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The effectiveness of nano-system probiotics in enhancing the growth of broiler chickens infected with *Escherichia coli*

Indra Saptiama[1], Marlina Marlina[1], Rondius Solfaine[2], Riedho Aulia Anggara[3], Alfian Mahardika Forentin[1], Veronika Yulianti Susilo[1], Luhur Sediyoadi[4], Isa Mahendra[1], Ahmad Kurniawan[1], Muhamad Basit Febrian[1], Iwan Sahrial Hamid[4]✉

[1] Research Center for Radioisotope, Radiopharmaceutical, and Biodosimetry Technology, Research Organization for Nuclear Energy, National Research and Innovation Agency of the Republic of Indonesia, South Tangerang 15314, Indonesia
[2] Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma, Surabaya 60225, Indonesia
[3] Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 60115, Indonesia
[4] Centra Biotech Indonesia, Klaten 57465, Indonesia
iwan-s-h@fkh.unair.ac.id

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Abstract

Introduction: Probiotics have been studied as feed additives that can improve the growth performance of broiler chickens. The increasing prevalence of avian pathogenic *Escherichia coli* (APEC) and restrictions on antibiotic use in poultry have increased the interest in probiotics as alternatives to antimicrobials. Their effectiveness often being limited by gastrointestinal tract conditions and extreme pH variations, support for their survival and a nano-system for encapsulated delivery in drinking water were investigated in APEC-infected poultry. **Material and Methods:** A chitosan-based nano-system was developed for the encapsulation of a probiotic formulation comprising *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Candida pintolopesii*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, and *Bacillus megaterium*. It was tested for stability at pH 2.0 and 8.0 to simulate the digestive environment of broiler chickens. An acute safety assessment was made in liver, lung, heart, and intestine sections and by complete blood count. A total of 50 Cobb CP 707 broiler chickens of both sexes were enrolled at 2 d old. They were divided into five groups of 10 birds each: one of untreated controls and four dose-differentiated encapsulated-probiotic-administered birds. The doses were 1.75×10^{13}, 3.5×10^{13}, 1.05×10^{14}, and 1.75×10^{14} colony-forming units per kg body weight (CFU/kg b.w.). A challenge study with APEC was also conducted in 50 Cobb CP-707 broiler chickens, which were challenged at 7 d old and subsequently treated with nano-system probiotics for 21 d. This study used five groups: untreated controls, birds treated with unencapsulated probiotics, birds treated with a half dose (7×10^{11} CFU/kg b.w.) of encapsulated probiotics and birds treated with a full dose (1.4×10^{12} CFU/kg b.w.). Body-weight gain, histopathological and immunohistochemical analyses were conducted. In addition, the intestinal tract probiotic accumulation was evaluated using autoradiography with technetium-99m radiolabelling. **Results:** The nano-system exhibited high physicochemical stability under both pH conditions. No adverse effects were shown in the acute safety assessment, as indicated by the absence of mortality and the maintenance of normal haematological profiles in treated broiler chickens. The autoradiography results showed specific accumulation of nano-system probiotics in the intestinal tract, confirming effective targeted delivery. Body-weight gains were statistically significantly greater in the groups which received probiotics. **Conclusion:** This chitosan-based nano-system may represent a safe and efficient means of probiotic delivery in broiler chickens, potentially improving gut health and reducing reliance on antibiotics.

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Keywords: antibiotics, broilers, growth promoter, nano-systems, probiotic.

Introduction

Poultry production contributes significantly to the global supply of animal protein. In Asia, the broiler chicken is among the cheapest sources of meat available, and its consumption is considered one of the easiest ways to meet dietary protein requirements (17). The increasing demand for broiler chicken meat has driven the rapid expansion and intensification of broiler production systems, which in turn has heightened their susceptibility to pathogenic bacteria, particularly avian pathogenic *Escherichia coli* (APEC). This is the primary causative agent of colibacillosis, which is one of the primary diseases threatening the health and productivity of poultry. Colibacillosis causes significant health problems and is characterised by conditions such as perihepatitis, airsacculitis, pericarditis, egg peritonitis, salpingitis, coligranuloma, omphalitis, cellulitis, and osteomyelitis or arthritis. As a result, colibacillosis leads to enormous economic losses in the global poultry industry (12, 13).

Conventionally, antibiotics are the primary agents used to control bacterial diseases and enhance growth performance in broilers. However, the current scenario suggests that the indiscriminate use of antibiotics has contributed to the emergence of multidrug-resistant bacteria, underscoring the urgent need for sustainable alternative strategies (21). Under such circumstances, probiotics, defined as live microorganisms, are increasingly investigated as potential feed additives capable of restoring gut microbial balance, enhancing immune function and suppressing the growth of pathogenic bacteria, thereby improving poultry growth performance and reducing the incidence of diseases (16, 24).

The efficacy of probiotics in enhancing growth performance is often constrained by challenges in the digestive tract where pH levels range from 2.0 to 7.0, significantly affecting their viability (2, 11). Therefore, numerous efforts have been made to protect probiotics from the harsh conditions of the digestive system by encapsulating the bacteria in protective coating shells at the micro- and nanoscale (1, 9). Furthermore, while nanotechnology-based delivery systems, including probiotic encapsulation strategies, may enhance stability and bioavailability, their implementation in the broiler industry will ultimately depend on demonstrated cost-effectiveness and scalability. These important considerations, however, are beyond the scope of the present study.

