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NANOEMULSIFIED CHIVE (*Allium schoenoprasum* L.) ESSENTIAL OIL IN DRINKING WATER IMPROVES GROWTH AND GUT HEALTH OF BROILERS UNDER COLD-HUMID STRESS

Hoang Van SON[1], Ngo Mau DUNG[2], Phan Vu HAI[2]

[1]*Department of Animal Science, Faculty of Agriculture, Forestry and Fisheries, Hong Duc University, Thanh Hoa province, Vietnam*
[2]*Faculty of Animal Science and Veterinary Medicine, University of Agriculture and Forestry, Hue University, Hue city, Vietnam*

Email: phanvuhai@hueuni.edu.vn

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ABSTRACT: This study evaluated whether supplementing drinking water with a nanoemulsion of chive (*Allium schoenoprasum* L.) essential oil could improve growth performance and intestinal health of broiler chickens reared under cold and humid stress conditions. One hundred and eighty one-day-old colored Rilai male broiler chicks were brooded for four weeks and then allocated to five treatments (four replicates, nine birds/replicate) for an eight-week trial: an unsupplemented negative control, a positive control receiving tetracycline (50 ppm), and three nanoemulsion doses (25, 50, and 100 µL/L). Chicks were housed on wet litter during a cold-humid period (approximately 12-15°C; 68-75% relative humidity). Compared with the negative control, supplementation at 50 µL/L improved final body weight and weight gain and reduced feed conversion ratio ($P < 0.05$), while feed intake, water consumption, and mortality were unchanged ($P > 0.05$). Nanoemulsion supplementation increased lactic acid bacteria counts in ileocecal digesta and reduced *Salmonella* spp., with no detectable colonies in supplemented groups. Jejunum histomorphology results showed greater villus height and villus width and deeper crypts in the 50 µL/L group ($P < 0.01$). These findings indicate that nanoemulsified chive essential oil supplied via drinking water, particularly at 50 µL/L, can support performance and intestinal health in broilers under cold-humid stress and may help reduce reliance on antibiotic growth promoters. Further studies are required to evaluate safety, dose tolerance, and possible adverse effects before broad field application.

Keywords: *Allium schoenoprasum*; Broiler chickens; Cold stress; Essential oil; Gut morphology; Intestinal microbiota.

INTRODUCTION

In intensive broiler production, housing microclimate fluctuations represent key stress factors that directly impact productivity, health, and animal welfare. In addition to heat stress, cold stress (environmental temperatures below the thermoneutral zone) causes substantial losses through elevated thermogenesis, metabolic alterations, and immune suppression (Akinyemi and Adewole, 2021). Notably, cold stress can impair gut structure, compromise epithelial barrier integrity, and suppress antioxidant and inflammatory responses, thereby facilitating intestinal pathogen proliferation; these effects have been documented in recent broiler studies (Gao et al., 2025).

Meanwhile, high relative humidity exacerbates litter moisture and air contamination (e.g., NH_3), adversely affecting growth and feed utilization efficiency (Weaver and Meijerhof, 1991). Physiologically, high humidity at constant temperature can reduce growth of the chicks and modify mitochondrial glucose/ATP consumption in broilers (Zhou et al., 2019). The combination of low temperature and high humidity (cold-humid) serves as a realistic stress model, commonly encountered by producers during cold seasons in subtropical tropical regions, where ventilation is restricted to conserve heat, leading to moisture and gas buildup.

Amid efforts to reduce the use of growth-promoting antibiotics, phytogenic additives and essential oils are gaining attention as natural solutions to enhance digestion, immunity, and pathogen control. Chive (*Allium schoenoprasum* L.) is a spice herb from the *Allium* genus, rich in sulfur-containing compounds (sulfides, thiosulfinates), flavonoids, and antioxidants (Hai et al., 2025), with reported antibacterial and antioxidant properties (Dai et al., 2024; Hai and Khuong, 2025). Essential oils from the *Alliaceae* family generally (including chive) demonstrate antibacterial activity by interacting with bacterial cell membranes and increasing permeability; studies on local chive varieties also describe sulfide-rich essential oil compositions with antimicrobial effects (Hai et al., 2020). Broader Allium literature indicates that organosulfur compounds can interfere with microbial membranes and metabolism while also modulating host redox and inflammatory pathways (Putnik et al., 2019; Singh et al., 2025). This is particularly relevant under cold–humid stress because the proposed stress cascade involves two main processes: (i) oxidative damage and activation of inflammatory signaling in the intestinal epithelium, and (ii) disruption of the gut microbial balance (dysbiosis) that favors the expansion of opportunistic pathogens. In principle, chive essential oil could help attenuate cold–humid stress through convergent

