Salmonella Enteritidis に対する酵母細胞壁の採卵鶏への飼料添加の免疫応答増強効果

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Dietary supplementation of yeast cell wall (YCW) may slightly enhance immune responses to *Salmonella Enteritidis* infection in layers

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Summary

YCW was supplemented intermittently (for three weeks after one week interval) at a rate of 0.05% to starter, grower, and formula feed of a layer flock (YCW-F) and its effect was evaluated by comparison with a control flock fed non-YCW feed (Cont-F). Ten birds of each flock were challenged with 10^-7 of *Salmonella Enteritidis* (SE) orally and cloacal swab and blood of 5 birds from each flock were cultured every 14 days. The serum antibody to SE was checked by means of rapid plate agglutination test of *Salmonella Pullorum* (RPA-SP). On SE colonization no significant difference was recognized between the 2 flocks. However, the positive rate of RPA-SP in YCW-F was higher than that in Cont-F. YCW addition may have an effect on antibody production.

Keywords: antibody production, feed additive, *Salmonella Enteritidis* (SE), YCW (mannan and β-1,3-glucan)

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Introduction

Yeast cell wall (YCW) consists of two layers. The outside layer contains a lot of the polysaccharide mannan, which mainly consists of mannose, and the inner layer contains the polysaccharide glucan, which mainly consists of glucose. Mannan can form mannan oligosaccharides (MOS).

YCW has anti-tumor activity9, 12, 13, 14, 15), which is thought to be the effect of activating non-specific cell immunity, such as the enhancement of macrophage activity due to β-1,3-glucan8, 12). Moreover, YCW also has the activity of eliminating pathogens such as *Salmonella spp.*, by competitive exclusion4, 8. Pathogens that invade animals cannot adhere to the carbohydrate connecting part of animal cells because polysaccharide mannan or the oligosaccharide form of the molecule adheres to the carbohydrate connecting part of the pathogen. Thus, YCW is expected to both activate non-specific cell immunity9, 12, 13, 14, 15) and eliminate5 pathogens such as *Salmonella spp.*

We performed an administration of YCW to the hen at a farm at which *Salmonella Enteritidis* (SE) contamination had been previously occurred. Moreover, we carried out SE challenge trials to birds fed YCW-supplemented formula feed.

The purpose of this study is to identify the both activities of YCW in layers. The farm was not invaded by SE during the experimental period and SE invasion was not found for the controls at the farm. Few findings in the SE challenge trials were recognized.

Materials and Methods

The dry yeast cell wall used in this study was the commercial product YCW (Tanabe Seiyaku Co. Ltd., Osaka). The product contains 13.9 ~ 15.3% polysaccharide mannan, of which the principal ingredient is mannose, and 21.5 ~ 22.8% macromolecular polysaccharide β-1,3-glucan. YCW is used for food and is highly safe. This experi-
ment was conducted in a farm of HACCP system. The farm consists of 1 growing-chicken house and 4 adult henhouses. The chicks were raised on the floor with brooders initially and then raised in a traditional cage of an open-type chick house while growing. Adult layers were fed on the floor with a nest in open-type adult henhouses. A henhouse accommodates about 1,000 birds. The floor of the henhouse was paved with concrete and sawdust was used as litter. Automatic chain feeder and bell drinker were used in the henhouses.

Marek's disease (MD) and fowl pox (FP) live vaccines were injected into the experimental birds at the hatchery. Infectious bursal disease live vaccine (IBD-L) (Nisseiken Co. Ltd., Tokyo) was administered with drinking water at 15 days of age in the growing-chick house. Newcastle disease and infectious bronchitis mixture live vaccine (NB-L) (Gen Corporation and Nisseiken Co. Ltd., Tokyo) was administered by spraying at the ages of 21 and 28 days. Afterwards, FP (Gen Co.) was injected at 31 days of age. Oil-adjuvant Newcastle disease and infectious bronchitis mixed inactivation vaccine (NB-K-Oil) (Nihon Pharmacy Co. Ltd., Tokyo) were injected to the leg muscle at 64 days of age. The inoculation quantity of each vaccine was applied for the dosage regimen.

Neo-teramix (Kokin Chemical Ltd, Osaka) was administered to 22 days of age for 3 days to prevent respiratory disease (mycoplasmosis). At 31 days of age, Tyrocin (Shionogi Medicine Manufacture Ltd, Osaka) was administered for 4 days to prevent mycoplasmosis, and Exuteshin (Daichii Seiyaku Ltd, Tokyo) were injected to the leg muscle at 64 days of age. The inoculation quantity of each vaccine was applied for the dosage regimen.

