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Original article

COMPARATIVE STUDY OF PURIFICATION METHODS FOR INFLUENZA-SPECIFIC IgY FROM CHICKEN EGGS

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Summary

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Chicken eggs are widely used for different purposes including as a source of specific immunoglobulin (IgY). Therefore, this study aimed to evaluate various purification methods for influenza-specific IgY from chicken egg yolk. Three laying hens were vaccinated with an influenza vaccine. Influenza-specific IgY was purified from the chicken egg yolk using four different purification techniques including (1) water-soluble sodium chloride (WS-NaCl), (2) water-soluble polyethylene glycol (WS-PEG), (3) WS-PEG-ammonium sulfate (WS-PEG-AS) precipitation, and (4) a commercial IgY purification kit. These methods were compared in terms of protein concentration, IgY structure, and specificity against the influenza virus. The results showed that all purification methods successfully obtained influenza-specific IgY, as confirmed by the agar gel precipitation (AGP) test against influenza antigen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that the WS-PEG precipitation method was unable to purify the entire IgY structure, while the other three methods were successful. The highest protein concentration was obtained using the commercial kit (9.931±0.214 mg/mL), followed by the WS-NaCl method (6.944±0.979 mg/mL). Although the commercial kit yielded the highest protein concentration, it may not be cost-effective for large-scale IgY production. The WS-NaCl method appeared to be a promising and feasible option for generating large quantities of IgY from chicken eggs.

Key words: chicken, egg yolk, IgY, influenza, purification methods

INTRODUCTION

Pneumonia is a form of acute respiratory infection, commonly caused by influenza virus types A and B (Cavallazzi & Ramirez, 2018; WHO, 2024). In many countries, pneumonia remains a significant public health concern, as it is a leading cause of death across all age groups (Pangesti *et al.*, 2014). Effective strategies for controlling transmission include the administration of annual vaccines and the use of antiviral medications (Rudraraju & Subbarao, 2018). However, several challenges persist, such as the efficacy limitations of some antivirals due to influenza virus mutations that create new variants, leading to antiviral resistance. Another obstacle is the relatively long preparation time required for vaccine development, which struggles to keep pace with the rapid mutations of the virus. An alternative therapy is the use of specific immunoglobulin (IgY) (Abbas *et al.*, 2019). According to several studies, IgY prophylaxis is reportedly a potential treatment for viral infections that invade the respiratory tract (Artman *et al.*, 2021; El-Kafrawy *et al.*, 2021; Lee *et al.*, 2021; Agurto-Arteaga *et al.*, 2022; Saputri *et al.*, 2022).

IgY is used as an alternative to mammalian serum antibody (IgG) and the application offers several advantages, including the avoidance of procedures that cause stress and pain in mammals, including blood collection and the sacrifice of animal donors (Pereira *et al.*, 2019; Constantin *et al.*, 2021). Since IgY is harvested from egg yolk, it is consistent with animal welfare principles in laboratory animal use. The concentration of antibodies that can be collected from chicken eggs is higher compared to that from mammals. The average volume of egg yolk per egg is approximately 15 mL, containing 2–10% IgY. A laying hen can produce around 14 eggs, yielding a total yolk volume of about 210 mL in just two weeks and this equates to approximately 1.120 mg of antibody content. In contrast, using a mammal such as a rabbit for antibody collection provides only about 200 mg of antibodies from 40 mL of whole blood (Narat, 2003; Pereira *et al.*, 2019).

Several IgY purification methods have been proposed in the past decade. A major challenge in purifying IgY from chicken eggs is the removal of lipids, which are abundant in the yolk. Therefore, purification methods typically require an initial delipidation step (Sudjarwo *et al.*, 2017). There are various methods for IgY purification, including water dilution (WD), extraction with 3.5% polyethylene glycol (PEG) 6000, caprylic acid, chloroform, phenols, and ammonium sulfate (Bizhanov, 2017; Primacella *et al.*, 2018; Madera-Contreras *et al.*, 2022; Shimelis *et al.*, 2022). The majority of the methods rely on PEG precipitation (Munhoz *et al.*, 2014; Madera-Contreras *et al.*, 2022; Shimelis *et al.*, 2022). However, this method is often associated with impurities in the precipitate, which might be challenging to remove (Marcet *et al.*, 2011). A new purification method using tap water and NaCl appears promising, as it uses only natural ingredients to purify IgY (Hodek *et al.*, 2013).

