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ARTICLE

Determination of the Administration Routes, Doses and Appropriate Age to Vaccinate With Ornitin Triple Vaccine For Cross-breed Colored Broilers in Vietnam

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ARTICLE INFO

Article history
Received: 9 September 2019
Accepted: 10 October 2019
Published Online: 30 November 2019

Keywords:
ORT disease
swelling reaction
titer IDEXX
bacteria vaccine

ABSTRACT

The study was to determine the appropriate dose and administration route of Ornitin Triple vaccine in cross-breed coloured broilers in Vietnam by evaluating the antibody titer against *Ornithobacterium rhinotracheale* (ORT) and local reactions at injection sites on chickens after vaccination. The study was divided into 2 trials. Both trials were designed with 3 vaccine dose groups: 0.0ml (control group), 0.25ml and 0.5ml and 2 different administration routes: subcutaneous at neck (SC) and intramuscular at breast (IM) injection. The result showed that, no statistically significant difference was found between antibody titer of two administration routes as well as 2 vaccine doses until 13-week-old. Local reactions at the injection sites of IM route was less severe than SC at neck and in higher dose would produce a more severe swelling reaction. Daily weight gain was found to have a slight decrease in the vaccinated groups within 2 weeks after vaccination, however, no statistically significant difference was found in later stage ($P > 0.05$). In conclusion, Ornitin Triple can be used to vaccinate by IM with the dose of 0.25ml for coloured broilers at early age (3-week-old), or 0.5ml for older birds and should be careful for some reactions at the injection sites.

1. Introduction

Respiratory diseases are the most common among the considerably difficult problems and account for a high proportion in poultry diseases. There are many different causes for respiratory diseases including microorganism factors and farms' management [8]. Among those, bacteria *Ornithobacterium rhinotracheale* (ORT) plays an important part in causing complex respiratory syndromes with high mortality [15]. Diversity of ORT with 18 serotypes (type A to type R), serotype A was the most common with 94% [4,7]. Beside that, a survey on random samples, Numee et al found that 3 common serotypes were A (35.5%), B (19.4%), and C (12.9%) [11]. Unlike other repiratory causes, ORT was discovered rather late; it was not known until 1981; the first case of ORT infection was found in turkeys in Germany [3]. Mortality rate of ORT was from 1% to 15% in chickens aged 2-8 weeks old; however, in cases where ORT was followed by a sencondary infection, mortality rate could reach up to 50% [5]. Common clinical signs caused by ORT include coughing, nasal discharge, labored breathing, decreased

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 DOI: https://doi.org/10.30564/vsr.v1i2.1190

feed and water intake and could lead to death [9]. In breeders, the disease could cause the flock to decrease its egg production as well as produce weak offsprings [5]. As ORT is a slow grower and require a really special growing environment, other bacteria (such as *E.coli*), which have the ability to grow fast and easily, would be dominant in the infected organs [12]. Moreover, ORT is usually a secondary infection to other diseases such as Infections bursal disease (IBD), Newcastle (ND) and Infectious Bronchitis (IB). These are all factors contributing to the late or misdiagnosis and hinder prevention of ORT infection and therefore would leave a negative impact on the economy. In 1998, according to *John R. Glisson*, treatment with antibiotics was more efficient than using vaccine [8]. However, one year later, Van Empel and Hafez (1999) found that treatment of ORT with antibiotics would be extremely difficult due to the fact that most ORT strains had already developed antibiotic resistance [16]. In 2001, Devries et al found that ORT bacteria strains were already resistant to 80-100% of commonly used antibiotics in treating poultry, namely ampicillin, ceftiofur, tylosin, spiramycin, lincomycin, tilmicosin, flumequine, enrofloxacin and doxycycline [6]. As a result, deeper research into ways to prevent the disease might be a more effective solution against ORT. Van Empel et al. (1999) carried on a study on the ORT vaccine with an oil adjuvant capable of reducing clinical signs of the disease. From that, this study carried on to evaluate the serum HGA and the reaction at the injection site of the chicken after vaccination [16]. In 2002, Cauwerts et al conducted an experiment on vaccine against ORT in Belgium and brought about good results such as a decrease in mortality rate and an increase in poultry production performance [2]. In 2005, Schuijffel et al proved the ability to generate cross protection against different serotypes of ORT [14]. However, bacterial vaccines are often seen causing strong reactions in poultry as well as the level of immune response largely depends on the reaction at the injection sites and vaccine antigen [13]. As a result, this research was the first in Vietnam to determine the appropriate doses, administration routes and age in order to simultaneouly minimize the side effects and be able to produce a high and prolonged antibody titer to protect local cross-breed broilers from ORT.

