Effects of dietary supplementation with antibiotic, organic acid, probiotic and prebiotic on the intestinal morphology and Newcastle disease virus titers of broilers in commercial farms

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ABSTRACT

This experiment was carried out to survey the antibody levels against Newcastle disease virus (NDV) and the morphology of ileal villi of broilers in commercial farms. Based on antibiotics and feed additives used, farms were classified in 3 groups as follows (1) Group I was supplied with antibiotic, probiotics and prebiotics; (2) Group II were supplemented with antibiotic, probiotics, prebiotics and organic acids; and (3) Group III was supplied with antibiotic and probiotics. In each farm, ten chicks were sacrificed at day 1, and five chicks were sacrificed at 7, 14, and 28 days old. A total number of 225 Ross 308 broilers at 1, 7, 14 and 28 days of age were selected randomly from nine farms for the titration of antibody against NDV by using Hemagglutination inhibition assay (HI). Furthermore, total 54 ileum samples of chickens on 14 and 28 days old were also collected for measurement of intestinal morphology. The present study showed there were significant differences about the body weights of broilers across farms within the same antibiotics, feed additives and vaccination programs or among different groups at 7, 14 and 28 days of age. However, at the age of 28 days, except Farm 1, 9 (Group I); 4 (Group II) and 3 (Group III), the remaining farms did not meet the criterion of chicken's body weight. After ND vaccination for broilers at one day old, the mean value of HI antibody titers gradually declined in the first two weeks. Except for Farm 7 and 8, at 14 days old, the remaining farms showed the low antibody titers under $3\log_2$. No significant differences about the antibody titers against ND virus were found in broilers at 28 days of age ($P > 0.05$) among farms. The findings suggested that the supplementation of antibiotics, probiotics and/or prebiotics and/or organic acids did not have any consistent effects on immune response to NDV and body weights of broilers. However, the morphometric parameters of ileal villi were improved and the positive correlations between body weight and villi height or villi area in ileum segment were found in these chickens.

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1. Introduction

Newcastle disease (ND) has been known as one of the most important worldwide disease of poultry which could impact on the potential economic of poultry industry. Newcastle disease virus (NDV), the causative agent, was an avian paramyxovirus type 1, belonging to the family *Paramyxoviridae* (Mayo, 2002). The virulence of NDV strains was specified by mean death time of inoculated embryonated eggs, resulting in three major pathotypes - lentogenic, mesogenic and velogenic, of which the velogenic strains could cause up to 100% morbidity and mortality in infected birds (Munir et al., 2012). The World Organization for Animal Health (OIE) indicated ND as an OIE notifiable disease when it met certain criteria of virulence (OIE, 2018). According to Dimitrov et al. (2017), to prevent ND virus from contacting poultry, strict biosecurity and administration of efficacious vaccines should be used. The use of antibiotics combined with strict biosecurity and hygiene measures has helped the poultry industry to grow by preventing the negative impacts of many avian diseases (Gadde, 2018). However, the failure of ND vaccination program and suboptimal levels of growth and productivity in poultry were found in the poultry farms where antibiotics have continued to be used widely (Ambali et al., 2017). The over-use and misuse of antibiotics in animals and humans has led to increased problem of antibiotic resistance (WHO, 2017). To minimize the use of antibiotics in the poultry industry, alternative applications have been reported with an emphasis on providing nutrients, modulating host immunity, inhibiting/preventing pathogen intestinal colonization, and improving intestinal barrier function. Besides preventing infection or disease, those applications have been indicated to improve the body weight, feed conversion, and carcass yield (Houshmand et al., 2012). This study was performed to investigate the NDV titers and the morphology of intestinal villi of broilers which were raised with diet containing antibiotics, probiotics, prebiotics and/or organic acids in field trip.

