The effect of a selected yeast fraction on the prevention of pullorum disease and fowl typhoid in commercial breeder chickens

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ABSTRACT A selected yeast fraction (**SYF**) was tested for the purpose of preventing pullorum disease and fowl typhoid in breeder chickens. In a challenge-protection experiment, commercial Three-Yellow breeder chicks were initially divided into groups A, B (challenged, treated), C (challenged, untreated), and D (unchallenged, untreated). The group A diet was supplemented with SYF and group B was supplemented with *Acidipure* via drinking water. At 7 D, birds of groups A, B, and C were divided into 2 equal subgroups (A1-A2, B1-B2, and C1-C2). Subgroups A1, B1, and C1 were challenged with *Salmonella pullorum* (**SP**), while subgroups A2, B2, and C2 were challenged with *Salmonella gallinarum* (**SG**). Clinical signs and mortality were recorded daily. At intervals, antibodies against SP and SG were detected by a plate agglutinate test (**PAT**). At 42 D, all birds were weighed and necropsied, lesions were recorded and challenge pathogens were isolated. Results showed that SP and SG isolation positive rates of groups A1-A2 were significantly lower ($P < 0.05$) than those of B1-B2 and C1-C2, respectively. The average body weight (**BW**) of groups A1-A2 was significantly higher ($P < 0.05$) than that of B1-B2 and C1-C2, respectively. In the field trial, chicks were randomly divided into 3 groups. Group 1 birds were fed a diet supplemented with SYF, group 2 diet was supplemented with *Acidipure* via drinking water, and group 3 was fed the same but un-supplemented diet as the control group. Antibodies against SP and SG were detected by PAT at 120 D. The antibodies positive rate of group 1 was significantly lower ($P < 0.05$) than those of groups 2 and 3, while no significant difference ($P > 0.05$) was found between groups 2 and 3. The results demonstrated that SYF supplementation could significantly decrease SP and SG infection rates, improve the BW of birds challenged with SP and SG, and was more effective than *Acidipure* via drinking water.

Key words: selected yeast fraction, *Acidipure*, pullorum disease and fowl typhoid, infection, plate agglutination test

INTRODUCTION

Avian salmonellosis is a widespread and harmful infectious disease that can spread not only in the environment but also in the flock. Pullorum disease (**PD**) and fowl typhoid (**FT**), caused by *Salmonella pullorum* (**SP**) and *Salmonella gallinarum* (**SG**), respectively, are two major salmonellosis diseases that seriously harm the health of the poultry flocks, especially in the local breeds of the brooding breeder flocks in China (Wei and Cui, 2015). Infection with SP and SG will lead to high mortality rates at an early age in poultry (Dhillon et al., 2001). The survivors are usually the carriers of *Salmonella*, although they usually display no obvious disease. The production performance of the breeder flock could also be adversely affected by SP and SG and may pass the pathogens through the egg to the offspring in the egg-producing period (Beaudette, 1925; Berchieri et al., 2001). The Chinese Three-Yellow chicken is one of the most popular local breeds in southern China for producing meat, due to its unique flavor, and is highly desired by consumers. However, most of these chicken farms are still endangered by SP and SG due to inadequate controls and/or decontaminations (Barrow and Freitas Neto, 2011; Wei and Cui, 2015; Filho et al., 2016; Guo et al., 2016). This can cause a major economic loss to the poultry industry. In the past years, antibiotics were used to prevent and treat the bacterial diseases like salmonellosis. In recent years, however, the abuse of antibiotics has led to the excessive growth of antibiotic resistance,

even the emergence of the multi-drug resistance strains, which has led to serious difficulties in prevention and treatment of salmonellosis in the poultry industry (Pan et al., 2009; Gong et al., 2013; Samanta et al., 2014; Penha et al., 2016). This trend also has a negative impact on food safety and public health, which prompts people to actively seek alternatives to antibiotics (Shu-Kee et al, 2015).

Currently, yeast cell-wall polysaccharide, a natural and high-efficiency immunopotentiator, is gradually replacing antibiotics in poultry production. Its major effective components are mannanoligo-saccharides (**MOS**) and β-1,3/1,6-glucans (Oyofo et al., 1989a,b). With the continuous improvement of technology and production processes, a high-quality yeast polysaccharide called *Safmannan*, was obtained. *Safmannan*, also known as a selected yeast fraction (**SYF**), is mainly composed of MOS, β-1,3/1,6-glucans, and D-Mannose. It has been reported that yeast β-D-glucans interferes with the adhesion of *Salmonella* in the gut and allows bacteria to aggregate together through the intestine without compromising the intestinal tract (Shao et al., 2016). It is speculated that due to the versatile nature of yeast fractions (reducing the effects of harmful pathogens in feed), less energy will be used to overcome disease challenges and to support the immune system, thus allowing more energy to be available for growth development (Corrigan et al., 2017). Reports using refined functional carbohydrates from enzymatically hydrolyzed yeast to prevent and control major food-borne pathogens such as *Salmonella enteritis* (**SE**) in chickens has shown good results (Walker et al., 2017). However, there is no report on beneficial effects of SYF on prevention and control of SP and SG infections in the Chinese local chicken breeds. At present, organic acids, such as those found in *Acidipure* are commonly used to control *Salmonella* infections in the local breeds of chickens (Pande et al., 2017; Polycarpo et al., 2017), but a comparison of effects on chicken *Salmonella* between SYF and organic acids has not been reported so far. Therefore, the objectives of the present study are to evaluate the beneficial effects of SYF against the infection of SP and SG in Three-Yellow chicken breeders and to compare it with the effects of using *Acidipure*.

