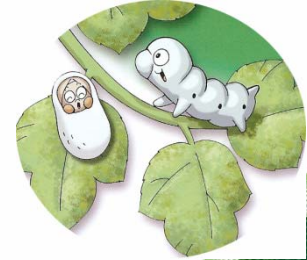


The Research Trend of Functional Sericulture in Korea



*Department of Agricultural Biology
National Academy of Agricultural Science,
Rural Development Administration, Korea*



Kang-Sun Ryu

Contents

1. Genetic resources of silkworm and mulberry
2. Silkworm powder for antidiabetes
3. Tonic effect of the silkworm
4. Codyceps from silkworm
5. Utilization of mulberry leaves
6. Utilization of silkprotein
7. Silkworm Bio-factory
8. Silver agriculture and experience study

The Genetic Resources of silkworm



Special silkworm variety for golden silk production

■ Silkworm variety JS169×CS186 breeding and characteristics

- For produce of golden colored silk
- DNJ content : spring 4.34mg/g, 14% more than control 3.81mg/g
- Productivity of Dongchunghacho : 1.41% less than control raw weight 1.20g



Special variety for golden silk production and study experience

Colored silkworm of each stage



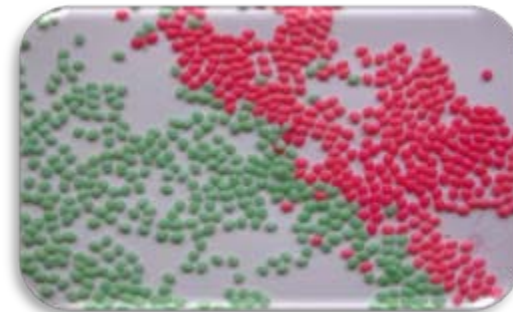
5th instar



Colored cocoon



Colored larvae



Colored eggs

Study experience through colored silkworm



Garden and flower made from colored cocoon



<mini-garden>



<flower-1>



<flower-2>

Research trend of special variety breeding

- ◆ Sex limited marking varieties
- ◆ Sex limited yellow cocoon color varieties
- ◆ Sex limited black eggs varieties
- ◆ Varieties for various larval marking of silkworm for study experience
- ◆ Special varieties for various colored silk production

The Genetic Resources of mulberry



Mulberry variety breeding for fruit production



Daesungbong

Mulberry variety breeding for fruit production



Gugsang No 20 4X
For raw fruit



Turkey - D
White colored

Effect of Mulberry fruits(One cup daily morning)



Before



After

Mulberry tree form for fruits



**Middle stemmed
pruning**

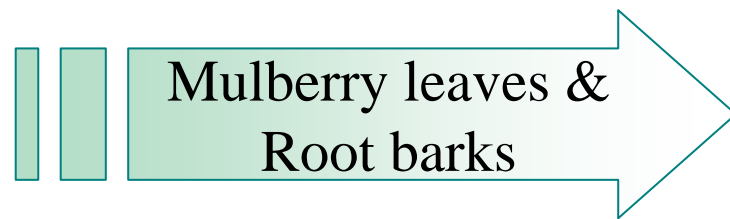


Low stemmed pruning

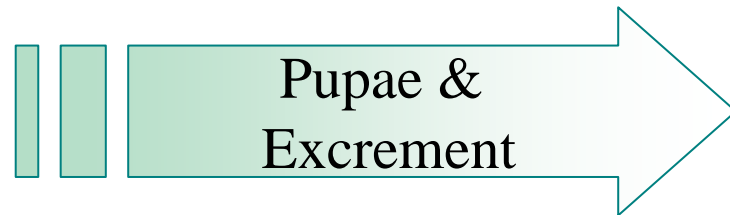
Manufactured goods of mulberry frits(Jam, Juice, Wine)



Functional effect of sericultural products in oriental medicine



- ❖ Diabetes
- ❖ Cough
- ❖ Brain disease
- ❖ Hypertension



- ❖ Diabetes
- ❖ Palsy
- ❖ Alergy

Pupae +Excrement+Mulberry

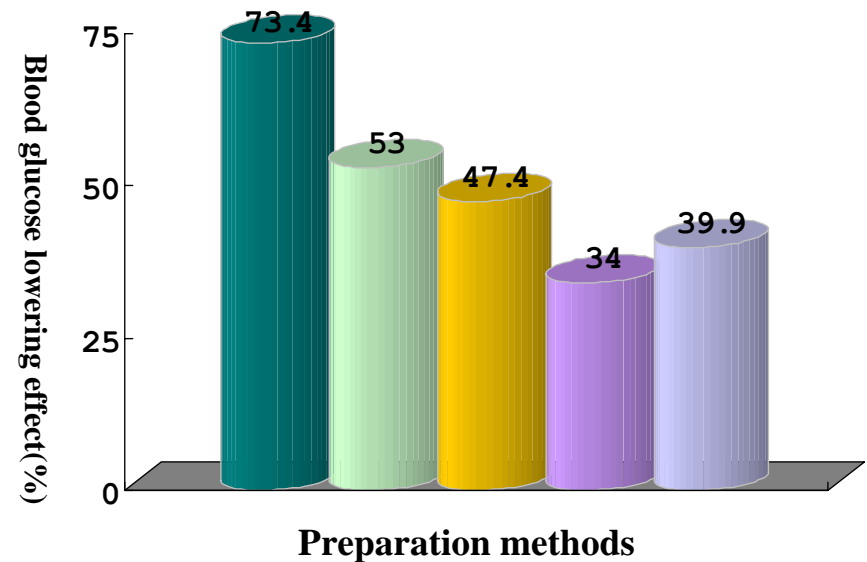


Chemical content of Powdery Silkworm

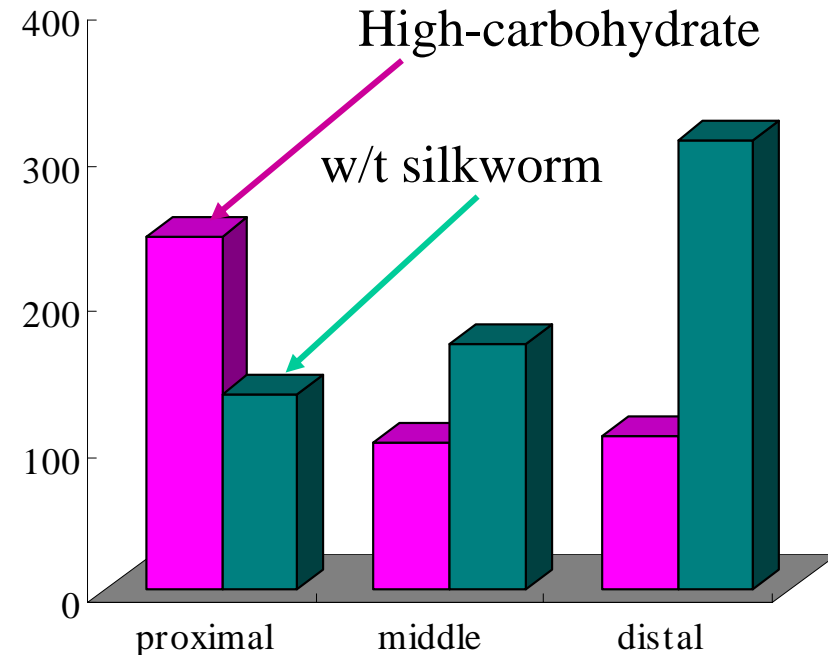
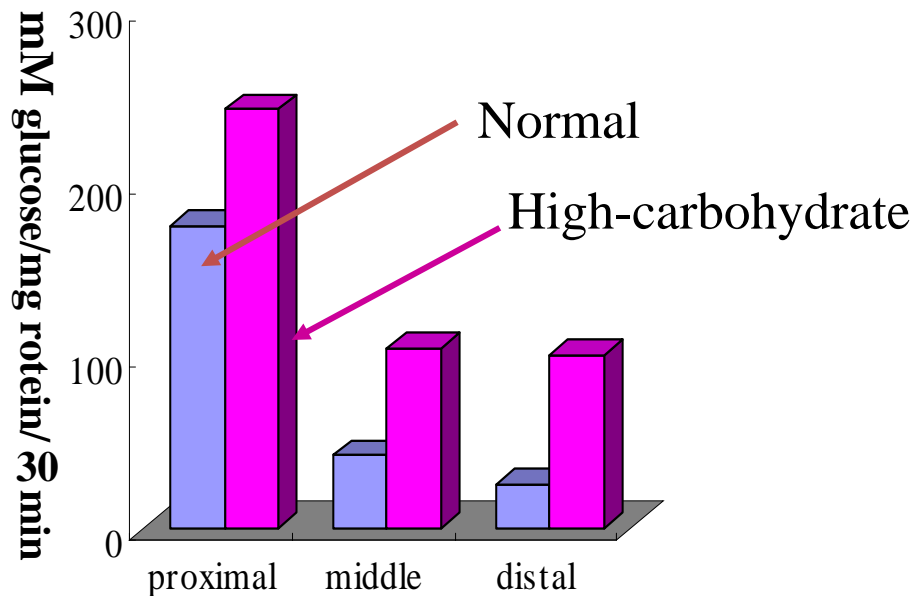
	crude content				
	water	protein	fat	fiber	ash
5 th instar 3rd day	4.77	56.76	9.27	6.62	9.14
5 th instar 6th day	3.98	62.10	13.28	4.59	6.52
	Ca	P	K	Na	Mg
5 th instar 3rd day	0.44	0.86	6.38	0.06	0.38
5 th instar 6th day	0.37	0.70	5.38	0.04	0.25

