Original Article

Development of Inactivated Newcastle Disease Vaccine using Palm Oil as an Adjuvant

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Abstract

The experiments aimed to develop the inactivated Newcastle disease (ND) vaccine in the form of water in oil (WO) and water in oil in water (WOW) using palm oil, which is easily found in tropical countries, and to study the effect of vitamin E in the form of dl-α-tocopheryl acetate for improving the efficacy of inactivated ND vaccine prepared from palm oil. Two hundred and twenty, thirty five days old male layer chickens were divided into 11 groups each of 20 chickens. The chickens in group 1 were an unvaccinated control group. The chickens in group 2 were a live La Sota strain vaccine control group. The chickens in group 3-10 were vaccinated with a live La Sota strain vaccine combined with inactivated Newcastle disease virus (NDV) WO or inactivated NDV WOW vaccines. The chickens in group 11 were vaccinated with a live La Sota strain combined with a commercial inactivated vaccine. The viscosity of WOW vaccines was significantly less than that of WO and commercial inactivated vaccines ($p<0.05$). The stability test of WOW vaccines at room temperature was longer than WO vaccines. The tissue reaction of WOW vaccines was less severe than WO vaccines. The HI antibody titers and disease resistance against very virulent NDV (vvNDV) of chickens receiving palm-WO or palm-WOW vaccine combined with live vaccine or mineral-WO or mineral-WOW combined with live vaccine, were not significantly different than those of chickens receiving only live vaccine but they were higher than those of the chickens in the unvaccinated control group ($p<0.05$).

Keywords : inactivated Newcastle disease vaccine, palm oil, vitamin E, water in oil, water in oil in water

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บทคัดย่อ

การพัฒนาวัคซีนนิวคาสเซิลชนิดเชื้อตายโดยใช้น้ำมันปาล์มเป็นสื่อวัคซีน

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การทดลองมีวัตถุประสงค์ในการพัฒนาวัคซีนนิวคาสเซิลชนิดเชื้อตายในรูปแบบของน้ำในน้ำมันและน้ำในน้ำมันในน้ำโดยใช้น้ำมันปาล์มที่ซึ่งจัดหาได้ง่ายในประเทศเขตร้อนและศึกษาประสิทธิภาพของวิตามิน อี ในรูป dl-α-tocopheryl acetate ในการปรับปรุงประสิทธิภาพของวัคซีนนิวคาสเซิลชนิดเชื้อตายที่เตรียมจากน้ำมันปาล์ม ทำการแบ่งไก่ไข่เพศผู้จำนวน 220 ตัว ที่อายุ 35 วันออกเป็น 11 กลุ่ม กลุ่มละ 20 ตัว ไก่กลุ่ม 1 เป็นกลุ่มควบคุม ไม่ได้รับวัคซีนและไวรัสนิวคาสเซิล ไก่กลุ่ม 2 เป็นกลุ่มควบคุม ได้รับวัคซีนชนิดเชื้อเป็นเสตรนลา โซตา ไก่กลุ่ม 3-10 ได้รับวัคซีนชนิดเชื้อเป็นเสตรนลา โซตา ร่วมกับวัคซีนนิวคาสเซิลชนิดเชื้อตายแบบน้ำในน้ำมันหรือน้ำในน้ำมันในน้ำ ไก่กลุ่ม 11 ได้รับวัคซีนชนิดเชื้อเป็นเสตรนลา โซตา ร่วมกับวัคซีนนิวคาสเซิลชนิดเชื้อตายเชิงพาณิชย์ ความหนืดของวัคซีนชนิดน้ำในน้ำมันในน้ำน้อยกว่าวัคซีนชนิดน้ำในน้ำมันและวัคซีนเชื้อตายเชิงพาณิชย์อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) การทดสอบความคงตัวที่อุณหภูมิห้อง พบวัคซีนน้ำในน้ำมันในน้ำ มีระยะเวลานานกว่าวัคซีนแบบน้ำในน้ำมัน ปฏิกิริยาต่อเนื้อเยื่อของวัคซีนแบบน้ำในน้ำมันในน้ำ พบความรุนแรงน้อยกว่าวัคซีนชนิดน้ำในน้ำมัน ไม่พบความแตกต่างอย่างมีนัยสำคัญของแอนติบอดีและความต้านทานโรคของไก่ที่ได้รับวัคซีนที่เตรียมจากน้ำมันปาล์มแบบน้ำในน้ำมันหรือแบบน้ำในน้ำมันในน้ำร่วมกันวัคซีนชนิดเชื้อเป็นหรือวัคซีนที่เตรียมจากน้ำมันแร่แบบน้ำในน้ำมันหรือแบบน้ำในน้ำมันในน้ำร่วมกันวัคซีนชนิดเชื้อเป็นต่อไวรัสนิวคาสเซิลเสตรนรุนแรงมาก เมื่อเปรียบเทียบกับไก่ที่ได้รับวัคซีนชนิดเชื้อเป็นเพียงอย่างเดียว แต่พบแอนติบอดีและความต้านทานโรคของไก่ที่ได้รับวัคซีนสูงกว่าไก่กลุ่มควบคุมที่ไม่ได้รับวัคซีนอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$).