MATERIALS AND METHODS

Materials

Safmannan, a commercial SYF product, was kindly supplied by Phileo-Lesaffre Animal Care (Marcq-en-Baroeul, France). *Acidipure*, a commercial product, was produced by Shanghai Frontan Animal Health Co., Ltd., China. One-day-old chicks of Three-Yellow chicken breeders, negative for PD and FT, from the grandparent flock, were kindly provided by Guangxi Hongguang Agricultural and Animal Husbandry Ltd., China. Plate agglutination test (**PAT**) antigens of SP and SG were purchased from Beijing Zhonghai Biotech Co. Ltd., China. *Salmonella* A to F group-specific diagnostic sera and *Salmonella* serotype-specific monovalent sera were purchased from the Ningbo Tianrun Bio-pharmaceutical Co. Ltd., China. Finally, SP and SG reference strains were provided by the Institute for Poultry Science and Health, Guangxi University, China.

Laboratory Challenge-Protection Experiment

One hundred and sixty 1-day-old commercial chicks were divided into groups A and B, along with a group C, which served as the challenged but non-treated control group, and these groups had 50 birds each. Also, another group of 10 birds, designated group D, served as the non-treated and non-challenged control (see Table 1). The control and the different challenged bird groups were housed separately, in identical pens in the same building, with a solid wall partition dividing them. Each pen was 1 m wide by 1.2 m long, resulting in a total pen area of 1.2 m^2 at a stocking density of 20.8 chicks/m^2. Pens contained one drink dispenser and one feeder. Room temperature was approximately 35°C for the first week. Ambient temperature was thereafter gradually reduced to 26°C by 2°/wk. The house temperature was kept uniform throughout by opening the window to ventilate in the daytime and then closing it at night. A photoperiod of 24 h of light was provided through the first week by 30-Watt heat lamps, then followed by 15-Watt incandescent lamps for 22 h through the 14th day, 21 h through the 21st day, and 14 h on the 22nd day and beyond. Natural light alone was used during daylight hours after 14 D of age. Each group was fed the same amount of feed daily. All diets were provided by the Guangxi Hongguang Agriculture and Animal Husbandry Co., Ltd.

During the entire one 42-D experiment, group A was fed a commercial diet (without any antibiotics, see Table 2) supplemented with 250 g/metric ton (MT) of SYF, group B was fed the same commercial diet as group A but *Acidipure* (1.5 mL/L) was added into their drinking water, and groups C and D were fed the same commercial diet without supplementation. Pure challenge strains were originally isolated from the distribution of *Salmonella* in Pullorum-Gallinarum antibody positive hens or in dead embryos and preserved at −80°C. The challenge strain was revived before the trial began as follows. The cryopreserved challenge strain was inoculated with a 5 mL broth tube and incubated for 16 h in a 37°C shaking water bath. The number of *Salmonellae* per milliliter of culture medium was determined by the plate-count method. According to the Spearman–karber method, the 50% infective dose (**ID50**) of *Salmonella* infection in chicks was determined. At 7 D, birds in groups A, B, and C were divided into 2 subgroups, equal in number, designated groups A1, A2, B1, B2, C1-, and C2. Groups A1, B1, and C1 were orally challenged with

Table 1. Arrangement of laboratory challenge-protection experiment and the field trial.

Groups		Treatments	Challenge strains	No. of birds	Groups	Treatments	No. of birds
		Challenge-protection experiment				The field trial	
A	A1	SYF[1] (250 g/MT[3] Added in the feed)	*Salmonella pullorum*	25	1	SYF[1] (250 g/MT[3] Added in the feed)	2833
	A2		*Salmonella gallinarum*	25			
B	B1	*Acidipure*[2] (1.5 mL/L Added in the drinking water)	*Salmonella pullorum*	25	2	*Acidipure*[2] (1.5 mL/L Added in the drinking water)	2489
	B2		*Salmonella gallinarum*	25			
C	C1	No SYF[1] and no *Acidipure*[2]	*Salmonella pullorum*	25	3	Control	3513
	C2		*Salmonella gallinarum*	25			
D	Non-treated and non-challenged	No SYF[1] and no *Acidipure*[2]	–	10			

[1]Selected yeast fraction (SYF) product was supplied by Phileo-Lesaffre Animal Care (Marcq-en-Baroeul, France).