Activity by different preparation of Silkworm Powder

- 5th instar3days freezing dry : 73.4%
- 5th instar3days heating dry : 53.0%
- Fasting for 24hours : 47.4%
- Artificial diet : 34.0%
- During molting : 39.9%

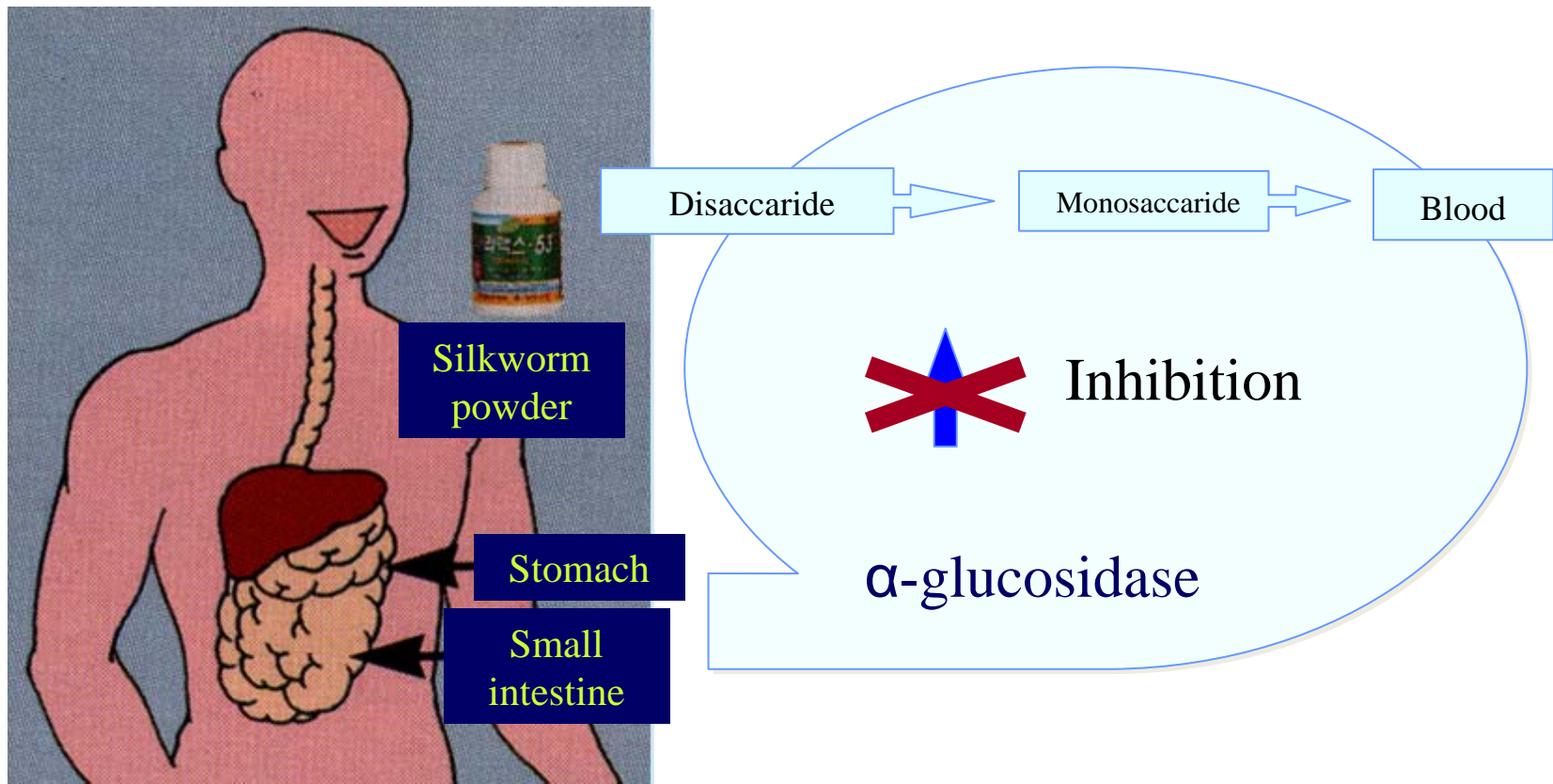


Maltase activities in the small intestine parts

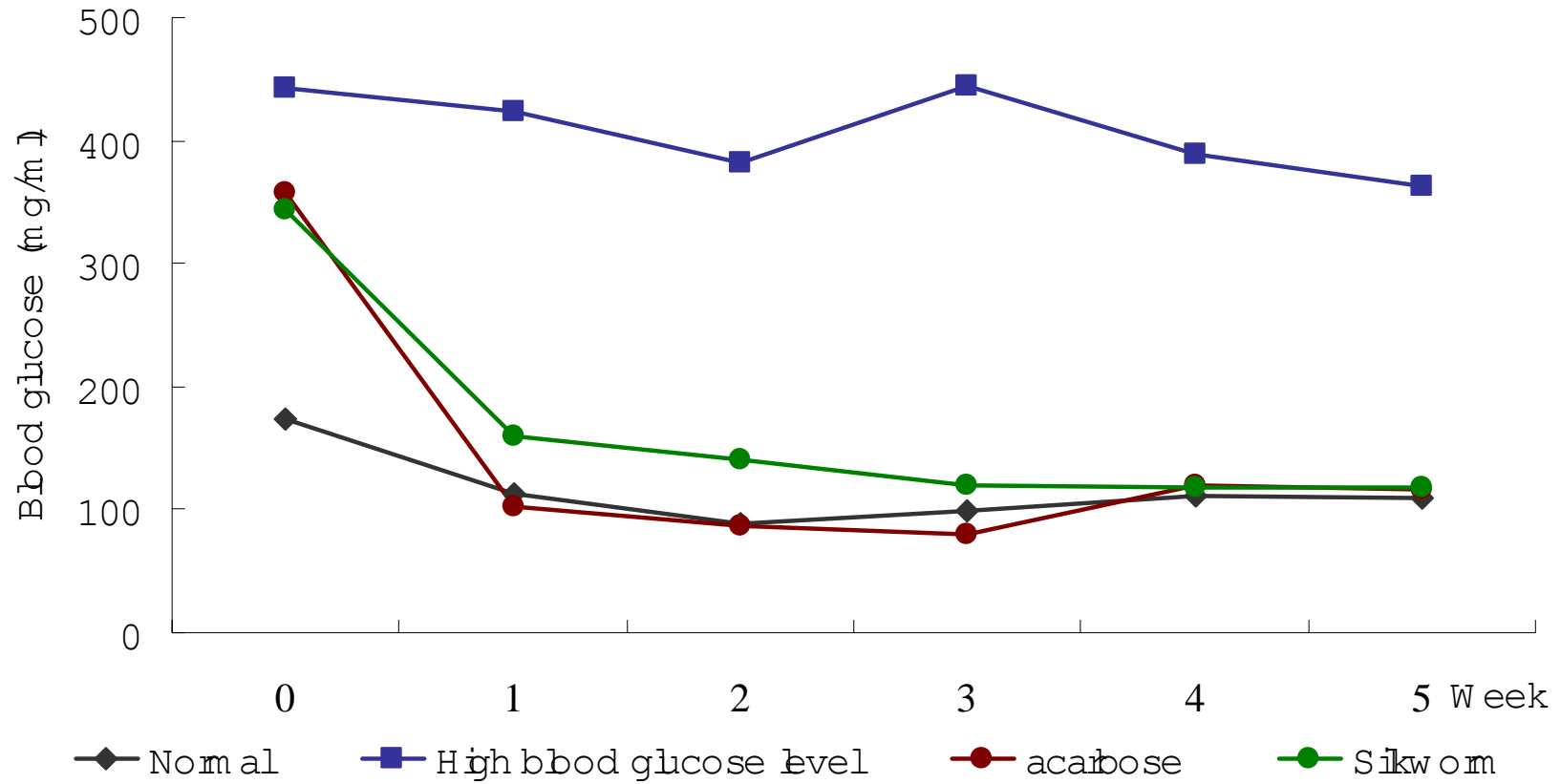


- Maltase activities in the small intestine of lean and high-carbohydrate administered mice(A) and effect of silkworm on the maltase activity of control high-carbohydrate administered mice(B). Difference between lean and control are significant with $p < 0.01 (**)$ and between high-carbohydrate control and silkworm treated significant with either $p < 0.01 (**)$ or $p < 0.05 (*)$.

