IgY+4 계란의 산업적 이용

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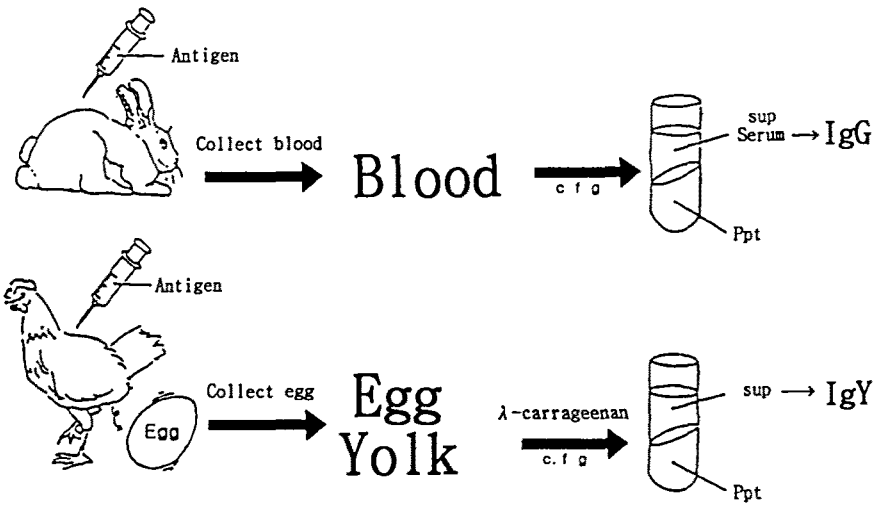
1. What's IgY?

IgY is the immunoglobulin found in chicken egg yolk and is the avian homologue of mammalian IgG.



2. What is IgY technology?

IgY Technology is a very wide area and covers all aspects from basic science and research to industrial applications of IgY, the major immunoglobulin (antibody) produced by birds.

3. Why IgY?

Antibodies are very useful tools in research and IgY have many advantages over conventional antibodies derived from rabbits, mouse, rats and other mammals.

4. Definition of IgY+4

Fresh Eggs with anti-*Helicobacter pyroli* IgY, anti-*E.coli*(ETEC) IgY, anti- *Salmonella enteritidis* IgY, and anti-*Salmonella typhimurium* IgY, simultaneously (Free Salmonella Eggs)

1) Anti-*Helicobacter pyroli* IgY(anti-gastritis antibody)

2) Anti-*E.coli*(ETEC) (anti-diarrhea antibody)

3) Anti- *Salmonella enteritidis* IgY(anti-food poisoning antibody)

4) Anti-*Salmonella typhimurium* IgY(anti-food poisoning antibody)

5. Contents of specific IgY (per egg)

*** This results is analyzed from Korea Food Research Institute(KFRI)**

- Sample : HEL▒P▒ZERO(IgY+4)
- Date Requested : 2001. 9. 4
- Date Tested : 2001. 9.20
- File No. AF20010920FS004

1) Anti-*Helicobacter pyroli* IgY : 86.4mg/per egg

2) Anti-*E.coli*(ETEC) : 13.3mg/per egg

3) Anti- *Salmonella enteritidis* IgY : 38.2mg/per egg

4) Anti-*Salmonella typhimurium* IgY : 42.1mg/per egg

6. Microorganism detection

*** This results is analyzed from Korea Food Research Institute(KFRI)**

- Sample : HEL▒P▒ZERO(IgY+4)
- Date Requested : 2001. 8. 14
- REF. No. AO2001-08-28-157

1) *Salmonella typhimurium* : Negative

2) *Salmonella enteritidis* : Negative

7. Chemical analysis

*** This results is analyzed from Korea Food Research Institute(KFRI)**

- Sample : IgY yolk powder(IgY+4)
- Date Requested : 2001. 8. 30
- REF. **No. AO2001-09-12-175**

Item	IgY yolk powder	unit
Heat capacity	666	Kcal/100g
Moisture	1.8	g/100g
Crude lipid	57.4	g/100g
Crude protein	29.4	g/100g
Crude ash	3.4	g/100g
Carbohydrate	8.0	g/100g

8. Activity of IgY : ELISA TEST

IgY(Immunoglobulin in yolk) against *Helicobacter pylori, E. coli*(ETEC), *Salmonella enteritidis* and *Salmonella typhimurium* was produced by immunizing hen with some antigens