[2]*Acidipure*, a commercial product, was produced by Shanghai Frontan Animal Health Co., Ltd., China.

[3]MT = metric ton.

Table 2. Composition and nutrient levels of the experimental basal diet.

Items (g/kg)	Starter[3]	Grower A[4]	Grower B[5]
Corn	596	688	750
Soybean meal (78.0 g/kg)	342	266	214
Soybean oil	22	9	0
Limestone (370 g/kg)	12.7	13.4	13.2
Calcium hydrogen phosphate	18	15	15
Sodium chloride	2.5	2.5	2.5
Methionine (998 g/kg)	2.5	2	1.6
Lysine HCL (780 g/kg)	2	1.8	1.4
Vitamin premix[1]	0.3	0.3	0.3
Mineral premix[2]	1	1	1
Choline chloride (500 g/kg)	1	1	1
Total	1,000	1,000	1,000
Calculated nutrient content			
Metabolizable energy (kcal/g)	2,950	2,950	2,920
Crude protein (g/kg)	198	171	152
Calcium (g/kg)	0.95	0.95	0.95
Available phosphorus (g/kg)	4.5	4.5	4
Lysine (g/kg)	12.1	10	8.6
Methionine (g/kg)	5.4	4.6	4

[1]Vitamin premix supplied the following per kg of diet: 10,500 IU vitamin A, 3000 IU vitamin D3, 25 IU vitamin E, 0.03 mg vitamin B12, 0.15 mg biotin, 4 mg menadione (K3), 4 mg thiamine, 6 mg riboflavin,18 mg D-pantothenic acid, 6 mg vitamin B6, 50 mg niacin, and 1.5 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese,100 mg; zinc, 95 mg; iron, 95 mg; copper, 12 mg; iodine, 0.8 mg and sodium selenite 0.2 mg.

[3]Starter diet was fed from approximately 0 to 42 D of age.

[4]GrowerA diet was fed from approximately 43 to 84 D of age.

[5]GrowerB diet was fed from approximately 85 to 120 D of age.

0.5 mL of SP (2.83×10^8 CFU/bird), while groups A2, B2, and C2 were orally challenged with 0.5 mL of SG (2.0×10^9 CFU/bird).

Field Trial

A total of 8,835, day-old Three-Yellow chicken breeder chicks, from the PD and FT negative grand-parent flock, were randomly divided into 3 groups with no less than 2,000 birds for each (see Table 1 for details). During the 1 to 120-D trial, group 1 was fed a commercial diet (without any antibiotic) supplemented with 250 g/MT of SYF, group 2 was fed the same diet as group 1 but *Acidipure* (1.5 mL/L) was added into their drinking water, and group 3 was fed the same commercial diet, without any supplement, as a control. Each group was fed the same amount of feed daily. Housing conditions and bird management followed standard recommendations. The trial was carried out on a commercial parent-stock farm of Guangxi Hongguang Agricultural and Animal Husbandry Ltd., China.

Parameters of the Laboratory Challenge-Protection Experiment

Clinical Signs, Gross Lesions and the Body Weight (BW) The clinical signs of tested birds and the gross lesions of the necropsied birds were observed daily and recorded. At the end of the experiment at 42 D, all birds in each group were weighed and data were recorded.

Detection of Agglutination Antibodies Against SP and SG At 1, 2, 3, 4, and 5 wk post-challenge (**WPC**), all the birds were evaluated to detect antibodies against SP and SG by PAT with the antigens of SP and SG according to the described method (Gast, 1997) and positive rates of the groups were calculated.

Recovery of the Challenged Bacteria At the end of the experiment at 42 D, all the remaining birds were euthanized and the organs were excised aseptically. The cloacal swabs, cecum, and cecum contents and the liver tissues were sampled, respectively for the isolation of the challenged *Salmonella* according to the China
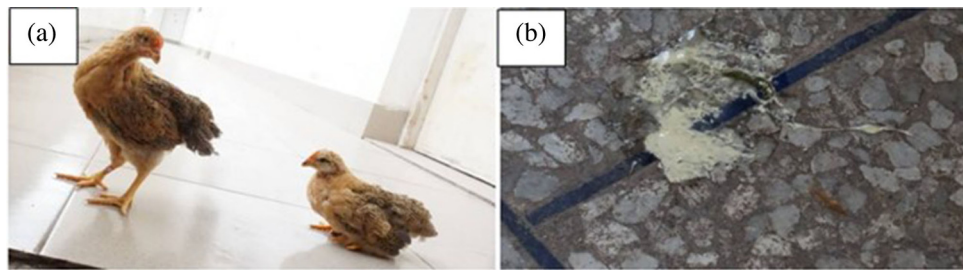
Figure 1. Clinical signs of the challenged birds. (a) Clinical normal chick on the left was from group A1 and the chick on the right was from group C1, showing weakness and poor growth. (b) White diarrhea from chicks in group C1.