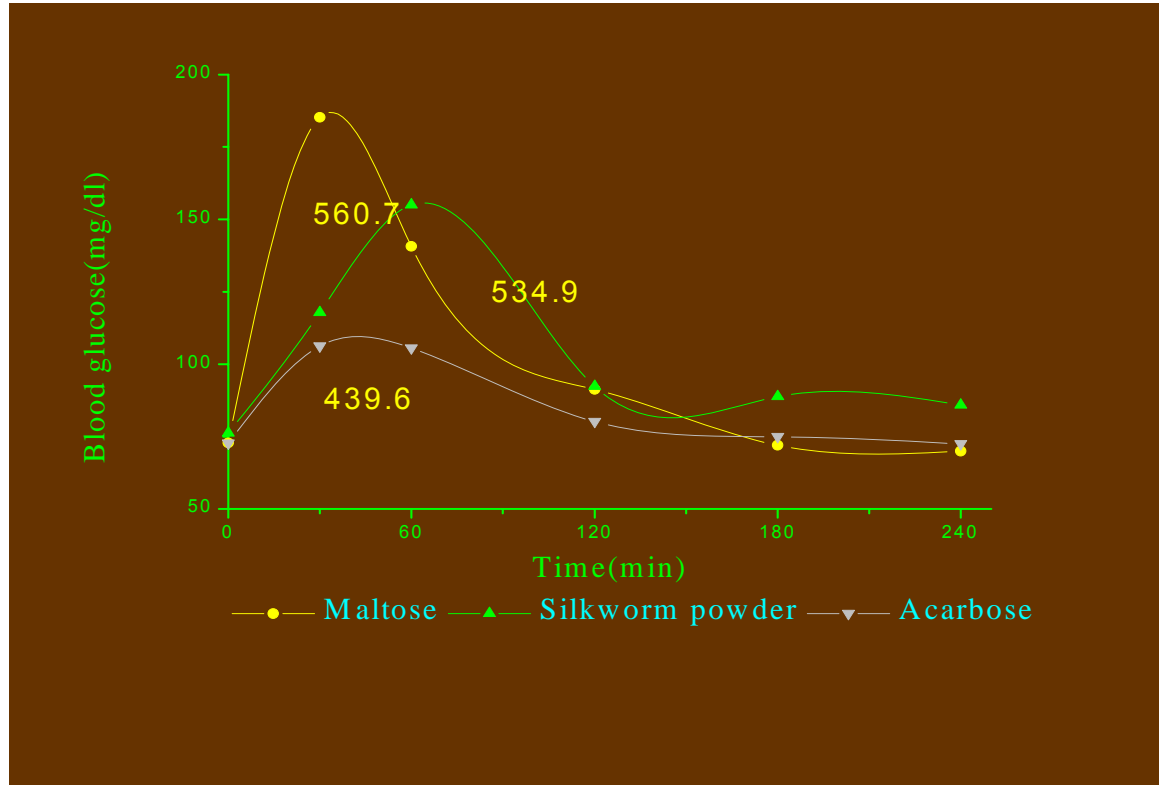
The mechanism of silkworm powder



The change of blood glucose levels with long term feeding

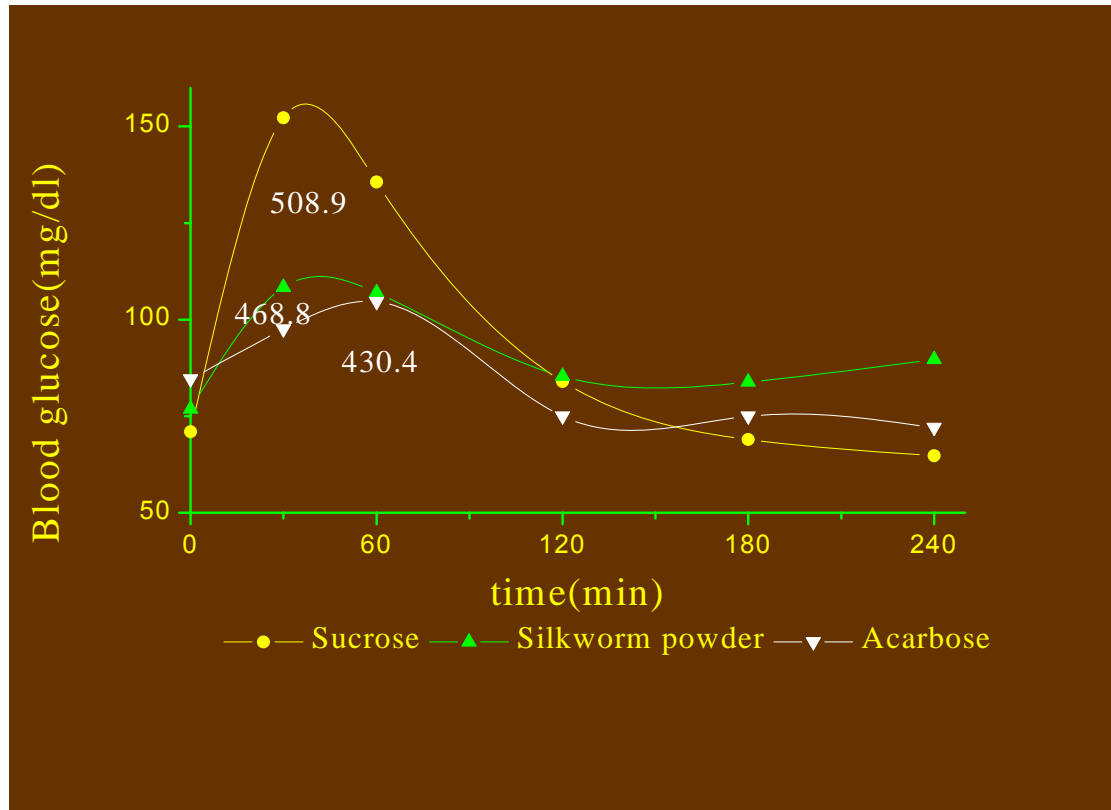


Pharmaco-dynamic of S/W



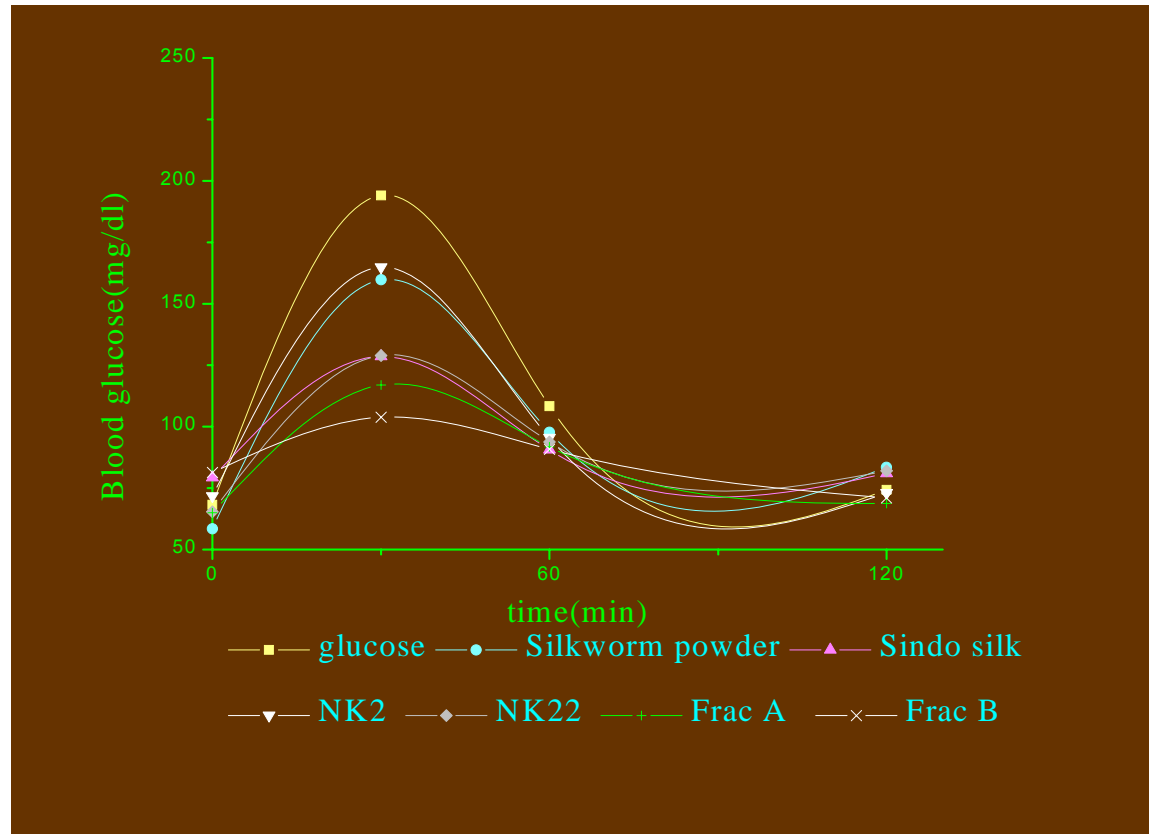
■ Total amount of blood glucose in mice administered to maltose and silkworm powder extract