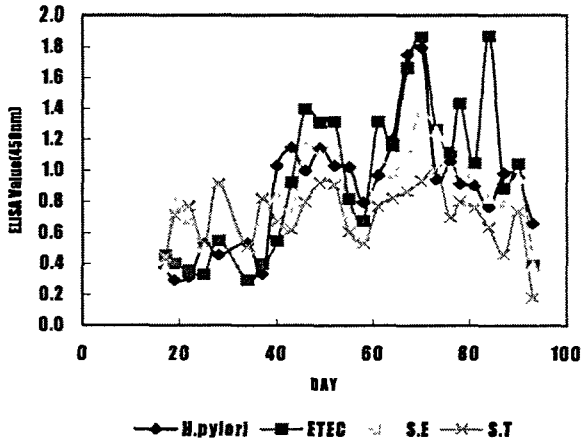


Fig. 1. Changes of various specific IgY activity.

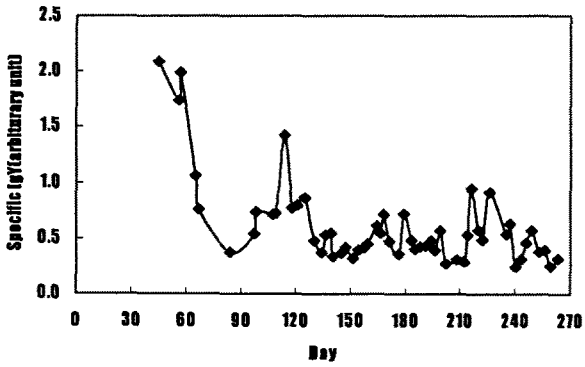


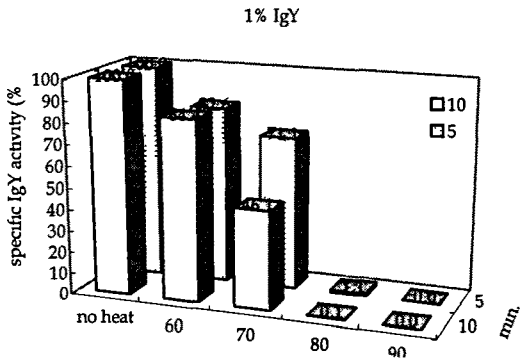
Fig. 2. Changes of anti-*H. pylori* IgY contents for nine month(IgY+4).

9. Heat stability

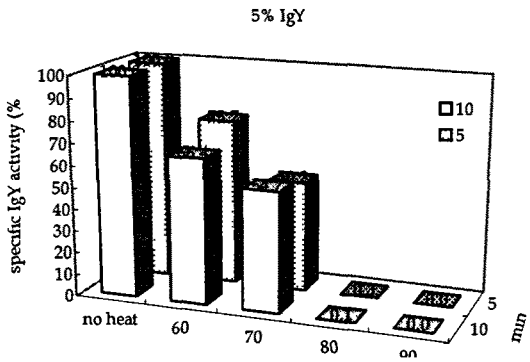
Anti-*H. pylori* specific IgY activity of IgY suspension after sterilization was stable at 63℃ for 30 minutes. However, Specific IgY activity was decreased below 50% at 70℃ for 10minutes.

Table 1. Anti-H.pylori specific IgY activity of IgY suspension after heat treatment

	1%		5%		10%		WSF	
	5 min.	10 min.	5 min.	10 min.	5 min.	10 min.	5 min.	10 min.
no heat	0.470		3.377		4.655		0.770	
60℃	0.388	0.397	2.557	2.241	3.331	2.833	0.576	0.532
70℃	0.334	0.217	1.694	1.847	2.548	2.241	0.519	0.437
80℃	0.005	0.001	0.012	0.003	0.009	0.002	0.006	0.000
90℃	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000



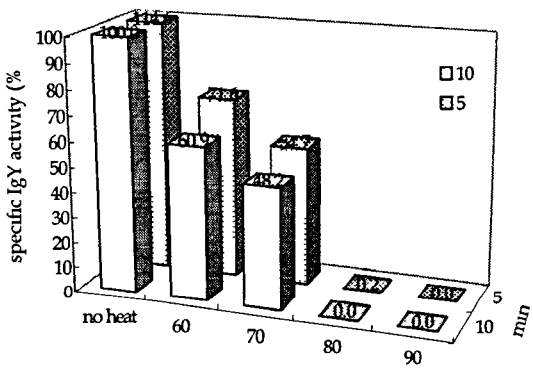
(a)