Bacterial encapsulation has shown potential in protecting bacterial viability during feed processing, storage, and gastrointestinal passage, thereby enhancing their stability and bioavailability (22). According to a previous study, a probiotics nano-system was successfully synthesised and characterised. The developed nano-system probiotics were shown to promote nutrient absorption and induce immune responses. However, their effect on growth performance was not significant during the finisher phase of broiler chickens (5).

Despite these promising results, limited investigations have focused on the role of encapsulated probiotics in mitigating the negative impacts of APEC on the growth performance and health parameters of broiler chickens. Most existing studies have investigated either the general benefits of probiotics or the application of encapsulation technologies in feed delivery systems, without assessing their combined potential to mitigate pathogenic bacterial infections (9). Therefore, this study aimed to evaluate the effectiveness of nano-system probiotics in enhancing growth performance, immune response, and overall health of broiler chickens experimentally infected with APEC. Additionally, the objective of this study is to assess the acute toxic effects of the test substance following a single oral administration by monitoring clinical signs, haematological parameters, and both macroscopic and microscopic changes, as well as to provide a preliminary estimation of the LD_{50} in the event of mortality. These results are expected to contribute to the development of safe and effective alternatives to antibiotics, thereby supporting sustainable poultry production practices.

Material and Methods

A schematic overview of the experimental design, probiotic supplementation, APEC challenge, and subsequent sample collection and analyses is presented in the graphical abstract (Supplementary Fig. S1).

Nano-system probiotics encapsulation. Nano-system probiotics encapsulation was prepared according to the guidelines described by Febrian *et al.* (5). Briefly, 1 L of multistrain bacterial stocks (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus thermophillus*, *Candida pintolopesii*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, and *Bacillus megaterium*) with a total concentration of 1.4×10^{12} colony-forming units (CFU)/mL was added to a bio-activator and then homogenised.

Low-molecular-weight nanochitosan (1,000 mg) was added to 1,000 mL of multistrain bacterial stock suspension at room temperature and stirred at 600 rpm for 30 min. Subsequently, 1,300 mg of sodium tripolyphosphate dissolved in 10 mL of distilled water was added dropwise at a rate of 1 mL/min. The resulting mixture was further homogenised at 1,000 rpm for 30 min using a magnetic stirrer at room temperature and then stored at room temperature for 24–48 h before use.

pH-dependent stability analysis. The pH of nano-system probiotics solution was adjusted to 2 and 8 to represent the range of acidity and alkalinity in the broiler chicken gastrointestinal tract. Adjustments were with 0.1 M hydrochloric acid (HCl) or sodium hydroxide (NaOH). The particle size of the nano-system probiotics was then measured.

Acute safety assessment studies. Two-day-old commercial broiler chickens were orally administered nano-system probiotics with doses of 1.75×10^{13}, 3.5×10^{13}, 1.05×10^{14} and 1.75×10^{14} CFU/kg b.w., and one parallel group were left unadministered as controls (n = 10 per group). The mortality was observed for 24 h post-administration of nano-system probiotics. At the end of the observation period, blood samples were collected from the birds *via* the brachial vein for complete blood count (CBC) analysis. The birds were euthanised by cervical dislocation, performed by trained personnel in accordance with the approved animal experimental protocol. Following sacrifice, liver, lung, heart, and intestine tissue samples were collected, fixed in 10% neutral buffered formalin (NBF) for 24 h, and routinely processed for paraffin embedding and sectioning. Complete blood count and histopathology analyses were also performed. The tissue sections were stained with H&E, subjected to histopathological examination and assessed for necrosis, inflammation and degeneration. The histopathology semiquantitative scoring system was independently evaluated by pathologists (14).

Radiolabelling of nano-system probiotics with technetium-99m. Radiolabelling of nanochitosan with technetium-99m [^{99m}Tc] is in a coordination process initiated by the reduction of the pertechnetate ion. Since the initial ^{99m}Tc is non-reactive with the polymer, stannous chloride ($SnCl_2$) is typically used to convert the technetium into lower, more reactive oxidation states. Reduced cations then interact with amino and hydroxyl groups on the nanochitosan surface, forming stable chelate complexes. Optimal labelling efficiency and best nanoparticle integrity are provided by maintaining the reaction at a pH of 6.0 (14, 15).

An indirect radiolabelling study was carried out to determine the uptake of the probiotics. A short-lived low-energy gamma emitter was selected as the radiolabelling agent to monitor the biodistribution of nano-formulated probiotics across various organs, specifically the intestine. Nano-system probiotics were radiolabelled with ^{99m}Tc using a direct-labelling method. Briefly, ascorbic acid (3 mg) and 1 mL of stannous (II) chloride solution (1 mg mL^{-1} in distilled water) were added consecutively to the nano-system probiotic suspension. Subsequently, 500 µL of Na[^{99m}Tc]TcO_4 solution (37 MBq), obtained from a ^{99}Mo/^{99m}Tc generator (irradiated and manufactured by Badan Riset dan Inovasi Nasional/National Research and Innovation Agency, South Tangerang, Indonesia), was added to the mixture. The resulting suspension was shaken for 30 s and then incubated at room temperature for 15 min. The reaction environment was maintained at a pH between 6 and 8 (8, 18). The radiochemical purity (RCP) of the ^{99m}Tc-labelled nano-system probiotics was evaluated by radio–thin-layer chromatography (radio-TLC) using two complementary solvent systems. Subsequently, 30 min after incubation, a 5-µL aliquot of the radiolabelled preparation was applied to chromatography paper. In the first system, chromatographic separation was carried out using 100% acetone as the mobile phase. Following development, the strips were air-dried and analysed using an AR-2000 radio-TLC imaging scanner (Eckert & Ziegler Berlin, Germany) and Gina Star software (Elysia Raytest, Ougrée, Belgium). Under these conditions, free Na[^{99m}Tc]TcO_4 migrated with the solvent front (Rf ≈ 1.0), while the ^{99m}Tc-labelled nano-system probiotics and reduced hydrolysed technetium (radiocolloid) remained at the origin (Rf ≈ 0.0). The percentage of free Na[^{99m}Tc]TcO_4 was calculated according to the equation given below, as described in the literature (4):

$$\% \text{ free Na } [^{99m}\text{Tc}]TcO_4 = \{100 - (\text{radiocolloid } (\%) + \text{radiolabelled nano-system probiotic } (\%))\}$$

