Effect of Dietary Nano-encapsulated Mindi (*Melia azedarach* Linn.) Leaf Extract on Growth Performance and Intestinal pH of Broiler Chickens

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Abstract. A four weeks study was conducted to evaluate the effects of nano-encapsulated Mindi (*Melia azedarach* Linn.) leaves extract inclusion in drinking water on growth performance and intestinal pH in broiler chickens. A hundred and ninety two chicks were allotted into eight treatment groups: a negative control group without any treatment in the drinking water (T1), a positive control group with tetracycline 100 ppm (T2), groups with 100 mg (T3), 200 mg (T4), 400 mg (T5) mindi leaves extract per litre, or groups with 100 mg (T6), 200 mg (T7), 400 mg (T8) nano-encapsulated mindi leaves extract per litre (L). Each treatment was replicated four times with six birds per pen. Oneway ANOVA was used to analyse collected data, continued with Duncan's new Multiple Range Test for data with significant difference. No treatment effect were detected on feed intake, body weight gain, feed conversion ratio, water intake, or final weight. However, inclusion with 200 mg mindi leaves extract per L reduced pH (P<0.05) in the jejunum and ileum. Current research indicated that addition of low dose Mindi leaves extract – nanocapsulated or non-nanoencapsulated – might give significant effect on improving intestinal health condition in broiler chickens.

1. Introduction

Phytobiotics are plant-derived natural bioactive compounds that can be added into diets to improve the growth performance of livestock. Bioactive compounds found in the phytobiotics were reported to have some beneficial properties, such as: growth promoter, digestive enzymes stimulator, intestinal microflora balancer [1], as well as antibacteria, antifungi [2, 3, 4] antihelmimtic [5], antioxidants [6, 7], and anti-stress [8]. Compared with synthetic antibiotics or inorganic chemicals compounds, plants derived feed additives have been proven to be natural, low in toxicity, free of residue, cheaper, and an ideal feed additive in animal feed [9]. Huyghebaert et al. [10] indicated that bioactive components in phytobiotics are mostly secondary plant constituents, such as: terpenoids (steroids), phenolics (flavonoids and tannins), glycosides and alkaloids (alcohols, aldehydes, ketones, esters, ether etc.). One candidate that contain bioactive compounds and possibly can be used as alternative for antibiotic is mindi (*Melia azedarach* L.) leaves.

Mindi was known for many years in some countries as a medicinal herbs to fight malaria, diabetes, coughs, and skin diseases [11]. As a member of Meliaceae family, mindi leaves were reported to contain many bioactives components, such as: phenols, flavonoids, tannins, alkaloids, polisteroids, and saponins. Phenol compounds were reported to have biological functions, such as: antioxidants, anti-inflammatory, anti-carcinogen, and anti-atherosclerotic [12]. Munir et al. [13] indicated that secondary metabolite contents in mindi leaves were reported to have antimicrobial properties by reducing the
activities and populations of pathogenic bacteria in both gram-positive bacteria (Bacillus thuringiensis and Staphylococcus aureus) and gram-negative (Escherichia coli). On the other hand, many bioactive compounds in mindi leaves have lipophilic properties, low in water solubility, low bioavailability, easily damaged by environmental influences in the digestive tract and limited absorption in the digestive tract [14].

One of potential strategies to increase the efficacies of herbal active compounds is by using nano-capsule technologies. Nanotechnology maximizes the process of transportation for effective absorption, as in chitosan nanoparticles that is cross-linked with STPP [15]. Nano-encapsulation can be made with ionic gelation method using chitosan and sodium tripolyphosphate (STPP). In scientific perspective, ionic gelation method is based on the different charges between positive charges of chitosan and negative charges of STPP [16]. Chitosan is known as a biocompatible, natural, biodegradable, and bioadhesive polymer. STPP is polyanion that can be used as cross-link agent. The interaction between chitosan and STPP as nano-encapsulated agent leads to the formation of biocompatible cross-linked chitosan nanoparticles, which can efficiently deliver bioactive of phytobiotics as antimicrobial agent in broiler chicken intestine. Therefore, purpose of this study was to investigate the likely beneficial effects of nano-encapsulated mindi leaves extract inclusion in drinking water on growth performance and intestinal pH in broiler chickens

