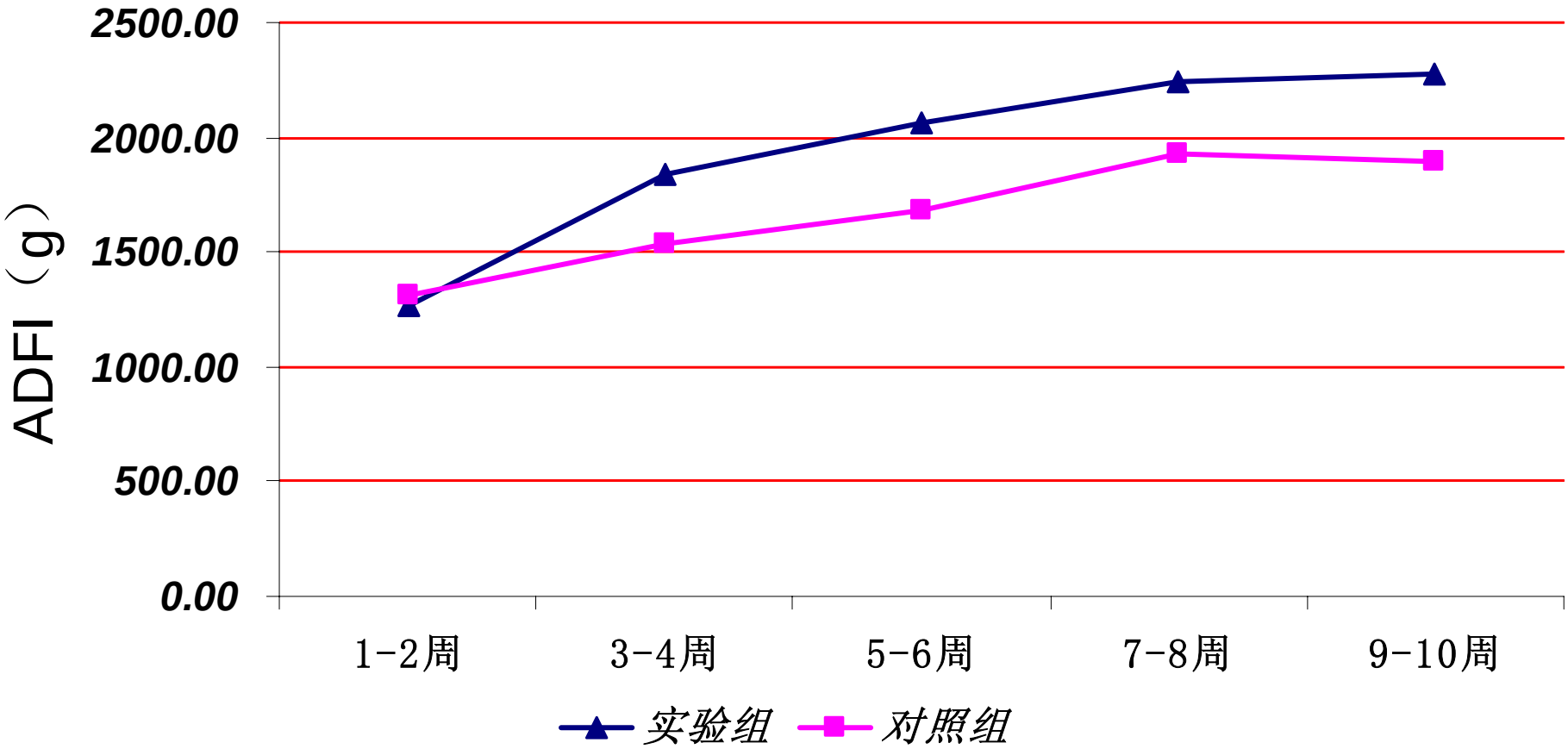
  } better growth

--- immunization against

- IGF-1、IGF-2:
- faster growth， less fat deposition
- adrenocorticotropin: better growth
- LH: removing boar taint
  avoiding surgical castration
  maintaining high leanness and G/F
- adipose plasma membrane:
- more lean gain, less fat gain

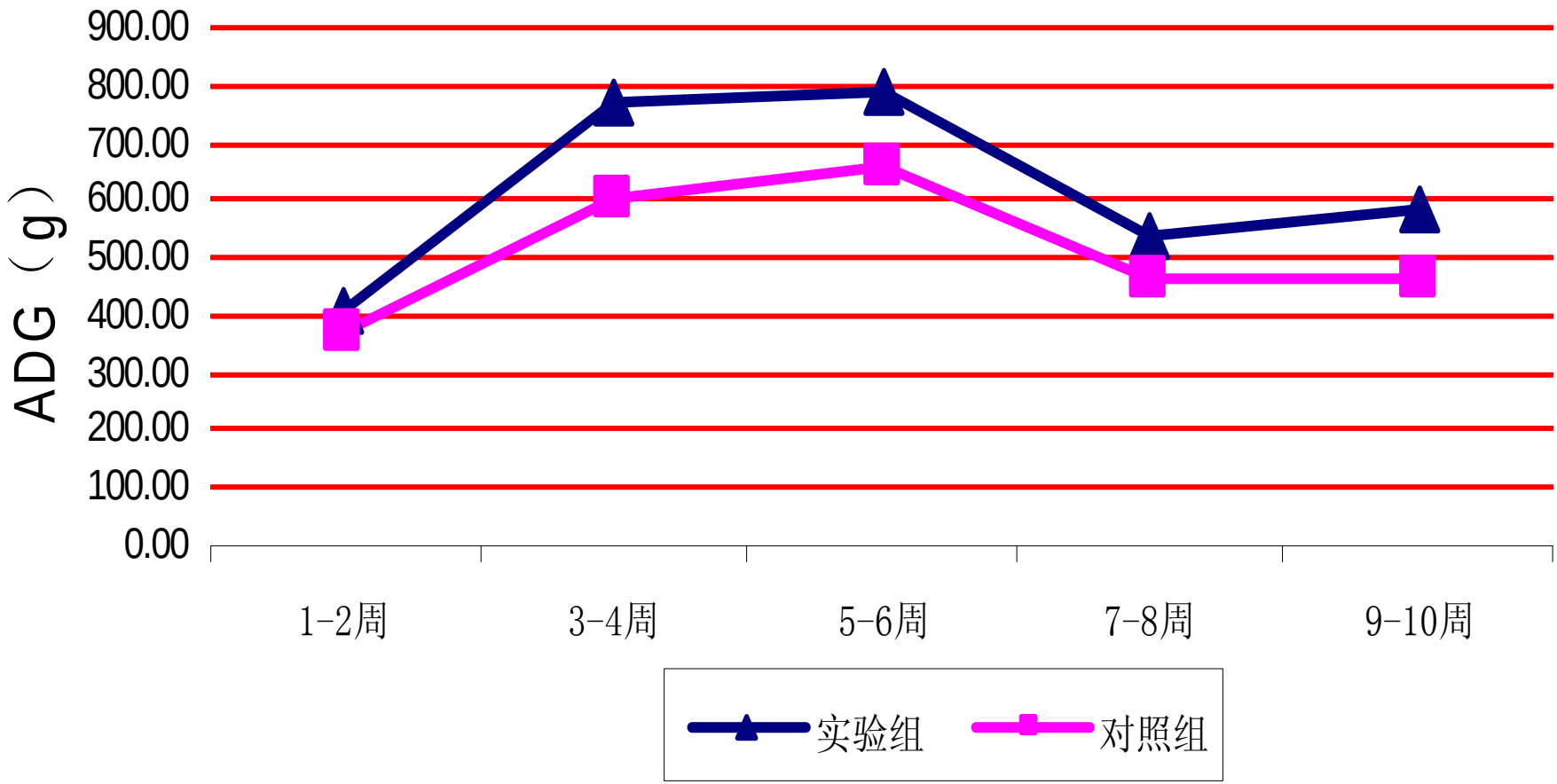
Active immunization against CCK in pigs 0f 27-90 kg