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mechanisms: (i) exerting direct antimicrobial activity against enteric pathogens, thereby reducing pathogen-driven inflammation; (ii) supporting a more favorable gut microbiota, which indirectly strengthens colonization resistance; and (iii) modulating redox status and inflammatory responses, potentially preserving intestinal barrier integrity and improving nutrient absorption during stress exposure (Putnik et al., 2019). However, despite promising bioactivity, essential oils face practical constraints in poultry production due to volatility, poor water solubility, and instability during storage and mixing.

To address these delivery limitations, nanoemulsion technology has emerged as an enabling platform for lipophilic phytochemicals. Nanoemulsions can enhance dispersion and apparent solubility, protect actives from degradation, and improve contact with biological membranes, thereby increasing functional efficacy following oral administration (Buya et al., 2020). Reviews of nanoemulsions further highlight that nanometric droplet size increases surface area and can improve stability and bioavailability, helping to overcome the intrinsic limitations of essential oils (Barradas et al., 2021; Omidian et al., 2025). From an application standpoint, delivering phytogenic compounds through drinking water is attractive because it enables easy oral administration and may also help reduce microbial contamination in the water system, thereby complementing their gut-directed effects.

Accordingly, this study aimed to (i) characterize chive essential oil nanoemulsion (NC) and (ii) assess the effects of its supplementation in daily drinking water on growth performance, ileocecal microbiota, and intestinal morphology in broilers under cold-humid stress. It was hypothesized that NC, owing to its antibacterial and antioxidant activities, could alleviate the negative effects of cold-humid stress, enhancing feed efficiency and gut health parameters.

MATERIALS AND METHODS

Animals, diet, and experimental design

A total of 180 one-day-old male colored Rilai broilers were used. The chicks were brooded for 4 weeks, then randomly divided into five treatments to ensure uniformity in age, male-female ratio, initial body weight, care and feeding regime, and disease prevention. The coops were made of wire mesh (1 m x 1 m x 1 m), with a floor made of rice husk microbial bedding. The chicks were provided with sufficient feed and water throughout the experiment (3 months). The basic diet was managed in the form of a homemade mixed feed for chickens meeting the feed standards for broiler chickens of the Vietnam Ministry of Agriculture and Rural Development (10 TCN 661-2005). The feed composition included main ingredients such as corn, rice bran, anchovy meal, soybean meal 48%, oyster meal, vitamin premix, mineral premix, $CaCO_3$ powder, L-lysine, and DL-methionine. The experiment commenced at 1 month of age and continued until the end of 12 weeks of age. Birds were randomly assigned to 5 treatments with 4 replicates (9 birds/replicate):

CON: negative control (unsupplemented drinking water); AB: positive control (drinking water supplemented with 50 ppm tetracycline); 25NC: drinking water supplemented with NC at 25 μL/L; 50NC: drinking water supplemented with NC at 50 μL/L; 100NC: drinking water supplemented with NC at 100 μL/L

Temperature (°C) and humidity (%) in the coop were recorded daily using a Testo thermometer/humidity meter (Model 608-H1), suspended at a height of 0.3m in each coop. This data was automatically recorded every 5 minutes throughout the experiment using a HOBO data logger (Onset Computer Corporation, Pocasset, MA, USA) (Figure 1). According to Weaver and Meijerhof (1991), weeks with relative humidity (RH) ≥ 70% were classified as high-humidity conditions (orange alert), whereas weeks featuring prolonged low environmental temperatures combined with high RH were deemed severe cold-humid episodes (red alert). Based on recorded data, broilers experienced pronounced cold-humid stress during weeks with markedly low temperatures (11.6–16.6°C) and RH ~71–73% (e.g., weeks 5–6 and 8–10). Conversely, certain periods reflected humidity-induced stress even at rising temperatures (e.g., week 11: 22.2°C; RH 71.6%).