As for the competitive exclusion treatment, Broilact (Fujisawa Pharmaceutical Company Limited, Osaka) was administered to the one-day-old chicks by fog scattering at the hatchery and Aviguard* (Bayer Ltd, Tokyo) was administered to the feed in fodder at the age of 71 days.

**Experiment 1 (YCW supplementation in a laying flock and SE monitoring)**

Two thousand birds of one-day-old layer chicken for these experiments were divided into 2 groups (flock) of 1,000 birds. One was fed 0.05% YCW-supplemented starter and grower (YCW-F) and the other was fed non-YCW starter and grower (Cont-F) in the same growing-chicken house until 120 days of age. Then, the groups of adult layer birds were transported to 2 of the 4 adult henhouses, and YCW-F were fed with 0.05% YCW-supplemented formula feed and Cont-F were fed with non-YCW formula feed until 338 days of age. Both flocks were consistently monitored for SE invasion.

At the beginning of the experiment, feces attached to the litter (wood slices) in the 10 percent of transportation box, and 1 male chick and 10 female chicks were supplied for bacterial isolation (SE inspection). Eleven chickens were necropsied and the liver, the heart, the vermiform appendix, and the vestigial remnant yolk were checked to be salmonella-free according to the approved standard method for isolation of salmonella. Moreover, rapid plate agglutination test of Salmonella Pullorum (RPA-SP) was also performed with the serum of 11-day-old chicks.

During the experimental periods, cloacal swab (CS) and blood (whole blood and serum) of 10 birds (21 days of age), 50 birds (42, 71, 117, and 148 days of age), and 30 birds (338 days of age) were collected. Five pooled samples of CS were incubated together. On the day of investigation, we observed the health condition and checked for clinical signs such as diarrhea for both flocks, and measured the weight of 10 birds of each flock. To investigate potential SE invasion (environmental pollution inspection) for both flocks, dust samples (D) and drug swab samples (DS) of both flocks were also collected on each investigation day.

**Examination 2 (SE challenge trials)**

Ten birds of each flock at 120 days of age were transported from the growing henhouse to our laboratory and fed 0.05% YCW-supplemented formula feed and non-YCW formula feed. Ten birds from each flock were inoculated with 10^4-7 of SE in our laboratory experimental room. The organism was SE that had been detected on the farm before.

Ten birds (120 days of age) of each flock were inoculated 10^4-7 of SE orally and observed clinically. CS and blood samples (whole blood with heparin and serum) of 5 birds each were collected every 14 days. SE inspections were performed with the whole blood and CS samples. RPA-SP was also performed with the serum every 14 days. On the 7th and 14th days after SE inoculation, 5 birds of each flock were necropsied and the liver, the heart, the kidney, the lung, and the ovary were cultured with DHL agar using novobiocin directly, and mixed organs and intestine were cultured with the same agar after enrichment culture with TTB.
Method of Salmonella inspection

As for the monitoring test of the poultry farm for Salmonella contamination, about 10 g samples of dust [D] from the cage, facilities, and ales (containing feather, dried rat excrement, and insects) were collected from more than 30 points of the henhouse into sample bags (WHIRL-PAK BAGS : Nasco Co., Ltd, Dallas.) by hand using disposable plastic gloves. Drug swab [DS] technique was also performed. Gauze pad with 1.5 m strings dipped in 20% skimmed milk was dragged through the ales (when chicks were growing) or on the floor (soon after chicks were born and for laying hens) for more than 15 minutes. CS samples were collected with a sterile cotton applicator from 30 or 50 birds per group. Five samples were put together in 1 tube (SP tube : Eiken Kizai Co., Ltd, Tokyo.). Salmonella isolation culture technique was as follows. Before enrichment culture, D, DS, and CS samples were suspended in peptone solution. Subsequently, enrichment culture was performed with tetra-tionate broth (TTB DIFCO Co., Ltd, NJ). After enrichment culture, samples were inoculated on DHL agar to which novobiocin was added. The culture of blood was performed with the above-mentioned heparin blood. 0.5 ml of heparin blood samples was dropped on the DHL with a micropipette and spread on the agar with a glass stick. The incubation time and temperature were 24 hours and 37.8°C, respectively. Identification and serotyping of Salmonella isolates was accomplished with commercial 0 and H antisera (Denka Seiken, Tokyo, Japan) according to the method of the Japanese Society on Poultry Disease.

The cross-reaction to SE was detected by RPA-SP. The antigen for the pullorum disease rapidity diagnosis “Chiva” (Chiba Prefectural Serum Laboratory) was used for the detection of the cross antibody of SE.

To compare the detective rate of RPA-SP antibody between YCW-F and Cont-F, Student’s t test were performed.