---- feed intake



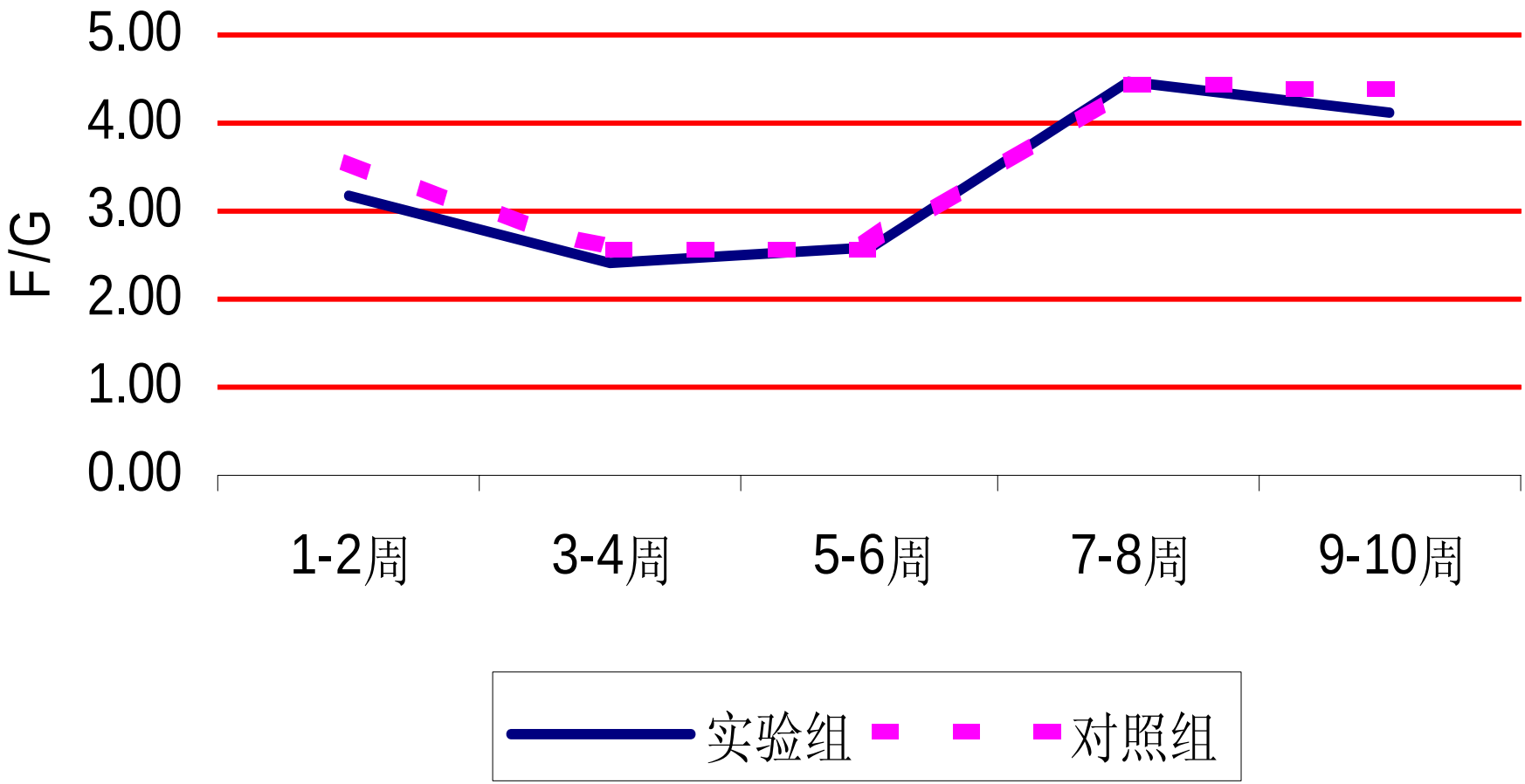
Active immunization against CCK in pigs 0f 27-90 kg

----ADG



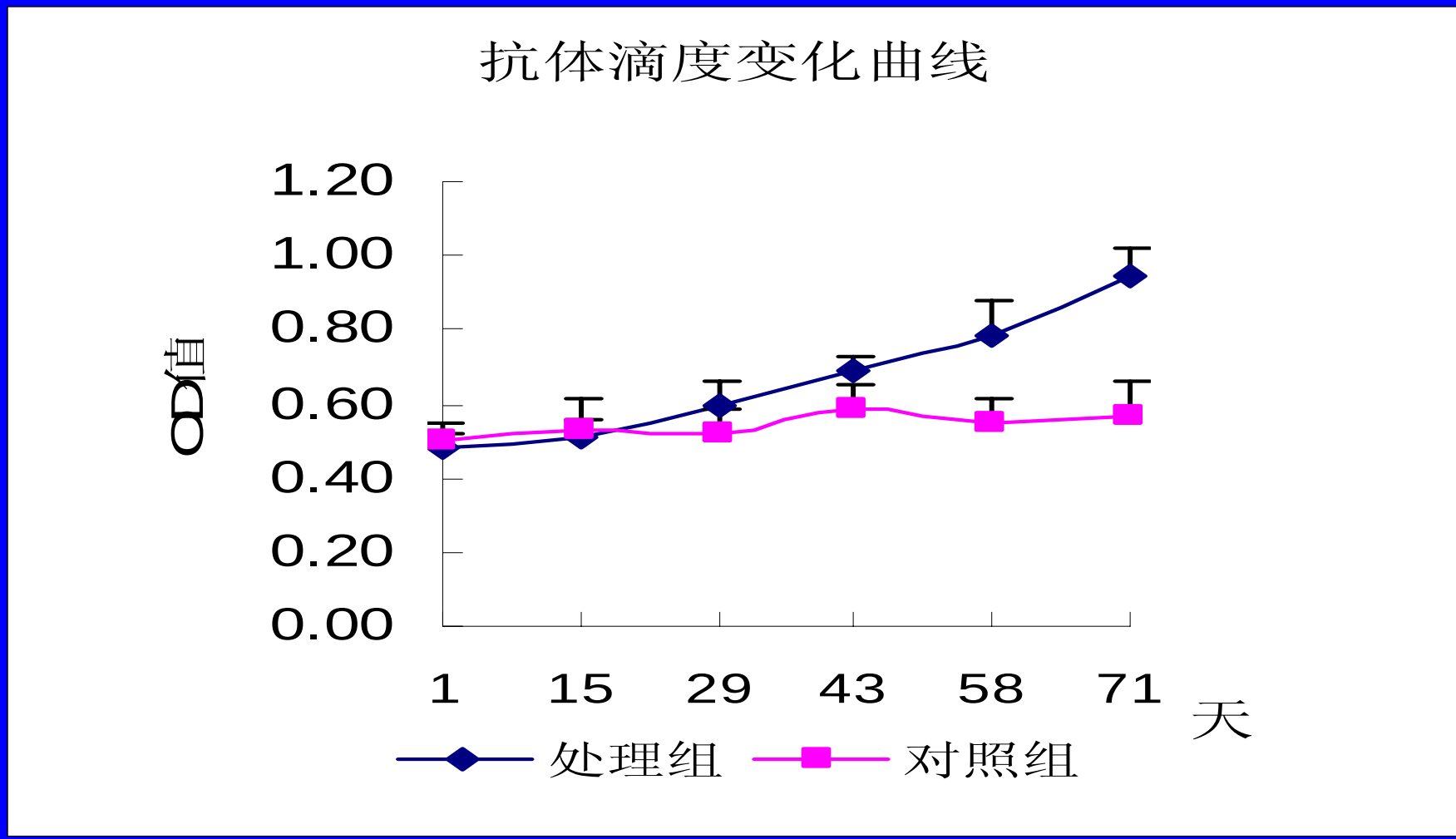
Active immunization against CCK in pigs 0f 27-90 kg