This study produced influenza-specific IgY and tested four purification methods including (1) water-soluble sodium chloride (WS-NaCl), (2) water-soluble polyethylene glycol (WS-PEG), (3) WS-PEG-ammonium sulfate (WS-PEG-AS) precipitation, as well as (4) a commercial IgY purification kit. The objective was to evaluate IgY purification methods from chicken egg yolks to determine which

achieves the highest production yield for commercial applications. The results were assessed for purity, protein concentration and content, IgY structure, and specificity against influenza.

MATERIALS AND METHODS

This study complied with Article 80 on "Research in Animal Health" of the Indonesian Law on Livestock and Animal Health UU/18/2009. Animal ethics approval was received from the Veterinary Ethics Commission of the School of Veterinary Medicine and Biomedical Sciences (SKHB), IPB University, on November 3, 2020, with clearance number 014/KEH/SKE/XI/2020.

Chickens

Three Hy-Line Brown chickens were used for IgY production. The chickens were introduced to the rearing process at 19 weeks of age, with a two-week acclimatisation period, then housed in battery cages and were provided with food and water *ad libitum*.

Vaccine and vaccination

The vaccine used in this study was an inactivated quadrivalent influenza (Quadrivalent® Influenza Vaccine Types A and B, Sanofi Pasteur Inc., USA) and included four influenza virus strains namely A virus H1N1 pdm09 (A/Brisbane/02/2018), A H3N2 (A/Kansas/14/2017), B strain B/Colorado/06/2017, and B strain B/Phuket/3073/2013. The chicken were vaccinated three times using a 0.25 mL dose per bird through the intramuscular (IM) route into the chest muscles, with vaccinations spaced four weeks apart. The first vaccination was administered at 24 weeks of age (Saputri *et al.* 2024).

Serum and egg collection

Serum and eggs were collected weekly from the first up to 20th week after the initial vaccination. The samples were stored at −20 °C until analysis. Eggs were collected daily and grouped by week, then the yolks were separated from the whites and stored at −20 °C until use.

Agar gel precipitation (AGP) test

The presence of influenza-specific IgY in serum and egg yolk, as well as the specificity of purified IgY, was determined using the agar gel precipitation (AGP) test (Poetri *et al.*, 2008; Saputri *et al.*, 2024). Egg yolks showing an AGP antibody titre of ≥1:23 were pooled for purification. The yolk was diluted with phosphate saline buffer (PBS) at a 1:1 ratio, centrifuged at 13,500 rpm for 15 minutes at 4 °C, and the supernatant was collected for use (Ruhi *et al.*, 2018).

Influenza antigens for the AGP test were prepared from vaccine (Quadriflu®) by freeze-thawing and sonication processes (Saputri *et al.*, 2024). Agar plates were prepared using 1% agarose and 0.1% sodium azide in 50% (v/v) PBS. A positive result was indicated by the formation of a precipitation line between the antigen and the serum or egg yolk (Tizard, 2004). The samples were subjected to serial 1:2 dilutions, and the antibody titre was determined based on the highest dilution that produced a positive result in the AGP test.

IgY purification using WS-NaCl method

IgY from chicken egg yolk was purified using the water-soluble (WS) fraction method followed by NaCl precipitation (Wibawan *et al.*, 2018). Egg yolks (3 mL) were diluted with distilled water at a 1:7 ratio and pH adjusted to 5.0. The mixture was then homogenised using a stirrer and incubated at 20 °C overnight. After incu-

bation, the solution was thawed to 4 °C and centrifuged at 13,500 g for 15 min at 4 °C. The supernatant was filtered using filter paper to obtain a clear water-soluble fraction (WSF). Next, 8.8% (w/v) NaCl was added to the WSF, and the solution was homogenised with a stirrer for two hours at room temperature. Subsequently, the solution was centrifuged at 3,700 g for 20 min at 4 °C, and the resulting pellets were pooled and dissolved in 2 mL PBS. The purified IgY was then stored at −20 °C until further use.

IgY purification using WS-PEG method

IgY from chicken egg yolk was purified using the water-soluble (WS) fraction method followed by PEG precipitation (Hussain, 2017). Egg yolks were diluted with PBS at a 1:2 ratio, and 3.5% (w/v) PEG-6000 was added and homogenised. The solution was then centrifuged at 2,264 g for 30 min at 4 °C. The supernatant was collected and further treated with 8.5% (w/v) PEG-6000, homogenised, and centrifuged again. The pellets obtained were resuspended in PBS to a total volume of 10 mL, then 12% (w/v) PEG-6000 was added, homogenised, and re-centrifuged. The final pellets were collected, dissolved in 2 mL of PBS, and the purified IgY was stored at −20 °C until further use.