Results and Discussion

Experiment 1

The result of SE monitoring test at the farm is shown in Table 1. No Salmonella spp. were detected from the feces attached to the litter in the transportation boxes or from 11 hatched chicks (male 1, female 10) in the pre-experimental inspection. No Salmonella spp. were detected in the samples of CS from both flocks during the experimental period. Moreover, no Salmonella spp. were detected in the environmental monitoring test of DS and D samples of both flocks at 0, 21, 42, 71, and 117 days of age in the growing-chicken house, and at 148 and 338 days of age in the adult henhouse. No clinical signs such as diarrhea were observed in both flocks throughout the experimental period. The average body weight (BW) of YCW-F was greater than that of Cont-F, but it was not significantly different. The antibody of SE by RPA-SP was not detected in both flocks until 42 days of age. One positive bird from 50 or 30 inspected birds was detected in YCW-F after 71 days of age, and 2 suspected positive birds at 71 days of age and 5 suspected positive birds at 148 days of age were detected. Only one positive bird was detected in Cont-F at

Table 1. Salmonella Enteritidis (SE) monitoring result with dust, drug swab, and cloacal swab, and RPA-SP antibody detection in YCW-F and Cont-F.

<table>
<thead>
<tr>
<th>Inspected day</th>
<th>4/23</th>
<th>5/14</th>
<th>6/4</th>
<th>7/3</th>
<th>8/18</th>
<th>9/25</th>
<th>3/2</th>
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<tbody>
<tr>
<td>Days of age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YCW-F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cont-F</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Drug swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YCW-F</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cont-F</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cloacal swab</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>YCW-F</td>
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<td>0/10</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/30</td>
</tr>
<tr>
<td>Cont-F</td>
<td>0/10</td>
<td>0/10</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/30</td>
</tr>
<tr>
<td>RPA-SP antibody positive</td>
<td>YCW-F</td>
<td>0/10</td>
<td>0/10</td>
<td>0/50</td>
<td>1/50 (± 2/50)</td>
<td>1/50</td>
<td>1/50 (± 5/50)</td>
</tr>
<tr>
<td>Cont-F</td>
<td>0/10</td>
<td>0/10</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/50</td>
<td>0/30</td>
</tr>
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RPA-SP: Rapid plate agglutination test of Salmonella Pullorum
YCW-F: 0.05% YCW-supplemented starter, grower, and formula feeds were fed.
Cont-F: Non-YCW starter, grower, and formula feeds were fed.
102 days of age.

**Experiment 2 (SE challenge trials in the laboratory)**

Before inoculation, SE was not detected from CS of all 10 tested birds of each flock. SE was detected from the CS samples of both flocks after SE inoculation. At the 4th day after inoculation with SE, SE was detected in all the CS samples of both flocks. In Cont-F, SE was not detected in the CS samples at 8, 9, and 10 days of age. No significant difference between the 2 flocks was observed. SE was not detected from the CS samples of both flocks on the 14th day. SE was not detected in the 5 heparin blood samples of both flocks for 1114 days.

As for the antibody of RPS (SE cross antibody), positive birds were not detected until the 7th day after inoculation. The antibody-positive rate of the 5 birds in YCW-F was assumed to be higher than that of the 5 birds in Cont-F after the 9th day (figure.1).

SE detection rates from CS and RPA-SP antibody-positive rates in 5 other birds of both flocks at 7 days of age were almost the same as the result mentioned above (SE detection in CS : YCW-F 4/5 and Cont-F 3/5, RPA-SP : YCW-F 1/5 and Cont-F 1/5).

The result of SE detection is shown in Table 2. At the necropsy on the 14th day, no gross lesions were found in the birds of both flocks.

In YCW-F, SE was detected from 1 kidney (1 bird) and 1 ovary (1 other bird) directly, and from mixed organ and intestine of 1 other bird after enrichment culture (3 of 5 birds were positive in YCW-F). In Cont-F, SE was detected from 1 spleen (1 bird) and 1 ovary (1 other bird) directly, and from 1 intestine of first 1 bird after enrichment culture (2 of 5 birds were positive in Cont-F). No significant difference between the 2 flocks was found.

At the necropsy on the 7th day, SE was only detected from 1 spleen of the birds in YCW-F.

We studied SE control measure with YCW in a farm that had experienced SE contamination, referring to the effect of guar-bean-enzyme-hydrolysates on the colonization of Salmonella Enteritidis in hen\(^2\). We cleaned up the farm and removed SE contamination, with an all-in-all-out system, repeated disinfection, and Salmonella spp. inspection for poultry houses and introduced chicks, putting years for two years. We planned this experiment on the condition that SE remained on the farm. However, this was fortunately not the case as SE was not detected and did not invade during the experimental period.