2. Materials and Methods

2.1 Materials

Ornitin Triple (Phibro Animal Health, USA) is an oil emulsion vaccine. In the dose of 0.5ml, the antigen amount consists of 10^9 CFU for each serotype A, B and C. Vaccine is recommended to use in prevention of respiratory diseases caused by ORT. Information of vaccine bottle was used in this study: the batch number: 24461034 and expiry date: 23-05-2020.

Cross-breed colored broilers chickens in Vietnam or namedly backyard chickens or native chickens are raised for 14-16 weeks, with live body weigh at slaughter time in average for 2 – 2.5 kg/bird.

2.2 Methods

2.2.1 Experimental Design

The research consisted of 2 trials:

Trial I: 108 3-week-old local cross-bred broilers

Trial II: 120 5-week-old local cross-bred broilers

All chickens in both trials were tagged individually on the legs. They were initially weighed, measured for ORT antibody before vaccinated and randomly allocated into six groups according to 2 factors shown in Table 1.

(1) 0.0ml represented control group, injected with 0.5ml saline solution (NaCl 0.9%).

(2) 0.25ml represented test group injected with 0.25ml Ornitin Triple vaccine.

(3) 0.5ml represented test group injected with 0.5ml

Table 1. Experimental design

	Trial I: ORT vaccination at 21 days old							**Trial II: ORT vaccination at 35 days old**					
	Subcutaneous (SC)			**Intramuscularly (IM)**				**Subcutaneous (SC)**			**Intramuscularly (IM)**		
Dose (ml) Group	0.0	0.25	0.5	0.0	0.25	0.5		0.0	0.25	0.5	0.0	0.25	0.5
Bird/ cage	9	9	9	9	9	9		10	10	10	10	10	10
No. cage	2	2	2	2	2	2		2	2	2	2	2	2
Total bird	18	18	18	18	18	18		20	20	20	20	20	20
No. blood sample	10	10	10	10	10	10		10	10	10	10	10	10
Average BW (g/bird)	312.7	312.1	312.3	312.5	312.1	312.5		760.1	759.6	763.1	761.5	760.4	759.2

DOI: https://doi.org/10.30564/vsr.v1i2.1190

Ornitin Triple vaccine.

(4) SC represented subcutaneous injection at neck.

(5) IM represented intramuscular injection at breast.

Experimented chickens were weighed and put into groups in each trial. Blood samples were taken from 10 chickens in each group.

2.2.2 Effects of Administration Routes, Doses and Age to ORT Antibody Titer

In each experiment, a total of 18-20 chickens were numbered and 10 were randomly selected for serum sample. Those 10 chickens were recorded of their tag number for later reference. Until they reached the age of 13 weeks old, in both experiments, 5 serum samples were collected in each cage. There were 30 serum samples in each trial collected each collecting time. A total of 600 serum samples from both trials were taken during the study.

ORT antibody titer in chickens' serum samples was tested using ELISA technique by IDEXX commercial kit, IDEXX ORT Ab Test, USA, product code 99-43600. According to the recommendation of the manufacturer, grouping of ORT antibody titer was shown in Table 2 and samples with titer ≤ 844 would be considered as negative, and samples with titer ≥ 844 would be considered as positive (IDEXX, USA).

2.2.3 Evaluation of the Safety of Ornitin Triple vaccine

The safety of the vaccine was evaluated by chickens' general body reactions and local reactions at the injection sites after vaccination. Chickens' general body reactions of each group were assessed by body temperature and feed intake. Body temperature of chickens was measured using Amrus thermometer from 4PM to 6PM on day 1, 2, 4, 5, 6, 8, 10, 14 after vaccination. Each group's feed intake was also recorded for 14 continuous days after vaccination. Chickens' local reactions at the injection sites were observed and scored: no swelling = 0 point, swelling less than 1cm = 1 point, swelling from 1-2 cm = 2 points, and swelling more than 2 cm = 3 points.

2.2.4 Evaluation of Vaccine's Effects to Chickens' Performance

Chickens were weighed and recorded individually; their daily average weight gain (DAWG) was calculated using the following formula:

$$DAWG = \frac{Weight\ (after) - Weight\ (before)}{Age\ (after) - Age\ (before)}$$

2.3 Experimental Data Analysis

Data was collected and managed by MS Excel 2010 (Microsoft, USA). Analyzed by T-student test for 2 group of trials, and by ANOVA variance analysis method of software R version 6.3.1.

3. Results and Discussions

3.1 Evaluation of the Level of ORT Antibody Titer before and after Vaccination

Before vaccination, ORT antibody titer of chickens from all groups were recorded as negative (titer ≤ 844). According to IDEXX, chickens that were not vaccinated against ORT and not infected with ORT by field strains would be considered as negative against ORT and therefore, be classified into group 0 (Figure 1, Figure 2) (Table 2).