To determine the percentage of radiocolloidal species, a second radio-TLC system was used. A 5-µL aliquot of the sample was applied to chromatography paper and developed using a pyridine/acetic acid/water (3 : 5 : 1.5, v/v/v) solvent mixture as the mobile phase. Following the development, the strips were dried and scanned using the radio-TLC scanner. In this system, free Na[^{99m}Tc]TcO_4 and the ^{99m}Tc-labelled nano-system probiotics migrated with the solvent front, while radiocolloids remained at the point of application. The percentage of radiocolloid was calculated using the equation given below:

% radiocolloid = {100 − (free$Na[^{99m}Tc]TcO_4$ + radiolabelled nano-system probiotic (%))}

After quantification of free $Na[^{99m}Tc]TcO_4$ and radiocolloidal impurities, the labelling efficiency of the ^{99m}Tc-labelled nano-system probiotics was calculated using the appropriate equation:

% RCP = {100 − (free radiolabelled nano-system probiotic (%) + radiocolloid (%)}

***Ex vivo* autoradiography.** The target specificity of nano-system probiotics was further confirmed by *ex vivo* autoradiography technique by following the protocol from Griem-Krey *et al.* (7). The tissue sections were incubated with the $[^{99m}Tc]Tc$–nano-system probiotics 0.5 MBq in 1 mL PBS for 1 h, and were subsequently washed every 5 min using ice-cold PBS. The tissue was then dried, covered and further exposed to the phosphor screen imager (Perkin Elmer, Shelton, CT, USA) for 15 min.

APEC challenge study. Seven-day-old commercial broiler chickens (Cobb CP-707) were infected with 3×10^7 CFU of *Escherichia coli* for two days. Broiler chickens were then supplemented with nano-system probiotics for 21 d through drinking water. The experimental chickens were divided into five groups: (1) untreated controls; (2) APEC-infected birds without probiotic treatment; (3) birds treated with unencapsulated probiotics; (4) birds treated with a half dose of nano-system probiotics (7×10^{11} CFU/kg b.w.); and (5) birds treated with a full dose of nano-system probiotics (1.4×10^{12} CFU/kg b.w.). The mean weight gain, intestinal uptake of the encapsulated probiotics and CD3 expression as indication of were then evaluated. At the end of the 24-d observation period, the broilers were euthanised by cervical dislocation, performed by trained personnel in accordance with the approved animal experimental protocol. Following euthanasia, jejunal tissue samples were collected and fixed in 10% NBF for 24 h. The fixed tissues were subsequently processed, embedded in paraffin and sectioned for histopathological and immunohistochemical analyses.

Throughout the study, broiler chickens were provided with a commercial feed (BR1-1 511B, Charoen Pokphand, Jakarta, Indonesia), and its nutritional composition is presented in Supplementary Table S1.

Immunohistochemistry. The expression of CD3 in formalin-embedded intestinal tissue sections was analysed by immunohistochemistry as an indicator of inflammatory changes in the intestinal mucosa. The tissue was stained using Mouse Anti-Chicken CD3 (cat. No. MCA1473; Bio-Rad Laboratories, Hercules, CA, USA) and a secondary antibody, followed by detection with 3,3'-diaminobenzidine substrate solution. The immunohistochemistry scoring system was independently evaluated by pathologists using the immunoreactive score (IRS) Remmele method (19).

Statistical analyses. Data were analysed using IBM SPSS Statistics v. 30 (IBM, Armonk, NY, USA). Haematological and growth parameters were compared using one-way ANOVA with *post-hoc* tests. Histopathological results were assessed using Kruskal–Wallis and Mann–Whitney. The median lethal dose was estimated when mortality exceeded 50%. A significance level of P-level < 0.05 was used.

Results

Stability study. In these studies, nano-system probiotics were successfully synthesised as described, and the *Lactobacillus* spp., *Saccharomyces* sp., and *Streptococcus* sp. were identified after the encapsulation process. The stability of nano-formulated probiotics was good, and no significant difference in their particle size was noted under conditions of pH 2.0 (1,261.9 ± 768.5 nm) or 8.0 (1,407.1 ± 176.9 nm), as shown in Fig. 1.