--- F/G



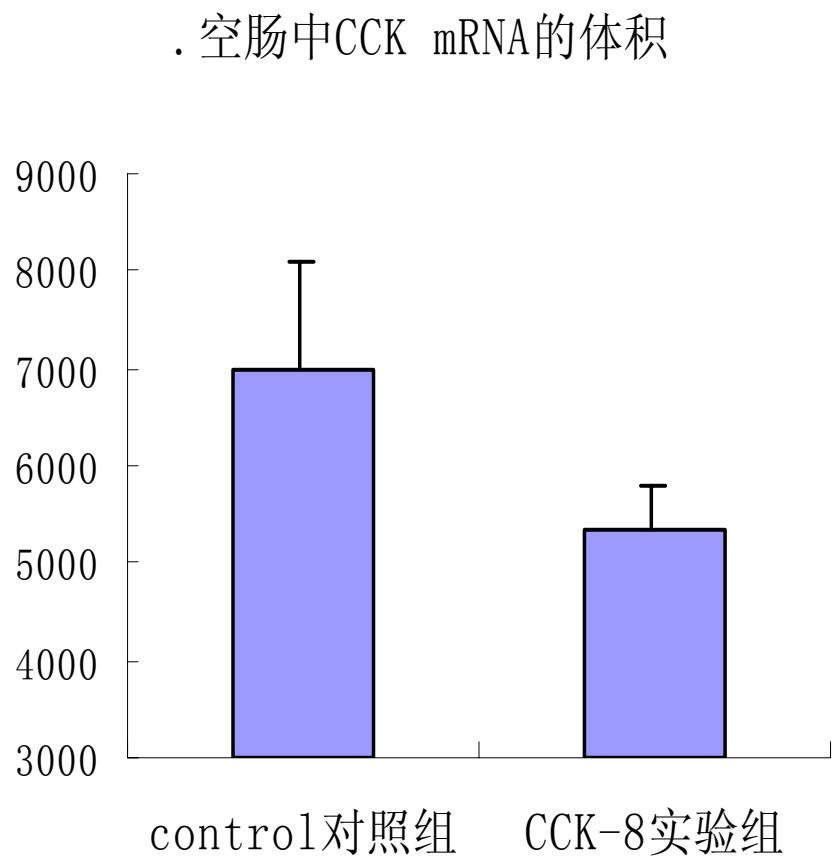
Active immunization against CCK in pigs 0f 27-90 kg

