



Growth Performance and Intestinal Health of Broiler Chickens Supplemented with Coriander Oil Nanoemulsion in Drinking Water

K. Sholiha^a, N. D. Dono^a, B. Ariyadi^b, & Zuprizal^{a,*}

^aDepartment of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada

^bDepartment of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada

Jalan Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia

*Corresponding author: zuprizal@ugm.ac.id

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ABSTRACT

This research was conducted to investigate the effects of coriander (*Coriandrum sativum*) essential oil nano emulsion (NCSEO) in drinking water on growth performance and intestinal health (intestinal microbiota and histomorphology) of broiler chickens. A total of 180 one-day-old male broiler chickens were randomly allocated into five treatments with four replicates and 9 chicks per replicate pen. Birds were provided NCSEO via drinking water. The treatments applied were as follows: without any addition (T0), drinking water added with 50 ppm antibiotic tetracycline (T1), drinking water added NCSEO at 25 $\mu\text{L/L}$ (T2), 50 $\mu\text{L/L}$ (T3), and at 100 $\mu\text{L/L}$ (T4). Variables observed were characteristics of NCSEO, growth performance, intestinal microbiota (LAB and *Salmonella* spp.), and histomorphology of intestine (villus height, villus width, crypt depth, villus height to crypt depth ratio). All collected data were analyzed using ANOVA in a completely randomized design. Results showed that the particle size of NCSEO was 13.1 nm with PDI 0,257, zeta potential -5.65 mV, stable, and spherical shape. The addition of NCSEO improved ($p < 0.05$) final body weight, weight gain, and FCR, while no significant differences in feed intake and water intake, increased the LAB population ($p < 0.05$) whereas *Salmonella* spp. was not found, and stimulated ($p < 0.05$) villus height and width. The addition of NCSEO at 50 $\mu\text{L/L}$ resulting the highest promoting effect on growth performance, intestinal microbiota, and intestinal histomorphology of broiler chickens. It can be concluded that 50 $\mu\text{L/L}$ of NCSEO as an alternative to antibiotics can be supplemented in drinking water to improve the growth performance and intestinal health of broiler chickens.

Keywords: broiler chicken; coriander oil nanoemulsion; growth performance; histomorphology; microbiota

INTRODUCTION

The increasing growth of the world population and its food economy has resulted in a significant proportion of the increase in animal production and consumption of poultry meat. The OECD-FAO Agricultural Outlook 2020–2029 predicted that global chicken meat consumption will increase to 145 million tons in 2029 and contribute to approximately 50% of the total increased global meat consumption (FAO, 2020). Consequently, the poultry industry faces an enormous challenge in maintaining optimum productivity. In the poultry industry, the gastrointestinal system's health plays a significant role in reaching optimal production. Gut health affects digestion, absorption, transportation, and secretion of nutrients, intestinal barrier and homeostasis, immune response, protection from pathogen, and inflammatory balance (Kallam & Sejian, 2021).

For several decades, antibiotic growth promoters (AGPs) became essential to the growing poultry industry to support healthy and efficient chicken production. Nevertheless, the prolonged use of antibiotics has

resulted in bacterial resistance and the accumulation of residues in animal products. Indonesian government has banned the use of antibiotics as a growth promoter in animal since January 1, 2018, based on the Regulation Law of the Minister of Agriculture No. 14/PERMENTAN/PK.350/5/2017. Therefore, several alternatives to growth-promoting antimicrobials have been investigated in recent years. Among those possible alternatives, Phyto biotic feed additives (PFA) are the compounds derived from the plants, including herbs, spices, and essential oils that have shown promising effects on poultry production. Some of the Phyto biotics stimulate the growth and development of beneficial bacteria, improve immunity and antioxidant status, protect intestinal cells by altering the membrane permeability, increase feed intake, and have antibacterial effects (Fascina *et al.*, 2012; Cho *et al.*, 2014).