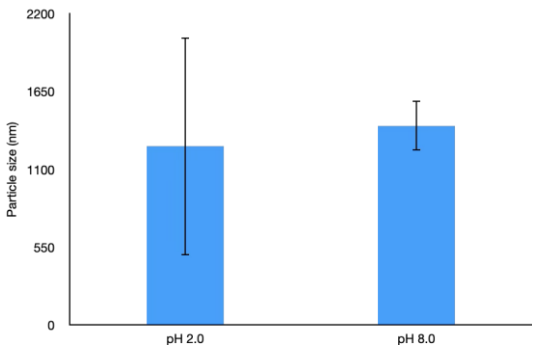

Fig. 1. Hydrodynamic particle size of nano-system probiotics for broiler chickens in pH 2.0 and 8.0

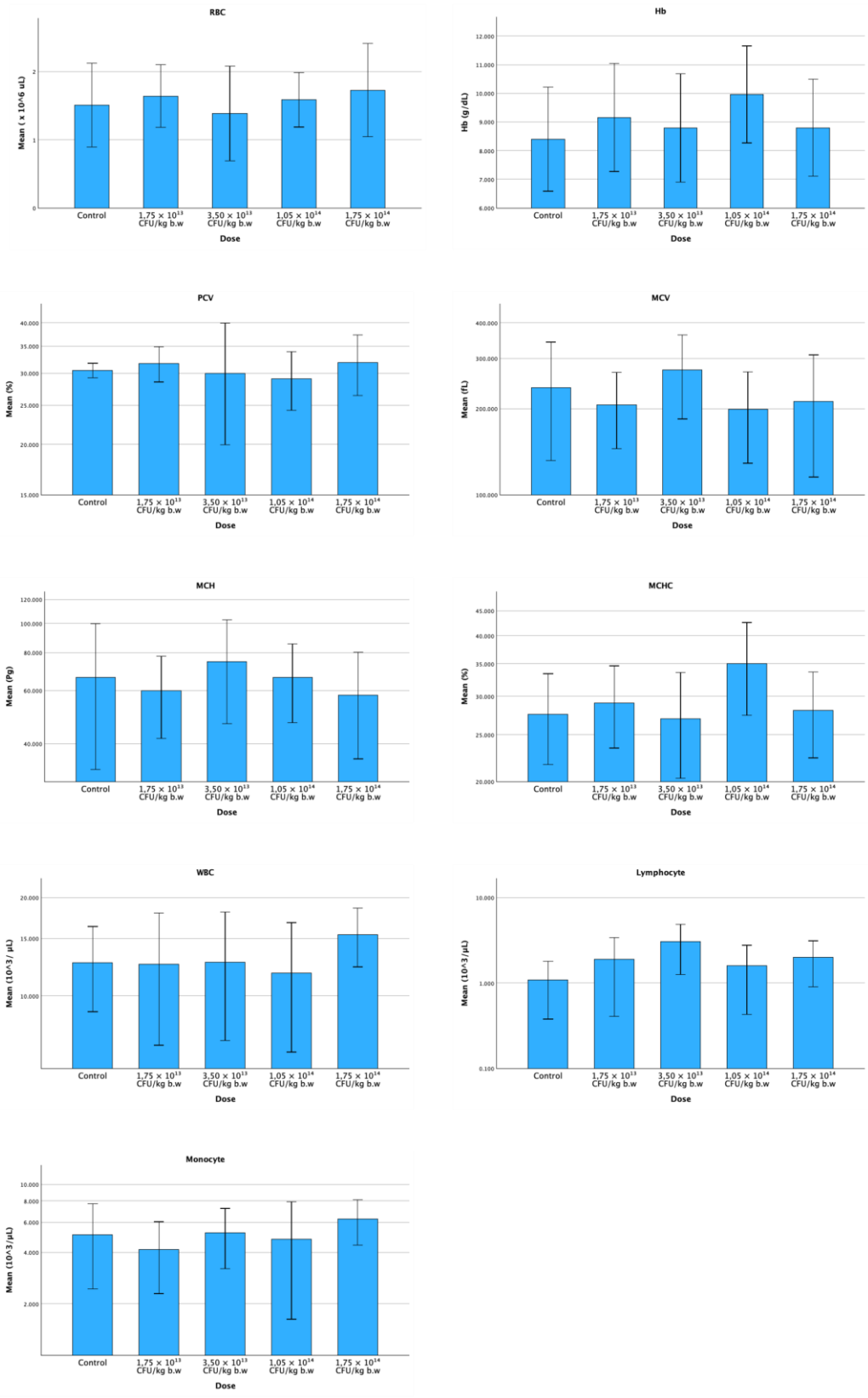

Fig. 2. Complete blood count of broiler chickens in an acute toxicity study of nano-system probiotics. CFU – colony-forming units; b.w. – body weight; Hb – haemoglobin; PCV – packed cell volume (haematocrit)

Acute safety assessment. No mortality was observed in broiler chickens after the administration of nano-system probiotics at lower doses than 1.75×10^{14} CFU/kg b.w. In addition, haematological parameter following probiotic supplementation showed the erythrocyte profile, including red blood cell (RBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), showed no variations across the different supplementation groups with no clear dose-dependent pattern was evident from the observed values. Similarly, no variations in total white blood cell (WBC), lymphocyte, and monocyte counts were observed among the experimental groups (Fig. 2). The results of histopathological summaries, as presented in Table 1, showed that no adverse effects were observed at the dose of 1.75×10^{13} CFU/kg b.w. Representative haematoxylin and eosin (H&E)-stained sections of the liver, intestine, heart, and lungs are presented in Supplementary Fig. S2, further supporting the absence of histopathological abnormalities.

Radiolabelling and *ex vivo* autoradiography. The labelling efficiency of [^{99m}Tc]Tc–nano-system probiotics is summarised in Table 2. Radio-TLC analysis confirmed that the majority of the radioactivity was associated with nano-system probiotics, with only minor contributions from free pertechnetate and radiocolloidal impurities, as shown in Fig. 3. Consequently, the calculated labelling efficiency exceeded 90%, indicating effective coordination of ^{99m}Tc to the functional groups present on the nano-system probiotic particle surface.

