

## Potency of Green Cincau Leaves (*Premna oblongifolia* merr) Juice as Prebiotics and its Effect on Laying Hen Performances

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### Abstract

This study investigated the potency of green cincau leaves (*Premna oblongifolia* merr) juice as prebiotics and its effect on laying performances (feed intake, egg weight, feed conversion and hen day production) based on pH and digestive viscosity. The potency of green cincau leaves juice as prebiotic was tested. The result showed that green cincau leaves juice was effectively support growth of Lactic Acid Bacteria (LAB) with  $4.3 \times 10^2$  CFU.g<sup>-1</sup> samples but did not support the growth of *Escherichia coli*. The effect of green cincau leaves juice on laying performances was examined. Two hundred fifty 56-wk-old Hisex Brown layers received different diets namely 0, 5, 7.5, 10 and 12.5 ml of green cincau leaves juice added in the basal feed. The method used in this study was experiment based on Completely Randomized Design and followed by Duncan's Multiple test if significant effect occurs. The result showed that increase level of *P. oblongifolia* merr in feed did not significantly affect ( $P>0.05$ ) feed intake, egg weight, feed conversion and hen day production.

**Keywords:** feed additives, laying hens performance, prebiotic, *Premna oblongifolia* merr juice.

### INTRODUCTION

Fiber is one of important component in diet which gives beneficial effect to the development of gastrointestinal tract and, therefore, performance in monogastrics. Green cincau leaf (*Premna oblongifolia* merr) which contains soluble and insoluble fibers, could be promoted to manipulate the digestive physiological function. The soluble fiber optimizes growth of anaerobic bacteria which modifies and/or changes villous characteristics [1]. Insoluble fiber, on the other hand, increases retention time of the digesta in upper part of digestive tract (crop to gizzard) and gizzard function. The soluble fiber of green cincau leaf consist of mostly hemiselulose and pectin. While the insoluble fiber contents are selulosa, lignin and silica. The ratio of insoluble to soluble fiber is important for more efficient production [2].

The use of plant phytonics has been found to be a good candidate to replace antibiotics. Antibiotics are used to treat specific health problems (therapeutic use) and to improve the animal performances (growth promoter use). This practical use of antibiotics has been avoided due to the development of antibiotic-resistant

strains of bacteria that contribute to the presence of antibiotic-resistant pathogens in humans. Previous report indicated that aqueous extract of green cincau leaf (*Cyclea barbatas* merr) 3 mL.day<sup>-1</sup> in rats did not affect on the acetylsalicylic acid-induced gastric acid, but it showed protective effect against acetylsalicylic acid induced gastric mucosal lesion [3], and green cincau leaf (*P. oblongifolia* merr) extract of 5.4 mL.day<sup>-1</sup> significantly decreased triglycerides of Sprague Dawley rats dyslipidemia for about 18% [4]. The current research was intended to examine effect of added various level of green cincau juice in diets on laying hen performances and digesta characteristics (TPC for Lactic acid bacteria and *Escherichia coli*, pH and viscosity), and performances.

### MATERIALS AND METHODS

#### Preparation of *Premna oblongifolia* merr juice

Total 10 g of selected leaves of *P. oblongifolia* merr plant were mixed with 250 ml of water, stirred the mixture by using a medium speed (250 rpm for 1 minute) of kitchen blender. The results were then filtered by using muslin cloth.

#### Chemical analysis

Analysis of soluble fiber was by gravimetric method and pectin analysis was by Bayes method. Crude fiber was analyzed by using Van Soest method [5].

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### Microbial analysis

MRSA were used for isolation of Lactic Acid Bacteria, and EM agar for *E. coli*. The total plate count lactic acid bacteria and *E. coli* were counted after incubation in aerobic chamber at 37°C for 48 hours with colony counter [6].

### Bird and Diet

A total 250 fifty-six-weeks old laying hens were used in this experiment and randomly allotted to 5 treatments and 5 replications, 10 hens for each replication. The addition of different levels of *P. oblongifolia* merrj uices into the basal diet were arranged into 5 treatments (Table 1).

Table 1. Diet for the Hens

| Treatments     | Basal diet | Green cincau leaves extract<br>(ml.bird <sup>-1</sup> .day <sup>-1</sup> ) |
|----------------|------------|--|
| P <sub>0</sub> | ✓          | -  |
| P <sub>1</sub> | ✓          | 5.0  |
| P <sub>2</sub> | ✓          | 7.5  |
| P <sub>3</sub> | ✓          | 10.0   |
| P <sub>4</sub> | ✓          | 12.5   |

The basal diet (Table 2 and 3) was formulated to meet the layer chicken requirements (Table 4) based on SNI [7]. The layer chicken had free access to feed and water. Basal Diet was analyzed in Feed and Nutrition laboratory Faculty of Animal Husbandry-UB.