Coriander seed essential oil (*Coriandrum sativum*) is a type of Phyto biotics that contains the major natural compound of linalool (60%–80%). It has been reported in previous studies for its antibacterial activity against *Escherichia coli*, *Salmonella typhimurium*, *Salmonella*

enterica, and *Campylobacter jejuni* (Lalitha *et al.*, 2011). However, there are some limitations to their oral use as an efficient antimicrobial agent, such as volatility, less solubility, and high instability.

The recent application of efficient delivery strategies is in a processed product called a nano emulsion. Self-nano emulsifying drug delivery system (SNEDDS) is a nanocarrier for poorly water-soluble that forms spontaneously from the mixture of oil, surfactant, co-surfactant, and an active compound in a homogenous oil-in-water nano emulsion liquid form (Buya *et al.*, 2020). The ability of this system, with relatively ranges between 1-100 nm in size, could improve the solubility, provide a larger interfacial area while enhancing the rate of absorption, and provide a better enzymatic and chemical stability (Patel *et al.*, 2011; Singh *et al.*, 2015).

Accordingly, this present research was conducted to investigate the effects of *Coriandrum sativum* essential oil nano emulsion (NCSEO) in drinking water on growth performance, intestinal microbiota, and histomorphology of broiler chickens.

MATERIALS AND METHODS

Animal, Diets, and Experimental Design

All experimental procedures applied in this study were approved by the Research Ethics Committee of Universitas Gadjah Mada with approval number 00098/EC-FKH/Eks./2021.

The research was carried out at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. A total of 180 one-day-old male broilers of the New Lohmann (MB 202 Platinum) strain were used in this study. The initial body weight of each chick (40.35±0.5 g) was taken, recorded, and randomly allocated into 5 treatment groups with 4 replicates (9 chicks in each replicate). Broilers were housed in an electrically heated, thermostatically controlled, and maintained on a 24-h constant light schedule. Broilers were reared in clean pens under standard hygienic conditions and equipped with a feeder and drinking water bowl. They were allowed *ad libitum* access to the experimental diets

and drinking water starting from days 8-35. The experimental drinking water was administered every day. The diets were fed in the mash form. The basal corn-soybean meal diet (Table 1) was formulated to meet or exceed nutrient requirements for broilers based on the recommendation of the National Research Council (1994). Birds were provided NCSEO via drinking water. The treatments applied were as follows: without any addition (T0), drinking water added with 50 ppm antibiotic tetracycline (T1), drinking water added NCSEO at 25 µL/L (T2), 50 µL/L (T3), and at 100 µL/L (T4).

Formulation of Nano Emulsion

The nano emulsion of NCSEO was formulated by stirred (Cimarec Digital Stirring Hot Plate SP131320-33Q, Waltham, USA) at 300 rpm for 15 minutes and conditioned in a water bath (Mettler, Schwabach, Germany) at 45 °C for 15 minutes (Abouelkassam *et al.*, 2015; Ali & Hussein, 2017) using 12.68% of coriander oil, 4.22% virgin coconut oil, 68.81% Tween 80, and 14.29% polyethylene glycol 400, respectively. The optimum formula was determined using Design Expert® software version 11x.1.5. (Stat-Ease, Inc., Minneapolis, MN USA).

Characterization of Nano Emulsion

Stability test. The stability test was carried out in 3 stages: heating-cooling, freeze-thaw cycle, and centrifugation (Agrawal *et al.*, 2015). Heating-cooling was executed by storing the formula at the temperature of approximately 45 °C and then cooling at -21 °C with a storage time of 24 hours each. The freeze-thaw cycle was accomplished by storing at a freezing temperature of -21 °C, then thawed at 25 °C, with 24 hours of storage for each temperature. The stability test was carried out by centrifuging the formula using centrifugation (Hettich Zentrifugen EBA 20, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 3500 rpm for 30 minutes. Observation of stability parameters such as separation, precipitation, creaming, and cracking was carried out (Kassem *et al.*, 2016).