IgY purification using WS-PEG-AS method

IgY from chicken egg yolk was purified using the water-soluble (WS) fraction method followed by PEG and ammonium sulfate (AS) precipitation. Egg yolks were diluted with PBS at a 1:3 ratio, and then 3.5% (w/v) PEG-6000 was added. The mixture was homogenised and centrifuged at 1,008 g for 25 min at 4 °C. The supernatant was collected, treated with 12% (w/v) PEG-6000, and centrifuged. The pellet was resuspended in PBS to the original volume, and ammonium sulfate $(NH_4)_2SO_4$ was added to a final concentration of 40%. The solution was centrifuged at the same speed as in the previous steps and this process was repeated three times. The final pellet was dissolved in 2 mL PBS and dialysed against PBS pH 8 with the buffer volume 150 times the initial volume of IgY. The dialysed IgY was then collected (Polson *et al.*, 1980).

IgY purification using a commercial kit

IgY from chicken egg yolk was purified using a commercial chicken IgY purification kit (Pierce™, Thermo Fisher Scientific, Waltham, USA), following the manufacturer instructions. The egg yolk was diluted with the delipidation reagent solvent at a 1:5 ratio, then homogenised and incubated overnight at 4–8 °C. The solution was centrifuged at 13,500 g for 15 min at 4 °C. The supernatant, which should be clear and translucent, was transferred to a clean beaker. While stirring gently, an equal volume of cold IgY precipitation reagent was added, and the mixture was incubated overnight at 4–8 °C. Subsequently, the solution was centrifuged at 10,000 g for 15 min at 4 °C, and the supernatant was discarded. PBS, in a volume equal to the initial volume of the egg yolk, was added to the pellet and mixed gently until completely dissolved.

Protein characterisation of purified IgY using SDS-PAGE

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method was used to determine the molecular weight and purity of the resulting IgY. About 12% running gel and 5% stacking gel were prepared along with loading and electrode buffer. For sample preparation, protein samples were mixed with loading buffer (4:1) and heated at 60 °C for 3 min.

About 10 µL of protein samples and marker samples (ExcelBand™) were loaded into each well, then electrophoresis was performed at 100 V for 80 min. The protein bands were stained with 0.1% (v/v) Coomassie Blue for 3 h, after which the gel was submerged in a de-staining solution for 24 h (Laemmli, 1970).

Western blot assay to confirm influenza-specific IgY

Western blotting (WB) was performed to confirm the presence of IgY. Initially, the electrophoresis gel was transferred to a nitrocellulose membrane using the wet transfer method at 100 V and 0.25 A for 180 min. After transfer, the nitrocellulose membrane was washed with phosphate-buffered saline-Tween 20 (PBST) for 10 min. Blocking was carried out with 5% Blotto solution (5 g skim milk in 100 mL PBS) for 24 h, followed by a wash with PBST.

The nitrocellulose membrane was incubated with 250 µL of human influenza virus mixed with 10 mL of 1% Blotto solution for 24 h and washed again. Subsequently, the membrane was incubated with 150 µL of HRP-conjugated anti-chicken IgY (Jackson ImmunoResearch®, USA) (1:5000) mixed with 15 mL of 1% Blotto solution for 60 min. The substrate solution, consisting of 0.02 g of 3,3-diaminobenzidine tetrahydrochloride, 30 mL of Tris-buffered saline, and 24 µL of 0.02% H_2O_2, was added and the solution was incubated for 30 min. A positive result was indicated by the presence of a brown band (Bollag & Edelstein, 1991).

Protein quantification

Protein concentration was quantified using the Bradford method which comprised four key steps namely (1) using Bovine Serum Albumin (BSA) as the standard protein, (2) applying Bradford reagent, (3) generating standard curves and linear equations, (4) calculating the protein concentration. Absorbance values were measured using a spectrophotometer at a wavelength of 595 nm. The results for each sample were then substituted into the linear equation derived from the standard curves to determine the protein concentration (Saputri *et al.*, 2022; 2024).

Data analysis

Protein concentrations were analysed using ANOVA with Tukey *post-hoc* test for group comparisons. Other data were analysed descriptively.