The results of chickens' ORT antibody titer 3 weeks after vaccination were displayed in Figure 1 and 2. There was a significant difference between vaccinated groups and the control group in terms of ORT antibody titer. In trial I, 3 weeks after vaccination (chickens reached the age of 6 weeks), their ORT antibody titers would be classified into group 8 and above, with the average titer belonging to group 13 or 14, equivalent to 19000 – 20000 titer unit. In trial II, 3 weeks after vaccination (chickens reached the age of 8 weeks), the lowest ORT antibody titer was recorded at group 15 range with the average titer falling into group 16 or 17. In figure 1, titers ranged from group 8 to 16 while in figure 2, titers ranged from group 15 to 18. This result showed that older chickens can produce higher

Table 2. Grouping of ORT antibody titer (xChekPlus software, IDEXX, USA)

Grouping	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Antibody titer	0-844	845 -999	1000 -1999	2000 -2999	3000 -3999	4000 -4999	5000 -5999	6000 -7999	8000 -9999	10000 -11999
Grouping	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	
Antibody titer	12000 -13999	14000 -15999	16000 -17999	18000 -19999	20000 -21999	22000 -23999	24000 -27999	28000 -31999	≥32000	

and more uniform ORT antibody titers 3 weeks after vaccination.

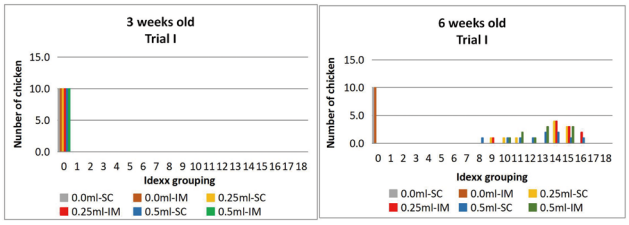

Figure 1. Grouping of ORT antibody titer of chickens at 3 weeks old (before vaccination) and 3 weeks after vaccination in Trial I

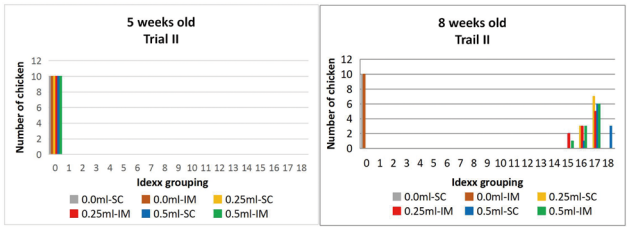

Figure 2. Grouping of ORT antibody titer of chickens at 5 weeks old (before vaccination) and 3 weeks after vaccination in Trial II

3.2 ORT Antibody Titer Post Vaccination

Each group's ORT antibody titers at different ages from trial I and II were recorded in Table 3 and 4, respectively. Before vaccination, ORT antibody titers of all groups were similarly low and not significanly difference ($P > 0.05$). After vaccination, both trials' vaccinated groups experienced an increase in antibody titer compared to the control groups ($P < 0.001$). In addition, 2 weeks after vaccination, both trial I and trial II had ORT antibody titers surged significantly, while in control groups, all were in negative group (Table 3, Table 4).

3.2.1 Effects of Administration Routes to ORT Antibody Titer

According to Bermudez (2008), vaccines needed to be administered at the correct sites in order to achieve the best immune response for poultry [1]. Different type of vaccine would require a different optimal injection site. The effects of administration routes were evaluated based on assessing groups with the same administration dose of each trial. In trial I, with the vaccine dose of 0.25 ml, ORT antibody titer of SC route was not different from that of IM route; similarly, there was no statistical significance between 2 administration routes with the vaccine dose of 0.5 ml ($P > 0.05$) (table 3). The same situation applied to trial II when assessing 2 administration routes with no difference found ($P > 0.05$) (table 4). It can be concluded that, SC or IM injection does not affect the ORT antibody titer of coloured broilers, as there was no significant difference found between the 2 injection routes. Although, there was no statistically difference between SC and IM, it should be carefully to use IM at breast route for broilers to be slaughtered less than 6 weeks after vaccination because IM injection will damage inside the muscle and leave consequences on the carcass quality.

3.2.2 Effects of Vaccine Doses to ORT Antibody Titer

The effect of injection dose on the antibody titer was as-

Table 3. Effects of administration routes and doses to ORT antibody titer after vaccinating with Ornitin Triple vaccine – Trial I