Table 1. Ingredients and chemical composition of the broiler chicken basal diet

Ingredients	Proportion (%)	CP (%)	ME (kcal/kg)	Ca (%)	Pav (%)	Lys (%)	Met (%)	Thr (%)
Yellow corn	58.00	5.17	1954.60	0.01	0.13	0.17	0.11	0.21
Soybean meal	28.50	12.72	734.45	0.08	0.18	0.83	0.19	0.46
Meat bone meal	7.00	3.27	177.10	0.67	0.33	0.19	0.04	0.11
Rice brand	2.60	0.31	75.07	0.00	0.03	0.01	0.00	0.01
Palm oil	2.00	0.00	176.80	0.01	0.01	0.00	0.01	0.00
Vitamin premix*	0.25	0.00	0.00	0.08	0.00	0.00	0.00	0.00
L-Lysine HCl (98%)	0.20	0.00	0.00	0.00	0.00	0.16	0.00	0.00
DL-methionine (99%)	0.20	0.00	0.00	0.00	0.00	0.00	0.20	0.00
CaCO ₃	1.00	0.00	0.00	0.34	0.00	0.00	0.00	0.00
NaCl	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.00	21.46	3118.02	1.19	0.67	1.35	0.54	0.78

Note: CP= Crude protein; ME= Metabolizable Energy, Ca= Calcium; Pav= Available Phosphor; Lys= Lysine; Met= Methionine; Thr= Threonine. Chemical compositions of the basal diet determined in a laboratory analysis based on AOAC (2005) and applied result for nutritious composition.*Vitamin premix chemical composition was Vit. A= 12,500,000 IU; Vit. D3= 2,500,000 IU; Vit. E= 10000 mg; Vit. K3= 2000 mg; Vit. B1= 2000 mg; Vit. B2= 4000 mg; Vit. B6= 1000 mg; Vit. B12= 12000 mcg; Vit. C= 40000 mg; Niacin= 40000 mg; Biotin= 200 mg.

Transmittance. Transmittance determination of NCSEO formulations was diluted with distilled water. Percent transmittance was measured using UV-vis spectrophotometer (Optima SP-3000 nano, Optima, Tokyo, Japan) at 650 nm using purified water as a blank (Patel *et al.*, 2011).

Self-emulsification time. Self-emulsification time of NCSEO formulations was carried out by adding 0.2 mL of NCSEO to 50 mL of Artificial Gastric Fluid (AGF) at 41 ± 0.5 °C, then rotated at 100 rpm. Emulsification time was assessed visually and recorded as the time required to obtain a clear dispersion (Balakumar *et al.*, 2013).

Particle size, polydispersity index, and zeta potential. Particle size, polydispersity index, and zeta potential were measured using a particle size analyzer (Horiba Scientific SZ-100, Horiba, Kyoto, Japan) by applying the dynamic light scattering (DSL-PSA) method. A total of 500 µL NCSEO was put into 5 mL distilled water (ratio 1:10 v/v). Light scattering was monitored at 25 °C with a 90° angle (Patel *et al.*, 2011; Abouelkassem *et al.*, 2015).

Nano morphology. The morphology of NCSEO was observed under transmission electron microscopy (JEM-1400, JEOL, Tokyo, Japan). The formula samples were prepared by diluting NCSEO with water (1: 1000 v/v). A sample drop was stained with 2% aqueous solution of phospho-tungstic acid for 30 s and placed on a copper grid. After natural drying, the samples were placed under TEM for observation (Dash *et al.*, 2015; Singh *et al.*, 2021).

Broiler Growth Performance

The initial broiler weights were determined at the beginning of the experiment. Feed intake and water consumption were recorded daily from days 8 to 35 throughout the study for each replicate within each treatment. Body weight was recorded weekly to compute body weight gain. Values of feed intake and body weight gain were used to calculate the feed conversion ratio (FCR). The mortality of birds was also recorded daily (day 8 to 35).