Pharmaco-dynamic of S/W



■ Total amount of blood glucose in mice administered to sucrose and silkworm powder extract

Effects of Glucose Uptake



■ Effect of silk products on blood glucose level from an oral load of glucose(2g/kg) in mice

Structure of 1-deoxynojirimycin



1-deoxynojirimycin (DNJ)

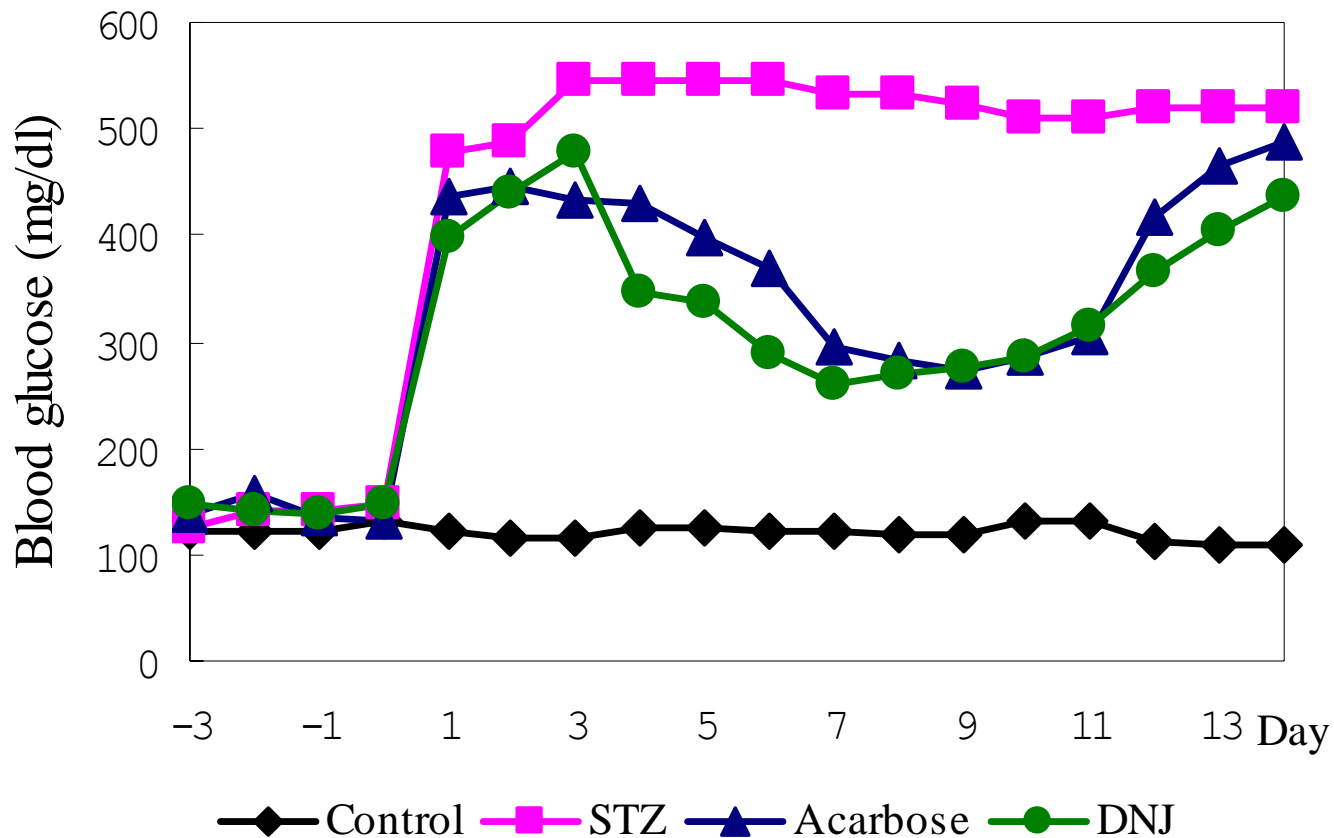
Polyhydroylated alkaloid content of sericultural materials

Alkaloid	(mg/200g powder)		
	Mulberry fruits	Mulberry leaves	Silkworms
DNJ(1)	168	138	376
Me-DNJ(2)	1.0	None	None
Gal-DNJ(3)	2.8	61	None
Gal-DNJ(4)	2.8	1.2	None
FAG(5)	3.6	37	25
Glc-FAG(6)	None	1.1	None
epi-FAG(7)	None	4.2	4.2
DAB(8)	2.4	11	13
Glc-DAB(9)	4.0	13	8.2
CAL (10)	3.6	5.2	None
Gal-CAL(11)	1.6	1.8	None
HNT(12)	2.0	None	None

Purified DNJ from Silkworm Powder



Activity of Purified DNJ from Silkworm Powder



Effects of Silkworm Powder on four body types

A brief information on the Sasangurhak

Sasangurhak=Medical science based on the four body types

Tae-Yang: More energy from light

Tae-Um: More energy from shade

So-Yang: Small energy from light

So-Um: Small energy from shade

Yang: light, sky

Um: shade, ground

Sasangurhak was first advocated at the end of 19th century by Je-Ma Lee.

He divided human body into four types.

Based on these body types, physiology and pathology of the disease, disease susceptibility, and development are different due to different physical constitution.

General characteristics of participants

	Tae-um		So-yang		So-um	
	Drug	Non-drug	Drug	Non-drug	Drug	Non-drug
No (M/F)	22 (13/9)	10 (6/4)	12 (7/5)	8 (4/4)	10 (6/4)	6 (3/3)
Age (yrs)	61.2±9.4	58.4±8.7	63.2±8.9	60.6±8.7	62.4±7.9	60.1±8.8
Duration (yrs)	8.5±3.2	6.4±2.8	7.5±3.5	6.8±2.7	7.1±3.7	6.0±3.4
height(cm)	165.7±22.3	161.8±16.7	164.3±18.1	160.9±19.7	164.0±18.6	161.7±19.5
Weight (kg)	78.8±15.6	75.1±14.8	76.5±16.2	72.5±14.2	75.0±15.8	71.8±16.2
BMI (kg/m ²)	28.2±5.6	28.7±4.8	28.0±4.6	27.8±4.9	27.2±5.6	27.0±4.9
Fat (%)	26.5±4.7	24.5±5.1	26.9±5.0	24.8±4.7	25.1±5.0	26.0±5.3
WHR	0.99±0.04	0.98±0.04	0.98±0.04	0.98±0.04	0.97±0.04	0.98±0.04
BP systolic	148.2±23.5	150.0±21.8	149.5±22.7	150.5±20.7	152.2±23.1	150.8±21.9
diastolic	95.5±14.8	96.0±13.0	96.1±16.2	94.0±13.4	93.8±11.5	94.4±13.5

Effects of Silkworm Powder on four body type

■ Levels of blood glucose before and after taking silkworm powder

	0	2 weeks	4 weeks	8 weeks
Fasting Blood sugar				
Tae-um Drug	142.4±32.5	123.3±35.4	120.3±36.8(15.5)	133.7±31.6
Non-drug	153.5±42.7	140.7±33.8	135.4±38.2(11.8)	144.8±36.1
So-yang Drug	147.9±32.6	132.3±32.7	125.7±34.3(15.0)	135.9±35.3
Non-drug	150.1±40.2	141.5±35.2	131.3±38.6(12.5)	142.6±36.5
So-um Drug	138.1±30.7	130.9±32.5	126.7±33.4(8.3)	133.3±35.4
Non-drug	145.2±32.5	138.4±30.6	134.8±33.5(7.2)	139.2±35.0
Postprandial 2 hour: □ Miglitol : FPG(6.8□12.5%), PPG(7.3□21.1%) 1783□				
Tae-um Drug	256.1±58.4	215.8±72.6	192.7±62.6(24.8)	223.4±58.4
Non-drug	268.0±68.2	236.5±57.3	212.1±72.6(20.9)	246.5±63.1
So-yang Drug	261.1±62.8	230.8±58.6	210.4±63.0(19.4)	233.6±62.2
Non-drug	273.0±67.4	245.3±62.7	230.1±60.9(15.7)	246.5±63.8
So-um Drug	250.7±66.1	223.6±64.5	214.1±65.8(14.6)	227.1±58.4
Non-drug	264.3±65.2	237.1±62.9	223.6±66.1(15.4)	240.9±60.6

