EDITORIAL BOARD OF THE JOURNAL OF AGRICULTURE AND DEVELOPMENT

EDITORIAL SECRETARIAT

Contact information:
Nong Lam University
Room 404, Thien Ly Building
Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
Tel: (84-28)37245670
Email: jad@hcmuaf.edu.vn

The Journal of Agriculture and Development
Volume 21 - Issue 6 (2022)
pISSN 2615-9503
eISSN 2615-949X

CONTENT

Effects of nitrogen and potassium rates on growth and yield of red turmeric (*Curcuma longa* L.) on the gray soil in Ho Chi Minh City

Trang T. H. Nguyen[1*], Binh V. Tran[1], Tri D. Q. Phan[1], Linh D. Dinh[1], Son T. T. Le[1], Thao X. Nguyen[1], Quang T. Le[2], Truong V. Nguyen[3], & Thinh V. Tran[1]

[1]Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Forest Science Institute of South Vietnam, Ho Chi Minh City, Vietnam
[3]Department of Plant Bioscience, Life and Industry Convergence Research Institute, Pusan National University, Miryang, South Korea

ARTICLE INFO

Research Paper

Received: June 23, 2022
Revised: July 18, 2022
Accepted: July 19, 2022

Keywords

Cow manure
Gray soil
Nitrogen
Potassium
Red turmeric

[*]**Corresponding author**

Nguyen Thi Huyen Trang
Email:
nthtrang@hcmuaf.edu.vn

ABSTRACT

The objective of this study was to determine the appropriate rates of nitrogen and potassium fertilizers for growth, yield and economic efficiency of red turmeric cultivated on the gray soil in Ho Chi Minh City. The field experiment was conducted at the Agronomy Research Station in Nong Lam University, Ho Chi Minh City from December 2020 to October 2021. The experiment was laid out in a split-plot design with three replicates. The main plots included four nitrogen rates 60, 90 (control), 120 and 150 kg N/ha. The subplots included four potassium rates 90, 120 (control), 150, and 180 kg K_2O/ha. All treatments were basally applied with 500 kg lime, 10 tons cow manure and 60 kg P_2O_5/ha. The results showed that growth attributes and yield were significantly affected by the rates of nitrogen and potassium. Red turmeric applied with 150 kg N/ha combined with 180 kg K_2O/ha obtained the outstanding results in growth, yield and profit, such as the plant height of 43.0 cm, stem diameter of 19.7 mm, a number of leaves of 8.6 (180 DAP), soil plant analysis development index of 42.1 (120 DAP), actual fresh yield of 33.9 tons/ha, the profit of VND 370.17 million/ha and the benefit-cost ratio of 2.68.

Cited as: Nguyen, T. T. H., Tran, B. V., Phan, T. D. Q., Dinh, L. D., Le, S. T. T., Nguyen, T. X., Le, Q. T., Nguyen, T. V., & Tran, T. V. (2022). Effects of nitrogen and potassium rates on growth and yield of red turmeric (*Curcuma longa* L.) on the gray soil in Ho Chi Minh City. *The Journal of Agriculture and Development* 21(6), 1-9.

1. Introduction

In Vietnam, red turmeric (*Curcuma longa* L.) is popularly grown in various types of soil. Turmeric rhizomes are often harvested and used as a spice, cosmetic, and medical plant based on traditional methods (Do, 2004). Besides, turmeric is a horticultural crop demanding heavy fertilization for increasing yield and quality (Yamgar et al., 2001).

Nitrogen (N) plays an important role in accumulating dry matter, which enables to increase the yield and quality of turmeric rhizomes. Besides, N is involved in chlorophyll formation, and its influences stomatal conductance and photosynthetic efficiency (Marschner, 2002). Potassium (K) plays catalytic roles in the plant rather becoming an integral part of plant components. Plants with an inadequate supply of K show the poor fruit or seed formation, yellowing of the leaves, poor growth, and low resistance to coldness and drought. A supply of K promotes N up-

take efficiency of plants due to its stimulant effect on plant growth (Oya, 1972).

Although potassium is not an element that engages in any transfer processing, it regulates the permeability of cell wall and activities of various mineral elements as well as neutralizes physiologically important organic acids (Marschner, 2002; Akamine et al., 2007). Therefore, combining nitrogen and potassium in growing red turmeric can enhance the yield of rhizome and the quality of plants.

In term of cultivation, proper fertilizer application is a crucial method that affects not only vegetative parameters, and yield but also economic factors. Hence, enhancing efficiency of red turmeric production on gray soil by combining between organic fertilizers and inorganic ones is necessary (Rao et al., 1975; Gopalakrishna et al., 1997).

Regarding the necessary in practical production in local fields of the research "Effects of nitrogen and potassium rates on growth and yield of red turmeric (*Curcuma longa* L.) on the gray soil in Ho Chi Minh City" was conducted.

2. Materials and Methods

2.1. Time and location

The field experiment was conducted at the Agronomy Research Station in Nong Lam University, Ho Chi Minh City, Vietnam from December 2020 to October 2021.

2.2. Planting material

The red turmeric tuberous roots were collected from a farm in Bu Gia Map district, Binh Phuoc province, Vietnam. Red turmeric rhizomes were then treated with 0.5% chlorine for 30 min before dried and incubated for 2 weeks. When the red turmeric seedlings reached 10 - 15 cm in height and were produced with 1 - 3 leaves, each plant was separated before growing (Mai et al., 2000).

2.3. Fertilizers

Fertilizers used in the experiment included cow manure (1% N, 2% P_2O_5, 1% K_2O); Phu My urea (46.3% N); Lam Thao superphosphate (16% P_2O_5, 10% S, 12 mg Cd/kg); Canadian potassium chloride (61% K_2O).

Before the establishment of the experiment in December 2020, the soil sample from surface (0 - 20 cm depth) was taken about 0.5 Kg to determine soil texture, pH_{KCl} of the soil, the soil organic matter content, total nitrogen, phosphorus and potassium in the soil. The properties of the initial soil are given in Table 1. The data showed that the soil is classified as clay texture (USDA, 1960), moderately acidic, high in soil organic matter, and low in total nitrogen but high in total phophorus and potassium (Slavich & Petterson, 1993; Rayment & Lyons, 2011).

2.4. Experimental design

Two-factor experiment was laid out in split-plot design (SPD) with three replicates. The main plots included four nitrogen rates 60, 90 (control), 120, and 150 kg N/ha. The subplots included four potassium rates 90, 120 (control), 150, and 180 kg K_2O/ha. All treatments were applied basally with the same rate of 500 kg lime, 10 tons cow manure and 60 kg P_2O_5/ha.

Entire amount of cow manure, lime and phosphorus were applied in respective plots as per treatment during the final soil preparation. The amount of nitrogen and potassium were split into three installments: $\frac{2}{4}$ N + $\frac{1}{4}$ K_2O (30 days after planting - hereafter referred to as DAP), $\frac{1}{4}$ N + $\frac{1}{4}$ K_2O (90 DAP), $\frac{1}{4}$ N + $\frac{2}{4}$ K_2O (150 DAP).

The plot size was 6.3 m^2 (4.5 m in length, 1.4 m in width). The health primary rhizomes of red turmeric were planted at the distance of 35 cm × 25 cm. The spacing between blocks and plots were 1.0 and 0.5, respectively.

2.5. Data collection

Growth attributes were collected at 180 DAP, while biomass (leaves, stem, roots, rhizomes), yield and yield component data were collected at 270 DAP where plant leaves started by drying and withering. Growth attributes such as plant height, stem diameter, number of leaves per plant and soil plant analysis development (SPAD) index were recorded from ten randomly selected plants from middle rows. At 270 DAP, red turmeric was removed from the field, washed free of sand and then separated at the crown into three parts: aboveground (leaves and stem), roots and rhizomes. Once separated, aboveground, roots and rhizomes were washed, dried and weighed (g/plant). The dry biomass data

Table 1. Physical and chemical properties of soil sample used in the experiment

Texture (%)			$pH_{1:5}$	Organic	Total N	Total P_2O_5	Total K_2O
Clay	Silt	Sand	(KCl)	matter (%)	(%)	(%)	(%)
5.3	9.3	85.4	4.85	1.71	0.07	0.06	0.06

Soil samples were analyzed at the Forestry Science Institute of Southern Vietnam, 2021.

was determined after weighing separately fresh biomass data. Actual fresh rhizome yield was calculated from fresh rhizome weight (g per net plot area) and converted onto an ha basis, and then the data were expressed as tons/ha. Economic efficiency including total cost, total revenue, profit and benefit cost ratio (BCR) were computed.

2.6. Statistical analysis

All variables were subjected to analysis of variance as a SPD using R software (R 4.1.0 GUI 1.76, 2021). Differences between treatments were tested using the least significance difference (LSD) test at the probability of 0.05.

3. Results and Discussions

3.1. Growth attributes

The analysis of variance indicated that the growth attributes such as plant height, stem diameter, number of leaves per plant were all significantly ($P < 0.05$) influenced by N and K rates. As shown in Table 2, a non-significant nitrogen × potassium interaction was also observed for all growth attributes, except stem diameter.

The general trend showed that increasing N rate from 60 to 150 kg N/ha increases the growth of the red turmeric plant in this study (Table 2). At 180 DAP, the highest plant height (43.8 cm) was recorded at treatment of 150 kg N/ha application for red turmeric, while the lowest value of plant height was recorded at the rate of 60 kg N/ha. Stem diameter varied in N rate where the largest stem diameter (18.5 mm) was found from plants which received 150 kg N/ha, whereas the smallest value of stem diameter (15.8 mm) was recorded at 60 kg N/ha.

Similar results were also observed in the number of leaves per plant where the greatest number of leaves per plant was also produced when 150 kg N/ha was applied for red turmeric, followed by 120 and 90 kg N/ha, and the lowest number of leaves per plant was recorded at 60 kg N/ha in Table 2.

An increase in red turmeric vegetative growth attributes such as plant height, stem diameter and a number of leaves per plant in treatments receiving higher N rates in current study was consistent with the results reported by Mekonnen & Garedew (2019). This trend could be probably due to its marked influence on the capacity of plants to absorb and utilize optimum amount of N in build up of plant tissue and vegetative growth (Leva et al., 2013). It can also attribute to the rapid conversion of synthesized carbohydrates into protein, which increases in number and size of growing cells, resulting ultimately in increased overall growth (Singh et al., 2001). Similar result has also been reported by Thomas & Utietiang (2019) who indicated that the maximum growth parameters was obtained with the application of N up to 200 kg/ha on turmeric.

Regarding K rates, plant height and stem diameter were significantly affected by K rates. In general, increasing K rate from 90 to 180 kg K_2O/ha decreased plant height, but increased stem diameter and the number of leaves in this study (Table 2). The highest plant height (44.8 cm) was obtained when 90 kg K_2O/ha was applied for red turmeric, and significantly different from other treatments. In contrast, the lowest plant height (42.5 cm) was obtained at the rate of 180 kg K_2O/ha.

A different trend was observed in stem diameter and the number of leaves. The largest stem diameter (18.4 mm) was recorded when 180 kg K_2O/ha was applied for red turmeric, followed by 150 and 120 kg K_2O/ha, and the smallest stem diameter (16.0 mm) was recorded at 90 kg K_2O/ha. A similar trend was also observed in the number of leaves per plant, but there was not significantly different in the number of leaves per plant among K treatments (Table 2).

A significant interaction between N and K was found in stem diameter, particularly the largest one (19.7 mm) recorded when applying a combination of 150 kg N/ha and 180 kg K_2O/ha (Table 2). This was possibly explained that a sufficient supply of K promotes N uptake efficiency of plants due to its stimulant effect on plant growth

Table 2. Effect of N and K rates on plant height, stem diameter and number of leaves of red turmeric at 180 days after planting

Parameters	K rate (kg K_2O/ha) (K)	N rate (kg N/ha) (N)				Average (K)
		60	90[(1)]	120	150	
Plant height (cm)	90	44.0	44.7	45.3	45.3	44.8^{a}
	120[(2)]	43.0	43.3	43.7	43.7	43.4^{b}
	150	42.0	42.7	42.7	43.0	42.6^{c}
	180	42.0	42.3	42.7	43.0	42.5^{c}
	Average (N)	42.8^{c}	43.3^{b}	43.6^{a}	43.8^{a}	
	CV (%) = 0.8	$F_N = 119.1^{**}$	$F_K = 9.6^{**}$	$F_{N*K} = 0.5^{ns}$		
Stem diameter (mm)	90	15.4^{c}	15.7^{c}	16.1^{c}	16.7b[c]	16.0^{c}
	120	15.5^{c}	17.0^{abc}	16.2^{c}	17.8^{abc}	16.7^{c}
	150	15.6^{c}	17.1^{abc}	17.9^{abc}	19.1^{ab}	17.6^{b}
	180	16.7^{bc}	17.6^{abc}	19.6^{a}	19.7^{a}	18.5^{a}
	Average (N)	15.8^{c}	16.8^{b}	17.7^{a}	18.5^{a}	
	CV (%) = 8.5	$F_N = 89.3^{**}$	$F_K = 16.3^{**}$	$F_{N*K} = 3.2^{*}$		
Number of leaves (leaves/ plant)	90	7.7	7.9	7.9	8.2	7.9
	120	7.8	8.0	8.2	8.3	8.1
	150	8.1	8.2	8.3	8.4	8.3
	180	8.2	8.3	8.3	8.6	8.4
	Average (N)	8.0^{c}	8.1^{b}	8.2^{b}	8.4^{a}	
	CV (%) = 4.0	$F_N = 5.9^{*}$	$F_K = 0.7^{ns}$	$F_{N*K} = 0.7^{ns}$		

Within a group of means, values followed by the same letter are not significantly different at 5% level; **: significant at 1% level; *: significant at 5% level; ^{ns}: non significant; [(1)] 90 kg N/ha (control), [(2)] 120 kg K_2O/ha (control).

(Oya, 1972).

3.2. SPAD index

SPAD index, which could be converted to chlorophyll content of plant was only significantly influenced by N rates in this study (Table 3). The general trend indicated that SPAD index of the red turmeric increases with increasing N rate from 60 to 150 kg N/ha. At 60 DAP, the highest SPAD (37.5) was recorded when 150 kg N/ha was applied for red turmeric, whereas the lowest value of SPAD (36.1) was recorded at 60 kg N/ha. Similar result was also recorded at 120 DAP. However, SPAD index has not been affected by different rates of K application as well as the combination of N and K treatments. This could be explained on the basis of the physiological fact that N plays an essential role in chlorophyll formation, it influences stomatal conductance and photosynthetic efficiency. The decreases in growth with reduced N rates could be the reason for a decline in the net photosynthesis. In addition, it was evident that the plants grown without or less N application withered earlier resulting in a poorer vegetative growth. N deficiency results in lower chlorophyll in leaves which ultimately causes earlier plant death (Sarker et al., 2002).

3.3. Yield components and yield

The results revealed that both of application N and K were significantly influenced to fresh weight of aboveground, roots and rhizomes per plant of red turmeric at 270 DAP, however the experiment also revealed that there was not interacted between N and K treatments for all above measurements.

In general, an increasing N or K rates increased the fresh weight of aboveground, roots and rhizomes per plant of red turmeric in current study (Table 4). At 270 DAP, the highest fresh weight of aboveground biomass per plant (2091.0 g) was recorded when 150 kg N/ha was applied for red turmeric, while the lowest value was recorded at the rate of 60 kg N/ha. Similarly, a highest fresh weight of roots and rhizomes per plant was obtained from the treatment which had received 150 kg N/ha with the values of 304.3 mg and 406.3 g, respectively, followed by the application of 120, 90 and 60 kg N/ha. This result clearly indicated that the increase fresh biomass and rhizomes due to N application could be ascribed to their roles in growth and tissue differentiation (Marschner,

Table 3. Effects of N and K rates on soil plant analysis development (SPAD) index of red turmeric at 60 and 120 days after planting

Days after planting	K rate (kg K_2O/ha) (K)	N rate (kg N/ha) (N)				Average (K)
		60	90[(1)]	120	150	
60	90	35.5	36.0	36.3	36.5	36.1
	120[(2)]	35.5	36.8	36.9	37.6	36.7
	150	36.6	36.9	37.4	37.7	37.2
	180	36.7	37.1	37.8	38.1	37.4
	Average (N)	36.1^{c}	36.7^{b}	37.1^{a}	37.5^{a}	
	CV (%) = 13.3	$F_N = 1.8^{**}$	$F_K = 0.6^{ns}$	$F_{N*K} = 1.1^{ns}$		
120	90	39.5	40.3	40.5	41.1	40.4
	120	39.9	40.7	40.7	42.1	40.9
	150	40.6	41.1	41.5	42.1	41.3
	180	41.0	41.9	41.9	42.1	41.7
	Average (N)	40.3^{c}	41.0^{b}	41.2^{a}	41.9^{a}	
	CV (%) = 13.2	$F_N = 3.1^{**}$	$F_K = 1.4^{ns}$	$F_{N*K} = 1.0^{ns}$		

Within a group of means, values followed by the same letter are not significantly different at 5% level; **: significant at 1% level; ^{ns}: non significant; $^{(1)}$ 90 kg N/ha (control), $^{(2)}$ 120 kg K_2O/ha (control).

2002). It can be also explained on the basis of the physiological fact that N, which is the principal nutrient of plant significantly increased vegetative growth attributes of turmeric comparison with any other nutrients (Behura, 2001).

The decreases in growth with reduced N rates could be the reason for a decline in growth attributes and the number of rhizomes on the treatment which reveived the lowest N rate as 60 kg N/ha, for example in this study. With such reduced growth components, net photosynthesis would be lower so that it was difficult for the plant to supply adequate amounts of substrate to the sinks, i.e., the rhizomes. In contrast, the plants applied with increasing N stimulated vegetative growth and remained green for longer, which contributed to longer photosynthesis and resulted in a higher biomass.

Similarly to N rates, the fresh weight of aboveground, roots and rhizomes per plant of red turmeric were significantly affected by K rates (Table 4). The highest fresh weight of aboveground biomass (2398.7 g per plant) was obtained at the treatment of 180 kg K_2O/ha for red turmeric, and significantly different from other treatments. Similarly, the highest fresh weight of roots per plant was recorded at the rate of 180 kg K_2O/ha whilst the lowest fresh weight of that was recorded from the treatment of 90 kg K_2O/ha application. Similarly, results were also recorded in the fresh weight of rhizomes (Table 4). A non-significant nitrogen × potassium interaction was also observed in fresh weight of aboveground, roots and rhizomes, but the maximum biomass attributes was always obtained when 150 kg N/ha was applied in combination with 180 kg K_2O/ha for red turmeric (Table 4).

Both the main effects of N and K rates had a significant effect ($P < 0.05$) on the dry weight of aboveground, roots and rhizomes of red turmeric, but there was non-significant nitrogen × potassium interaction for dry biomass attributes (Table 5).

Aboveground dry weight increased with increasing N rates from 60 to 150 kg N/ha. The red turmeric plants which were applied with 150 kg N/ha achieved the highest aboveground dry weight, whereas the lowest aboveground dry weight was recorded from the treatment which received 60 kg N/ha. Similarly, the maximum dry weight of roots and rhizomes per plant was also obtained at the rate of 150 kg N/ha with the respective values of 46.6 mg and 62.4 g, while the minimum dry weight of those values was always obtained at the rate of 60 kg N/ha (Table 5).