2. Materials and Methods

2.1. Animals and diets

Total of 225 Ross 308 broilers from nine different farms in Ho Chi Minh City and surrounding areas were sampled. Chickens were reared on intensive indoor farms and fed ad libitum with commercial feed (De Heus Vietnam feed company. Ltd, Vietnam) ad libitum by nipples drinkers and plastic feeders. Depending on antibiotics, feed additives and vaccination programs were used, three groups of farms were divided including Group I with chickens from Farm 1, 2, 6, 7, and 9. The diet in Group I contained antibiotics; prebiotics consisting of 45% beta glucans, 27.3% malto - oligosaccharides, > 20% d-Galactosamine, and < 10% d-Glucosamine (Celmanax® Liquid, USA); probiotics (Clostat® SP Dry, USA, consisting of at least 4.0 × 10^{11}CFU *Bacillus subtilis*/kg); Group II including Farm 4 and 8 were supplied with antibiotics; prebiotics (Celmanax® Liquid, USA); probiotics (Clostat® SP Dry, USA); and organic acids including aqueous formaldehyde 37% solution and propionic acid (Sal curb®, USA); and Group III including Farm 3 and 5 were supplied with antibiotics and probiotics (Nutrilaczym, Vietnam) contained at least 3 × 10^{10}CFU *Bacillus subtilis*/kg); and Group IV or Farm 5 was supplied with antibiotics and probiotics (Pnbio-superzym, Vietnam), which contained at least 1 × 10^8CFU *Bacillus spp.*/g, at least 1 × 10^6CFU *Lactobacillus sp.*/g and at least 1 × 10^7CFU *Saccharomyces sp.*/g). All details of age of feed additives and vaccination programs were shown in Table 1 and 2, respectively.

2.2. Sample collection, antibody titers against NDV and histopathological examination

Birds were collected on 4 different days of age for sampling. Day 1, ten chicks were collected upon arrival from each farm; Day 7, 14 and 28, five birds were randomly taken from each farm. Each bird was weighed before sample collection. A 2 mL volume of blood was collected from the wing vein of each bird and the serum was collected. There were 225 samples in total that were used to detect antibodies against NDV using Hemagglutination Assay (HA) and Hemagglutination Inhibition Assay (HI). The tests were carried out following the OIE instruction (OIE, 2018). A lentogenic ND antigen (LaSota strain) was used as a standard antigen in the HA-HI test for detection and titration of specific antibodies against NDV. For convenience, the titer was recorded as just the log index. For example, the titer of $2\log_2$ was recorded as two. The geometric mean titers (GMT) were calculated. In this study, the published cut off value for the protective HI antibody titer (HI titer $\geq 3\log_2$; i.e. GMT ≥ 3) for ND vaccination in chickens was used (Alexander et al., 2004; OIE, 2018).

Furthermore, ileum samples from 3 chickens of 14 and 28 days of age from each farm were also collected. Each sample was stored in a plastic jar containing 10% buffered formalin solution at room temperature for histopathologic examination. From each tissue sample, 10 well-oriented villi and crypts were examined using ImageJ software (Schneider, 2012). These morpho-

Table 1. Feed additives and period of distribution

Groups (Farms)	Probiotic	Prebiotic	Organic acids	Antibiotics					
				Enrofloxacin; Gentamycin	Florfenicol	Tilmicosin	Tylosin	Amoxycillin + Colistin	Doxycycline
I (1, 2 , 6, 7, and 9)	1st - 8th 13th - 16th 22th - 28th	11th - 13th 15th - 18th 23rd- 26th	6th; 14th; 22th 27th - 28th	1st - 5th	8th - 11th 18th - 21st	15th - 19th	-	1st - 5th 23rd- 26th	18th - 21st
II (4 and 8)	1st - 28th	1st - 5th 12th - 14th 23rd - 28th	1st - 28th	-	8th- 11th 18th - 21st	15th - 19th	1st - 5th	1st - 5th	9th - 11th 15th - 19th
III (3 and 5)	1st - 28th	-	-	-	-	-	18th - 21st	1st - 5th	8th - 11th

$\bar{X}$: The mean of number larvae recovered.

metric measures were utilized for the calculation of the mean value of the height and width of ileal villi; the depth and width of crypts; and the villus height to crypt depth ratios. Villus absorptive surface area was calculated by using the formula: Villus absorptive surface area = $2\pi \times$ (average villus width/2) $\times$ villus height (Sakamoto et al., 2000).