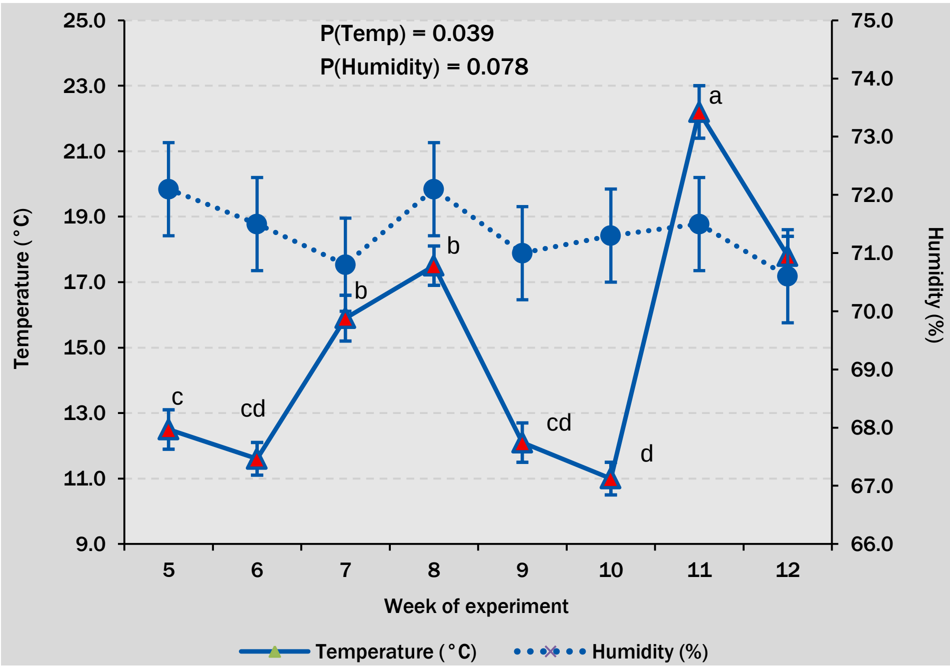

Figure 1 - Temperature and humidity throughout the experiment

Preparation of chive essential oil nanoemulsion

Chive (*Allium schoenoprasum*; GenBank ID: NC_057575.1), aged ~4.5 months and cultivated under VietGAP standards (TCVN 11892-1:2017), was used as a raw material. Essential oil was extracted from the bulbs following Hai et al. (2020) with minor modifications. Briefly, 200 g of fresh bulbs were macerated in Meizan Gold vegetable oil at a 1:5 (w/v) ratio and incubated at 60 °C with shaking (120 rpm) for 48 h. After filtration, the essential oil was separated using ethanol and recovered by vacuum evaporation under reduced pressure. The oil was dried over anhydrous Na_2SO_4 to obtain purified chive essential oil, yielding 2.04% (w/w). The nanoemulsion was prepared via magnetic stirring with an optimized ratio of 16.90% pure chive essential oil, 68.81% Tween 80, and 14.29% polyethylene glycol 400; the mixture was stirred at 300 rpm for 15 min and stabilized in a 45°C water bath for 15 min (Yani et al., 2020). Stability tests (heating–cooling cycles, freeze–thaw cycles, centrifugation), transmittance (650 nm), self-emulsification time in artificial gastric fluid, particle size/PDI/zeta potential (DLS), and morphology (TEM) followed standard nanoemulsion protocols (Vladisavljević 2019; Zhao et al., 2023).

Performance parameters

Feed and water intake were recorded daily per replicate; body weight was measured weekly. Weight gain, feed conversion ratio (FCR), and mortality rate were calculated for the experimental period (Quintana-Ospina et al., 2023).

Ileocecal microbiota

At experiment end, ileocecal digesta from representative birds per replicate were sampled to quantify LAB on MRS agar and *Salmonella* spp. on SSA agar, incubated at 37°C for 24 h, and expressed as log CFU/g, following ISO standards (ISO compilation, 2019).

Intestinal histology

Approximately 2 cm of the middle jejunum was excised, flushed with saline, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin (H&E). Villus height (VH) and crypt depth (CD) were measured using an optical microscope with image analysis software (Phan et al., 2025a).

Statistical analysis

Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS software (Version 22.0). Differences among treatment means were separated using Tukey's Honestly Significant Difference (HSD) test, with statistical significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of chive essential oil nanoemulsion

The nanoemulsion (NC) showed high clarity (99.90 ± 0.05% transmittance) and rapid self-emulsification (48.08 ± 0.57 s), with a mean droplet size of 13.05 ± 0.07 nm (PDI = 0.257 ± 0.05) and zeta potential of −5.65 ± 0.07 mV. TEM images indicated near-spherical droplets without visible aggregation. These properties confirm a well-dispersed nanoscale system, which is expected to improve dispersion of hydrophobic essential-oil constituents in drinking water and promote intestinal exposure. This interpretation is consistent with reports that nanoemulsion/self-emulsifying systems can enhance dispersion and oral delivery of lipophilic bioactive (Buya et al., 2020).