2. Materials and methods

2.1. Animal, Diets, and Experimental Design

The study was conducted at the Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. A hundred and ninety two of eight days old New Lohmann MB 202 broiler chickens were plotted into eight treatment groups: a negative control group without any treatment in the drinking water (T1), a positive control group with tetracycline 100 ppm (T2), groups with 100 mg (T3), 200 mg (T4), 400 mg (T5) mindi leaves extract per L, or groups with 100 mg (T6), 200 mg (T7), 400 mg (T8) nano-encapsulated mindi leaves extract per L, respectively. The treatments were replicated four times with six birds in each replicate pen. Feed formulation and chemical compositions for the experimental diets are shown in Table 1. The diets and drinking water were supplied ad libitum for the 35 consecutive experimental days.

2.2. Preparation and Extraction of Mindi Leaves

Fresh mindi leaves were collected from Sleman Regency, Yogyakarta, Indonesia. The leaf samples were oven-dried at 55°C for 2-3 days. The dried samples were milled with Thomas-Willey mill (Arthur H. Thomas Co., Philadelphia, USA) to pass through a 1 mm sieve and stored with room temperature. Two hundred grams of mindi leaves powder were placed in beaker glass and added with 600 ml ethanol (70%). The mixture was macerated for 3 days and stirred regularly. After 3 days all of the macerate was filtered and the filtrate was evaporated using a waterbath. The thick extract was then diluted with distilled water to 2%

2.3. Preparation of Nano-encapsulation Mindi Leaves Extract: Chitosan: STPP

The nano-encapsulation was processed using ionic gelation method by mixing mindi leaves extract: chitosan: STPP with optimum formulation (1: 7: 1/175%) w/v. Two grams chitosan were added into 100 ml acetic acid (1% b/v) and dissolve 0.04 ml into distilled water and are stirred for one hour or until it appeared clear. Mindi leaves extract and chitosan were mixed with optimum formulation (chitosan dissolved in acetic acid pH 4 solution), and stirred using a magnetic stirrer for an hour. STPP was added and stirred for 30 minutes. After the solution became clear, the sediment were separated. The precipitate was removed and the solution of nano encapsulation was used in the in vivo study.
Table 1. Ingredients composition and nutrients calculation of the experimental basal diet

| Items                                      | Contents |
|--------------------------------------------|----------|
| Yellow corn                                | 55.70    |
| Soybean meal                               | 26.80    |
| Meat bone meal                             | 7.00     |
| Rice bran                                  | 6.50     |
| Palm oil                                   | 2.80     |
| Premix vitamin*                            | 0.25     |
| L-Lysine HCl                               | 0.20     |
| DL-Methionine                              | 0.20     |
| Calcium carbonate                          | 0.30     |
| Sodium chloride                            | 0.25     |
| **Total**                                  | 100.00   |

| Calculated nutrients and energy, %         |          |
|-------------------------------------------|----------|
| Crude Protein                             | 21.34    |
| Metabolizable energy, kcal/kg              | 3089.69  |
| Crude fiber                               | 3.31     |
| Available phosphorus                      | 0.72     |
| Calcium                                   | 0.95     |
| Lysine                                    | 1.32     |
| Methionine                                | 0.53     |

*Premix vitamin (Masamix-Bro) supply per kg diet: Vit. A: 12,500,000 IU; Vit. D3: 2,500,000 IU; Vit. E: 10,000 mg; Vit. K3: 2,000 mg; Vit. B1: 2,000 mg; Vit. B2: 4,000 mg; Vit. B6: 1,000 mg; Vit. B12: 12,000 mcg; Vit.C: 40,000 mg; Niacin: 40,000 mg; Biotin: 200 mg.

2.4. Growth Performance
Feed intake (FI) and water intake (WI) were recorded daily. Body weight gain (BWG) were recorded weekly. Feed conversion ratio (FCR) were calculated daily. Final weight were weighted on day 35.

2.5. Intestinal pH
At days 35, one bird with body weight close to the median weight of each pen were selectively chosen. The selected birds were slaughtered according to the Islamic law for measurement of intestinal pH. The pH of digesta from 3 intestinal segments (duodenum, jejunum, and ileum) was determined immediately by inserting the needle tip of pH meter (PT 370, Boeco, Hamburg, Germany).