In addition, dry biomass attributes increased with increasing K rates from 90 to 180 kg K_2O/ha where aboveground dry weight increased by 137.6 g, root dry weight by 19.7 mg and rhizome dry weight by 12.8 g. The maximum weight of dry aboveground was recorded when 180 kg K_2O/ha was applied, followed by 150 and 120 kg K_2O/ha. The minimum weight of dry aboveground was recorded from the treatment which had received 90 kg K_2O/ha. Similar results were also observed in the dry weight of roots and rhizomes of red

Table 4. Effects of N and K rates on fresh weight of aboveground, roots and rhizomes per plant of red turmeric at 270 days after planting

Parameters	K rate (kg K_2O/ha) (K)	N rate (kg N/ha) (N)				Average (K)
		60	90[(1)]	120	150	
Above-ground fresh weight (g)	90	1458.3	1604.3	1646.7	1743.3	1613.2^{c}
	120[(2)]	1540.7	1679.7	1690.7	1916.7	1707.0^{c}
	150	1679.7	1699.7	1843.0	2014.7	1809.3^{b}
	180	2120.0	2348.7	2436.7	2689.3	2398.7^{a}
	Average (N)	1699.7^{c}	1833.1^{b}	1904.3^{b}	2091.0^{a}	
	CV (%) = 18.2	$F_N = 9.0^{*}$	$F_K = 0.07^{*}$	$F_{N*K} = 0.8^{ns}$		
Root fresh weight (mg)	90	164.7	168.7	187.3	206.0	181.7^{c}
	120	216.7	253.3	278.0	290.3	259.6^{b}
	150	250.0	261.0	290.7	320.0	280.4^{b}
	180	266.3	287.0	314.0	401.0	317.1^{a}
	Average (N)	224.4^{c}	242.5^{bc}	267.5^{b}	304.3^{a}	
	CV (%) = 24.9	$F_N = 3.4^{*}$	$F_K = 1.4^{*}$	$F_{N*K} = 0.9^{ns}$		
Rhizome fresh weight (g)	90	256.8	299.9	332.5	386.1	318.8^{c}
	120	308.1	346.7	360.5	406.4	355.4^{b}
	150	314.7	346.8	375.8	406.4	360.9^{ab}
	180	326.1	351.0	381.2	426.4	371.2^{a}
	Average (N)	301.4^{c}	336.1^{b}	355.0^{b}	406.3^{a}	
	CV (%) = 14.1	$F_N = 3.2^{*}$	$F_K = 1.9^{*}$	$F_{N*K} = 0.5^{ns}$		

Within a group of means, values followed by the same letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant; (1) 90 kg N/ha (control), (2) 120 kg K_2O/ha (control).

Table 5. Effects of N and K rates on dry weight of aboveground, roots and rhizomes per plant of red turmeric at 270 days after planting

Parameters	K rate (kg K_2O/ha) (K)	N rate (kg N/ha) (N)				Average (K)
		60	90[(1)]	120	150	
Above-ground dry weight (g)	90	213.3	247.3	255.7	288.0	251.1^{c}
	120[(2)]	245.0	254.0	290.0	292.0	270.3^{bc}
	150	253.0	291.0	291.0	330.3	291.3^{b}
	180	335.7	369.0	384.3	466.3	388.7^{a}
	Average (N)	261.8^{c}	290.3^{b}	305.3^{b}	344.2^{a}	
	CV (%) = 25.5	$F_N = 11.6^{**}$	$F_K = 0.08^{*}$	$F_{N*K} = 1.0^{ns}$		
Root dry weight (mg)	90	23.7	25.7	29.7	33.7	28.2^{c}
	120	34.0	39.3	41.7	46.0	40.3^{b}
	150	35.7	42.0	43.0	49.3	42.5^{b}
	180	42.0	44.3	48.0	57.3	47.9^{a}
	Average (N)	33.9^{c}	37.8^{b}	40.6^{b}	46.6^{a}	
	CV (%) = 22.6	$F_N = 3.4^{*}$	$F_K = 1.2^{*}$	$F_{N*K} = 0.5^{ns}$		
Rhizome dry weight (g)	90	36.5	48.4	48.7	56.4	47.5^{d}
	120	46.2	50.6	52.9	60.0	52.4^{c}
	150	46.3	55.3	59.8	65.7	56.8^{b}
	180	53.0	57.4	63.1	67.5	60.3^{a}
	Average (N)	45.5^{c}	52.9^{b}	56.1^{b}	62.4^{a}	
	CV (%) = 18.7	$F_N = 2.6^{*}$	$F_K = 2.6^{*}$	$F_{N*K} = 0.8^{ns}$		

Within a group of means, values followed by the same letter are not significantly different at 5% level;**: significant at 1% level; *: significant at 5% level; ns: non significant; (1) 90 kg N/ha (control), (2) 120 kg K_2O/ha (control).

Table 6. Effects of N and K rates on actual fresh yield (tons/ha) of red turmeric

K rate (kg K_2O/ha) (K)	N rate (kg N/ha) (N)				Average (K)
	60	90[(1)]	120	150	
90	20.4	23.8	24.3	26.4	23.7^{c}
120[(2)]	24.5	27.5	28.6	30.6	27.8^{c}
150	25.0	27.5	29.8	32.3	28.7^{b}
180	25.9	27.9	30.3	33.8	29.5^{a}
Average (N)	24.0^{d}	26.7^{c}	28.3^{b}	30.8^{a}	
CV (%) = 19.6	$F_N = 18.9^{*}$	$F_K = 17.0^{*}$	$F_{N*K} = 6.0^{ns}$		

Within a group of means, values followed by the same letter are not significantly different at 5% level; *: significant at 5% level; ns: non significant; (1) 90 kg N/ha (control), (2) 120 kg K_2O/ha (control).

Table 7. Economic efficiency of red turmeric at the different rates of N and K

N rate (kg N/ha)	K rate (kg K_2O/ha)	Actual fresh yield (tons/ha)	(million VND/ha)			BCR
			Total revenue	Total cost	Profit	
60	90	21.3	319.50	136.53	182.97	1.34
	120[(2)]	21.8	327.00	136.86	190.14	1.39
	150	22.1	331.50	137.19	194.31	1.426
	180	22.5	337.50	137.52	199.98	1.45
90[(1)]	90	22.2	333.00	136.80	196.20	1.43
	120[(2)]	23.0	345.00	137.13	207.87	1.52
	150	23.9	358.50	137.46	221.04	1.61
	180	24.8	372.00	137.79	234.21	1.70
120	90	23.4	350.55	137.07	213.48	1.56
	120[(2)]	24.3	364.50	137.40	227.10	1.65
	150	25.3	379.50	137.73	241.77	1.76
	180	32.3	484.50	138.06	346.44	2.51
150	90	24.3	364.50	137.34	227.16	1.65
	120[(2)]	24.4	366.00	137.67	228.33	1.66
	150	26.8	402.00	138.00	264.00	1.91
	180	33.9	508.50	138.33	370.17	2.68

(1) 90 kg N/ha (control), (2) 120 kg K_2O/ha (control).

turmeric (Table 5).

Actual fresh yield of red turmeric in responses to N and K rates was shown in Table 6. The general trend showed that increasing N or K rates increases the yield of red turmeric in current study. The highest actual fresh yield was recorded when 150 kg N/ha was added while the lowest value was recorded at the rate of 60 kg N/ha. Further, potassium application of the rate of 180 kg K_2O/ha produced the highest actual fresh yield (28.3 tons/ha) and significantly different from other treatments (Table 6).

Although there was a non significant interaction between N and K rates, the maximum actual fresh yield (33.9 tons/ha) was obtained at the combination of 150 kg N/ha and 180 kg K_2O/ha (Table 6). The greater rhizome yield from high rates of N application could be associated with more luxuriant growth parameters and the higher supply of substrate which helped in producing larger rhizomes, consequently resulting in higher yields (Imas et al., 1999). The greater yields at higher N rates may also be due to increased stem diameter and the weight of rhizomes per plant, which might result from an increase in the number of leaves per plant.

3.4. Economic efficiency of red turmeric

The results showed that red turmeric plants were treated at 150 kg N/ha combined with 180 kg K_2O/ha achieved the highest revenue of VND 508.5 million/ha, the highest profit of VND 370.17 million/ha and the highest cost benefit ratio of 2.68 (Table 7).

4. Conclusions

The study clearly showed that the higher application rates of nitrogen and potassium fertilizers increased on the growth attributes and yield of turmeric plant on the gray infertile soil in Ho Chi Minh City. Co-application of 150 kg N/ha with 180 kg K_2O/ha obtained the outstanding results in the growth and yield of red turmeric. The maximum yield and yield components of red turmeric were also obtained in this treatment such as plant height of 43.0 cm, stem diameter of 19.7 mm, a number of leaves of 8.6 (180 DAP), SPAD-index of 42.1 (120 DAP), actual fresh yield of 33.9 tons/ha, the profit of VND 370.17 million/ha and the benefit cost ratio of 2.68. Therefore, an application of 150 kg N/ha in combination with 180 kg K_2O/ha can be suggested for the farmers in this area to maximize rhizome yield. Further research should be conducted in the future to determine the optimum doses of nitrogen and potasium to achieve maximum yield and economic of red turmeric crops.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The author would like to express their deep gratitude to Nong Lam University, Ho Chi Minh City, Vietnam for funding this study. These works were supported by the student belonging to the Faculty of Agronomy and staff officers.

References

Akamine, H., Hossain, A., Ishimine, Y., Yogi, K., Hokama, K., Iraha, Y., & Aniya, Y. (2007). Effects of application of N, P and K alone or in combination on growth, yield and curcumin content of turmeric (Curcuma longa L.). Plant Production Science 10(1),151-154. https://doi.org/10.1626/pps.10.151.

Behura, S. (2001). Effect of nitrogen and potassium on growth parameters and rhizomatic characters of mango-ginger *(Curcuma amada). Indian Journal Agronomy* 46(4), 747-751.

Do, L. T. (2004). *Vietnamese medicinal plants and herbs*. Ha Noi, Vietnam: Medical Publishing House.

Gopalakrishna, V., Reddy, M. S., & Kumar, T. V. (1997). Response of turmeric to FYM and N fertilization. *Journal of Research ANGRAU* 25(3), 58-59.

Imas, P., Bansal, S. K., Khurana, S., Shekhawat, G. S., & Pandey, P. (1999). Potassium and integrated nutrient management in potato. In Paul Khurana, S. M. (Ed.). *Potato, global research and development*, (744-754). Shimla, India: Indian Potato Association.

Leva, R., Thanki, J. D., Patel, D. D., & Patel T. (2013). Growth and yield of turmeric (*Curcuma longa* L.) as influenced by planting methods and fertigation under vertisols of South Gujarat condition. *Research on Crops* 14(3), 964-967.

Mai, Q. V., Le, N. T. V., Ngo, V. Q., Nguyen, H. T., & Nguyen, K. T. (2000). *Popular herbs and spices in Vietnam*. Ho Chi Minh City, Vietnam: Agricultural Publishing House.

Marschner, H. (2002). *Mineral nutrition of higher plants* (2nd ed.). New York, USA: Academic Press Inc.

Mekonnen, B., & Garedew, W. (2019). Growth, yield and responses of turmeric (*Curcuma longa* L.) to nitrogen fertilizer rate and timing of its application. *Journal of Acta Agrobotanica* 72(3), 1-11. https://doi.org/10.5586/aa.1781.

Oya, K. (1972). *Evaluation of potassium availability of four Michigan soils* (Unpublished doctoral dissertation). Michigan State University, Michigan, USA.

Rao, M. R., Reddy, K. R. C., & Subbarayudu, M. (1975). Promising turmeric types of Andhra Pradesh. *Arecanut and Spices Bulletin* 2(2), 2-5.

Rayment, G. E., & Lyons, D. J. (2011). *Soil chemical methods – Australasia*. Melbourne, Australia: CSIRO publishing.

Sarker, M. A. Z., Murayama, S., Akemine, H., & Nakamura, I. (2002). Effect of nitrogen fertilization on photosynthetic characters and dry matter production in F1 hybrids of rice (*Oryza sativa* L.). *Plant Production Science* 5(2), 131-138. https://doi.org/10.1626/pps.5.131.

Singh, P., Srivastava, R., Sharma, A., Hore, D., & Panwar, B. (2001). Genetic variability and correlation in turmeric (*Curcuma longa* L.). *Indian Journal of Hill Farming* 14, 24-28.

Slavich, P. G., & Petterson, G. H. (1993). Estimating the critical conductivity of saturated paste extracts from 1:5 soil, water suspensions and texture. *Australian Journal of Soil Research* 31(1), 73-81. https://doi.org/10.1071/SR9930073.

Thomas, O. O., & Utietiang, L. U. (2019). Growth, yield and quality of turmeric (*Curcuma longa* Linn.) as influenced by nitrogen rates and nitrogen split application in Obubra, South-South China. *European Journal of Agriculture and Forestry Research* 7(4), 1-13.

USDA (United States Department of Agriculture). (1960). *Soil classification, a comprehensive system*. Washington DC, USA: Government Printing Office.

Yamgar, V. T., Kathmale, D. K., Belhekar, P. S., Patil, R. C., & Paul, P. S. (2001). Effect of different levels of nitrogen, phosphorus and potassium and split application of N on growth and yield of turmeric (*Curcuma longa* L.). *Indian Journal of Agronomy* 46(2), 372-374.

Effects of post-hatch feeding time and pre-starter feeds on growth performance and relative weight of visceral organs in slow-growing chickens

Phung T. K. Bui*, Tran P. U. Cao, Khang N. Duong, & Tung M. Che
Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: December 08, 2021
Revised: November 24, 2022
Accepted: November 30, 2022

Keywords

Commercial
Pre-starter
Slow-growing chickens
Vi-start

***Corresponding author**

Bui Thi Kim Phung
Email: phung.buithikim@hcmuaf.edu.vn

ABSTRACT

The objective of the experiment was to evaluate effects of post-hatch feeding time and two different pre-starter diets on growth performance and relative weight of visceral organs and yolk sac in slow-growing chickens. A total of 480 one-day-old chicks (Luong Phuong breed) were randomly assigned into 4 treatment groups in a completely randomized design of 2 × 2 factorial arrangement with 10 chicks per pen. The two factors consisted of post-hatch feeding time (immediate access to feed after hatching (0 h) and delayed access to feed for 30 h after hatching) and pre-starters (Vi-start and Commercial 1). Birds were fed 2 different pre-starter diets from 0 to 7 days of age, and then all birds were fed the same commercial diets from 8 to 56 days of age. The results showed that during 0 - 7 days of age, chicks that were not fed for 30 h after hatch were significantly lower in body weight, average daily feed intake, average daily gain and feed efficiency (FE) than those fed immediately right after hatch ($P < 0.05$). In this period, chicks fed Vi-start had better growth performance and FE than those fed Commercial 1. Over a 56-d study, there were no main effects of post-hatch feeding time or pre-starter feed on growth performance of chickens ($P > 0.05$). However, the post-hatch feeding time × pre-starter feed interaction was significant for final BW at 56 days of age ($P = 0.01$), suggesting that within commercial feed, delayed access to feed for 30 h increased the final BW of chickens as compared with immediate access to feed after hatch. In brief, Vi-start fed to chicks improved the growth performance of chicks during the first week after hatch. Feeding pre-starter feeds to chicks immediately right after hatch would be beneficial.

Cited as: Bui, P. T. K., Cao T. P. U., Duong, K. N., & Che, T. M. (2022). Effects of post-hatch feeding time and pre-starter feeds on growth performance and relative weight of visceral organs in slow-growing chickens. *The Journal of Agriculture and Development* 21(6), 10-16.

1. Introduction

In contrast to newborns of other species, the newly hatched chicks do not receive any direct nutritional support from their mothers. Particularly in commercial hatchery production, the hatching time could last from 24 to 48 h. Therefore, the first hatched chicks will not be exposed to feed and water for a long time. Recent studies have said that delaying chick using feed for 48 h can reduce the body weight of chickens at finished age (Gonzales et al., 2003; Bhanja et al., 2009; Abed et al., 2011). Moreover, the gastrointestinal tract of newly hatched chicks is immature and in a process of development. According to Nitsan (1991), the growth rate of the digestive system during the first week is three to five times faster than the rest of the chick's body. The markedly rapid development occurs in duodenum, colon and pancreas. Thus, after hatching, chicks have very high nutritional requirements to meet their potential growth and development of digestion and immune systems, help them achieve optimal productivity (Noy & Sklan, 2001). However, there is no evidence on effects of delayed feeding or pre-starter feeds on growth performance and intestinal mor-

phology of chickens, especially for slow-growing local breeds of chickens in Vietnam. Therefore, the objective of the experiment was to evaluate the effects of post-hatch feeding time and two different pre-starter diets on growth performance and relative weight of visceral organs and yolk sac in slow-growing chickens from 0 to 56 days of age.

2. Materials and method

2.1. Materials

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Nong Lam University of Ho Chi Minh City (NLU), Vietnam. The experiment was conducted at the Applied Research Farm of Department of Animal Production at NLU.

2.2. Experimental design

A total of 480 day-old unsexed chicks (Luong Phuong breed; initial BW: 42.36 ± 0.18 g/chick) were used in this experiment. Chicks were randomly assigned to each of four treatments in a 2 x 2 factorial arrangement (feeding time after hatching: immediate access to feed vs. delayed access to feed for 30 h; Pre-starter diet: Vi-start vs Commercial 1) in a completely randomized design. There were 12 replicate cages per treatment and 10 birds per cage. Birds were exposed to different pre-starters during 0-7 days of age (Vi-start, Commercial 1) and then all birds were fed the same commercial diets from 8 to 56 days of age (Commercial 2 and 3) (Table 1).

The Vi-start feed was obtained from the Department of Animal Production of Nong Lam University Ho Chi Minh City and Vi-Start diet was formulated and mixed at the Applied Research Farm located on the campus of Nong Lam University (Table 2). Vi-start's chemical composition (crude protein, crude fiber, phosphorous, calcium and ether extract) and other ingredients were analyzed at the Upscience company, Binh Duong province, Vietnam. The commercial feeds 1, 2, 3 were supplied by Cargill Feed Company, Dong Nai province, Vietnam. Birds were reared in multi-tiered cages (120 cm × 50 cm × 40 cm) in an open-sided house for eight weeks, and each cage had a feeder and a drinker.

Table 1. Nutrient contents of Vi-start and Commercial for different stages*

Constituents	Vi-start[1] (0-7 days)	Commercial 1[2] (0-7 days)	Commercial 2[3] (8-21 days)	Commercial 3[4] (22-56 days)
Dry matter, %	89.11	89.14	88.73	89.19
Protein, %	20.88	21.53	16.84	14.31
Crude fat, %	5.02	4.26	5.57	6.04
Crude fiber, %	0.70	2.59	3.20	4.43
Total mineral, %	4.42	5.40	5.16	4.89
Calcium, %	1.08	0.82	0.65	0.57
Phosphorus, %	0.49	0.62	0.56	0.51

(*)Chemical composition (crude protein, ether extract, crude fiber, phosphorous and calcium) of Vi-start and feed Commercial 1, 2, 3 which were used in this experiment were analyzed at the Upscience company, Binh Duong province, Vietnam

(1)Vi-start diet was formulated and mixed at the Applied Research Farm located on the campus of Nong Lam University, Vietnam

(2,3,4)Commercial feeds 1, 2 & 3 were supplied by Cargill Feed Company, Dong Nai province, Vietnam.

Table 2. Ingredient composition of Vi-start diet used in the experiment*

Feed Ingredients (%)	Vi-start
Corn	6.00
Broken rice	55.10
Soybean meal	14.60
Egg powder	17.00
MCP (15.23)	1.21
Limestone	1.90
Salt	0.20
Mineral premix	0.10
Vitamin premix	0.10
Phytase	0.02
Lysine, 78.8%	0.26
Dextrose	3.00
Antioxidants	0.10

*Vi-start diet was formulated and mixed at the Applied Research Farm located on the campus of Nong Lam University, Vietnam.