Growth performance

Cold-humid stress reduced growth performance in the negative control (CON), reflected by the lowest ADG and poorer feed efficiency, which agrees with stress-driven shifts in nutrient partitioning from growth to maintenance and impaired gut function (Akinyemi and Adewole, 2021; Gao et al., 2025). Supplementing NC via drinking water improved performance in a dose-responsive manner. Compared with CON, ADG increased by 12.5% and 14.2% in 50NC and 100NC, respectively ($P < 0.05$), and the 100NC response was comparable to the antibiotic group (AB; 50 ppm tetracycline). Feed efficiency improved accordingly, with significantly lower FCR in 50NC and 100NC ($P < 0.05$) (Figure 2). The improvement under cold-humid challenge likely reflects gut-mediated effects, supported by microbiological and histological outcomes below.

Ileocecal microbiota

NC supplementation increased beneficial LAB populations, with 50NC and 100NC showing significantly higher LAB counts than CON and 25NC ($P < 0.05$). In parallel, *Salmonella* spp. counts were highest in CON and declined dose-dependently with NC (Figure 3). Such shifts are biologically relevant under cold-humid stress, where immune competence and gut barrier function may be compromised, increasing susceptibility to enteric pathogens (Akinyemi and Adewole, 2021; Gao et al., 2025). The antibacterial activity of Allium essential oils has been linked to sulfur-containing compounds that disrupt bacterial membranes and key metabolic pathways (Phan et al., 2025b), and nano-dispersion may strengthen these effects by increasing contact between actives and bacterial cells. The LAB increase may also contribute to colonization resistance through competitive exclusion and local acidification, thereby limiting *Salmonella* spp. proliferation.