Table 2. Vaccination schedules used in nine farms

Age of vaccination (days of age)	Vaccines (route)	Groups
1	ND (SC)	I, II, III
1	ND + IB (Spray)	I, II, III
9	IBDV (SC)	II
12	IBDV (DW)	III
14	IBDV (DW)	I
16	ND + IB (DW)	I, III
18	ND + IB (DW)	II

ND (SC): inactivated oil emulsion vaccine, Newcastle disease strain La Sota (subcutaneous)
ND + IB (Spray): live attenuated vaccine, NDV strain Clone 30 + Infectious bronchitis virus strain Ma5
IBDV (SC): live immune complex infectious bursal disease virus vaccine, Winterfield 2512 strain + antibodies against Infectious Bursal Disease Virus (subcutaneous)
IBDV (DW): live vaccine strain Cu-1M; ND + IB (DW): live attenuated vaccine, NDV strain B1 + IBV strain Mass H120 (drinking water).

2.3. Statistical Analysis

Descriptive data were obtained and analyzed by using Microsoft Excel. Data were expressed as the mean values ± SD and analyzed by One-way ANOVA using SPSS14 and Duncan's multiple range test that were used to compare the means. Pearson correlation test was used to determine the relationships between body weight and villi height or villi area in ileum segment. Multivariate regression was used to analyze the relationships between body weight, antibody titers against NDV, morphometric parameters and types of feed additives. Significance was accepted at the level of $P < 0.05$.

3. Results and Discussion

The day old body weight of broiler chicks ranged from 44.2 ± 1.6 to 49.3 ± 3.0 g. As the age increased, there were significant differences about the body weights of broilers among farms in one group and among groups ($P < 0.05$), (Table 3). Compared to the reference for body weight of chickens by Aviagen (2019), Farm 1 (Group I) and 4 (Group II) got the criterion of chicken's body weight at different age groups.

At the first day of age, the maternally antibody titers ranged from 4 to 8.4 ($\log_2$). All birds of 1 day old and 7 days old were protected from NDV; but the mean value of HI antibody titers of 7 days old chickens of Farm 4 was 1.4 $\log_2$. At the age of 14 days, Farm 7 (Group I) and Farm 8 (Group II) had the mean HI antibody titers were $\geq 3\log_2$, while the remaining farms had the mean value of HI antibody titers under $3\log_2$. The obtained results revealed that, the mean value of HI antibody titers gradually declined in the first two weeks post vaccination when vaccination take place at 1 day old (Table 2). No significant differences about the antibody titers against ND virus were found across different farms in the same group or among different groups at 14 and 28 days of age ($P > 0.05$). The seroconversion of birds at 28 days of age after the second innoculation (around 16 and 18 days of age) were found in all these farms.

Antibodies against NDV were detected in the serum starting at six days after live virus vaccination and peaks 2-3 weeks after vaccination (Al-Garib et al., 2003) (Table 4). The lower the % CV, the more uniform the distribution of titers and the better the vaccination. At 28 days of age, the proportions of birds protected from ND at these farms were under 80% and the large CV (Coefficients of Variance) ranging from 48.0% to 77.8% were found (except Farm 2, 3 and 7). In addition, enrofloxacin showed no negative effect on the immune response to NDV in chickens of Farm 7 during this study (Group I). On the contrary, Sureshkumar et al. (2013) confirmed that the significant reduction in HI antibody titers against NDV of enrofloxacin administered birds was found. According to previous studies, a high proportion of birds ($> 85\%$) with a high antibody titre ($\geq 3\log_2$) after vaccination will ensure that no epidemic spread is possible in vaccinated population (Kapczynski & King, 2005; Boven et al., 2008). Deka et al. (2020) reported that the vaccination program for broilers should be tailored according to the endemicity of the disease, biosecurity level of the farm premises, and level of passively transferred immunity of the birds. Therefore, the findings of this study suggested that the proper time to start the first vaccination against ND in flocks of broilers with maternal antibodies should be considered.