2.3. Data collection and sampling

After hatching, chicks were weighed and randomly allocated into treatment groups. Subsequent weights of chickens and feed consumption were recorded at 7, 28, 42 and 56 days of age. The average body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated on a per-cage basis. At 7 and 56 days old, 12 birds were randomly selected from each treatment for measurements of relative weight of visceral organs. Feed was withdrawn 10 h before slaughter, but birds were allowed free access to water. Right after slaughtering, abdominal cavity of each bird was opened for collection of visceral organs and then their weights were measured.

2.4. Statistical analysis

Data were analyzed as a completely randomized design with a 2 × 2 factorial treatment arrangement by ANOVA using the general linear model (GLM) procedure of SAS (SAS/STAT Version 9.2, SAS Institute Inc., Cary, NC) to determine the effects of post-hatch feeding time and two different pre-starter diets and their interaction. Treatment effects were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Average body weight, average daily body weight gain, average daily feed intake, feed conversion ratio

The chickens' weight increased gradually, consistent with the development stages of poultry (Table 3). At 7 days of age, BW of birds fed right after hatching was significantly higher than that of birds held 30 h and birds fed Vi-start pre-starter which weighs more than that fed Commercial 1, corresponding (9.73%, 5.08%, respectively). However, there was no statistically significant effects of between two post-hatch feeding time or two pre-starter feeds on BW of chickens at 56 days old although their interaction was found ($P < 0.05$). Chickens fed Commercial 1 pre-starter later post-hatching (30 h) were heavier than that exposed the same pre-starter at 0 h ($P < 0.05$) whereas with Vi-start × 0 h, BW of birds was equivalent with Commercial 1×30 h treatment ($P > 0.05$). This result showed that, if the chickens were fed early, they should choose the right pre-starter feed and the nutrient of Vi-start was suitable for early and late feeding.

From the results of Table 4, at stage 1 - 7 days of age; the ADG, ADFI and FCR of birds fed Vi-start were significantly better ($P < 0.01$) than that of birds fed the Commercial 1 diet. Although average daily feed intake was low, daily weight gain of chicken in Vi-start was better than in Commercial 1, so the ability metabolize of feed was quite good, proving that the Vi-start with high nutritional would promote early stimulation of intestinal development.

The results indicated that some indicators (ADG, ADFI) have increase trend when comparing with bird feeding after 30 h hatching with $P < 0.001$ (Table 4). This is consistent with previous studies, in the early stage after hatching, the loss weight of chick was approximately 4 g per 24 h, because of moisture loss as well as yolk and pectoral muscle utilisation (Halevy et al., 2003; Tona et al., 2003; Careghi et al., 2005). Other authors argued that birds did not access to feed for initial 48 h or more which could have lasting adverse effects (Batal and Parsons, 2002; Noy and Sklan, 2001; Juul-Madsen et al., 2004), despite no FCR significant correlation was detected ($P > 0.05$) at this stage. The results of this study did not find any correlation between post-hatch holding time and starter feeds on ADG, ADFI and FCR

Table 3. Average body weight of chickens at different ages* (g/bird)

Age	0 day	7 days	28 days	56 days
Pre-starter feeds				
Vi-start	42.41	101.82[a]	563.13	1534.35
Commercial	42.36	96.65[b]	566.24	1558.77
SEM[1]	0.037	1.384	7.387	14.472
P	0.331	0.000	0.756	0.215
Post-hatch feeding times				
0 h	42.36	104.31[a]	573.77	1554.3
30 h	42.40	94.16[b]	555.56	1538.8
SEM[1]	0.053	1.043	7.146	14.613
P	0.506	0.000	0.078	0.428
Interaction				
Vi-start × 0 h	42.37	106.88	579.50	1566.9[ab]
Vi-start × 30 h	42.44	96.76	546.66	1541.8[ab]
Commercial × 0 h	42.36	101.74	568.03	1501.8[b]
Commercial × 30 h	42.36	91.55	564.46	1575.8[a]
SEM[1]	0.038	1.289	10.085	19.422
P	0.525	0.979	0.154	0.014

*Mean values represent averages each pen (Pre-starter feeds, n=24; Holding times, n=24; Interactive effects, n=12)
[1]SEM = Standard error of means
[ab] Means within the same column without common letters are statistically different ($P < 0.05$).

of chickens at stage of 0 - 7 days ($P > 0.05$).

At stage 8 - 28 days of age (Table 5), effect of ADG, ADFI and FCR of chicken in the use of starter feeds, feeding time and the interaction between the two experimental factors was unknown ($P > 0.05$). However, chicks that were held 30 h had lower ADG, ADFI and FCR.

The ADG of birds fed Vi-start (26.16 g/d) was lower than that of birds fed the Commercial 1 (26.49 g/d) and that of the birds that were fed immediately after hatching (26.36 g/d) had a higher than those chicks held for 30 h (26.28 g/d) during the experimental period, but all different were not statistically significant ($P > 0.05$) (Table 6). Similarly, the final ADFI, FCR of chicken in the use of starter feeds, post-hatch feeding time and the interaction between the two experimental factors were unknown ($P > 0.05$) at stage 0 - 56 days of age.

3.2. Heart, liver and yolk sac

As presented in Table 7, at 7 days and 56 days of age, the relative weight (%) of heart, liver and yolk sac were not statically different ($P > 0.05$). There has been study showing that 30% of the nutrients for maintenance and growth come from yolk sac (O'Sullivan et al., 1991). On day 7th of the experiment, although the difference was not statistically significant, the survey resulted that yolk sac utilization of starter feed Vi-start was faster than that of treatment Commercial 1. Besides, the yolk sac of birds that was fed immediately after hatching was disappeared faster than that of birds feeding late, too. As Feher & Gyuru (1971) reported the consumption of feed increases gastrointestinal activity and bird metabolism, this helps to explain that when the birds were fed, the yolk sac contents would be excreted through the vitelline duct to enter the intestines. So combined ADFI results, our yolk sac results are also consistent with this report.

There was a trend of higher relative weight of heart and liver in chickens which consumed Vi-start pre-starter than another (1.03 vs 0.99%, and 4.95 vs 4.98%) but the differences were not significant ($P > 0.05$). The impacts of Pre-starter diets on heart and liver weight also changed depending on the feeding time post-hatching at early period (1.03 vs 0.99% and 5.06 vs 4.88%, respectively) although the impact was not clear ($P > 0.05$), and at day 7th, there were interaction between the experimental factors on relative weight of heart, specifically, chickens that were fed Vi-start at 0h had relative weight of heart which was higher than that at 30h ($P > 0.05$) (Table 7). There has an evidence that first two weeks after hatching, heart growth is more variable than other (Phelps et al., 1987). Post-hatching at five

Table 4. Effects of post-hatch feeding times and pre-starter feeds on ADG, ADFI and FCR of chickens from 0 to 7 days*

Age	ADG, g/d	ADFI, g/d	FCR
Pre-starter feeds			
Vi-start	8.38[a]	9.64[b]	1.15[b]
Commercial	7.76[b]	10.10[a]	1.31[a]
SEM[1]	0.197	0.677	0.011
P	0.003	0.004	0.000
Post-hatch feeding times			
0 h	8.75[a]	10.65[a]	1.22
30 h	7.39[b]	9.09[b]	1.24
SEM[1]	0.152	0.416	0.019
P	0.000	0.000	0.427
Interaction			
Vi-start × 0 h	8.99	10.31	1.15
Vi-start × 30 h	8.50	10.98	1.30
Commercial × 0 h	7.76	8.96	1.16
Commercial × 30 h	7.03	9.23	1.31
SEM[1]	0.198	0.270	0.015
P	0.560	0.196	0.673

*Mean values represent averages each pen (Pre-starter feeds, n = 24; Holding times, n = 24; interactive effects, n = 12)
[1]SEM = Standard error of means.

Table 5. Effects of post-hatch feeding times and pre-starter feeds on ADG, ADFI and FCR of broiler chicks from 8 to 28 days*

Age	ADG, g/d	ADFI, g/d	FCR
Pre-starter feeds			
Vi-start	21.29	44.53	2.10
Commercial	21.65	45.05	2.08
SEM[1]	0.313	0.510	0.014
P	0.422	0.467	0.502
Post-hatch feeding times			
0 h	21.58	45.25	2.10
30 h	21.36	44.33	2.08
SEM[1]	0.314	0.504	0.014
P	0.611	0.210	0.240
Interaction			
Vi-start × 0 h	21.73	45.32	2.09
Vi-start × 30 h	21.43	45.17	2.11
Commercial × 0 h	20.85	43.73	2.10
Commercial × 30 h	21.87	44.94	2.06
SEM[1]	0.440	0.659	0.020
P	0.143	0.351	0.140

*Mean values represent averages each pen (Pre-starter feeds, n = 24; Holding times, n = 24; interactive effects, n = 12)
[1]SEM = Standard error of means.

Table 6. Effects of post-hatch feeding times and pre-starter feeds on ADG, ADFI and FCR of broiler chicks from 0 to 56 days*

Age	ADG, g/d	ADFI, g/d	FCR
Pre-starter feeds			
Vi-start	26.16	62.15	2.38
Commercial	26.49	63.03	2.38
SEM[1]	0.277	0.195	0.009
P	0.390	0.321	0.870
Post-hatch feeding times			
0 h	26.36	62.72	2.38
30 h	26.28	62.46	2.38
SEM[1]	0.279	0.120	0.009
P	0.846	0.767	0.761
Interaction			
Vi-start × 0 h	26.56	62.93	2.37
Vi-start × 30 h	25.75	61.37	2.39
Commercial × 0 h	26.16	62.52	2.38
Commercial × 30 h	26.82	63.55	2.37
SEM[1]	0.385	0.881	0.013
P	0.066	0.149	0.224

*Mean values represent averages each pen (Pre-starter feeds, n = 24; Holding times, n = 24; interactive effects, n = 12)
[1]SEM = Standard error of means.

Table 7. Effects of post-hatch feeding times and pre-starter feeds on the ratio of heart, liver and yolk sac (%)

Age	7 days			56 days	
	Heart	Liver	Yolk sac	Heart	Liver
Pre-starter feeds					
Vi-start	1.03	4.95	0.73	0.59	1.91
Commercial	0.99	4.98	0.82	0.54	1.89
SEM[1]	0.024	0.186	0.233	0.021	0.039
P	0.219	0.901	0.767	0.132	0.711
Post hatch times					
0 h	1.03	5.06	0.70	0.56	1.88
30 h	0.99	4.88	0.85	0.57	1.91
SEM[1]	0.024	0.185	0.233	0.022	0.039
P	0.148	0.499	0.643	0.564	0.658
Interaction					
Vi-start × 0 h	1.09[a]	4.98	0.82	0.57	1.88
Vi-start × 30 h	0.96[b]	4.93	0.63	0.61	1.93
Commercial × 0 h	0.97[b]	5.14	0.57	0.54	1.89
Commercial × 30 h	1.01[ab]	4.83	1.08	0.54	1.89
SEM[1]	0.031	0.267	0.332	0.031	0.056
P	0.010	0.626	0.296	0.478	0.643

*Mean values represent averages each pen (Pre-starter feeds, n = 24; Holding times, n = 24; interactive effects, n = 12)
[1]SEM = Standard error of means
[ab]Means within the same column without common letters are statistically different ($P < 0.05$).

to six days later, heart growth rate will be at peak then stabilizes. This explains our results at day 56^{th}. The results of relative weight of heart were almost similar.

4. Conclusions

During 0 - 7 days of age, feed consumption and weight gain of chicks were improved if they were fed immediately after hatching. The Vi-start pre-starter diet given to chicks for one week enhanced their growth rate and feed efficiency only during the first week after hatching as compared with a commercial pre-starter diet. Over a 56-d study, however, post-hatch feeding time and pre-starter diet did not clearly affect the growth performance of slow-growing chickens.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was financially supported by the Nong Lam University Ho Chi Minh City research funding (the research code: CS-CB22-CNTY-01). We also express our sincere gratitude to the staff of Centre for Applied Research Farm (ARF), University of Nong Lam, Ho Chi Minh city, Viet Nam for helping with the management of chickens during the study period.

References

Abed, F., Karimi, A., Sadeghi, A., Shivazad, G., Dashti, S., & Sadeghi-Sefidmazgi, A. (2011). Do broiler chicks possess enough growth potential to compensate for long-term feed and water depravation during the neonatal period? *South African Journal of Animal Science* 41 (1), 33-39.

Batal, A. B., & Parsons, C. M. (2002). Effect of fasting versus feeding oasis after hatching on nutrient utilization inchick. *Poultry Science* 81, 853-859.

Bhanja, S. K., Anjalidevi, C., Panda, A. K., & ShyamaSunder, G. (2010). Effect of post hatch nutrient incubation on performance, intestinal growth, meat yield and immune response in broiler chickens. *Asian-Australasian Journal of Animal Sciences* 23 (4), 515–520.

Careghi, C., Tona, K., Onagbesan, O., Buyse, J., Decuypere, E., & Bruggeman, V. (2005). The effects of the spread of hatch and interaction with delayed feed access after hatch on broiler performance until seven days of age. *Poultry Science* 84 (8), 1314-1320.

Feher, G., & Gyuru, F. (1971). Data on the postembryonal changes of the yolk sac in the domestic fowl. Postembryonal changes of the yolk sac in chickens. *Magy Allatorv Lapya* 6, 353-360.

Gonzales, E., Kondo, N., Saldanha, E. S., Loddy, M. M., Careghi, C., & Decuypere, E. (2003). Performance and physiological parameters of broiler chickens subjected to fasting on the neonatal period. *Poultry Science* 82(8), 1250 - 1256.

Halevy, O., Nadel, Y., Barak, M., Rozenboim, I., & Sklan, D. (2003). Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in turkey poults. *Journal of Nutrition* 133(5) 1376-1382.

Juul-Madsen, H. R., Su, G., & Sorensen, P. (2004). Influence of early or late start of first feeding on growth and immune phenotype of broilers. *Bristish Poultry Science* 45(2), 210-222.

Nitsan Z., Dunnington, E. A., & Siegel, P. B. (1991). Organ growth and digestive enzyme levels to 15 days of age in lines of chickens differing in body weight. *Poultry Science* 70(10), 2040-2048.

Noy, Y., Geyra, A., & Sklan, D. (2001). The effect of early feeding on growth and small intestinal development in the posthatch poult. *Journal of Poultry Science* 80, 912-919.

O'Sullivan, N., Dunnington, E., & Siegel, P. (1991). Relationships among age of dam egg components embryo lipid transfer and hatchability of broiler breeder eggs. *Poultry Science* 70(10), 2180-2185.

Phelps, P. V., Edens, F. W., & Christensen, V. L. (1987). The posthatch physiology of the turkey poultry. *II. Hematology. Comparative Biochemistry Physiology* 86A, 745-750.

Tona, K., Onagbesan, O., De Ketelaere, B., Decuypere, E., & Bruggerman, V. (2003). Effects of turning duration during incubation on corticosterone and thyroid hormone levels, gas pressures in air cell, chick quality, and juvenile growth. *Poultry Science* 82, 1974-19.

Effects of dietary seaweed supplementation on milk productivity, milk quality and health of dairy cows

Hai T. Nguyen[1], Nhan T. M. Nguyen[1], Phuong H. Ngo[2], & Chanh V. Nguyen[1*]

[1]Department of Animal Production, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Department of Animal Nutrition, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: April 22, 2022
Revised: July 26, 2022
Accepted: August 22, 2022

Keywords

Blood biochemical parameters
Digestive disease
Lameness
Milk yield and quality
Seaweed

***Corresponding author**

Nguyen Van Chanh
Email: chanh.nguyenvan@hcmuaf.edu.vn

ABSTRACT

The objective of this study was to evaluate effects of dietary supplementation of a seaweed-originated product (SOP) on milk productivity, milk quality and health of milking cows under Vietnam weather conditions at the dairy farm of One Member Dairy and Beef Joint Stock Company HCMC, Vietnam from October 2019 to February 2020. A total of 40 Holstein Friesian crossbred cows were randomly allotted into 2 treatments in a randomized complete block design. The 2 dietary treatments included (1) cows fed a basal ration without SOP supplementation (control) and (2) control plus 70 g SOP/cow per day (about 0.35% dry matter intake/day). Parity, days in milk, body weight, and milk yield of cows in both treatments were almost equal ($P > 0.05$). The results showed that the average milk yield of cows over the experimental period was not different between the two treatments ($P > 0.05$), but the lactation stability curve was better in SOP group. The SOP supplementation also did not improve milk quality indicators (fat, protein, solids not fat, lactose, somatic cell count) as compared with the control ($P > 0.05$). The blood ketone level of cows in the control group was higher than that of cows in the SOP group ($P < 0.05$), although there were no differences in the blood levels of AST, ALT, protein, glucose, cholesterol, cortisol ($P > 0.05$). The SOP supplementation did not affect BW, body condition score, and locomotion score as well as the prevalences of lameness and digestive diseases ($P > 0.05$). Briefly, these results suggest that the dietary SOP addition of 70 g/cow per day appears not to improve milk productivity, milk quality and health of milking cows.

Cited as: Nguyen, H. T., Nguyen, N. T. M., Ngo, P. H., & Nguyen, C. V. (2022). Effects of dietary seaweed supplementation on milk productivity, milk quality and health of dairy cows. *The Journal of Agriculture and Development* 21(6), 17-25.

1. Introduction

In tropical developing countries, dairy production systems are affected by many factors (Chu et al., 2004; Nguyen et al., 2016) including genetics, nutrition, infectious, parasitic diseases, or heat stress caused by high temperature and humidity. The development of dairy production in Vietnam requires the enhancement of knowledge and skills of farmers (Nguyen, 2021) related the general husbandry such as genetics (Bang et al., 2021c), nutrition (Nguyen & Diep, 2020b; Bang et al., 2021a), and heat stress (Bang et al., 2021b) management. In addition to the primary step from breed selection for improvement of milk productivity and feed efficiency, nutritional factors also affect milk yield and compositions (Hristov et al., 2004; Lee et al., 2014; Olika, 2021), so nutrient balance plays an important role in dairy production.

In recent years, seaweed (known as marine algae or marine macroalgae) has been interested in

applied scientific fields, especially in nutritional composition and its benefits in the health improvement of humans and animals (Brown et al., 2014; Shi et al., 2019; Shimazu et al., 2019). In fact, it is very rich in several polysaccharides and complex carbohydrates (Makkar et al., 2016); or in useful metabolites and necessary minerals, being considered as a natural source of additives in various animals (Morais et al., 2020). It was indicated that seaweed addition into daily diets for milking cows significantly improved milk production (Baek et al., 2004; Lee et al., 2005; Cruywagen et al., 2015). Franklin et al. (1999) also demonstrated that lactation cow diets supplemented 910 g/cow per day of marine algae increased concentration of beneficial fatty acids in milk fat. Besides, Cruywagen et al. (2015) indicated a marine algae product at 90 g/cow per day improved not only milk yield but also ruminal pH and feed efficiency. In addition, Baek et al. (2004) revealed that brown seaweed residues supplementation at 800 g/cow per day (4%) into dairy cow diets stabilized rumen pH, improved milk yield and linoleic acid content. In Vietnam, however, the practical benefits of seaweed in dairy production are still limited and this study is needed to clarify this point.

Therefore, the objective of this study was to determine the effects of dietary supplementation of a seaweed product on milk yield and quality, some blood parameters, body condition score, lameness and digestive diseases of lactation cows under Vietnam weather conditions.

2. Materials and methods

2.1. Location

The experiment was conducted at the dairy farm of One Member Dairy and Beef Joint Stock Company HCM City, Vietnam from 10/2019 to 02/2020.

2.2. Experimental design, animals, and housing

The study was arranged into a randomized complete block design (block: parity) with two treatments of rations, including (1) cows fed a basal ration without seaweed-originated product (SOP, Oceanfeed^{TM} bovine product) supplementation (control) and (2) control plus 70 g SOP/cow per day (about 0.35% dry matter intake/day, DMI/day). Cows were housed in the same cubicle shed containing rubber mats with continual access to water. The study was conducted on a total of 40 Holstein Friesian (HF) crossbred cows with at least 3/4 HF blood, with parities at 1^{st} - 4^{th}, divided into two treatments (20 cows/treatment), and lasted 90 days. Cows in two treatments prior to experimental period were almost equal at parity, days in milk (DIM), body weight and milk yield ($P > 0.05$; Table 1).

