CHAPTER 4 RESULTS AND DISCUSSION

In order to produce anti-chicken coccidia IgY, 855 chickens of six months old were immunized intramuscularly 3 times. For the first immunization, the hens were injected at two different sites of breast muscle with extracted coccidia antigen (as described in 3.2.1) emulsified with equal volume of Freund's complete adjuvant (30 μg protein antigen/site). For the second and third immunization, Freund's incomplete adjuvant were used and performed at 2 and 6 weeks after first immunization, respectively. After the third immunization, level and specificity of anti-chicken coccidia IgY in immunized chicken eggs was determined. Eggs containing anti-chicken coccidia IgY from immunized chickens were collected and the pooled homogenized egg yolk was spray dried. Finally, the IgY product in form of dried yolk powder was characterized for animal feed supplement purpose.

4.1 Anti-chicken coccidia IgY production and its specificity

4.1.1 Detection of anti-chicken coccidia IgY

Anti-chicken coccidia IgY production in immunized chicken egg was determined by an indirect ELISA (as described in 3.2.4). 200 eggs from immunized hens from week 13 and 14 after first immunization were collected randomly. The egg yolk was individually separated (as described in 3.2.3). The fresh yolk samples were subjected to IgY preparation as then used as a primary antibody in ELISA assay. In this assay, negative control was prepared from yolk from chickens in the same farm but were immunized with other non related antigen, adipocyte membrane protein. Due to a large number of IgY samples, the test could not be achieved using one ELISA plate. Thus, 200 IgY samples were divided into ten sets. Each set consists of 20 IgY samples and one negative control. In order to assess efficacy of immunization, the optical density (OD) of all ten negative controls were averaged and used to compare with OD of all 200 samples since the ODs of both samples and negative control are proportional to the amount of primary antibody bound to the tested antigens.

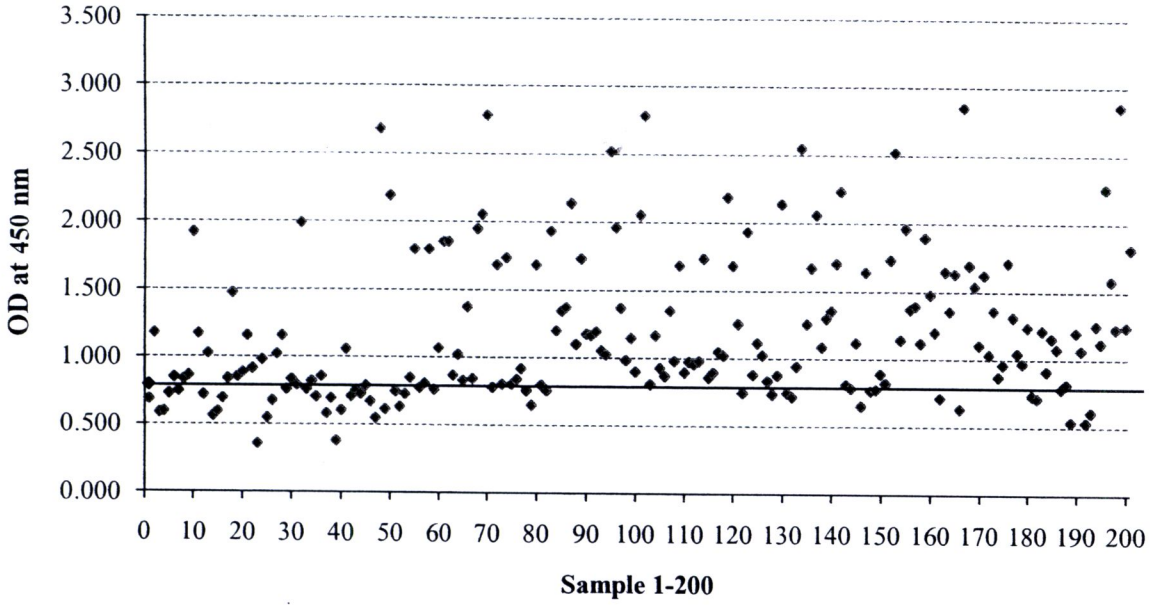

Figure 4.1 Anti-chicken coccidia IgY determination from 200 fresh yolk samples using indirect ELISA (Solid line indicated average OD at 450 of negative control samples)

It was found that average OD_{450} of negative control was 0.683 (solid line), while OD_{450} of IgY samples varied, ranging from 0.35–2.86 (perforated spot) as shown in Figure 4.1.There are several reports indicating that IgY levels in the egg yolk are not always consistent and may vary within and between chicken populations. Carlander *et al.* (2001)investigated IgY production using 10 Single Comb White Leghorn hens and found that concentration of IgY varied from 2.8 ± 0.3 to 7.0 ± 0.5 mg/mL.There was also day to day variation in IgY concentration of eggs produced by single individual laying hen but this variability was less than what was seen among hens.