คำสำคัญ : วัคซีนนิวคาสเซิลชนิดเชื้อตาย น้ำมันปาล์ม วิตามิน อี น้ำในน้ำมัน น้ำในน้ำมันในน้ำ

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Introduction

Newcastle disease (ND) is an important avian disease that had an economic impact on the poultry industry (Eidson et al., 1982). This disease had affected the international trading of poultry and poultry products around the world (Bennejean, 1988). In some countries, this disease can be controlled; nevertheless, it can cause economic loss due to the expense of vaccination and disease surveillance (Alexander, 1997). ND can be prevented using efficient vaccines. In Thailand, the major control measures of Newcastle disease virus (NDV) are vaccination both live and inactivated vaccines and strictly biosecurity. Nowadays, commercially available inactivated vaccines are frequently prepared from mineral oil as an adjuvant. This mineral oil can cause an adverse effect including tissue reaction, vaccine residues in poultry tissue and the mineral can act as a carcinogen on consumers. Owing to these adverse effects of the mineral oil, much research had been initiated to overcome these problems by finding other kinds of oil that can be substituted for the mineral oil (Stone and Xie, 1990; Gupta et al., 1993; Yamanaka et al., 1993; Stone, 1993; Stone, 1997). In 1996, an inactivated vaccine in the form of water in oil in water (WOW) had been developed by using a subunit of virus, acquired from complete or partial disruption by Tween 80, as an antigen (Cajavec et al., 1996). This vaccine provided less viscosity, more stability and reduced the ratio of mineral oil in the vaccine to support the practical vaccination and easy cleaning of vaccination equipment (Cross, 1988). Previous report revealed that the WOW vaccine seemed to cause fewer tissue reactions than water in oil (WO) vaccine because

of the lower concentration of liquid paraffin (Fukanoki et al., 2001). The vitamin E in the form of dl-α-tocopheryl acetate at a ratio of 20-30% of the mineral oil could elicit the better avian immunity and increase the level of Hemagglutination Inhibition (HI) antibody production (Franchini et al., 1995). This effect may be explained by the combination of the inflammatory reaction from mineral oil and the immuno-stimulation effects of vitamin E to lymphocytes, macrophages and plasma cells at the site of vaccination. Moreover, several indicators of immune responsiveness are depressed when chicks are vitamin E and/or selenium deficient. Since these nutrients serve as antioxidants, cellular integrity may be affected by a deficiency. Cellular integrity is very important for receiving, and responding to the messages needed to coordinate an immune response. High levels of vitamin E (greater than 10 times the required level) have been found to be immunostimulatory (Latshaw, 1991). The vaccines prepared from natural oil provided less efficiency of HI antibody stimulation and more viscosity compared to vaccines prepared from mineral oil (Stone, 1993). The objective of this experiment was to study the properties of inactivated ND vaccine in the form of WO and WOW prepared from palm oil which is easily found in tropical countries and to study the effect of vitamin E in the form of dl-α-tocopheryl acetate for improving the efficacy of inactivated ND vaccine prepared from palm oil.