All birds had increased villus height and crypt width compared to the results of birds raised with basal diet (Alshamy, 2018), (Table 5). The highest villi of broilers in Farm 1 (762; 845) and the shortest villi of broilers in Farm 3 (441, at 14 days old) and Farm 6 (491, at 28 days old) were found (Table 5; Figures 1 and 2). The elongation of the villi increased the area of nutrient absorption (Awad et al., 2009); meanwhile, Adibmoradi et al. (2006) reported that the ratio of the crypt depths to the villi height was an indicator of the digestive potential of the gut and might indicate the maturity of the intestinal mucosa. The positive correlation between body weight and villi height or villi area in ileum segment were investigated in chickens at 14 (r = 0.3; 0.3) and 28 days of age (r = 0.6; 0.5) among these farms. However, at the age of 28 days, except Farm 1, 9 (Group I); 4 (Group II) and 3 (Group III), the remaining farms were not met the criterion of chicken's body weight (Aviagen, 2019). In this study, the supplementation of antibiotics, probiotics and/or prebiotic and/or organic acid were supplemented for these birds during the whole rearing period (Table 1). The findings of this study showed the fluctuation of the ratios of these birds was protected from NDV, body weights as well as the morphometric parameters of ileal villi across diferent farms in the same groups or among groups. It was likely that the supplementation of antibiotics, probiotics, organic acids and prebiotics had no clear effects on the increasing immune response to NDV and body weights. Previous study investigated the the dietary supplementation with probiotics (*Bacillus subtilis*) or prebiotics or organic acid or the combination of probiotics and prebiotics improved the growth performance in broilers (Bagal, 2016). However, Fernandes et al. (2014) stated that the morphometric parameters of intestine and broiler performance were not improved by the supplementation of probiotic, prebiotic and organic acid during the rearing period.

Meanwhile, Santos & Turnes (2005) stated that if probiotics, prebiotics, and organic acid were used correctly along with nutritional, managerial and biosecurity measures, they could be a powerful tool in maintaining the health of the gastrointestinal tract of poultry, thus improved their zootechnical performances.

Table 3. Body weight of chickens [(1)] of nine farms (g)

Groups/ Farms		1 day of age ($\overline{X} \pm SD$)	7 days of age ($\overline{X} \pm SD$)	14 days of age ($\overline{X} \pm SD$)	28 days of age ($\overline{X} \pm SD$)
I	1	$44.4^{a} \pm 2.6$	$198.8^{ab} \pm 17.5$	$521.4^{ab} \pm 60.5$	$1530.0^{b} \pm 130.4$
	2	$43.8^{a} \pm 3.0$	$190.3^{bc} \pm 12.0$	$458.2^{bcd} \pm 26.2$	$1310.0^{cd} \pm 89.4$
	6	$43.4^{a} \pm 6.2$	$93.8^{g} \pm 4.8$	$384.1^{de} \pm 14.6$	$1280.0^{d} \pm 130.4$
	7	$48.5^{a} \pm 2.8$	$108.4^{fg} \pm 11.3$	$436.2^{cd} \pm 18.7$	$1340.0^{bcd} \pm 89.4$
	9	$46.3^{a} \pm 2.7$	$156.1^{de} \pm 14.4$	$512.6^{abc} \pm 54.3$	$1510.0^{bc} \pm 102.5$
II	4	$46.7^{a} \pm 3.4$	$225.1^{a} \pm 17.8$	$579.3^{a} \pm 52.2$	$1760.0^{a} \pm 89.4$
	8	$44.2^{a} \pm 1.6$	$131.7^{ef} \pm 14.0$	$509.0^{abc} \pm 45.6$	$1290.0^{d} \pm 74.2$
III	3	$49.3^{a} \pm 3.0$	$162.2^{cd} \pm 22.8$	$467.7^{bcd} \pm 20.7$	$1419.0^{bcd} \pm 85.8$
	5	$46.4^{a} \pm 2.3$	$173.7^{bcd} \pm 5.8$	$333.4^{e} \pm 42.3$	$980.0^{e} \pm 75.8$
Reference [(2)]		43	177	458	1413

[(1)] Numbers of birds sampled per farm: 1-day old, ten; the others groups of age, five

[(2)] Body weight of chicken by Aviagen (2019)

*Values represented with same superscript letters for body weight of chickens did not differ significantly ($P > 0.05$).