National Food Safety Standard GB4789.4–2010 (food microbiological examination: *Salmonella*). According to this examination procedure, samples of visceral organs and cloacal swabs were first cultured for *Salmonella*. Then, suspected bacterial colonies were detected with A to F *Salmonella* diagnostic sera by using the slide agglutination test. Next, positive bacterial strains were confirmed by PCR. Finally, *Salmonella* isolates were further classified by *Salmonella* A to F group-specific diagnostic sera and *Salmonella* serotype-specific mono-valent sera, by the motility test and finally by a bio-chemical test. Confirmation entailed obtaining a 10 μL disposable inoculation loop full of solution from the sample and streaking that onto selective media until a pure culture was obtained.

Parameters of the Field Trials

At the age of 120 D, all pullets were tested for an-tibodies against SP and SG by the described PAT method and positive rates of the groups were calculated (Gast, 1997).

Statistical Analysis

Data were processed by Microsoft Excel and sub-jected to the GLM procedure of the Statistical Prod-uct and Service Solutions (**SPSS**), 19.0, statistical soft-ware. Factor analysis of variance and chi-square test were used to test the difference between groups. A value of $P < 0.05$ was used to indicate statistical significance.

RESULTS

The Clinical Signs and Gross Lesions Observed

Birds in group C1 and C2 exhibited weakness, ruffled feathers, poor growth, and white diarrhea (Figures 1a and 1b). By the necropsy of the birds, liv-ers with necrotic white foci of 1 to 3 mm in diameter (Figure 2b) and hemorrhage (Figure 2c), as well as a softened heart with pericardial effusion containing yel-low cellulose exudate (Figure 2d) could be observed. No bird death occurred during the test. Birds from group A1 were clinically normal (Figure 1a) and no lesions

in the liver were observed (Figure 2a). The percentage of birds exhibiting clinical signs and lesions consistent with SP and SG has been shown in Table 3.

Body Weight

In the SP challenge test, the average BW of chicks in group A1 was greater ($P < 0.05$) than that of groups B1 and C1 (Figure 3a) and no significant difference ($P > 0.05$) was observed between groups B1 and C1 even though the average BW of group B1 was slightly more than that of group C1. In the SG challenge test, the average BW of groups A2 and B2 was greater ($P < 0.05$) than that of group C2 but no significant difference ($P > 0.05$) was observed between groups A2 and B2. The BW of group D was significantly greater ($P < 0.05$) than the average BW of any of the other groups.

The Positive Rate of PAT in the Challenge-Protection Experiment

In the SP challenge, as shown in Table 4 and Figure 4, the positive rates of groups A1 and B1 were significantly lower than those of group C1 at 1 and 5 WPC ($P < 0.05$), but there was no significant difference between the 3 groups at 2 and 3 WPC ($P > 0.05$). The positive rate of group B1 was lower than those of groups A1 and C1 at 4 WPC, and the rate was significantly different from group C1 ($P < 0.05$).

In the SG challenge, the positive rate of group B2 was significantly greater than those of groups A2 and C2 at 1 WPC ($P < 0.05$), the positive rate of group A2 was significantly less than those of groups B2 and the C2 group at 2 WPC ($P < 0.05$), but there was no significant difference between the 3 groups at 3 WPC ($P > 0.05$). At 4 and 5 WPC, the positive rate of group A2 was significantly less than those of groups B2 and C2 ($P < 0.05$), and the positive rate of group B2 was significantly lower than that of group C2 ($P < 0.05$).

The Positive Rate of PAT in the Field Trial

The results of the field trial showed that the serum PAT positive rate of the SYF group (group 1, including
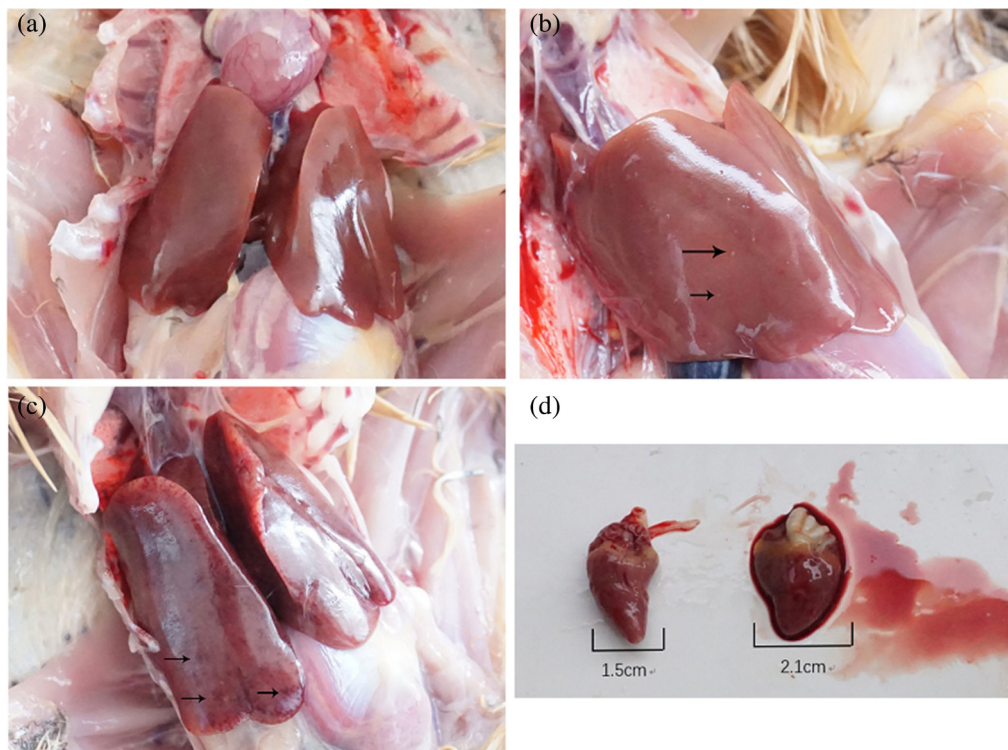
Figure 2. The gross lesions of the challenged birds. (a) Liver of group A1, no lesion was observed; (b) liver of group C1, necrostic white foci on the surface; (c) hemorrhage of liver from group C1; and (d) the heart from group A1 (on the left) showed no lesion and the heart from group C1 (on the right) showed a distorted shape.