(b)

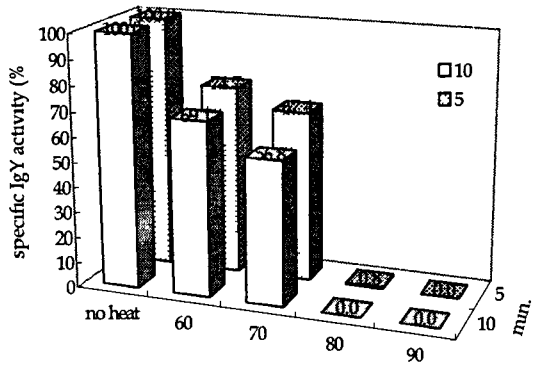
Fig. 3. Anti-*H. pylori* specific IgY activity of IgY suspension after heat treatment.
(a) 1% suspension of freeze-dried IgY, (b) 5% suspension of freeze-dried IgY

10% IgY



(c)

WSF IgY



(d)

Fig. 4. Anti-*H. pylori* specific IgY activity of IgY suspension after heat treatment.

10. pH stability

Specific IgY activity in a variety of pH conditions was studied. Residual IgY activity at pH 3.0 was 59.9%.

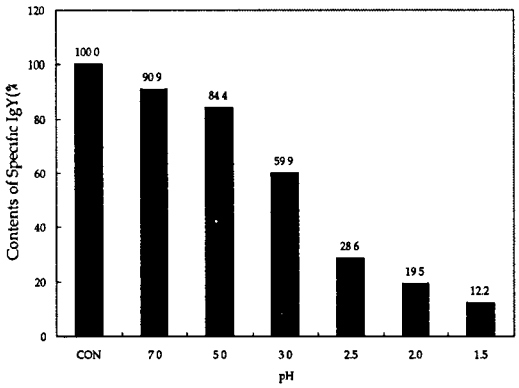


Fig. 5. Specific IgY activity in a variety of pH conditions

11. Enzyme stability

IgY activity in egg yolk after pepsin (100 unit) digestion on pH 2.0 and pH 4.0 was no differences.

IgY activity in water soluble fraction after pepsin (100 unit) digestion on pH 2.0 was totaly destroyed but IgY activity in water soluble fraction on pH 4.0 relatively stable.

IgY activity in egg yolk fraction after trypsin digestion on pH 7.2 was over 80% but

IgY activity in water soluble fraction was less than 50%.

(1) Pepsin treatment

1) Pepsin treatment on pH 2.0 with egg yolk

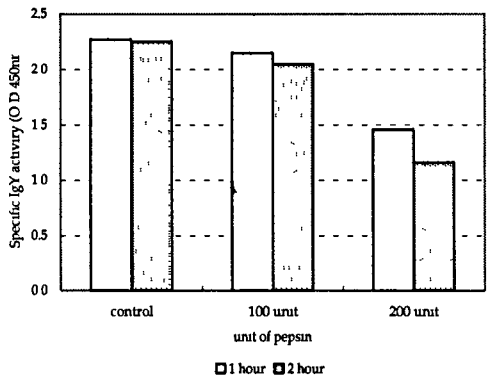


Fig. 6. IgY activity after pepsin digestion (egg yolk on pH 2.0)

2) Pepsin treatment on pH 2.0 with WSF(water soluble fraction)

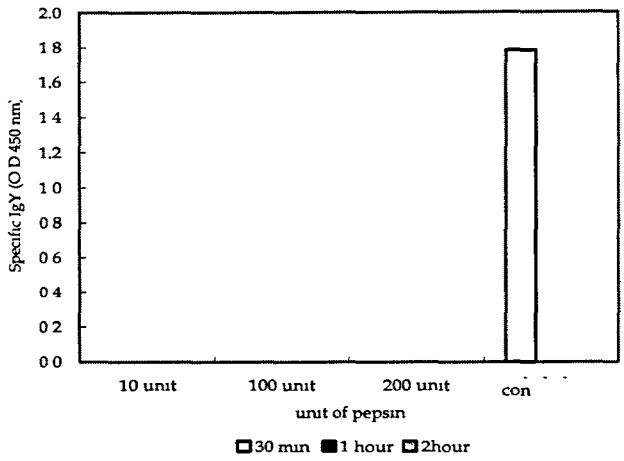


Fig. 7. IgY activity after pepsin digestion (WSF on pH 2.0)