RESULTS

The presence of specific influenza IgY

Influenza antibodies were detected in the serum and egg yolk starting one week after the first vaccination and were monitored for 20 weeks. The highest antibody titre, reaching 1:23, was observed in both serum and egg yolk at 9 and 10 weeks following the first vaccination. The results of the AGP test evaluating the binding of purified IgY produced by different purification methods to influenza antigen are shown on Fig. 1. Purified IgY from all purification methods yielded positive results on the AGP test indicating specificity for influenza virus.

Protein characterisation of purified IgY

The results of purified IgY were analysed by SDS-PAGE and are presented in Table 1 and Fig. 2. The molecular weight patterns of all purification methods showed the expected molecular mass for the heavy chains, approximately ±67 kDa. The WS-NaCl precipitation, WS-PEG-AS precipitation, and commercial IgY purification

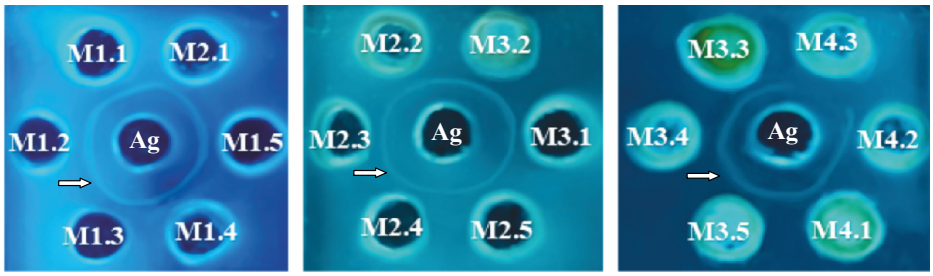

Fig. 1. The presence of specific influenza IgY using AGP test. Ag is influenza antigen; white arrow pointing to the precipitation line; M1.1 – 5 (purified IgY using WS-NaCl); M2.1 – 5 (purified IgY using WS-PEG); M3.1 – 5 (purified IgY using WS-PEG-AS); M4.1-2 (purified IgY using commercial kit); M4.3 (IgY control).

Table 1. Characterisation of purified IgY based on band structures formed

Purification method	Details	Band formed	Western-blot results	Details
M1	WS-NaCl precipitation	189.78 kDa	+	IgY
		135.82 kDa	–	Apolipoprotein-LDL
		95.41 kDa	–	Apolipoprotein-LDL
		67.33 kDa	+	heavy chain / Fc
		27.98 kDa	+	light chain / Fab
M2	WSF-PEG precipitation	95.41 kDa	–	Apoliprotein-LDL
		64.28 kDa	+	heavy chain / Fc
		58.84 kDa	–	α-Livetin
		19.75 kDa	-	Apolipoprotein-LDL
M3	WSF-PEG-ammonium sulfate precipitation	189.78 kDa	+	IgY
		95.41 kDa	–	Apoliprotein-LDL
		67.02 kDa	+	heavy chain / Fc
		64.28 kDa	–	Apoliprotein-LDL
		28.11 kDa	–	β-livetin
M4	IgY commercial purification kit	189.78 kDa	+	IgY
		64.88 kDa	+	heavy chain / Fc
		25.61 kDa	–	β-livetin
M4	IgY commercial purification kit	189.78 kDa	+	IgY
		108.16 kDa	–	Apolipoprotein-HDL
		67.33 kDa	+	heavy chain / Fc
		28.11 kDa	–	β-livetin
Control	IgG	154.69 kDa	–	IgG
		49.55 kDa	–	heavy chain / Fc
		28.11 kDa	–	light chain / Fab
Control	IgY commercial purification kit (Non-vaccinated chicken)	180.33 kDa	–	IgY
		118.70 kDa	–	Apolipoprotein-LDL
		61.93 kDa	–	heavy chain / Fc
		29.45 kDa	–	light chain / Fab

kit methods effectively purified the entire IgY molecule and light chain, with SDS-PAGE results showing the expected molecular mass for the whole IgY at ±180 kDa

and the light chain at ±27 kDa. However, SDS-PAGE results for all methods showed impurities, with bands corresponding to molecular masses of ±108−135 kDa, ±95 kDa, and ±19 kDa (Fig. 1). The purification results using the commercial kit had the least impurities compared to other methods. The WS-NaCl and WS-PEG-AS precipitation methods had two unexpected molecular masses, while the WS-PEG showed three unexpected molecular masses.