		Subcutaneous (SC)			Intramuscularly (IM)			F_{-value} P_{-value}
		0.0 ml	**0.25ml**	**0.5 ml**	**0.0 ml**	**0.25 ml**	**0.5 ml**	
3 weeks old (N = 10)	**X±SD CV%**	176.7 ± 101.5 57.4	235.0 ± 185.7 79	251.6 ± 297.3 118.2	138.9 ± 134.3 96.7	116.4 ± 57.2 49.1	189.5 ± 173.1 91.4	0.902 0.487
5 weeks old (N = 10)	**X±SD CV%**	113.4 ± 182^{b} 160.5	16269.5 ± 493.9^{a} 39.9	16081.1 ± 416.6^{a} 39.9	115.9 ± 214.5^{b} 185.1	14364.2 ± 6408.3^{a} 44.6	14343.0 ± 6261.3^{a} 43.7	22.679 0.000
6 weeks old (N = 10)	**X±SD CV%**	168.3 ± 173.9^{b} 103.3	19093.0 ± 654.3^{a} 24.4	18581.8 ±4520.9^{a} 24.3	187.9 ± 246.2^{b} 131	21182.5 ± 4179.3a 19.7	18809.0 ± 3667.5^{a} 19.5	81.705 0.000
8 weeks old (N = 10)	**X±SD CV%**	192.7 ± 247.6^{c} 128.5	26537.8 ± 85.7^{ab} 11.6	27238.5 ± 1762^{a} 6.5	260.4 ± 286.7^{c} 110.1	22994.3 ± 4875.7^{b} 21.2	24741.4 ± 4059.8^{ab} 16.4	193.35 0.000
10 weeks old (N = 5)	**X±SD CV%**	995.6 ± 558.1^{b} 56.1	26365.2 ± 248.9^{a} 23.7	30085.2 ± 831.7^{a} 9.4	1360.6 ± 520.4^{b} 38.2	28194.5 ± 7686.1^{a} 27.3	32200.5 ± 2424.5^{a} 7.5	39.159 0.000
13 weeks old (N = 5)	**X±SD CV%**	571.2 ± 443.2^{b} 77.6	22692.8 ± 8876^{a} 39.1	25960.0 ± 198.1^{a} 8.5	767.4 ± 504.8^{b} 65.8	20615.4 ± 7680.7^{a} 37.3	25425.4 ± 8390.4^{a} 33	20.357 0.000

Note: ^{abc}Means within a row with different superscripts differ ($P < 0.05$).

DOI: https://doi.org/10.30564/vsr.v1i2.1190

Table 4. Effects of administration routes and doses to ORT antibody titer after vaccinating with Ornitin Triple vaccine – Trial II

		Subcutaneous (SC)			Intramuscularly (IM)			F_{-value} P_{-value}
		0.0 ml	0.25 ml	0.5 ml	0.0 ml	0.25 ml	0.5 ml	
5 weeks old (N = 10)	X±SD CV%	198.9 ± 186.8 93.94	177.0 ± 132.3 74.73	197.0 ± 207.6 105.38	155.8 ± 88.0 56.44	196.9 ± 96.7 49.13	201.9 ± 80.5 39.85	0.164 0.975
7 weeks old (N = 10)	X±SD CV%	209.0 ± 262.0^{b} 125.34	25745.0 ± 5957^{a} 23.14	26458.0 ± 6798^{a} 25.69	270.1 ± 295.8^{b} 109.53	26125.0 ± 5388^{a} 20.62	26008.0 ± 5290^{a} 20.34	76.982 0.000
8 weeks old (N = 10)	X±SD CV%	224.7 ± 244.4^{b} 108.75	28669.0 ± 2005^{a} 6.99	29748.0 ± 2364^{a} 7.95	227.5 ± 234.3^{b} 102.99	27168.0 ± 2767^{a} 10.18	27985.0 ± 2665^{a} 9.52	520.28 0.000
10 weeks old (N= 10)	X±SD CV%	219.6 ± 222.4^{c} 24.41	24576.0 ± 2501^{ab} 10.18	27403.0 ± 1609^{a} 6.17	361.2 ± 286.9^{c} 79.43	23909.0 ± 4467^{b} 18.68	26746.0 ± 1150^{ab} 4.3	340.39 0.000
12 weeks old (N = 5)	X±SD CV%	1161.5 ± 706.5^{b} 60.8	39781.0 ± 8101^{a} 20.36	33063.0 ± 10749^{a} 32.51	821.0 ± 425.0^{b} 51.77	33788.0 ±10710^{a} 31.70	38160.0 ± 3462^{a} 9.07	32.767 0.000
13 weeks old (N = 5)	X±SD CV%	632.0 ± 233.0^{b} 36.91	28182.0 ± 5003^{a} 17.75	25839.0 ± 7878^{a} 30.49	672.0 ± 329.0^{b} 49.00	24533.0 ± 8752^{a} 35.67	25827.0 ± 3439^{a} 13.32	24.829 0.000

Note: ^{ab}Means within a row with different superscripts differ (P < 0.05).