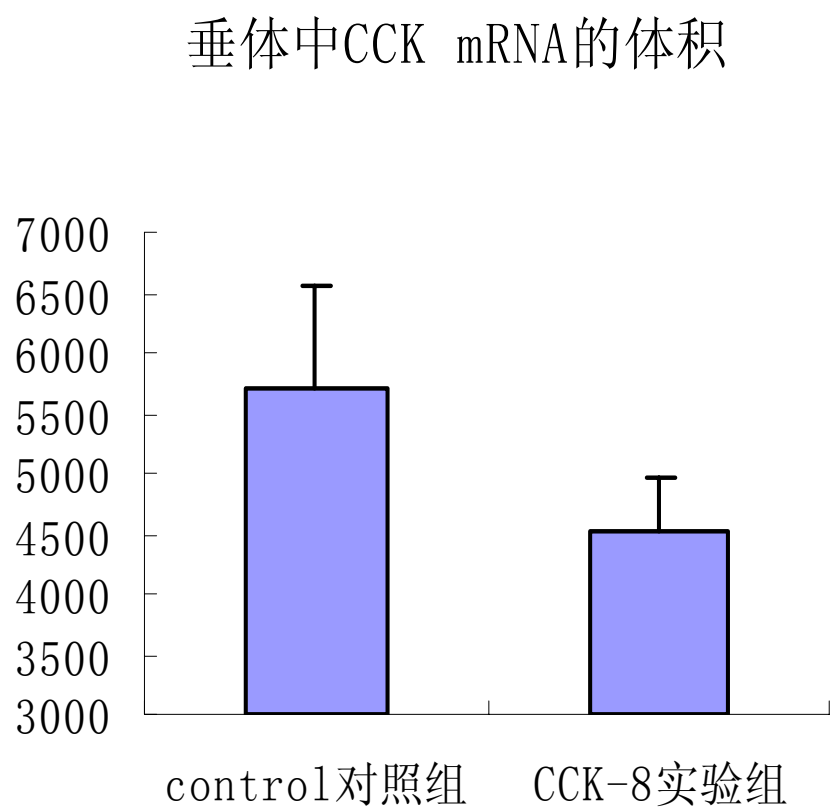
----CCK antibody titer in serum

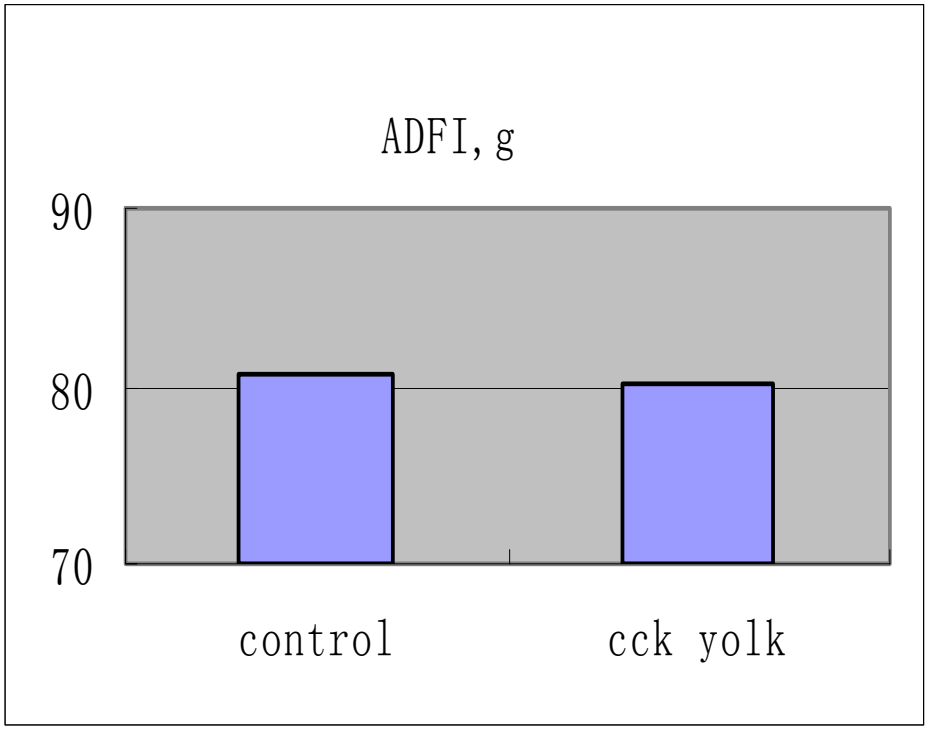


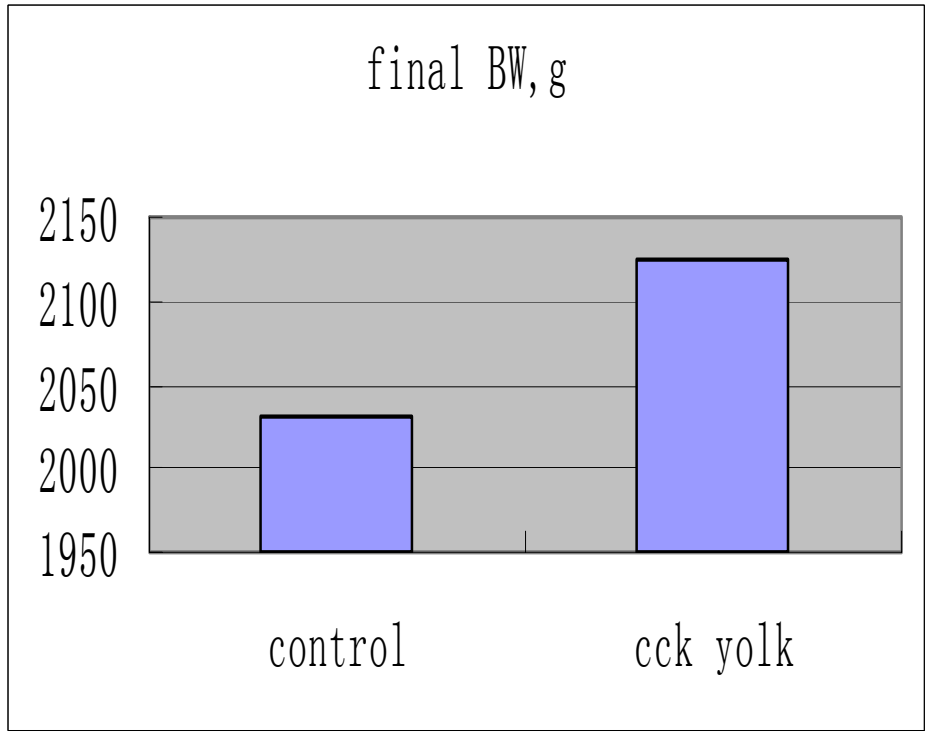
Active immunization against CCK in pigs 0f 27-90 kg

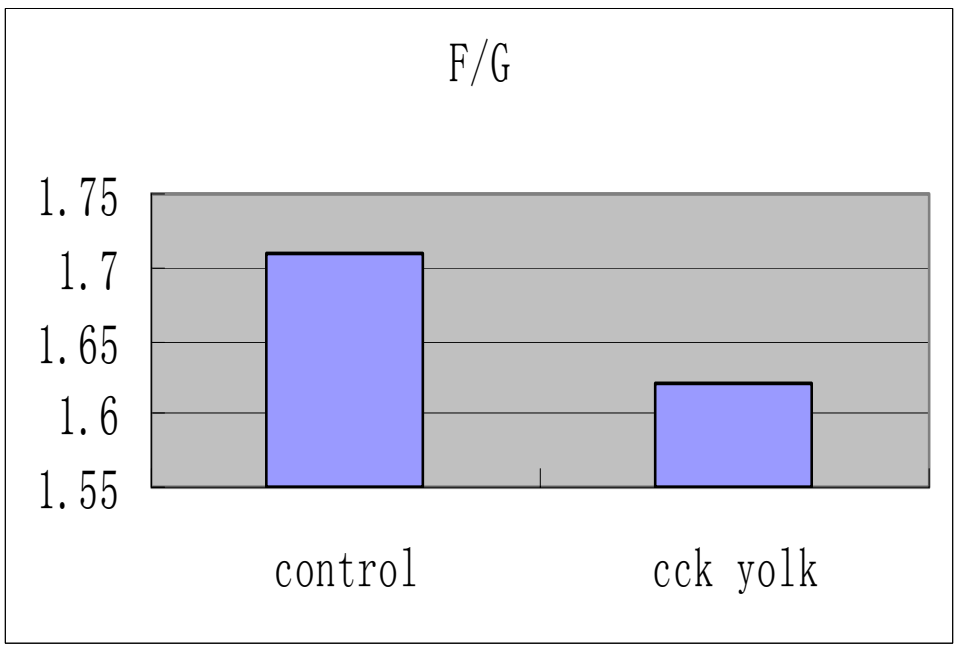
-----CCK gene expression











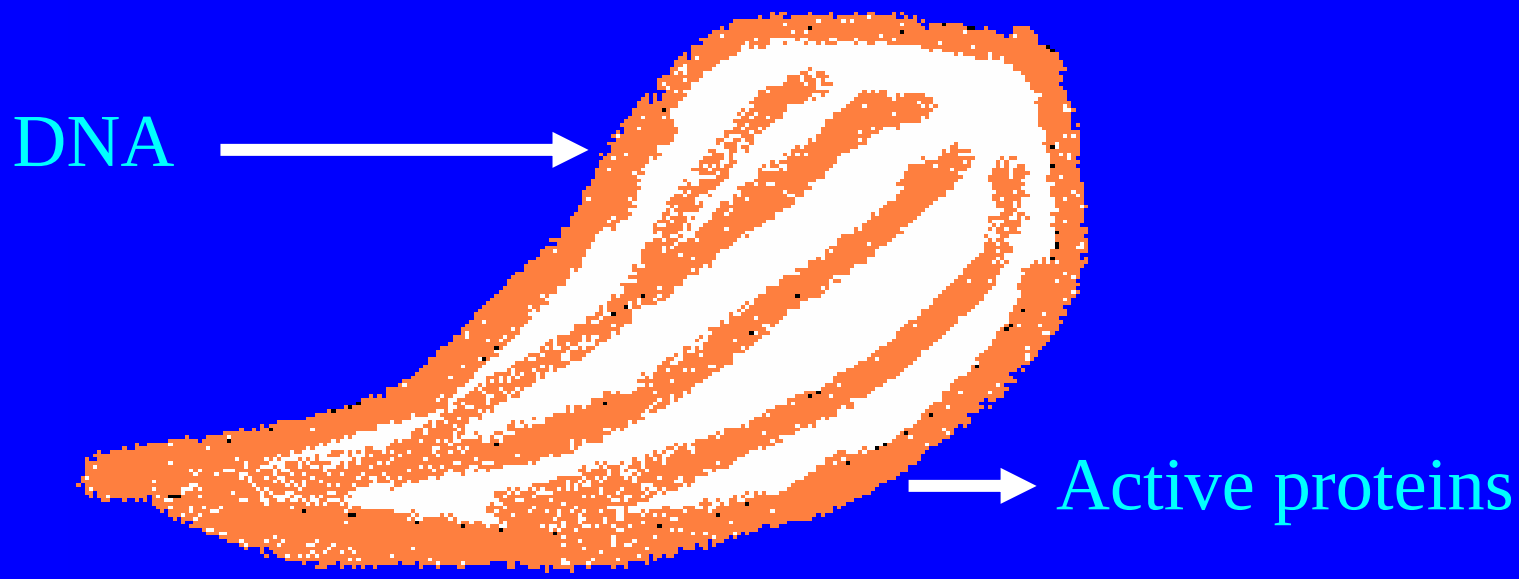
Effect of 100 ppm CCK yolk in the diet on performance of broiler of d 1-42

Immunomodulation

- advantage：
  -- no side effects
  -- more acceptable than GH injection
- disadvantage：
  -- active immunization： poor control of level and duration of immune response
  -- passive immunization: more expensive