4.1.2 Determination of extracted anti- chicken coccidia IgY titer

Because egg yolk contains high amount of lipid, to determine activity and specificity of anti-chicken coccidia IgY in immunized chicken eggs, it is preferable to extract the IgY from the yolk first. Anti-chicken coccidia IgY was extracted from fresh yolk of ten eggs collected from week 13 after first immunization using chloroform-polyethylene glycol procedure as described in 3.2.7. Total protein in the extracted IgY then was determined using Bradford protein assay. Finally, the extracted IgY was serially diluted and subjected to indirect ELISA to determine titer of the extracted IgY. The titer is defined as the reciprocal of highest dilution of sample whose OD_{450} was higher than OD_{450} obtained from the same dilution of negative control (IgY against adipocyte membrane protein). OD_{450} of the extracted IgY at various dilutions were shown in Table 4.1

Table 4.1 Extracted IgY titer determined in fresh yolk sample collected at 13 weeks after the first immunization

Sample	Dilutions	Concentration of total protein (ug/mL)	Av. OD at 450 nm
Negative Control (Extracted IgY against adipocyt membrane proteins)	1:160	35	0.638
	1:320	17.5	0.504
	1:640	8.75	0.358
	1:1280	4.38	0.243
	1:2560	**2.12**	**0.174**
Extracted anti-chicken coccidia IgY	1:160	35	2.078
	1:320	17.5	1.550
	1:640	8.75	1.001
	1:1280	4.38	0.560
	1:2560	**2.12**	**0.316**

Results obtained using indirect ELISA showed that all dilutions of the extracted IgY have antigen binding activity higher than negative control. The titer of extracted IgY in this experiment was 2560 (bold letter) since it was the highest dilution whose OD_{450} was higher than negative control. This information could be used further for preparing of antibody dilution in immunofluoresence assay.

4.1.3 Specificity of anti-chicken coccidia IgY

Specificity of anti-chicken coccidia IgY extracted from fresh yolk sample to coccidia antigen was studied. Immunofluorescence technique was used to visualize specific binding of the IgY to coccidia cells at two different stages under fluorescence microscope. In this study, antigens employed were *Eimeria* oocysts at various stages i.e., unsporulated oocysts, sporulated oocysts and extracted sporulated oocysts. Anti-chicken coccidia IgY extracted from pooled fresh yolk sample (as described in 3.2.7) was used as primary antibody whose activities at various dilutions were determined by indirect ELISA before subjected to immunofluorescence assay. The result from indirect ELISA (Table 4.1) was used as a basis to select the suitable dilution of the extracted IgY to be employed. Although the result from Table 4.1 showed that all dilutions of the extracted IgY (1:160 - 1:2560) have antigen binding activity higher than that of negative control, not all dilutions showed strong fluorescence. Preliminary results of immunofluorescence showed that the highest dilution of the extracted IgY yielding strong fluorescence was found to be 320. Thus, the extracted IgY at dilution 320 was

adopted. IgY diluted to 1:320 was dispersed onto slides spotted with different stages of *Eimeria* oocysts and incubated to allow binding of the specific IgY antibodies to antigens. Non specific antibodies and other particulates were removed by washing. Subsequently a secondary antibody specific to IgY antibodies (rabbit anti-IgY FITC) was then dropped. Since the secondary antibody employed are linked to fluorescent dye therefore a bright fluorescence can be observed under fluorescent microscope. Egg yolk from chickens from the same farm immunized with adipocyte membrane protein as well as PBS was employed as primary antibody in negative control. They were introduced in order to be certain that coccidia cells were not autofluorescent.

Immunofluorescence results showed that the extracted anti-chicken coccidia IgY produced in this work bound to coccidia cells only at sporulated stage. Both non extracted and extracted sporulated oocysts were bound by extracted anti-chicken coccidia IgY at dilution 320 which appeared as bright green fluorescence as shown in Figures 4.2(b3) and 4.2(c3), respectively, while binding was not observed with the unsporulated oocysts (Figure 4.2(a3)). In addition, the extracted IgY bound to all component of extracted sporulated oocysts which consist of sporozoites, sporocysts, oocysts and other debris (Figure 4.2(c3)). The extracted sporulated oocysts were tested became it was the antigen used for immunization of hens. These extracted sporulated oocysts include sporozoites, sporocysts and small amount of oocysts as well as cell debris. Thus, the immunized hens appeared to produce IgY specific to all components of these extracted sporulated oocysts but not to unsporulated oocysts as can be anticipated.

For negative control, no binding was noted for unsporulated stage of coccidia when IgY against adipocyte membrane proteins as primary antibody was employed (Figure 4.2 (a2)). However, it was found to bind partially to extracted sporulated oocysts (Figure 4.2(c2)). Although non specific IgY was extracted from yolk of hens immunized with adipocyte membrane protein, it is possible that there are tiny amount of IgY against sporulated oocysts contain in those yolks. Since the hens used in this study were raised in open farm such that they might be naturally infected with sporulated oocysts of coccidia *spp.* in the environment rendering the production of IgY antibodies specific to sporulated oocysts. When PBS was employed as negative control it was found that coccidia cells in this experiment were not autofluorescent (Figure 4.2 (a1-c1)).