Based on the autoradiography results (Fig. 4), the radiolabelled nano-system probiotics showed a marked accumulation in the intestine, indicating effective targeting. In contrast, free ^{99m}Tc exhibited a weaker intestinal signal under identical acquisition conditions.

APEC challenge study. Following the APEC challenge, broiler chickens treated with nano-formulated probiotics gained more body weight than those receiving non-encapsulated probiotics, infected untreated broiler chickens and the untreated control group (P-value < 0.05), as shown in Fig. 5.

Histopathological examination showed substantial restoration of the jejunal mucosa previously affected by erosion in the unencapsulated probiotics group and in both the 1-dose and 0.5-dose probiotic nano-system groups. Importantly, APEC-induced villus necrosis was markedly attenuated in both unencapsulated probiotics-treated and nano-formulated probiotics groups. In parallel, infiltration of mononuclear inflammatory cells and the presence of erythrocytes in the lamina propria were significantly reduced (Fig. 6).

As shown in Fig. 7, CD3 expression was markedly reduced following probiotics administration. The immunoreactivity score was 1 in the unencapsulated probiotics group, 4 in the nano-system probiotics 1-dose group, and 6 in the 0.5-dose group, while it was 8 in the control group.

Table 1. Results of acute safety assessment of nano-system probiotics in broiler chickens

Dose	Toxicity	Interpretation
0	Non-toxic	Non-toxic
1.75×10^{13}	Minimal and reversible toxicity	No observed adverse effect level
3.50×10^{13}	Light–mild toxicity	Low observed adverse effect level
1.05×10^{14}	Mild–heavy toxicity	Moderately toxic
1.75×10^{14}	Heavy toxicity	Severe toxic effect

Table 2. Radiochemical composition of bulk technetium-99m [^{99m}Tc] eluate and [^{99m}Tc]Tc–nano-system probiotics and their labelling efficiency

Sample	% Free $^{99m}TcO_4^-$	% Radiocolloid	% Labelling efficiency
^{99m}Tc solution	99.81	0.29	—
[^{99m}Tc]Tc–nano system probiotic	4.69	2.69	92.62

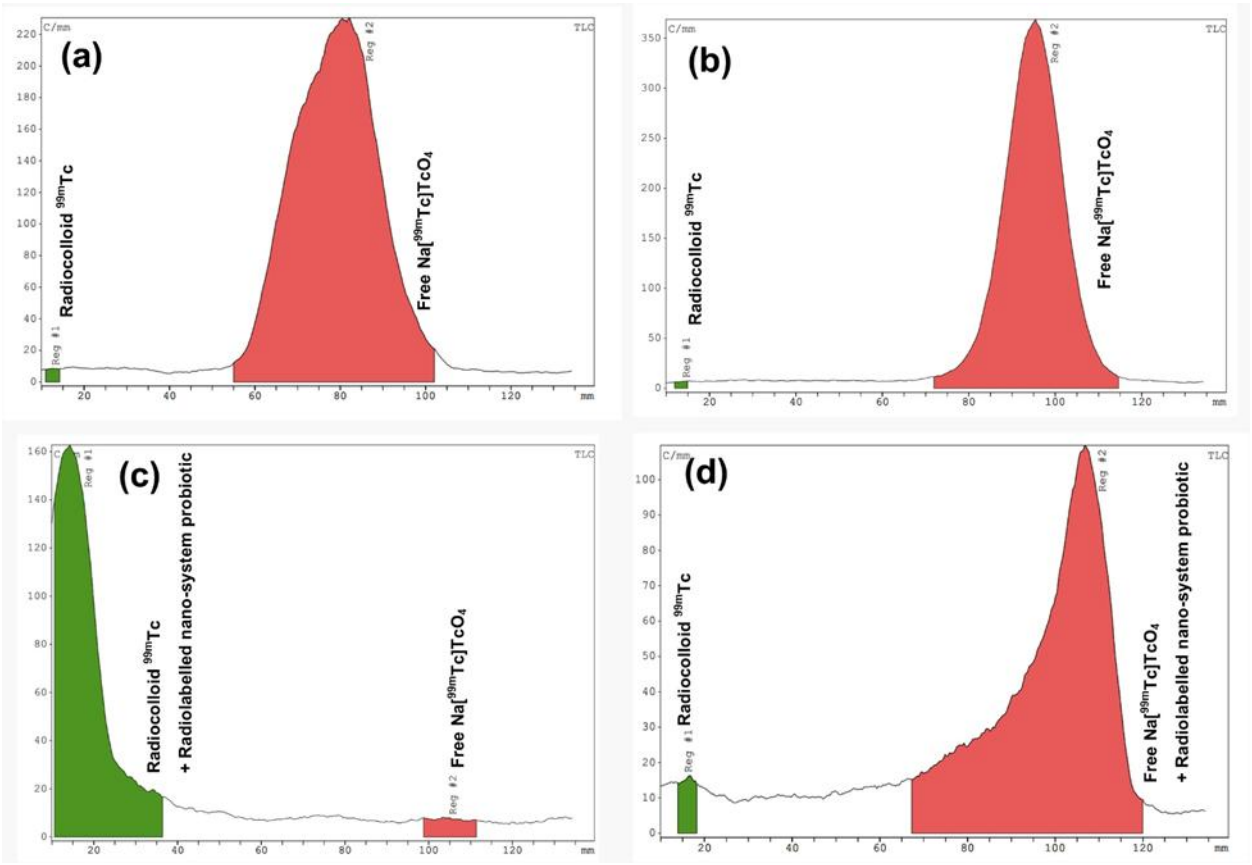

Fig. 3. Radiochromatograms of the bulk technetium-99m [^{99m}Tc] solution developed in (a) acetone and (b) pyridine/acetic acid/water (3 : 5 : 1.5, v/v/v), and of [^{99m}Tc]Tc–nano-system probiotics developed in (c) acetone and (d) pyridine/acetic acid/water (3 : 5 : 1.5, v/v/v)

