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Research Article
Effect of Nano-encapsulation of Noni (*Morinda citrifolia*) Fruit Extract on Jejunal Morphology and Microbial Populations in Laying Hens

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Abstract
Objective: This study aimed to analyze the effect of nano-encapsulation of noni (*Morinda citrifolia*) fruit extract in drinking water on antibacterial activity and the morphology of intestinal villi in laying hens. **Materials and Methods:** The experiment was conducted in a completely randomized design consisting of 6 treatments and 5 replicates, with 12 chickens (20 weeks) in each replicate pen. The treatments were: P0 = Water without an additive (negative control), P1 = Water+50 mg/hen of tetracycline (positive control), P2 = Water+0.5% extract of noni fruit, P3 = Water+0.5% nano-encapsulation of noni fruit extract, P4 = Water+1% nano-encapsulation of noni fruit extract and P5 = Water+1.5% nano-encapsulation of noni fruit extract. At the end of the experiment (5 weeks), parameters included bacterial populations in the small intestine (*Escherichia coli*, *Bacillus subtilis* and *Salmonella* sp.) and the morphology of intestinal villi (villus height, villus width, crypt depth and villus height: Crypt depth) were observed. Data were analyzed statistically using one-way ANOVA. Orthogonal contrasts were used to analyze all data with significant differences. **Results:** The results showed that the addition of 1% nano-encapsulation of noni fruit extract in drinking water did not affect the morphology of the intestinal villi or the populations of *Bacillus subtilis* and *Salmonella* sp., but it reduced the population of *Escherichia coli* ($p<0.05$). **Conclusion:** Supplementation with nano-encapsulation of noni fruit extract in the drinking water of laying hens reduced colonization by *Escherichia coli* and might be used as an alternative to antibiotics in laying hens.

Key words: Laying hens, nano-encapsulation, noni fruit, intestinal bacteria, jejunal morphology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The presence of pathogenic bacteria in the digestive tract of laying hens decreases the quality of eggs and harms consumers[1]. The use of antibiotics to control pathogenic bacteria in laying hens has resulted in residues that can be found in eggs up to several weeks after administration[2]. One of the potential replacements for antibiotics is noni fruit (*Morinda citrifolia*). Noni fruit reportedly has some antibacterial compounds, including anthraquinones, alizarin, aucubin, saponin, L-asperuloside and scopoletin[3,4]. However, bio-active compounds such as polyphenols have low bioavailability. Polyphenol compounds are highly sensitive to gastrointestinal conditions (alkaline pH, enzymes and the presence of other nutrients) and are difficult to dissolve in water, making them difficult to absorb[5]. One of the efforts that can be taken to maintain the activity of compounds with low bioavailability is the nano-encapsulation method[6]. The advantages of this method are that particle size can be manipulated, a drug can be carried to the target organ, the activity of bio-active compounds can be maintained and the bio-active compounds can be absorbed faster[7,8]. Therefore, this study was conducted to observe the critical functions of nano-encapsulation of noni fruit extract, namely, the reduction of colonization by harmful intestinal microbes and the effect on jejunal morphology in laying hens.

MATERIALS AND METHODS

A total of 360 female ISA Brown chickens (20 weeks of age) obtained from a local company in Kediri, Indonesia, were used in the current study. Noni fruits were obtained from the Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia. Chitosan and sodium tripolyphosphate (STPP) were purchased from Bratachem, Yogyakarta, Indonesia. The experiment was conducted in a completely randomized design consisting of 6 treatments and 5 replicates, with 12 chickens in each replicate pen. The treatments were as follows: P0 = Drinking water without an additive (negative control), P1 = Drinking water+50 mg/hen of tetracycline (positive control), P2 = Drinking water+0.5% noni fruit extract (NFE), P3 = Drinking water+0.5% nano-encapsulation of NFE, P4 = Drinking water+1% nano-encapsulation of NFE and P5 = Drinking water+1.5% nano-encapsulation of NFE. The observed parameters were the microbial populations of the jejunal intestine, including *Bacillus subtilis*, *E. coli* and *Salmonella* sp. and the histomorphology of the jejunal intestine, including villus height, villus width, crypt depth and villus height: Crypt depth.