5 Gene Therapy

Skeletal muscle



artificial endocrine tissue

- plasmid DNA-encoding function genes into muscle tissue

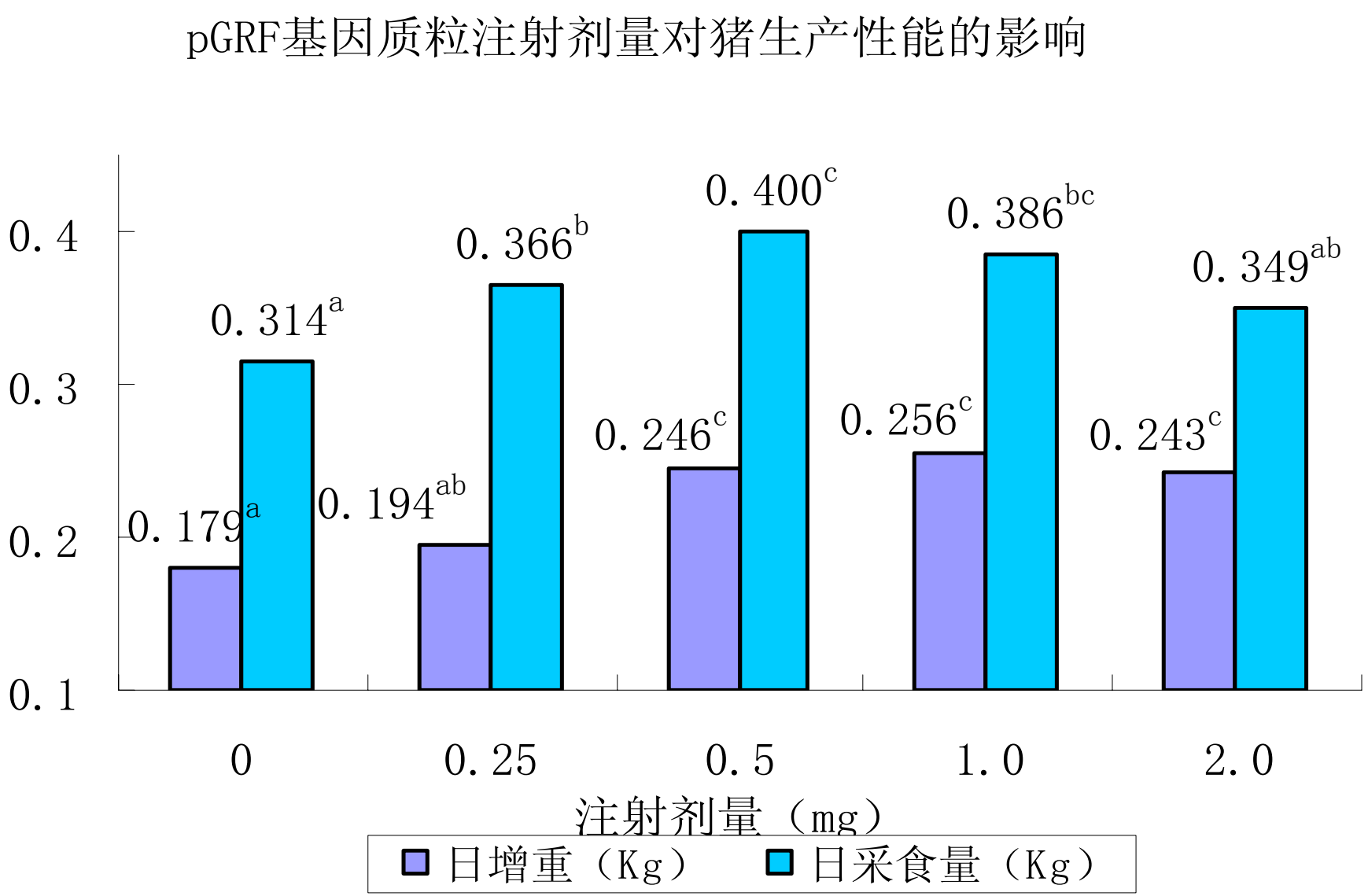
- In mice（DraghiaAkli et al.,1997）

  injection of 100 ug GHRH-pDNA

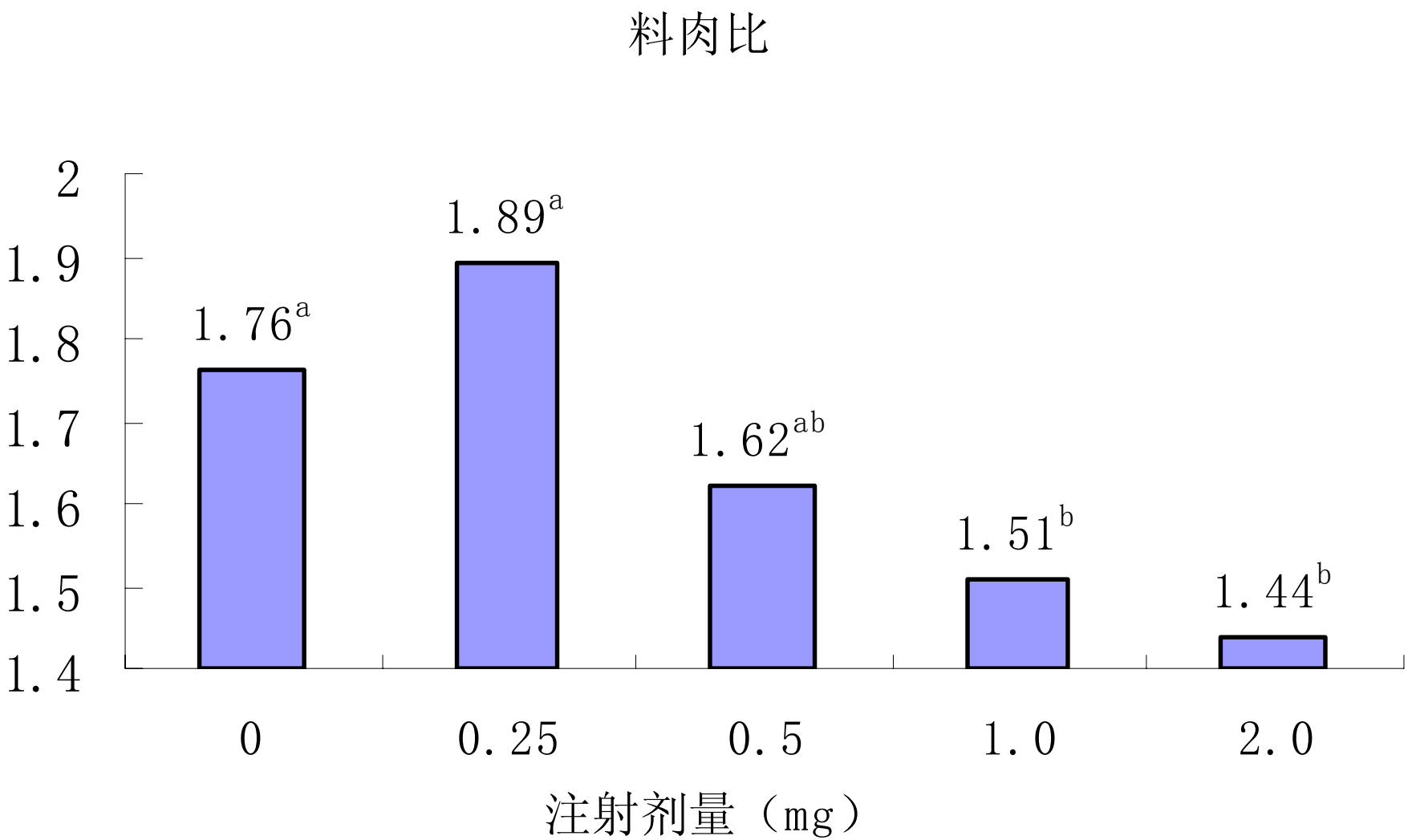
  serum GH: 3-4 fold for 2 wks；