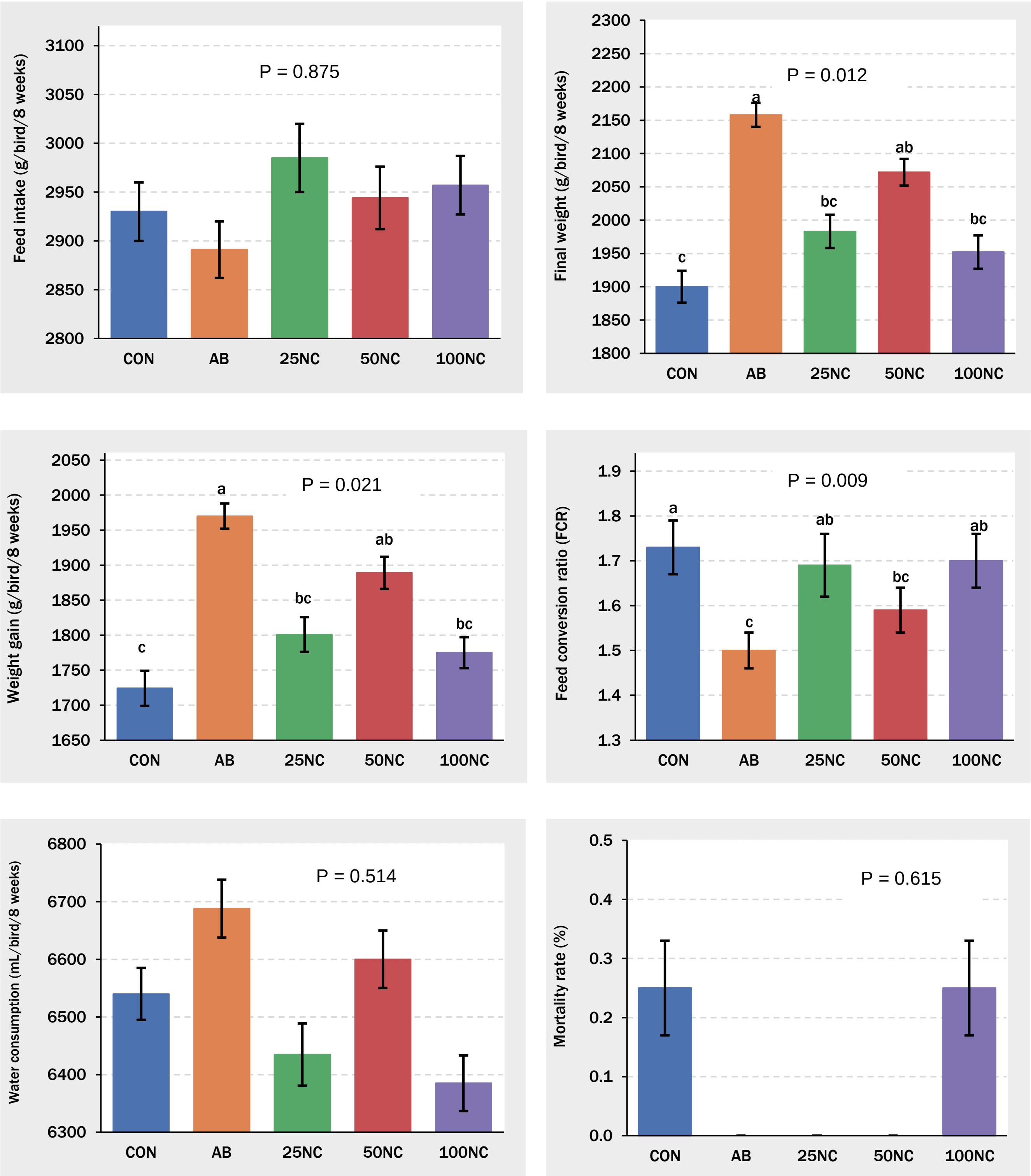

Figure 2 - Performance of broiler chickens supplemented with NC in drinking water. Note: CON = negative control; AB = 50 ppm tetracycline; 25NC = 25 μL/L NC; 50NC = 50 μL/L NC; 100NC = 100 μL/L NC. Values in the bars with different superscripts differ significantly ($P < 0.05$).

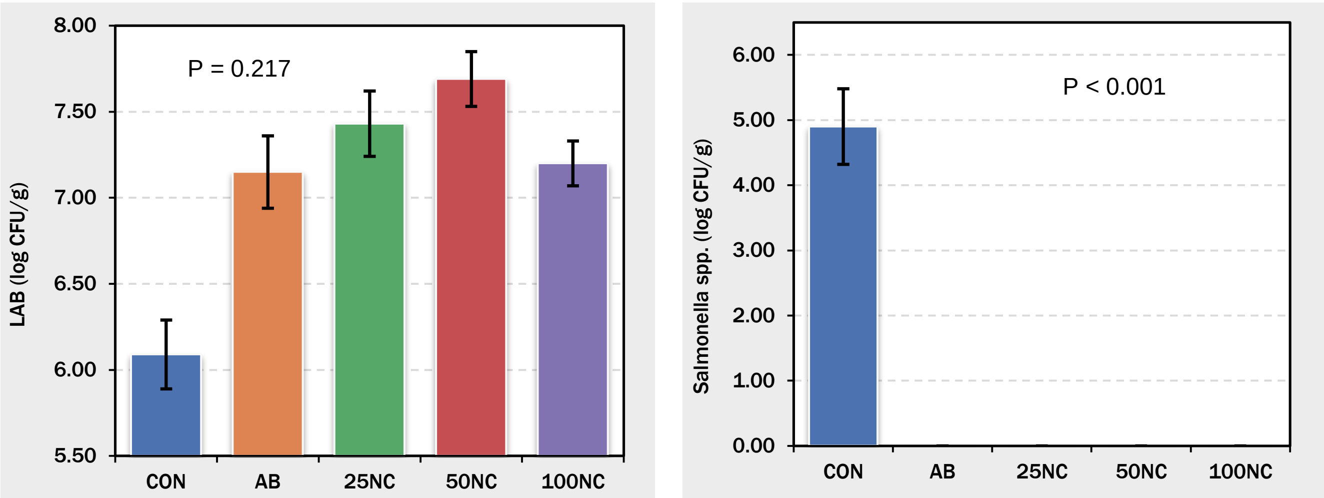

Figure 3 - Ileocecal microbiota of broiler chickens supplemented with NC in drinking water. Note: Treatment designations as in Figure 2; values in the bars with different superscripts differ significantly (P < 0.05).