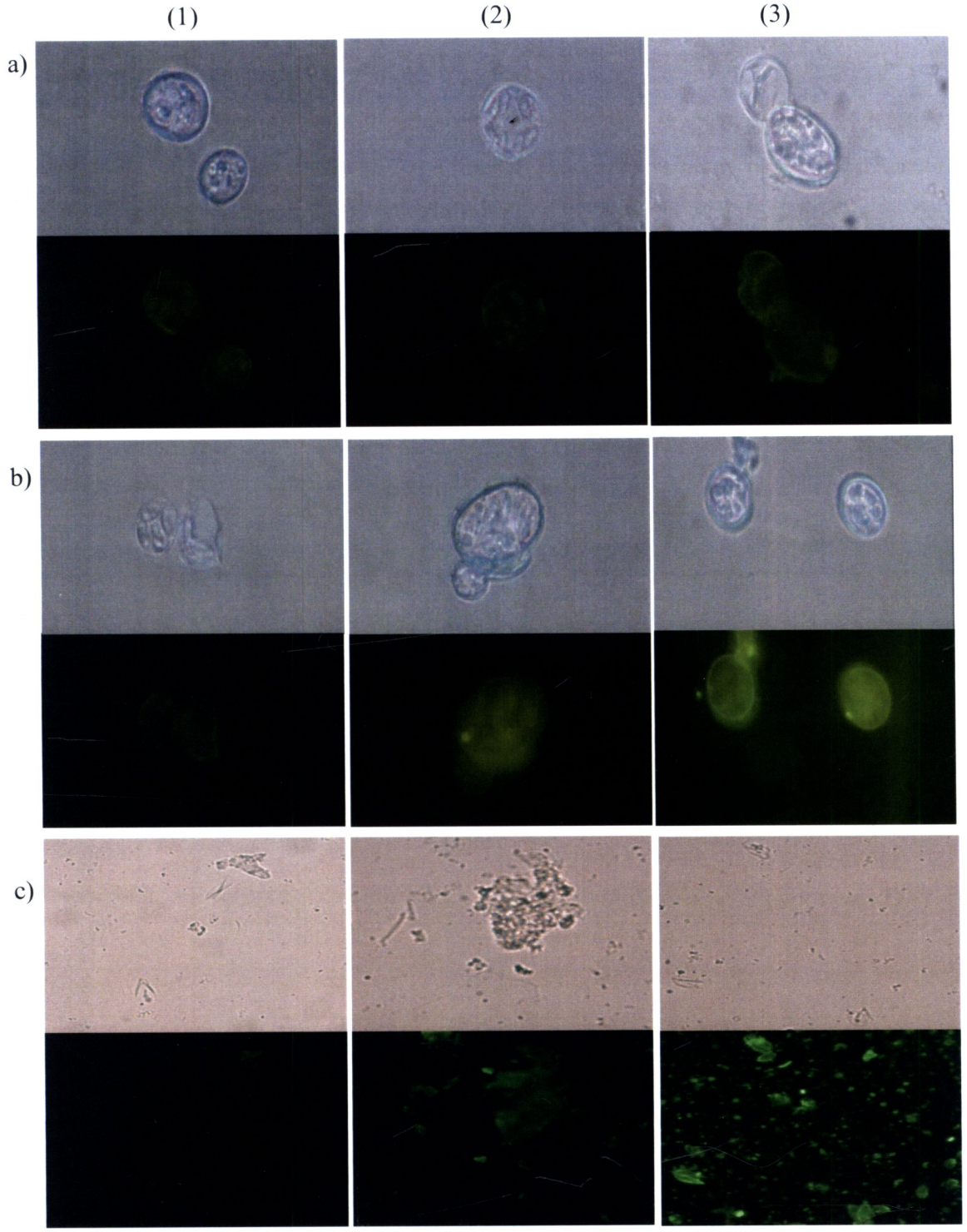

Figure 4.2 Immunofluorescence showing specific binding of anti-chicken coccidia IgY (at dilution 320) to coccidia cells at sporulated stage. (a) Unsporulated oocysts (b) Sporulated oocysts (c) Extracted sporulated oocysts (1) Negative control using PBS as primary antibody (2) Negative control using IgY against adipocyte membrane proteins as primary antibody (3) Anti-chicken coccidia IgY as primary antibody

4.2 Large scale production of anti-chicken coccidia IgY

4.2.1 Immunization efficiency

Because 855 hens were immunized, it is important to study the immunization efficiency of those hens. Anti-chicken coccidia IgY in 200 randomly selected eggs was detected individually using indirect ELISA to determine the percentage of eggs that contain the specific IgY.

IgY level in 200 egg yolk samples found varied as shown in Figure 4.2. The samples could be classified according to their OD_{450} as followed; two positive groups, moderately positive and strongly positive. For the former, it was found that 152 samples (76 %) showed OD_{450} higher than the average OD_{450} of negative control whereas, for the later, 28 samples (14%) whose OD_{450} were 2-folds higher than the average OD_{450} of negative control were identified. It was found further that 21 samples (10%) had OD_{450} lower than negative control indicating no IgY production against immunized antigens. Results on efficacy of IgY production is provided in Table 4.2.

Table 4.2 Classification of 200 eggs from coccidia immunized hens using indirect ELISA

Types of sample	**Negative (eggs not contain IgY)**	**Moderately positive (eggs contain IgY)**	**Strongly positive (eggs contain IgY)**
OD	≤ 0.683	0.684 – 1.366	> 1.366
No. of sample	20	152	28
Percentage (%)	10	76	14

It can be concluded that 14 % of immunized hens produced high amount of anti-chicken coccidia IgY whilst the majority of the hens (76%) produced moderately amount of the IgY. 10% of the hens was found negative. Factors such as chicken health and vaccine distribution might cause variation in IgY production among hens. It was probable that unhealthful chickens as well as chickens receiving insufficient vaccine might produce eggs containing low concentration of IgY or even no IgY production.

