Research Article

Effect of *Allium fistulosum* L. Powder on Immune Status in Chickens Vaccinated Against Newcastle Disease Virus

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Abstract | This study investigated the efficacy of *Allium fistulosum* L. powder supplemented in the basal diet on the immune response against the Newcastle Disease (ND) virus and the growth performance of the local chickens. One-day-old chicks (n=400) were allocated to a completely randomized design with four replicates of five treatments. Chickens in treatment T1 were not supplemented with any powder in the basal diet. Chickens in treatment T2 were supplemented with chlortetracycline with 10 mg/kg feed. Chickens in treatment T3, T4, and T5 were supplemented with *A. fistulosum* L. powder with 5 g/kg, 10 g/kg, and 15 g/kg feed, respectively. The experiment lasted 14 weeks. All chickens were vaccinated against Newcastle Disease on days 7, 21, and 60. Sixteen blood samples were taken from each treatment on days 49, 74, and 81. The HI test measured the serum antibody level against the Newcastle disease virus. The results showed that *A. fistulosum* L. powder supplementation (10 g/kg) in the basal diet increased the antibody titer against, while chlortetracycline (10 mg/kg) in the basal diet had a low antibody titer against ND virus in chickens on 49 days of age. Feed intake and feed conversion ratio were similar among the five treatments. Weight gain was significantly different among the five treatments. Weight gain was significantly higher in T2, T3, and T5 treatments than in control treatment T1. The supplementation of *A. fistulosum* L. powder at 5 g/kg and 15 g/kg feed improved the weight gain of chickens.

Keywords | *Allium fistulosum* L., Antibody titer, Chicken growth, Local chickens, Newcastle disease

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INTRODUCTION

In livestock production, antimicrobial compounds are commonly used for controlling diseases and promoting growth. Antibiotic growth promoters have been banned due to their cross-resistant and the risks of multiple drug resistance in human pathogenic bacteria. Therefore, selecting medicinal plants that have efficacy in preventing and treating animal diseases can replace the role of antibiotics, which are being resisted by bacteria is necessary. Many studies were done to find alternative sources of antibiotics in animal diets through phytobiotics from herbs or plant extracts (Goodarzi *et al*., 2013). Many plant products contain bioactive compounds with broad antimicrobial, antifungal, and antioxidant properties (Chang and Cheong, 2008; Vlase *et al*., 2013; Țigu *et al*., 2021). The genus *Allium* includes several species, a few of which are essential as food plants and as drugs in folk medicine, notably garlic *Allium sativum* L., onion *Allium cepa* L., and scallion *Allium fistulosum* L. Scallions are herbaceous perennials with a strong onion or garlic scent, easy to grow and commonly used as a food additive, and they

are always present in every Vietnamese cuisine. In Vietnam, scallions are also one of the herbs used as traditional medicine in treating the common flu, feeling hot or cold for people; the chopped plant or extract juice is mainly used as a traditional medicine to treat or prevent common flu for local chickens. The medicinal properties of *Allium fistulosum* are linked to flavonoids, furostanol saponins, cinnamic acid derivatives, thiolane-type sulfide, and various other bioactive compounds. Studies have shown it possesses anti-obesity, anti-viral, antimicrobial, anti-tumor, antioxidant, anti-inflammatory, and immunomodulatory effects (Balkrishna *et al*., 2023). Although scallions have been well known for their medicinal properties, there is very little available information emphasizing the growth performance and immune-stimulating effect of scallions in chicken.