Protein concentrations of purified IgY were determined using the Bradford assay, and the data are presented in Table 2. The highest protein concentration was observed in the purification results using commercial kits (9.931±0.214 mg/mL), followed by WS-NaCl (6.944±0.979 mg/mL), WS-PEG (4.402±0.319 mg/mL),

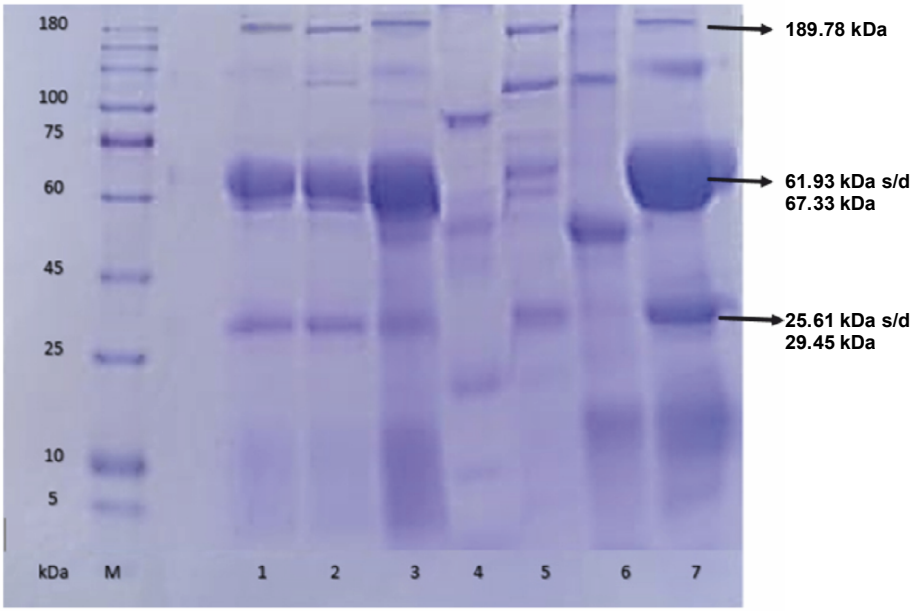

Fig. 2. Protein characterisation of purified egg yolk using SDS-PAGE. M: Marker; 1: IgY purified by commercial kit; 2: IgY purified by commercial kit; 3: IgY purified by WS-NaCl method; 4: IgY purified by WS-PEG method; 5: IgY purified by WS-PEG-AS method; 6: IgG control; 7: IgY control.

Table 2. Protein concentration

Purification method	Details	Protein concentration (mg/mL) mean ± SD
M1	WS-NaCl precipitation	6.944±0.979[a]
M2	WSF-PEG precipitation	4.402±0.319[b]
M3	WSF-PEG-ammonium sulfate precipitation	3.684±0.337[b]
M4	IgY commercial purification kit	9.931±0.214[a,b]

[a, b]The same superscript in the same column did not show a significant difference (P>0.05).

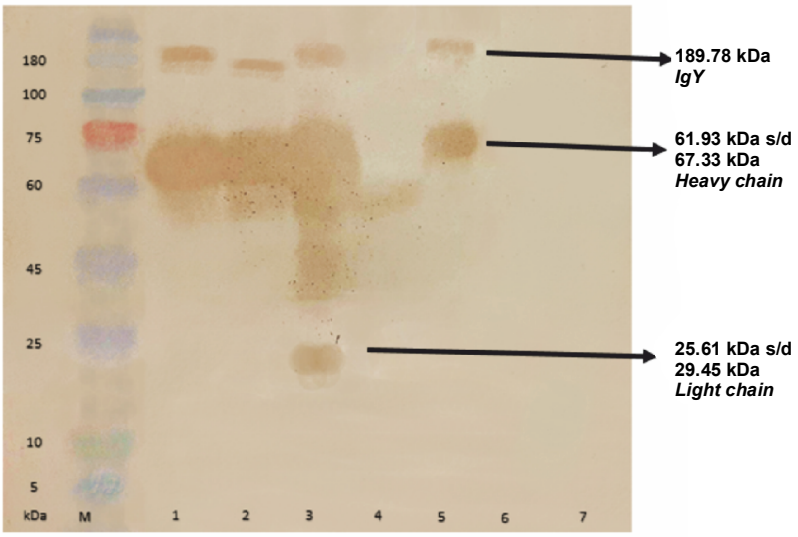

Fig. 3. IgY confirmation using Western blot assay. M: Marker; 1: IgY purified using commercial kit; 2: IgY purified using commercial kit; 3: IgY purified by WS.

and WS-PEG-AS precipitation (3.684± 0.337 mg/mL). There were significant differences in protein concentrations between the methods ($P<0.05$).