Preparation of noni fruit extract: A total of 1.5 kg of fresh noni fruits was blended and mixed thoroughly with 1900 mL of aqua dest for 15 min. The mixture was then filtered using nylon fabric. The liquid extract was heated at 90°C for 4 h to reduce the water content to form a paste. The paste was mixed with 575 mL of 96% alcohol using a magnetic stirrer (C-MAG HS 7, IKA, Selangor, Malaysia) for 20 min. Sediment precipitated from the solution after 20 min, at which point the solution was ready for nano-encapsulation.

Nano-encapsulation of noni fruit extract: Nano-encapsulation procedures were modified from Sundari *et al.*[9]. A total of 3.816 g of chitosan was dissolved in 610 mL of 2.5% acetate using a magnetic stirrer for 30 min. The chitosan solution was combined with 382 mL of noni fruit extract and stirred for 20 min. In a separate container, 0.125 g of STPP was dissolved in 16.7 mL of aqua dest. The STPP solution was then added to the chitosan-noni fruit extract solution and the mixture was homogenized using a magnetic stirrer for 20 min. The resulting solution was heated at 70°C for 1 h to evaporate the alcohol.

Populations of microbial intestinal pathogens

***Bacillus subtilis*:** Jejunal digesta were planted in brain heart infusion agar (BHIA) medium according to Koshikawa *et al.*[10]. The sample was then incubated upside down at 37°C for 24-48 h. The number of bacteria was determined by the total plate count (TPC) method.

Escherichia coli: Eosin methylene blue agar (EMBA) medium was used according to Leininger *et al.*[11]. Jejunal digesta were inoculated on EMBA by the spread method. After being inoculated, the sample was stored upside down in an incubator overnight at a temperature of 37°C. The number of *E. coli* was calculated as a total plate count (TPC) by counting greenish colonies with black spots in the middle.

***Salmonella* sp.:** One gram of jejunal digesta was inserted into nutrient broth and incubated for 24 h at 37°C and then planted on *Salmonella shigella* agar (SSA) medium according to Al-Lahham *et al.*[12]. Jejunal digesta were inoculated on SSA by the spread method and then incubated for 24 h at 37°C. The number of *E. coli* was calculated by the total plate count (TPC) method[13].

Histomorphology of jejunal villi: The samples used for histomorphology were taken a 6 cm section of the jejunum. The jejunal digesta were removed and the intestinal mucosa was cleaned by physiological sodium chloride (0.89% NaCl in

sterile water) and then stored in a 10% formalin buffer solution. The sample was then dehydrated in a series of acetone concentrations (35, 50, 70 and 95%)[14] and embedded in paraffin. Villi tissues were then cut into 4 µm slices using a microtome and placed on slides to be stained by the hematoxylin-eosin method[15]. The observations of histomorphology of jejunal villi, including villus height, villus width, crypt depth and villus height: Crypt depth, were conducted using a light microscope with 4X magnification equipped with an Optilab digital camera (Optilab Advance, Miconos, Yogyakarta, Indonesia).

Statistical analysis: Microbial populations and histomorphology of jejunal intestine were compared between groups following a completely randomized design in a one-way arrangement[16]. The statistical analysis was conducted in Statistical Package for the Social Sciences (SPSS for Windows Version 16, SPSS GmbH, Munich, Germany). All significant differences were tested further using orthogonal contrasts. Indications of apparent differences in this study were based on a probability of less than 5%.