Poultry is sensitive to several pathogens; among them, Newcastle disease occurs frequently in chickens and causes economic loss for the farmers. Khatun *et al*. (2018) reported a huge financial loss in Bangladesh due to the outbreak of Newcastle disease in village chickens. The study found an adverse impact on household dietary diversity and animal-source food consumption. Newcastle disease is a severe contagious viral disease causing up to 100% mortality in susceptible chickens during devastating outbreaks. Although the vaccine for Newcastle disease has been used for a long time in poultry farming, the effectiveness of vaccination has been affected by several factors, including vaccine virus strains, hosts, etc. (Dimitrov *et al*., 2017). Thus, supportive methods are essential to improve the ability of chickens to be immune against Newcastle disease. This study evaluated the impact of different levels of scallion powder supplementation in the basal diet on growth performance and antibody titers against Newcastle disease virus in chickens vaccinated against Newcastle disease.

MATERIALS AND METHODS

Experimental design

Four hundred one-day-old local chickens (Vietnamese crossbred Noi) were used; they were allocated according to a completely randomized design with four treatments and four replicates. In each treatment, 20 one-day-old chickens were placed in a cage and freely accessed feed and water. Those chickens were purchased from the Center of Animal Breed in Ben Tre province, Vietnam. During the starter phase, the chickens were used the same basal diet and water. The basal feed of DeHeus code 6930 (Netherlands) was supplied for those chickens. The introduction of the treatments began when the chicks reached 15 days of age. The experiment lasted 14 weeks (98 days of age). The experimental treatments were arranged as follows:

T1: ND vaccination and the basal diet without chlortetracycline and *A. fistulosum* L. powder

T2: ND vaccination and the basal diet with chlortetracycline 10 mg/kg

T3: ND vaccination and the basal diet with *A. fistulosum* L. powder 5 g/kg

T4: ND vaccination and the basal diet with *A. fistulosum* L. powder 10 g/kg

T5: ND vaccination and the basal diet with *A. fistulosum* L. powder 15 g/kg.

Vaccination of chickens

All chickens in the experiment were vaccinated against Newcastle Disease on days 7, 21, and 60. Newcastle disease vaccines included two types of freeze-dried vaccines, Newcastle strain Lasota and M (Navetco, Viet Nam). Each dose of Newcastle strain Lasota contains at least 10^6 EID_{50} of the ND strain Lasota, and the vaccine was administered by ocular and nasal instillation for chickens on days 7 and 21. Newcastle strain M contains at least 10^5 EID_{50} of the ND strain M, and the vaccine was administered by intramuscular injection for chickens on day 60.

Collection and storage of blood serum

To determine antibody titers against Newcastle Disease, the blood samples were randomly collected from 240 chickens aged 49, 74, and 81 days (16 chickens were randomly collected from each treatment for each age stage) according to the recommendation of Vietnamese standard TCVN 8400-4:2010 of the diagnostic of Newcastle disease. Blood samples were collected from the wing vein and immediately transferred into 1 mL eppendorf tubes. The blood samples were allowed to clot at room temperature for about 1 hour and in the refrigerator for another 1 hour. The serum was separated by centrifugation at 3,000 rpm for 15 minutes. The serum was aspirated into a serum collecting tube with a micropipette. It was stored at -20°C until antibody titer against ND was determined.

Determination of antibody titers

Antibody titer against ND virus in serum was determined by hemagglutination inhibition (HI) test as described by (OIE, 2021). The geometric mean titer (GMT) was presented as reciprocal log2 values of the highest dilution that displayed HI. GMT was calculated mathematically using the following formula (Villegas, 2008): GMT = antilog (average endpoint tube number - 1) x (log of the factor) + (log of reciprocal of first dilution).

Statistical analysis

Statistical analysis was performed using the Minitab 16.0 software program. The variance analysis was conducted using the general linear model for the growth performance of the local chickens. Differences between dietary treatments were determined by using Tukey's pairwise

comparisons. Fisher's exact probability test was used for the immune response of chickens. $P \leq 0.05$ was considered statistically significant, and $P \leq 0.01$ was considered enormously significant.

Table 1: Effect of *A. fistulosum* L. powder on antibody titer of chickens vaccinated against Newcastle virus.