Table 4. Newcastle disease virus hemagglutination inhibition antibody titer ($\log_2$)

Group	I					II		III	
Farm	1	2	6	7	9	4	8	3	5
1 day of age (n = 10)									
$\overline{X}$	8.2^{a}	8.4^{a}	6.2^{bc}	4.7^{cd}	7.1^{ab}	4.0^{d}	7.5^{ab}	7.4^{ab}	7.6^{ab}
SD	1.2	1.0	1.6	0.8	1.1	0.5	1.4	1.4	1.5
CV%	14.9	11.5	26.1	17.5	15.5	13.4	19.1	18.2	19.8
Protection (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
7 days of age (n = 5)									
$\overline{X}$	4.4^{a}	5^{a}	5^{a}	3.8^{ab}	4.6^{a}	1.4^{b}	4.4^{a}	5.8^{a}	3.6^{ab}
SD	1.1	1.2	1.6	1.3	2.1	0.5	1.3	0.8	0.9
CV%	26.0	24.5	31.6	34.3	45.0	39.1	30.5	14.4	24.9
Protection (%)	100.0	100.0	100.0	80.0	100.0	0.0	80.0	100.0	40.0
14 days of age (n = 5)									
$\overline{X}$	1.8^{a}	1.4^{a}	0.8^{a}	3.4^{a}	2.0^{a}	1.0^{a}	3.0^{a}	2.6^{a}	2.4^{a}
SD	1.1	0.5	1.3	2.0	0.7	0.0	3.36	0.9	1.5
CV%	60.9	39.1	163.0	61.0	35.0	0.0	98.9	34.4	63.2
Protection (%)	20.0	0.0	20.0	60.0	20.0	0.0	60.0	40.0	40.0
28 days of age (n = 5)									
$\overline{X}$	3.6^{a}	4.6^{a}	4.8^{a}	5.6^{a}	3.0^{a}	3.4^{a}	4.8^{a}	5.6^{a}	4.0^{a}
SD	2.8	0.5	2.2	2.4	1.9	2.3	2.7	2.2	2.5
CV%	77.6	11.9	45.2	43.0	62.4	67.7	55.9	39.1	61.2
Protection (%)	60.0	100.0	80.0	100.0	60.0	60.0	80.0	100.0	80.0

*Values represented with same superscript letters for a HI antibody titre ($\log_2$) did not differ significantly ($P > 0.05$)

n: numbers of birds sampled per farm.

Table 5. Morphometric parameters of ileal villi (height and width) and crypts (depth and width)

Groups/ Farms		Villi (μm)		Crypt (μm)		Villus/ crypt ratio	Villus absorptive surface area
		Height ($\overline{X} \pm SD$)	Width ($\overline{X} \pm SD$)	Depth ($\overline{X} \pm SD$)	Width ($\overline{X} \pm SD$)		
14 days old (n = 3)							
I	1	$762^{a} \pm 136$	$113^{bc} \pm 19$	$120^{cd} \pm 31$	$34^{b} \pm 8$	6.4	270.5
	2	$593^{bc} \pm 171$	$97^{abc} \pm 25$	$205^{ab} \pm 40$	$44^{ab} \pm 11$	2.9	180.7
	6	$544^{cd} \pm 67$	$94^{abc} \pm 15$	$154^{bcd} \pm 29$	$41^{ab} \pm 7$	3.5	160.6
	7	$581^{bcd} \pm 100$	$94^{abc} \pm 22$	$167^{abc} \pm 28$	$42^{ab} \pm 14$	3.5	171.6
	9	$626^{abc} \pm 62$	$120^{a} \pm 26$	$213^{a} \pm 44$	$43^{ab} \pm 7$	2.9	235.9
II	4	$706^{ab} \pm 81$	$101^{abc} \pm 23$	$201^{ab} \pm 33$	$36^{ab} \pm 9$	3.5	224.0
	8	$619^{bc} \pm 63$	$80^{c} \pm 11$	$196^{ab} \pm 44$	$39^{ab} \pm 15$	3.2	155.6
III	3	$441^{d} \pm 70$	$88^{bc} \pm 27$	$109^{d} \pm 28$	$37^{ab} \pm 11$	4.0	121.9
	5	$613^{bc} \pm 77$	$112^{ab} \pm 24$	$206^{ab} \pm 50$	$49^{a} \pm 11$	3.0	215.7
Reference (1)		300		70		3.6	
28 days old (n = 3)							
I	1	$845^{a} \pm 112$	$95^{abc} \pm 14$	$222^{bc} \pm 50$	$39^{a} \pm 10$	3.8	252.2
	2	$754^{ab} \pm 149$	$109^{ab} \pm 20$	$192^{bcd} \pm 57$	$37^{a} \pm 9$	3.9	258.2
	6	$491^{de} \pm 65$	$105^{abc} \pm 33$	$231^{b} \pm 62$	$46^{a} \pm 13$	2.1	161.9
	7	$551^{de} \pm 99$	$98^{abc} \pm 20$	$347^{a} \pm 72$	$42^{a} \pm 11$	1.6	169.6
	9	$584^{cd} \pm 80$	$96^{abc} \pm 25$	$147^{cd} \pm 38$	$39^{a} \pm 5$	4.0	176.1
II	4	$769^{ab} \pm 40$	$104^{abc} \pm 14$	$181^{bcd} \pm 56$	$43^{a} \pm 11$	4.2	251.3
	8	$580^{cd} \pm 77$	$91^{bc} \pm 21$	$193^{bcd} \pm 28$	$38^{a} \pm 5$	3.0	165.8
III	3	$686^{bc} \pm 60$	$126^{a} \pm 21$	$137^{d} \pm 46$	$47^{a} \pm 9$	5.0	271.5
	5	$533^{de} \pm 51$	$113^{ab} \pm 23$	$233^{b} \pm 51$	$38^{a} \pm 9$	2.3	189.2
Reference (1)		390		75		4.8	