Confirmation of influenza-specific IgY

The Western blot results (Fig. 3) showed three protein bands corresponding to the whole IgY structure, the heavy chain, and the light chain. The IgY structure, approximately 189 kDa, was identified in purification results using WS-NaCl, WS-PEG-AS, and the commercial kit. The heavy chain, approximately 67 kDa, was identified in all purification methods, while the light chain, approximately 27 kDa, was identified only in the WS-NaCl precipitation results.

The WS-NaCl precipitation method yielded two unexpected bands at 95.45 kDa and 135.48 kDa, identified as apolipoprotein-LDL and apolipoprotein-HDL, respectively. The WS-PEG precipitation method produced three unexpected bands namely apolipoprotein-LDL at 19.74 kDa and 95.5 kDa, as well as α-livetin at 58.9 kDa. The WS-PEG-AS method showed two unexpected bands at 64.32 kDa and 28.02 kDa, identified as apolipoprotein-LDL and β-livetin. The commercial kit produced two unexpected bands at 108.85 kDa and 27.98 kDa, identified as apolipoprotein-HDL and β-livetin. The Western blot results indicated that all purification methods were capable of purifying IgY except for the WS-PEG precipitation method that did not successfully purify intact IgY.

DISCUSSION

Influenza-specific IgY can be generated from eggs obtained from chickens that have been vaccinated. The presence of specific IgY was confirmed by the AGP test then egg yolks with positive results were collected for purification. All meth-

ods used for purification successfully purified influenza-specific IgY, as demonstrated by the results.

The AGP test is a serological assay that is relatively easy to perform compared to others assay such as the hemagglutination inhibition (HI) assay or enzyme-linked immunosorbent assay (ELISA). However, a limitation of the AGP test is that results can only be observed after at least 18 hours (de Wit *et al.*, 1992). In this test, antigens and antibodies diffuse passively out of the well into the agar. Specific serum-purified IgY influenza antigens form an interlaced antigen-antibody complex that precipitates in the agar (Darniati *et al.*, 2016). A white precipitation line represents the antigen-antibody complex, which is visible to the naked eye (Tizard, 2004). According to Wibawan *et al.* (2009), a precipitation line in the AGP test appears when the IgY HI is ≥ 27. In this study, the highest AGP titre observed in sera and egg yolk was 23, indicating that the influenza antibodies present were substantial, although slightly below the threshold.

A significant obstacle in producing antibodies from chicken eggs is removing the lipid content (Tang *et al.*, 2015). In this study, all purification methods used showed impurities in addition to intact IgY, as indicated by SDS-PAGE. Intact IgY molecules and the light chain were only identified in the purification methods using WS-NaCl, WS-PEG-AS, and commercial kits, while the heavy chain was detected in all purification methods.

In chicken, IgY serves a function equivalent to IgG in mammals and is similarly composed of two light chains as well as two heavy chains. However, a structural difference exists between the two, particularly in the heavy chains. IgG features three constant region arrangements (CH1-CH3), while IgY has four (CH1-CH4). Consequently, IgY has a molecular weight of approximately 180 kDa, which is larger than the 150 kDa of IgG (Marcet *et al.*, 2011; Pereira *et al.*, 2019). In this study, intact IgY was identified at approximately 189 kDa, consistent with the results of Munhoz *et al.* (2014), who reported the composition of IgY as two heavy chains (68 kDa), two light chains (27 kDa), and the whole IgY molecule (approximately 190 kDa).

The results differed slightly from those of Sudjarwo *et al.* (2017), who identified two prominent bands at 112 kDa and 29 kDa as the heavy and light chains, respectively. In this study, heavy chains were identified at approximately 67.33 kDa in the purification results from WS-NaCl, WS-PEG-AS, and the commercial kit. The WS-PEG precipitation method showed a heavy chain at 64.28 kDa, while light chains were consistently identified around 27–28 kDa across the WS-NaCl precipitation, PEG-ammonium sulfate precipitation, and the commercial kit. These results are consistent with those of Pereira *et al.* (2019) and Madera-Contreras *et al.* (2022), stating that IgY consists of heavy chains with molecular weights of 67-70 kDa and light chains with a molecular weight of approximately 25 kDa.

