A Novel Beef Production System Based on Grass Resources by Applying Metabolic Imprinting

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Background of Agriculture

Marbled beef culture and its strong market

Containing more than 50% intramuscular fat
Beef marbling standard (B.M.S.)

Beef color standard (B.C.S.)

Beef fat standard (B.F.S.)

6,700 Euro/head
After the Second World War:

We have to historically purchase Feed:
Our main dish is “Rice”.
70 - 80 % Mountain area in Japan
→ Difficult to produce feed
→ Dependent on imported feeds
→ 90 % of feed (grain) are imported
Imported feed in Japan: total more than 20 million ton/year

**Materials of concentrate**
- Corn 12,168,387 ton (USA 96%)
- Grain sorghum 1,439,889 ton (USA 47%, Australia 43%)
- Barley 1,209,300 ton (Australia 50%, USA 20%, Canada 20%)
- Wheat 164,797 ton (Australia 70%, USA 30%)
- Soybean meal 983,342 ton (China 50%, USA25%, India 10%)
- etc…

**Roughage**
- Hay 2,211,891 ton (USA74%, Canada10%)
- Hay cube 446,095 ton (USA85%, Canada 12%)
Feeding a considerable amount of Imported Concentrate

Foot and mouth disease

Bovine spongiform encephalopathy (BSE)

A large amount of excrement
Soil pollution (more than 4,500kg)

And...a large amount of wasted fat from carcass: subcutaneous fat, abdominal fat... (More than 200kg/head)

Foot and mouth disease
Bovine spongiform encephalopathy (BSE)

Beef production in Japan

Problem of beef production in Japan

Marbled beef..... But not always produced

Beef production in Japan

Marbled beef...
The role of ruminant: Material circulation

Cattle make protein (meat and milk) from grass resource

Beef

Rumen: microorganism

Special stomach

muscle

excrement

10%

90%
Weak points: in the case of fattening by grass, Meat quality and quantity.... Even if in Wagyu....

If we can get over against these weak points by grass fattening, in meat quality: if moderate intramuscular fat is contained, its meat become tasty, and its intramuscular fat includes functional fatty acid and good vitamins. Its good!!
Background

Concept of Metabolic Imprinting

The hypothesis:

Developmental origins of health and disease (DOHaD)
Background DOHaD

Nutritional stimuli or environment during early growth stage

Getting fatty constitution

Keep fatty constitution

Metabolic imprinting

Metabolic syndrome
Obesity, Diabetes, arteriole sclerosis etc.

Metabolic Imprinting
Many kinds of researches are investigating about this topics.

Results of “irreversibility” regarding obesity, abnormal hormone secretion, and methylation of DNA etc., have been proved.
What is “metabolic imprinting”?

Nutrition during early growth stage

Keep fatty constitution

Fatty constitution

Metabolic imprinting

Metabolic syndrome
Obesity arterial sclerosis etc.

Applying to cattle…..

High nutrition during calf

Higher potential to absorb nutrition

Earning fatty constitution

Effect of metabolic imprinting

Absorption of nutrition from grass

up
Science of livestock production in Food chain

A new beef production system...

Metabolic imprinting technology

New Breeding technology

Nature
  domestic roughage
  and crops

Marketing technology

Japanese & Asian Food Chain

ECOLOGY
  Organisms and Environment

Utilization of domestic resources:
  Grass resources of Abandoned Agricultural land,
  Domestic pasture

Return or Recycle

Reconstruction of Novel beef production based on Asian grass resources

Quality and Quantity → Safety and function
  Brand Beef : Q beef
Aim:

In order to study the effects of metabolic imprinting on beef production, a fattening experiment was conducted.

In this experiment, we feed mainly roughage and investigated how metabolic imprinting affects the expressions of meat quality or quantity and related genes in Japanese Black cattle (Wagyu).
Materials and Methods

Animals
Japanese Black steers (Same father, sire) (n=14)

Treatment
Roughage group (R) (n=7); Feeding only hay (Italian ryegrass) after 2 months of age
Concentrate group (C) (n=7); Feeding concentrate till 10 months of age

only hay after 11 months of age

Sampling
2, 5, 10, 17, 22 and 26 months of age by biopsy

Slaughter
26 months of age

Analysis:
Real time RT-PCR analysis of IGF-I concentration in serum of blood, histochemistry