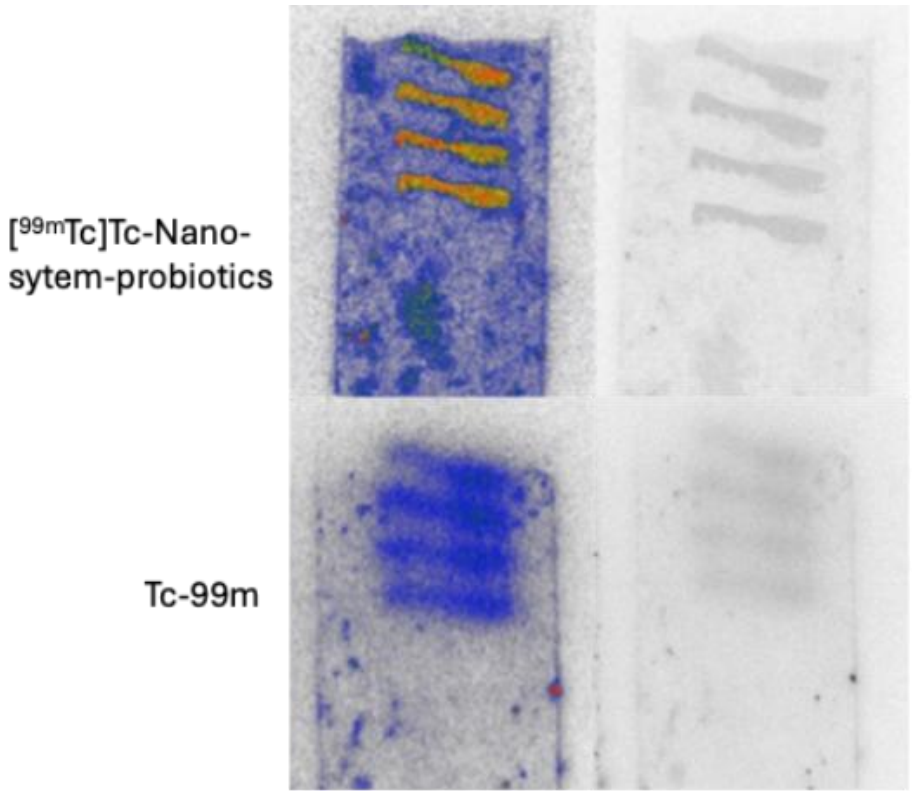

Fig. 4. *Ex vivo* autoradiography of technetium-99m (Tc-99m/[^{99m}Tc])Tc–nano-system probiotics in broiler chicken intestine sections

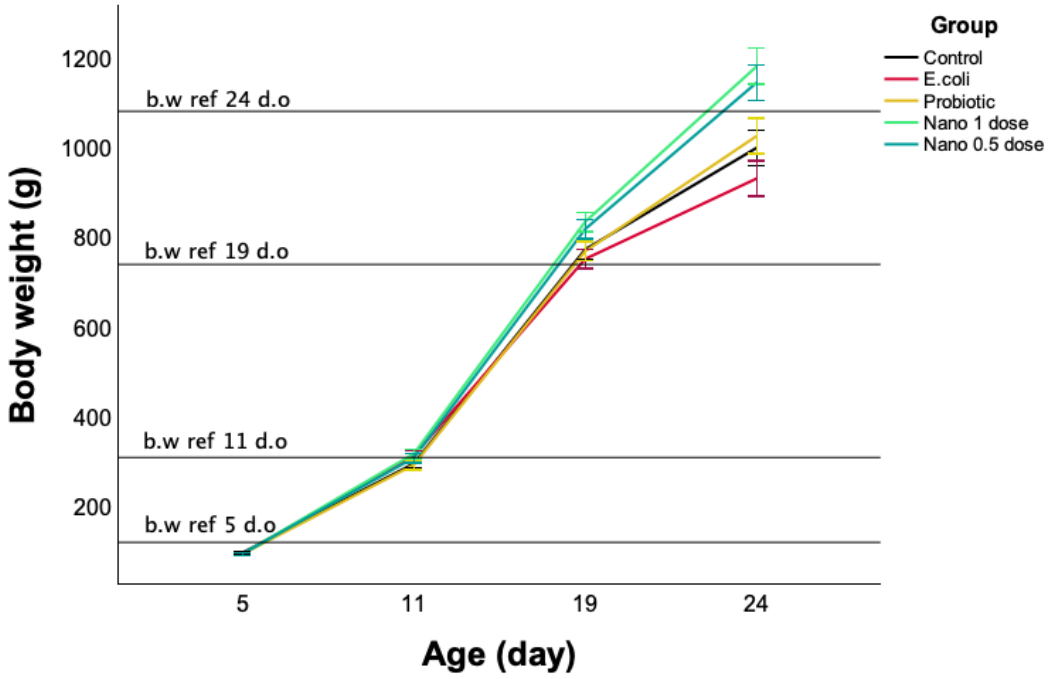

Fig. 5. Body weight (b.w.) changes over 24 d of observation during the administration of nano-system probiotics for 21 d after broiler chickens were challenged with avian pathogenic E. coli (APEC). Horizontal lines are indicate the reference body weight (b.w.) at 5, 11, 19, and 24 d old and error bars/whiskers show the mean ±SD. Control – broilers neither infected with APEC nor treated; *E. coli* – broilers infected with APEC but not treated; Probiotic – broilers infected with APEC and treated with unencapsulated probiotics; Nano 1 dose – broilers infected with APEC and treated with nano-system probiotics at 1.4×10^{12} CFU/kg b.w.; Nano 0.5 dose – broilers infected with APEC and treated with nano-system probiotics at 7×10^{11} CFU/kg b.w.; d.o. – days old