Table 3. The percentage of birds exhibiting clinical signs and lesions consistent with *Salmonella pullorum* and *Salmonella gallinarum*.

| Challenged strains | Groups | Clinical signs white diarrhea | Lesions | |
			Necrostic white foci on the liver surface	Distorted shape of the heart
Salmonella pullorum	A1	26.09%	8.70%	21.74%
	B1	30.43%	26.09%	26.09%
	C1	36.00%	44.00%	36.00%
Salmonella gallinarum	A2	21.74%	26.09%	8.70%
	B2	28.00%	36.00%	16.00%
	C2	43.48%	43.48%	17.39%

both hens and cocks) was significantly lower ($P < 0.05$) than those of the *Acidipure* group (group 2) and the control group (group 3), respectively, while there was no significant difference ($P > 0.05$) between groups 2 and 3 (Table 5). No chicken death occurred during the field trial.

Recovery of Bacteria Used for Challenge From Challenged Birds

In the SP challenge test, the total positive rate of *Salmonella* isolation in group A1 was significantly lower ($P < 0.05$) than those of groups B1 and C1 (Figure 3b), and no significant difference was observed between groups B1 and C1. There was the same trend

noted in the SG challenge test. No *Salmonella* was isolated in group D.

Figure 3c indicates the positive rates of *Salmonella* isolation in different organs in the SP and SG challenge-protection experiments. The positive rates of liver and cecum were significantly higher ($P < 0.05$) than that of cloaca swab in both the SP and SG challenge-protection experiments, and the positive rate of the liver was slightly higher ($P > 0.05$) than that of the cecum.

In the SP challenge, the cecum positive rate of group A1 was significantly lower ($P < 0.05$) than those of groups B1 and C1 (Figure 3d). In the SG challenge, the cecum positive rate of group A2 was significantly lower ($P < 0.05$) than that of C2, and the positive rate of group B2 was slightly less ($P > 0.05$) than that of group C2. No *Salmonella* was recovered from group D.
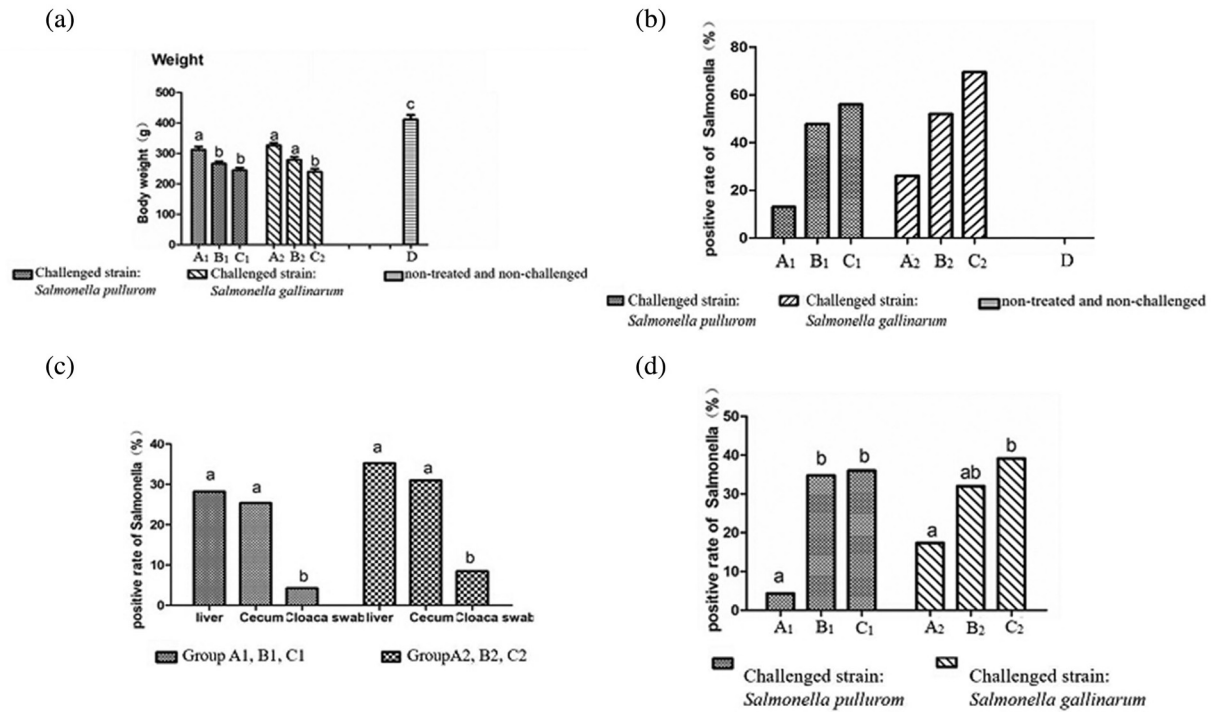
(a)