Table 5. Effects of vaccination age to ORT antibody titer

	Dose of 0.25 ml		Df	T *P-value*	Dose of 0.5 ml		Df	t *P-value*
	21 Days old	35 Days old			21 Days old	35 Days old		
Vaccination day	175.7	186.9	35.69	-0.2703 0.7885	220.6	199.5	32.37	0.3319 0.7421
2 weeks post	15316.9^{b}	25935.2^{a}	37.29	-5.6364 0.0000	15212.1^{b}	26232.9^{a}	37.91	-5.7269 0.0000
3 weeks post	20137.8^{b}	27918.5^{a}	29.78	-6.8496 0.000	18695.4^{b}	28866.6^{a}	32.68	-9.5063 0.0000
5 weeks post	24766.5	24242.6	36.44	0.4164 0.6794	25990.0	27074.5	26.02	-1.3446 0.1904
7 weeks post	21654.1^{b}	36784.6^{a}	17.43	-3.8738 0.0012	31142.9	35611.3	11.06	-1.6732 0.1223
13 weeks old*	20403.4	26560.0	16.95	-1.4636 0.1616	25692.7	25833.1	18.00	-0.0545 0.9571

Note: ^{ab}Means within a row with different superscripts differ (P < 0.05); *11 weeks post for Trial I & 8 weeks post for Trial II.

sessed similarly to that of the injection routes. In trial I (Table 3), there was no difference between the injection dose of 0.25 and 0.5 ml of the same SC or IM injection route (P> 0.05). Trial II provided similar results to trial I (Table 4). It was clear from comparing results of both trials that the antibody titer of experimental chicken groups was not dependant on the injection doses up to 13 weeks old (Table 3, Table 4). This results were obtained only until 13 weeks, it is possible that there is simply not sufficient time to reflect a long of immunity response to higher injection doses (0.5ml vs 0.25ml). Therefore, for chickens raising in longer than 13 weeks, 0.5ml dose should be considered.

3.2.3 Effects of Vaccination Age to ORT Antibody Titer

The comparison of ORT antibody titer after vaccination between trial I and trial II was conducted by T test and the results were shown in Table 5.

These 2 trials was conducted on chickens of 2 different age groups (Table 5), trial I used 3-week-old chickens and trial II used 5-week-old chickens. According to the results of comparing ORT antibody titers of these 2 trials, it was found that the antibody titer after vaccination with the vaccine dose of 0.25 ml in trial I was lower than that of trial II at the times of 2, 3 and 7 weeks after vaccination (P < 0.05).

Likewise, at week 2 and 3 after vaccination with the vaccine dose of 0.5 ml, the ORT antibody titer of trial I was also lower than trial II (P < 0.001). However, after that and when the chickens reached the age of 13 weeks, no statistical difference was found between the trials (P > 0.05). As a result, it could be concluded that with the same

 DOI: https://doi.org/10.30564/vsr.v1i2.1190

vaccine doses, ORT antibody titer of 3-week-old chickes would increase at a slower rate compared to 5-week-old chickens; however, there would be no difference found between chickens of older age groups.

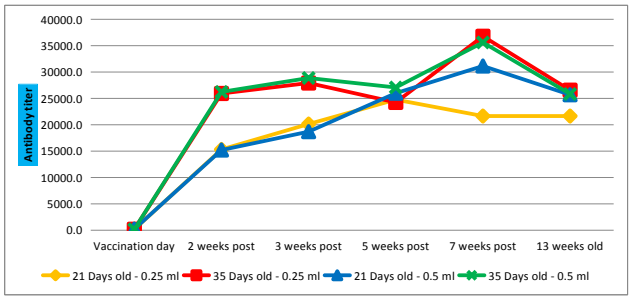

Figure 3. Effects of vaccination age and dose to ORT antibody titer

As recommended by vaccine manufacturer Ornitin Triple, the vaccine is suitable for turkeys 6 weeks of age and the appropriate vaccine dose is 0.5ml. However, there have been no specific recommendations for the local chicken breeds in Vietnam, which are raised for 13-16 weeks (turkeys are 20 weeks or more) and were especially susceptible to ORT at 5-6 weeks of age (Vo et al, 2019).

The appropriate age for vaccination of chickens was in accordance with the body weight, the level of completion of the immune system, health status, disease pressures and especially the effects of bacterial vaccines which often caused a strong reaction in vaccinated chickens (Nguyen Ba Hien et al, 2007).

Figure 3 showed that if chickens can wait until 5-week-old to be vaccinated would be better than 3-week-old chickens. However, if ORT outbreak happen about 5-6 weeks of age then chickens should be vaccinated at least 2 weeks before that, which is 3-week-old. Figure 3 also showed that for chickens raised less than 13 weeks, 0.25ml dose would be good enough, however, for chickens raised more than 13 weeks, 0.5ml dose should be considered.