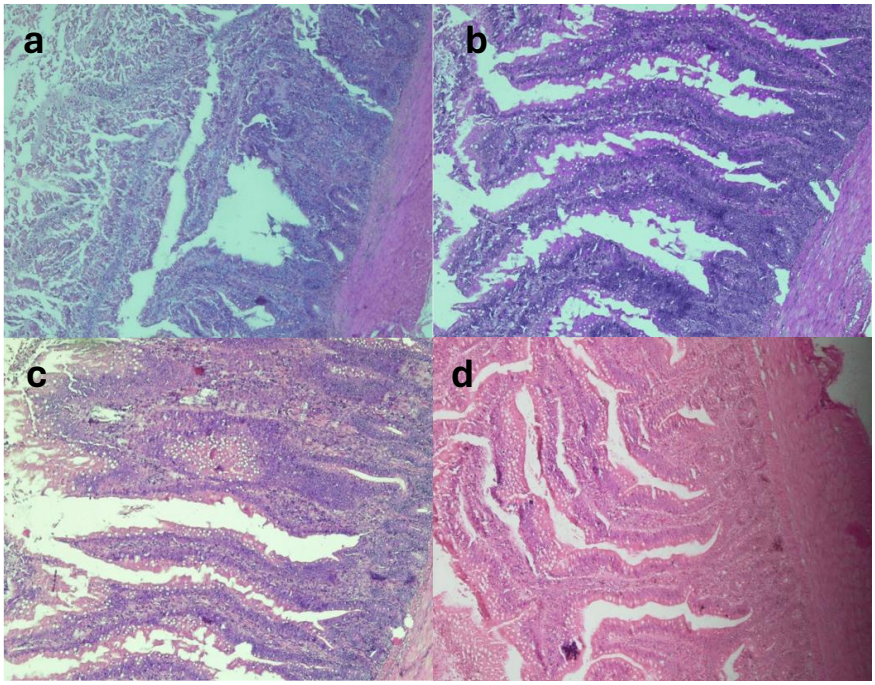

Fig. 6. Histopathology images of 24-day-old broiler chicken jejuna after a 21-d challenge with avian pathogenic *E. coli* (APEC). a) Untreated APEC infection; b) APEC + unencapsulated probiotics; c) APEC + nano-system probiotics at 1.4×10^{12} CFU/kg b.w. (1 dose); d) APEC + nano-system probiotics at 7.0×10^{11} CFU/kg b.w. (0.5 dose). HE, 100× magnification

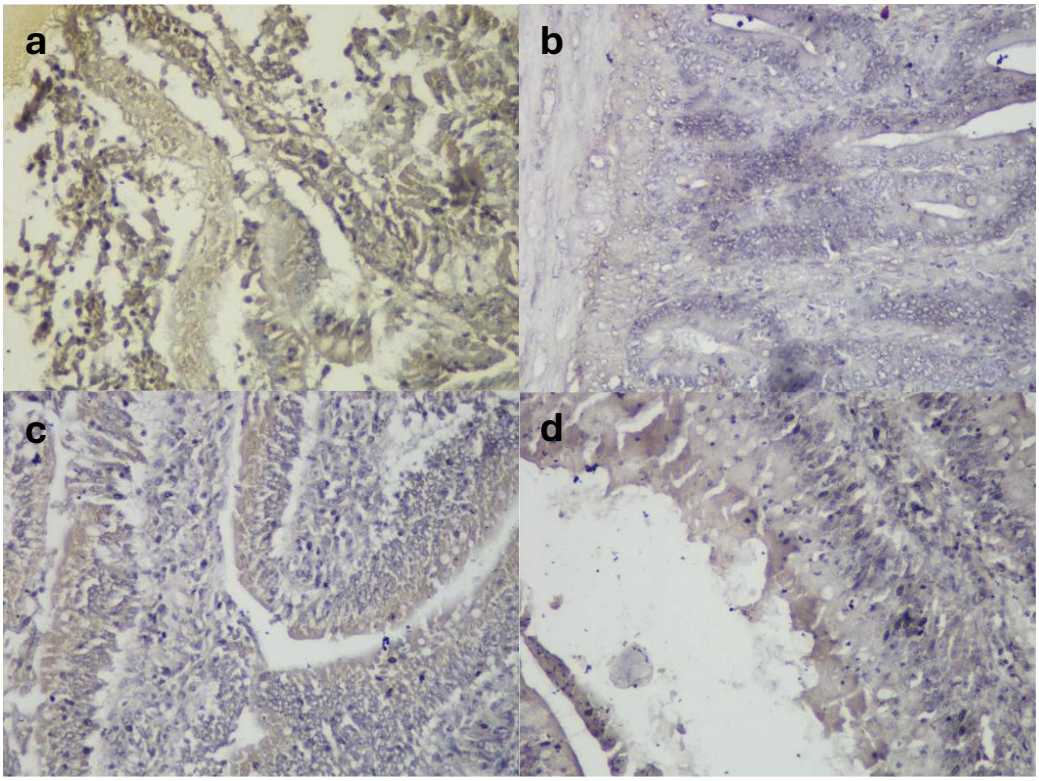

Fig. 7. CD3 expression in 24-day-old broiler chicken jejuna after a 21-d challenge with avian pathogenic *E. coli* (APEC). a) Untreated APEC infection; b) APEC + unencapsulated probiotics; c) APEC + nano-system probiotics at 1.4×10^{12} CFU/kg b.w. (1 dose); d) APEC + nano-system probiotics at 7.0×10^{11} CFU/kg b.w. (0.5 dose). 100× magnification