Day	Criteria	Treatments					P value
		T1	T2	T3	T4	T5	
49	Total chickens	16	16	16	16	16	
	Positive chickens	16	16	16	16	16	
	Protected chickens	12	8	12	16	12	
	Proportion of chickens (%)	75.0	50.0	75.0	100	75.0	0.435
	GMT	13.45	6.73	11.31	19.03	11.31	
74	Total chickens	16	16	16	16	16	
	Positive chickens	16	16	16	16	16	
	Protected chickens	16	16	16	16	16	
	Proportion of chickens (%)	100	100	100	100	100	
	GMT	45.25	26.91	45.25	32.00	26.91	
81	Total chickens	16	16	16	16	16	
	Positive chickens	16	16	16	16	16	
	Protected chickens	16	16	16	16	12	
	Proportion of chickens (%)	100	100	100	100	75.0	0.541
	GMT	22.63	19.03	26.91	22.63	9.51	

RESULTS AND DISCUSSION

The efficacy of different levels of *Allium fistulosum* L. powder (*A. fistulosum* powder) supplemented in the basal diet on immune status against Newcastle Disease (ND) virus

The results in Table 1 showed that on day 49, the chickens in treatments T3, T4, and T5 had the high ND antibody titer with GMT from 11.31 to 19.03 and achieved the high proportion of chickens from 75% to 100% in which treatment T4 (10 g/kg *A. fistulosum* L. powder supplementation in the basal diet) achieved 100%. On the contrary, the chickens in treatments T2 (10 mg/kg chlortetracycline supplementation in the basal diet) had lower ND antibody titer with GMT 6.73 and achieved a low proportion of chickens at 50%. Results of the present study indicated that antibody titers against Newcastle Disease virus antigen were better in chickens receiving *A. fistulosum* L. powder supplementation than in chickens receiving chlortetracycline supplementation. The result of the previous study indicated that herd immunity could only be achieved if a high proportion of chickens (> 85%) have a high antibody titer (log2 HI titer≥ 3) after vaccination (Van Boven *et al*., 2008). Therefore, the third vaccination is necessary. On day 74 (14 days after the third vaccination), ND antibody titers of the chickens in all treatments increased with GMT from 26.91 to 45.25 and achieved a high proportion of chickens (100%), and the herd immunity lasted 81 days of age. The present study showed that high antibody titers were developed 49 days after the second vaccination against ND. The GMT of experimental chickens reached the protection level against the Newcastle virus according to Vietnamese standard TCVN 8400-4:2010.

Growth performance of the local chickens

Besides, the results in Tables 2 and 4 showed the average feed intake and feed conversion ratios were similar among the five treatments. However, the results in Table 3 showed that the weight gain of chickens among the five treatments was significantly different (P ≤ 0.001).

Table 2: The effect of *A. fistulosum* L. powder supplementation on feed intake of chickens (g/chicken/week).

Week of age	Treatments					P
	T1	T2	T3	T4	T5	
1-2	280.3	280.3	280.3	280.3	280.3	-
3-10	2531.5	2525.6	2481.8	2392.4	2540.2	0.952
11-14	2031.5	1964.1	2037.0	1884.0	2044.2	0.851
Total (1-14)	4843.3	4769.9	4799.1	4556.7	4864.7	0.914

Table 3: The effect of *A. fistulosum* L. powder supplementation on weight gain of chickens (g/chicken/week).

Week of age	Treatments					P
	T1	T2	T3	T4	T5	
1-2	93.4	97.3	94.3	94.8	96.6	0.190
3-10	927.1^{c}	1061.7^{a}	1042.9^{ab}	955.4^{bc}	1015.0^{abc}	0.002
11-14	538.6^{b}	552.8^{ab}	582.4^{a}	518.3^{b}	588.2^{a}	0.001
Total (1-14)	1559.1^{b}	1711.8^{a}	1719.7^{a}	1568.5^{b}	1699.8^{a}	≤0.001

Means with different letter (row) superscripts are significantly different (P<0.05).