RESULTS AND DISCUSSION

The basal diet composition and nutrient content are represented in Table 1 and the data for bacterial populations in the jejunum are presented in Table 2. There was a significant effect ($p<0.05$) of supplementing with nano-encapsulation of NFE on the population of *E. coli* in laying hens. Supplementation with 1% nano-encapsulation of NFE in the drinking water of laying hens reduced the population of *E. coli* in the jejunum. The results of this study were in line with those of Kurniawan *et al.*[17], who reported that supplementation with noni fruit reduced the amount of *E. coli* bacteria in the digestive tract of ducks. The presence of antibacterial compounds in noni fruit was thought to be the cause of the decline in *E. coli* populations. Saponins could change the permeability of microbial cell walls[18]. Aucubin inhibited the synthesis of RNA and microbial proteins and decreased the production of IL-6 and TNF in mast cells[19]. Flavonoids had an inhibitory effect on nucleic acid synthesis and energy metabolism and destructed the cytoplasmic membrane of pathogenic bacteria[20].

The effects of supplementation with nano-encapsulation of NFE on the population of *B. subtilis* are summarized in Table 2. Initially, there was no significant effect ($p>0.05$) of treatment on the population of *B. subtilis* in the laying hens. The low population size of *B. subtilis* in all groups might be due to the sensitivity of *B. subtilis* to all treatments, including the controls. P0 had the largest *E. coli* population of all the treatments, so competition would occur between *E. coli* and *B. subtilis* for attachment to the intestinal wall[21]. The use of tetracycline in group P1 might have suppressed the growth of *B. subtilis*[22]. Tetracycline inhibits bacterial protein synthesis at the translation stage on the ribosome[23]. The presence of phenolic compounds in noni fruit could also have inhibited colonization by *B. subtilis* in groups P2, P3 and P4. Phenolic compounds have small molecules that penetrate the cell wall[24]. The *Salmonella* sp., bacterial population was not detected in any treatment. This might be caused by the effective application of a biosecurity program, such that *Salmonella* sp. was not able to grow in the digestive tract of laying hens. Good biosecurity programs such as maintaining the cleanliness of the cage and burying dead, infected chickens reduced *Salmonella* infections[25].

The effects of supplementation with nano-encapsulation of NFE on villus characteristics are summarized in Table 3. Initially, there was no significant effect ($p>0.05$) of treatment on the villus characteristics of the jejunum, including villus height, villus width, crypt depth and villus height: Crypt depth. This result differed from that of Kurniawan *et al.*[17], who reported that supplementing feed with noni fruit could increase villus height. This difference in results is thought to be due to a difference in the types of livestock, since the study of Kurniawan *et al.*[17] used ducks. The results of this study were also different from the results of the study by Sunder *et al.*[26], which showed that noni fruit juice could increase the height of intestinal villi in the duodenum. Supplementation with 1%

Table 1: Basal diet composition and nutrient content

Items	Content
Diet composition (%, as fed)	
Corn	59.00
Rice bran	11.00
Meat and bone meal	4.00
Dicalcium phosphate	1.00
Soybean meal	18.00
Phytase	0.02
Sodium bicarbonate	0.30
DL-methionine	0.10
L-lysine HCl	0.05
Salt	0.25
Limestone	4.28
Vitamins and minerals premix[a]	2.00
Total	100.00
Analyzed nutrient content	
Metabolic energy (kcal kg^{-1})[b]	2700.00
Crude protein (g kg^{-1})	182.00
Moisture (g kg^{-1})	124.00
Ash (g kg^{-1})	67.40
Ether extract (g kg^{-1})	40.00
Crude fiber (g kg^{-1})	33.70

[a]Vitamins and minerals premix content per kg: Calcium 32.5%, Phosphorus: 1.0%, Iron: 6 g, Manganese: 4 g, Iodine: 0.075 g, Copper: 0.3 g, Zinc: 3.75 g, Vitamin B12: 0.5 mg, Vitamin D3: 50,000 IU, [b]Conversion values from 70% gross energy

Table 2: Microbial populations in the jejunum of laying hens given drinking water with nano-encapsulation of noni fruit extract for 35 days