Normal suckling
2 months 10 months 17 months 26 months slaughtered

13 ~ 16 months second sexual character

<table>
<thead>
<tr>
<th>group R</th>
<th>roughage</th>
</tr>
</thead>
<tbody>
<tr>
<td>group C</td>
<td>concentrate roughage</td>
</tr>
</tbody>
</table>
Target genes 1: intramuscular fat

- Marbling (intramuscular fat)
  - Important factor of Meat quality
  - Tastiness, accumulation of fat-soluble vitamins and functional fatty acid

- Development of intramuscular fat
  - mRNA expression
  - Adipocyte differentiation factors PPARgamma2, C/EBPs
  - Leptin
  - PGC1-alpha
    - Fatty acids synthesizing enzymes
      - G6P glucose-6-phosphate dehydrogenase
      - ADRP adipose differentiation-related protein
      - SCD stearoyl-CoA desaturase
      - FASN fatty acid synthase

Fat cell diameter, intramuscular fat contents percentage
Adipocyte differentiation regulation and fatty acid synthesize enzyme related genes

C/EBPβ+δ
ADD1/SREBP1

C/EBPα

Pref-1

aP2

leptin

SCD
stearoyl-CoA desaturase

G6P
glucose-6-phosphate dehydrogenase

3T3-L1 cell line

white fat tissue

PPARγ2

Faculty of Agriculture

KYUSHU UNIVERSITY
Target genes 2: Muscle growth regulation related genes

Myogenic regulatory factors: MRFs

- Myf5
- MyoD
- Myostatin
- Myogenin
- MRF4
- Myostatin

IGF-I
IGF-I receptor

Satellite cell (stem cell?) → Myoblast → Myotube → Myofiber

Maintain and mature myotube
Maintain differentiation from myoblast to myotube and myotube

Final differentiation specific factor
PGC-1α (PPARγ coactivator-1) is a cold inducible coactivator of nuclear receptors and stimulates mitochondrial biogenesis and respiration within myofibers. PGC-1α is an important factor driving the formation of slow-twitch (Type-I) myofibers (Lin et al., 2002).
Results body weight

*** mean significantly differences between groups at P < 0.001
Roughage group. Concentrate group.

Same father
Same age

12 months of age

26 months of age

metabolic Imprinting?
Weaning 10 months of age 26 months of age

**Roughage**

- B.W. $63 \pm 6.3$ kg
- Height - $93.9 \pm 2.4$ cm
- B.L. - $94.2 \pm 5.5$ cm

1.9 times

- B.W. 17.3$ \pm 12.5$ kg
- Height 93.9$ \pm 2.4$ cm
- B.L. 94.2$ \pm 5.5$ cm

4.3 times

- B.W. 357$ \pm 25.5$ kg
- Height 125.9$ \pm 3.5$ cm
- B.L. 135$ \pm 19.1$ cm

**Concentrate**

- B.W. $61 \pm 7$ kg
- Height - $110.6 \pm 4.1$ cm
- B.L. - $110.8 \pm 17$ cm

1.8 times

- B.W. 264.9$ \pm 17.9$ kg
- Height 133.8$ \pm 7.1$ cm
- B.L. 150.9$ \pm 53.1$ cm

3.0 times
Meat production

<table>
<thead>
<tr>
<th></th>
<th>Sirloin (kg)</th>
<th>Rib loin (kg)</th>
<th>Fillet (kg)</th>
<th>Ramp (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrate</td>
<td>9.89±0.76</td>
<td>7.20±0.95</td>
<td>5.03±0.58</td>
<td>10.33±2.1</td>
</tr>
<tr>
<td>Roughage</td>
<td>5.8±0.63</td>
<td>4.54±0.84</td>
<td>3.45±0.26</td>
<td>7.14±1.19</td>
</tr>
</tbody>
</table>

**P<0.01, ***P<0.001

Meat production (70% up)
(60% up)
(50% up)
(40% up)
Results: PPARgamma2 C/EBPs

![Graph of PPARgamma2](image)

![Graph of C/EBPalpha](image)

![Graph of C/EBPbeta](image)

![Graph of C/EBPdelta](image)

* means significantly differences between groups (P < 0.05)
Results: Leptin PGC-1 alpha

* and *** mean significantly differences between groups at $P < 0.05$ and $P < 0.001$
Results: G6P ADRP SCD FASN

* , ** and *** mean significantly differences between groups at P < 0.05, P < 0.01 and P < 0.001.
Results: Fat cell diameter

*** mean significantly differences between groups at P < 0.001
Results: Intramuscular fat content

** mean significantly differences between groups at $P < 0.01$
Results: Gene expressions in intramuscular fat

Metabolic Imprinting Treatment

2 months

Intensive feeding of concentrate: becoming fatty constitution

Grass Fattening

months

Feeding only hay:

Slaughter

26 months

G6P
IGF-I
Serum IGF-I

5 mo.