n: numbers of birds sampled per farm

Reference (1): villus height and crypt width of birds raised with basal diet (Alshamy, 2018)

*Values represented with same superscript letters for morphometric parameters of ileal villi (height and width) and crypts did not differ significantly ($P > 0.05$).

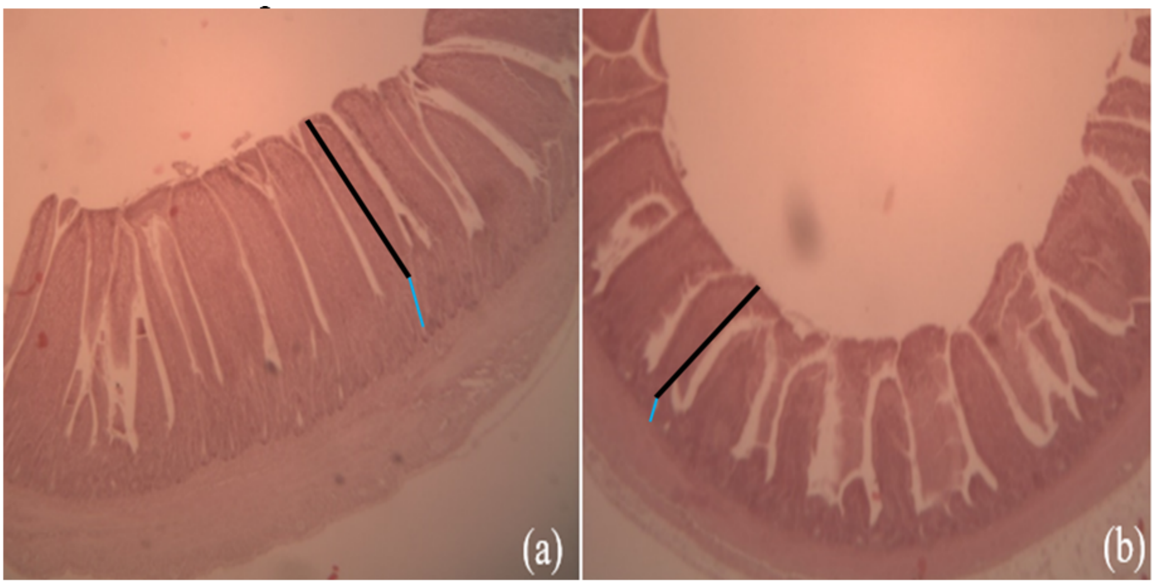

Figure 1. Microscopic appearance of the highest villi height of (a) 14 days old chicken at Farm 4; and the shortest villi of (b) 14 days old chicken from Farm 3. Villi were short and thinned distribution (H&E, original magnification X40, Olympus CX40). Black line: villi height; blue line: crypt depth.

