Production of IgY by layers injected with *Salmonella typhimurium*

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Abstract

In the present study, production of IgY and performance of layers immunised with *Salmonella typhimurium* were evaluated. Thirty 23-wk old layers (Lohmann Brown) with average body weight of 1.6 ± 0.03 kg. were randomly and equally divided into two groups. Both groups were placed in a specific pathogen free room equipped with an exhaust fan. The layers were placed in single battery cages with slightly tilted floor which allowed eggs to roll straight into the collecting tray. Lyophilized, attenuated whole cells of *S. typhimurium* strain [serotype: Somatic (O), antigen 1, 4, (5), 12, flagella (H) N12 phase I: 1; phase II: 1, 2 (Group B)] that were emulsified with Freund's complete adjuvant were used to immunise the layers intramuscularly 3 times at 2-wk interval (treatment group). Layers in the control group were injected with a solution of sterilized phosphate-buffered saline (PBS) emulsified with an equal volume of Freund's complete adjuvant. The experimental period lasted for 14 wks. Specific antibodies were detected by enzyme-linked immunosorbent assay. The results showed that the birds lost weight during the injection period, but the weights slowly increased in the following weeks. However, the weight of layers in the treated group remained lower ($P<0.05$) when compared to the period before injection. The loss in body weight of layers was the result of reduced feed intake, in particular during the injection weeks. Layers in the control group had a higher egg production, by about 10.1% than layers in the treatment group throughout the experimental period. The egg production for layers in treatment group showed a significant decrease ($P<0.05$) during the immunization period by 28.8%. The egg production significantly increased ($P<0.05$) to 71.9% after this period, but the production did not reach the level before immunisation. The weight of eggs of treated layers were significantly lower ($P<0.05$) than the control layers during the injection weeks. The egg mass was significantly reduced ($P<0.05$) in layers during the injection period, but increased during the postprandial period. IgY titre continued to increase after the first booster and reached a high level at about 4 wk postprandial and IgY level was maintained until the end of the experimental period. The specific IgY was about 9.6 mg/g of yolk, approximately 1% w/w of egg yolk. The present study showed that specific IgY antibodies were successfully raised against *S. typhimuirum* through injection of attenuated *S. typhimurium* whole cells to layers.

Key words: Antibody production, layers, egg production, egg mass, specific IgY

Introduction

Salmonella spp. remain a major public health problem for the whole world. Salmonella infection, or salmonellosis causes an intestinal inflammation and most people develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 h after infection. Salmonellosis is usually a foodborne disease, spread through consumption of contaminated foods, especially eggs, and poultry (CDC, 2014). Among the over 1800 known serovars which under current classification are considered to be different species of *Salmonella*, *S. typhimurium* is one of the leading causes of salmonellosis in humans (Derache *et al.*, 2009).

Oral administration of antibodies is a treatment strategy that plays an important role in preventing infectious diseases and diarrhea in animals as well as humans. There are several potential sources of antibodies for oral immunotherapy, which include human IgG, monoclonal antibodies, bovine colostrums (Chen and Chang, 1998) and chicken egg yolk immunoglobulin Y (IgY) antibodies (Kassaify and Mine, 2004). Immunoglobulin Y is the major antibody found in eggs from chickens which plays a similar biological role as mammalian IgG, the major immunoglobulin providing defense against infectious agents (Munhoz et al., 2014). Immunoglobulin Y has distinct properties which can be exploited in various applications in diagnostics and therapy and in the development of an mmunocapture assay in a variety of clinical samples (Shin et al, 2009) and for detection or quantification of antibodies and specific antigens associated with various diseases (Spillner et al., 2012; Munhoz et al., 2014).

The production of IgY or the IgY-technology refers to the non-invasive method involving the immunisation of chickens and the extraction of specific IgY antibodies from egg yolk (Schade et al., 2005). This alternative method supports the animal welfare as it reduces painful manipulations of the animals and it is recommended as a replacement for the usage of IgG.

The IgY yields have been reported to range from 60 to 150 mg IgY per egg (Cook and Trott, 2010; Pauly et al., 2009), of which 2% to 10% is antigen-specific (Schade et al., 1991; Tini et al., 2002). The amount of IgY deposited into the yolk is dependent on several factors, including age, breed of chicken, antigens, and methods of immunisation. Nonetheless, this technology offers economical advantages because the cost of hens is lower than that of larger animals.