In experiment 1, antibodies of RPA-SP were detected from the birds in both flocks, but SE and Salmonella Pullorum were not detected in the monitoring test, although Proteus spp. were detected from some CS sam-

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**Figure 1.** Salmonella Enteritidis (SE) detection and rapid plate agglutination test of Salmonella Pullorum (RPA-SP) antibody detection in YCW-F and Cont-F after inoculation with 10\(^{4.7}\) of SE orally. YCW-F : 0.05% YCW-supplemented formula feed was fed.

Cont-F : Non-YCW formula feed was fed.
samples in both flocks. RPA-SP cross-reacts with SE (Kim) and with Citrobacter spp. (Sato, Sonobe). The positive antibody result in experiment 1 is assumed to be a cross-reaction to Proteus spp. or Citrobacter spp. because Proteus spp. were detected and the antibody-positive rate was low from the beginning to the end.

In experiment 2, the rate of detection of SE from CS samples and necropsied organs in YCW-F was higher than that of Cont-F. Therefore, effects of YCW to eliminate SE from the intestine and prevent adherence to the intestine were not found like the result of Akachi. In the report of Fukata, a control effect due to Salmonella by the 0.5% or 1.0% mannan oligosaccharide supplement was also not found.

Kaneko et al. evaluated an enzyme-linked immunosorbent assay (ELISA) using a pair of heat-extracted antigens of SE and Citrobacter Freundii to improve the cross-reaction of SE antibody with other serotypes having a common antigen. They expected that their ELISA system could detect the cross antibodies to SE more sensitively. Imai et al. also developed ELISA for detecting cross antibody to SE in experimentally infected chicken and determined that a lipo-polysaccharide antigen for use in ELISA was more sensitive than whole blood cell agglutination test, the tube agglutination test, and bacteriological examination, and was suitable for screening sera for antibodies to SE. Antibody test by ELISA system might evaluate the effect of production of this antibody more exactly.

We could not detect SE from the 28 birds in this farm whose RPA-SP with serum was positive before. In experiment 2, SE was hardly detected from CS in RPA-SP-positive birds after SE inoculation. It is better to choose samples from birds whose RPA-SP antibody result is negative for salmonella detection in this monitoring. Moreover, SE was not detected from blood at all. It might be necessary to improve the technique of Salmonella isolation.

A preventive effect to SE infection due to YCW supplementation could not be expected only from the result of our experiment 2 for 14 days, although Akachi reported a significant result for SE elimination from the 13th day to the 21st day. Our experiment might have to be prolonged for 7 more days because secondary antibody production was found.

However, some immune-enhancing effects of YCW (improvement of antibody production) were found in this experiment. YCW-F and Cont-F were separate groups from the same flock and were vaccinated in the same program. In the antibody detection in the farm, the movements of antibody on ND and IB were in accordance with the program and the movement on IBD reflected the vaccine and natural infection. However, antibody production on ND, IB, and IBD in YCW-F was higher than that in Cont-F (except for 71 days of age on ND-HI) as we reported in another journal.

Moreover, in experiment 1, the antibody-positive rate of RPA-SP that seemed to be due to Proteus spp. or

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Table 2. *Salmonella* Enteritidis (SE) detection from the necropsied birds of YCW-F and Cont-F

<table>
<thead>
<tr>
<th>Inspection</th>
<th>Direct culture</th>
<th>Enrichment culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>YCW-F</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Cont-F</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Positive rate

YCW-F: 0.05% YCW-supplemented starter, grower, and formula feeds were fed.
Cont-F: Non-YCW starter, grower, and formula feeds were fed.
Citrobacter spp. was higher in YCW-F than in Cont-F. In addition, in experiment 2, antibody production rate was slightly high in YCW-F compared with that in Cont-F.

Akazawa described in the book Marvel of the Barn that YCW induced the enhancement of cellular immunity, such as macrophage activation, but an increase in immunoglobulin was not found.

The enhancing effect of YCW on antibody production in layers should be investigated to clarify the influence of the immunoglobulin production as a secondary effect.

Acknowledgements

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References


要約

「Salmonella Enteritidisに対する酵母細胞壁の採卵鶏への飼料添加の免疫応答増強効果」

酵母細胞壁「YCW」を0.05%飼料添加したYCW区と無添加対照区の採卵鶏にSalmonella Enteritidis（SE）を
10^4個経口投与し、SE検出状況とひら白喉速達平板凝集反応によるSE交叉抗体の出現状況を14日間調査した。両区ともクロアカからSEが6日後まで高率に検出され、14日後には検出されなくなった。SE検出率や抗体陽性率に有意差はなかったが、陽性率はYCW区が若干高かった。

キーワード：抗体重生、Salmonella Enteritidis（SE）、飼料添加物、YCW（mannan and β-1,3-glucan）