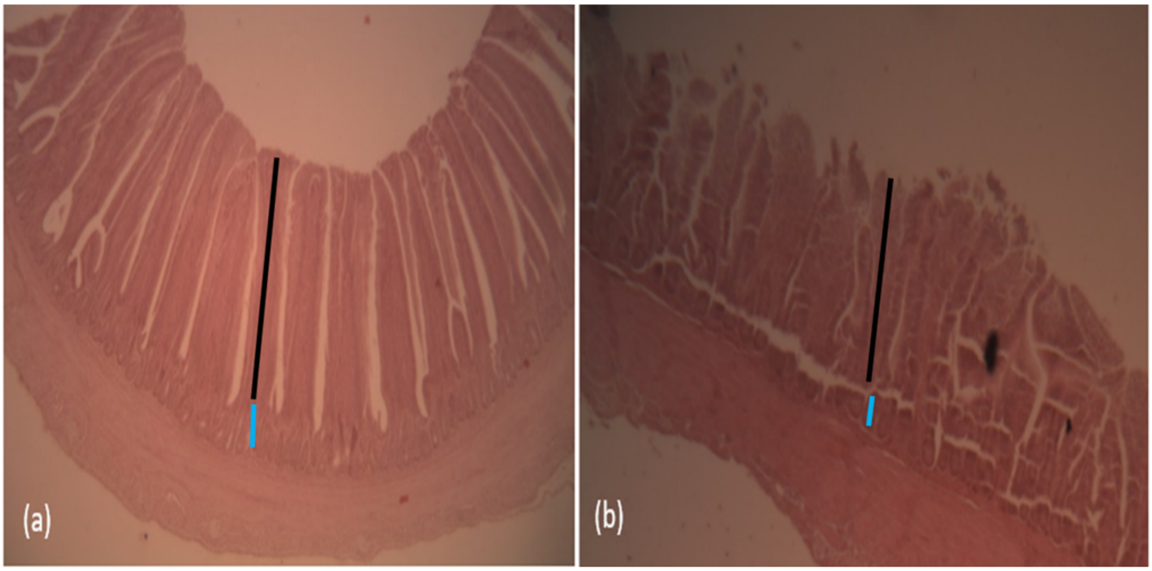

Figure 2. Microscopic appearance of the highest villi of (a) 28 days old chicken from Farm 1 and the shortest villi of (b) 28 days old chicken from Farm 6. Villi were intact and finger-shaped (H&E, original magnification X40, Olympus CX40). Black line: villi height; blue line: crypt depth.

4. Conclusions

During this study, both Farm 1 and 4 got the criterion of chicken's body weight. At the age of 14 days, this study showed the level of flock immunity against NDV was the low risk of NDV infection. These current results indicated that the morphometric parameters of intestine were improved and the positive correlations between body weight and villi height or villi area in ileum segment were investigated in these chickens; but it was hard to explain whether the supplementation of antibiotics, probiotic, prebiotic and organic acids for chickens in these farms had positive effect on the body weights of chickens as well as the antibody titers to ND or not.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or fi-nancial relationships that could be construed as a potential conflict of interest.

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References

Adibmoradi, M., Navidshad, B., Seifdavati, J., & Royan, M. (2006). Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *Journal of Poultry Science* 43(4), 378-383. https://doi.org/10.2141/jpsa.43.378.

Alexander, D. J., Bell, J. G., & Alders, R. G. (2004). *A technology review: Newcastle disease.* Rome, Italy: Food and Agriculture Organization of the United Nations.

Al-Garib, S. O., Gielkens, A. L. J., Gruys, E., & Kochi, G. (2003). Review of Newcastle disease virus with particular references to immunity and vaccination. *World's Poultry Science Journal* 59(2), 185-200. https://doi.org/10.1079/WPS20030011.

Alshamy, Z., Richardson, K. C., Hünigen, H., Hafez, H. M., Plendl, J., & Masri, S. A. (2018). Comparison of the gastrointestinal tract of a dual-purpose to a broiler chicken line: a qualitative and quantitative macroscopic and microscopic study. *PLoS ONE* 13(10), e0204921. https://doi.org/10.1371/journal.pone.0204921.