4.2.2 Productivity of IgY egg yolk powder

Anti-chicken coccidia IgY was produced in form of egg yolk powder. Eggs of chickens immunized with coccidia *spp.* were collected weekly from week 12 after first immunization onwards. Egg yolks were separated from egg white, pooled and homogenized then subjected to spray drying process. Subsequently, the anti-chicken coccidia IgY titer of pooled samples was determined. The numbers of eggs collected, weight of fresh and dried yolks and the IgY titer of pooled product each week were provided in Table 4.3.

Table 4.3 Numbers of egg, weight of fresh yolk and yolk powder derived from 855 immunized hens each week after immunization

Weeks after first immunization	No. of eggs laid by immunized hens/week	Weight of fresh yolk/week (Kg)	Av. Volume of fresh yolk/an egg (mL)	Weight of yolk powder/week (Kg)	% loss of yolk powder	IgY titer of pooled product
12	1800	29.2	16.2	4.5*	-	2560
13	3500	58.5	16.7	20	31.62	2560
14	2400	39.4	16.4	12	39.08	2560
15	2500	40.7	16.3	10	50.86	2560
16	2300	37.3	16.2	11.8	36.73	2560
17	2190	36.8	16.8	5.68*	-	2560
18	1965	32.7	16.6	10.86	33.58	2560
19	2370	37.2	16.0	12.7	31.73	2560
20	1695	29.8	17.6	10.8	27.52	1280
21	2375	38.2	16.1	12	37.17	1280

* The machine was stopped due to technical error during spray drying process

Yield of the IgY product in the form of yolk powder produced each week depended on the number of eggs laid by the immunized hens. Usually, one egg is laid per day or around six eggs are laid per week, the yolk comprises about 31% of the total weight of an egg or average volume of egg yolk is 15 mL providing that the water content of egg yolk is approximately 51%. Thus, 855 immunized hens produced around 5,100 – 5,900 eggs per week. This could yield around 76.50 – 88.50 kg of fresh yolk per week and subsequent 38.25 – 44.25 kg of yolk powder per week. However, it was found that a number of eggs laid from the immunized hens collected each week was lower than the

expected number. This might be because of the fact that the age of the hens used in this study was around 6 month old at the time of first immunization which was usually considered late for egg laying stage.

The number of eggs laid from the immunized hens varied from approximately 1,700 – 3,500 eggs per week and consequent total volume of yolk separated from those eggs per week varied from around 30-38 kg corresponding to the number of eggs derived. Average volume of yolk/an egg in each week ranged from 16.0-17.6 mL which was consistent with those reported in literature. Yield of IgY product, the dried yolk powder, varied from 10-20 kg which was less than the expected yield. Because of the rapid agglomeration of semi-dried yolk droplets forming a gel adhered to the inner wall of the drying machine during the drying process. It was found that after spray drying process, a lot of yolk powder remained within the spray drying machine which was estimated about 27-50% loss each week (% loss of yolk powder was calculated as weight loss of yolk powder yield divided by expected weight of yolk powder yield multiplied by 100). The anti-chicken coccidia IgY titer of pooled dried product was constant (at dilution 2560) from week 12 until week 19 after first immunization and dropped afterward (Figure 4.4).

4.2.3 Determination of anti-chicken coccidia IgY production period

The IgY production period is an important economical factor for producing the IgY at large scale because maximum IgY can be harvested during this period. It is important to monitor this period in order to set up production strategies.

Anti-chicken coccidia IgY production period of the 855 immunized hens was investigated. Titer of the anti-chicken coccidia IgY in pooled fresh yolk was monitored weekly beginning at 6 weeks after first immunization until the IgY titer dropped. Production period of the anti-chicken coccidia IgY was the time period when level of the titer remained high. The titers of IgY monitored after the 3th immunization are given in Figure 4.3.

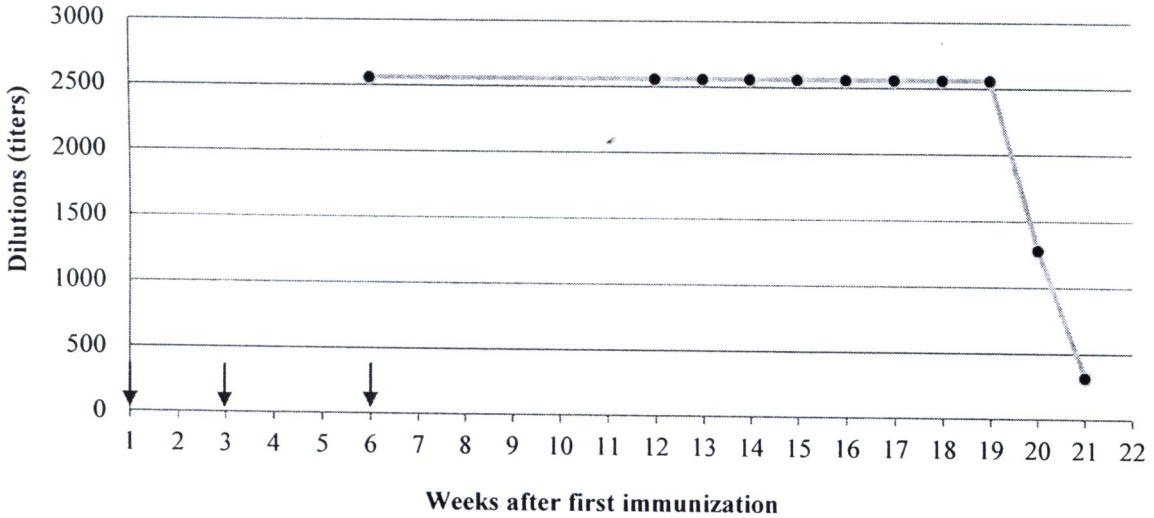

Figure 4.3 IgY titer of pooled fresh yolk monitored weekly after the third immunization (arrow indicate the time of immunization)