3) Pepsin treatment on pH 4.0 with egg yolk

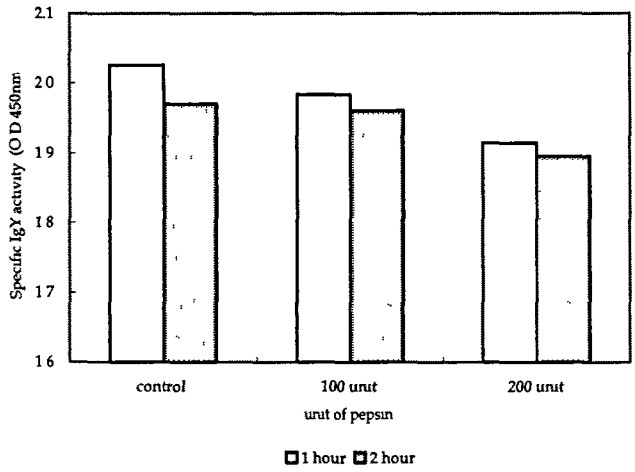


Fig. 8. IgY activity after pepsin digestion (egg yolk on pH 4.0)

4) Pepsin treatment on p H 4.0 with WSF(water soluble fraction)

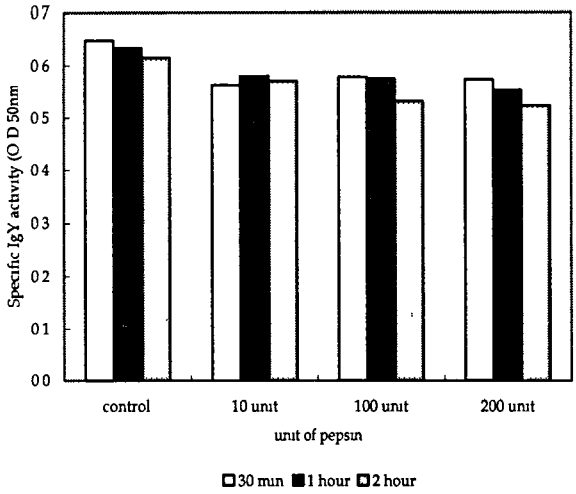


Fig. 9. IgY activity after pepsin digestion (WSF on pH 4.0)

(2) Trypsin treatment

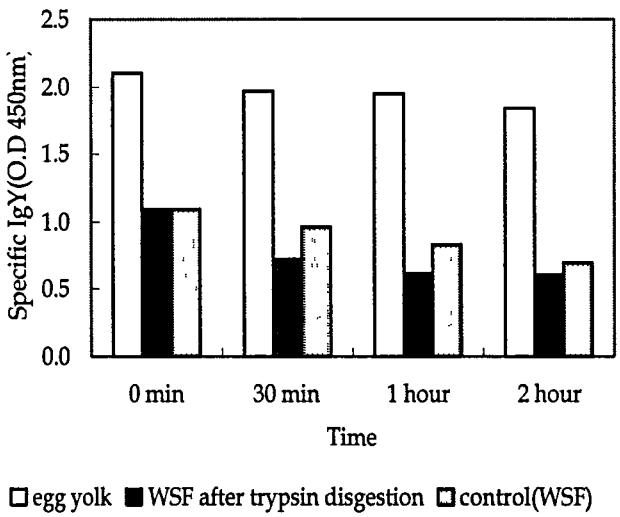


Fig. 10. IgY activity after trypsin disgestion (egg yolk and WSF on pH 7.2)

12. Separtion of crud IgY(water soluble protein fraction) by ionized water and concentration

* Specific IgY titer was proportional to concentration rate

	Concentration rate	WSF (water soluble protein fraction)	Freeze dried WSF after concentration *(2.512g/63ml)
Specific IgY (ELISA value 450nm)	control	0.285	0.282
	2	0.485	0.480
	3	0.645	0.654
	4	1.009	0.968
	5	1.002	1.110
	6	1.300	1.494
	7	1.526	1.498
	8	1.648	1.632
	9	1.859	1.836