The deposition of IgY into the egg yolks of the immunised bird provides an elegant source of polyclonal immunoglobulins. Hence, the objective of the present study was to evaluate the production of specific IgY and the performance of layers immunised with *S. typhimurium*.

Materials and Methods

Layers and their management

Thirty 23-wk old layers (Lohmann Brown) were obtained from a local hatchery. The average body weight of layers was 1.6 ± 0.03 kg. The layers were randomly and equally divided into 2 groups. Each group was placed in a specific pathogen free room equipped with an exhaust fan. The layers were placed in single battery cages with a slightly tilted floor which allowed eggs to roll straight into the collecting tray. Fourteen h of light were provided by 2 14KW light bulbs with an automated system. The feed at 180 g per day (about 20% above the *ad libitum* intake) was provided in a feeder (10.5 cm length x 16.5 cm width x 6.0 cm height) for each bird.

Each layer was prevented from cross eating by putting a wire-mesh between each cage. The composition of the basal diet is presented in Table 1. Automatic drinkers were used to provide water *ad libitum*.

Table 1: Composition of the basal diet

Ingredient	Amount (%)
Ground yellow corn	58.48
Soya bean meal	28.25
Fish meal	1.00
Palm oil	1.15
60% choline chloride	0.10
Vitamin premix[1]	0.10
Salt (NaCl)	0.16
DL-methionine	0.12
Limestone	9.10
Dicalcium phosphate	1.50
Total	**100.00**
Calculated analysis (%)	
Crude protein	18.20
Crude fat	3.68
Crude fibre	3.05
Calcium	3.96
Available phosphorus	0.45
Methionine	0.38
Lysine	1.04
Metabolisable energy (Kcal/kg)	2691.0

[1]Vitamin premix (per kg diet): 100mg iron; 110mg manganese; 20mg copper; 100mg zinc; 2mg iodine; 0.2mg selenite; 0.6mg cobalt; 0.6mg santoquin; 0.33mg folic acid; 0.83mg thiamin; 1.33mg pyridoxine; 0.03mg biotin; 2mg riboflavin; 0.03mg cyanocobalamin; 3.75mg D-calcium pantothenate; 23.3mg niacin; 2000mg vitamin A; 25mg vitamin D; 23,000mg vitamin E; and 1.33mg vitamin K_3.

The feed was changed daily at 0900 h and feed intake was recorded daily. Temperature and relative humidity of the closed-house system was within 25 °C to 33 °C and 84 to 90%, respectively. The experiment complied with the guidelines of the *Consortium Guide* (FASS, 1988) with respect to animal experimentation and care of animals under study.

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Preparation of S typhimurium

The stock culture of *S. typhimurium* strain [serotype: Somatic (O), antigen 1, 4, (5), 12, flagella (H) N12 phase I: 1; phase II: 1, 2 (Group B)] was obtained from Veterinary Research Institute, Ipoh, Malaysia and stored in a freezer at -80 °C. This stock culture was then sub-cultured in brain heart infusion broth, BHI (ICN) and incubated at 37 °C with shaking at 200 rpm by using Psycro Therm™ for overnight. Sub-culturing was repeated 3 times to optimize the growth of *S. typhimurium* to reach the colony forming unit (cfu) of approximately 10^9 cfu/ml. The culture was incubated in 2 separate conical flasks, each containing 500 ml of BHI broth and incubated at 37°C with shaking at 200 rpm for overnight.

Cells were harvested by centrifugation at 12,000 x g for 20 min at 4°C by using Beckman Coulter™. The cells were treated with 0.5% formalin for 3 h at room temperature to attenuate the phatogenicity of the cells. The attenuated cells were washed 3 times by suspension with sterilised phosphate buffer saline (PBS), consisting of 0.12M phosphate and 0.04M NaCl with a pH value of 7.2. Then, the cell pellets were collected by centrifugation at 5,000 x g for 15 min at 4°C. The cell pellets were frozen for 24 h at -20°C and then freeze-dried in a freeze-dryer (Labconco) for 5 h. Viability counts were determined after freeze-drying. These lyophilised cells were stored at -20°C before use. Determination of colony forming unit (cfu) for these lyophilised cells was done regularly before they were used in the experiment.