Levels of HBA1C before and after taking silkworm powder

		0	4 weeks	8 weeks
Tae-um	Drug	7.3±1.6	6.2±2.0(15.0)	5.7±1.8
	Non-drug	8.1±1.9	7.6±2.2(6.2)	7.7±2.3
So-yang	Drug	6.9±1.7	6.4±2.1(7.2)	6.8±1.9
	Non-drug	8.3±2.2	7.9±2.1(4.8)	7.6±2.4
So-um	Drug	7.2±1.9	6.7±2.0(6.9)	6.7±1.9
	Non-drug	7.7±2.4	7.0±2.5(9.1)	7.1±2.3

Levels of fasting blood insulin before and after taking silkworm powder

	0	4 weeks	8 weeks
Tae-um Drug	21.4±10.9	16.7±9.4(22.0)	18.7±10.5
Non-drug	6.9±3.4	6.2±2.8(10.1)	7.1±3.3
So-um Drug	19.6±11.0	17.8±10.7(9.2)	18.8±9.7
Non-drug	5.8±2.8	6.1±2.7(0)	6.0±3.1
So-um Drug	20.9±10.6	19.7±11.3(5.7)	20.6±10.4
Non-drug	5.9±2.5	5.7±2.8(3.4)	6.1±3.0

Anti-diabetes Drink from Extract of Silkworm Powder

- Comparison of TG and lipid peroxide content between silkworm extract and Daonil

	control	silkworm extract	Daonil -80
TG	89.13±8.00	74.80±3.40	62.20±4.39
	100.0%	83.9%	69.8%
lipid peroxide	0.38±0.01	0.33±0.04	0.32±0.03
	100.0%	86.8%	84.1%

Anti-diabetes Drink from Extract of Silkworm Powder

- Effect of silkworm extract and Daonil on the free radical and Superoxide Dismutase

	control	silkworm extract	Daonil -80
Free radical	3.83±0.48	3.08±0.30	3.35±0.30
	100.0%	80.6%	87.7%
Superoxide	0.35±0.02	0.40±0.04	0.45±0.04
Dismutase	100.0%	114.3%	128.6%

Effects on the Gastro-intestine Function

- Transit time and speed and lengths of small and large intestine of S.D. rat applied with silkworm powder

	Transit time(min)	Small intestine(cm)	Large intestine(cm)	Transit speed($\frac{S}{min}$)
Control	644.00 (100)	114.70 ^ŷ 8.51 (100)	19.05 ^ŷ 8.17 (100)	20.77 (100)
T1	555.85 (86)*	125.15 ^ŷ 8.80 (109)*	19.75 ^ŷ 8.52 (104)	26.07 (126)*

* Significantly different from control at $p < 0.05$ *

Hepatotoxicity of Silkworm Powder

- Effects of extracts of *Bombyx mori* larvae on glutamic-pyruvic transaminase activity on CCl₄-intoxicated primary cultured rat hepatocytes

CCl ₄	Test materials	Treatment (\$ μg / ml / h)	GPT (U/ml serum) (%)
0	Control	0	29.1 ± 3.5(100)
10	Reference	0	147.2 ± 3.8(0)
10	Total MeOH extract	50	106.9 ± 3.2(34)
10	Hexane fraction	50	170.3 ± 0.5(-)
10	CH ₂ Cl ₂ fraction	50	104.9 ± 4.2(36)
10	BuOH fraction	50	96.9 ± 0.5(43)
10	H ₂ O fraction	50	106.2 ± 0.9(35)

[별지 제2호 서식]

(앞쪽)

국내	수입
○	

제 2009-67 호


건강기능식품 기능성 원료 인정서

대표자 : 박 동 철
 업 소 명 : (사)대한감사회
 소 재 지 : 서울시 영등포구 여의도동 17-9 감사회관 901
 원 료 명 : 동결건조누에분말

수 출 국 : 수출국 제조회사명 :
 수출국 제조회사 소재지 :

건강기능식품 기능성 원료 인정에 관한 규정 제10조의 규정에 의하여 건강기능식품의 기능성 원료로 인정합니다.

2009 년 8 월 2 일

식품의약품안전청장 

* 붙임서류
 1. 원료명
 2. 제조기준(원재료, 제조방법, 기능성분(또는 지표성분), 규격)
 3. 제품의요건(기능성 내용, 일일 섭취량, 섭취 시 주의사항)

210mm×297mm(보존용지(1종) 120g/ m²)

*Certificate of
 Functional Health
 Food from
 KFDA(2009)*

*** ‘11 ~ : Apply for new herbal medicine of silkworm powder**

Main Books about Silkworm powder for antidiabetes



Japanese



Korean

Discrimination method of silkworm

5th instar larva

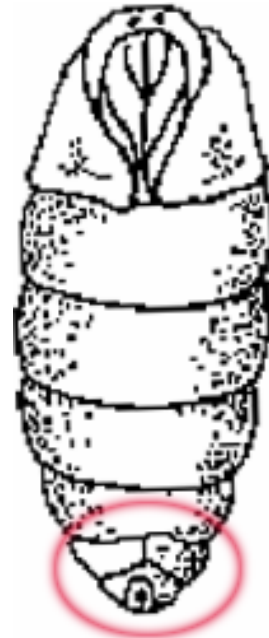
Pupa



♀



♂



♀



♂

Breeding of Sex limited silkworm variety

Sex chromosome in human



men(♂) : XY

women(♀) : XX



Sex determined

Sex chromosome in silkworm



male(♂) : ZZ

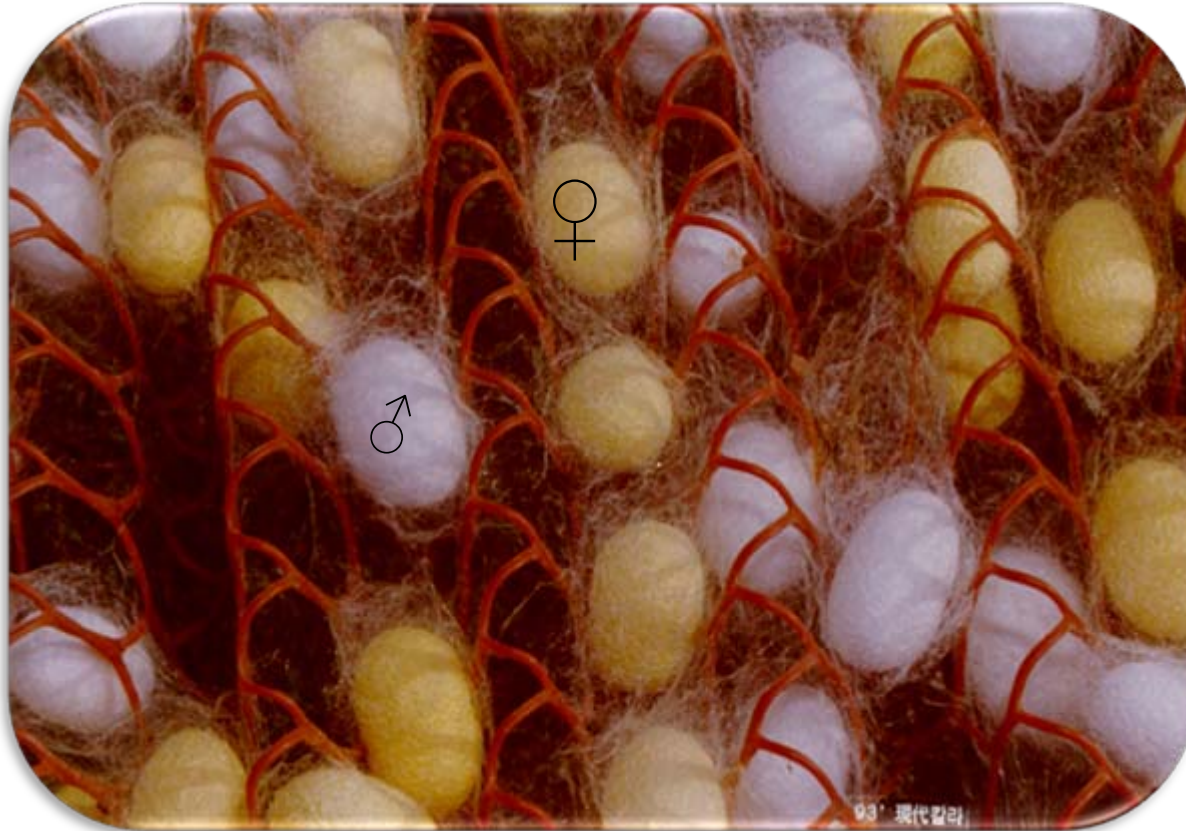
female(♀) : ZW



Sex determined

□ W chromosome : yellow cocoon, larval marker

Breeding of Sex limited silkworm variety with cocoon color



Breeding of Sex limited silkworm variety with larval marker



Comparison of testosterone contents

Treatments	ng/mg protein	Percent increase (%)
control	14.34 ± 1.63	100.0
Viagra	15.76 ± 1.83	109.9
Kissometer	13.20 ± 0.94	92.1
Silkworm moth extract 100mg	17.66 ± 1.13	123.2
Silkworm moth extract 250mg	19.04 ± 1.72	132.8
Silkworm moth extract 500mg	16.41 ± 1.14	114.4