Materials and Methods

Inactivated oil adjuvant vaccines: The vaccines were prepared by using the local strain of very virulent ND virus (vvNDV), namely NDV-CU-1, propagated in 9 days old embryonated eggs. The intracerebral pathogenicity index (ICPI) of the local NDV strain was 1.8 (Chansiripornchai and Sasipreeyajan, 2006). The concentration of $10^{5.7}$ ELD_{50}/dose or $10^{7.7}$ EID_{50}/dose was used as the stock virus for vaccine preparation. The concentration of virus vaccine was identified by hemagglutination (HA) test (OIE, 2004). The NDV crude antigen for producing vaccines in these studies had HA titer of 256 HAU/50 μl. The virus was inactivated by treatment with 0.05% formaldehyde according to OIE (2004) and inoculated into the chicken embryonic eggs for 2 passages intended to assure the inactivation efficacy.

Preparation of water in oil vaccines: The first formulation of WO vaccine was prepared. The aqueous phase of emulsion vaccines consisted of NDV. The aqueous phase of emulsion contained of 19.2 ml viral stock solution and 0.8 ml Tween 80 and was mixed in a magnetic stirrer (Nuova II stir plate, Iowa, USA) adjusted at the level 9 for 2 min. The oil phase contained 72.0 ml mineral oil and 8.0 ml Span 80. Then, the aqueous phase was added dropwise to the oil phase while the oil phase was constantly stirred with the magnetic stirrer for 2 min. After the addition was complete, this mixture was emulsified by homogenization with blender cups for 2 min (Stone, 1997). The second formulation of WO vaccine was performed by the 14.4 ml (±)-α-tocopheryl acetate (Sigma®) (20% of mineral oil) and 57.6 ml mineral oil was performed instead (Franchini et al, 1995; Stone, 1997). The third formulation of WO vaccine was prepared the same way as the first formulation but palm oil was used instead of mineral oil. The forth formulation was performed in the same way as the first formulation but palm oil was used instead of mineral oil and 20% of vitamin E in palm oil was added.

Preparation of water in oil in water vaccines: The fifth formulation of WOW vaccine was prepared (Cajavec et al., 1996). The aqueous phase I contained, the virus antigen of solubilized NDV 25.0 ml and PBS pH 7.0 25.0 ml which was then mixed in a magnetic stirrer for 2 min. The aqueous phase I was added to 16.6 ml of 5% Tween 80 and mixed in a magnetic stirrer for 2 min to make aqueous phase II. The 30 ml of mineral oil was added to 3.4 ml Span 80 and mixed in the magnetic stirrer for 2 min in order to prepare the oil phase II. After this, the oil phase II was added to the aqueous phase I and homogenized in blender cups (Waring Commercial Blender, Connecticut, USA) for 5 min for the preparation

of water in oil phase I. The water in oil phase I was added to the aqueous phase II and homogenized in blender cups to make WOW vaccine. The sixth formulation of WOW vaccine was performed in the same way as the fifth formulation and 6 ml of vitamin E (20% of mineral oil) was added. The seventh formulation of WOW vaccine was prepared in the same way as the first formulation but palm oil was used instead of mineral oil. The eighth formulation was performed in the same way as the first formulation but palm oil was used instead of mineral oil and 20% of vitamin A had been added. A commercial inactivated vaccine (Chick N-K, Kimber strain, Fort Dodge, USA) was used for the ninth formulation.