Table 4: The effect of *A. fistulosum* L. powder supplementation on the feed conversion ratio of chickens.

Week of age	FCR					
	T1	T2	T3	T4	T5	P
1-2	3.01	2.88	2.97	2.96	2.90	0.192
3-10	2.74	2.37	2.38	2.50	2.50	0.355
11-14	3.76	3.56	3.50	3.63	3.47	0.852
Average (1-14)	3.11	2.78	2.79	2.90	2.86	0.511

Immune response of chickens to ND vaccine

On 49 days after the second vaccination against ND,

the chickens receiving 10 g/kg *A. fistulosum* L. powder supplementation (treatment T4) had high titers and consistently high titers throughout three sampling periods. It seems that 10 g/kg *A. fistulosum* L. powder supplementation in the basal diet could improve vaccinated chickens' immunity against Newcastle disease at a young age. *Allium fistulosum* L. is a common *Allium* plant with antimicrobial properties due to its high concentration of sterols and sulfuric compounds (Vlase *et al*., 2013). Besides, *A. fistulosum* contains bioactive components, including allicin, quercetin, and isoquercitrin (Țigu *et al*., 2021). Han *et al*. (1995) reported that *allicin* enhanced immune response. According to (Rahman, 2007), *allicin* has high permeability across bacterial cell membranes to inhibit bacterial proliferation. Allicin and its derivatives have been shown to have an inhibitory effect against Gram-positive and Gram-negative organisms, fungi, and viruses, including influenza viruses (Chang and Cheong, 2008). Moreover, quercetin also inhibited the growth of bacteria because quercetin could damage the structure of bacterial cell walls and inhibit protein synthesis and DNA replication of bacteria (Calderon-Montano *et al*., 2011). Isoquercitrin had antifungal solid activity (Yun *et al*., 2015). Ueda *et al*. (2013) also reported that the *A. fistulosum* mucus was found to enhance natural immunity by increasing interferon (IFN)-γ production from spleen cells and natural killer (NK) activity. These findings indicate that oral administration of mucus can augment the body's innate immune response in experimental mice. Thus, it can be assumed that *A. fistulosum* L. powder in this study effectively enhanced the immune status of chickens, especially in preventing Newcastle disease.

Performance of chickens

The weight gain of chickens in treatments T2, T3, and T5 was higher than in treatments T1 and T4. This indicated that chlortetracycline supplementation (10 mg/kg) and *A. fistulosum* L. powder supplementation (5 g/kg and 15 g/kg) in the basal diet could increase the weight gain of chickens. Although chicken groups supplied with chlortetracycline and *A. fistulosum* L. powder gained weight, using antibiotics in feed was prohibited in actual animal husbandry. Moreover, using alternative methods, including herbs, can bring more benefits to the animals. Yazing *et al*. (2021) indicated that supplementation with *Allium mongolicum* Regel (AM) and AM extracts could increase growth performance, carcass quality traits, and some healthier fatty acids in sheep. In this study, chickens in the treatments supplemented with *A. fistulosum* L. powder had a higher weight gain because scallions contained many beneficial ingredients for chickens. Compounds in *A. fistulosum* L., including disulfide, trisulfide, thiosulfinate, and thiosulfonate could kill roundworms (Tada *et al*., 1988). The diallyl sulfides, flavonoids, vitamin C, and carotenoids in *A. fistulosum* L. are believed to have a beneficial effect on the growth performance of chickens (Rattanachaikunsopon and Phumkhachorn, 2009). Active substances in scallions stimulate the body to create antibodies against influenza A H1N1 viruses (Lee *et al*., 2012). Besides, sulfur-containing compounds stimulated intestinal cell development, increasing the contact area of intestinal microvilli (Saeid *et al*., 2013). In addition, fructo-oligosaccharides in scallions improved growth performance by maintaining beneficial microorganisms in the intestinal tract and enhancing nutrient absorption for chickens (Farahani *et al*., 2015). Ferulic acid has many vital biological properties, such as growth-enhancing, antioxidant, antibacterial, and immunomodulatory effects (Saeed *et al*., 2019). *A. fistulosum* L. had a high content of ferulic acid (Țigu *et al*., 2021). Thus, it can be assumed that *A. fistulosum* L. powder in the diet improved the growth performance of chickens.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, *A. fistulosum* L. powder supplementation in the basal diet well affected the weight gain of chickens. It impacted the immune response of chickens vaccinated against Newcastle disease at an early age. Thus, using *A. fistulosum* L. powder supplementation could support chickens' performance and immune response against Newcastle disease in poultry farming.