Intestinal Microbiota

At 35 days of age, chicken from each replicate was selected and slaughtered, the jejunal was dissected, and digesta was collected in sterilized sampling tubes. The jejunal digesta contents were kept on ice until inoculation and incubation. Digesta samples with approximately 1 g were squeezed from jejunum into 9 mL peptone water solution. The solution was mixed using vortex. The suspension was prepared from dilutions 10^{-1} and serial dilutions were done (10^{-2} to 10^{-9}). A total of 1 mL solution was removed from three dilutions and poured into petri dishes containing the medium. Lactic acid bacteria were counted on the MRS agar (Merck, Darmstadt, Germany, 2013), and plates were incubated at 37 °C for 24 h. and *Salmonella* spp. was quantified on the SSA agar (Merck, Darmstadt, Germany, 2013). Plates were incu-

bated for 24 h at 37 °C. Bacterial colony forming units (CFU) in the Petri dishes were counted using a colony counter (Taha *et al.*, 2019).

Intestinal Histomorphology

At the end of the experiment, on day 35, tissue samples were taken from mid-jejunum (approximately 2 cm). The jejunum sample was separated from the end duodenal loop up to 1 cm close to the junction of Meckel's diverticulum. The samples were fixed in 10% buffered formalin for 48h. Tissue samples of jejunum were dehydrated by transferring to a series of alcohol with increasing concentrations (70%, 80%, 90%, and 100%), placed into xylol solution, and embedded in paraffin wax. Transverse and longitudinal sections with 4 µm thickness were stained with Hematoxylin-Eosin, and examined under the electron transmission microscope (Optilab Advance, Miconos, Yogyakarta, Indonesia). Villus height was measured from the tip to the crypt junction, and crypt depth was defined as the depth of the invagination between adjacent villi (Ariyadi *et al.*, 2013).

Statistical Analysis

All collected data were subjected to statistical analysis using one-way analysis of variance, and mean differences among treatments were evaluated by Duncan's multiple range test using the IBM SPSS Statistics Version 25 (SPSS Inc., Chicago, IL) statistical software. The significance was considered at 5% probability.

RESULTS

Characterization of Nano Emulsion

Observations of stability indicated that NCSEO passed three stages of stability and remains stable, characterized by the formation of sediment; there was no separation, precipitation, cracking, or creaming. The NCSEO formula was good to have a clear visual sighting with a transmittance $99.90\pm 0.05\%$ and self-emulsification time 48.08 ± 0.57 s. The average particle size was 13.05 ± 0.07 nm, while the PDI obtained was 0.257 ± 0.05 . The NCSEO resulted in zeta potential of -5.65 ± 0.07 , and the droplets appeared dark and the surroundings were bright. No signs of drug precipitation were observed, indicating the stability of the formed nano emulsion, which were in the nano-size range, relatively uniform in shape, and existed as spherical particles.

Broiler Growth Performance

The effects of NCSEO supplemented in drinking water on growth performance are shown in Table 2. The NCSEO treatments improved ($p<0.05$) final body weight, weight gain, and FCR compared to broiler chickens receiving the negative control. Supplementation of 50 µL/L NCSEO resulting the best growth-promoting effect, whereas antibiotics as a positive control were the highest. No differences were found

in feed intake, water intake, as well as mortality rates during the experimental period.

Intestinal Microbiota

The addition of NCSEO in drinking water influenced ($p < 0.05$) the LAB population in broiler chickens (Table 3). Drinking water containing 50 $\mu\text{L/L}$ of NCSEO showed the highest LAB population compared to other groups. The antibiotic as a positive control had a lower jejunal microbiota population in comparison with the NCSEO treatments. The population of *Salmonella* spp. in jejunum of broiler chickens was not found in all treatments except in negative control.

Intestinal Histomorphology

Table 4 shows that supplementation of NCSEO significantly ($p < 0.001$) stimulated villus height and width, whereas there was no difference on V:C ratio. The addition of NCSEO showed the highest crypt compared to the other treatments due to the increasing villus height and width. Broiler chickens receiving 50 $\mu\text{L/NCSEO}$ resulted in the greatest villi height and width.