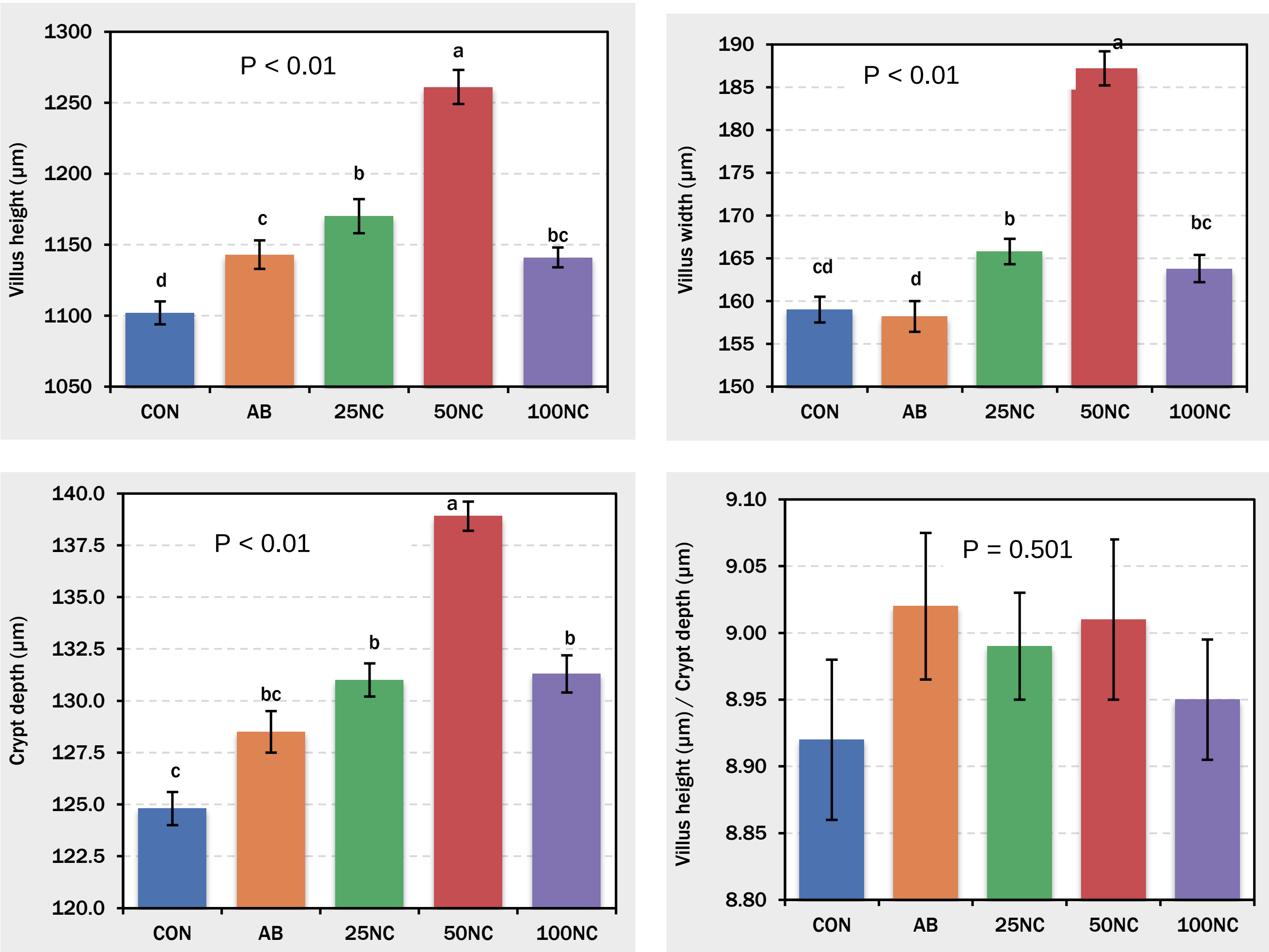

Figure 4 - Ileum histology parameters of broiler chickens supplemented with NC in drinking water. Note: Treatment designations as in Figure 2; values in the bars with different superscripts differ significantly (P < 0.05).

Intestinal morphology

Stress-associated villus atrophy was observed in CON, whereas NC improved intestinal architecture. The 100NC group showed the greatest villus height (approximately 18% higher than CON; P < 0.05), and the VH:CD ratio was significantly higher in NC-supplemented groups (Figure 4). Villus development generally indicates an improve in the absorptive surface area and more efficient nutrient uptake (El Sabry et al., 2013), providing a plausible mechanism for the improved FCR and ADG observed in 50–100NC. Overall, the findings support the view that phytogenic additives can help maintain gut structure under stress by reducing enteric pathogen burden and stabilizing epithelial turnover and function.