Immunisation of chickens

Fifteen layers in the treatment group were injected with lyophilised, attenuated whole cells of *S. typhimurium*, to obtain *S. typhimurium*-specific IgY loaded eggs. Lyophilised *S. typhimurium* (500 µg of cell/mL) whole cells were suspended in sterilised phosphate–buffered saline (PBS, which was a mixture of 0.14M NaCl, 0.0027M KCl, 0.0081M Na_2HPO_4, and 0.0015M KH_2PO_4, pH 7.2) and emulsified with an equal volume of Freund's complete adjuvant (Sigma). Fifteen hens in the control group were injected with a solution of sterilised PBS emulsified with an equal volume of Freund's complete adjuvant (Kapoor et al., 2000). All hens in both the treatment group and the control group were intramuscularly injected at 4 different sites (0.25mL per site) of breast muscles (2 sites per left and right breast muscles). Two booster inoculations given in the same way and same amount were carried out at 2nd week and 4th week after the initial injection, except the complete Freund's adjuant was replaced with Freund's incomplete adjuvant (Sigma).

Data collection

Feed consumption (g/hen) was recorded daily at 0900 h. Eggs produced were collected, and total number of eggs per wk was recorded. Egg production was based on the percentage of egg produced per wk by each hen (7 eggs/wk/hen were equivalent to 100%). Eggs were weighed individually and stored at 4ºC until used on a weekly basis. Egg mass (g/hen/wk) was calculated weekly by multiplying the percentage of egg production by average weight of eggs for each hen.

Isolation and partial purification of IgY

Crude IgY was extracted according to the method described by Hatta *et al.* (1990) with minor modification. Each egg yolk was separated from the white and washed with distilled water to remove adhering albumin. The yolk membrane was pierced and the yolk

was allowed to flow into a measuring cylinder. The volume of the yolk was recorded as VmL. The same volume of chilled distilled water (VmL) was added and homogenised for 30 sec. Carrageenan (Sigma) at 6V was dissolved in 4V volume of chilled distilled water. The 2 solutions were mixed and left for 30 min at room temperature. The mixture was centrifuged at 10,000 x g for 15 min at 4ºC. The total volume of supernatant (water-soluble fraction) was measured and stored at -20ºC until further analysis.

The supernatant was then filtered by using Whatman filter paper, No. 2 to remove lipid droplets of yolk. DEAE chromatography was used to isolate IgY from the protein contaminants by using different isoelectric points of the protein in the samples. Waters Protein-Pak DEAE 8HR weekly anion exchange column was used with the Waters HPLC system (Waters 2690 Separations Module). A gradient of buffer system was set in a duration time of 30 min. The initial mobile phase for the column was 20mM Tris-Cl buffer, pH 8.5 and ended up with 20mM Tris-Cl buffer, pH 7.0 with 0.3M NaCl. The flow rate was set at 1mL/min.

The elute that contained IgY, with pI value of 6.58 ± 0.86, was pooled for further purification,15% (w/v) of sodium sulfate anhydrous powder (Sigma) was added to the pooled elute at 20ºC. Then, the solution was gently stirred for 30 min at 20ºC. The solution was then centrifuged at 10,000 x g for 15 min at 4ºC. The resulting precipitate was dissolved in 100 mL of 0.1M, pH 8.0 phosphate buffer (PB). The salting-out procedure was repeated twice. The final precipitate was dissolved in 100 mL of PB and dialysed against the same cool buffer overnight. The dialysed solution was filtered through 0.45 µm membrane filter and the volume of the filtrate was measured and recorded.