	Treatments						Statistics	
Species	P0	P1	P2	P3	P4	P5	SEM	p-value
Escherichia coli (10^4 CFU)	62.67[b]	16.00[a]	19.00[a]	53.00[a]	9.67[a]	32.33[a]	10.48	0.043
Bacillus subtilis (10^4 CFU)	0.33	1.67	10.00	2.67	4.33	4.00	1.49	0.286
Salmonella sp. (10^4 CFU)	Ud	Ud	Ud	Ud	Ud	Ud	-	-

Ud: Undetected, SEM: Pooled standard error of means from column-wise comparisons

Table 3: Jejunal morphology responses to administration of nano-encapsulation of noni fruit extract via drinking water in laying hens for 35 days

	Treatment						Statistics	
Morphological parameters	P0	P1	P2	P3	P4	P5	SEM	p-value
VH (μm)	1031.3	1071.5	1231.3	1236.7	1299.7	1164.60	114.64	0.558
VW (μm)	50.9	63.7	42.7	39.5	32.2	45.10	6.35	0.062
CD (μm)	162.2	242.0	226.4	256.6	343.0	286.30	38.84	0.094
V:C ratio	7.48	4.83	5.70	4.91	3.85	4.14	0.99	0.202

VH: Villus height, VW: Villus width, CD: Crypt depth, V:C ratio: Villus height: crypt depth, SEM: Pooled standard error of means from column-wise comparisons

nano-encapsulation of noni fruit extract in drinking water reduced the population of *E. coli* but did not affect the population of *Salmonella* sp. or *B. subtilis*, nor did it affect the histomorphology of the jejunal intestine. The current study showed a beneficial function of the nano-encapsulation technique, namely, to increase the efficacy of phytobiotics administration through drinking water to reduce populations of potentially pathogenic microbes. The reduction of harmful microbes may help to maximize nutrient uptake for daily body requirements.

CONCLUSION

Supplementation with nano-encapsulation of noni fruit extract in the drinking water of laying hens reduced colonization by *E. coli* and might be used as an alternative to in-feed antibiotics in laying hens.

SIGNIFICANCE STATEMENTS

This study discovered that supplementation with nano-encapsulation of noni fruit extract in the drinking water of laying hens can be beneficial by reducing colonization by *E. coli*. This study will help researchers to explore the use of nano-encapsulation for protection using plant extracts, especially from noni fruit. Therefore, a new theory may develop regarding the efficacy of nano-encapsulation of noni fruit extract.

REFERENCES

1. Hafez, H.M., 2011. Enteric diseases of poultry with special attention to *Clostridium perfringens*. Pak. Vet. J., 31: 175-184.
2. Goetting, V., K.A. Lee and L.A. Tell, 2011. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: A review of the literature. J. Vet. Pharmacol. Ther., 34: 521-556.
3. Satwadhar, P.N., H.W. Deshpande, I.H. Syed and K.A. Syed, 2004. Nutritional composition and identification of some of the bioactive components in *Morinda citrifolia* juice. Food Chem., 52: 58-59.
4. Wang, M.Y., B.J. West, C.J. Jensen, D. Nowicki, C. Su, A.K. Palu and G. Anderson, 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacol. Sin., 12: 1127-1141.
5. Munin, A. and F. Edwards-Levy, 2011. Encapsulation of natural polyphenolic compounds: A review. Pharmaceutics, 3: 793-829.
6. Turk, C.T.S., Z.S. Bayindir and U. Badilli, 2009. Preparation of polymeric nanoparticles using different stabilizing agents. J. Fac. Pharm. Ankara, 38: 257-268.
7. Bunglavan, S.J., A.K. Garg, R.S. Dass and S. Sameer, 2014. Use of nanoparticles as feed additives to improve digestion and absorption in livestock. Livest. Res. Int., 2: 36-47.
8. Mohanraj, V.J. and Y. Chen, 2006. Nanoparticles-A review. Trop. J. Pharm. Res., 5: 561-573.
9. Sundari, S., Z. Zuprizal, T. Yuwanta and R. Martien, 2014. The effect nanocapsule of turmeric extracts in rations on nutrient digestibility of broiler chickens. Anim. Prod., 16: 107-113.
10. Koshikawa, T., M. Yamazaki, M. Yoshimi, S. Ogawa, A. Yamada, K. Watabe and M. Torii, 1989. Surface hydiophobicity of spores of *Bacillus* spp. Microbiology, 135: 2717-2722.
11. Leininger, D.J., J.R. Roberson and F. Elvinger, 2001. Use of eosin methylene blue agar to differentiate *Escherichia coli* from other Gram-negative mastitis pathogens. J. Vet. Diagn. Invest., 13: 273-275.
12. Al-Lahham, A.B., M. Abu-Saud and A.A. Shehabi, 1990. Prevalence of *Salmonella*, *Shigella* and intestinal parasites in food handlers in Irbid, Jordan. J. Diarrhoeal Dis. Res., 8: 160-162.