* 2.512g/(one egg) of freeze dried WSF was solved into 63ml of PBS(Phosphate Buffer Solution)

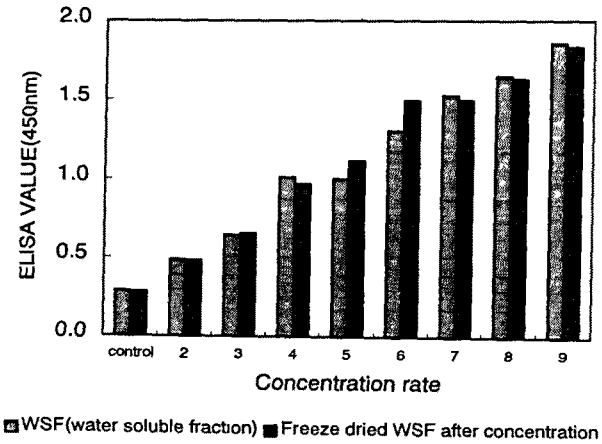


Fig. 11. IgY activity of crud IgY(WSF) and concentrated IgY

13. *In vitro* test

1) Tube agglutination test : antigen-antibody reaction

(This examination evaluated that specific IgY block *H. pylori* infection)

- *H. pylori* : $8x10^7$cell
- Specific anti-*Helicobacter pylori* IgY : 6.5mg
- *H. pylori* and specific IgY were mixed in glass tube and incubated at 37℃ overnight.
- The agglutinated complex of *H. pylori* and IgY was precipitated on the bottom of tube, if specific antigen-antibody reaction occurred.

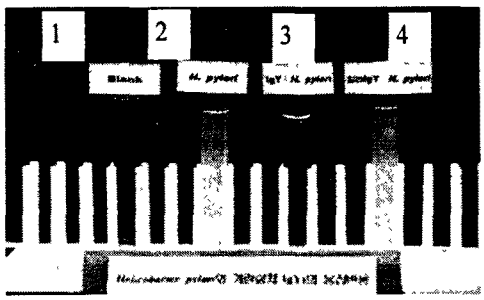
2) Growth inhibitory activity of specific anti-*H. pylori* IgY

(This examination was investigated that specific anti-H.pylori IgY have inhibitory activity against *H. pylori*)

- *Helicobacter pylori* : 1 x 10^7 CFU
- IgY concentration: 0 mg/ml, 0.16 mg/ml, 0.32 mg/ml,1.6 mg/ml, 8 mg/ml, 40 mg/ml)
- The mixture of *H. pylori* and IgY was Spread on TSA(Tryptic soy agar) and incubated anaerobic condition(10%CO_2)
- Count CFU(colony forming unit) : count the colony *of H. pylori* in agar plate

3) Result

1) Tube agglutination test : specific antigen-antibody reaction

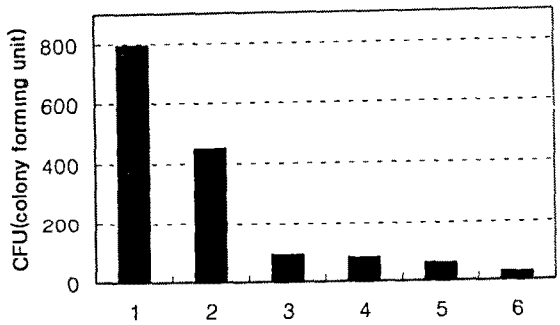


1. BLANK : saline
2. *H. pylori* : *H. pylori* only (anti-*H. pylori* IgY free)
3. IgY + *H. pylori* : *H. pylori* mix with specific anti-*H. pylori* IgY
   : *H. pylori* agglutinated with anti-*H. pylori* IgY and precipitated, clear supernatant.
4. non-specific IgY + *H. pylori* : *H. pylori* mix with non-specific anti-*H. pylori* IgY
   : *H pylori* didn't agglutinated with non-specific IgY.

This results appeared that specific anti-*H. pylori* IgY blocked antigenicity of *H. pylori*, but non-specific IgY didn't block.