Determination of specific anti-S. typhimurium IgY concentration

The concentration of specific IgY against *S. typhimurium* was measured by using ELISA, as described by Sunwoo *et al.* (2000) with some modifications and 150 µL of rabbit anti-chicken IgG (Sigma) at a concentration of 3.75 µg/mL and lyophilized *S. typhimurium* whole cells (1 mg/mL) were coated into wells of a microtiter plate, respectively. Two-fold serial dilutions of re-suspended specific (25.00 to 1.56 µg/mL) and non-specific (25.00 to 1.56 µg/mL) IgY powder in PBS were added to wells coated with *S. typhimurium*. Meanwhile, two-fold serial dilution of purified chicken IgG (Sigma) (1 mg/mL) in PBS (10.000-0.008 µg/mL) were added to wells coated with rabbit anti-chicken IgG (Sigma) and 150 µL of rabbit anti-chicken IgG conjugated with horseradish peroxidase (diluted 1:1000 in PBS-Tween 20, pH 7.2) was coated into each well. The reaction was carried out at 37ºC for 60 min. The freshly prepared substrate solution, 2-2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) in 0.05M phosphate citrate buffer (pH 5.0) containing 30% hydrogen peroxidase was used as substrate, respectively. The mixture was incubated at 37ºC for 60 min. Absorbance of the mixture was read at 405 nm by a kinetic microplate reader (Opsys MR, DYNEX).

Determination of total IgY concentration

Total IgY concentration was determined by comparing the OD values to a standard curve. A standard curve was prepared by using ELISA as described above, except the microtiter plate was coated with 150 µL of rabbit anti-chicken IgG at a final concentration of 3.75 µg/mL. Then, a serial dilution of purified chicken IgG in PBS was used as the reference antibodies. On the other

hand, samples of the water soluble fraction were diluted 1:90,000 with PBS, pH 7.2 before coated into a microtiter plate. Specific or non-specific IgY powder was reconstituted and serially diluted with PBS (64 mg/mL to 16 µg/mL).

Statistical analysis

Values of each parameter measured were compared by analysis of variance (one-way ANOVA). These tests were used to test the differences between mean values throughout the whole experimental period. The computation was done using the SPSS program version 11.5. All statements of significance were based on the probability level of 0.05 ($P<0.05$).

Results and Discussion

Performance of layers

Mortality was not observed throughout the study period as the lyophilised *S. typhimurium* whole cells that were used to inject layers intra-muscularly were attenuated with 0.5% formalin. This attenuation reduced the pathogenicity of *S. typhimurium* to the host. The overall results of layers performance throughout the experimental period are summarised in Table 2, which shows the weight of layers, their feed intake, egg production, mean weight of egg and egg mass. Generally, birds lost weight during the injection period, but the weight slowly increased in the postprandial period. However, the weight of layers in the treated group remained lower ($P<0.05$) when compared to the period before injection. The loss in body weight was the result of reduced feed intake, in particular during the injection wk. The feed intake of layers in the control group was higher ($P<0.05$) than layers in the treatment group for the period after immunisation. This may have been caused by the different health status of layers in each group. Layers in the treatment group developed diarrhea during the immunisation period (injection of *S. typhimurium* whole cells at 10^9 cfu/mL) whereby the feed intake was only about 98.2 ± 6.72 g per day. Then, feed intake of layers in this group significantly increased to 127.3 ± 6.33g per day. Figure 1 shows the changes in feed intake of control and treated birds during the experimental period. The results showed that layers in both groups required 1-2 wk to recover from the stress caused by the cells and PBS+adjuant injection, to return to the normal feed intake. However, the mean feed intake (Table 2) was not significant during the period before and during immunisation for the control layers. Henceforth, the feed intake increased significantly after immunisation for both groups of layers. It has been reported by Gast and Beard (1990) that the injection of *S. enteritidis* to layers would cause anorexia that result in the reduction of feed intake. Shivaprasad *et al*. (1990) had also reported that an inoculation of 2-4 x 10^8 cfu of *S. enteritidis* whole cells to layers could cause depression, anorexia, reduced egg production, diarrhea and some mortality.