(b)

(c)

(d)

Figure 3. (a) Body weight of the birds from different groups. (b) The total positive rates of Salmonella isolation in different organs of the birds in Salmonella pullorum and Salmonella gallinarum challenge-protection experiment. (c) The positive rates of *Salmonella* isolation in the bird's different organs in challenge-protection experiment. (d) The isolation positive rate of the challenged *Salmonella pullorum* and *Salmonella gallinarum* in the cecum of each group. A1, B1, C1, A2, B2, C2, and D are the names of the experimental groups according to Table 1. [a,b,c]Means in a row with different superscripts are significantly different ($P < 0.05$).

Table 4. Comparison of PAT[1] positive rate in the breeders of each group in challenge-protection experiment.

Challenged strains	Groups	Weeks post-challenge (days)				
		1(14 D)	2(21 D)	3(28 D)	4(35 D)	5(42 D)
Salmonella pullorum	A1	0%[a]	39.13%	73.91%	69.57%[b]	39.13%[d]
	B1	0%[a]	47.83%	65.22%	47.83%[b]	47.83%[d]
	C1	60.00%	60.00%	76.00%	76.00%[c]	84.00%
Salmonella gallinarum	A2	10.00%[A]	21.74%	69.57%	43.48%	47.83%
	B2	30.00%	48.00%[B]	68.00%	60.00%[C]	60.00%[E]
	C2	10.00%[A]	56.52%[B]	69.57%	86.96%[D]	86.96%[F]

[1]PAT = plate agglutinate test.
[a–d]Means in a column with different superscripts are significantly different ($P < 0.05$) for groups A1, B1, and C1.
[A–F]Means in a column with different superscripts are significantly different ($P < 0.05$) for groups A2, B2, and C2.

Comparison of the Positive Rates of Challenged Salmonella Isolation With the PAT Results

Experiment data were subjected to correlation analysis by EXCEL, and the correlation coefficient r value was calculated by the CORREL formula. The range of the r-value was from -1 to $+1$ ($r > 0$ is a positive correlation, $r < 0$ is a negative correlation, and $r = 0$ means no correlation). The greater the absolute value of r is, the higher the correlation is. As seen from Table 6, a group with a higher positive rate of plate agglutination antibodies also had a higher positive rate

of *Salmonella* isolation. The r value calculated by the CORREL formula was 0.85, which was highly positively correlated.

DISCUSSION

Effect of SYF on the Prevention of Salmonella Infection in Challenged Young Chickens

PD and FT caused by *Salmonella* are serious diseases that endanger the poultry industry in China by causing adverse effects throughout the whole life of the bird and
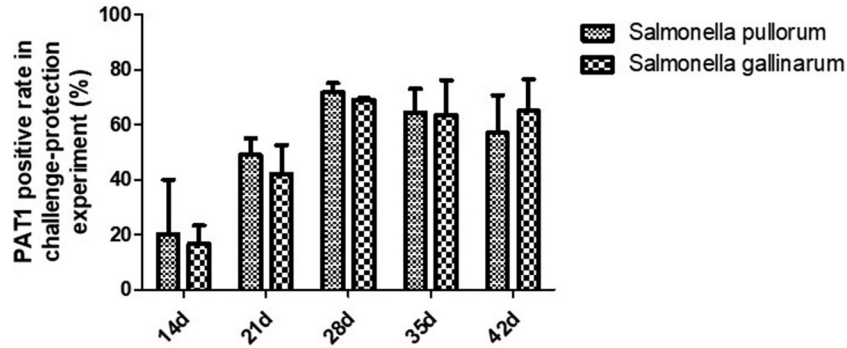
Figure 4. Comparison of PAT positive rate in the breeders of each group in challenge-protection experiment. PAT = plate agglutinate test.

Table 5. PAT[1] positive rates in the commercial breeders of each group at 120 D.