2) Growth inhibitory activity of specific anti-*H pylori* IgY

< Growth inhibitory activity of specific IgY and natural extract mixture>



Concentration: 1) 0 mg/ml, 2) 0.16 mg/ml, 3) 0.32 mg/ml
4) 1.6 mg/ml , 5) 8 mg/ml, 6) 40 mg/ml

***In vitro* examination, anti-*H. pylori* IgY inhibited *H. pylori* growth in the treatment concentration more than 320mg/ml.**

14. *In vivo* test(animal examination)

: Passive protective effect of anti-*H. pylori* IgY and natural extracts against *H. pylori* infection.

- Materials & method
- Animals: C57BL/6 mice
- Dosage of Challenge *H. pylori* : 5x 10^7 cell
- Feed containing 5%, 10% mixture
(Mixture components: IgY(88%), aloe(2%), green tea(2.5%) and *Perilla frutescens*(1%))

→ Daily dosage of specific IgY : 5% feed -> 0.39mg/mice (1/20 egg)
10% feed -> 0.78mg/mice (1/10egg)

- experimental groups

A : feed containing 10% mixture(IgY) powder
B : feed containing 5% mixture(IgY) powder
C : feed containing 10% mixture(IgY)powder after 3 week-post-inoculation
D : feed containing 5% mixture(IgY) powder after 3 week-post-inoculation
E : infected control group

- Mice infected *H. pylori* and fed the mixture
- Stomach was done biopsy(3, 7weeks after) and than tested
- *Helicobacter pylori* detection method (in the experimental stomach)

A) Urease test : determined urease activity of *H. pylori*

B) PCR test (Polymerase chain reaction test) : detected ureA gene of *H. pylori*

* This method was used for detecting the *H. pylori* in the experimental stomach.

■ PCR test

PCR test is widely used for detecting the gene of organism. Gene is amplified by the specific primers, and confirmed by electrophoresis system.

Briefly, DNA is extracted from organism and PCR is processed by PCR cycling machine. PCR step consists of denaturation step(DNA was denatured linearly), annealing step(specific primer attach the specific site of DNA) and polymerization step(single DNA is polymerized by polymerase(enzyme) and then double DNA produced. This PCR step is progressed cyclically and DNA is amplified. The PCR product is electrophoresed on agarose gel and stained with EtBr and visualized under ultraviolet light..

- UreA gene specific primers (used in the examination)
  forward primer ;5'-ATTACTGACGCTGATTGTGC-3'
  reverse primer ;5'-CTGGAGAGACTAAGCCCTCC-3'
- Amplified PCR product : 109bp (DNA size)

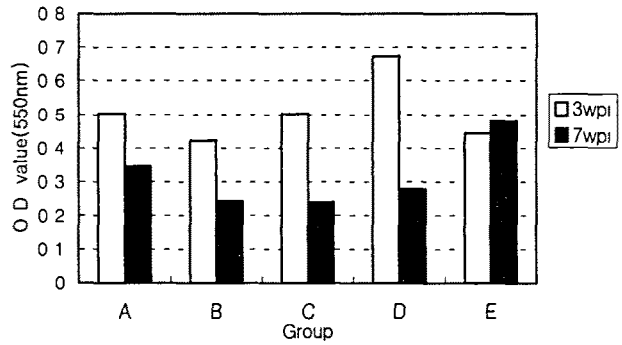
C) Histological test : histological change of stomach (H-E stain)This test was evaluated the protective effect of specific IgY using histological change of the experimental stomach. The stomach of infection control group and specific IgY feeding group were treated for histological test and stained H-E(Hematocylin-Eosin chromogen).

D) Results

1) Urease activity test

〈The urease activity of *Helicobacter pylori* in the stomach〉

absorbent of reacted broth, measured on O.D.550nm(mean)



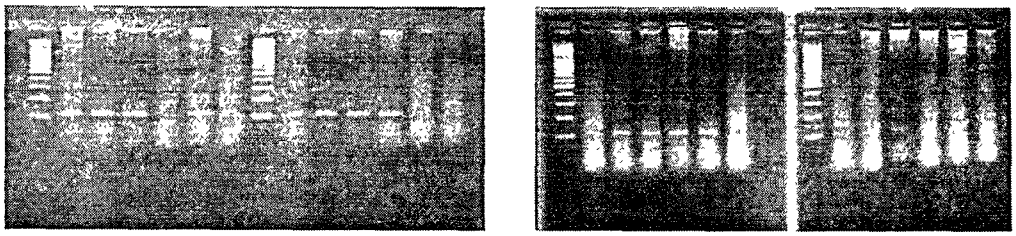
Urease activity of groups fed IgY containing feed decreased as 50% at 3wpi, and 67% at 7wpi(week-post-inoculatin). Urease activity of infected control group maintained infected level. This result proved protective effect of specific IgY.