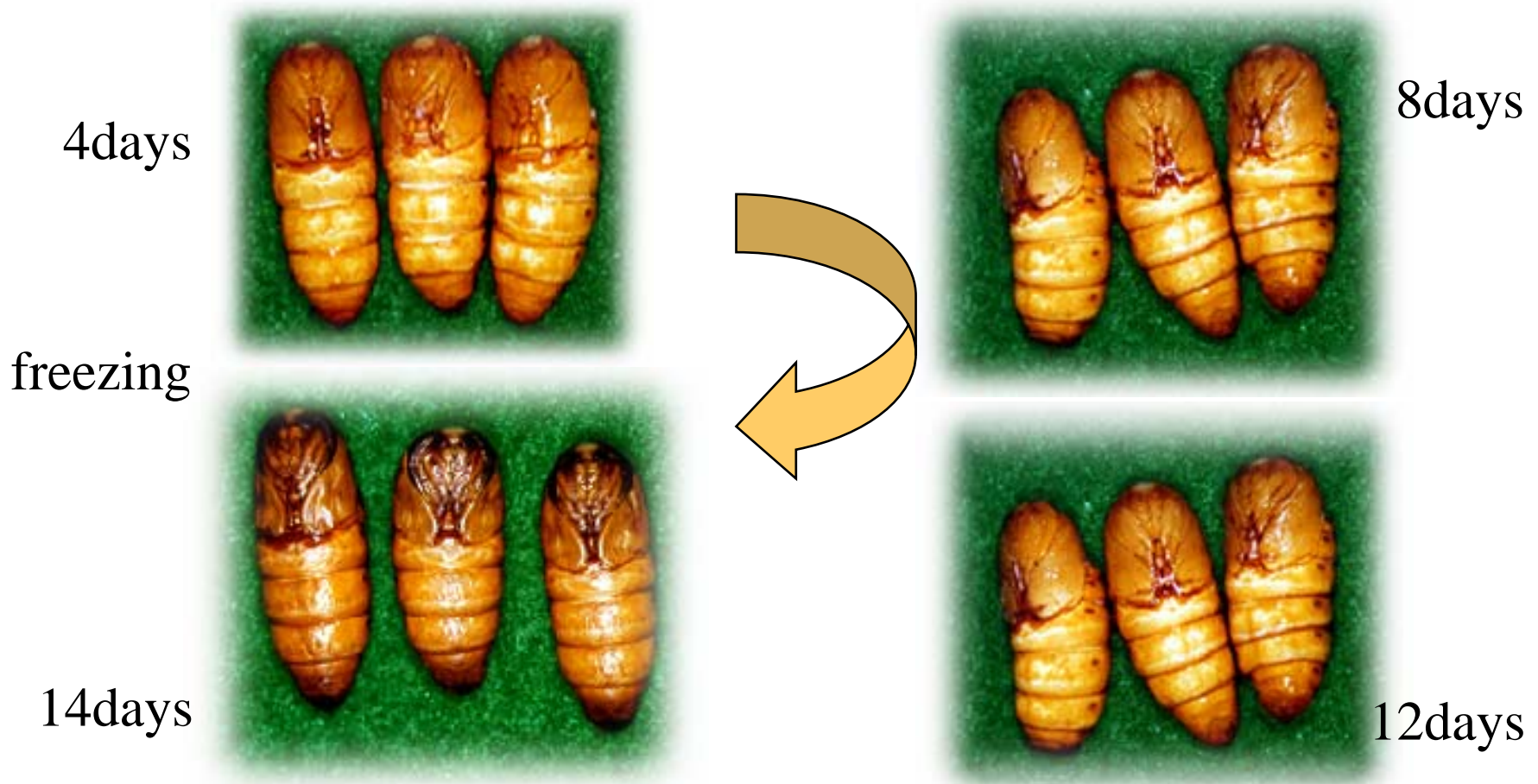
Comparison of Nitric Oxide contents

Treatments	nmol/ng protein	percent (%)
control	20.21 ± 1.12	100.0
Viagra	21.28 ± 2.01	105.3
Kissometer	21.33 ± 0.42	105.5
Silkworm moth extract 100mg	21.62 ± 2.23	106.9
Silkworm moth extract 250mg	23.56 ± 1.35	116.6
Silkworm moth extract 500mg	23.42 ± 0.93	115.9
Botany 1	23.98 ± 0.65	118.7

Comparison of Sperm count

Treatments	Sperm count (X10 ⁶ /g epididymis)	Percent(%)
control	53.38 ± 2.18	100.0
Viagra	59.72 ± 0.80	111.9
Kissometer	51.51 ± 1.68	96.5
Silkworm moth extract 100mg	70.22 ± 2.37	135.6
Silkworm moth extract 250mg	75.47 ± 0.09	141.4
Silkworm moth extract 500mg	66.47 ± 4.71	124.5
Botany 1	70.95 ± 2.05	132.9 45/73

Optimal growth stage of male pupa



Nuegra for stamina from male silk-moth



* '09 ~ : Clinical test for new functional health food

The effects of codyceps

- Anti-cancer
- Enhance of immunity
- Anti-fatigue
- Anti-stress



Codyceps from silkworm

Pacilomyces japonica (*P. tenuipes*)



The other type of Codyceps in China

Cordyceps sinensis



The development of Mini-kit Codyceps for home cultivation



Infection of codyceps



Cultivation



Mini-kit

The codyceps wine “Bullhee”



Healthy foods utilizing mulberry leaves

■ Mulberry leaves ice-cream

- Simple prepared powdery mulberry leaves : green smell
- Preservation of original color
- Finely ground mulberry leaves : below 200 meshes

■ Effect of mulberry leaves ice-cream on the blood glucose level

	Before	After 45 min	Lowering effect(%)
General	87.4±14.8	107.8±10.9	27.8
mulberry leaves	113.4±7.8	111.4±14.0	-1.6

Mulberry leaves ice-cream



Mulberry leaves jelly



The utilization of silkprotein

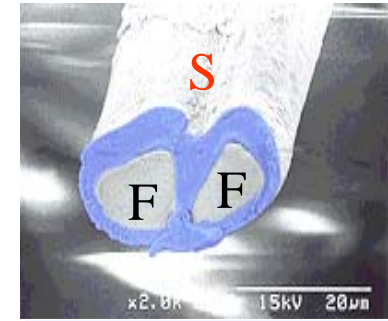


Silk fibroin
(75%)