3.3 Vaccine Reaction

3.3.1 General Body Reactions of Chickens

Body temperature

There was a fluctuation in body temperature after vaccination from day 5 of trial I and day 8 of trial II. There was a statistically significant difference between the control and vaccinated groups ($P < 0.05$). According to Nguyen Ba Hien et al, 2007, after vaccination, body temperature can increase by 0.5-1°C due to the immune responses that occur in the body. In this study, vaccination for younger chickens (3-week-old) would give a change in body temperature following the Ornitin Triple vaccination earlier (day 5 vs 8) than vaccination for older chickens (5-week-old) (Table 6, Table 7).

Table 6. Effects of administration routes and doses to chickens' body temperature – Trial I

	Subcutaneous (SC) (°C)			P F	Intramuscularly (IM) (°C)			P F
Dose	**0.0 ml**	**0.25 ml**	**0.5ml**		**0.0ml**	**0.25ml**	**0.5ml**	
1 days post	40.9 ± 0.4	41.1 ± 0.3	41.1 ± 0.3	**0.086 2.58**	41 ± 0.3	41.1 ± 0.4	41.1 ± 0.3	**0.3445 1.09**
2 days post	41.3 ± 0.3	41.4 ± 0.2	41.4 ± 0.3	**0.3 1.23**	41.4 ± 0.2	41.4 ± 0.2	41.3 ± 0.3	**0.4756 0.75**
3 days post	41.1 ± 0.2	41.2 ± 0.3	41.3 ± 0.4	**0.071 2.79**	41.2 ± 0.3	41.1 ± 0.3	41.2 ± 0.4	**0.7590 0.28**
4 days post	41.3 ± 0.2	41.3 ± 0.3	41.5 ± 0.3	**0.108 2.32**	41.3 ± 0.3	41.5 ± 0.3	41.4 ± 0.3	**0.4105 0.91**
5 days post	41.1 ± 0.4^{b}	41.4 ± 0.4^{ab}	41.5 ± 0.3^{a}	**0.013 4.75**	41.3 ± 0.2^{b}	41.5 ± 0.3^{a}	41.5 ± 0.3^{ab}	**0.0398 3.44**
6 days post	41.1 ± 0.3^{b}	41.4 ± 0.4^{a}	41.3 ± 0.4^{ab}	**0.035 3.59**	41.1 ± 0.2	41.3 ± 0.3	41.4 ± 0.5	**0.0642 2.90**
10 days post	41.7 ± 0.3	41.7 ± 0.3	41.7 ± 0.3	**0.735 0.31**	41.3 ± 0.2^{b}	41.7 ± 0.3^{a}	41.8 ± 0.3^{a}	**0.0000 14.58**

Note: ^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Table 7. Effects of administration routes and doses to chickens' body temperature – Trial II

	Subcutaneous (SC) (°C)			P F	Intramuscularly (IM) (°C)			*P F*
Dose	**0.0 ml**	**0.25 ml**	**0.5ml**		**0.0ml**	**0.25ml**	**0.5ml**	
1 days post	41.6 ± 0.2	41.6 ± 0.2	41.7 ± 0.3	**0.503 0.70**	41.5 ± 0.2	41.6 ± 0.2	41.6 ± 0.2	**0.141 2.03**
2 days post	41.6 ± 0.2^{b}	41.7 ± 0.2^{ab}	41.8 ± 0.2^{a}	**0.005 5.92**	41.6 ± 0.3	41.6 ± 0.3	41.6 ± 0.3	**0.713 0.34**
4 days post	41.6 ± 0.2	41.7 ± 0.2	41.7 ± 0.3	**0.296 1.24**	41.6 ± 0.3	41.7 ± 0.2	41.6 ± 0.3	**0.580 0.55**
7 days post	41.5 ± 0.2	41.5 ± 0.3	41.6 ± 0.2	**0.084 2.59**	41.4 ± 0.4	41.6 ± 0.4	41.6 ± 0.3	**0.099 2.40**
8 days post	41.7 ± 0.2	41.8 ± 0.3	41.8 ± 0.3	**0.133 2.09**	41.7 ± 0.2^{b}	41.9 ± 0.3^{a}	41.8 ± 0.2^{ab}	**0.029 3.76**
10 days post	41.5 ± 0.2^{b}	41.8 ± 0.3^{a}	42 ± 0.3^{a}	**0.000 14.15**	41.5 ± 0.2^{b}	41.9 ± 0.2^{a}	41.8 ± 0.3^{a}	**0.000 11.85**
14 days post	41.6 ± 0.2	41.6 ± 0.2	41.7 ± 0.3	**0.876 0.13**	41.6 ± 0.2	41.7 ± 0.3	41.8 ± 0.3	**0.051 3.14**

Note: ^{ab}Means within a row with different superscripts differ ($P < 0.05$).

Feed intake

The effects of the vaccination to chickens' feed intake were evaluated in the same rearing environment in order to produce the most accurate result. The feed intake (g/chicken/day) after vaccination of each group was recorded every 2-3 days. The results showed that the feed intake of chickens in both trials decreased after vaccination. However, around 3-4 weeks afterwards, no significant difference was found (table 8 and 9).