Discussion

This study evaluated the effectiveness of nanochitosan encapsulation in preserving probiotics under highly acidic conditions (pH 2.0). Although no statistically significant difference was observed in the hydrodynamic size of nano-system probiotics, a wide standard deviation was reported after exposure to acidic conditions. Particle-size analysis showed the presence of two distinct fractions at pH 2.0, corresponding to the nanochitosan fraction and the nano-system probiotics fraction, indicating that acidic pH had a direct effect on nano-system probiotics. However, the geometric mean particle size of nano-system probiotics (1020.4 ± 288.6 nm) suggests that encapsulation with nanochitosan was able to protect probiotics and improve the acid tolerance. Furthermore, the physicochemical stability of nanochitosan was afforded by its fabrication with a polyelectrolyte complex (PEC) driven by electrostatic interactions between positively charged chitosan and negatively charged tripolyphosphate. This PEC configuration enhances the stability of nanochitosan over a wide pH range in acidic, neutral, and mildly alkaline conditions (3, 10, 15). In the present study, we did not evaluate a broad range of pH conditions, focusing instead on acidic and alkaline environments (pH 2 and pH 8), as these pH values were considered representative and stable. Reported pH values across different segments of the broiler chicken gastrointestinal tract generally range from approximately pH 2.5 to 8.0. Therefore, future studies investigating a wider range of pH conditions that more closely mimic the physicochemical environments of specific gastrointestinal segments would be valuable for further understanding the behaviour of the compound under physiological conditions (23).

Ex vivo autoradiography demonstrated significant accumulation of nano-formulated probiotics in the intestine, indicating their capacity to effectively localise and interact with intestinal tissue. In addition, when used as an encapsulating material for probiotics, nanochitosan possesses a positive surface charge that facilitates electrostatic interactions with negatively charged mucin, thereby enhancing mucoadhesive properties (6). However, the precise mechanism by which nano-formulated probiotics internalise into the intestine of broiler chickens remains unclear and warrants further *in vivo* investigation to achieve comprehensive mechanistic characterisation.

The safety assessment using high doses of nano-system probiotics that was conducted in these studies aimed to determine an acceptable

dose that would not interfere with the outcomes of the challenge studies using APEC. The results of this study indicated that the no adverse observed effects level was 1.75×10^{13} CFU/kg b.w. At this dose, reversible mild pathological changes were observed in the liver, lung, heart, and intestine. Therefore, the treatment dose was further reduced, and a dose of 1.4×10^{12} CFU/kg b.w. was used in subsequent experiments. Although this study did not directly evaluate the potential adverse effects of nano-formulated probiotics in broiler chickens, the results provide preliminary safety data that may serve as a basis for future comprehensive toxicity assessments.

In previous studies, nano-system probiotics exhibited a direct improving effect on the systemic immune function in normal conditions (5). In the present study, we further investigated the effects of nano-system probiotics in broiler chickens challenged with APEC as the pathogenic agent. Based on the body weight gain change, there was a difference in the average weight difference between the nano-system probiotics group with 1 dose application and the APEC infection and control groups, which were 84.3 and 65.6 g, respectively. In addition, the body weight gain in the nano-system probiotic group was higher than that reported for the Cobb CP-707 reference group from a commercial broiler breeding farm. However, we recognise that the difference in body weight between the control group and the Cobb CP-707 reference group may reflect differences in rearing conditions rather than the effects of the treatments alone. While the experimental chickens in the present study were raised under laboratory-scale conditions, the Cobb CP-707 reference data were obtained under commercial breeding conditions. We assume that the different conditions of the laboratory-scale facility compared to the industrial scale at the Cobb CP-707 breeding farm may have influenced this difference. Furthermore, because the standard deviation of the Cobb CP-707 reference group established by the poultry breeding company was not reported, it is not possible to determine whether the observed difference reaches statistical significance. Therefore, this comparison should be interpreted descriptively rather than as a direct statistical comparison. Based on these results, nano-system probiotics could provide potential economic benefits when applied in a large broiler chicken population.

The results of histopathology and immunohistochemistry of CD3 expression in the jejunum showed that nano-system probiotics improved the damage recovery and decreased the inflammatory factor after APEC infection. However, the results were inconsistent with those for body weight gain, as the unencapsulated-probiotics group demonstrated greater jejunal damage recovery than the nano-formulated probiotics group. Probiotic-mediated intestinal repair effectively reduced local mucosal damage, but it was insufficient to fully counteract the systemic inflammatory and metabolic disturbances induced by APEC infection. As a result, improvements in growth performance were only partial and did not scale proportionally with the extent of intestinal recovery. Other studies on the treatment of APEC also reported similar phenomena (20). These results suggest that additional systemic pathways may limit the overall efficacy of probiotic intervention. The mechanism of action of nano-system probiotics in the treatment of APEC infections requires further investigation.

Conclusion

The nano-system for encapsulating multi-strain probiotics effectively protected them under acidic conditions and promoted their accumulation in the intestine. Moreover, nano-encapsulated probiotics demonstrated beneficial effects on growth performance in broiler chickens challenged with APEC.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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