3. Statistical Analysis
The data were statistically analyzed by one-way ANOVA with completely randomized design, using Statistical Package for Social Science or SPSS (SPSS GmbH, Munich, Germany). The data with significant differences were further separated using Duncan's new Multiple Range Test. All indications of significant differences were based on the probability of less than 5%.
4. Results and Discussion

4.1. Broiler Growth Performance
There was no significant difference between groups on growth performance (FI, FCR, WI, BWG, and final weight) parameter on weeks 1, 2, 3, or 4. As shown in Table 2, no effect was found in feed intake data following 100-400 mg/l mindi leaves extract (MLE) or nano-encapsulation mindi leaves extract (NMLE) supplementations. The lack effect on feed intake in current study might be attributed to the improper supplemental dose of the extracts or the quality of mindi leaves used in extraction process. The both limitations might reduce the contents of bioactive compounds, such as phenols and flavonoids, and efficacies of the green additives. Result in this study was similar with the findings of Windisch et al. [3] that also reported non-significant effect on feed intake following improper dose supplementations. Al-Dhanki et al. [17] also showed a same result that non significant different in feed intake was shown following 10 and 15 mg/ml mindi leaves extract supplementations in drinking water for 49 days study.

Table 2. Feed intake data of broiler chickens fed basal diet supplemented with MLE and NMLE through drinking water

| Treatments² | Weeks       |       |       |       |
|-------------|-------------|-------|-------|-------|
|             | 1           | 2     | 3     | 4     |
| T1          | 419.18±18.43| 702.29±17.38 | 1054.81±70.09 | 1239.97±114.90 |
| T2          | 405.77±12.92| 713.20±33.72 | 1023.01±58.59 | 1118.89±111.74 |
| T3          | 406.50±8.14  | 709.58±41.96 | 1066.23±109.19| 1239.82±134.08 |
| T4          | 391.64±17.84| 701.81±39.07 | 1017.93±87.32 | 1081.51±141.44 |
| T5          | 388.82±17.50| 660.22±64.73 | 1020.57±32.44 | 1158.57±182.35 |
| T6          | 402.46±13.02| 719.38±38.78 | 1084.04±29.92 | 1204.18±121.82 |
| T7          | 401.50±14.12| 689.18±36.79 | 1020.37±109.53| 1241.39±137.02 |
| T8          | 416.56±9.58 | 727.15±8.95 | 994.71±88.37  | 1221.67±103.88 |

SEM  | 2.86       | 6.93  | 13.24 | 23.12 |
P-value| 0.074 | 0.359 | 0.768 | 0.550 |

¹Means represent 4 pens of 6 birds each per treatment.
²T1=negative control (NC); T2=positive control + 100 ppm tetracycline; T3=NC+100 mg/l MLE; T4=NC+200 mg/l MLE; T5=NC+400 mg/l MLE; T6=NC+100 mg/l NMLE; T7=NC+200 mg/l NMLE; T8=NC+400 mg/l NMLE.

The non significant different result in body weight gain (Table 3) might be caused by insignificant difference result on the feed intake between supplemented and non-supplemented birds. As reported in Mohammadrezaei et al. [18] study, body weight gain was positively correlated with the intake of feed and fulfillment of nutrient and energy requirements. Therefore, the lack of difference in BWG could be initiated by the similarity in the amount of feed consumed by the birds. Result in this study was also corroborate with the findings of Al-Dhanki et al. [17] which reported a non-significant difference result between control and herb treatments on BWG after 10 mg/ml MLE supplementations, however decreased BWG after 15 mg/ml MLE supplementations.
Table 3. Body weight gain data of broiler chickens fed basal diet supplemented with MLE and NMLE through drinking water