Limitations and future directions

This study relied on culture-based bacterial enumeration and morphometric endpoints; therefore, mechanistic interpretation would be strengthened by integrating microbiome profiling (e.g., 16S rRNA sequencing) and intestinal inflammatory/oxidative stress biomarkers. Because the zeta potential was relatively low, future work should quantify physical stability of NC under commercial waterline conditions (time, temperature, minerals, and disinfectants) and verify active-compound retention. Dose optimization should also be evaluated across genotypes and production systems, together with a cost–benefit assessment to support practical adoption as an antibiotic alternative under cold-humid stress.

CONCLUSION

Daily supplementation of NC in drinking water, particularly at 50 µL/L, enhanced final body weight, weight gain, and FCR in broilers under cold-humid stress, while boosting LAB, eliminating *Salmonella* spp., and improving ileum morphology. The findings propose NC as a promising phytogenic additive for antibiotic reduction strategies and gut health enhancement in challenging microclimatic conditions.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Phan Vu Hai, 102 Phung Hung Street, Phu Xuan Ward, Hue city, Viet Nam. E-mail: phanvuhai@hueuni.edu.vn; ORCID: https://orcid.org/0000-0002-0161-8430

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

Phan Vu Hai: development of the concept and design of the study, analysis of the obtained data, writing the original text of the manuscript. Hoang Van Son: statistical processing of data, literature review, editing of the text. Ngo Mau Dung: participation in the experiment, sample collection and laboratory analysis, correction of the manuscript. All authors approved the final version of the article and are responsible for the accuracy of the presented data.

Ethical regulations

All procedures involving animals complied with Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14) and institutional guidelines. All processes of experiments were in accordance with animal welfare rules under monitoring of Hue University ethical board.

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Competing interests

The authors have not declared any competing interests.

Abbreviations: NC: nanoemulsion of chive essential oil; FCR: feed conversion ratio; LAB: lactic acid bacteria; VH: villus height; CD: crypt depth; ADG: average daily gain; RH: relative humidity; TEM: transmission electron microscopy; PDI: polydispersity index; DLS: dynamic light scattering; HE: hematoxylin and eosin; CFU: colony-forming unit; ANOVA: analysis of variance; SPSS: Statistical Package for the Social Sciences; HSD: honestly significant difference; CON: negative control; AB: antibiotic control; ppm: parts per million; SSA: Salmonella Shigella agar; MRS: de Man Rogosa Sharpe agar; ISO: International Organization for Standardization.

REFERENCES

Akinyemi F and Adewole D (2021). Environmental stress in chickens and the potential effectiveness of dietary vitamin supplementation. Frontiers in Animal Science, 2: 775311. https://doi.org/10.3389/fanim.2021.775311

Barradas TN and de Holanda e Silva KG (2021). Nanoemulsions of essential oils to improve solubility, stability and permeability: a review. Environmental Chemistry Letters, 19: 1153-1171. https://doi.org/10.1007/s10311-020-01142-2

Buya AB, Witika BA, Bapolisi AM, Tuyishime J, Bukuru D, Makoni PA, Matsabisa MG, Chikukwa MT, Memvanga PB and Nkanga CI (2020). Self-nanoemulsifying drug delivery systems: from development to current applications and challenges in oral drug delivery. Pharmaceutics, 12(12): 1194. https://doi.org/10.3390/pharmaceutics12121194

Dai X, Jia C, Lu J and Yu Z (2024). Metabolism of phenolic compounds and antioxidant activity in different tissue parts of post-harvest chive (*Allium schoenoprasum* L.). Antioxidants, 13(3): 279. https://doi.org/10.3390/antiox13030279

El Sabry MI, Yalçın S and Turgay-İzzetoğlu G. (2013). Interaction between breeder age and hatching time affects intestine development and broiler performance. Livestock Science, 157(2): 612-617. https://doi.org/10.1016/j.livsci.2013.07.012

Gao H, Wang Y, Zhao X, Yu Y, Guo Y, Li Z and Zhou Z (2025). Growth performance and gut health of cold-stressed broilers in response to supplementation with a combination of sodium butyrate and vitamin D3. Animals, 15(6): 861. https://doi.org/10.3390/ani15060861

Hai PV, Anh LX and Hoa NX (2025). Fermented chive (*Allium schoenoprasum*) with *Lactobacillus plantarum*: A potential antibiotic alternative feed additive for broilers challenged with *Escherichia coli*. Fermentation, 11(5): 277. https://doi.org/10.3390/fermentation11050277