2.3. Daily ration of cows

All cows were fed twice a day (6:30 and 15:00, *ad libitum*) as Total Mixed Ration (TMR) method including Hamil grass (25 kg/cow per day), alfalfa hay (2 kg/cow per day), complete feed (7 kg/cow per day), molasses (0.5 kg/cow per day), and brewers's grains (7 kg/cow per day). The seaweed-originated product (SOP) was mixed with new rice bran and mixed well with TMR for the SOP group twice a day. TMR feed was available at all positions of the feeding trough for the same consumption per cow. All cows were adapted to the experimental condition for two weeks in advance. All cows were washed and cleaned two times a day.

2.4. Sample collection and measurements

Milk yield (kg/cow per day): All cows were milked by milking system into specialized container two times a day (5:30 and 16:00), using the recording machine in the milking system, and then merging two times into the average milk yield/cow per day.

Milk quality: About 100 mL of milk were taken in the morning milking time to determine concentrations of milk fat, protein, solids not fat (SNF), lactose, somatic cell count (SCC), stored in the 2 – 6°C condition and transported quickly to an analytical laboratory. Milk quality was analyzed by Ekomilk Total machine (BULTEH 2000, Bulgaria) for about 60 sec/sample for the testing result. The SCC was quickly analyzed by Soma Ekomilk Scan (BULTEH 2000, Bulgaria) for about 4 min/sample with a limitation between 90 and 1.500×10^3 cells/mL.

Blood biochemical parameters: Some blood biochemical parameters analyzed in this study included aspartate aminotransferase (AST), alanine aminotransferase (ALT), protein, glucose,

Table 1. Experimental design

Treatment	Control (No dietary SOP addition)	SOP (Dietary SOP addition)
Cows (n)	20	20
Parity ($P = 0.324$)	2.20 ± 1.15	2.15 ± 1.03
DIM (days) ($P = 0.840$)	119.42 ± 32.71	124.34 ± 27.42
Body weight (kg/cow) ($P = 0.487$)	487.21 ± 52.84	496.74 ± 48.76
Milk yield (kg/cow per day) ($P = 0.798$)	15.68 ± 4.75	15.33 ± 3.96
Daily dietary SOP addition (g/cow per day)	0	70

cholesterol, cortisol and ketone. The blood samples of 2 mL/cow were taken from separate individuals at the farm and added to the tube without anticoagulant, but contained micronized silica granules. To ensure the same condition for all samples, they were collected from 9:00 to 11:00. After collection, samples were stored at 2 - 8°C and transferred to Medlatec hospital and analyzed for blood biochemistry (AST, ALT, protein, glucose, and cholesterol) by Accelerator A3600 system with 60 min/sample and cortisol by Cobass 8100 system for 90 min/sample. Ketone level was measured by FreeStyle Optium Neo Ketone Monitoring System at farm by which a 6 μL of blood dropped to FreeStyle Optium blood β-Ketone test strips in 10 sec/sample.

Body weight (BW, kg/cow): Cows were weighed individually at the beginning and the end of the experimental period by a specialized electronic scale (Electronic scale, VNS China) in the morning before feeding.

Body condition score (BCS): Individual cow was evaluated for BCS ranging from 1 to 5 according to the official method described by Wildman et al. (1982) at the beginning and the end of the experimental period.

Locomotion score (LS): Based on locomotion scoring method determined by Sprecher et al. (1997) from 1 to 5 with non to severe lameness, respectively. The LS of all cows was recorded every week to have a total of 12 recording times of LS per cow during 90 days of experiment. Cows with score more than 3 were assigned into lameness group.

Digestive diseases (%): All cows were observed and recorded for all issues related to digestive diseases in the experimental period to calculate the percentage of digestive diseases per treatment. The signals of digestive diseases were recognized from feces with its height, color, consistency, bubbles, mucous and foamy (Hall, 2002; Heinrichs et al., 2016).

2.5. Statistical analysis

Data were analyzed as a randomized complete block design by ANOVA using the GLM procedure of Minitab Software version 16.2. The individual cow was considered an experimental unit for all parameters. The average values were compared by Tukey test and the percentages were compared by χ^2 test, the differences were consid-

ered significant at $P \leq 0.05$.

3. Results

3.1. Milk yield and lactation stability curve

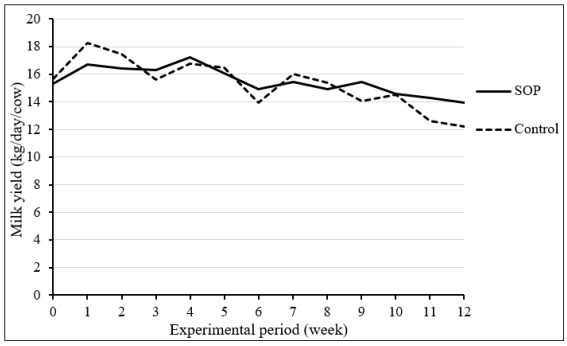

Figure 1. Effect of seaweed-originated product (SOP) addition on lactation stability curve during experiment.

The milk yield of cows in both groups had a downward trend in the mild lactation stage, but the lactation curve of cows fed the dietary SOP supplementation was more stable than that of cows fed control diet without SOP product with large oscillations (Figure 1). However, there was no significant difference in average milk yield between two treatments ($P = 0.740$; Table 2).

3.2. Milk quality

The percentages of milk quality indicators (fat, SNF, protein, lactose) of cows fed the farm-based ration were not different from those of cows fed diet supplemented SOP product ($P > 0.05$; Table 3). The level of somatic cell count (SCC) in milk from control group was 327.10×10^3 cells/mL and higher than that in milk of SOP group of 274.70×10^3 cells/mL ($P = 0.574$).

3.3. Blood biochemical parameters

The level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, glucose, total cholesterol and cortisol in blood of cows fed the farm-based ration were not different from those of cows fed diet supplemented SOP product ($P > 0.05$; Table 4). However, the average blood ketone level of cows in control group was 0.81 mmol/L and significantly higher than that of cows in SOP group of 0.68 mmol/L a ($P = 0.017$).

3.4. Body weight, lameness and digestive diseases

The average body weight (BW), body condition score (BCS) and locomotion score (LS) of cows fed the current farm-based ration after 90 days of experiment were not different from those of cows fed the daily SOP supplementation diet ($P > 0.05$; Table 5). The total of 12 times of LS evaluations during the 90-day period per cow, there were 35 cases of lameness in control treatment which accounted for 14.58% and were not different from those of SOP treatment which had 27 cases of lameness which accounted for 11.25% ($P = 0.341$; Table 6). Besides, there were two cases of diarrheas with bubbles in feces and one case of light rumen acidosis in control group which accounted for 15.00% and were not different from those of SOP group which had one case of diarrhea with bubbles in feces which accounted for 5.00% ($P = 0.605$). There were not any signals of other diseases related to the gastrointestinal tract during the experimental period.

4. Discussion

The present study showed that although the dietary SOP addition helped the lactation curve more stable during 90 days of the experiment, it could not significantly improve ($P > 0.05$) the average milk yield as compared with control group without SOP addition. Although there was a tendency to improve milk quality in all five indicators (fat, SNF, protein, lactose and SCC) from the dietary SOP supplementation group, these tested parameters did not differ significantly between two treatments ($P > 0.05$). Therefore, the dietary supplementation with SOP at 70 g/cow per day (about 0.35% DMI/day) could not remarkably improve milk productivity and quality under Vietnam dairy husbandry conditions. In agreement with our study, Newton et al. (2021) showed that the diet with a small amount of seaweed supplementation (0-158 g/cow per day) for dairy cows was not adequate to improve considerably these parameters. It was also demonstrated that the dietary seaweed supplementation for dairy cows did not affect milk productivity and basic compositions (Lopez et al., 2016; Antaya et al., 2019; Hein, 2021). In addition, Karatzia et al. (2012) reported a lack of effect of

Table 2. Effect of dietary seaweed-originated product (SOP) supplementation on average milk yield

Treatment/Milk yield	n (cows)	$\overline{X}$ ± SD (kg/cow per day)	SEM	*P*
Control	20	16.08 ± 3.12		
			1.051	0.740
SOP	20	16.15 ± 2.56		

Table 3. Effect of dietary seaweed-originated product (SOP) supplementation on milk quality

Milk compositions	Control	SOP	SEM	*P*
n (cows)	20	20		
Fat (%)	3.50 ± 0.45	3.61 ± 0.44	0.122	0.478
Solids not fat (SNF) (%)	9.14 ± 0.25	9.17 ± 0.38	0.087	0.804
Protein (%)	3.57 ± 0.09	3.61 ± 0.14	0.029	0.265
Lactose (%)	4.89 ± 0.21	4.89 ± 0.21	0.058	0.967
SCC ($\times 10^3$ cells/mL)	327.10 ± 34.00	274.70 ± 24.80	77.82	0.574

SCC: somatic cell count.

Table 4. Effect of dietary seaweed-originated product (SOP) supplementation on some blood biochemical parameters

Blood biochemical parameters	Control	SOP	SEM	*P*
n (cows)	20	20		
AST (U/L)	74.69 ± 12.67	75.12 ± 9.12	2.367	0.915
ALT (U/L)	18.16 ± 6.94	18.39 ± 5.30	1.639	0.903
Protein (g/L)	75.68 ± 4.69	76.60 ± 5.17	1.208	0.526
Glucose (mmol/L)	2.04 ± 0.40	2.06 ± 0.38	0.106	0.898
Glucose (mg/dL)	43.05 ± 3.03	45.75 ± 5.93	1.273	0.083
Cholesterol total (mmol/L)	5.40 ± 1.19	5.79 ± 3.03	0.646	0.607
Cortisol (nmol/L)	27.24 ± 12.03	24.36 ± 8.41	1.130	0.199
Ketone (mmol/L)	0.81^{a} ± 0.19	0.68^{b} ± 0.18	0.044	0.017

[ab]Means in the same column without common letter are different at $P \leq 0.05$

Table 5. Effect of dietary seaweed-originated product (SOP) supplementation on body weight (BW), body condition score (BCS) and locomotion score (LS)

Parameters	Control	SOP	SEM	*P*
n (cows)	20	20		
BW day 1 (kg/cow)	482.97 ± 49.42	491.52 ± 43.96	14.186	0.548
BW day 90 (kg/cow)	475.90 ± 71.80	498.00 ± 65.00	16.821	0.276
BCS day 1	2.81 ± 0.51	3.08 ± 0.57	0.140	0.120
BCS day 90	3.09 ± 0.55	3.14 ± 0.67	0.173	0.198
LS day 1	2.45 ± 1.34	2.30 ± 1.28	0.242	0.720
LS day 90	2.24 ± 1.10	2.19 ± 1.07	0.220	0.850

Table 6. Effect of dietary seaweed-originated product (SOP) supplementation on prevalences of lameness and digestive diseases

Prevalence of lameness based on locomotion score (LS)				
Treatment	n (Evaluation times of LS)	Evaluation times with LS > 3	Percentage of disease case (%)	*P*
Control	240	35	14.58	0.341
SOP	240	27	11.25	
Digestive diseases				
Treatment	n (cows)	Cows with digestive disease	Percentage of disease case (%)	*P*
Control	20	3	15.00	0.605
SOP	20	1	5.00	

dietary seaweed supplementation at 80 g/cow per day on the average daily milk production, milk protein and fat. Moreover, Hong et al. (2015) reported that the addition of seaweed by-products (2 – 4% DM) in Holstein cattle diet during transition did not affect daily milk yield and compositions. In contrast, it was illustrated that milk yield and some milk basic constituents were remarkably improved by daily dietary seaweed addition at 90 g/cow per day (0.4% DM) (Cruywagen et al., 2015) or 910 g/cow per day (Franklin et al., 1999). In addition, Baek et al. (2004) and Lee et al. (2005) indicated that seaweed added 4% DMI/day (800 g/cow per day) into diets of dairy cows significantly increased milk yield, but not milk compositions. Interestingly, Newton et al. (2021) revealed that the low supplementation of seaweed (13-40 g/cow per day) did not increase milk yield and quality whereas the dietary high seaweed concentration (26-158 g/cow per day) improved considerably some milk quality parameters (milk protein and casein) without a difference in milk yield and other milk compositions (milk fat, lactose, free fatty acids, SCC). Therefore, there was a wide variation in the effectiveness of SOP supplementation on milk performance and quality in the various supplemented concentrations in recent studies (Baek et al., 2004; Lee et al., 2005; Newton et al., 2021) and only a noticeable enhancement with seaweed supplementation from 90 g/cow per day (equivalent to 0.4% DMI/day) (Cruywagen et al., 2015). As a result, no effect of dietary seaweed supplementation on dry matter intake and feed efficiency of dairy cows (Hong et al., 2015; Hein, 2021) from a relatively small consumption amount (Newton et al., 2021) could be the reason for no differences between two groups in this study.

Regarding results of blood biochemical parameters, there was a considerable improvement of average ketone level in specific ($P < 0.05$) and ketosis disease in general in cows fed daily diet supplemented SOP product at 70 g/cow per day compared with control group without SOP, although the other parameters (AST, ALT, protein, glucose, cholesterol, cortisol) were relatively similar between two treatments ($P > 0.05$). The SOP group showed better stability of blood ketone levels in the normal range (< 1.4 mmol/L) (Oetzel, 2004; Nguyen & Diep, 2020a) for dairy cows during the experiment and this could be the main reason for no case of ketosis disease in SOP group while the subclinical ketosis disease appeared in control group. Interestingly, there was a downward trend in blood cortisol from SOP group with an average reduction of 2.88 nmol/L in comparison with control group, in spite of no considerable difference. It was demonstrated that cortisol concentrations could be increased by effects of stressors such as pain, heat stress or high ambient temperature (Chaiyabutr et al., 2008; Aggarwal & Upadhyay, 2013). Therefore, it would be of interest in future studies to determine precisely whether dietary SOP supplementation with various concentrations could alleviate the stress status for dairy production under Vietnam weather conditions. Ibrahim et al. (2020) showed the effect of dietary seaweed addition in lamb diets in heat stress conditions was significantly different at a level of 2 or 4%; in particular, a significant increase in total protein at 4% but not at 2%, a considerable rise in blood glucose at 2% but not at 4%, and a remarkable decrease in total cholesterol at both levels, as compared with con-

trol. Besides, Karatzia et al. (2012) also revealed that seaweed supplementation at 80 g/cow per day for dairy cows markedly improved blood glucose, but not for the other parameters. In fact, the current results indicated that the blood glucose levels in both groups were slightly lower than the normal level of dairy cows ($\leq$ 2.22 mmol/L) (Dubuc & Buczinski, 2018). Hence, it manifested that the current farm ration could be unbalanced with key nutrients such as protein and energy, suggesting that the unbalanced diets might be one of the important factors affecting the SOP product efficacy, although studies with a larger number of samples will be needed to clarify this point.

The current study revealed that BW, BSC, and LS of lactation cows were not affected by SOP supplementation. This is totally consistent with the results of previous studies about no effect of seaweed supplementation on BW and BSC of dairy cows (Hong et al., 2015), possibly because of no effect of it on feed intake (Baek et al., 2004; Lee et al., 2005; Hein, 2021) and feed efficiency (Hong et al., 2015). However, Cruywagen et al. (2015) indicated that seaweed supplementation at 90 g/cow per day (0.4% DM) had a greater effect on the efficiency of feed conversion into milk. Besides, the figures in this study also exhibited that the cows were healthy with addition of dietary SOP product, leading to a decrease on the prevalences of lameness and digestive diseases with the positively improved trend, despite no statistically significant differences.

5. Conclusions

The dietary SOP addition of 70 g/cow per day was not sufficient to yield a significant improvement on important parameters in dairy production such as milk yield, basic compositions, BW, BSC, LS, some blood biochemical indicators, or reduced prevalences of lameness and digestive diseases; except for a remarkedly improvement in the lactation stability curve and blood ketone level. Therefore, further investigations are urgently required to accurately assess the effectiveness of dietary SOP supplementation under practical dairy husbandry conditions of Vietnam through an extensive study with different supplemental concentrations. Besides, it would also be interesting in future studies to examine whether the dietary nutrient balance affects the efficacy of SOP addition into dairy cattle diets.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Authors acknowledge Pham Thi Thu Trang, Ngo Hai Trieu, and Hoang Thi Huong Giang for assistance with data collection.

References

Aggarwal, A., & Upadhyay, R. (2013). *Heat stress and animal productivity*. Haryana, India: Springer. https://doi.org/10.1007/978-81-322-0879-2_2.

Antaya, N. T., Ghelichkhan, M., Pereira, A. B. D., Soder, K. J., & Brito, A. F. (2019). Production, milk iodine, and nutrient utilization in Jersey cows supplemented with the brown seaweed *Ascophyllum nodosum* (kelp meal) during the grazing season. *Journal of Dairy Science* 102(9), P8040-8058. https://doi.org/10.3168/jds.2019-16478.

Baek, I. K., Maeng, W. J., Lee, S. H., Lee, H. G. Lee, S. R., Ha, J. K., Lee, S. S., & Hwang, J. H. (2004). Effects of the brown seaweed residues supplementation on in vitro fermentation and milk production and composition of lactating dairy cows. *Journal of Animal Science and Technology* 46(3), 373-386. https://doi.org/10.5187/JAST.2004.46.3.373.

Bang, N. N., Chanh, N. V., Trach, N. X., Khang, D. N., Hayes, B. J., Gaughan, J. B., Lyons, R. E., Hai, N. T., & McNeill, D. M. (2021a). Issues of feeding strategy for lactating cows in Vietnamese smallholder dairy farms. *Animals* 11(3), 729. https://doi.org/10.3390/ani11030729.

Bang, N. N., Gaughan, J. B., Hayes, B. J., Lyons, R. E., Chanh, N. V., Trach, N. X., Khang, D. N., & McNeill, D. M. (2021b). Characteristics of cowsheds in vietnamese smallholder dairy farms and their associations with microclimate-a preliminary study. *Animals* 11(2), 351. https://doi.org/10.3390/ani11020351.

Bang, N. N., Hayes, B. J., Lyons, R. E., Randhawa, I. A. S., Gaughan, J. B., & McNeill, D. M. (2021c). Genomic diversity and breed composition of Vietnamese smallholder dairy cows. *Journal of Animal Breeding and Genetics* 139(2), 145-160. https://doi.org/10.1111/jbg.12651.

Brown, E. M., Allsopp, P. J., Magee, P. J., Gill, C. I. R., Nitecki, S., Strain, C. R., & McSorley, E. M. (2014). Seaweed and human health. *Nutrition Reviews* 72(3), 205-216. https://doi.org/10.1111/nure.12091.

Chaiyabutr, N., Chanpongsang, S., & Suadsong, S. (2008). Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *International Journal of Biometeorology* 52(7), 575-585. https://doi.org/10.1007/s00484-008-0151-x.

Chu, L. T. K., Yokogawa, H., & Kawaguch, T. (2004). Dairy Production in Vietnam: Opportunities and Challenges. *Journal of the Faculty of Agriculture, Kyushu University* 49(1), 179-193. https://doi.org/10.5109/4578.

Cruywagen, C. W., Taylor, S., Beya, M. M., & Calitz, T. (2015). The effect of buffering dairy cow diets with limestone, calcareous marine algae, or sodium bicarbonate on ruminal pH profiles, production responses, and rumen fermentation. *Journal of Dairy Science* 98(8), 5506-5514. https://doi.org/10.3168/jds.2014-8875.