| Treatments | 1             | 2             | 3             | 4             |
|------------|---------------|---------------|---------------|---------------|
| T1         | 296.38±6.57   | 486.66±39.32  | 610.80±21.40  | 616.56±79.66  |
| T2         | 297.95±7.18   | 473.15±40.23  | 583.85±30.86  | 567.05±80.09  |
| T3         | 287.00±11.50  | 487.70±31.96  | 564.15±52.65  | 581.25±112.95 |
| T4         | 292.54±4.87   | 478.35±15.23  | 562.14±56.64  | 513.64±74.65  |
| T5         | 283.04±6.01   | 447.36±5.52   | 576.01±36.93  | 603.69±98.51  |
| T6         | 293.35±8.82   | 473.55±21.90  | 599.23±103.92 | 661.54±55.63  |
| T7         | 283.30±17.40  | 462.64±29.50  | 599.23±103.92 | 609.79±93.43  |
| T8         | 296.84±3.88   | 478.70±25.80  | 584.65±55.42  | 609.79±93.43  |

SEM 1.76 4.95 8.94 15.74  P-value 0.151 0.545 0.914 0.524  

1Means represent 4 pens of 6 birds each per treatment.  
2T1=negative control (NC); T2=positive control + 100 ppm tetracycline; T3=NC+100 mg/l MLE; T4=NC+200 mg/l MLE; T5=NC+400 mg/l MLE; T6=NC+100 mg/l NMLE; T7=NC+200 mg/l NMLE; T8=NC+400 mg/l NMLE.

Table 4. Feed conversion ratio of broiler chickens fed basal diet supplemented with MLE and NMLE through drinking water

| Treatments | 1             | 2             | 3             | 4             |
|------------|---------------|---------------|---------------|---------------|
| T1         | 1.41±0.043    | 1.45±0.096    | 1.72±0.068    | 2.02±0.172    |
| T2         | 1.36±0.027    | 1.51±0.067    | 1.75±0.092    | 1.98±0.127    |
| T3         | 1.42±0.033    | 1.45±0.030    | 1.89±0.104    | 2.16±0.199    |
| T4         | 1.34±0.046    | 1.46±0.040    | 1.81±0.078    | 2.11±0.149    |
| T5         | 1.38±0.050    | 1.48±0.144    | 1.78±0.106    | 1.95±0.448    |
| T6         | 1.37±0.025    | 1.52±0.056    | 1.84±0.082    | 2.05±0.24     |
| T7         | 1.42±0.052    | 1.49±0.032    | 1.72±0.127    | 1.88±0.200    |
| T8         | 1.40±0.033    | 1.52±0.079    | 1.72±0.302    | 2.02±0.192    |

SEM 0.008 0.012 0.024 0.040  P-value 0.055 0.777 0.550 0.776  

1Means represent 4 pens of 6 birds each per treatment.  
2T1=negative control (NC); T2=positive control + 100 ppm tetracycline; T3=NC+100 mg/l MLE; T4=NC+200 mg/l MLE; T5=NC+400 mg/l MLE; T6=NC+100 mg/l NMLE; T7=NC+200 mg/l NMLE; T8=NC+400 mg/l NMLE.

Table 4 showed drinking water supplementations with MLE or NMLE did not affect FCR on weeks 1, 2, 3, and 4. Our finding agreed with Al-Dhanki et al. [17] who observed that no significant different was found in FCR value following 49 days drinking water supplementations with 10 and 15 mg/ml mindi leaves extract. In other study, Beg et al. [19] also reported that supplementation of 2.5% neem leaves meal, which contained similar bioactive compound with mindi, did not affect FCR.

Supplementations of MLE and NMLE were also did not affect water intake of broiler in week 1, 2, 3, 4 (Table. 5). This result was also similar with the result of Zanu et al. [20] study which reported a non-significant effect on water intake following drinking water supplementations with neem leaves extract. However, Durrani et al. [21] showed that drinking water treatment with 50 ml/l neem leaves extract reduced daily feed and water intakes. Difference results between our study and the other study might be attributed to the differences of material and methods used in the studies.
The secretion of microbial metabolites, such as: or colonization of beneficial microbes. Treatment with antimicrobial agents like MLE might reduce pathogenic microbial population in the gut, which could be favored by increased population of beneficial microbes in the jejun and ileum. However, treatment with MLE and NMLE might increase the population of beneficial microbes in the jejun and ileum, and might reduce the effect to reduce the pathogenic bacteria.