DOI: https://doi.org/10.30564/vsr.v1i2.1190

Table 8. Chickens' daily feed intake after vaccination – Trial I

Days old	Subcutaneous (SC) *(gam/bird/day)*			Intramuscularly (IM) *(gam/bird/day)*		
	0.0ml	0.25 ml	0.5 ml	0.0 ml	0.25 ml	0.5 ml
35* - 37	65.6	64.2	62.2	68.2	73.0	63.6
38 - 40	72.6	67.8	65.9	69.6	74.9	67.1
41 - 43	79.4	56.9	63.3	83.8	68.3	66.2
44 - 47	89.4	69.6	53.9	85.7	71.4	71.8
48 - 50	90.2	79.3	79.1	95.1	78.0	75.7
51 - 53	91.8	90.8	94.2	93.9	90.5	82.2

Note: *vaccination time

Table 9. Chickens' daily feed intake after vaccination – Trial II

Days old	Subcutaneous (SC) *(gam/bird/day)*			Intramuscularly (IM) *(gam/bird/day)*		
	0.0ml	0.25 ml	0.5 ml	0.0 ml	0.25 ml	0.5 ml
21* - 24	47.8	44.8	42.4	46.9	43.9	45.2
25 - 28	52.7	42.4	41.1	55.8	48.3	42.7
29 - 35	92.4	71.8	64.6	99.1	83.5	81.0
36 - 44	64.2	51.6	45.8	66.8	55.5	48.0
45- 49	73.0	67.6	59.2	75.9	63.4	62.5
50 - 52	98.4	95.6	89.4	92.3	89.5	94.5

Note: *vaccination time

3.3.2 Local Reactions at the Injection Sites

In the control groups, no reaction at the injection sites was recorded. In the vaccinated groups, swelling reaction started to increase 4-7 days after vaccination and peaked at 9-10 days after vaccination. In both trials, swelling reaction was found to develop the strongest in vaccinated groups with the dose of 0.5 ml by SC at neck and then 0.25 ml by SC at neck (Figure 4, Figure 5). In general, in both trials, IM route would produce a less severe swelling reaction compared to SC route; and at some time, the swelling at the injection sites of chickens with the vaccine dose of 0.5 ml would be more severe than those with the dose of 0.25 ml.

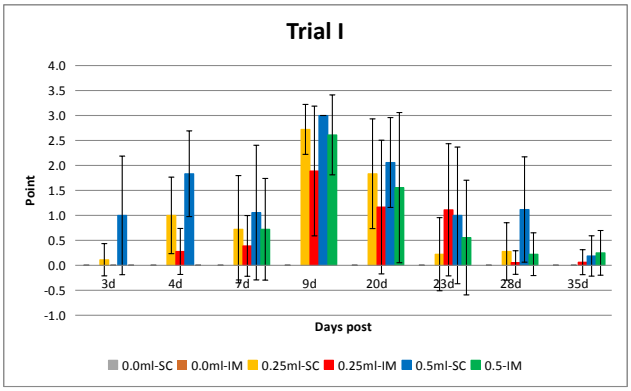

Figure 4. Effects of vaccine to swelling reaction at injection sites - Trial I

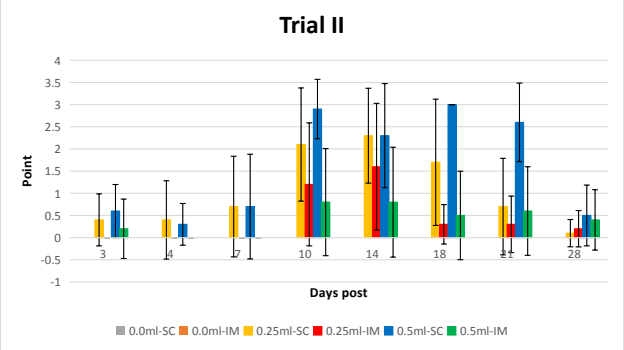

Figure 5. Effects of vaccine to swelling reaction at injection sites - Trial II

3.4 Growth Performance

The performance of chickens in each group was evaluated by their daily weight gain and the results were shown in Table 8 and 9. In both trials, within 2 weeks after vaccination, the chickens' performance had been influenced by the vaccine ($P < 0.001$), especially the SC-0.5ml group was most seriously affected. However later on (up to 91 days old), no difference was found between vaccinated groups and control groups ($P > 0.05$).

A comparison between groups with the same administration routes or the same vaccine doses was performed in order to compare the effects of administration routes or vaccine doses to the perfomance of chickens.