DISCUSSION

Characterization of Nano Emulsion

Stability is the important factor that determines the quality of nano emulsions. NCSEO was stable under heating-cooling, freeze-thaw cycle, and centrifugation, indicated by the absence of precipitation, cracking, or creaming. Senapati *et al.* (2016) reported that stability is examined by exposing the formulations to a heating-cooling cycle as well as a freeze-thaw cycle expressed by the absence of turbidity, phase separation, or drug precipitation signs. A high transmittance value is representative of the clarity level of the dispersion system based on the absorbance of the water solution. Evaluation of NCSEO formulations produced transmittance values above 95%, confirming formulations have fulfilled the requirement of nano emulsion (Costa *et al.*, 2014). Hence, the rate of emulsification becomes a principal sign for the assessment of the efficiency of self-emulsification. The efficacy of self-emulsification determined by the formula should disperse completely and spontaneously when subjected to water dilution under mild stirring in a simulated gastric environment.

Table 2. The performance of broiler chickens fed with different levels of *Coriandrum sativum* essential oil nano emulsion (NCSEO)

Variables	Treatments					SEM	P-value
	T0	T1	T2	T3	T4		
Feed consumption (g/bird/35 days)	2932.89	2889.44	2983.21	2942.38	2956.38	31.79	0.937
Final body weight (g/bird/35 days)	1903.61 ^c	2157.92 ^a	1982.08 ^{bc}	2070.34 ^{ab}	1952.14 ^{bc}	26.41	0.004
Weight gain (g/bird/35 days)	1722.11 ^c	1969.89 ^a	1801.11 ^{bc}	1888.84 ^{ab}	1774.59 ^{bc}	25.72	0.004
Feed conversion ratio	1.70 ^a	1.47 ^c	1.66 ^{ab}	1.56 ^{bc}	1.67 ^{ab}	0.020	0.008
Water consumption (L/bird/35 days)	6540.02	6687.11	6434.23	6599.52	6381.79	55.82	0.454
Mortality rate	0.25	0.00	0.00	0.00	0.25	0.068	0.573

Note: T0= Negative control without feed additive, T1= Positive control with antibiotic tetracycline, T2= 25 $\mu\text{L/L}$ of NCSEO, T3= 50 $\mu\text{L/L}$ of NCSEO, T4= 100 $\mu\text{L/L}$ of NCSEO. ^{abc}Means with different superscripts in the same row differ significantly ($p < 0.05$).

Table 3. The jejunal microbiota of broiler chickens fed with different levels of *Coriandrum sativum* essential oil nano emulsion (NCSEO)

Species	Treatments					SEM	P-value
	T0	T1	T2	T3	T4		
LAB (Log cfu/g)	6.09 ^b	7.14 ^{ab}	7.43 ^{ab}	7.69 ^a	7.20 ^{ab}	0.227	0.217
<i>Salmonella</i> spp. (Log cfu/g)	4.90 ^a	0 ^b	0 ^b	0 ^b	0 ^b	0.651	0.035

Note: T0= Negative control without feed additive, T1= Positive control with antibiotic tetracycline, T2= 25 $\mu\text{L/L}$ of NCSEO, T3= 50 $\mu\text{L/L}$ of NCSEO, T4= 100 $\mu\text{L/L}$ of NCSEO. ^{abc}Means with different superscripts in the same row differ significantly ($p < 0.05$).

Table 4. The histomorphology of jejunal intestine of broiler chickens fed with different levels of *Coriandrum sativum* essential oil nano emulsion (NCSEO)

Item	Treatments					SEM	P-value
	T0	T1	T2	T3	T4		
Villus height (μm)	1097.86 ^d	1139.08 ^c	1170.41 ^b	1259.94 ^a	1143.86 ^{bc}	12.911	<0.001
Villus width (μm)	161.05 ^{cd}	157.18 ^d	165.87 ^b	186.07 ^a	163.59 ^{bc}	2.371	<0.001
Crypt depth (μm)	124.92 ^c	128.61 ^b	131.96 ^b	139.89 ^a	130.27 ^b	1.277	<0.001
V:C ratio	8.82	9.06	8.89	9.09	8.85	0.057	0.468

Note: T0= Negative control without feed additive, T1= Positive control with antibiotic tetracycline, T2= 25 $\mu\text{L/L}$ of NCSEO, T3= 50 $\mu\text{L/L}$ of NCSEO, T4= 100 $\mu\text{L/L}$ of NCSEO. ^{abc}Means with different superscripts in the same row differ significantly ($p < 0.05$).