The viscosity, stability and tissue reaction tests: The viscosity of the vaccines was determined as the number of seconds using stop watch (Citizen, QT9017-A) it took for the meniscus of a vaccine drawn up into a 1-ml serological pipette (HBG, Germany) to descend vertically from the 0.0-ml mark to the 0.4-ml mark at room temperature (Stone et al., 1978; Stone and Xie, 1990). The stability of the vaccine was tested by the preparation vaccine was observed at 4°C and 37°C for 4 weeks in the tightly screw-capped tubes until the emulsion vaccine has clearly separated between the water phase and emulsion phase (Stone, 1997). The tissue reaction was determined by observing the breast muscle of 5 chickens per treatment at the injection sites. The lesion has been scored in 3 levels. The mild level meant pale muscles around 1 cm and no vaccine left at the injection site. The intermediate level meant pale to red muscles around 1-3 cm in the superficial muscles at the injection site and the small droplets of vaccine left. The severe level meant red and inflammable muscles around 3-4 cm in the superficial and deep muscles at the injection sites and the vaccine was observed when the muscle was dissected (Stone et al., 1978).

Experimental designs: Male layer type chickens (Babcock 500) were used. Two hundred and twenty, one day old chickens were kept in an isolated unit. At 35 days old, the chickens were divided into 11 groups each of 20 chickens. The chickens in group 1, the unvaccinated control group, received PBS 0.5 ml, subcutaneous (s/c) route at the nape of the neck. The chickens in group 2, the NDV live vaccine control group, was received NDV live vaccine, La Sota strain of one dose each via eye drops. The chickens in group 3-10 received NDV live La Sota strain vaccine of one dose each combined with inactivated vaccine preparation formulation 1-8 in the dose of 0.5 ml/

Table 1. Inactivated NDV vaccine formulation 1-9 for total volume of 100 ml

Formulation	Type	Oil phase (ml)			Aqueous phase (ml)		
		Mineral oil	Palm oil	Vit.E	Span 80	Antigen	Tween 80
1	Control (PBS)	-	-	-	-	-	-
2	ND live vaccine	-	-	-	-	-	-
3	WO (mineral)	72.0	-	-	8.0	19.2	0.8
4	WO (mineral) + Vit E	57.6	-	14.4	8.0	19.2	0.8
5	WO (Palm)	-	72.0	-	8.0	19.2	0.8
6	WO (Palm) + Vit E	-	57.6	14.4	8.0	19.2	0.8
7	WOW (mineral)	30.0	-	-	3.4	63.3	3.3
8	WOW (mineral) + Vit E	24.0	-	6.0	3.4	63.3	3.3
9	WOW (palm)	-	30.0	-	3.4	63.3	3.3
10	WOW(palm) + Vit E	-	24.0	6.0	3.4	63.3	3.3
11	Commercial Vaccine	Commercial inactivated vaccine					

bird s/c route at the nape of the neck, respectively. The chickens in group 11 received NDV live La Sota strains vaccine combined with commercial inactivated vaccine (Chick N-K, Kimber strain, Fort Dodge, USA) one dose/bird following the company suggestions (Table 1). Guidelines and legislative regulations of Chulalongkorn University, Bangkok, Thailand on the use of animals for scientific purposes were followed.

Sample collection: All chickens were bled from wing veins. Sera were collected at 35, 42, 49, 56, 63 and 73 days old and stored at -20°C until used.

Hemagglutination Inhibition (HI) tests: HI test using the Beta method following Thayer and Beard (1998) was used. Virus antigen was prepared from live La Sota strain vaccine. One percent of chicken red blood cells were used.

Disease resistance test: Four weeks after vaccination (63 days old), the chickens were challenged orally with local strain of vvNDV (Chansiripornchai and Sasipreeyajan, 2006.) at the concentration of $10^{5.7}$ ELD_{50}/dose or $10^{7.7}$ EID_{50}/dose according to British Pharmacopoeia (Veterinary) (1998). Morbidity and mortality rates were observed for 10 days.

Statistic analysis: The HI antibody levels were analyzed by Analysis of Variance: ANOVA and Duncan's multiple range test at 0.5 (SPSS version 9.0 for window). Morbidity, mortality and disease resistance were performed using chi-square.