2) PCR test

- *H. pylori* infectivity after feeding was determined by PCR test.

(A) Feed contained 5% mixture(IgY), 3 week-post-inoculation
Feed-point(3 wpi) After feeding(7 wpi)

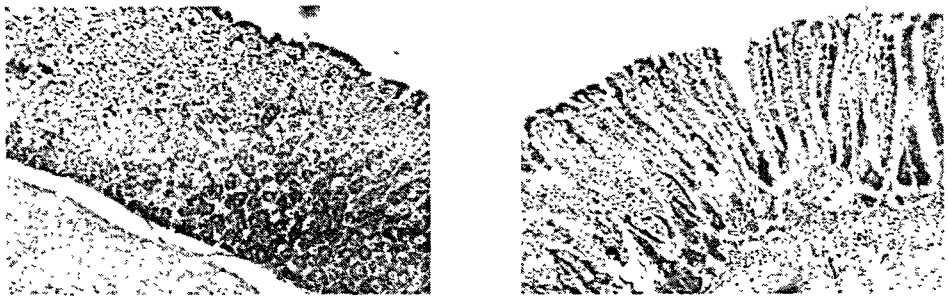
(B) Infected control group
3 wpi 7wpi



(A) Infectivity was decreased by feed containing 5% mixture(IgY); 66% -> 16%.(at 7wpi) (B) Infectivity of Infected control group didn't change between 3wpi and 7wpi.
This results identifed that IgY block the infection of *H pylori* ; the passive protective effect of IgY.

Electrophoresis of PCR product of ureA gene (*H. pylori*). PCR products was ,electrophoresed on 2.5% agarose gel and stained with EtBr. PCR product size is 109bp.

3) Histological test



(A) (B)

(A) The stomach of infected control group: infiltration of mononuclear cells on the lamina propria and submucosa and erosions in many parts of the epithelium. (B) The stomach of feeding (IgY containing feed) group: normal stomach.

Histological change of *H. pylori* infected group and IgY feeding group

15. Clinical examination

: Protection from H.pylori by feeding of anti-*H. pylori* IgY and natural extracts in human

A) Materials & method

-Volunteers: *H. pylori* infected volunteers(n=16)

-Groups :

1) feed IgY egg yolk powder and natural extracts mixture (n=8)

-> Total daily dosage of mixture : 14g/day

->Daily dosage of specific IgY : 110.5mg/day (12.32g egg yolk, 1.76 eggs)

(♠ specific IgY in egg yolk powder : 8.97mg/g)

2) feed WSF (n=8)

(water soluble protein franction -> clude purified anti-*H. pylori* IgY)

->Daily dosage of specific IgY : 70.4mg/day (3.2g WSF, 3.7 eggs)

▶specific IgY in water soluble protein fraction : 20mg/g

-Examination period : 21 days

-Protective effect of mixture(IgY) was evaluated by UBT test(Urease breast test)

UBT test determined urease activity of *H. pylori* in the stomach, and higher UBT value indicate severe infection of *H. pylori*.

-Measured at pre-examination and 21days after feedding.

B) Result

*Results of UBT test(urease breast test)

	Group of feeding IgY and natural extraction mixture		Group of feeding WSF (water soluble protein franction : Clude purified IgY)	
	Pre-xamination	21day-post-feed	Pre-examination	21 day-post-feed
Mean of UBT(DOB:/mil)	17.7 - 64.2 45.1	6.4 - 54.2 18.5	33.8 -76.6 59.8	26.7 - 59.3 33
% of volunteers :UBT value decreased more than 50%	71.4%		62.5%	

UBT test was determinative method of *H. pylori* infection in human. The UBT value of group of feeding IgY and natural extract mixture decreased from 45.1% to 18.5% and WSF group decreased from 59.8% to 33.0%. Therefore, it approved protective effect of IgY(anti-*H pylori*) against *H. pylori* infection.