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NOVELTY STATEMENT

This research was the first report to implicate the effectiveness of using scallion (*A. fistulosum* L.) in improving the immune response in Vietnamese local chickens against the Newcastle disease virus and enhancing chickens' performance. Therefore, herbs, including scallion, could be an effective alternative to treating and preventing animal diseases.

AUTHOR'S CONTRIBUTION

MTLB, TML and TKN conceptualized, designed, and supervised the research. TKN critically reviewed the study. MTLB and TML collected samples and processed the data. MTLB analyzed and interpreted the data generated. All authors revised and approved the final manuscript.

Ethical approval

This study was conducted following the guidelines outlined in the Helsinki Declaration and the animal welfare and safety procedures of Can Tho University, Vietnam.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Balkrishna A, Chaudhary M, Sharma H, Srivastava D, Kukreti A, Kumar A, Arya V (2023). Phytochemistry, pharmacology, and medicinal aspects of *Allium fistulosum* L.: A narrative review. J. Appl. Pharm. Sci., 13(10): 107–118. https://doi.org/10.7324/JAPS.2023.142822

Calderon-Montano JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M (2011). A review on the dietary flavonoid kaempferol. Mini-Rev. Med. Chem., 11(4): 298-344. https://doi.org/10.2174/138955711795305335

Chang KJ, Cheong SH (2008). Volatile organosulfur and nutrient components from garlic by cultivating areas and processing methods. FASEB J., 22: 1108-1112. https://doi.org/10.1096/fasebj.22.1_supplement.1108.2

Dimitrov KM, Afonso CL, Yu Q, Miller PJ (2017). Newcastle disease vaccines. A solved problem or a continuous challenge? Vet. Microbiol., 206: 126-136. https://doi.org/10.1016/j.vetmic.2016.12.019

Farahani AM, Goodarzi M, Nanekarani S (2015). The effects of aqueous extract of onion on performance and some blood biochemical parameters of the Cobb and Ross broilers. Int. J. Adv. Biol. Biomed. Res., 3: 370–377. https://doi.org/10.18869/IJABBR.2015.370

Gokarna G, Nabaraj S, Shishir B (2017). Effect of *Allium sativum* on immune status against Newcastle Disease Virus and productive performance of broiler chicken. Int. J. Poult. Sci., 16(12): 515-521. https://doi.org/10.3923/ijps.2017.515.521

Goodarzi M, Landy N, Nanekarani S (2013). Effect of onion (*Allium cepa* L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks. Health, 5: 1210-1215. https://doi.org/10.4236/health.2013.58164

Han J, Lawson L, Han G, Han P (1995). Spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. Anal. Biochem., 225: 157-160. https://doi.org/10.1006/abio.1995.1124

Khatun M, Islam I, Ershaduzzaman M, Islam HMS, Yasmin S, Hossen A, Hasan M (2018). Economic impact of Newcastle disease on village chickens-a case of Bangladesh. Asian Inst. Res. J. Econ. Bus., 1: 358-367.