Dubuc, J., & Buczinski. S. (2018). Cow-and herd-level prevalence of hypoglycemia in hyperketonemic postpartum dairy cows. *Journal of Dairy Science* 101(4), 3374-3379. https://doi.org/10.3168/jds.2017-13773.

Franklin, S. T., Martin, K. R., Baer, R. J., Schingoethe, D. J., & Hippen, A. R. (1999). Dietary Marine Algae (Schizochytrium sp.) Increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. *The Journal of Nutrition* 129(11), 2048-2054. https://doi.org/10.1093/jn/129.11.2048.

Hall, M. B. (2002). Characteristics of manure: What do they mean? In Eastridge, M. L. (Ed.), *Proceedings of the Tri-State Dairy Nutrition Conference* (141-147). Indiana, USA. Retrieved July 8, 2020, from https://20fd2ea1-0814-41a9-868d-fa06f395c8c2.filesusr.com/ugd/36a444_e86c8527f0794013b88cea957e3890f9.pdf.

Hein, T. (2021). *Red seaweed as a feed additive to stop methane production*. Retrieved May 4, 2021, from https://www.allaboutfeed.net/all-about/new-proteins/red-seaweed-stops-methane-production/.

Heinrichs, J., Varga, G. A., & Kononoff, P. (2016). *Using manure evaluation to enhance dairy cattle nutrition*. Retrieved November 22, 2019, from https://extension.psu.edu/using-manure-evaluation-to-enhance-dairy-cattle-nutrition.

Hong, Z. S., Kim, E. J., Jin, Y. C., Lee, J. S., Choi, Y. J., & Lee, H. G. (2015). Effects of supplementing brown seaweed by-products in the diet of Holstein cows during transition on ruminal fermentation, growth performance and endocrine responses. *Asian-Australasian Journal of Animal Sciences* 28(9), 1296-1302. https://doi.org/10.5713/ajas.15.0235.

Hristov, A. N., Price, W. J., & Shafii, B. (2004). A meta-analysis examining the relationship among dietary factors, dry matter intake, and milk and milk protein yield in dairy cows. *Journal of Dairy Science* 87(7), 2184-2196. https://doi.org/10.3168/jds.S0022-0302(04)70039-9.

Ibrahim, N. H., Ellamie, A. M., Fouda, W. A., & Younis, F. E. (2020). Physiological and behavioral responses of growing barki ram lambs exposed to heat stress and fed brown seaweed as additives under semiarid conditions. *Journal of Animal and Poultry Production* 11(2), 55-65. https://doi.org/10.21608/jappmu.2020.84566.

Karatzia, M., Christaki, E., Bonos, E., Karatzias, C., & Florou-Paneri, P. (2012). The influence of dietary Ascophyllum nodosum on haematologic parameters of dairy cows. *Italian Journal of Animal Science* 11(2), 169-173. https://doi.org/10.4081/ijas.2012.e31.

Lee, H. G., Hong, Z. S., Li, Z. H., Xu, C. X., Jin, X., Jin, M. G., Lee, H. J., Choi, N. J., Koh, T. S., & Choi, Y. J. (2005). Effect of brown seaweed waste supplementation on lactational performance and endocrine physiology in Holstein lactating cows. *Journal of Animal Science and Technology* 47(4), 573-582. https://doi.org/10.5187/JAST.2005.47.4.573.

Lee, J., Seo, J., Lee, S. Y., Ki, K. S., & Seo, S. (2014). Meta-analysis of factors affecting milk component yields in dairy cattle. *Journal of Animal Science and Technology* 56(1), 5. https://doi.org/10.1186/2055-0391-56-5.

Lopez, C. C., Serio, A., Rossi, C., Mazzarrino, G., Marchetti, S., Castellani, F., Grotta, L., Fiorentino, F. P., Paparella, A., & Martino, G. (2016). Effect of diet supplementation with Ascophyllum nodosum on cow milk composition and microbiota. *Journal of Dairy Science* 99(8), 6285-6297. https://doi.org/10.3168/jds.2015-10837.

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology* 212, 1-17. https://doi.org/10.1016/j.anifeedsci.2015.09.018.

Morais, T., Inácio, A., Coutinho, T., Ministro, M., Cotas, J., Pereira, L., & Bahcevandziev, K. (2020). Seaweed potential in the animal feed: A review. *Journal of Marine Science and Engineering* 8(8), 559. https://doi.org/10.3390/jmse8080559.

Newton, E. E., Pétursdóttir, Á. H., Ríkharðsson, G., Beaumal, C., Desnica, N., Giannakopoulou, K., Juniper, D., Ray, P., & Stergiadis, S. (2021). Effect of dietary seaweed supplementation in cows on milk macrominerals, trace elements and heavy metal concentrations. *Foods* 10(7), 1526. https://doi.org/10.3390/foods10071526.

Nguyen, H. M., Jean-Daniel, C., Pham, K. D., Hoang, Q. V., & Guillaume, D. (2016). Which is a sustainable development of dairy sector in Vietnam: Dairy household farms or intensive and large scale farms. *Journal of Animal Science and Technology* 61, 12-21.

Nguyen, T. H., & Diep, T. T. (2020a). Efficacy of PCDairy application in optimal ration formulation for milking cows on milk productivity and quality. *Can Tho University Journal of Science* 12(1), 1-7. https://doi.org/10.22144/ctu.jen.2020.001.

Nguyen, H. T., & Diep, T. T. (2020b). Efficacy of propylene glycol on prevention and treatment of ketosis for dairy cows in lactation stage. *The Journal of Agriculture and Development* 19(2), 28-35. https://doi.org/10.52997/jad.4.02.2020.

Nguyen, N. B. (2021). *Strategies to improve dairy cow productivity and welfare in Vietnam* (Unpublished doctoral dissertation). The University of Queensland, Queensland, Australia.

Oetzel, G. R. (2004). Monitoring and testing dairy herds for metabolic disease. *Veterinary Clinics of North America: Food Animal Practice* 20(3), 651-674. https://doi.org/10.1016/j.cvfa.2004.06.006.

Olika, C. D. (2021). Review on effect of nutrition on milk composition and yield of dairy cows. *European Journal of Science, Innovation and Technology* 1(2), 24-31.

Shi, H., Kim, S. H., & Kim, I. H. (2019). Effect of dietary inclusion of fermented sea mustard by-product on growth performance, blood profiles, and meat quality in broilers. *Journal of the Science of Food and Agriculture* 99(9), 4304-4308. https://doi.org/10.1002/jsfa.9663.

Shimazu, T., Borjigin, L., Katoh, K., Roh, S., Kitazawa, H., Abe, K., Suda, Y., Saito, H., Kunii, H., Nihei, K., Uemoto, Y., Aso, H., & Suzuki, K. (2019). Addition of Wakame seaweed (Undaria pinnatifida) stalk to animal feed enhances immune response and improves intestinal microflora in pigs. *Animal Science Journal* 90(9), 1248-1260. https://doi.org/10.1111/asj.13274.

Sprecher, D. J., Hostetler, D. E., & Kaneene, J. B. (1997). A lameness scoring system that uses posture and gaitto predict dairy cattle reproductive performance. *Theriogenology* 47(6), 1179-1187. https://doi.org/10.1016/S0093-691X(97)00098-8.

Wildman, E. E., Jones, G. M., Wagner, P. E., Boman, R. L., Troutt, H. F., & Lesch, T. N. (1982). A dairy cow body condition scoring system and its relationship to selected production characteristics. *Journal of Dairy Science* 65(3), 495-501. https://doi.org/10.3168/jds.S0022-0302(82)82223-6.

Experimental *Toxocara canis* infection in chickens

Mai T. Duong*, Han N. N. Vu, Giang T. Tran, & Mai C. Duong

Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 10, 2022
Revised: September 26, 2022
Accepted: September 29, 2022

Keywords

Chicken
Larvae infection
Toxocara canis

***Corresponding author**

Duong Tieu Mai
Email:
mai.duongtieu@hcmuaf.edu.vn

ABSTRACT

The objective of this study was to determine the development phases of *Toxocara canis* eggs outside the host and the migration of larvae in the internal organs of chickens infected by ingestion of embryonated *T. canis* eggs. Under a microscope observation of *T. canis* eggs which were incubated in a petri dish containing 5 mL of distilled water at 30 – 33°C with regularly aerated, this study showed the development of *T. canis* egg through various stages, including one cell, two cells, three cells, four cells, early morula, late morula, blastula, gastrula, tadpole, pre-larva, embryonated larva. In addition, it took approximately 9 days for *T. canis* eggs to hatch and for infective larvae to develop at 30 - 33°C. A total of 50 chickens were randomly assigned to 3 groups including group I (10 chicks/group) was served as control group without *T. canis* eggs inoculation; two treatment groups II and III (20 chicks/group) were orally inoculated with 500 or 1000 *T. canis* eggs, respectively. On 1, 3, 6, 15 and 30 days post inoculation (dpi), two chickens/control group and 4 chickens/treatment group were necropsied. The results showed that the percentage of larvae recovered varied from 14.00 to 33.93% and 13.07 to 32.00% in treatment groups II and III, respectively. After 1, 3, 6, and 30 dpi, the significant differences about the number of larvae recovered in two treatment groups were found ($P < 0.05$). In both treatment groups, the percentage of larvae recovered from livers was higher than that in lung tissue. For 6 dpi, small white foci on the liver's surface were seen. Interstitial pneumonia, petechial hemorrhages, dark or gray inflammatory nodules in the lung tissue and the atelectatic area were observed. Histopathology examination revealed infiltrations of leukocytes and eosinophil scattered in the liver and lung tissue.

Cited as: Duong, M. T., Vu, H. N. N., Tran, G. T., & Duong, M. C. (2022). Experimental *Toxocara canis* infection in chickens. *The Journal of Agriculture and Development* 21(6), 26-31.

1. Introduction

Toxocara canis and *Toxocara cati* are typically gastrointestinal helminths found in canids and felids, respectively. *T. canis* is distributed worldwide and is a major zoonotic helminth, causing human toxocarosis. Female *Toxocara* spp. worm can produce up to 200,000 eggs which are shed into the environment and caused the potential risk for human infection (Glickman & Schantz, 1981). While dogs and cats are the main hosts, larvae may also persist and even cause serious sickness in a number of paratenic hosts including other mammals, birds and earthworms. While hatching larvae behave similarly to definitive larvae in paratenic hosts, development into the adult stage does not occur, and infectious third-stage larvae survive in tissues as a developmentally arrested state (Bruňaská et al., 1995). The potential zoonotic risks of *T. canis* should not be underestimated, as toxocarosis is one of the most frequently reported zoonotic helminth infections globally, this led to an increasing interest in the biology and epidemiology of the genus *Toxocara* (Magnaval et al., 2001). Humans and other mammals are at risk of infection from paratenic

chicken hosts. Previous studies described the larval distribution, persistence, the risks of infection for humans including the consumption of undercooked chicken infected with *T. canis* (Ito et al., 1986; Nagakura et al., 1989). Furthermore, *T. canis* larvae in chicken meat were demonstrated to be exceedingly infectious even after prolonged period of times/low temperatures, hens infected with *Toxocara* spp. offer a possible health risk (Taira et al., 2011). Chickens raised in free-range environments are more likely to consume embryonated eggs or infected paratenic hosts, such as earthworms (Pahari & Sasmal, 1991). According to Taira et al. (2003), *T. canis* larvae can migrate via the chicken's hepatopulmonary pathway, reinforcing the idea that poultry harboring migratory *T. canis* larvae may pose a zoonotic risk, particularly if the liver is ingested. Moreover, Beaver (1956) discovered that *T. canis* larvae remained viable in the livers of chickens and pigeons for at least three months. In previous studies, Nakamura et al. (1991) and Taira et al. (2003) discovered considerable differences in total *T. canis* larval recoveries among hosts. In addition, Taira et al. (2003) also suggested further investigations in chickens inoculated with small doses of *T. canis* embryonated eggs, which would more nearly imitate the natural way of infection to investigate the larval migration behavior.

Therefore, the objective of this study was to determine the morphology of the developmental stages from non-embryonated egg to infective egg. Additionally, *T. canis* larval distribution in organs of chickens was also investigated within this research.

2. Materials and methods

2.1. *In vitro* hatching of *T. canis* eggs

In this study, *T. canis* eggs were collected by using sedimentation method and counted by using Mc Master method from fresh faeces of infected dog with *T. canis*. Then, these *T. canis* eggs were raised in a petri dish containing 5 mL of distilled water and incubated as described of Abou-El-Naga (2018). *T. canis* eggs were sucked into a petri dish containing 5 mL of distilled water to raise eggs, incubating at 30 – 33°C. Every day, the egg dishes were provided oxygen for 15 - 30 min and refilled with distilled water equal to the initial water level (Abou-El-Naga, 2018). Using light microscope observation and recording were performed daily until all observed embryos were in L2 larvae (infected larvae).

2.2. Experimental *T. canis* infection in chickens

A total of 50 1-day-old Luong Phuong chicks weighing from 30 to 45 g were purchased from the Cu Chi Chicken Farming Enterprise. When these chickens were 7 days old, total 50 chickens were randomly assigned to 3 groups (1) group I (10 chickens/group) was served as control group without *T. canis* embryonated eggs inoculation; (2) two treatment groups II and III (20 chickens/group) were inoculated orally under 1 mL distilled water contained 500 or 1000 *T. canis* embryonated eggs, respectively, according to instructions of Oshima (1961). All birds were housed under similar conditions. Food and water were given ad libitum. At 1, 3, 6, 15 and 30 days post-inoculation (dpi), two chickens/control group and 4 chickens/treatment groups were chosen randomly for the gross pathology findings. In each treatment group, of 4 chickens, three chickens were used for the number of larvae recovered using digestion method and one chicken was used for histopathological examination. Prior to necropsy, blood samples from two treatment groups collected from the heart were smeared and stained with Giemsa for detection of larvae in blood. Subsequently, these chickens were euthanized by cervical dislocation for gross pathology findings, recovery of larvae and histological investigations. The entire duodenum, spleen, liver, heart, lungs, right inner pectoral muscle, and brain samples from three chickens per each treatment group were collected and kept separately. These samples were separately digested in HCl-pepsin solution for counting the number of larvae recovered. Each organ was chopped up and digested with 20 mL pepsin solution (5 g pepsin, 7 mL hydrochloric acid 36%, and 1000 mL distilled water) were added in sample tube. After that, the samples were incubated at 37°C for 24 h. After the digestion, the samples were centrifuged for 2 min at 1500 rpm and the supernatant was removed. Finally, the number of larvae recovered from each organ was counted under a microscope (Santos et al., 2009).

The percentage of relative larval tissue distribution in the liver and lungs was compared using the Kruskal – Wallis test for paired samples by groups, with a significance level of 5%.

3. Results and Discussion

3.1. *In vitro* hatching of *T. canis* eggs

This study showed that early morula, late morula, blastula, gastrula, tadpole, pre-larva, embryonated larvae were among the developmental stages documented. It took 7 to 9 days of rearing *T. canis* eggs in distilled water at 30 – 33°C and regularly aerated, the eggs from the one-embryo stage have developed to infective larvae eggs (Figure 1).

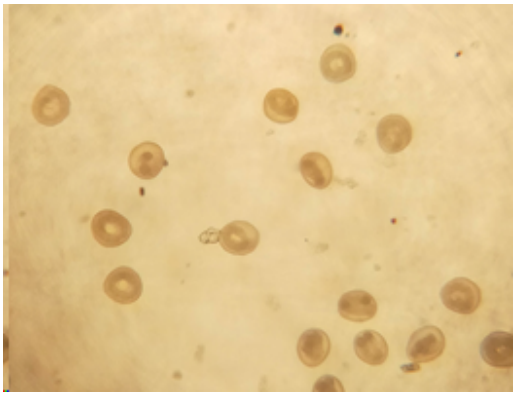

Figure 1. The morphology of T. canis infective larvae eggs (×10).

Meanwhile, if *T. canis* eggs were cultivated in distilled water at 28°C, it took 14 days for *T. canis* eggs to develop into first-stage larvae and 19 days for second-stage larvae (Abou-El-Naga, 2018). At the temperature range of 25°C - 30°C and humidity of 85 – 90%, it took 9 - 15 days for *T. canis* eggs to develop into infective larvae (Schacher, 1957; Okoshi & Usui, 1968). According to Cruz et al. (2012) and Abou-El-Naga (2018), temperature is the main factor that affects the development of roundworm eggs, the average daily growth rate increases significantly with raising temperature at 33°C. The average length and width of 50 *T. canis* larvae in this study were 383 μm and 19 μm, respectively.

3.2. Experimental *T. canis* infection in chickens

Table 1 showed the average percentage of total *T. canis* larvae collected from two treatment groups. At the time of examination, the majority of the larvae were still alive. No larvae were found in chickens of control group. After 1 day, 3, 6, and 30 days of inoculation, the significant differences in the numbers of larvae recovered between two treatment groups were found ($P < 0.05$). In consistent with previous study, Taira et al. (2003) demonstrated that the higher infective dose of larvae was used, the higher T. canis larvae recovered would be collected from animals. However, a failure of innoculation may occur in some chickens of treatment group III, this led to the significant differences about the number of larvae recovered between two treatment groups were not found after 15 days of inoculation. Oshima (1961) stated that larval inoculation results that are inconsistent can be attributed to the eggs' stickiness, which causes an unknown number of them to adhere to the walls of containers, syringes, and residual food.

In this study, most of larvae were only found in the liver and lung. Significant differences about the number of larvae recovered in livers between two treatment groups were found at 1 day, 3, 6, and 30 dpi ($P < 0.05$). Of these, liver is the most favorite site of localization of the *T. canis* larvae. In consistent with Okoshi & Usui (1968), *T. canis* larvae were mainly collected in the livers of chickens infected with 3000 embryonated ascarid eggs. Furthermore, all blood samples were negative with *T. canis* infection in this study. Burren (1972) indicated that larvae can enter the liver from the abdominal cavity (with no injury to the neighboring liver tissue), this explained why no larvae were found in blood samples after 24 h infection. According to Taira et al. (2003), the migration of *T. canis* larvae in chickens is mainly concentrated in the liver and lungs; meanwhile, a few of *T. canis* larvae was found in different organs including the pancreas, spleen, muscle, brain. However, no fatality and clinical signs were found in chickens in this study. In agreement with Taira et al. (2003), no clinical indications or abnormal behavior occurred in chickens inoculated with 3000 *T. canis* larvae.