Table 5. Water intake of broiler chickens fed basal diet supplemented with MLE and NMLE through drinking water

| Treatments | Weeks | 
|------------|-------|
|            | 1     | 2     | 3     | 4     |
| T1         | 875.7±30.54 | 1527.19±123.29 | 2464.18±271.67 | 3407.02±436.45 |
| T2         | 827.75±39.5 | 1417.39±99.89  | 2220.17±198.82 | 3015.83±327.79 |
| T3         | 836.58±52.11| 1464.37±106.78 | 2124.03±116.68 | 3120.28±594.56 |
| T4         | 821.92±39.92| 1432.47±88.74  | 2226.04±132.73 | 3029.09±449.00 |
| T5         | 820.56±19.31| 1426.34±97.03  | 2357.62±66.65  | 3583.68±436.20 |
| T6         | 839.16±19.04| 1450.09±58.28  | 2281.98±226.63 | 3252.63±197.85 |
| T7         | 848.19±41.19| 1481.73±89.93  | 2341.51±202.34 | 3283.83±491.46 |
| T8         | 875.75±30.28| 1524.64±73.95  | 2046.87±216.14 | 3252.18±134.74 |

Means represent 4 pens of 6 birds each per treatment.

4.2. Intestinal pH

Table 6 showed no effect of MLE or NMLE supplementations on duodenal pH. However, supplementation with 200-400 mg/l MLE or 100-400 mg/l NMLE reduced jejunal pH (P<0.05) and ileal pH (P<0.01). Reductions of the jejunal and ileal pH due to the MLE or NMLE supplementations might be attributed to the antimicrobial properties of the secondary metabolites in the MLE, such as: phenols, flavonoids, and tannins.

Table 6. Effect of nano-encapsulation of MLE and NMLE supplementations on intestinal pH

| Treatments | Duodenum | Jejunum | Ileum |
|------------|----------|---------|-------|
| T1         | 5.75±0.36 | 5.98±0.22 | 6.39±0.37 |
| T2         | 5.64±0.19 | 6.02±0.53 | 6.39±0.32 |
| T3         | 5.74±0.21 | 6.13±0.44 | 6.38±0.25 |
| T4         | 5.49±0.29 | 5.69±0.34 | 6.06±0.16 |
| T5         | 5.06±0.05 | 5.35±0.36 | 5.51±0.52 |
| T6         | 5.67±0.23 | 5.60±0.28 | 5.85±0.39 |
| T7         | 5.49±0.66 | 5.72±0.50 | 5.90±0.53 |
| T8         | 5.48±0.59 | 5.32±0.08 | 5.26±0.18 |

Means represent 4 pens of 6 birds each per treatment.

Munir et al. [13] stated that bioactive compounds in herbs, such as phenols and flavonoids, have antimicrobial activities which can decrease the growth and population of pathogenic bacteria. Reduction of pathogenic microbial population in the gut could have a favorable effect to stimulate the growth and colonization of beneficial microbes. The increased population of beneficial microbes might increase secretion of microbial metabolites, such as: organic acid that might reduced pH in the jejunal and ileum. Reductions of the pH in the part of intestine that have responsibilities for micro-nutrients
absorption might produce triple benefits: reduce the colonization of pathogenic microbes, reduce over production of intestinal mucous barrier by Goblet cells, and maximize the alteration and growth of micro-villi cells for optimization of micro-nutrients absorption.

Reductions of jejunal and ileal pH following NMLE supplementation might be also due to the presence of bioactive compounds and chitosan or STPP used in the encapsulation. This result was in agreement with the result of Kaur et al. [22] study which observed that nano-encapsulation technology can involved crosslinking process between chitosan and STPP which protect MLE bioactive compound. In addition, chitosan as the media of nano-encapsulation, was also known to have antimicrobial activity. Fernandes et al. [23] and Shekhawat et al. [24] reported that chitosan has antibacterial activity against: Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella paratyphi B. Combination effects of the mendi bioactive compounds and chitosan synergistically reduced jejunal and ileal pH, which in turn will also reduce population of the pathogenic microbes and improve intestinal health.

5. Conclusion

Nano-encapsulation of mendi leaves extract could be a beneficial alternative for antibiotics in the diets of broiler chickens. Reduction of the jejunal and ileal pH might be an indicator for the improvement intestinal health of broiler chickens.

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