Figure 4.3 shows that the IgY titer was 2560 at week 6, the time for administering the third immunization. The titer remained constant until week 19 after which decreased to 1280 and 320 at week 20 and 21, respectively. Pooled fresh yolk collected each week was dried using spray dryer and its titer was determined. It was found that spray drying process is of no adverse effect to the IgY titers of pooled dried yolk as they maintain high level of IgY at 2560 from week 12 until week 19 after first immunization then decreased to 1280 at week 20 and 21. Anti-chicken coccidia IgY titers of pooled dried yolk are provided in Figure 4.4.

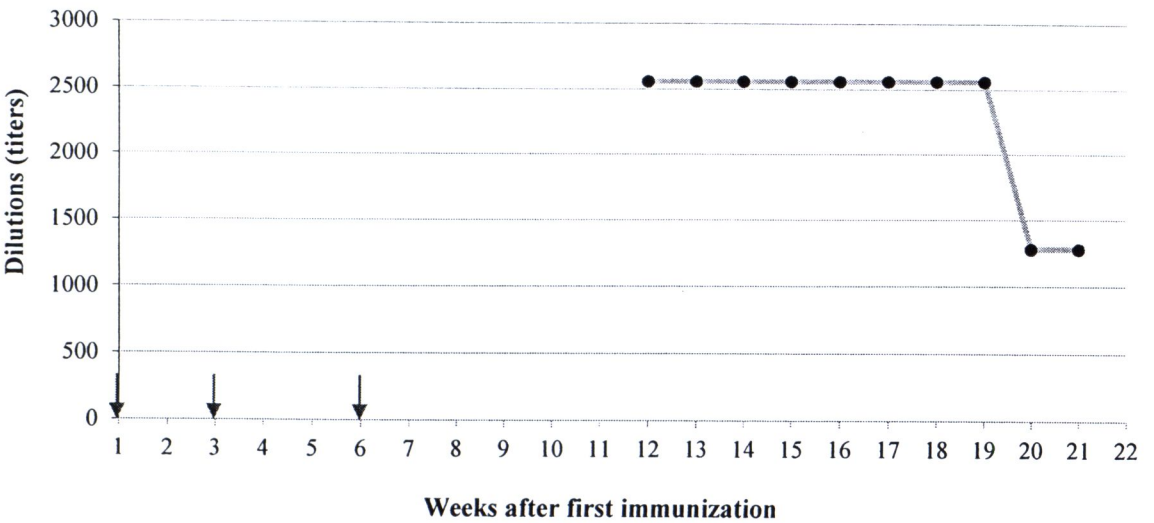

Figure 4.4 IgY titer of pooled dried yolk monitored weekly after third immunization (arrow indicate the time of immunization)

These results suggested that the immunized hens in this work had production period of anti-chicken coccidia IgY of approximately 14 weeks from week 6 to week 19 after first immunization. It is worthy to note that the chickens used in this study were in the late egg laying phase. It is, therefore, possible that the production period may prolong if using chickens at younger age.

Mahdavi *et al.* (2010) produced IgY against *E.coli* from forty 36 weeks old single comb white Leghorn chickens using similar immunization protocol used in this study. The authors found that specific antibody against the *E.coli* in egg yolk increased to high level within 2 weeks after first immunization and remained constant for at least 12 weeks which was of shorter production period compared to the anti-chicken coccidia IgY production 14 week period which is likely due to the chicken age.

Other methods can also be used to prolong production period of IgY production in chickens. Once the antibody titer reached high level in the production period, booster immunization may be given during the laying period to maintain the production of high levels of specific antibodies up to year (Schade *et al.*, 1996). However, it is important to find efficient immunization protocol for extending production period of IgY.

Since the use of chickens for polyclonal antibody production has increased during the past 20 years, many kinds of chicken egg yolk antibody (IgY) were produced against a number of antigens as reviewed in 2.4. However, all of those antibodies were produced in laboratory scale or for study purpose and information on production period of those IgY is limited. For these reasons, it is difficult to compare the IgY production period at large scale in our study with information available in literature.

4.2.4 Detection of bacterial contamination of the dried anti-chicken coccidia IgY product

Contamination of egg shells with aerobic bacteria is generally found for nest eggs from hens grew in open farm. Contamination of eggs via the cloaca may also occur with members of the *Salmonella* group. Thus, care must be taken to avoid the egg shell becoming contaminated with fecal matter. More than 90 percent of all eggs are free of contamination at the time they are laid. Contamination with either *Salmonella* or certain

spoilage organisms occur essentially afterward. Anti-chicken coccidia IgY in this study was prepared as dried yolk powder with which many production steps are prone to contamination. Although all eggs were cleaned by soaking in tap water before egg yolk separation step, it is essential to determine whether there is any pathogenic bacteria present in both fresh yolk and IgY yolk powder. Since the IgY product is intended to be used as in animal feed additive, it is important that the product conforms to the animal feed specification. Specification required for commercial feed product are as follows: Coliform germs should not be more than 10 cfu/g, *E.coli, S. aureus* and *Salmonella spp.* should be absent in 25 grams sample (AOAC). In this study, coliform germs, *E.coli* and *S.aureus* were determined by using 3M petrifilm^{TM} (a commercial prepared plate) while *Salmonella spp.* was detected using AOAC official method. Numbers of pathogenic microorganisms in both fresh and dried yolk are shown in Table 4.4 and 4.5.