13. Rahmi, E., D. Agustina and F. Jamin, 2014. Isolation and identification of genus *Salmonella* and *Shigella* from sumatran orangutan (*Pongo abelii*) feces in orangutan reintroduction center, Jantho. J. Med. Vet., 8: 5-8.
14. Zulkifli, I., H.S.I. Rahayu, A.R. Alimon, M.K. Vidyadaran and S.A. Babjee, 2009. Gut microflora and intestinal morphology of commercial broiler chickens and red jungle fowl fed diets containing palm kernel meal. Arch. Geflugelk., 73: 49-55.
15. Thanh, N.T., T.C. Loh, H.L. Foo, M. Hair-Bejo and B.K. Azhar, 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br. Poult. Sci., 50: 298-306.
16. Steel, R.G.D. and J.H. Torrie, 1993. [Principles and Procedures of Statistics: A Biometrical Approach]. 2nd Edn., PT Gramedia Pustaka Utama, Jakarta, Indonesia, Pages: 215-221.
17. Kurniawan, D., E. Widodo and I.H. Djunaidi, 2016. The effect of noni (*Morinda citrifolia*) fruit meal as feed additive on intestinal microfloras and villi characteristics of hybrid duck. Buletin Peternakan, 40: 34-39.
18. Godstime, C.O., O.E. Felix, O.J. Augustina and O.E. Christopher, 2014. Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens-a review. J. Pharm. Chem. Biol. Sci., 2: 77-85.
19. Shim, K.M., S.H. Choi, M.J. Jeong and S.S. Kang, 2007. Effects of aucubin on the healing of oral wounds. *In Vivo*, 21: 1037-1041.
20. Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.
21. Lloyd, D.P. and R.J. Allen, 2015. Competition for space during bacterial colonization of a surface. J. R. Soc. Interf., 12: 1-15.
22. Sharmila, P.S. and A.K. Vidya, 2015. Characterization and antibacterial activity of bacteriocin producing *Bacillus subtilis* isolated from raw milk. Int. J. Applied Bioeng., 9: 1-7.
23. Nguyen, F., A.L. Starosta, S. Arenz, D. Sohmen, A. Donhofer and D.N. Wilson, 2014. Tetracycline antibiotics and resistance mechanisms. Biol. Chem., 395: 559-575.
24. Russell, A.D., 1999. Bacterial resistance to disinfectants: Present knowledge and future problems. J. Hospital Infect., 43: S57-S68.
25. Donado-Godoy, P., I. Gardner, B.A. Byrne, M. Leon and E. Perez-Gutierrez *et al*., 2012. Prevalence, risk factors and antimicrobial resistance profiles of *Salmonella* from commercial broiler farms in two important poultry-producing regions of Colombia. J. Food Prot., 75: 874-883.
26. Sunder, J., T. Sujatha, N. Pazhanivel, A. Kundu and M.S. Kundu, 2014. Effect of *Morinda citrifolia* fruit juice and *Lactobacillus acidophilus* on broiler duodenal morphology. Adv. Anim. Vet. Sci., 2: 28-30.