After 24 h of infection, the livers of infected chickens appeared in yellowish color. More than 50% of the liver was damaged with small white spots after 3 dpi (Figure 2). At 30 dpi, although there were no milky white spots on the surface, these livers had pale color and hemorrhagic spots. Despite the presence of small white foci on the liver 6 days after administration, no gross lesion was found in the other organs (Maruyama et al., 1994). The fundamental lesion caused by larval migration comprised of a central zone of necro-

Table 1. Total larval burdens and relative larval tissue distribution of *T. canis* infected chickens

Days post infection	Egg doses	Total larval burdens		Relative larval tissue distribution (%)			
				Liver		Lung	
		$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%	$\overline{X}$ ± SD	%
1	500	70 ± 2.0	14.00	65 ± 1.0	13.00	5 ± 3.0	1.00
1	1000	320 ± 9.0	32.00	311.3 ± 6.0	31.13	8.67 ± 3.1	0.87
3	500	95 ± 6.2	19.00	93.3 ± 4.7	18.67	1.67 ± 2.9	0.33
3	1000	294 ± 23.2	29.43	290.7 ± 21.2	29.07	3.67 ± 2.1	0.36
6	500	82 ± 3.0	16.40	82 ± 3.0	16.40	0.00 ± 0.0	0.00
6	1000	304 ± 14.7	30.40	301.7 ± 14.4	30.17	2.33 ± 2.52	0.23
15	500	169.7 ± 6.1	33.93	169.7 ± 6.1	33.93	0.00 ± 0.0	0.00
15	1000	167 ± 2.5	16.73	167 ± 3.0	16.70	0.333 ± 0.6	0.03
30	500	58.3 ± 4.7	11.67	58.3 ± 4.7	11.67	0.00 ± 0.0	0.00
30	1000	130.7 ± 8.5	13.07	130.7 ± 8.5	13.07	0.00 ± 0.0	0.00

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

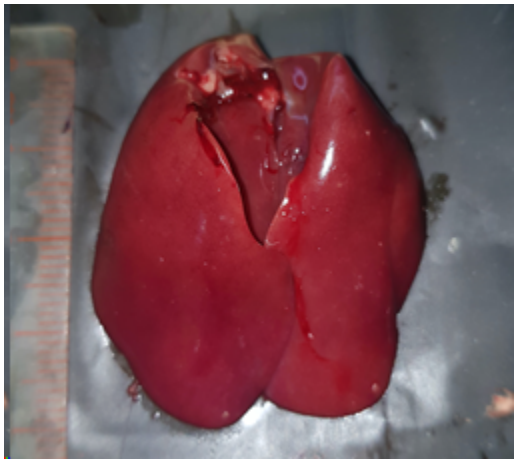

Figure 2. Gross lesions in liver (1000-egg dose on 3 day after infection: small white spots on the liver surface).

sis surrounded by a variable-intensity inflammatory zone. The necrosis usually involved the hepatic epithelium and, in some cases, the reticuloendothelial cells. The inflammatory response included varying degrees of reticuloendothelial cell hyperplasia as well as the infiltration of heterophiles and a lesser number of eosinophiles, monocytes, and lymphocytes (Galvin, 1964). No gross and histopathological changes were observed in control birds. At 15 dpi, the development of granulomas in areas surrounding the larvae was found (Figure 3A). According to Burren (1972), the majority of larvae penetrates the liver via the portal vein and is taken by the bloodstream to the "portal tracts" including a bile duct, portal vein, and artery; where they enter the lobules. However, these larvae were able to access the liver from the abdominal cavity, since no damage was observed in adjacent liver tissue, which could be attributed to internal migration, as shown in Figure 3A. The lesions were constituted of a central zone of necrosis and foreign body giant cells surrounded by a zone of reticular tissue containing a few leukocytes and ranging in size up to approximately 0.8 mm in diameter. The outermost layer was made up of fibrous connective tissue that formed a capsule containing a significant number of heterophile and eosinophils (a type of leukocyte often associated with parasites). In addition, the findings of this study are consistent with previous studies, the lungs from infected chickens appeared petechial hemorrhages on the surface. Interstitial pneumonia, the formation of dark to gray inflammatory nodules in the lung tissue as a result of migrating larvae, and the atelectatic area are also all observed lung abnormalities (Taira et al., 2003; Flecher et al., 2016). The lung injuries from infected chickens were unevenly distributed. On the 1st dpi, there was a significant quantity of eosinophils gathered and severe damage in alveolar wall structure. The gathering of eosinophils was enough evidence to prove *T. canis* larvae's presence in the lungs of inoculated chickens (Figure 3B).

4. Conclusions

This study showed the morphology changes of *T. canis* during the embryo development in the incubation *in vitro* at 30 - 33°C. The somatic migration of *T. canis* larvae was found in liver

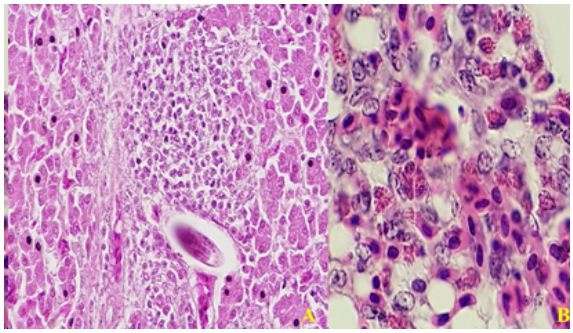

Figure 3. Microscopic lesions in the liver (A) and lung (B).

and lung of chickens. The results of this study provided the evidence that chickens can ingest *T. canis* eggs through the exposure to contaminated food/soil. Thus, the risk of eating raw or uncooked free- raising chicken especially chicken liver should be awareness.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported by fund of Nong Lam University through science and technology project (study protocol # CS-CB21-CNTY-09).

References

Abou-El-Naga, I. F. (2018). Development stages and viability of Toxocara canis eggs outside the host. Biom´edica: Revista del Instituto Nacional de Salud 38(2), 189-197. https://doi.org/10.7705/biomedica.v38i0.3684.

Beaver, P. C. (1956). Larva migrans. *Experimental Parasitology* 5(6), 587-621. https://doi.org/10.1016/0014-4894(56)90032-7.

Bruňaská, M., Dubinský, P., & Reiterova, K. (1995). *Toxocara canis*: ultrastructural aspects of larval moulting in the maturing eggs. *International Journal for Parasitology* 25(6), 683-690. https://doi.org/10.1016/0020-7519(94)00183-O.

Burren, C. H. (1972). The distribution of *Toxocara canis* larvae in the central nervous system of rodents. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 66, 937-942. https://doi.org/10.1016/0035-9203(72)90131-9.

Cruz, L. M., Allanson, M., Kwa, B., Azizan, A., & Izurieta, R. (2012). Morphological changes of *Ascaris* spp. eggs during their development outside the host. *Journal of Parasitology* 98(1), 63-68. https://doi.org/10.1645/GE-2821.1.

Flecher, C. M., Musso, C., Martins, I. V. F, & Pereira, F. E. L. (2016). Larval migration of the ascarid nematode *Toxocara canis* following infection and reinfection in the gerbil *Meriones unguiculatus*. *Journal of Helminthology* 90 (5), 569-576. https://doi.org/10.1017/S0022149X15000760.

Galvin, T. J. (1964). Experimental *Toxocara canis* infections in chickens and pigeons. *The Journal of Parasitology* 50 (1), 124-127. https://doi.org/10.2307/3276045.

Glickman, T. L., & Schantz, M. P. (1981). Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiologic Reviews* 3(1), 230-250. https://doi.org/10.1093/oxfordjournals.epirev.a036235.

Ito, K., Sakai, K., Okajima, T., Quchi, K., Funakoshi, A., Nishimura, J., Ibayashi, H., & Tsuji, M. (1986). Three cases of visceral larva migrans due to ingestion of raw chicken or cow liver. *Nippon Naika Gakkai Zasshi* 75, 759-766. https://doi.org/10.2169/naika.75.759.

Magnaval, J. F., Glickman, L. T., Dorchies, P., & Morassin, B. (2001). Highlights of human toxocariasis. *Korean Journal of Parasitology* 39(1), 1-11. https://doi.org/10.3347/kjp.2001.39.1.1.

Maruyama, S., Nino, T., Yamamoto, K., & Katsube, Y. (1994). Parasitism of *Toxocara canis* larvae in chickens inoculated with *Ascarid* eggs. *Journal of Veterinary Medical. Science* 56(1), 139-141. https://doi.org/10.1292/jvms.56.139.

Nagakura, K., Tachibana, H., Kaneda, Y., & Kato, Y. (1989). Toxocariasis possibly caused by ingesting raw chicken. *The Journal of Infectious Diseases* 160(4), 735-736. https://doi.org/10.1093/infdis/160.4.735.

Nakamura, S., Sotoyama, T., Hayasaka, S., Kameyama, Y., Maruyama, S., & Katsube, Y. (1991). Parasitism of *Toxocara canis* larvae in Japanese quails by inoculation of the ascarid eggs. *Journal of Veterinary Medical Science* 53(5), 865-872. https://doi.org/10.1292/jvms.53.865.

Okoshi, S., & Usui, M. (1968). Experimental studies on *Toxascaris leonina*. VI. Experimental infection of mice, chickens and earthworms with *Toxascaris leonina, Toxocara canis* and *Toxocara cati*. *The Japanese Journal of Veterinary Science* 30(3), 151-166. https://doi.org/10.1292/jvms1939.30.151.

Oshima, T. (1961). Standardization of techniques for infecting mice with *Toxocara canis* and observation on the normal migration routes of the larvae. *The Journal of Parasitology* 47(4), 652-656. https://doi.org/10.2307/3275079.

Pahari, T. K., & Sasmal, N. K. (1991). Experimental infection of Japanese quail with *Toxocara canis* larvae through earthworms. *Veterinary Parasitology* 39(3-4), 337-340. https://doi.org/10.1016/0304-4017(91)90051-V.

Santos, S. V., Lescano, S. Z., Castro, J. M., & Chieffi, P. P. (2009). Larval recovery of *Toxocara cati* in experimentally infected *Rattus norvegicus* and analysis of the rat as potential reservoir for this ascarid.

Memórias do Instituto Oswaldo Cruz 104(6), 933-934. https://doi.org/10.1590/S0074-02762009000600020.

Schacher, J. F. (1957). A contribution to the life history and larval morphology of Toxocara canis. *The Journal of Parasitology* 43(6), 599-612. https://doi.org/10.2307/3286548.

Taira, K., Permin, A., & Kapel, C. M. O. (2003). Establishment and migration pattern of Toxocara canis larvae in chickens. *Parasitology Research* 90(6), 521-523. https://doi.org/10.1007/s00436-003-0894-6.

Taira, K., Saitoh, Y., & Kapel, C. M. (2011). Toxocara cati larvae persist and retain high infectivity in muscles of experimentally infected chickens. *Veterinary Parasitology* 180, 287-291. https://doi.org/10.1016/j.vetpar.2011.03.020.

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

Vy T. L. Nguyen, Hoa T. K. Ho, Nha V. Nguyen, Ngoc H. Le, Tham H. Tran, & Mai C. Duong*

Faculty of Animal Science and Veterinary Medicine, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: April 06, 2022
Revised: July 18, 2022
Accepted: August 22, 2022

Keywords

Broiler
Newcastle disease
Organic acids
Prebiotics & probiotics
Villi height

***Corresponding author**

Duong Chi Mai
Email:
mai.duongchi@hcmuaf.edu.vn

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.

Cited as: Nguyen, V. T. L., Ho, H. T. K., Nguyen, N. V., Le, N. H., Tran, T. H., & Duong, M. C. (2022). 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. *The Journal of Agriculture and Development* 21(6), 32-39.

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 vel-

ogenic, 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^{8}CFU *Bacillus spp.*/g, at least 1×10^{6}CFU *Lactobacillus sp.*/g and at least 1×10^{7}CFU *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-

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

Table 1. Feed additives and period of distribution

Groups (Farms)	Antibiotics								
	Probiotic	Prebiotic	Organic acids	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

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

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 ($\overline{X}$ ± SD)	7 days of age ($\overline{X}$ ± SD)	14 days of age ($\overline{X}$ ± SD)	28 days of age ($\overline{X}$ ± SD)
I	1	44.4^{a} ± 2.6	198.8^{ab} ± 17.5	521.4^{ab} ± 60.5	1530.0^{b} ± 130.4
	2	43.8^{a} ± 3.0	190.3^{bc} ± 12.0	458.2^{bcd} ± 26.2	1310.0^{cd} ± 89.4
	6	43.4^{a} ± 6.2	93.8^{g} ± 4.8	384.1^{de} ± 14.6	1280.0^{d} ± 130.4
	7	48.5^{a} ± 2.8	108.4^{fg} ± 11.3	436.2^{cd} ± 18.7	1340.0^{bcd} ± 89.4
	9	46.3^{a} ± 2.7	156.1^{de} ± 14.4	512.6^{abc} ± 54.3	1510.0^{bc} ± 102.5
II	4	46.7^{a} ± 3.4	225.1^{a} ± 17.8	579.3^{a} ± 52.2	1760.0^{a} ± 89.4
	8	44.2^{a} ± 1.6	131.7^{ef} ± 14.0	509.0^{abc} ± 45.6	1290.0^{d} ± 74.2
III	3	49.3^{a} ± 3.0	162.2^{cd} ± 22.8	467.7^{bcd} ± 20.7	1419.0^{bcd} ± 85.8
	5	46.4^{a} ± 2.3	173.7^{bcd} ± 5.8	333.4^{e} ± 42.3	980.0^{e} ± 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 ($\overline{X}$ ± SD)	Width ($\overline{X}$± SD)	Depth ($\overline{X}$ ± SD)	Width ($\overline{X}$± SD)		
14 days old (n = 3)							
I	1	762^{a} ± 136	113^{bc} ± 19	120^{cd} ± 31	34^{b} ± 8	6.4	270.5
	2	593^{bc} ± 171	97^{abc} ± 25	205^{ab} ± 40	44^{ab} ± 11	2.9	180.7
	6	544^{cd} ± 67	94^{abc} ± 15	154^{bcd} ± 29	41^{ab} ± 7	3.5	160.6
	7	581^{bcd} ± 100	94^{abc} ± 22	167^{abc} ± 28	42^{ab} ± 14	3.5	171.6
	9	626^{abc} ± 62	120^{a} ± 26	213^{a} ± 44	43^{ab} ± 7	2.9	235.9
II	4	706^{ab} ± 81	101^{abc} ± 23	201^{ab} ± 33	36^{ab} ± 9	3.5	224.0
	8	619^{bc} ± 63	80^{c} ± 11	196^{ab} ± 44	39^{ab} ± 15	3.2	155.6
III	3	441^{d} ± 70	88^{bc} ± 27	109^{d} ± 28	37^{ab} ± 11	4.0	121.9
	5	613^{bc} ± 77	112^{ab} ± 24	206^{ab} ± 50	49^{a} ± 11	3.0	215.7
Reference (1)		300		70		3.6	
28 days old (n = 3)							
I	1	845^{a} ± 112	95^{abc} ± 14	222^{bc} ± 50	39^{a} ± 10	3.8	252.2
	2	754^{ab} ± 149	109^{ab} ± 20	192^{bcd} ± 57	37^{a} ± 9	3.9	258.2
	6	491^{de} ± 65	105^{abc} ± 33	231^{b} ± 62	46^{a} ± 13	2.1	161.9
	7	551^{de} ± 99	98^{abc} ± 20	347^{a} ± 72	42^{a} ± 11	1.6	169.6
	9	584^{cd} ± 80	96^{abc} ± 25	147^{cd} ± 38	39^{a} ± 5	4.0	176.1
II	4	769^{ab} ± 40	104^{abc} ± 14	181^{bcd} ± 56	43^{a} ± 11	4.2	251.3
	8	580^{cd} ± 77	91^{bc} ± 21	193^{bcd} ± 28	38^{a} ± 5	3.0	165.8
III	3	686^{bc} ± 60	126^{a} ± 21	137^{d} ± 46	47^{a} ± 9	5.0	271.5
	5	533^{de} ± 51	113^{ab} ± 23	233^{b} ± 51	38^{a} ± 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 ($P > 0.05$).

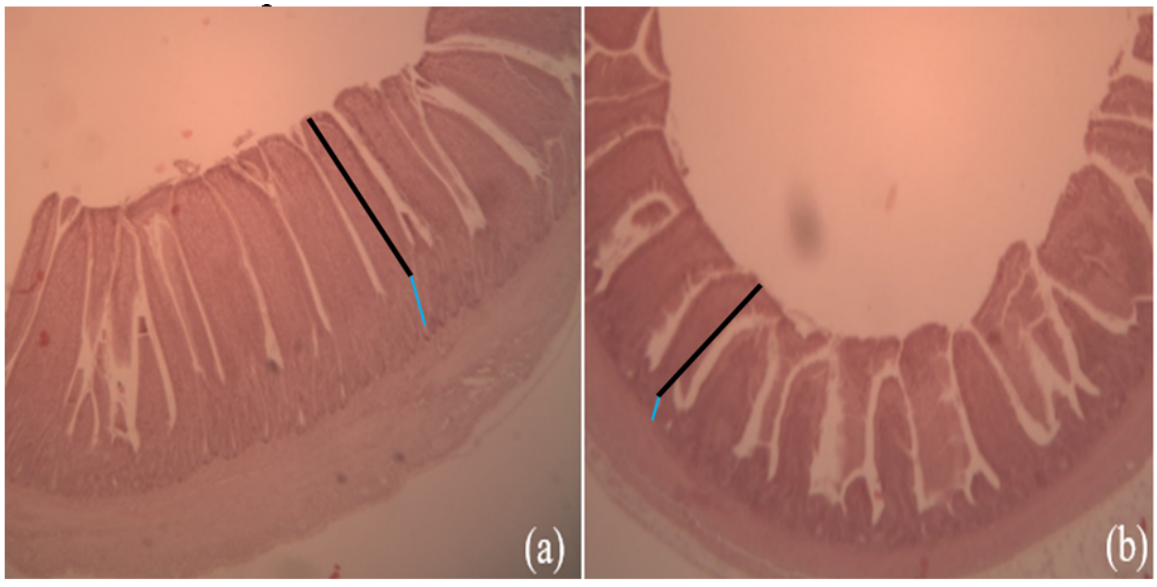

Figure 1. Microscopic appearance of the highest villi height of (a) 14 days old chicken at Farm 4; and the shortest villi of (b) 14 days old chicken from Farm 3. Villi were short and thinned distribution (H&E, original magnification X40, Olympus CX40). Black line: villi height; blue line: crypt depth.

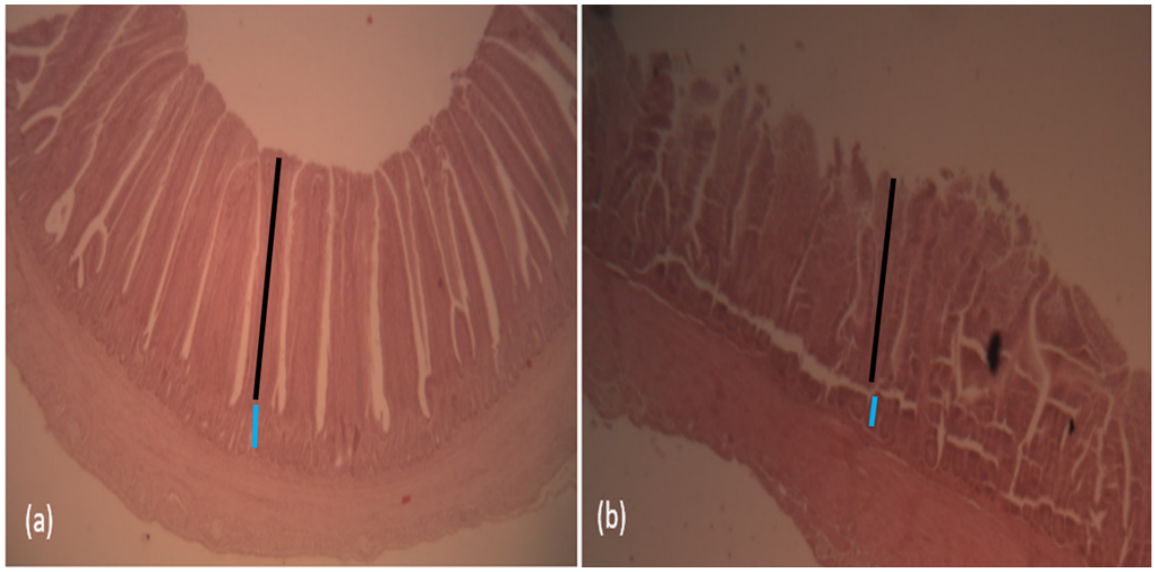

Figure 2. Microscopic appearance of the highest villi of (a) 28 days old chicken from Farm 1 and the shortest villi of (b) 28 days old chicken from Farm 6. Villi were intact and finger-shaped (H&E, original magnification X40, Olympus CX40). Black line: villi height; blue line: crypt depth.

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.

Acknowledgements

The authors gratefully acknowledge financial support from Nong Lam University, Ho Chi Minh City, Vietnam.

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.