Table 2: Performance of layers immunised by *S. thyphimurium*

Parameter	Experimental period					
	Before immunisation (Week-0 to Week-2)		During immunisation (Week-2 to Week-6)		After immunisation (Week-6 to Week-15)	
	Control	Treatment	Control	Treatment	Control	Treatment
Weight of layers (kg)	1.65 ± 0.06[a]	1.62 ± 0.04[a]	1.67 ± 0.06[b]	1.46 ± 0.04[c]	1.62 ± 0.06[b]	1.55 ± 0.04[d]
Feed intake per day (g)	117.2 ± 2.68[a]	112.9 ± 5.29[a]	112.3 ± 6.28[a]	98.2 ± 6.72[b]	139.9 ± 6.91[c]	127.3 ± 6.33[d]
Egg production (%)[1]	83.5 ± 2.76[a]	80.6 ± 2.99[a]	76.2 ± 6.06[b]	57.4 ± 7.46[c]	73.1 ± 5.88[b]	71.9 ± 5.80[d]
Mean weight of egg (g/egg)[2]	51.6 ± 1.73[a]	52.3 ± 1.33[a]	55.4 ± 3.15[b]	50.1 ± 5.04[a]	58.2 ± 2.21[c]	57.9 ± 2.45[c]
Egg mass (g/hen/day)[3]	43.9 ± 3.02[a]	42.5 ± 2.32[a]	45.3 ± 3.71[a]	34.6 ± 4.68[b]	43.9 ± 3.75[a]	44.5 ± 3.69[a]

Each value is a mean ± SEM (n=105).
Means with different superscripts in the same row were significantly different (P<0.05).
[1]Egg production (%) = egg produced per week by each hen ((7 eggs/ wk/ hen was equivalent to 100%).
[2]Mean weight of eggs (g/egg) = egg production x average weight of egg, calculated on a weekly basis.
[3]Egg mass (g/hen/day) = egg production x average weight of egg

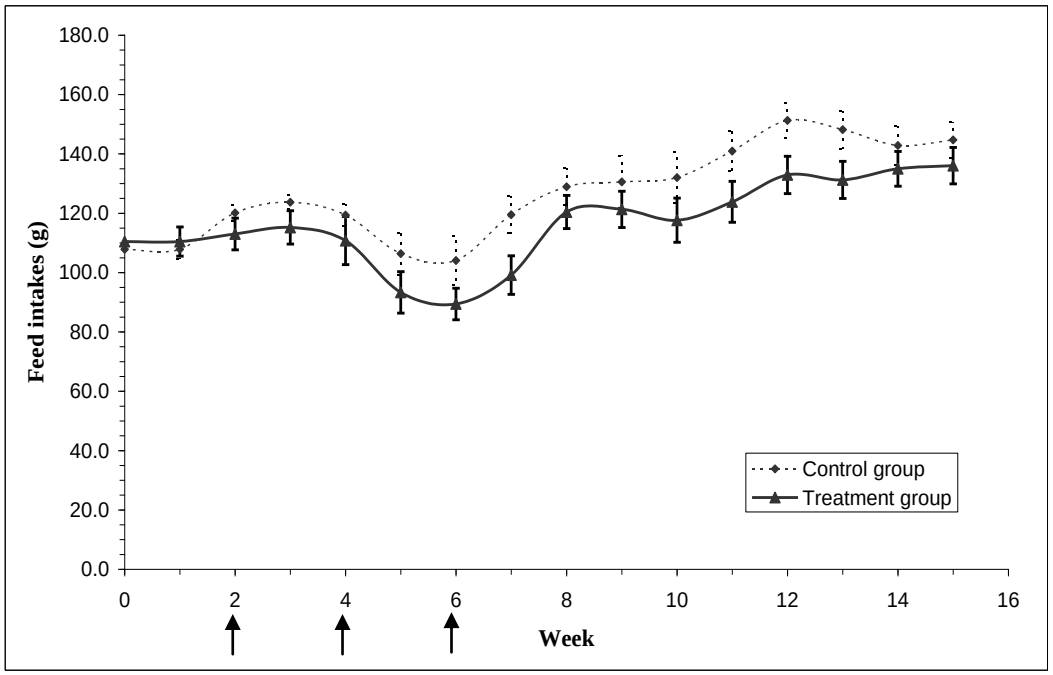

Figure 1: Feed intake of layers at different weeks.
Each control and treatment group consisted of 15 layers. Each value is a mean ± SEM of seven days for 15 chickens (n=105). Arrows indicate the week of injection. Control layers were injected with a solution of sterilised phosphate-buffered saline (PBS) emulsified with an equal volume of Freund's complete adjuvant, while treatment layers were injected with lyophilised, attenuated whole cells of *S. typhimurium* emulsified with Freund's complete adjuvant. Freund's complete adjuvant was replaced with Freund's incomplete adjuvant for the booster immunisations at week 4 and 6.