Ambali, H. M., Nwoha, R. I. O., & Abdu, P. A. (2017). Evaluation of antibody response to Newcastle disease vaccination in chickens in some commercial farms in two Local Government Areas in Lagos State, Nigeria. *Journal of Veterinary Medicine and Surgery* 1(2), 1-6. https://doi.org/doi: 10.4172/2574-2868.100010.

Aviagen. (2019). ROSS - Nutrition Specifications – Aviagen. Retrieved April 1, 2020, from https://en.aviagen.com/ Tech_Center/Ross_Broiler.

Awad, W. A., Ghareeb K., Abdel-Raheem, S. & Böhm, J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poultry Science* 88(1), 49-56. https://doi.org/10.3382/ps.2008-00244.

Bagal, V. L., Khatta, V. K., Tewatial, B. S., Sangwan, S. K., & Raut, S. S. (2016). Relative efficacy of organic acids and antibiotics as growth promoters in broiler chicken. *Veterinary World* 9(4), 377-382. https://doi.org/10.14202/vetworld.2016.377-382.

Boven, M. V., Bouma, A., Fabri, T. H. F., Katsma, E., Hartog, L., & Koch, G. (2008). Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology* 37(1), 1-5. https://doi.org/10.1080/03079450701772391.

Deka, P., Das, S., & Deka, P. (2020). Influence of maternal antibody on the efficacy of Newcastle disease vaccination in broilers. *Current Journal of Applied Science and Technology* 39(7), 108-114. https://doi.org/10.9734/CJAST/2020/v39i730581.

Dimitrov, K. M., Afonso, C. L., Yu, Q., & Miller, P. J. (2017). Newcastle disease vaccines-A solved problem or a continuous challenge? *Veterinary Microbiology* 206, 126-136. https://doi.org/10.1016/j.vetmic.2016.12.019.

Fernandes, B. C. S., Martins, M. R. F. B., Mendes, A. A., Milbradt, E. L., Sanfelice, C., Martins, B. B., Aguiar, E. F., & Bresne, C. (2014). Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. *Brazilian Journal of Poultry Science* 16(4), 417-424. https://doi.org/10.1590/1516-635X1604417-424.

Gadde, U. D., Oh, S., Lillehoj, H. S., & Lillehoj, E. P. (2018). Antibiotic growth promoters virginiamycin and bacitracin methylene disalicylate alter the chicken intestinal metabolome. *Scientific Reports* 8(3592). https://doi.org/10.1038/s41598-018-22004-6.

Houshmand, M., Azhar, K., Zulkifli, I., Bejo, M. H., & Kamyab, A. (2012). Effects of prebiotic, protein level, and stocking density on performance, immunity, and stress indicators of broilers. *Poultry Science* 91(2), 393-401. https://doi.org/10.3382/ps.2010-01050.

Kapczynski, D. R., & King, D. J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine* 23(26), 3424-3433. https://doi.org/10.1016/j.vaccine.2005.01.140.

Mayo, M. A. (2002). Virus taxonomy-Houston Arch. *Virology Division News* 1071-1076. https://ictv.global/ICTV/proposals/Ratification_2002a.pdf.

Munir, M., Abbas, M., Khan, M. T., Zohari, S., & Berg, M. (2012). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. *Virology Journal* 9(46). https://doi.org/10.1186/1743-422X-9-46.

OIE (World Organisation for Animal Health). (2018). *Manual of diagnostic tests and vaccines for terrestrial animals 2018*. Retrieved April 1, 2020, from https://www.oie.int/en/produit/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals-2018.

Sakamoto, K., Hirose, H., Onizuka, A., Hayashi, M., Futamura, N., Kawamura, Y., & Ezaki, T. (2000). Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. *Journal of Surgical Research* 94(2), 99-106. https://doi.org/10.1006/jsre.2000.5937.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9, 671-675. https://doi.org/10.1038/nmeth.2089.

Sureshkumar, V., Sarathchandra, G., & Ramesh, J. (2013). Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken. *Veterinary World* 6(6), 337-342. https://doi.org/10.5455/vetworld.2013.337-342.

WHO (World Health Organization). (2017). *Stop using antibiotics in healthy animals to prevent the spread of antibiotic resistance*. Retrieved April 1, 2020, from https://www.who.int/news/item/07-11-2017-stop-using-antibiotics-in-healthy-animals-to-prevent-the-spread-of-antibiotic-resistance.