Table 4.4 The numbers of pathogenic microorganism in fresh yolk samples

Weeks after first immunization	**No. of pathogenic microorganisms**			
	Coliform germs (< 10 cfu/g)	***E. coli* (absence/g)**	***S. aureus* (absence/g)**	***Sallmonella spp.* (absence/g)**
12	2	-	-	-
13	55	-	-	+
14	100	-	-	+
15	28	-	-	-
16	20	-	-	-
17	140	-	-	-
18	60	-	-	-
19	39	-	-	-
20	44	-	-	-
21	17	-	-	-

Symbol: – and + denote the absence and presence of microorganisms tested

Table 4.5 The number of pathogenic microorganism in dried yolk powder after spray drying process

Weeks after first immunization	No. of pathogenic microorganisms			
	Coliform germs (< 10 cfu/g)	*E. coli* (absence/g)	*S. aureus* (absence/g)	*Sallmonella spp.* (absence/g)
12	0	-	-	-
13	15	-	-	-
14	5	-	-	-
15	1	-	-	-
16	5	-	-	-
17	30	-	-	-
18	11	-	-	-
19	6	-	-	-
20	8	-	-	-
21	3	-	-	-

Symbol: – and + denote the absence and presence of microorganisms tested

It was found that 9 pooled fresh yolk samples collected during week 13 to 21 after first immunization contained coliform germs over the limitation. However, those coliform germs were significantly reduced after spray drying (Table 4.5). Seven dried yolk samples from week 12, 14, 15, 16, 19, 20 and 21 contained coliform germs under limitation. However, two samples from week 17 and 18 were still over limit. *E.coli* and *S.aureus* were not found in all fresh and dried yolk samples tested. Two fresh yolk samples collected from week 13 and 14 were contaminated with *Salmonella spp.* but it was not found in dried samples. It appeared that number of pathogenic organisms found in fresh yolk could be significantly reduced by spray drying process.

4.3 Characterization of dried anti-chicken coccidia IgY product

4.3.1 Anti-chicken coccidia IgY activity after spray drying

Since the anti-chicken coccidia IgY product was to be used as animal feed additive in dried powder form, thus, the pooled fresh yolk collected from each week was dried using spray dryer. The spray drying in this study was performed at 140°C for an air inlet temperature and 72°C for an air outlet temperature as suggested by Kakohki and Kanagawa (1991). Effect of spray drying process on anti-chicken coccidia IgY titer was monitored using indirect ELISA technique. Since fresh egg yolk contains approximately 51% water (Davis and Reeves, 2002), 300 mg of fresh yolk was dissolved in 1 ml PBS before subjecting to the indirect ELISA whereas 150 mg of dried yolk powder obtained after spray drying was dissolved in 1mL PBS and used for the same assay. The anti-chicken coccidia IgY titer of both fresh and dried yolk samples from week 15 to 17 after first immunization were compared as shown in Figure 4.5.

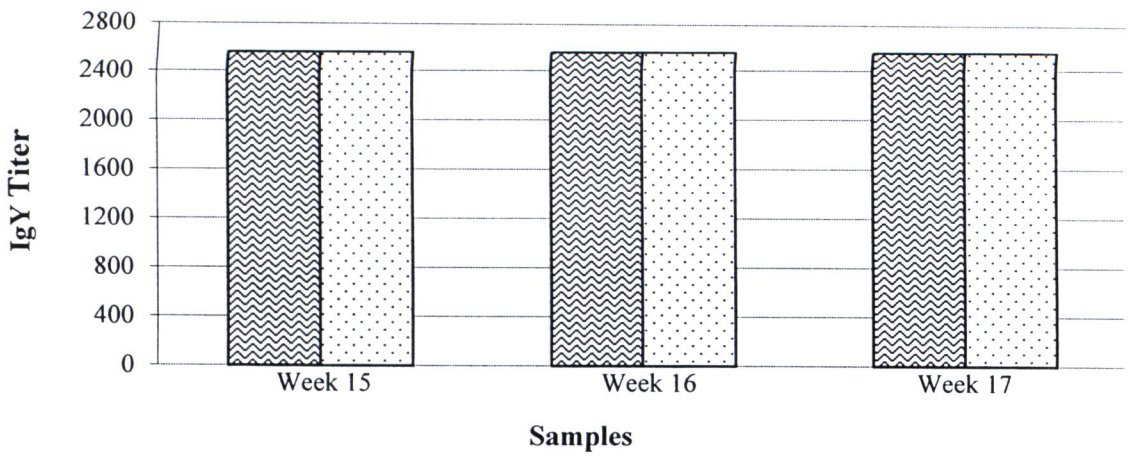

Figure 4.5 Comparison of IgY titer of pooled (▨) fresh yolk and (⬚) dried yolk powder from week 15 to 17 after first immunization

The result showed that anti-chicken coccidia IgY titers of all dried samples from spray drying process used in this study remained as high as that of the fresh yolk samples. The IgY titer of both fresh and dried yolk of all samples were constant at 2560, suggesting that there was no significant adverse effect on IgY activity when samples were prepared using spray dryer with the condition stated above.