Figure 2 shows the changes in egg production by layers in the control and treated groups. Egg production reflects the production capacity of the available birds in the house. A significant reduction in egg production for individual layers was observed after the fourth wk. The egg production in the control and treated groups showed a reduction of 8.7% and 28.8%, respectively, during the injection period (Table 2). Although egg production increased after immunisation, the values were still significantly lower when compared to the period before injection. The egg production was low compared to the desirable value of 85% or more on farm production. The influence of *S. typhimurium* injections in egg production was similar to the effect on egg production in laying chickens that had been inoculated with 2-4 x 10^8 cfu of *S. enteritidis* whole cells, as reported by Shivaprasad et al. (1990). In contrast, Chalghoumi et al. **(**2008) reported no clinical signs of distress in response to the immunisation procedure or to any of the six immune preparations which consisted of outer membrane proteins prepared from *S. enteritidis and S. typhimurium* for the induction of *Salmonella*-specific antibodies in egg yolk. These authors did not observe any significant difference between means of laying rate of the hens receiving the immune preparations as compared with that of hens injected with the Freund's complete adjuvant. These results also indicated that the use of the Freund's complete adjuvant did not alter the laying capacity of the hens. In the present study the use of PBS emulsified with Freund's complete/incomplete adjuvant (control group) caused a decrease in egg production, but increased the mean weight of eggs during the immunisation period. Several factors including species or strains being immunised, antigen properties and dosage, the route of administration, and types of adjuvant could influence the immunogenicity of an antigen (Marcq et al., 2015; Chalghoumi et al., 2008).

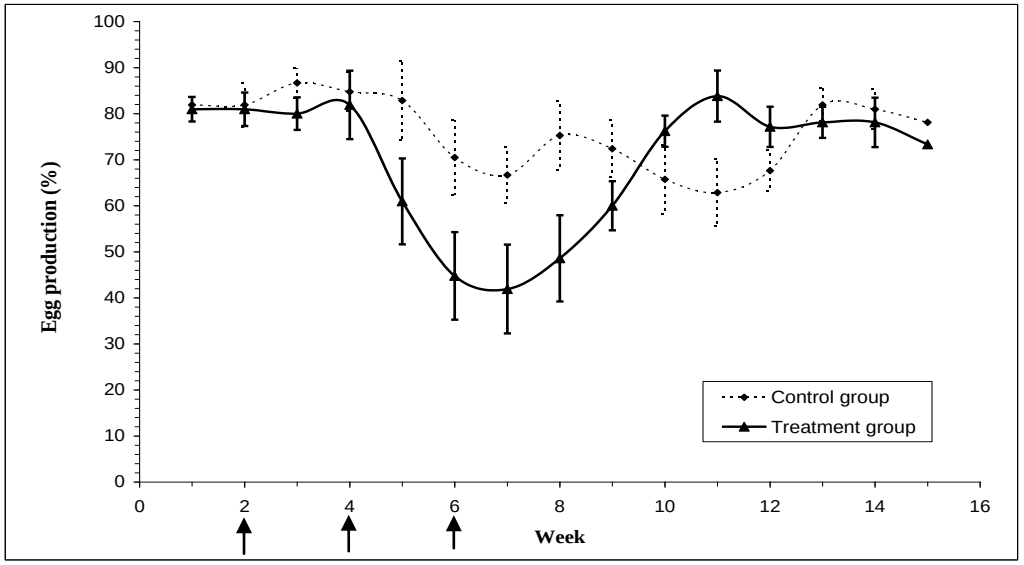

Figure 2: Percentage of egg production.
Each control and treatment group consisted of 15 chickens. Each value is a mean ± SEM of seven days for 15 chickens (n=105). Arrows indicate the week of injection. (Note: Production of seven eggs per hen per week was considered 100%)

The mean weight of eggs for the treatment group was reduced slightly during the injection weeks, but increased during the wk following injection to 57.9±2.45 g. As for the control group, the mean weight of eggs increased (P< 0.05) from 51.6±1.73 g to 58.2±2.21 g. Egg mass output is by far the best measure for matching nutrient intake to egg output. The results showed that a reduction in feed intake by treated layers affected egg mass production. Egg mass of treated layers during the immunisation period was lower (P< 0.05) compared to the period before injection. The egg mass for the layers in this group decreased during the immunisation period to 34.6 ± 4.68 g/hen/day. As feed intake increased, egg mass increased and returned to about the same value obtained before the injection period. Meanwhile, the egg mass for the layers in the control group did not change markedly throughout the experimental period. The values observed were within the range from 43.9 ± 3.02 to 45.3 ± 3.71 g/hen/day.