Gender	Groups	Treatments	No. tested	No. positive	Positive rate (%)
Hen	1	SYF[2]	1,941	107	5.51
	2	*Acidipure*[3]	1,582	238	15.04[a]
	3	Not supplemented	2,302	365	15.86[a]
Cock	1	SYF[2]	892	57	6.39
	2	*Acidipure*[3]	907	110	12.13[b]
	3	Not supplemented	1,211	144	11.89[b]

[1]PAT = plate agglutinate test.
[2]Selected yeast fraction (SYF) product, was supplied by Phileo-Lesaffre Animal Care (Marcq-en-Baroeul, France).
[3]*Acidipure*, a commercial product, was produced by the Shanghai Frontan Animal Health Co., Ltd., China.
[a,b]Means in a column with different superscripts are significantly different ($P < 0.05$).

Table 6. Comparison of the positive rates of the *Salmonella* isolation and the PAT.[1]

Challenged strains	Groups	Positive rate (%)	
		Salmonella isolation	PAT[1]
Salmonella pullorum	A1	13.04 (3/23)	39.13 (9/23)
	B1	47.80 (11/23)	47.83 (11/23)
	C1	56.00 (14/25)	84.00 (21/25)
Salmonella gallinarum	A2	26.09 (6/23)	47.83 (11/23)
	B2	52.00 (13/25)	60.00 (15/25)
	C2	69.57 (16/23)	86.96 (20/23)

[1]PAT = plate agglutinate test.

by even causing death in the early stages of life. Once infected with SP and SG, the bird will easily develop diarrhea, poor growth, weight loss, and possibly death, which consequently result in economic losses (Wei and Cui, 2015). In this study, the isolation rates of SP and SG in groups B1-B2 were slightly lower ($P > 0.05$) than those of groups C1-C2 at 42 D, while the isolation rates of SP and SG in groups A1-A2 were significantly lower ($P < 0.05$) than those of groups B1, B2 and C1, C2. The results showed that both SYF and *Acidipure* could reduce the infection of SP and SG in chicks, and the effect of SYF was more significant than that of *Acidipure*. In order to understand the prevalence of SP and SG in infected birds' organs, cloaca swabs, as well as cecum and liver samples were collected from the challenged birds for the isolation of challenged SP and SG (Figure 3c). Gast and Beard (1990a,b) demonstrated that the

isolation rate in the liver was the highest, and the isolation rate of cecum was slightly lower than that of the liver. Our results showed that the positive rates of SP and SG from the cloaca swabs were significantly lower ($P < 0.05$) than those of other organs, and the positive rate of livers was slightly higher ($P > 0.05$) than that of the cecum, these results coinciding with the previous study. Acute PD and FT are characteristically systemic infections with causative organisms that can be isolated from most internal organs. The liver, spleen, and ceca usually are involved and are the preferred organs to culture (Shivaprasad and Barrow, 2013). In this study, the fact that the positive rate of livers was slightly higher ($P > 0.05$) than that of the cecum has been attributed to the fact that the livers have white foci of infection, which measured 1 to 2 mm in diameter (Figure 2b).

SP and SG can be asymptomatically colonized in the intestine. Chickens that have long been infected with SP and SG may not exhibit any signs but their environment becomes polluted by the shedding with SP and SG in the feces. Environmental contamination may serve as a source of infection for other birds. SYF used in this study has the effect of reducing the probability of *Salmonella* from colonizing the intestine. Also, the addition of yeast β-glucans to the diet significantly ($P < 0.05$) reduced intestinal *Salmonella* (Shao et al., 2016). Mannanoligosaccharides (**SC-MOS**) can effectively inhibit *Salmonella* colonization in the intestine (Spring et al., 2000). A hydrolyzed yeast culture product can

reduce *Salmonella* colonization in the cecum of turkeys (Huff et al., 2013). In the present study, the SP and SG isolation rates of groups B1 and B2 were slightly lower ($P > 0.05$) than that of groups C1 and C2, while the SP and SG isolation rates of groups A1 and A2 were significantly lower ($P < 0.05$) than those of groups C1 and C2. The results indicated that SYF might inhibit *Salmonella* adhesion to the intestinal wall resulting in the clearance of *Salmonella* out of the body after adsorption with bacteria, thereby reducing the colonization of *Salmonella* in the intestine and the further invasion into the internal organs.

Clinical Signs, Gross Lesions, and Mortality in the Challenged Young Chickens

In the challenge-protection experiments, birds in groups C1 and C2 showed poor growth, white diarrhea, and severe liver and heart lesions, which were consistent with the classical clinical signs and gross lesions described in the literature (Shivaprasad and Barrow, 2013). Although the clinical signs and gross lesions were obvious, no chicken death occurred during the experiment, which differed from that of the literature (Shivaprasad and Barrow, 2013), and we speculated that it could be related to the breed of chicken used in the study that may have more resistance to the challenge.