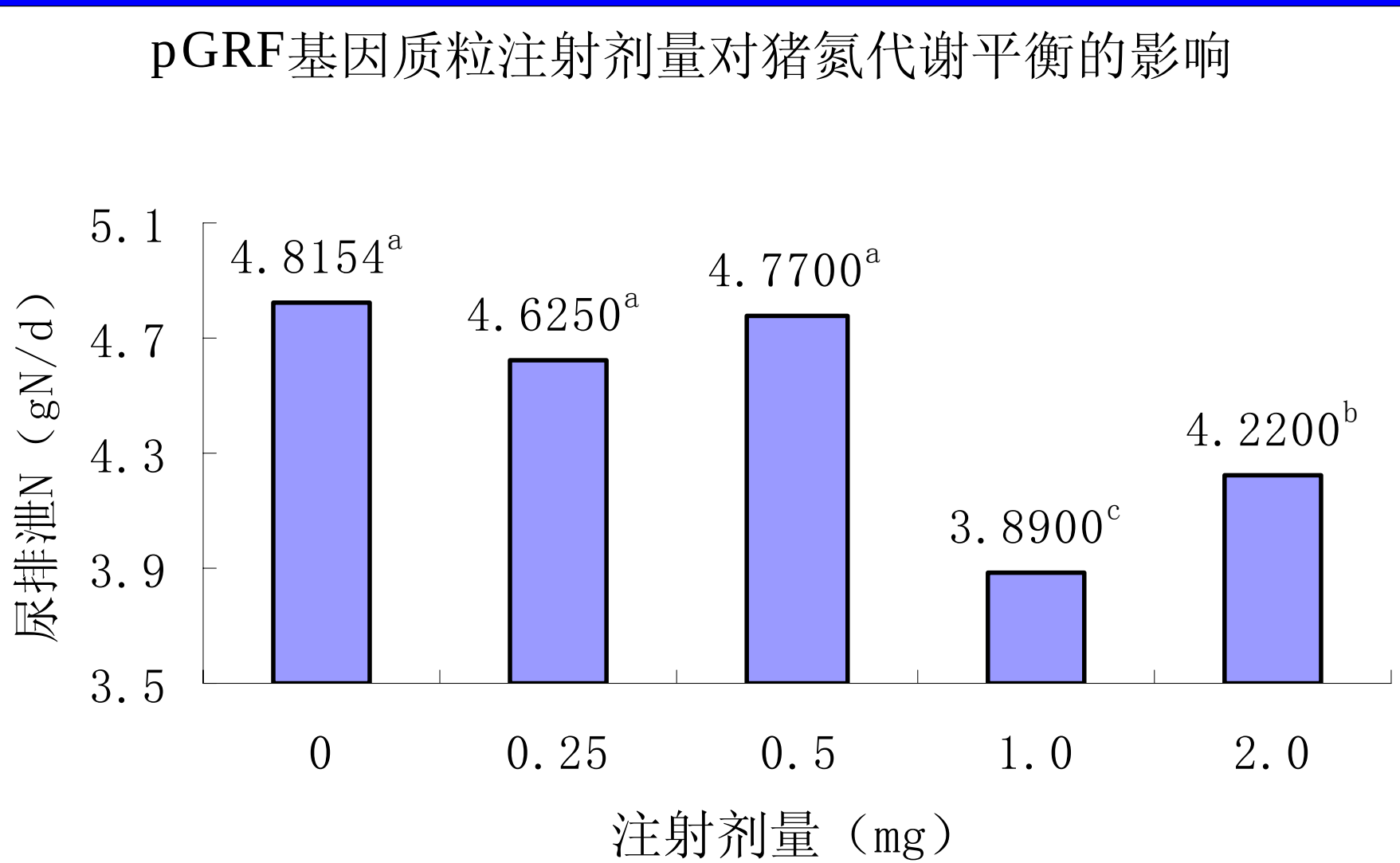
  ⟶ body gain: 10% higher。

Researches in Sichuan.Agri.Univ.

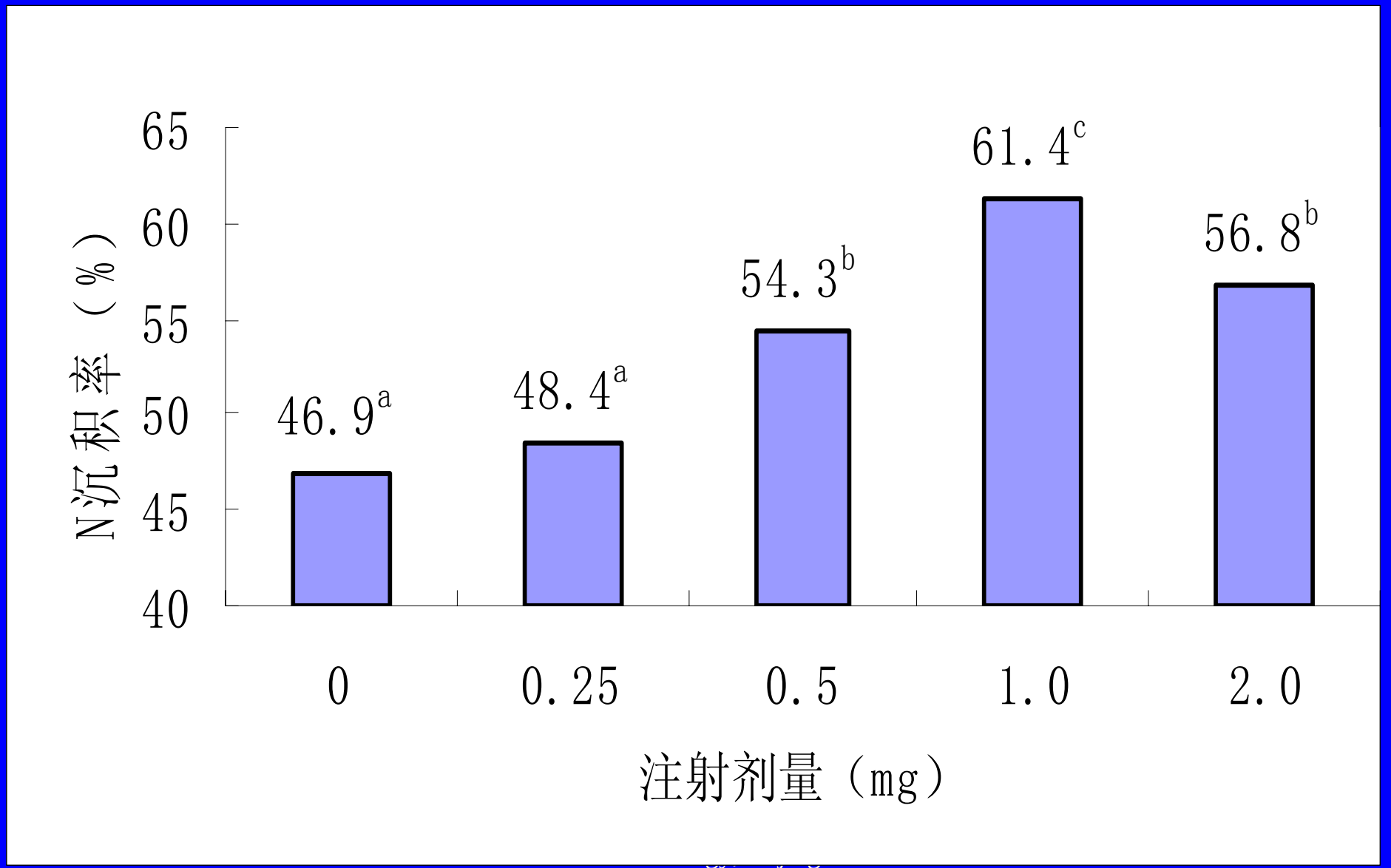


Researches in Sichuan.Agri.Univ.




pGRF基因质粒注射剂量对猪氮代谢平衡的影响
尿排泄N（gN/d）
5.1
4.7
4.3
3.9
3.5
4.8154a
4.6250a
4.7700a
3.8900c
4.2200b
0
0.25
0.5
1.0
2.0
注射剂量（mg）

Researches in Sichuan.Agri.Univ.