Results

The viscosity tests: All WOW vaccine formulations provided less viscosity compared to the commercial vaccine and WO vaccine ($p<0.05$). Also, all WO vaccine formulations had more viscosity than the commercial vaccine ($p<0.05$) (Table 2).

The stability tests: After vaccine preparation, all the vaccines showed very good emulsion. At 4°C, the 7th formulation (WOW, palm) and 8th formulation (WOW, palm+vit E) revealed a stability less than 1 week and the 3rd formulation (WO, palm) and 4th formulation (WO, palm+ Vit E) showed a stability less than 4 weeks. At 32°C, the 3rd formulation and 4th formulation revealed stability less than 1 week and the 7th formulation (WOW, palm) and 8th formulation (WOW, palm+vit E) revealed stability less than 4 weeks.

Table 2. Viscosity and Stability of each vaccine formulation

Formulation	Type	Time (second)*			MEAN ± SE	Stability (wk)	
		1st test	2nd test	3rd test		4°C	37°C
1	WO (mineral)	28.366	25.892	27.806	27.355 ± 0.749^{C}	> 4	> 4
2	WO (mineral) + Vit.E	55.656	53.146	54.486	54.429 ± 0.725^{D}	< 4	< 1
3	WO (palm)	54.911	50.721	56.278	53.970 ± 1.678^{D}	<4	< 1
4	WO (palm) + Vit.E	115.439	142.936	139.026	132.467 ± 8.589^{E}	> 4	> 4
5	WOW (mineral)	3.171	2.506	2.816	2.831 ± 0.192^{A}	> 4	> 4
6	WOW (mineral) + Vit.E	3.746	3.826	4.006	3.860 ± 0.077^{A}	< 1	< 4
7	WOW (palm)	2.186	2.206	2.229	2.207 ± 0.013^{A}	< 1	< 4
8	WOW(palm) + Vit.E	1.622	1.419	1.587	1.543 ± 0.063^{A}	> 4	> 4
9	Commercial Vaccine	16.336	16.092	15.776	16.068 ± 0.162^{B}	> 4	> 4

Note: The tests were performed at 23.8°C, 43% moisture. The different superscripts in the same column were statistically significant ($p<0.05$).

Tissue reaction tests: The tissue reactions were observed 12 days after vaccination. The 4th vaccine formulation vaccine revealed the most severe level of tissue reactions followed by the 2nd (WO, mineral+vit E) and 1st (WO, mineral) formulations. The 8th formulation and a commercial vaccine showed intermediate levels of tissue reaction (Table 3).

HI antibody titers: After receiving live NDV vaccine combined with inactivated NDV vaccine in each formulation, we found that all chickens in each experiment showed HI antibody titers at 49 days old or 2 weeks after vaccination. Later, we found that the HI antibody titer of chickens in all groups was reduced except for group 11, which received the commercial vaccine. Chickens in group 11, receiving a commercial vaccine, revealed an increase of HI antibody titers until 63 days old. The HI antibody titers of chickens in group 11 were higher than all other groups of the experiment ($p<0.05$) (Table 4). Furthermore, all chickens receiving combined vaccines from live and inactivated NDV vaccine revealed higher HI antibody titers than chickens that received single live vaccine. All chickens in group 1, the unvaccinated control group, died within 10 days after challenge (Table 4).

Table 3. The tissue reaction of chickens, 12 days after receiving vaccine in each formulation.