Effect of SYF on the Positive Rates of PAT of SP and SG in the Breeder Pullets

At present, SP and SG are still the dominant serotypes of pathogenic *Salmonella* in China (Gong et al., 2013). The Agriculture Ministry of China has already included the positive rate of PD and FT, as the most important mandatory index, into the standard of breeder health. Therefore, the elimination of PD and FT on the breeder farm has considerable economic significance to the chicken industry. The serum agglutination test is widely used in most of chicken farms for a PD and FT eradication procedure (Gast and Beard, 1990a,b; Gast, 1997). In the challenge-protection experiment (Table 4), the PAT positive was observed at 1 WPC (14 D), and the PAT rate of birds in each group was higher at 5 WPC (42 D) than at 1 WPC. At 3 WPC (28 D), the PAT positive rate in the *Acidipure* group was slightly lower than that of other groups, but the PAT positive rate in SYF group was lower than that in *Acidipure* group at 5 WPC (42 D), and significantly lower than that in the non-treated control group. In the field trial, according to the practice of the routine eradication program against PD-FT on the breeders, PAT was carried out at 120 D before the pullets were transferred to a laying house to make sure they are SG-free and SP-free. At this age, the breeder hens are in the stage of sexual maturity and immunologic maturity, so the PAT is a convenient and accurate

measurement method (Shivaprasad and Barrow, 2013). Serologic tests before the transfer of pullets is very important before their laying, which can ensure that the eggs are free of SP and SG. In our field trial, results showed that supplementation with SYF in the diet had a significant effect on decreasing the positive rate of PAT for PD-FT. The positive rate of the SYF group was lower than that of the *Acidipure* group, which was significantly lower than that of the non-treated control group, whether it was experimentally induced infection or natural infection in breeder flocks on the chicken farm due to an environmental source or PD-FT incomplete decontamination. In conclusion, both SYF and *Acidipure* can reduce the infections of SP and SG in the breeder flocks, and SYF had a greater beneficial effect than *Acidipure*.

The positive results of the serum agglutination test indicated that the tested birds had been infected with *Salmonella*. However, it could not provide accurate information on the infection status of the current flock (Waltman and Horne, 1993). Both the positive detection results of PAT and bacteria isolation were compared in our challenge-protection experiment at 42 D in order to understand the correlation between these 2 detections. The results showed that the positive numbers of serum PAT were higher than that of bacteria isolation. Both indices had a high positive correlation. However, *Salmonella* could not be isolated from infected birds with positive antibody scores in the PAT, indicating that the chicken antibody agglutination test has certain reference value, but deviations may exist. Therefore, it is not possible to rely solely on the PAT for the detection of positive birds. It is necessary to use the national standard method, bacteria isolation, for the detection of positive birds.

Effects of SYF on Body Weight Gain of Breeders

The poor growth performances following *Salmonella* infection have been reported (Marcq et al., 2011; Wang et al., 2012). The result of strong inflammatory responses of the intestinal mucosa may lead to excessive consumption of nutrients to fight infection rather than to promote normal growth. In the present study, the average BW of the 42-day-old breeders in the non-treated and non-challenged group was significantly greater than those of the challenged groups (see Figure 3a), indicating that *Salmonella* infection inhibited the normal growth of chicken breeders. The average BW of groups A1 and A2 at 42 D was significantly greater ($P < 0.05$) than those of groups B1-B2 and C1-C2. It is inferred that supplementation of SYF in the diet helps to improve the feed conversion before the breeding stage of pullets, and the decrease of BW caused by *Salmonella* infection in chicks was significantly eased, which is consistent with previous research reports listed here. Refined functional carbohydrate feed additive

derived from yeast supplemented in the diet increased the weight gain, daily gain and feed intake (Walker et al., 2018). As the dosage increased, there is an uptrend. Zhang et al. (2008) showed that the addition of β-1,3-1,6 glucan to the diet could improve the daily weight gain of chickens. Combination of an enzymatically hydrolyzed yeast and yeast culture with a direct-fed microbial in the feeds can improve feed conversion and nutrient digestibility (Gómez et al., 2012). Both β-glucans and MOS in yeast cell wall polysaccharides can improve the growth performance of chickens, and their effects related to the separation degree of functional components in yeast cell wall polysaccharides (Shao et al., 2016). SYF used in this study is a screened yeast cell wall with a special structure, in which the contents of β-glucans and MOS are more than 20%. Supplementation of SYF helps to increase the body weight gain and improve the feed conversion in chickens.

In summary, the supplementation of SYF in the diet effectively reduced the infection in Three-Yellow chicken breeders challenged with SP and SG, significantly reduced the positive culling rate of PAT of the pullets, reduced the BW decline in birds due to *Salmonella* infection, and its effect was obviously superior to the use of *Acidipure* supplemented via drinking water.

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ETHICS STATEMENT

The live animals described in this study were conducted according to the National Guidelines to Humanitarian Governance of Laboratory Animals Welfare (National Development and Reform Commission of the People's Republic of China, 2006) and were approved by the Animal Welfare and the Animal Experimental Ethical Committee of Guangxi University. All feeding procedures were conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. The animals were sacrificed by carbon dioxide narcosis.

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