Isolation and partial purification of IgY

The purification process started with water extraction (with a ratio of 1:6) which gave a purity of 52.8% of IgY against total percentage of protein content (Table 3). It was further purified to 63.1% by using a simple filtration procedure with a Whatman filter paper no. 2. This was further purified by using DEAE-Sephacel chromatography. The IgY antibody was eluted from the DEAE-Sephacel column when the pH of the running buffer was equivalent to the isoelectric point of IgY, which was 6.58. The salt precipitation gave a purity of 86.8% of IgY against total percentage of protein content (Table 3).

Table 3: Recovery values of protein IgY from immumnised layers

Purification steps	Protein		Total IgY		Purity of total IgY against protein, %	Specific IgY	
	Concentration (mg/g) of yolk	Yield %	Concentration (mg/g) of yolk	Yield, %		Concentration (mg/g) of yolk	Yield, %
Crude extract	45.8 ± 10.15 (N = 455)	100.0	24.2 ± 3.83 (N = 455)	100.0	52.8	12.9 ± 1.51 (N = 455)	100.0
Filtration	32.5 ± 1.38 (N = 65)	71.0	20.5 ± 2.69 (N = 65)	84.7	63.1	12.2 ± 0.98 (N = 65)	94.6
Salt precipitation *	13.6 ± 2.57 (N = 39)	29.7	11.8 ± 0.57 (N = 39)	48.8	86.8	9.6 ± 2.66 (N = 39)	74.4

All values for total protein, total IgY and specific IgY were measured using the freeze-dried yolk powder. Each value is a mean ± SEM.

1. Total protein concentration (mg/g) of yolk was measured using Bradford method (1976).
2. Total IgY concentration (mg/g) of yolk was determined using ELISA method with rabbit anti-chicken IgG as the reference.
3. Specific IgY concentration (mg/g) of yolk was measured using ELISA technique.

The standard curve was obtained by the titration between rabbit anti-chicken IgG and purified chicken IgG. The value was the difference between treated and control group.

4. N = number of egg yolk samples in treatment group.

* Sodium sulfate precipitation conducted after DEAE-Sephacel column chromatography

IgY Production

After the immunisation period, layers injected with *S. typhimurium* had higher concentration of specific anti-*S. typhimurium* IgY in their egg yolk compared to the period before injection (Figure 3). IgY titre continued to increase after the first booster and reached a high level at about 4 wk after injection. Specific anti-*S. typhimurium* IgY in the treatment group did not change markedly after the third injection of *S. typhimurium*, with an OD_{405nm} range of 0.8-0.9. The titre remained high until the end of the 14-wk experimental period. Injection of the antigen by intramuscular route frequently resulted in higher antibody levels by day 28th after immunisation (Wooley and Landon, 1995). It was reported that chickens immunised by the intramuscular route continued to produce specific antibodies during more than 200 d after immunisation or be used for the entire laying period depending on the antibody titres induced (Schade et al., 1996).