Removal of ammonium and phosphate from slaughterhouse wastewater by electrochemical method using magnesium electrodes

Nguyen T. T. Ho[1], Nhut T. Huynh[1*], Chat N. Tran[1], Thi Y. Ho[1], Vy T. H. Nguyen[1], Bang H. K. Nguyen[1], Manh C. Nguyen[2], & Hiep T. Nguyen[3]

[1]Faculty of Environment and Natural Resources, Nong Lam University, Ho Chi Minh City, Vietnam
[2]Department of Aquatic and Atmospheric Environment Research, Research Institute of Biotechnology and Environment, Nong Lam University, Ho Chi Minh City, Vietnam
[3]Research Institute for Sustainable Development, Ho Chi Minh University of Natural Resources and Environment, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: September 06, 2022
Revised: November 08, 2022
Accepted: November 11, 2022

Keywords

Ammonium removal
Electrochemical
Magnesium electrode
Phosphate removal
Slaughterhouse wastewater

***Corresponding author**

Huynh Tan Nhut
Email:
tannhut.env@hcmuaf.edu.vn

ABSTRACT

In this research, the treatability of slaughterhouse wastewater was investigated by using the electrochemical method with Mg electrodes. The influence of the variables such as initial pH (4, 5, 6, 7, 8, and 9), current density (15, 30, and 45 mA/cm^2), and reaction time (20, 25, and 30 min) on the removal efficiency of ammonium and phosphate was studied. The highest phosphate removal efficiency was reached at 100% after 20 min of electrochemical treatment with 30 mA/cm^2 of current density and initial pH 6. Meanwhile, the maximum removal percent of ammonium was approximately 52%. Thus, this method is feasible to apply for the removal of phosphate and ammonium in slaughterhouse wastewater.

Cited as: Ho, N. T. T., Huynh, N. T., Tran, C. N., Ho, T. Y., Nguyen, V. T. H., Nguyen, B. H. K., Nguyen, M. C., & Nguyen, H. T. (2022). Removal of ammonium and phosphate from slaughterhouse wastewater by electrochemical method using magnesium electrodes. *The Journal of Agriculture and Development* 21(6), 40-45.

1. Introduction

As the economy is growing, people's living standards are increasing day by day. Therefore, the basic needs also increase, especially food is one of the indispensable needs. Livestock and poultry slaughter takes place every day to meet the people's demand for meat products. Meat consumption has been steadily increasing in recent decades. The slaughtering of animals and the cleaning of slaughterhouse facilities and meat processing plantsgenerates substantial amounts of slaughterhouse wastewater (Bustillo-Lecompte & Mehrvar, 2015). The composition of slaughterhouse wastewater changes greatly depending on the various industrial processes and unique water requirements (Matsumura & Mierzwa, 2008; Bustillo-Lecompte et al., 2014). Because of the presence of organic materials such as blood, fat, grease, and proteins, these wastewaters have high levels of organics such as biochemical oxygen demand (BOD) and chemical oxygen demand

(COD), nitrogen, and phosphorus (Sirianuntapiboon & Manoonpong, 2001). As a result, slaughterhouse waste water requires treatment before they can be released into the environment in a safe and sustainable manner.

Traditionally, aerobic and anaerobic methods are applied to slaughterhouse wastewater treatment. Nonetheless, biological processes necessitate long hydraulic retention times and huge reactor capacities, as well as high biomass concentration and sludge loss control (Kobya et al., 2006). In recent years, the application of an electrochemical technique to successful wastewater treatment and resource recovery has attracted interest (Hand & Cusick, 2021; Salazar-Banda et al., 2021; Escobedo et al., 2022). Compared with conventional physico-chemical processes, electrochemical has many advantages such as simple equipment, easy operation and automation, a short retention time, low sludge production and no chemical requirement.

Electrode material can play a very important role in the electrolytic degradation of ammonium and phosphate present in wastewater. In this study, magnesium electrode material was used to examine the efficiency of the electrochemical removal of ammonium and phosphate in slaughterhouse wastewater. The process was examined under different values of current density, pH, and reaction time in order to determine optimum operating conditions.

2. Materials and Methods

2.1. Materials and characterization of wastewater

The wastewater was collected from a local slaughterhouse located in Di An city, Binh Duong province, Vietnam. The samples were eliminated hair and large suspended particles. The wastewater samples were stored at 4°C before use in experiments and further studies. The characteristics of slaughterhouse wastewater are shown in Table 1. The chemicals used in this study were purchased from Xilong Scientific Co., Ltd., China with a purity of 99%. Magnesium electrodes were chosen for use as both anode and cathode. Electrodes are manufactured according to Italian technology with chemical components including Al 2.5-5.5%, Zn 2.5-3%, Mn 0.2-4%, Cn 0.01%, Si 0.01%, Fe 0.006%, Ni 0.001%, the rest is Mg.

Table 1. The characterization of slaughterhouse wastewater used in this study

Parameters	Unit	Value
pH	-	7 - 7.2
COD	mg/L	863 - 935
TSS	mg/L	328 - 384
NH_4^+	mg/L	186 - 194
PO_4^{3-}	mg/L	34 - 35
Mg^{2+}	mg/L	12 - 16
Ca^{2+}	mg/L	18 - 23

COD: Chemical oxygen demand; TSS: Total suspended solids.

2.2. Experimental setup

According to previous studies (Ahmadian et al., 2012; Ozturk & Yilmaz, 2019) , the experiments were conducted by a laboratory-scale reactor using Berker 1000 mL (Figure 1). In the studies, 800 mL of wastewater was used. The cylindrical Mg electrodes had the following parameters: diameter of 1.8 cm, length of 10.5 cm, and effective area of 66.73 cm^2. All electrodes were arranged vertically in which the distance between the electrodes was 6 cm. They were connected to terminals of a direct current power supply (ROBOT®, model: 11JA, Vietnam) which is characterized as 10 A and 12/24 V. The laboratory-scale reactor has been stirred at 120 rpm by a magnetic stirrer (Thermostat magnetic stirrer 85-2, China).

Batch mode experiments were conducted to investigate the efficiency of ammonium and phosphate removal in slaughterhouse wastewater. The influence of varied reaction conditions on ammonium and phosphate efficiency were examined by altering the initial pH samples (viz., 4, 5, 6, 7, 8, 9), current density (viz., 15, 30, 45 mA/cm^2), and reaction time (viz., 20, 25, 30 min). At the end of each treatment, the samples were left to stand for 30 min, then filtered and analyzed. All of experiments were carried out in triplicates. The removal efficiency of pollutants was calculated using the following equation:

$$\text{Removal efficiency } (\%) = [(C_0 - C_t)/C_0]\times 100 \quad (1)$$

where C_0 is the initial concentration of the pollutants and C_t is the concentration of the pollutants at reaction time t.

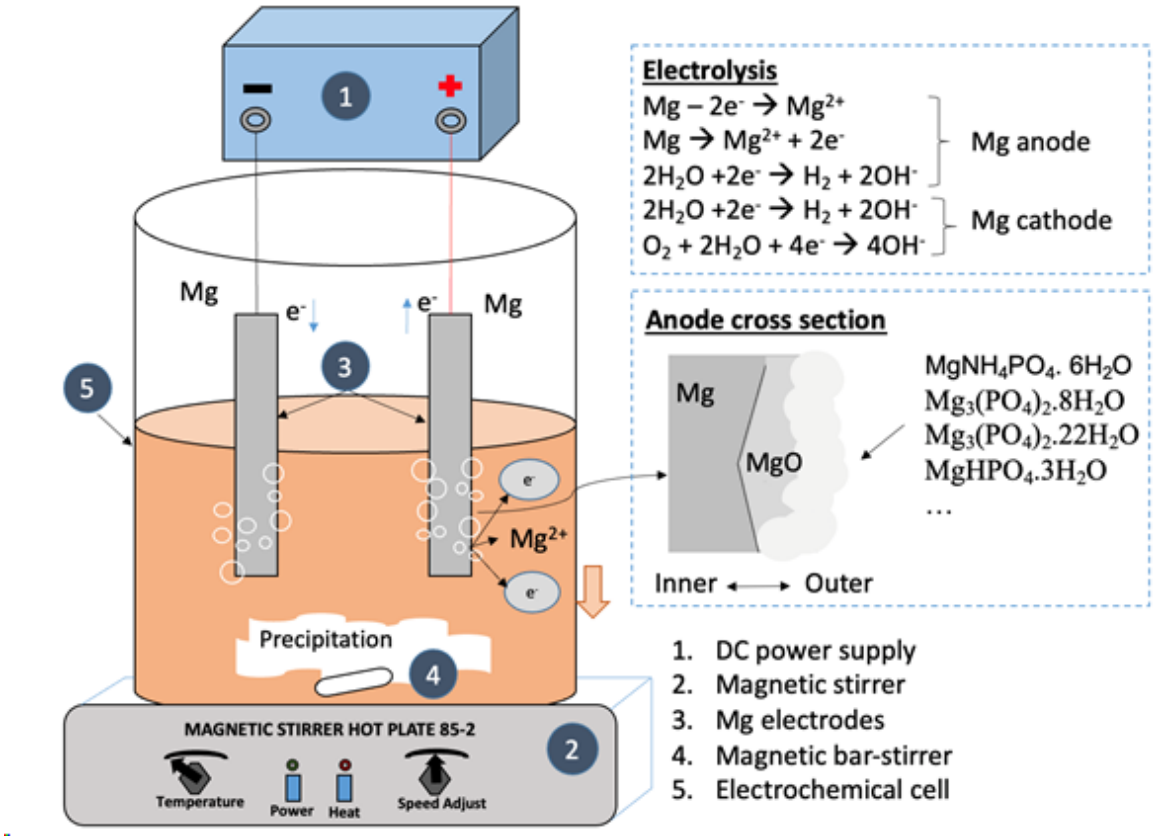

Figure 1. Electrochemical reactor schematic.

2.3. Analytical methods

The characterization of slaughterhouse wastewater including chemical oxygen demand (COD), total suspended solids (TSS), NH_4^+, PO_4^{3-}, Mg^{2+}, and Ca^{2+}+ was analyzed in accordance with standard methods for water and wastewater examination (Rice et al., 2012). The pH was measured using a pH meter (HI98107 Hanna). SPSS software was also used to analyze the data, as well as the variance test (one-way ANOVA) for mean comparisons.

3. Results and Discussion

3.1. Influence of initial pH

The effect of initial pH on the removal of ammonium and phosphate was investigated at a constant current density of 30 mA/cm^2 and reaction time of 20 min. As seen in Figure 2, the performance removal of ammonium gradually increased with the initial pH range of 4 – 6. However, it can be seen that the removal efficiency of ammonium decreased when the initial pH range of 7 – 9. The highest NH_4^+ removal efficiency (approximately 52%) is obtained when the initial pH is 6. In table 2, the results showed a significant difference in the treatment efficiency when increasing the pH value beyond 6 ($P < 0.05$). For phosphate, high PO_4^{3-} removal percent may be attained in the pH range of 4 – 8. At this pH range, the PO_4^{3-} removal completely occurred. When the pH is increased to 9, the PO_4^{3-} removal efficiency tends to slightly decrease, and the difference is statistically significant ($P < 0.05$).

Table 2. The removal efficiecy of ammonium and photphase at current density: 30 mA/cm^2, and reaction time: 20 min

Treatments	Removal efficiency (%)	
	NH_4^+	PO_4^{3-}
pH 4	48.4^a ± 1.4	100^a ± 0.0
pH 5	50.2^a ± 1.7	100^a ± 0.0
pH 6	51.6^a ± 1.4	100^a ± 0.0
pH 7	37.8^b ± 1.6	99.7^a ± 0.3
pH 8	35.0^b ± 2.3	99.5^a ± 0.2
pH 9	34.6^b ± 2.1	91.3^b ± 1.4

In the same raw, numbers with the same letter do not have a statistically significant difference; significant difference at the level of $\alpha = 0.05$.

This result is explained by the complicated reaction mechanism of ion Mg^{2+}, NH_4^+, and PO_4^{3-} in solution (Figure 1). According to the report of Carmona-Carmona et al. (2020), it is observed that from pH 5, the precipitation of solids were formed, which could correspond to $MgHPO_4.3H_2O$ (newberyite). The precipitates could correspond to $Mg_3(PO_4)_2.8H_2O$ (bobierrite) and $Mg_3(PO_4)_2.22H_2O$ (cattiite) when pH 8.5. Besides, the elimination of NH_4^+ could be related to the creation of struvite because it is feasible to stimulate the $MgNH_4PO_4.6H_2O$ formation, which is one of the most critical forms of precipitation of the dissolved phosphorus.

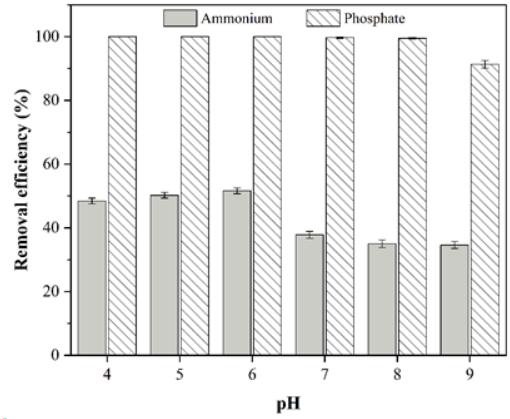

Figure 2. Influence of pH on ammonium and phosphate removal (current density: 30 mA/cm^2; reaction time: 20 min).

3.2. Influence of current density

The Figure 3 represents the effects of the current density on NH_4^+ and PO_4^{3-} removal efficiency, for magnesium electrode material, with a reaction time of 20 min and pH 6. In general, increasing current density improved processing efficiency. In the case of ammonium removal, the highest removal efficiency was obtained for the current density of 45 mA/cm^2 was approximately 52%. As can be seen in Figure 3, the experiment achieved PO_4^{3-} removal efficiency of above 95% when increased current density. At the current density of 30 mA/cm^2, the PO_4^{3-} removal efficiency was obtained approximately 100%. It is possible to argue that the rise in current density has accelerated the elimination of contaminants due to the generation of extra electrons (Ozturk & Yilmaz, 2019). Phosphate elimination was accelerated by increasing the current in wastewater as a result of increased Mg^{2+} generation to enhance precipitation. Nonetheless, the cur-

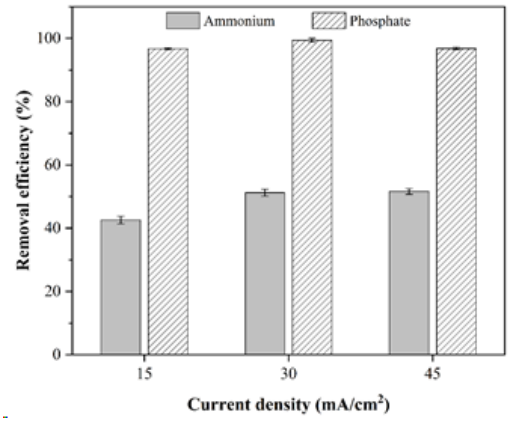

Figure 3. Influence of current density on ammonium and phosphate removal (initial pH: 6; reaction time: 20 min).

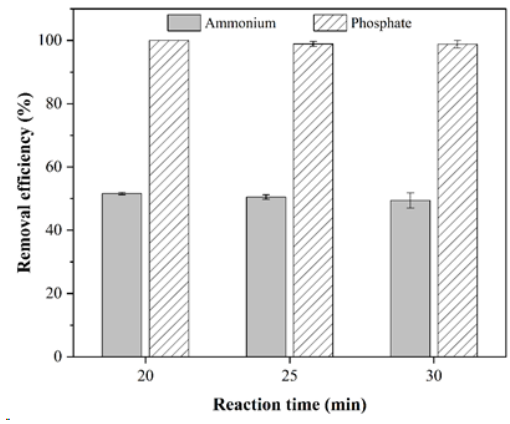

Figure 4. Influence of reaction time on ammonium and phosphate removal (initial pH: 6; current density: 30 mA/cm^2).

rent density also strongly influences the metal dissolution leading to a change in the mass of the electrodes. Therefore, in order to facilitate the removal of NH_4^+ and PO_4^{3-}, as well as reduce electrode wear, the optimal current density was selected as 30 mA/cm2. In Table 3, the results showed that the association between different current density was statistically significant ($P < 0.05$).

Table 3. The removal efficiecy of ammonium and photphase at pH 6, reaction time: 20 min

Current density	Removal efficiency (%)	
	NH_4^+	PO_4^{3-}
15 mA/cm^2	42.6^a ± 1.5	96.7^a ± 0.3
30 mA/cm^2	51.2^b ± 1.3	99.4^b ± 0.8
45 mA/cm^2	51.6^b ± 1.1	96.8^a ± 0.5

In the same raw, numbers with the same letter do not have a statistically significant difference; significant difference at the level of $\alpha = 0.05$.

3.3. Influence of reaction time

To investigate the effect of reaction time, the current density was set to 30 mA/cm^2 and the pH of the wastewater was adjusted to 6. The result of experiment is shown in Figure 4. In general, the removal effectiveness of NH_4^+ and PO_4^{3-} slightly decreased as the reaction time increased. In the case of ammonium removal, when reaction time was set to 20 min, the removal efficiency of NH_4^+ was approximately 52%. For phosphate, the PO_4^{3-} removal completely occurred (100%). In fact, the Mg^{2+} ion concentration increased as reaction time increased (Abdel-Shafy et al., 2020). After 20 min, however, it is obvious that removal efficiencies of both NH_4^+ and PO_4^{3-} have not significantly changed. This could be due to the discovery of dissolved metal ions and their hydroxides during the saturation stage of floc development. This result tends to be similar to the study of Luo et al. (2022). Thus, the optimum reaction time chosen was 20 min. Results of one-way ANOVA showed no difference between different reaction times ($P > 0.05$).

4. Conclusions

The current study explored the feasibility of simultaneous removing NH_4^+ and PO_4^{3-} from slaughterhouse wastewater using an electrochemical technique. The experimental results demonstrated that treating phosphate and ammonium from slaughterhouse wastewater is achievable by using magnesium as the electrodes. Moreover, the current density, reaction time, and initial pH are the key factors influencing the removal efficiency. On the other hand, when using Mg electrodes for electrolysis can form useful products in the form of fertilizers. Further research on the pilot plant size will disclose the economic feasibility of treating slaughterhouse wastewater with electrochemical method.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank for the re-search grant (Code: CS-SV21-MTTN-03) from Nong Lam University Ho Chi Minh City, Viet-nam. The research team also thanks to the Department of Aquatic and Atmospheric Environment Research (Research Institute of Biotechnology and Environment, Nong Lam University) for their support in analyzing wastewater samples.

References

Abdel-Shafy, H. I., Shoeib, M. A., El-Khateeb, M. A., Youssef, A. O., & Hafez, O. M. (2020). Electrochemical treatment of industrial cooling tower blowdown water using magnesium-rod electrode. *Water Resources and Industry* 23, 100121. https://doi.org/10.1016/j.wri.2019.100121.

Ahmadian, M., Yousefi, N., Van Ginkel, S. W., Zare, M. R., Rahimi, S., & Fatehizadeh, A. (2012). Kinetic study of slaughterhouse wastewater treatment by electrocoagulation using Fe electrodes. *Water Science and Technology* 66(4), 754-760. https://doi.org/10.2166/wst.2012.232.

Bustillo-Lecompte, C. F., & Mehrvar, M. (2015). Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *Journal of Environmental Management* 161, 287-302. https://doi.org/10.1016/j.jenvman.2015.07.008.

Bustillo-Lecompte, C. F., Mehrvar, M., & Quiñones-Bolaños, E. (2014). Cost-effectiveness analysis of TOC removal from slaughterhouse wastewater using combined anaerobic–aerobic and UV/H2O2 processes. *Journal of Environmental Management* 134, 145-152. https://doi.org/10.1016/j.jenvman.2013.12.035.