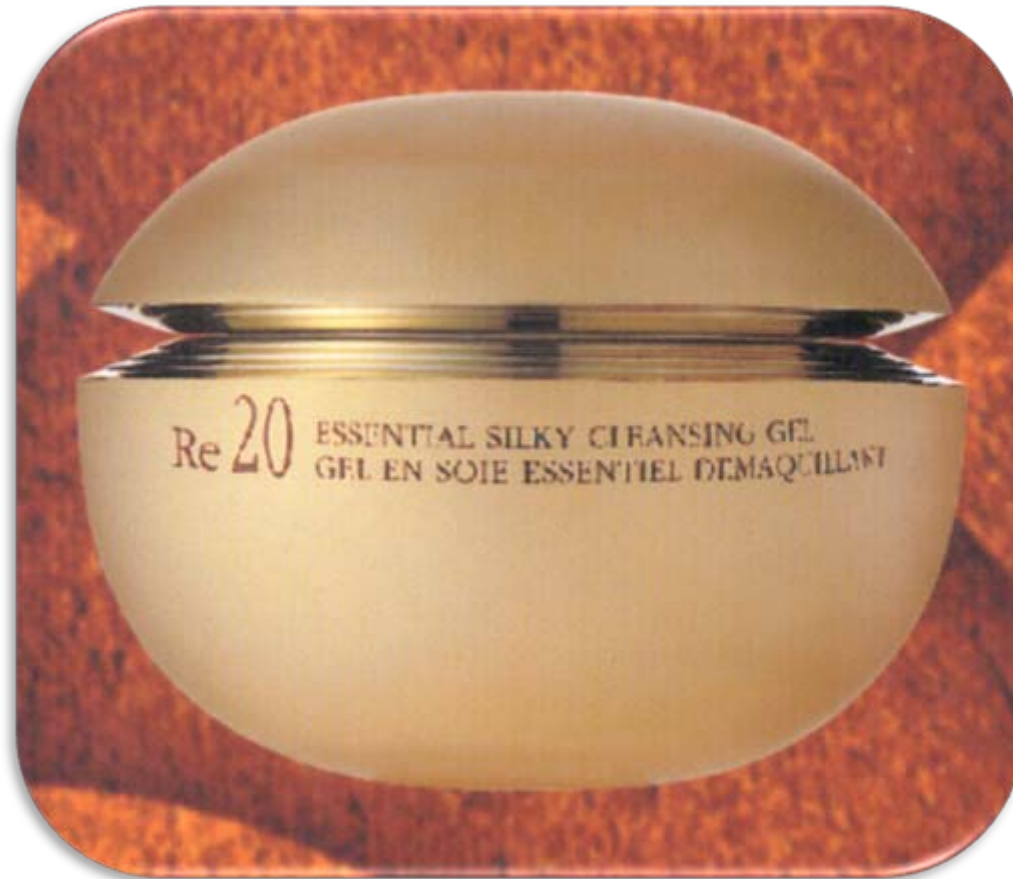
Dissolve & desalt

Bio-active sub.

Cosmetic materials



The silk cosmetics “Re20”



Silk Toothpaste (wound care in mouth)



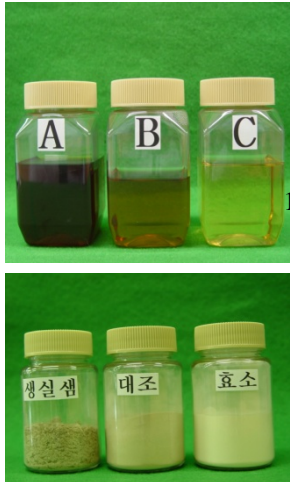
Functional analysis and extract of silkworm silk gland protein

- Active practical research of silk protein with skin compatible materials
- Labor and cost of degumming process and silkworm mounting in cocoon protein
- Mass production and functional analysis from silk gland protein (bio-film *et al*)

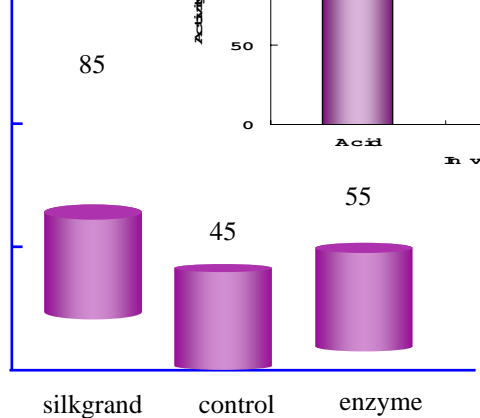
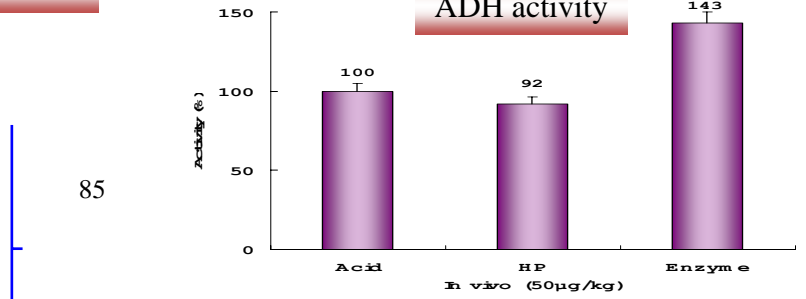
Extract of silkgrand



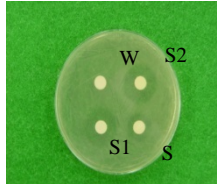
Dissolution and recovery of powder



ADH activity



Recovery ratio(%)



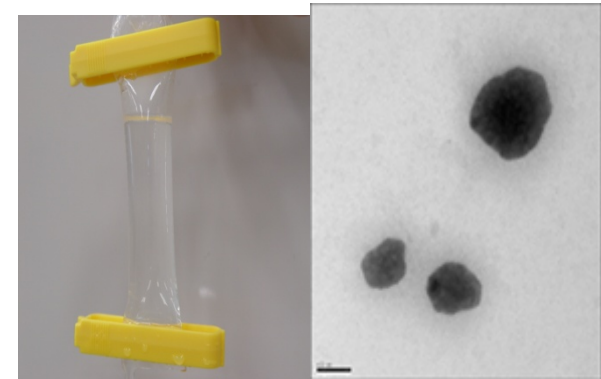
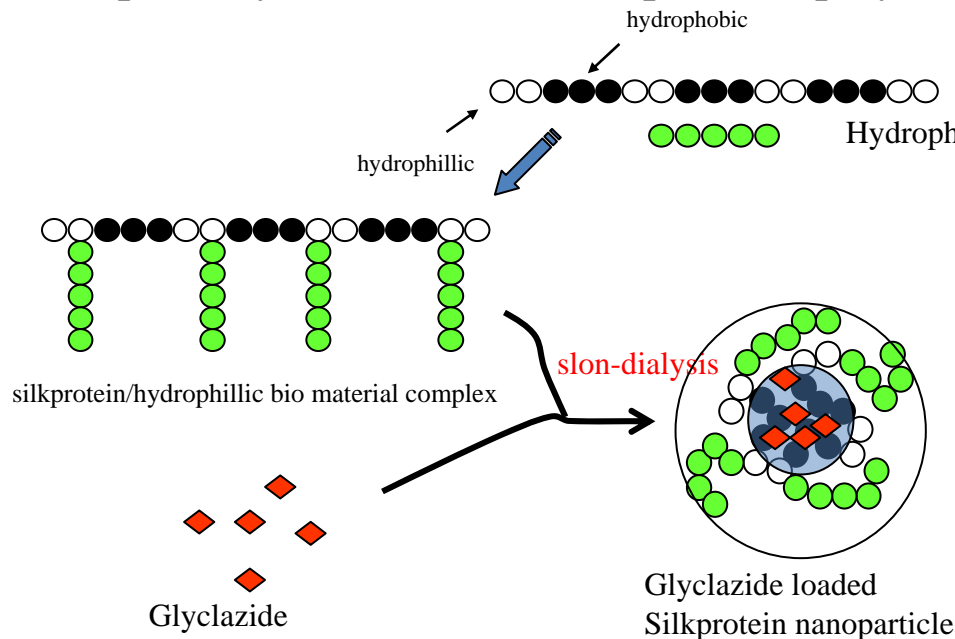
W:Water
S~S2:Silk

Decayed tooth

Nano-technology of silk-protein loaded

Trial of nano-particle development for transdermal delivery

- Produced nano-particle with loaded Glyclazide
- Shape analysis of loaded nano-particle (poly disperse spherical particle)

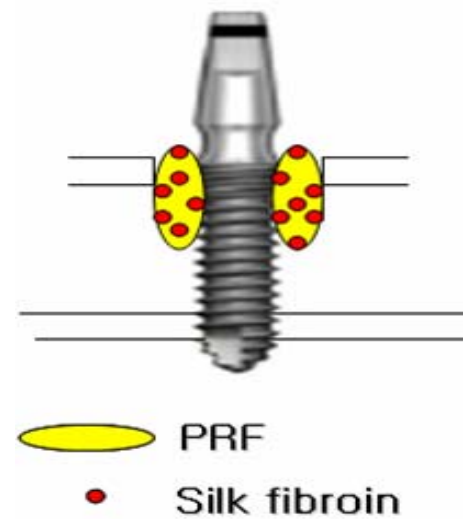


Nanoparticle soln and shape

Development of silk eardrum and silk artificial bone



Silk eardrum



Silk artificial bone

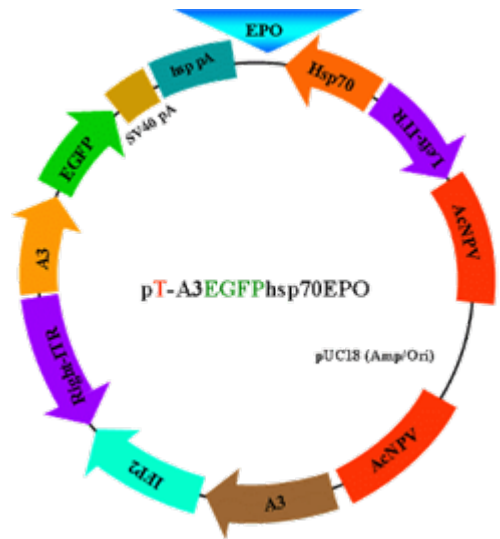
Research trend of new material with silkprotein

- ◆ Functional materials : food additive (soybean curd, ice-cream, rice water), cosmetics (recovery of skin, moisturizer)
- ◆ Medicine materials : wound dressing (bandage, toothpaste)
- ◆ High technology of biomaterial : recognition dementia, artificial internal organ, nano-technology (transdermal DDS)
- ◆ Research of production technology : silk gland extract, specific molecular weight, separation technology of peptides.