In both trials, at the beginning of the first 2 weeks after vaccination, all vacination groups had a significant weight gain lower than the control groups (Table 10, Table 11). At the same time, the first 2 weeks of both experiments, SC groups with the vaccine dose of 0.5 ml was documented to have a significant lower weight gain than those with the IM group with the vaccine dose of 0.25 ml ($P < 0.001$) (Table 10, Table 11). This could be the result of swelling reaction which was found to develop the strongest in vaccinated groups with the dose of 0.5 ml by SC at neck (Figure 4, Figure 5). The swelling reaction in the neck reduced feed intake especially during the first 2 weeks (Table 8, Table 9). Although, the vaccination groups did have an influence on chickens' performance at the beginning of both trials, however after two weeks, all vaccination groups recovered and no statistically significant influence on growth performance was documented ($P > 0.05$) (Table 10, Table 11).

4. Conclusion

In both trials, there was a development of ORT antibody titer by the 2 administration routes as well as with the 2 vaccine doses. The increase in antibody titer was quite stable in the vaccinated groups, which was statistically

Table 10. Chickens' average weight by age (week) – Trial I

Day-old	Subcutaneous (SC) (g/bird/day)			Intramuscularly (IM) (g/bird/day)			F_{-value} P_{-value}
	0,0 ml	0.25 ml	0.5 ml	0.0 ml	0.25 ml	0.5 ml	
20-35	29.8 ± 5.4^{ab} 17.99	24.3 ± 6.0^{cd} 24.61	20.4 ± 3.8^{d} 18.66	31.5 ± 5.1^{a} 16.24	26 ± 4.2^{bc} 16.07	23.2 ± 4.1^{cd} 17.55	13.000 0.000
36-49	31.3 ± 3.9 12.47	33.0 ± 4.4 13.43	31.4 ± 3.9 12.33	29.6 ± 8.0 26.88	28.6 ± 5.4 18.97	29.7 ± 4.3 14.53	1.6747 0.1475
50-63	30.9 ± 6.2 20.23	26.7 ± 5.2 19.44	30.9 ± 4.1 13.19	28.4 ± 7.9 27.68	30.4 ± 9.7 31.82	27.91 ± 7.4 26.53	0.5954 0.7035
64-91	33.3 ± 4.9 14.72	39.7 ± 6.6 16.73	35.3 ± 8.6 24.34	32.2 ± 3.5 10.7	35.2 ± 8.7 24.75	34.65 ± 5.2 15.13	0.2607 0.6123

Note: $^{a-d}$ Means within a row with different superscripts differ (P < 0.05).

Table 11. Chickens' average weight by age (week) – Trial II

Day-old	Subcutaneous (SC) (g/bird/day)			Intramuscularly (IM) (g/bird/day)			F_{-value} P_{-value}
	0.0 ml	0.25 ml	0.5 ml	0.0 ml	0.25 ml	0.5 ml	
36-49	30.7 ± 5.9^{a} 19.2	23.3 ± 5.7^{bc} 24.4	19.8 ± 7.9^{c} 40	30.3 ± 7.2^{a} 23.8	26.9 ± 8^{ab} 29.6	22.2 ± 6.8^{bc} 30.5	8.302 0.000
50-63	32.4 ± 5.6 17.3	34.6 ± 8 23.3	33.4 ± 7.3 21.7	31.3 ± 6.5 20.8	32.0 ± 12.4 38.6	31.3 ± 7.5 24	0.4887 0.7841
64-77	32.6 ± 9.6 29.6	29.2 ± 4.3 14.7	34.7 ± 8.6 24.7	28 ± 6.8 24.1	31 ± 4.3 13.9	28 ± 6.1 21.9	1.2812 0.2887
78-91	21.9 ± 5.6 25.7	24.6 ± 16.9 68.7	20.5 ± 3.8 18.5	26.4 ± 9.4 35.7	24.2 ± 5.1 20.9	23.7 ± 6.4 26.9	0.4469 0.8133

Note: ^{abc}Means within a row with different superscripts differ (P < 0.05).

significant compared to the control group.

There was no antibody titer statistically difference found between the two administration routes with the same dose and age (P> 0.05). ORT antibody titer of vaccine doses were different at the beginning but no difference when chickens reached the age of 13 weeks. Chickens vaccinated at 3 weeks old had a slower immune response than 5-week-old chickens.

Reactions at the injection site began to fluctuate 5-8 days after vaccination. The swelling reaction at the injection site by IM was less severe than SC route. Simultaneously, the smaller the vaccine dose, the less severe the swelling reaction.

Chicken growth index was affected 2-3 weeks after vaccination, however, no difference was found in later stages compared to the control group, which was reflected by feed intake and weight gain.

In conclusion, it was recommended that Ornitin Triple vaccine could be used for the young cross-breed coloured broilers in Vietnam at the dose of 0.25ml by IM or 0.5ml for older broilers which need to raise for more than 13 weeks. Moreover, it was also necessary to pay attention to the reaction at the injection sites after vaccination.

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