Formulation	Type	Tissue reaction levels		
		Mild[a]	Intermediate[b]	Severe[c]
1	Control (PBS)	5/5	0/5	0/5
2	WO (mineral)	1/5	1/5	3/5
3	WO (mineral) + Vit.E	0/5	2/5	3/5
4	WO (palm)	5/5	0/5	0/5
5	WO (palm) + Vit.E	0/5	1/5	4/5
6	WOW (mineral)	5/5	0/5	0/5
7	WOW (mineral) + Vit.E	5/5	0/5	0/5
8	WOW (palm)	5/5	0/5	0/5
9	WOW(palm) + Vit.E	2/5	2/5	1/5
10	Commercial Vaccine	2/5	3/5	0/5

Note: [a]pale muscles around 1 cm and no vaccine left at the injection site.
[b]pale to red muscles around 1-3 cm in the superficial muscles at the injection site and the small droplets of vaccine left.
[c]red and inflammable muscles around 3-4 cm in the superficial and deep muscles at the injection sites.

Discussion

The viscosity test revealed that the water in oil in water vaccine provided less viscosity than the water in oil vaccines and the commercial vaccine. The advantage of less viscosity vaccine is its benefit to practical vaccination and the cleaning of vaccine equipment. All vaccine preparations showed very good emulsion after preparation. After one week of observation at a low temperature (4°C), WOW (palm and palm+vit E) vaccines showed less stability than commercial and WO vaccines. However, at high temperatures (32°C), WOW (palm and palm+vit E) vaccines showed a higher stability than WO vaccines. This property of vaccines prepared from palm oil gives benefit for the vaccination process in tropical countries that have high temperature all year round. The tissue reaction after 12 days vaccination revealed that lesions and residues of the vaccines prepared from palm oil alone provided a mild degree that was pretty hard to observe with the naked eye. These results were according to Stone (Stone, 1993; Stone, 1997) which reported that the tissue reaction of vaccine prepared from animal and plant oil would be less compared to those of vaccines prepared from the mineral oil. However, when the vitamin E was added for palm oil vaccine formulation, the tissue reaction was found to be more severe than palm oil vaccine alone. This result could be explained by the fact that the tissue reaction came from the inflammatory response from vitamin E because a residue similar to vitamin E was found around the tissue reaction. From our experiments, we found that WOW vaccines had less

Table 4. HI antibody level of chickens that received live La Sota vaccine and/or inactivated local or commercial vaccine at 35 days old and challenge at 63 days old (4 weeks after vaccination)

Formulation	Type	GMT ± SE					Disease
		35 days	42 days	49 days	56 days	63 days	resistance
1	Control (PBS)	2 ± 0^{a}	2 ± 0^{a}	2 ± 0^{a}	2 ± 0^{a}	2 ± 0^{a}	0% (0/20)
2	ND live vaccine	2 ± 0^{a}	2 ± 0^{a}	$127\pm101^{a,b}$	$40\pm25^{a,b}$	$29\pm13^{a,b}$	100% (20/20)
3	WO (mineral)	2 ± 0^{a}	2 ± 0^{a}	$266\pm107^{a,b}$	$100\pm27^{a,b,c}$	$107\pm25^{b,c}$	95% (19/20)
4	WO (mineral) + Vit.E	2 ± 0^{a}	2 ± 0^{a}	358 ± 148^{b}	208 ± 68^{d}	127 ± 28^{c}	100% (20/20)
5	WO (palm)	2 ± 0^{a}	2 ± 0^{a}	$141\pm55^{a,b}$	$100\pm36^{a,b,c}$	$44\pm17^{a,b,c}$	100% (20/20)
6	WO (palm) + Vit.E	2 ± 0^{a}	2 ± 0^{a}	$107\pm25^{a,b}$	$82\pm26^{a,b,c}$	$48\pm14^{a,b,c}$	100% (20/20)
7	WOW (mineral)	2 ± 0^{a}	2 ± 0^{a}	$131\pm53^{a,b}$	$56\pm13^{a,b,c}$	95±18b,c	100% (20/20)
8	WOW (mineral) + Vit.E	2 ± 0^{a}	2 ± 0^{a}	$201\pm101^{a,b}$	$69\pm17^{a,b}$	127 ± 35^{c}	100% (20/20)
9	WOW (palm)	2 ± 0^{a}	2 ± 0^{a}	$87\pm50^{a,b}$	$29\pm7^{b,c,d}$	$44\pm8^{a,b,c}$	95% (19/20)
10	WOW(palm) + Vit.E	2 ± 0^{a}	2 ± 0^{a}	346 ± 114^{b}	$125\pm51^{c,d}$	$93\pm29^{b,c}$	100% (20/20)
11	Commercial Vaccine	2 ± 0^{a}	2 ± 0^{a}	$153\pm56^{a,b}$	156 ± 35^{a}	266 ± 53^{d}	100% (20/20)