Hai PV and Khuong NDT (2025). Antibacterial effects of probiotic fermented shallot and garlic chive extracts against multidrug-resistant *Proteus mirabilis* and *Escherichia coli* in a chicken model. Poultry Science and Management, 2(1): 7. https://doi.org/10.1186/s44364-025-00010-5

Hai PV, Hung PHS, Dung HT, Liem TN, Khuong NDT and Hoa NX (2020). The dietary supplement efficiency of essential oil of chive (*Allium macrostemon*) on the productivity and health performance of broilers. CTU Journal of Innovation and Sustainable Development, 12(3): 1–6. https://doi.org/10.22144/ctu.jen.2020.018

ISO (2019). Making food safer according to ISO methods. ISO, Geneva, Switzerland. https://www.analisisavanzados.com/modules/mod_tecdata/Metodos_ISO.pdf

Omidian H, Cubeddu LX and Gill EJ (2025). Harnessing nanotechnology to enhance essential oil applications. Molecules, 30(3): 520. https://doi.org/10.3390/molecules30030520

Phan VH, Hoang TAP, Pham HSH, Tran TN, Ngo HL, Ho TD and Nguyen XH. (2025a). *Lactiplantibacillus plantarum* fermentation boosts antioxidants and antibacterial effects in *Allium* species, potentially replacing chicken antibiotics. Journal of the Hellenic Veterinary Medical Society, 76(3): 9591-9600. https://doi.org/10.12681/jhvms.39235

Phan VH., Nguyen XH and Hoang TAP. (2025b). Fermented purple onion (*Allium cepa L.*) and chive (*Allium schoenoprasum*) bulb extracts as antibiotic alternatives against toxin-carrying bacteria: *In vitro* and pathogenicity assessment in chickens. Open Veterinary Journal, 15(6): 2355-2364. https://doi.org/10.5455/OVJ.2025.v15.i6.8

Putnik P, Gabrić D, Roohinejad S, Barba FJ, Granato D, Mallikarjunan K, et al. (2019). An overview of organosulfur compounds from *Allium* spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties. Food Chemistry, 276: 680–691. https://doi.org/10.1016/j.foodchem.2018.10.068

Quintana-Ospina GA, Alfaro-Wisaquillo MC, Oviedo-Rondon EO, Ruiz-Ramirez JR, Bernal-Arango LC and Martinez-Bernal GD. (2023). Data analytics of broiler growth dynamics and feed conversion ratio of broilers raised to 35 d under commercial tropical conditions. Animals, 13(15), 2447. https://doi.org/10.3390/ani13152447

Singh N, Gusain A, Nigam M and Mishra AP (2025). The pharmacological and therapeutic versatility of *Allium* species: a comprehensive exploration of bioactive constituents and biological activities. Discover Applied Sciences, 7: 349. https://doi.org/10.1007/s42452-025-06800-0

Yani K, Rurie Rh and Ronny M (2020). Optimization of soybean oil, Tween 80, PEG 400 in formulation of beta carotene nanoemulsion. Research Journal of Pharmacy and Technology, 13(12): 6027–6032. https://doi.org/10.5958/0974-360X.2020.00991.9

Vladisavljević GT (2019). Preparation of microemulsions and nanoemulsions by membrane emulsification. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 579: 123709. https://doi.org/10.1016/j.colsurfa.2019.123709

Weaver WD Jr and Meijerhof R (1991). The effect of different levels of relative humidity and air movement on litter conditions, ammonia levels, growth, and carcass quality for broiler chickens. Poultry Science, 70(4): 746–755. https://doi.org/10.3382/ps.0700746

Zhou Y, Zhang MH, Feng JH and Diao HJ (2019). Effect of relative humidity at chronic temperature on growth performance, glucose consumption, and mitochondrial ATP production of broilers. Journal of Integrative Agriculture, 18(6): 1321–1328. https://doi.org/10.1016/S2095-3119(19)62584-0

Zhao S, Wang Z, Wang X, Kong B, Liu Q, Xia X and Liu H. (2023). Characterization of nanoemulsions stabilized with different emulsifiers and their encapsulation efficiency for oregano essential oil: Tween 80, soybean protein isolate, tea saponin, and soy lecithin. Foods, 12(17): 3183. https://doi.org/10.3390/foods12173183

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