Lee JB, Miyake S, Umetsu R, Hayashi K, Chijimatsu T, Hayashi T (2012). Anti-influenza A virus effects of fructan from Welsh onion (*Allium fistulosum* L.). Food Chem., 134(4): 2164-2168. https://doi.org/10.1016/j.foodchem.2012.04.016

OIE Terrestrial Manual (2021). Newcastle disease (infection with newcastle disease virus). Chapter 3.3.14. In: Manual of diagnostic tests and vaccines for terrestrial animals 2021. OIE, Paris.

Rahman MS (2007). Allicin and other functional active components in garlic: Health benefits and bioavailability. Int. J. Food Prop., 10(2): 245-268. https://doi.org/10.1080/10942910601113327

Rattanachaikunsopon P, Phumkhachorn P (2009). Shallot (*Allium ascalonicum* L.) oil: Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria. Afr. J. Microbiol. Res., 3(11): 747-750. https://doi.org/10.1271/bbb.80482

Saeed M, Alagawany M, Fazlani SA, Kalhoro SA, Naveed M, Ali N, Kifayat-ullah, Arain MA, Chao S (2019). Health promoting and pharmaceutical potential of ferulic acid for the poultry industry. World's Poult. Sci. J., 75(1): 83-92. https://doi.org/10.1017/S0043933918000740

Saeid JM, Mohamed AB, Al-Baddy MA (2013). Effect of adding garlic powder (*Allium sativum*) and black seed (*Nigella sativa*) in feed on broiler growth performance and intestinal wall structure. J. Nat. Sci., 3(1): 35-41.

Tada M, Hiroe Y, Kiyohara S, Suzuki S (1988). Nematicidal and antimicrobial constituents from *Allium grayi* Regel and *Allium fistulosum* L. var. *caespitosum*. Agric. Biol. Chem., 52(9): 2383-2385. https://doi.org/10.1271/bbb1961.52.2383

Țigu AB, Moldovan CS, Toma VA, Farcaș AD, Moț AC, Jurj A, Fischer-Fodor E, Mircea C, Pârvu M (2021). Phytochemical analysis and *in vitro* effects of *Allium fistulosum* L. and *Allium sativum* L. extracts on human normal and tumor cell lines: A comparative study. Molecules, 26(3): 574. https://doi.org/10.3390/molecules26030574

Ueda H, Takeuchi A, Wako T (2013). Activation of immune responses in mice by an oral administration of bunching onion (*Allium fistulosum*) mucus. Biosci. Biotechnol. Biochem., 77(9): 1809-1813. https://doi.org/10.1271/bbb.130084

Van Boven M, Bouma A, Fabri TH, Katsma E, Hartog L, Koch G (2008). Herd immunity to Newcastle disease virus in poultry by vaccination. Avian Pathol., 37(1): 1-5. https://doi.org/10.1080/03079450701772391

Villegas P (2008). Titration of biological suspensions. In: Dufour-Zavala, L. (Ed), A laboratory manual for the isolation and identification of avian pathogens. Athens: The American Association of Avian Pathologists. pp. 217-221.

Vlase L, Parvu M, Parvu EA, Toiu A (2013). Phytochemical analysis of *Allium fistulosum* L. and *A. ursinum* L. Dig. J. Nanomater. Biostruct., 8(1): 457-467.

Yaxing Z, Erdene K, Changjin A, Zhibi B, Hongxi D, Zejun F, Umair A, Chen B (2021). Effects of *Allium mongolicum* Regel and its extracts supplementation on the growth performance, carcass parameters and meat quality of sheep. Ital. J. Anim. Sci., 20(1): 1899-1908. https://doi.org/10.1080/1828051X.2021.1971572

Yun J, Lee H, Ko HJ, Woo ER, Lee DG (2015). Fungicidal effect of isoquercitrin via inducing membrane disturbance. Biochim. Biophys. Acta Biomembr., 1848(2): 695-701. https://doi.org/10.1016/j.bbamem.2014.11.019