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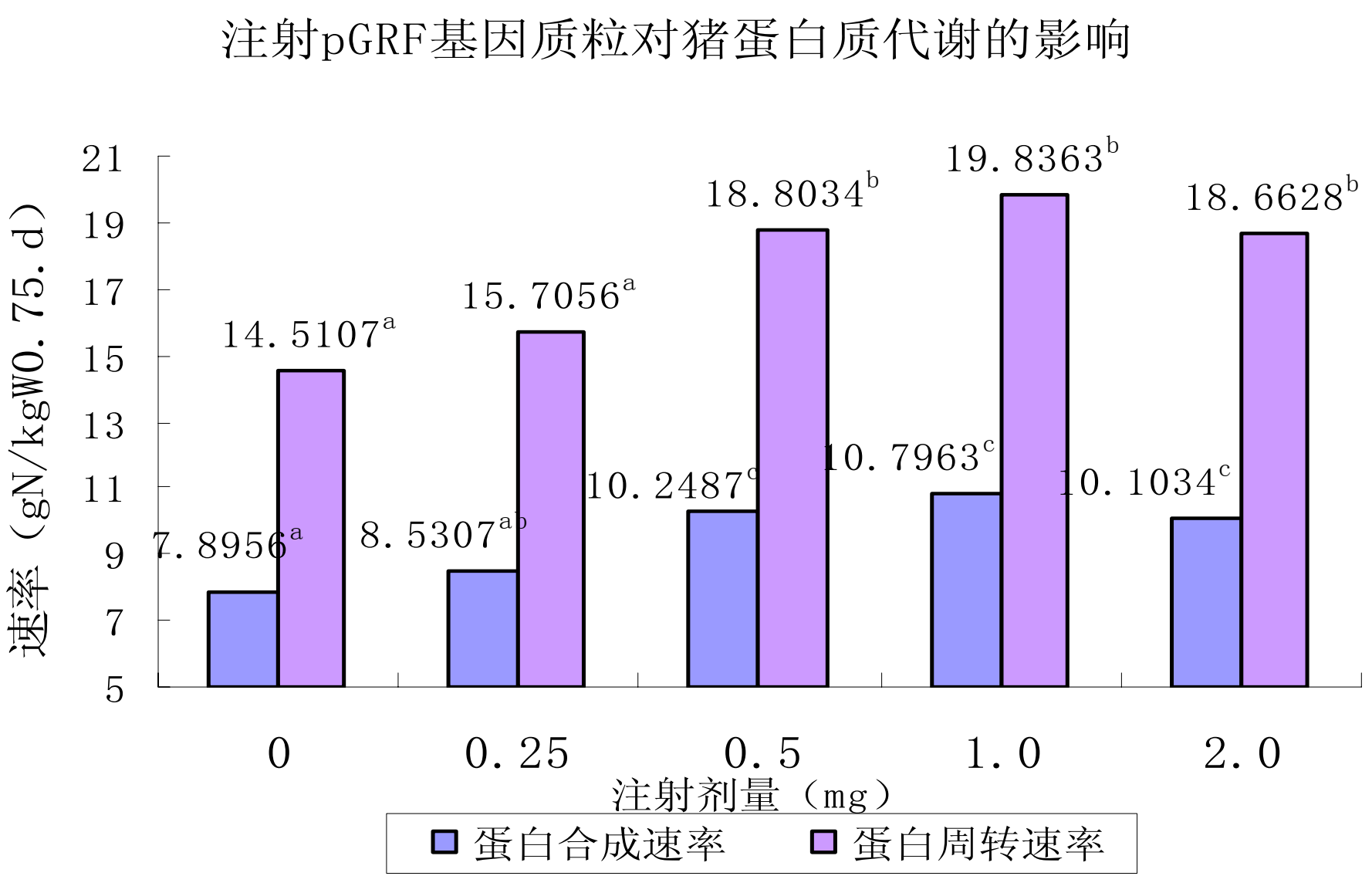

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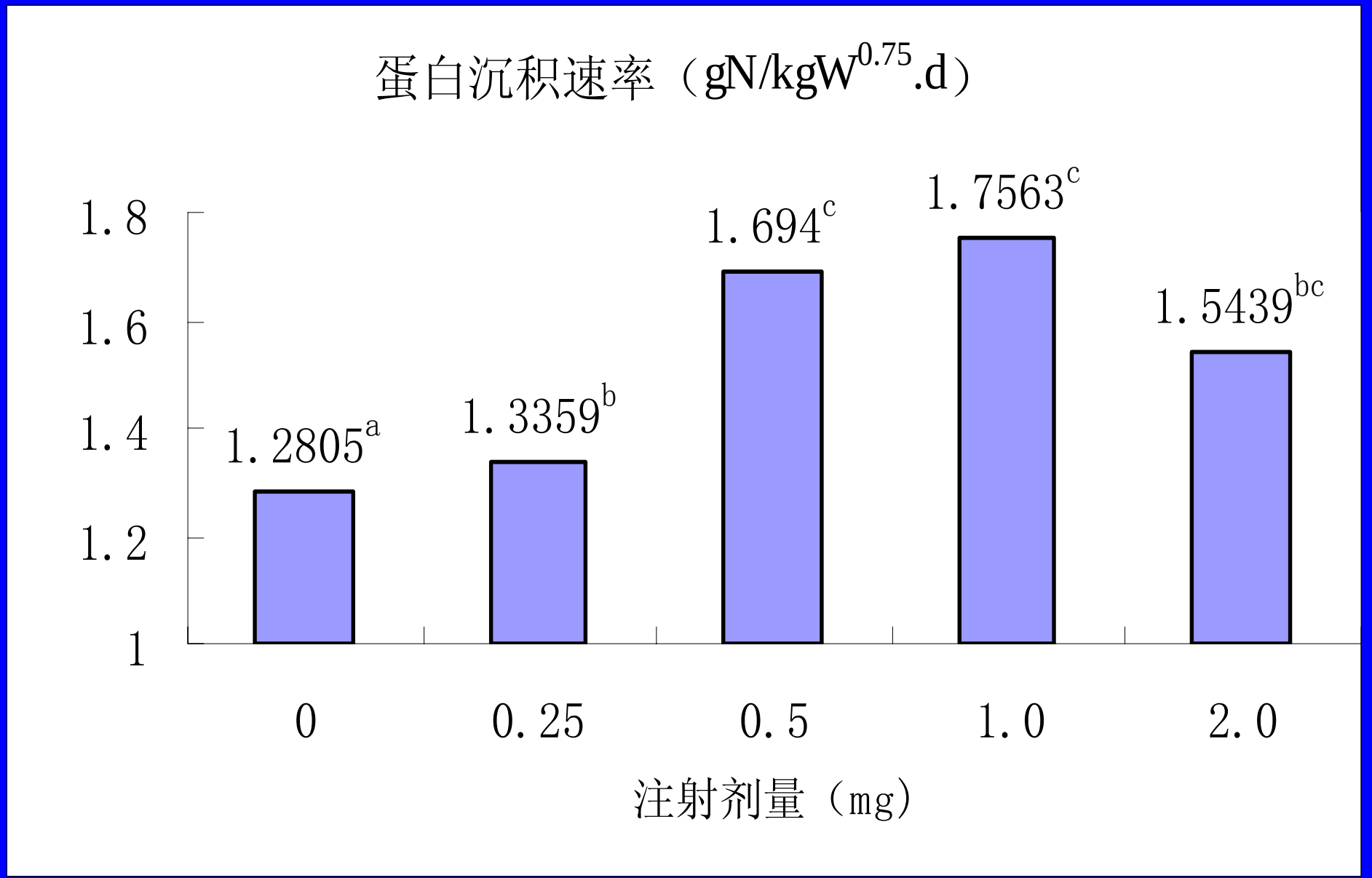