Silkworm Bio-factory

<Construction of piggy Bac vector fused with Ac NPV>

- Transgenic efficiency : 2% → 70%

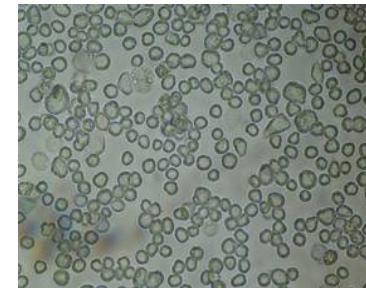


<improved piggy Bac vector>

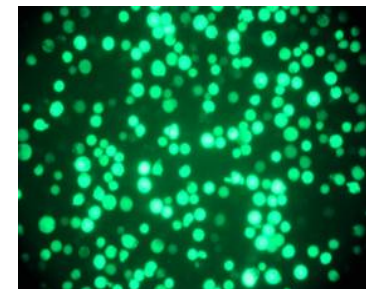
pG-A3-EGFP
-Hsp70-EPO

pT-A3-EGFP
-Hsp70-EPO

Brig



EGFP

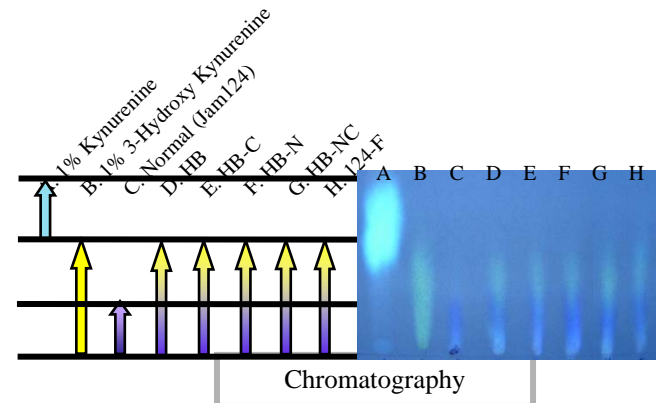
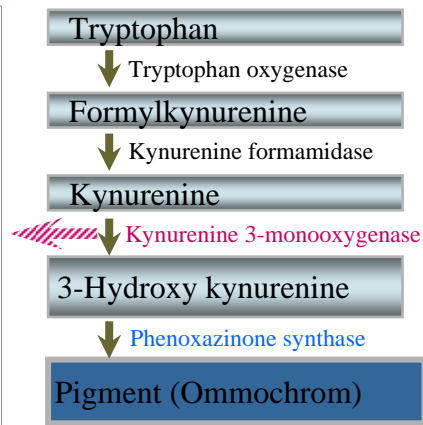
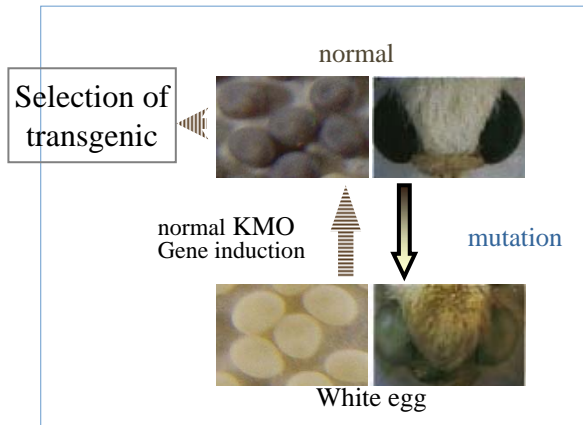


<comparison of pG and pT of transgenic vector>

Development of selection marker for transgenic silkworm

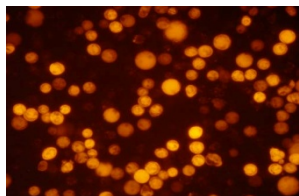
Development of marker to select transgenic silkworm under white light

- Using mutant silkworm strains : Hibakran

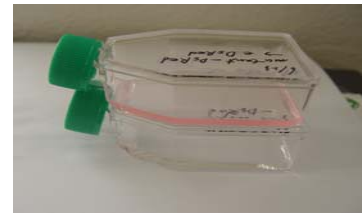
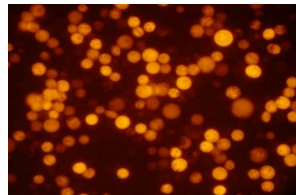


<possession of KMO gene in all 6 white egg variety, induction of white egg with mutation of phenoxiazinone synthase

- Point mutation of DsRed2 gene : detectable under white light



Normal DsRed2 gene

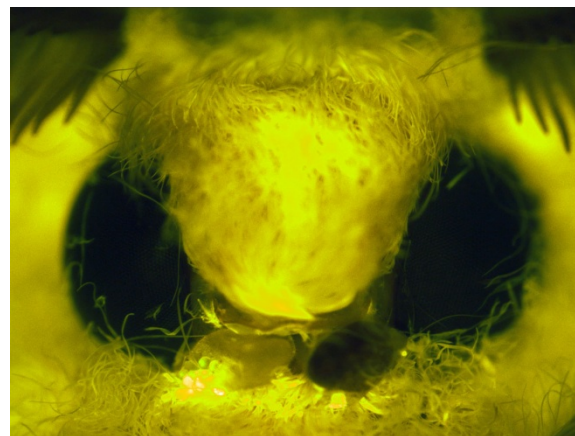


DsRed2 gene point mutation

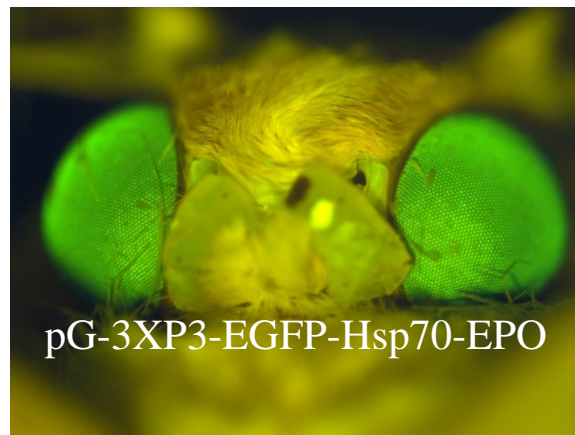
DsRed2 gene

Transgenic silkworms

Normal silkworm



Transgenic silkworm



Illumination under white light

EGFP-excitation-wavelength light

Research of Bio-factory of silkworm

- ◆ Development of highly efficient transgenic vector
 - Cloned piggyBac element, AcNPV and piggyBac transposase gene in only one vector (need not helper plasmid): native originality technology
- ◆ Construction of transgenic technology system :
 - more advanced technology than artificial insemination and microinjection
- ◆ Produce of materials with induction of valuable gene: EPO , anti-oxidants
 - in present, produce of new medicine for animals in future
- ◆ Expression control of specific gene : Mass production of specific materials and induction of mutation variety with blocking of produce

Silver agriculture and experience study with sericulture



Silver agriculture and experience study with sericulture



Buan Nue Town

Silver agriculture and experience study with sericulture



*See the inside silkworm in
Youngchon*

Silver agriculture and experience study with sericulture



Experience study in Youngchon

Current and Future of Korea Sericulture

■ Current Strategy

- Genetic resources of silkworm & mulberry : special var. breeding
- Silkworm Powder : Authorization with Health Functional Food and Herbal medicine from KFDA (now proceeding)
- New industry of mulberry fruit : Juice, Wine, Jam, Food additives
- Silk Protein : New bio-materials with anti-aging and silk-bone
- Activation of rural community : Good work for aged man

■ Future Strategy

- Bio-technology Sericulture : Production of Interferon, Lactoferrin with transgenic silkworm
- Silver agriculture and experience study with sericulture :
Attraction of retirees in city and experience study for children

THANK YOU