It was exhibited that NCSEO had emulsification time < 1 m and the transparent emulsion was discovered, thus approving the formation of good nano emulsion (Bandyopadhyay *et al.*, 2012). Agrawal *et al.* (2015) explained the rapid emulsification is likely related to the ease of water penetration into the various liquid formed on the surface of the globule.

Nano emulsion particle size is considered a decisive factor in self-emulsification performance because it determines the rate and extent of drug release and absorption (Parmar *et al.*, 2011; Kumar *et al.*, 2014). The mean droplet size of NCSEO formulation was found to be in nanometric <100 nm with PDI value close to 0, indicating narrow size distribution (Gupta *et al.*, 2011) and zeta potential range less than -30 mV and more than +30 conferring physical stability to the system (Kurpiers *et al.*, 2020). Therefore, the negative charge on zeta potential was possible due to the presence of fatty acids in the surfactant and co-surfactant, which were used to develop the formulation (Balakumar *et al.*, 2013; Tripathi *et al.*, 2016). The morphology of NCSEO exhibited a spherical shape, homogeneous droplets smaller than 50 nm, and no signs of drug precipitation, inferring the stable nature of formed nano emulsions.

Broiler Growth Performance

In this study, the body weight, weight gain, and FCR had significant effects of the supplementation of NCSEO. The addition of NCSEO improved 5% of body weight compared to the control group. These results correspond with previous studies (Hamodi *et al.*, 2010; Rashid *et al.*, 2014); this is probably due to the antibacterial properties which act as natural growth promoters (Pathak *et al.*, 2011). The difference in growth performance caused by nano emulsion of essential oils may be attributable to the concentrations of the active substances and their biological activities, respectively (Applegate *et al.*, 2010). In the current study, the main active compound of coriander essential oil is linalool, which is closely related to the growth, development, and health of the digestive tract. Moreover, essential oils stimulate saliva production, secretion of digestible enzymes such as amylase, trypsin, chymotrypsin, and lipase (Hashemipour *et al.*, 2013), also enhance bile production resulting in improving the performance and digestibility, enhancing the digestion and utilization of protein in the intestine, and increase *Lactobacillus* spp. count (Malayoğlu *et al.*, 2010; Masouri *et al.*, 2017).

The balance of the microflora population in the gut is the most important characteristic of a well-functioning gut, increasing nutrient absorption and utilization rate (Soumeh *et al.*, 2019). Essential oil containing linalool stimulates the secretion of the high amount of intestinal mucus covering the whole intestinal tract, protecting the intestinal surface from invasion by harmful pathogens and facilitating nutrient transport and absorption (Ghazanfari *et al.*, 2015). Therefore, dietary ingredients that affect microbiota can indirectly affect intestinal absorption and, thus, the production performance of the chickens (Pan & Yu, 2014).

In contrast, the feed intake, water intake, and mortality rate were not affected by NCSEO treatments, and these results were in agreement with the other studies (Abadi & Andi, 2014; Abou-Elkhair *et al.*, 2014; Rashid *et al.*, 2014; Khubeiz & Shirif, 2020). This finding may be attributed to the chemical composition, organoleptic such as flavor and palatability, the form of the basal diets, and also the applied dosage of the respective ingredients. In addition, the response of chickens to a PFA might be affected by other factors, such as diet type, animal age, hygiene, environmental factors, and product quality (Amad *et al.*, 2011).

Intestinal Microbiota

The present study clearly shows that broiler chickens supplemented with NCSEO increased the population of *Lactobacillus* spp. whereas *Salmonella* spp. was not found. This current study, in agreement with Ningsih *et al.* (2018), reported no population of *Salmonella* spp. was detected in the intestinal mucosa that received nano encapsulated *Phaleria macrocarpa* fruits extract in drinking water. The increasing LAB population may be related to the major active component in coriander essential oil, which is linalool has the ability to disintegrate the bacterial membrane, leading to the release of membrane-associated material from the cell to the external medium (Keskin & Toroglu, 2011). In line with the current findings, Hosseinzadeh *et al.* (2014) reported that treatments of coriander EO reduced intestinal lesions, and modulated intestinal health by promoting the growth of beneficial bacteria and inhibiting the growth of harmful bacteria.