Carmona-Carmona, P. F., Linares-Hernández, I., Teutli-Sequeira, E. A., López-Rebollar, B. M., Álvarez-Bastida, C., Mier-Quiroga, M. d. l. A., Vázquez-Mejía, G., & Martínez-Miranda, V. (2020). Industrial wastewater treatment using magnesium electrocoagulation in batch and continuous mode. *Journal of Environmental Science and Health, Part A* 56 (3), 269-288. https://doi.org/10.1080/10934529.2020.1868823.

Escobedo, E., Cho, K., & Chang, Y.-S. (2022). Electrochemical activation of hydrogen peroxide, persulfate, and free chlorine using sacrificial iron anodes for decentralized wastewater treatment. *Journal of Hazardous Materials* 423, 127068. https://doi.org/10.1016/j.jhazmat.2021.127068.

Hand, S., & Cusick, R. D. (2021). Electrochemical disinfection in water and wastewater treatment: identifying impacts of water quality and operating conditions on performance. *Environmental Science and Technology* 55 (6), 3470-3482. https://doi.org/10.1021/acs.est.0c06254.

Kobya, M., Senturk, E., & Bayramoglu, M. (2006). Treatment of poultry slaughterhouse wastewaters by electrocoagulation. *Journal of Hazardous Materials* 133 (1-3), 172-176. https://doi.org/10.1016/j.jhazmat.2005.10.007.

Luo, W., Fang, Y., Song, L., & Niu, Q. (2022). Production of struvite by magnesium anode constant voltage electrolytic crystallisation from anaerobically digested chicken manure slurry. *Environmental Research* 214, 113991. https://doi.org/10.1016/j.envres.2022.113991.

Matsumura, E. M., & Mierzwa, J. C. (2008). Water conservation and reuse in poultry processing plant - A case study. *Resources, Conservation and Recycling* 52 (6), 835-842. https://doi.org/10.1016/j.resconrec.2007.10.002.

Ozturk, D., & Yilmaz, A. E. (2019). Treatment of slaughterhouse wastewater with the electrochemical oxidation process: Role of operating parameters on treatment efficiency and energy consumption. *Journal of Water Process Engineering* 31, 100834. https://doi.org/10.1016/j.jwpe.2019.100834.

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *Standard methods for the examination of water and wastewater*. Washington DC, USA: American Public Health Association.

Salazar-Banda, G. R., Santos, G. d. O. S., Gonzaga, I. M. D., Dária, A. R., & Eguiluz, K. I. B. (2021). Developments in electrode materials for wastewater treatment. *Current Opinion in Electrochemistry* 26, 100663. https://doi.org/10.1016/j.coelec.2020.100663.

Sirianuntapiboon, S., & Manoonpong, K. (2001). Application of granular activated carbon-sequencing batch reactor (GAC-SBR) system for treating wastewater from slaughterhouse. *Science and Technology Asia* 16-25.

Survey of ornamental plants with medicinal values at The Saigon Zoo and Botanical Garden in Ho Chi Minh City

Thanh T. Nguyen*, & The T. M. Ngo
Faculty of Environment and Natural Resources, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 23, 2022
Revised: September 05, 2022
Accepted: September 21, 2022

Keywords

Medicinal plants
Ornamental plants
Saigon Zoo and Botanical Garden
Townhouse landscaping

***Corresponding author**

Nguyen Thien Thanh
Email: nguyenthienthanh@hcmuaf.edu.vn

ABSTRACT

This study investigated the diversity of ornamental plants with medicinal values at the Saigon Zoo and Botanical Garden in Ho Chi Minh City. The research was conducted through a comprehensive survey, including questionnaires, observation, taking pictures and notes on each sample. The study identified 223 species of ornamental plants, of which species with medicinal values accounted for 55.6% (124 species). Among the identified families, the *Fabaceae* and the *Zingiberaceae* were the most popular families and each had 7 species detected. We also categorized ornamental plants with medicinal values according to different approaches, including tree morphology, layout in the landscape, shade tolerance and light-loving ability, and medicinal uses for application in the landscaping of townhouses and apartments.

Cited as: Nguyen, T. T., & Ngo, T. T. M. (2022). Survey of ornamental plants with medicinal values at The Saigon Zoo and Botanical Garden in Ho Chi Minh City. *The Journal of Agriculture and Development* 21(6), 46-51.

1. Introduction

For thousands of years, medicinal plants have existed together with the forest ecosystem and agricultural field. There is a close correlation between the biodiversity of medicinal plants with cultural diversity and traditional medicine associated with medical knowledge of 54 ethnic groups, which is the cultural identity of the Vietnamese people. According to the World Health Organization (WHO), up to 80% of the population in developing countries still uses plants available in nature for health care needs. Currently, the global trend is to use herbal medicines with natural ingredients to treat diseases to ensure safety.

Medicinal plants are mostly exploited in nature (95%), when put into gardens and landscapes, they can both beautify the landscape and can be used as an urgent treatment for people (Haridasan & Ganesh, 2017). More than 30% of all plants have been used for medicinal purposes (Joy, 1998). More than three quarters of the world's population relies heavily on plants and plant extracts for health care. According to the World Health Organization, in Africa, more than 80% of people rely on traditional medicinal plants for health care and treatment (Hamilton, 2008).

According to the statistics of the special collection on biodiversity and potential of Vietnamese medicinal plants, Vietnam has more than 4,000 species of herbs, which can be used to treat many

different diseases. However, due to continuous exploitation and not paying attention to the conservation and regeneration, 144 species of medicinal plants are listed in the Vietnam Red Book 2007 (Nguyen, 2021). In addition, the current domestic source of medicinal herbs can only meet 10-20% of the demand, leaving more than 80% of the market to be imported from China.

Built in 1864 and operated for more than 150 years, the Saigon Zoo and Botanical Garden – Ho Chi Minh City is considered one of the rare old forests located in the center of a big city with a large number of heritage trees. In addition, it is a place to preserve animals and plants. The Saigon Zoo and Botanical Garden – Ho Chi Minh City is one of the eight oldest parks in the world that owns the record of the first flora and fauna collection garden in Vietnam with more than 2500 trees belonging to about 900 species of plants preserved (Tran, 2007).

One of the most compelling motivations to integrate native medicinals into the landscape is the multi-layered benefits that they provide to habitat, ecosystem services, and social and cultural value, as well as wellness for people. Typically, native medicinal plants are wild harvested. Landscapers can help sustain wild populations of medicinal plants, like ginseng and purple coneflower, by integrating those plants into their installations. Cultivation of rare and endangered plants can help to reduce pressure to harvest wild populations, while at the same time helping to ensure the survival of these species. Raising awareness of the threat of extinction in the wild through educating clients also can develop wild populations of plants as well as help people realize the many levels of value these plants provide. Organizations like United Plant Savers, as well as local native plant societies and botanic gardens, can be important resources to consult for guidance and education (Todd, 2014). The market for ornamental plants used in the landscaping in Vietnam in general and in Ho Chi Minh City, in particular, has not yet taken advantage of the use of medicinal plants. Research on surveying ornamental plants with medicinal value at the Zoo and Botanical Garden, Ho Chi Minh City was carried out in order to find out the benefits which can contribute to the beauty of the landscape and help people easily treat diseases quickly by growing them in-house. The results of the research have shown that medicinal plants are suitable for use in landscaping, contributing to bringing medicinal plants closer to people's lives.

2. Materials and Methods

2.1. Research subjects

Ornamental plants with medicinal value were grown at the Saigon Zoo and Botanical Garden in Ho Chi Minh City, Vietnam.

2.2. Research methods

Document reference methods: collecting documents and information related to ornamental plants with medicinal value through books and reports to select important information for research.

Field survey methods: a survey of ornamental plants with medicinal value at the Saigon Zoo and Botanical Garden in Ho Chi Minh City. The survey was conducted through survey sheets. The form of the survey includes survey sheet numbers, photo numbers, investigation dates, enumerators, common names, scientific species names, plant families, and morphological descriptions.

Identification: using pictures taken in the field to compare the morphological characteristics to identify the names of species and plant families based on reference databases such as Vietnam Bonsai Resources by Tran (2016), Vietnamese herbs by Pham (2002), Vietnamese medicinal plants and herbs by Do (2004), documents on Vietnamese medicinal plants by Vo (1998, 2012), Poisonous plants in Vietnam by Tran & Pham (2004).

Methods of data processing and visualization: the collected data were processed using Microsoft Excel. Descriptive statistics (e.g., the number and percentage (%) of different choices) together with visualization items were then provided to comprehensively communicate the results of the survey.

3. Results and Discussion

3.1. The composition of ornamental plants with medicinal value is popular at the Zoo and Botanical Garden

The survey results showed that ornamental plants in the Zoo and Botanical Garden were very diverse in species. Accordingly, ornamental plants

Table 1. Statistics on the number of ornamental plants with medicinal value in the Saigon Zoo and Botanical Garden

No.	Scientific Family	Number of Species	No.	Scientific Family	Number of Species
1	Acanthaceae	2	31	Lecythidaceae	1
2	Agavaceae	1	32	Liliaceae	1
3	Alismataceae	1	33	Magnoliaceae	1
4	Alliaceae	1	34	Maliaceae	2
5	Amaranthaceae	2	35	Malvaceae	3
6	Amaryllidaceae	3	36	Marantaceae	1
7	Anacardiaceae	2	37	Moraceae	5
8	Annonaceae	1	38	Myrtaceae	1
9	Apiaceae	2	39	Nephrolepidaceae	1
10	Araceae	3	40	Nymphaeaceae	2
11	Araliaceae	3	41	Orchidaceae	1
12	Aramanthaceae	1	42	Oxalidaceae	1
13	Asclepiadaceae	2	43	Papilionaceae	2
14	Asparagaceae	4	44	Piperaceae	1
15	Asteraceae	6	45	Plumbahinaceae	1
16	Bignoniaceae	2	46	Poaceae	1
17	Cactaceae	1	47	Polygalaceae	1
18	Campanulaceae	2	48	Polygonaceae	2
19	Caricaceae	1	49	Portulacaceae	1
20	Clusiaceae	1	50	Rosaceae	2
21	Combretaceae	1	51	Rubiaceae	5
22	Commelinaceae	2	52	Rutaceae	3
23	Dracaenaceae	1	53	Scrophulariaceae	3
24	Eucommiaceae	1	54	Solanaceae	1
25	Euphorbiaceae	3	55	Trilliaceae	1
26	Fabaceae	7	56	Tropaeolaceae	1
27	Ginkgoaceae	1	57	Verbenaceae	2
28	Iridaceae	1	58	Vitaceae	1
29	Lamiaceae	5	59	Zingiberaceae	7
30	Lauraceae	2			

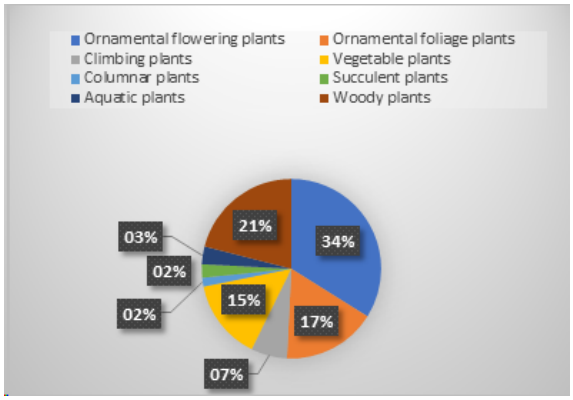

Figure 1. Percentage of ornamental plants with medicinal value in the Saigon Zoo and Botanical Garden – Ho Chi Minh City by morphological characteristics.

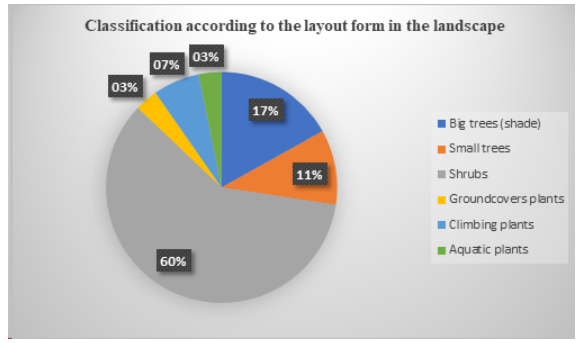

Figure 2. Percentage of ornamental plants with medicinal value at The Saigon Zoo and Botanical Garden according to the layout characteristics in the landscape.

in the Botanical Garden had 223 species belonging to 81 plant families, of which the most was the *Fabaceae* with 17 species. Of the 223 species, 124 species were medicinal plants, accounting for 55.6% of the total number of ornamental plants. The statistics in Table 1 show that, out of 124 medicinal plant species in the Zoo and Botanical Garden, belonging to 59 families, the families with the largest number of species were the *Fabaceae* and the *Zingiberaceae* family with 7 species each.

3.2. Grouping of ornamental plants with medicinal value

3.2.1. Grouping according to morphological characteristics

According to Tran (1998) and Pham (2002), ornamental plants with medicinal effects at the Zoo and Botanical Garden were divided into eight groups: ornamental flowering plants, ornamental foliage plants, climbing plants, vegetable plants, columnar plants, and succulent plants, aquatic plants, and woody plants. The main ornamental plants with medicinal properties were those in the group with ornamental flower plants, accounting for 33.9% with 42 species, the group with little medicinal properties was the columnar group, accounting for 1.6% with 2 species (Figure 1).

3.2.2. Classification according to the layout form in the landscape

Based on Han (1996) guideline on the arrangement of plants in the landscape, ornamental plants in the Botanical Garden were classified into six groups according to their arrangement in the landscape: big trees (shade), small trees, shrubs, groundcovers, climbing plants and aquatic plants. Through looking up the documents of Pham (2002), Tran (2016), the number of medicinal species according to the morphology planted in the landscape was calculated (Figure 2). Accordingly, the species with the highest proportion (59.7%) was the group of shrubs with 74 species, and the group with the lowest percentage is the group of groundcover plants (4 species) and aquatic plants (4 species) with the rate of 4 species (3.2%).

3.2.3. Classification according to the outdoor and indoor planting location

Based on plant morphology and physiology, combined with a quick interview survey, the research team classified plants into two groups: light-loving plants and shade-tolerant plants. The results of Figure 3 showed that the number of outdoor species was more dominant than the indoor species. There were 99 species of outdoor plants, accounting for 79.8%, while indoor plants had 25 species, accounting for 20.2%.

Knowing the ability of plants to live according to light needs and applying them to landscape design to arrange them in indoor or outdoor locations such as balconies, terraces, front gardens and behind the house (outdoor plants) are extremely important. With only 20.2% of the investigated medicinal plant species being shade-tolerant, this number was not diverse in the arrangement of indoor plants. However, through research on artificial light (led light), which can re-

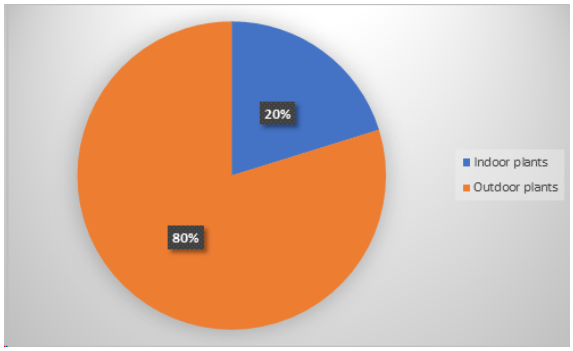

Figure 3. Percentage of ornamental plants with medicinal value at the Zoo and Botanical Garden according to their indoor and outdoor suitable characteristics.

place sunlight in providing light waves for photosynthetic plants, we can diversify indoor plant species. It is possible to arrange outdoor plants in the indoor environment with suitable positions by using lights to illuminate the plants. (Salama, 2021).

3.2.4. Classification according to the medicinal uses of the investigated ornamental plants

Based on medicinal plant books by Vo (1998, 2012) and Do (2004), the investigated medicinal plant species were classified into 14 different groups of medicinal lants (Table 2). The results in Table 2 showed that the group of ornamental plants with the effect of treating diseases of the stomach and digestive system accounted for the largest number with 25 plant species belonging to 18 plant families. The *Fabaceae* and *Zingiberaceae* families had the highest number with 3 species, the *Poaceae*, the *Moraceae*, the Asteraceae and the *Anacardiaceae* had 2 species. The group of plants that were used to treat nosebleeds and stop bleeding accounted for the smallest number with 1 species *Bletilla striata* (Thunb.) Reichb.f. belonging to the *Orchidaceae* family.

4. Conclusions

In the Saigon Zoo and Botanical Garden in Ho Chi Minh City, ornamental plants with medicinal values were very diverse in species. It was found that there were 124 species of ornamental plants with medicinal values belonging to 59 plant families at the Botanical Garden. Among them, *Fabaceae* and *Zingiberaceae* families were the most abundant with 7 species present. Ornamental plants with medicinal values can be grouped on the basis of plant morphology, landscape layout, outdoor and indoor suitability and medicinal uses so that they would be used in the landscaping of townhouses and apartments.

Table 2. Classification of ornamental plants at the Zoo and Botanical Garden according to medicinal uses

No.	Medicinal uses	Number of species
1	Sedation, insomnia	2
2	Fever	11
3	Women's disease	13
4	Skin Infections	8
5	Stomach and intestinal diseases	25
6	Blood pressure disease	5
7	Lung disease	12
8	Kidney disease	15
9	Diseases of the heart and circulatory system	3
10	Osteoarthritis	9
11	Inflammatory disease, swelling	3
12	Stop bleeding, nosebleed	1
13	Cure snake bites	2
14	Clear heat, detoxify	15

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by funding (Code: CS-CB21-MTTN-10) from Nong Lam University, Ho Chi Minh City, Vietnam. We thank Hong Xuan Do for his support and comments on the early draft of this manuscript.

References

Do, T. L. (2004). *Vietnamese medicinal plants and herbs.* Ha Noi, Vietnam: Medical Publisher.

Hamilton A. C. (2008). *Medicinal plants in conservation and development: case studies and lessons learn.* Retrieved April 1, 2018, from https://www.researchgate.net.

Haridasan, K., Ganesh Babu, N. M., Bhatti, R. D., UnniKrishnan, P. M., & Harirammoorthy, G. (2017). *Gardening and gardening options with medicinal plants.* Bangalore, India: Foundation for Revitalisation of Local Health Traditions.

Han, T. N. (1996). *Landscape architecture*. Ha Noi, Vietnam: Construction Publisher.

Joy, P. (1998). *Medicinal plants*. Kerala, India: Kerala Agricultural University.

Nguyen, M. K. (2021). *Conservation solutions of medicinal plant*. Retrieved August 1, 2021, from https://vietwiki.vn/

Pham, H. H. (2002). *Vietnamese plants*. Ho Chi Minh City, Vietnam: Young Publisher.

Salama, Y. (2021). *Can you grow plants in artificial light?* Retrieved November 13, 2021, from https://www.scienceabc.com.

Todd, L. (2014). *The case for native medicinal plants in the landscape*. Retrived August 14, 2014, from https://www.ecolandscaping.org.

Tran, C. H., & Pham, H. (2004). *Poisonous plants* in Vietnam. Ha Noi, Vietnam. Medical Publishing House

Tran, H. (2016). *Bonsai resources in Vietnam*. Ho Chi Minh City, Vietnam: Agricultural Publishing House.

Tran, H. (1998). *Trees and ornamental plants in Saigon*. Ho Chi Minh City, Vietnam: Epoch Publisher.

Tran, N. T. (2007). *Vietnam history Q & A*. Ho Chi Minh City, Vietnam: Young Publisher.

Vo, V. C. (2012). *Dictionary of Vietnamese medicinal plants*. Ha Noi, Vietnam: Medical Publishing House.

Vo, V. C. (1998). *Vegetables for medicinal purposes*. Dong Thap, Vietnam: Dong Thap General Publishing House.