4.3.2 Particle size distribution of dried anti-chicken coccidia IgY product

The dried IgY product could also be fed to chickens in suspension form as in drinking water. Generally, particle size of the powder should be smaller than 200 microns to ensure that the particles would not be easily settled down. Particle sizes of the IgY yolk powder was therefore analyzed by particle size analyzer (Model Mastersizer 2000).

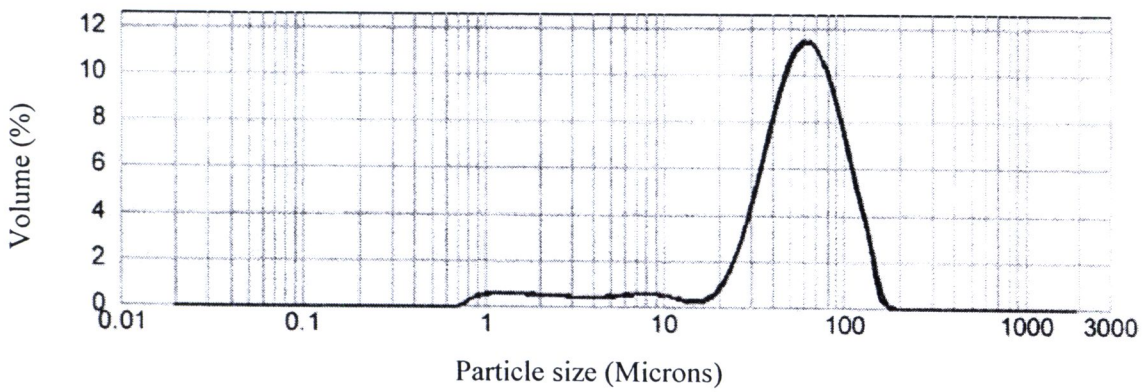

Figure 4.6 Particle size distribution of dried IgY product

Table 4.6 Particle size distribution of dried IgY product

Particle sizes (microns)	% of Particle size distribution			
	Rep 1	Rep 2	Rep 3	Average
0.1 – 0.5	-	-	-	-
0.5 – 1.0	0.86	0.63	0.94	0.81
1.0 – 5.0	4.51	3.76	4.74	4.34
5.0 – 10.0	2.94	2.10	2.34	2.46
10.0 – 50.0	**34.33**	**33.81**	**35.91**	**34.68**
50.0 - 100.0	**47.97**	**46.63**	**45.70**	**46.77**
100.0 – 200.0	9.39	13.07	10.37	10.94
Bigger than 200.0	-	-	-	-

Table 4.6 shows particle size of the anti-chicken coccidia IgY yolk powder which varied between 0.5 – 200.0 microns. Approximately 80% of sample (bold letter) have particle size distributed ranging from 10.0 to 100.0 microns while around 11% of sample have particle size distributed in range of 100.0 to 200.0 microns, and around 8% of sample have particle size less than 0.1– 10.0 microns. This suggests that the IgY yolk powder have smaller size than 200 microns and it is suitable to be used in water suspension form for feeding to chickens.

4.3.3 Sedimentation of dried anti-chicken coccidia IgY product in water suspension

Feeding of IgY via drinking water is an alternative delivery route for chicken. The IgY yolk powder must be kept in suspension form in drinking water. If the IgY yolk powder is settled down too rapidly, chickens might be fed with insufficient amount of IgY. It is therefore important to examine the duration which the anti-chicken coccidia IgY remained suspended in water. To prepare IgY suspension, Polysorbate 80, commercially known as Tween 80, is used as an emulsifier in foods. Adding this substance into the IgY suspension might delay sedimentation of IgY yolk powder. Thus anti-chicken coccidia IgY yolk powder was suspended in tab water containing 0.1%, 0.3% of Tween 80. Sedimentation of IgY yolk powder was observed every 30 min. Results are shown in Table 4.7.

Table 4.7 Sedimentation of dried IgY product in 0.1% and 0.3% Tween 80 solution.

Time (min)	Sedimentation		
	Control (tap water)	0.1% Tween 80	0.3% Tween 80
0	-	-	-
30	+	-	-
60	++	+	-
120	+++	++	-

Symbol: - no sedimentation, + sedimentation

It was found that Tween 80 could extend sedimentation time of anti-chicken coccidia IgY powder in suspension form. IgY yolk powder suspended in 0.1% and 0.3% Tween 80 solution took longer time to settle down than the IgY suspended in tap water. Anti-chicken coccidia IgY yolk powder suspended in tap water began to settle down to the bottom after 30 min while IgY yolk powder in 0.1% and 0.3% Tween 80 was still in suspension. Sedimentation of IgY yolk powder suspended in tap water containing 0.1% Tween 80 appeared after 60 min. After 120 min, IgY yolk powder in 0.3% Tween 80 was still in suspension form. This suggested that tween 80 could be used in drinking water to delay sedimentation of IgY yolk powder in suspension which give enhances

benefit of an alternative route for feeding anti-chicken coccidia IgY suspension to chickens.

4.3.4 Shelf life determination of dried anti-chicken coccidia IgY product

For commercial use, it is important that the shelf life of the product is determined. Shelf life of dried anti-chicken coccidia IgY product from spray drying was studied. Three sets of samples prepared from dried IgY product from week 15, 16 and 18 after first immunization (sample 1-3) were used in this study. Samples were filled in aluminum foil bags (approximately 30gram/bag) in vacuum condition and stored at 4°C and 30°C. Anti-chicken cocccidia IgY titer of samples kept in both temperatures were monitored monthly by indirect ELISA.