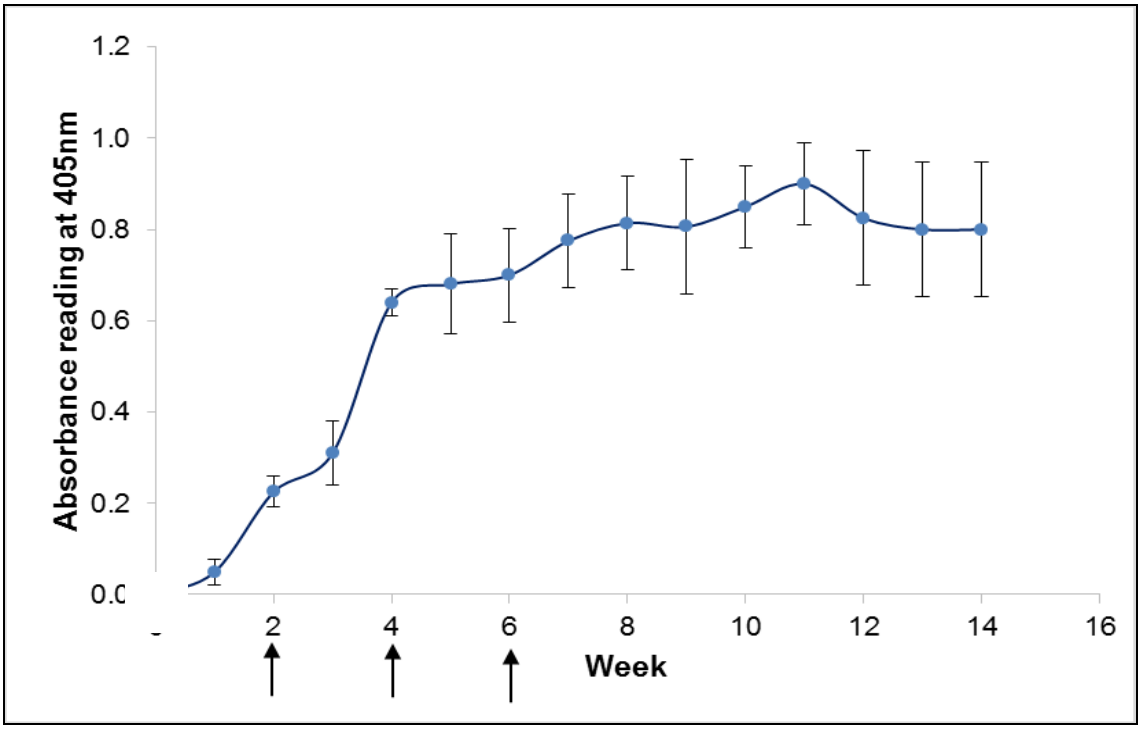

Figure 3: The changes in specific IgY detected in the water soluble fraction of egg yolk from layers immunised with *S. typhimurium* whole cells after subtracting the values of IgY in the egg yolk from layers in control group (mean ± SEM, n=5).
Arrows indicate the week of injection.

The amount of total IgY in yolk was about 11.8 mg per g of yolk. As the average weight of yolk was 15 g, the equivalent amount of IgY was 177 mg per egg; i.e., approximately 1.2% w/w of egg yolk was IgY. This amount was slightly higher that the range of 60 to 150 mg per egg given by Cook and Trott (2010) and Pauly et al. (2009). Laying hens that had been parentally hyperimmunised with formalin-inactivated *S. enteritidis* strain (phage type 4) also produced total IgY concentration ranging between 7.1-9.0 mg/g of yolk (Gürtler and Fehlhaber, 2004).

The specific IgY raised against *S. typhimurium* in the present study was about 9.6 mg/g yolk which was equivalent to 144 mg per egg, i.e., approximately 1% w/w of egg yolk. This value was less than the expected value of 2 to 10% (Schade et al., 1991; Tini et al., 2002). However, discrepancy in the level of specific IgY would arise as several factors, including age, breed of chicken, antigens, mode of immunisation and extraction procedures would affect the determination.

Traditionally, antibodies obtained from blood collected either by repeated bleeding or heart puncture would frequently result in distress or even death of the animal. On the other hand, specific IgY harvested from egg yolk of immunised chicken is an excellent alternative to the conventional methods.

Conclusion

Injection of attenuated *S. typhimurium* whole cells did not result in mortality, but caused significant changes to layers in terms of body weight, daily feed intake, egg production, mean weight of egg and egg mass. All these parameters were significantly lower during the immunisation period as compared to those before and after the immunisation periods. Isolation and partial purification of IgY by using µ-Carrageenan precipitation, filtration with a Whatman filter paper no. 2, partial purification with DEAE-Sephacel chromatography and salt precipitation produced IgY with 86.8% purity against total protein concentration. Specific IgY antibodies were successfully raised against *S. typhimuirum* through injection of attenuated *S. typhimurium* whole cells to layers.

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