The light chain was not identified in the purification results from the WS-PEG precipitation method, suggesting that the purity achieved was lower compared to other methodss. This may be due to the fewer precipitation steps in the method. SDS-PAGE can assess purity by showing the presence of proteins other than IgY, including heavy and light chains, as well as other structures that may affect purity (Primacella *et al.*, 2018). The additional structures found in the purified product

are lipoproteins, which is consistent with Primacella *et al.* (2018).

The IgY purification method is composed of two main stages, namely the separation of fat, and precipitation of IgY, with pH adjustment to an acidic level (pH 5) being a crucial factor (Bizhanov, 2017; Wibawan *et al.*, 2018). In the WS-NaCl method, fat separation was achieved by diluting IgY, freezing at –20 °C overnight, and adjusting the pH to 5. In the WS-PEG method, fat separation was performed by adding PEG-6000 at specific concentrations (3.5% and 8.5%). The WS-PEG-AS method used only 3.5% PEG-6000 for fat separation, while commercial kit using delipidation reagents in an acidic environment. Both WS-PEG and WS-PEG-AS methods did not include pH adjustment to an acidic environment (pH 5), which likely impacted the purification results.

Typically, egg yolk is composed of approximately 2/3 fat and 1/3 protein which are categorised into granules and plasma. The granule portion includes low-density lipoprotein (12%), phosvitin (16%), and α- and β-livetin (70%), while the plasma portion contains α-, β-, and γ-livetin, with α- and β-livetin serving as serum albumin in poultry (Kovacs-Nolan *et al.*, 2005).

Western blot analysis was conducted to confirm the presence of IgY in the purified egg yolk. The results showed that all purification methods successfully produced influenza-specific IgY. In a previous study by Amro *et al.* (2018), Western blot results showed positive bands at 62 kDa and 27 kDa, corresponding to the heavy and light chains, respectively. Eto *et al.* (2018) reported positive bands at 20 kDa and 75 kDa representing the light chain or Fab fragment, and heavy chain or Fc component respectively. The Fab fragment, a crucial part of the antigen-binding site, is essential for the ability of IgY to bind antigens. The fragment is formed by disulfide bonds between the heavy chains (VH and CH1) and a portion of the light chains (VL and CL) (Bates *et al.*, 2019).

The Bradford assay results showed that purified IgY obtained from the commercial kit had the highest protein concentration, followed by the WS-NaCl, WS-PEG, and WS-PEG-AS precipitation methods. Considering this study aimed to find an alternative to the commercial kit, the WS-NaCl precipitation method was considered a viable option, as it provided the highest concentration of purified IgY. Ren *et al.* (2016) found that the WS fraction method yielded higher concentrations of purified IgY compared to the PEG precipitation method. The addition of NaCl in the WS-NaCl precipitation method enhances the salting-out process, facilitating IgY precipitation.

The PEG precipitation method, which comprises two major steps, first using 3.5% PEG-6000 to remove fat, and second using 12% PEG to precipitate IgY, can produce IgY concentrations ranging from 2.87 to 9.7 mg/mL (Bizhanov, 2017; Hussain, 2017). The ammonium sulfate precipitation method yielded IgY concentrations between 3.8 and 13.0 mg/mL. Combining ionic and non-ionic molecules, as observed in the PEG-6000 and ammonium sulfate methods, offers the advantage of streamlining the purification process (Bizhanov, 2017).

This study successfully produced and purified influenza-specific IgY from the egg yolks of hyperimmunised chicken. The antibodies generated in this study are polyclonal and the chicken were immunised with the Quadriflu® vaccine, containing antigens from four influenza virus

strains. Consequently, the IgY produced may not be ideal for use as a diagnostic reagent due to polyclonal nature and broad specificity. For developing diagnostic kits, it is recommended to use antigens derived from a single virus species or microorganism to ensure specificity. The IgY produced in this study is well-suited for immunotherapy applications for individuals exposed to influenza.

CONCLUSIONS

In conclusion, this study showed that commercial IgY purification kits yield the highest protein concentration. However, due to the high cost, these kits might not be ideal for large-scale production. An effective and cost-efficient alternative is the WS-NaCl precipitation method, which has shown promising results in generating substantial quantities and high yields of pure influenza-specific IgY from chicken egg yolk.

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