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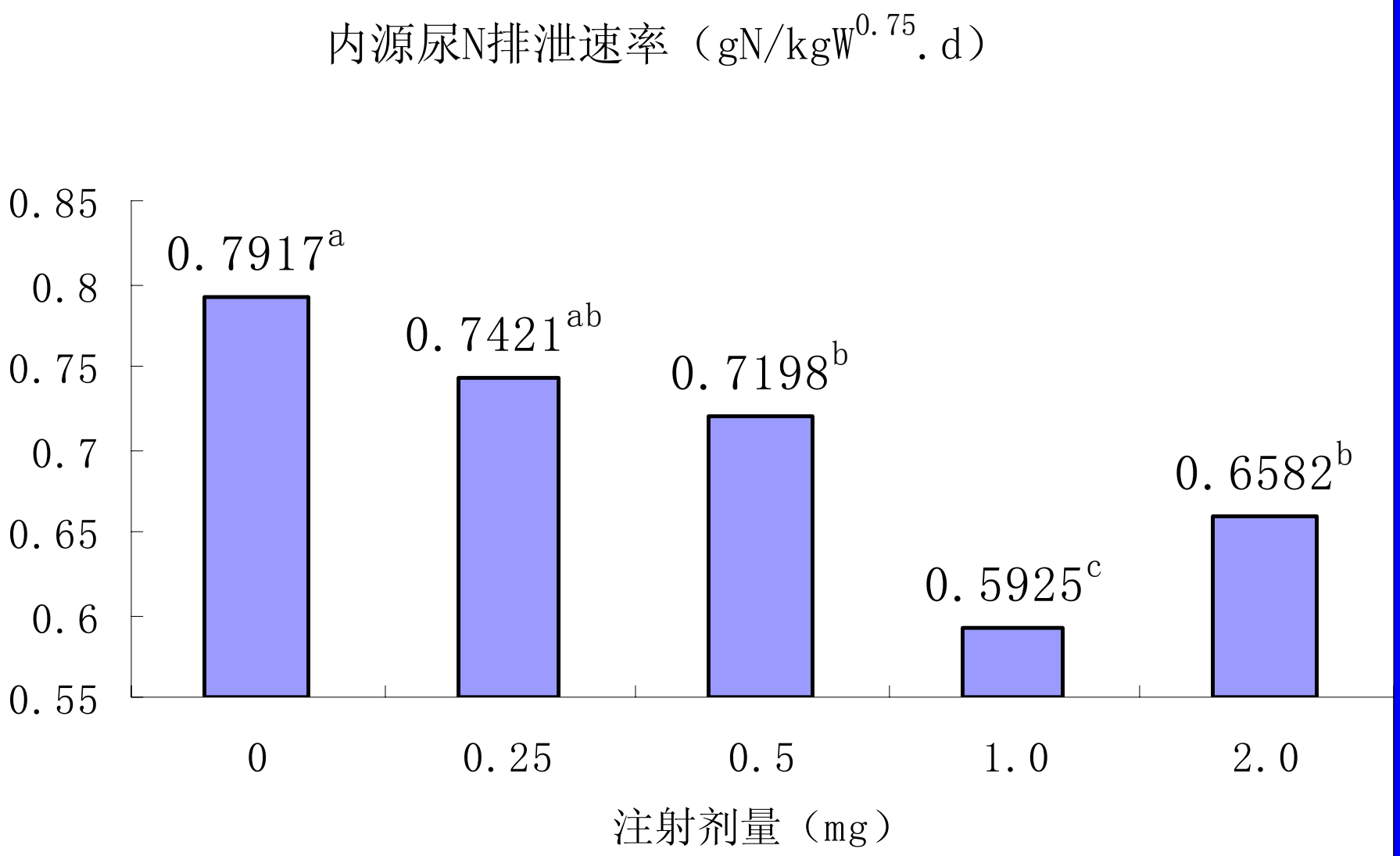

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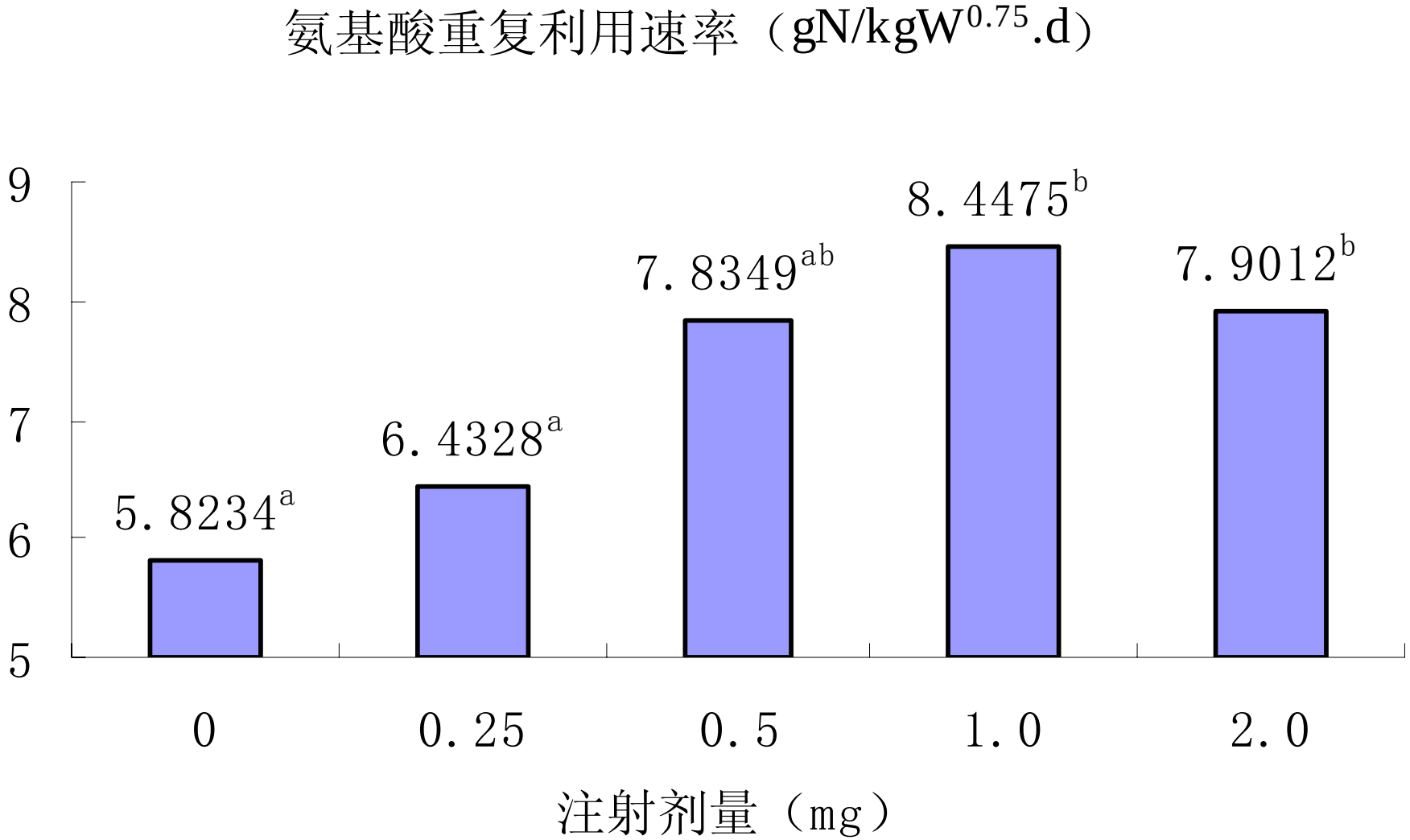

Conclusion

- Biotechnology is greatest potential,perhaps the key approach to improve animal growth potential and solve the problems of animal agriculture

Thanks and Goodbye !