Notes: The different superscripts in the same column are statistically significant ($p<0.05$).
Two hundred and twenty, one day old chickens were used in this experiment, and then at 35 days old, the chickens were divided into 11 groups each of 20 chickens as showed in this table.

viscosity and tissue reaction than WO vaccines. These results are in keeping with the report of Fukanoki et al. (2001) that a lower tissue reaction to WOW vaccine should arise from the reduction of the ratio of mineral oil in the vaccine. However, Franchini et al. (1991) reported that the lower tissue reaction of vaccines would induce less immune response. The chickens in groups 3-11 receiving the combined vaccines between live and inactivated vaccines revealed higher HI antibody titers than those of chickens receiving only single live vaccine ($p>0.05$) and the disease resistance was between 95-100%. Fukanoki et al.(2001) reported that WOW vaccines had less ability to stimulate HI antibodies than WO vaccines. According to our experiments, we found that vitamin E can elicit higher HI antibody titers in chickens receiving mineral oil or palm oil alone compared with chickens receiving mineral oil or palm oil combined with vitamin E ($p>0.05$) except for the chickens receiving WO (palm oil) vaccines. The WO vaccine combined palm oil and vitamin E elicited lower HI antibodies than that of WO vaccine made from palm alone but the HI antibody levels of both groups were not so different. According to this result, Franchini et al.(1995) reported that adding of 20-30% of vitamin E in mineral oil would promote more rapid and higher HI antibody levels compared to non-vitamin E, mineral oil vaccine. Comparing a singly live vaccine and combined live and inactivated vaccines, we found that chickens received a live and inactivated vaccine produced HI antibody titers higher than chickens received a singly live vaccine. It is according to Eidson et al.(1982) reported that vaccination of NDV live and subsequently NDV killed vaccines in chickens stimulated higher HI antibody titers than a NDV live vaccine alone. Furthermore, Folitse et al.(1998) explained the reasons for vaccination of an inactivated NDV vaccine (s/c route) combined with live NDV vaccine (intranasal route) provided the higher HI antibody titer because NDV from live vaccine can replicate rapidly on mucous membrane of ocular and nasal organs of chickens. It causes the primary immunity response and combines with the secondary immunity response slowly released from NDV inactivated vaccine in oil adjuvant. The immunity response of the preparation vaccines is different from the commercial vaccine. After vaccination, the HI antibody titer of chickens receiving WO and WOW vaccines were highest at 2 and 3 weeks, respectively after vaccination. After that, the HI antibody titers of chickens receiving both kinds of vaccines would be reduced gradually. The HI antibody titers of chickens receiving a commercial vaccine would be elicited 2 weeks after vaccination but this titer was still maintained until challenge. These results can be explained by the fact that the doses of NDV to prepare WO and WOW vaccines were $7.7x10^{7.3}$ ELD_{50}/bird and $7.1x10^{7.3}$ ELD_{50}/bird, respectively, which is lower than the standard dose of inactivated vaccine ($10^{8.4}$ ELD_{50}) (British Pharmacopoeia (Veterinary), 1998).

Additionally, the mixing process of vaccine preparation for making emulsion may take effect on this aspect because of a better quality of emulsifying process of the commercial vaccine.

In conclusion, the inactivated ND vaccine prepared from palm oil has the potential for replacing mineral oil, particularly when combined with vitamin E. The advantage of this plant oil vaccine is to reduce tissue reaction and vaccine residue in animal tissue.

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