Every month, 50 mg of yolk powder containing anti-chicken coccidia IgY was taken, dissolved in 1ml PBS and diluted before subjected to indirect ELISA. Anti-chicken coccidia IgY titer of the stored product was assayed beginning from 4 months after the beginning of storage. It was found that the samples kept in both temperatures maintain their activities at 2560 for at least 13 months. After 13 months, anti-chicken coccidia IgY titer of sample 1 and sample 3 stored at 30°C began to drop whereas anti-chicken coccidia IgY titer of sample 2 stored at 30°C dropped after 14 months. Anti-chicken coccidia IgY titer of all samples stored at 4°C remained the same at 2560 until 16 months after storage. The IgY titers of the three samples were shown in Figure 4.7. This indicates that IgY product stored at 4°C had shelf life at least 16 months which was longer than that of IgY product stored at 30°C which had shelf life around 12-13 months. This information will be useful for the development of dried anti-chicken coccidia IgY as feed supplement and its storage temperature.

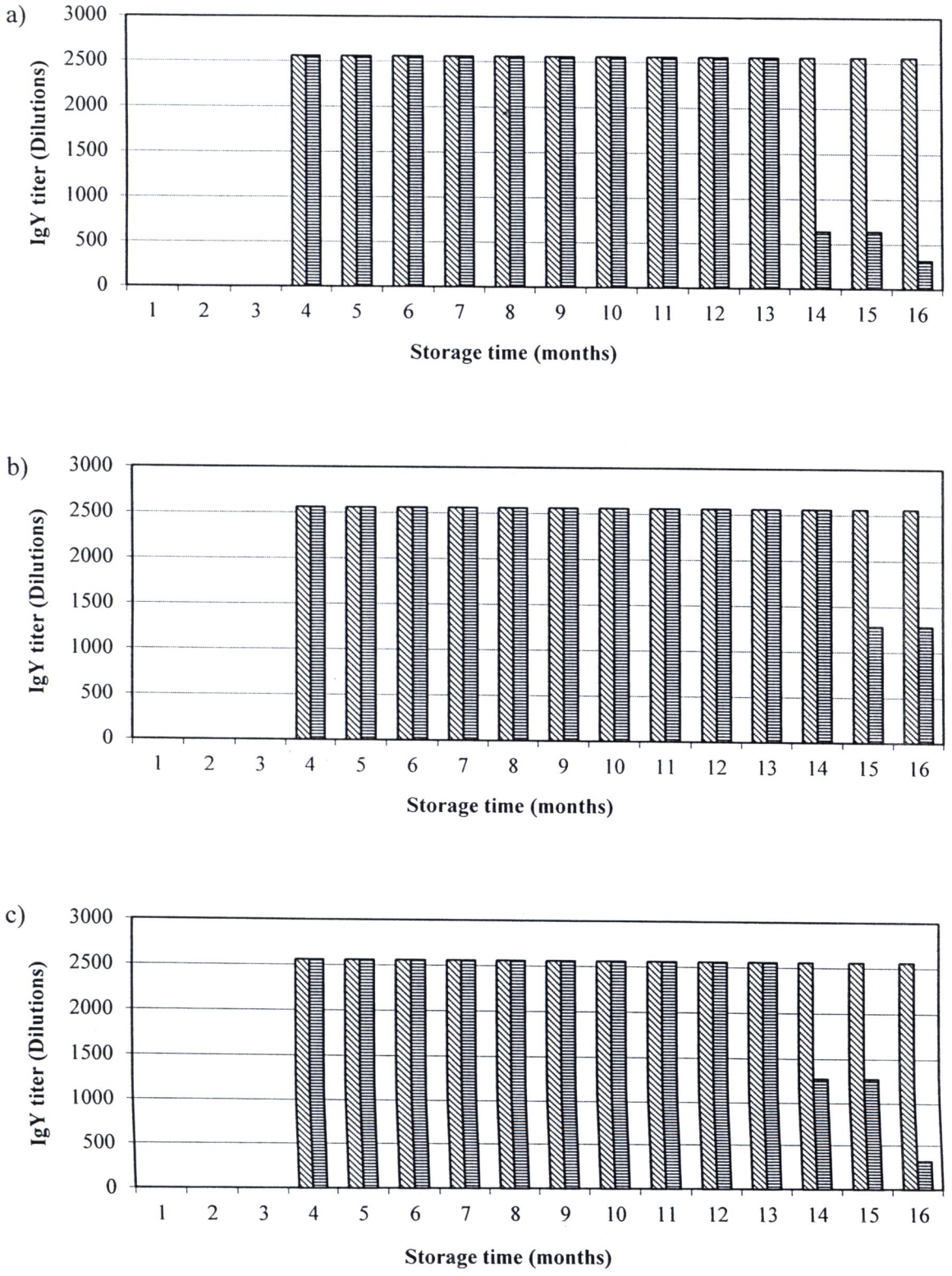

Figure 4.7 Anti-chicken coccidia IgY titer of dried IgY product from spray drying process stored at 4°C (▨) and 30°C (▤) a - c showed anti-chicken coccidia IgY titer of three samples of the dried IgY products