C/EBP
Leptin
G6P
SCD
FASN

10 %
Im. Fat
6 %

10 mo.

17 mo.

22 mo.

PPAR
C/EBP
Leptin
G6P
ADRP
SCD
FASN

HIGHLANDERS
Results 2: adipogenesis

2-10 months of age: imprinted by a high nutritional level → obtaining fatty constitution
11-17 months of age: adjusting grain feeding to grass feeding in rumen.
17 months of age – slaughter: restart fattening by grass
During myogenesis, the transcription factors: MyoD, Myf5, Myogenin and MRF4 for play important roles with developing skeletal muscle. Myostatin is the negative and IGF-I is the positive muscle growth regulation factors.
Results: MyoD family 1

Fig. 3. MyoD and Myf5 mRNA expressions in longissimus muscles of Japanese Black steers during growth. ○ group C, ■ group R, *p<0.05

- MyoD
- Myf5

% of G3PDH

2, 5, 10, 17, 22 months of age
Results: MyoD family 2

**myogenin**

**MRF4**

Same hay feeding

Fig. 4. Myogenin and MRF4 mRNA expressions in longissimus muscles of Japanese Black steers during growth. ○ group C, ■ group R, *p<0.05, **p<0.01
Results: IGF-I and myostatin

IGF-I mRNA in muscle

Fig. 5. IGF-I and myostatin mRNA expressions in longissimus muscles of Japanese Black steers during growth. ○ group C, ■ group R, **p<0.01 ***p<0.001
Results: Serum IGF-I concentration

Fig. 6. Serum IGF-I concentration during growth.

** P < 0.01, *** P < 0.001
Results: 1

Metabolic Imprinting  Treatment  Grass Fattening  Slaughter

2 months  months  26 months

Intensive feeding of concentrate: becoming fatty constitution

myogenin  IGF-I  Serum IGF-I
5 mo.  10 mo.
MRF4  MyoD  Myostatin  Serum IGF-I

myostatin
22 mo.

Feeding only hay:
Results 1: muscle development

The basic structure of muscle was constructed by a high nutritional level during the early growth stage??

Imprinting??  It is maintained after that .....??
Comparison of carcasses between commercial and imprinting Treatment cattle in Japanese Black

Commercial

Imprinting

Less subcutaneous fat

Commercial

Imprinting

Less abdominal fat
Fig. 2. Sirloin (left and right side) weight of carcass at 26 months of age.

***p<0.001
Cross-sectional Carcass of Imprinting treatment Japanese Black steers

Tasty red meat
And
Moderate i.m. fat

It is very important to produce this beef from almost domestic grass resources.

26 months of age, Japanese Black steer
Experiment: Grazing in Abandoned grove
*for beef production of metabolic imprinted cattle
Chef's Comments
Conclusion

The feeding level during the early growth stage influenced mRNA expressions in skeletal muscle.

The growth size, meat quantity and quality were markedly different between groups.

This may be caused by the effect of metabolic imprinting induced by a high feeding level during the early growth stage.
Science of livestock production in Food chain

A new beef production system...

Metabolic imprinting technology

New Breeding technology

Nature domestic roughage and crops

Marketing technology

ECOLOGY Organisms and Environment

Utilization of domestic resources: Grass resources of Abandoned Agricultural land, Domestic pasture

Return or Recycle

Japanese & Asian Food Chain

excrement (fertilization)

Reconstruction of Novel beef production based on Asian grass resources

Quality and Quantity – Safety and function
Brand Beef: Q beef

Japanese & Asian Food Chain
**Ideal beef cattle:**

1. Good quality beef from domestic grass resource
2. Good quantity beef from domestic grass resource
3. No wasted body fat
4. Production system is sustainable and based on material circulation and environment-friendly one.

**Future subjects:** Pinpoint feeding strategy by solving metabolic imprinting mechanism in ruminant.

What? When? How much? should we feed to create good constitution???

We have to make clear.....
Thank you for your kind Attention
2. **: $P<0.01$, *: $P<0.05$, †: $P<0.1$. 