Moreover, gut microbiota contributes to the maintenance of the normal physiological function of the intestine, providing a series of beneficial effects on their hosts, such as nutrient digestion and protection from invasive pathogens (Shang *et al.*, 2020). The mechanism of inhibition maybe due to the NCSEO, which partition lipids in the bacterial cell wall, disturbing the structures, permeating the cell membranes and mitochondria and their absorptivity to active compound, and dissipating the pH gradient in the bacterial cell (Mohebodini *et al.*, 2021). Reducing the populations of certain pathogenic microbial species may also be associated with acidification of the gut contents (Gopi *et al.*, 2014). In addition, Malayoğlu *et al.* (2010) reported that linalool is terpene alcohol and may display broad-spectrum antimicrobial activities; they are also able to stimulate digestive enzyme secretion and activity and increase bile synthesis. In this study, inhibiting harmful bacteria boosted the proliferation and metabolism of positive bacteria, providing the substrate for the growth and proliferation of lactic acid-producing bacteria in the intestinal such as *Lactobacillus* spp. which utilizes bioactive compounds of essential oils (Murugesan *et al.*, 2015). Further investigation, Choi *et al.* (2015) observed that essential oil products stimulate intestinal production of mucus in broiler chickens, an effect that was assumed to impair the adhesion of pathogenic bacteria such as coliforms and *Escherichia coli* and thus contribute to stabilizing the microbial eubiosis in the gut of the animals.

Intestinal Histomorphology

Supplementation of NCSEO in drinking water resulted in altering villus height and villus width. Barbarestani *et al.* (2020) explained that dietary essential oil could improve the gut structure, increasing the dendritic cells absorption capacity in the intestinal lumen and stimulating epithelial to release the mucosal cytokines. Bioactive substances of coriander essential oil may influence the digestive system by stimulating digestive enzymes and appetite as well as balancing the intestinal microbial population. The essential oil is assumed as antibacterial against *Salmonella* spp. growth which produces toxins that can disrupt the intestinal structure, as well as reducing pathogen bacteria, might also indirectly stimulate the improvement in villus and crypt structure (Hosseinzadeh *et al.*, 2014).

According to Hashemipour *et al.* (2013) reported that the essential oil increased bile acid secretion and influenced intestinal morphology by stimulating the release of digestive enzymes from the pancreas and intestinal mucosa, such as trypsin and amylase. Dietary supplementation with coriander oil increased amylase concentrations and found longer villi in the broiler intestine (Ghazanfari *et al.*, 2015). It is believed that the increased villus height and villus width by supplemented NCSEO were correlated to the increased epithelial cell mitosis by enhancing digestive and absorptive functions of nutrients.

The current study showed that supplementation of NCSEO resulted in the highest crypt as increasing villus height and width; otherwise V:C ratio was not affected. However, the addition of NCSEO increased V:C ratio compared to the other group. Reisinger *et al.* (2011) reported that a shortening of villi and low V:C ratio may indicate poor nutrient absorption and lower performance. An increased in the villus height and V:C ratio is directly correlated with activated cell mitosis and increased epithelial cell turnover. Decreased crypt depth was associated with effective nutrient absorption and performance. The beneficial effects of NCSEO on intestinal health also might be related to anti-inflammatory effects, dietary essential oil resulting in the reduction of oxidative tissue damage, and protection against disruption of the gut microbiota composition accompanying intestinal inflammation, thereby affecting intestinal morphology (Du *et al.*, 2016).

CONCLUSION

Supplementation of NCSEO in the drinking water enhances the final body weight, weight gain, FCR, increases LAB population, and stimulates intestinal morphology. Dietary 50 µL/L of NCSEO as an alternative to antibiotics had a positive impact and can be supplemented in drinking water to improve the growth performance and intestinal health of broiler chickens.

CONFLICT OF INTEREST

The researchers state that there is no conflict of interest with any financial, personal, or other relation-

ships with other people or organizations related to the material discussed in the manuscript.

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