Table 2. Formula of Basal Diet

| Ingredients | Total (%) |
|-------------|-----------|
| Corn grain  | 50.51     |
| Rice bran   | 15.78     |
| Concentrate | 31.57     |
| Mineral     | 1.89      |
| Premix      | 0.25      |

Table 3. Chemical composition of dietary ingredients

| Ingredients (%)  | Corn grain <sup>[8]</sup> | Rice bran <sup>[8]</sup> | concentrate <sup>[9]</sup> |
|------------------|---------------------------|--------------------------|----------------------------|
| Crude Protein    | 8.50                      | 12.20                    | 36.00                      |
| Crude Fat        | 3.80                      | 11.00                    | 5.00                       |
| Crude Fiber      | 2.20                      | 4.10                     | 8.00                       |
| Ash              | 1.50                      | 6.90                     | 35.00                      |
| Calcium          | 0.02                      | 0.05                     | 11.00                      |
| Total Phosphorus | 0.28                      | 1.31                     | 1.00                       |

Table 4. Basal Diet and Standard Quality of Layer Feed

| Ingredients                 | Basal Diet | Layer Feed Standard <sup>[7]</sup> |
|-----------------------------|------------|------------------------------------|
| ME (Kkal.kg <sup>-1</sup> ) | 2780*      | >2650                              |
| Crude Protein (%)           | 18.02      | 17.2-18.2                          |
| Crude Fat (%)               | 5.16       | <7.0                               |
| Crude Fiber (%)             | 4.36       | <7.0                               |
| Ash (%)                     | 13.41      | 14                                 |
| Calcium (%)                 | 3.68       | 3.25-4.25                          |
| Total Phosphorus (%)        | 0.66       | 0.6-1.0                            |

\* Estimating Metabolizable Energy (ME) as 70% of Gross Energy (GE)

### Sample

On 60 weeks old, one bird was randomly selected from each replication cage and was killed by cervical dislocation and immediately after that intestinal sample in the section between meckel diverticulum and ileocaecal junction was collected. The digesta of all above mentioned segment was then immediately brought to laboratory for microbial study.

### Performance parameter

Bird performance was evaluated through the average egg production within 60 days, in percentage per bird per day. It was obtained from the number of eggs produced, including cracked, broken or abnormal, and the number of birds per plot that produced them, the average egg weight at the end of each experimental week through the intact eggs collected during the day in each plot and weighed.

**Feed intake:** Food intake in grams consumed per bird per day.

**Egg weight:** The product of egg production and the average egg weight in grams.

**Feed conversion:** The average feed intake (kg) by the average eggs mass produced (egg weight, kg x egg production.100<sup>-1</sup>) expressed in kilograms of feed consumed per kilogram of egg production.

**Hen-Day Production (HDP):** Ratio between numbers of eggs on daily basis to number of bird available in the flock on that day x 100 %.

### pH and Viscosity of The Digesta

Digesta pH was then measured within using a digestive pH meter method [10]. Digesta were dissected and polled in collection tube, intestine digesta viscosity was measured according to the method [10].

### Statistical Analysis

Experiment based on Completely Randomized Design. It was followed by Duncan's multiple test if significant effect occurs.

## RESULT AND DISCUSSION

### Fiber Composition

Green cincau leaves (*P. oblongifolia merrj*) juice contains soluble fiber and insoluble fiber. Table 5 showed the composition and the fiber composition in green cincau leaves. The ½ of total fraction fiber was soluble fiber and ½ was insoluble fiber. In Green cincau leaves juice soluble fiber was 0.62%. The previous report indicated that leaves juice contained 1.94 g total

fiber with 1.45 g insoluble fiber and 0.48 g soluble fiber [11].

**Table 5.** Fiber Composition in Green Cincau Leaves

| Type of Fiber | Total (%) |
|---------------|-----------|
| Pectin        | 0.55      |
| NDF           | 67.68     |
| ADF           | 54.51     |
| Hemiselulosa  | 13.17     |
| Selulosa      | 35.71     |

### Microbial Population

TPC of digesta for Lactic Acid Bacteria (LAB) and *E. coli* were presented in Table 6 and 7. The result showed *P. oblongifolia* merr juice was effectively support growth of Lactic Acid Bacteria (LAB) with  $4.3 \times 10^2$  CFU.g<sup>-1</sup> samples but did not support the growth of *E. coli*. Previous report showed that Green cincau gel forming agent extract has a prebiotic effect with total lactic acid bacteria  $11.97 \times 10^{11}$  CFU.mL<sup>-1</sup> [12]. Pectin in green cincau leaves juice was suspect as substrat to support growth of Lactic Acid Bacteria (LAB). Carbon chain in galacturonic acid is converted to VFA (Volatil Fatty Acid) and became substrat that support growth of Lactic Acid Bacteria [13] but did not support the growth of *E. coli*.

**Table 6.** Total Plate Count (TPC) Lactic Acid Bacteria

| dilution         | Total LAB |    | median | Total<br>(CFU.g <sup>-1</sup> sample) |
|------------------|-----------|----|--------|---------------------------------------|
|                  | I         | II |        |                                       |
| 10 <sup>-1</sup> | 48        | 37 | 42.5   | 4.3 x 10 <sup>2</sup>                 |
| 10 <sup>-2</sup> | 19        | 12 | 15.5   |                                       |
| 10 <sup>-3</sup> | 5         | 3  | 4.0    |                                       |

**Table 7.** TPC (Total Plate Count) *Escherichia coli*

| Dilution         | Total <i>E.coli</i> |    | median | Total<br>(CFU.g <sup>-1</sup> sample) |
|------------------|---------------------|----|--------|---------------------------------------|
|                  | I                   | II |        |                                       |
| 10 <sup>-1</sup> | 0                   | 0  | 0      | 0                                     |
| 10 <sup>-2</sup> | 0                   | 0  | 0      |                                       |
| 10 <sup>-3</sup> | 0                   | 0  | 0      |                                       |

### pH of the Digesta

Table 8 showed that the addition of 12.5 mL.bird.<sup>-1</sup>day<sup>-1</sup> green cincau leaves juice in basal diet decreased pH of the digesta to 5.42 from 6.53.

**Table 8.** pH and viscosity of digesta

| Treatment      | Indicator                |                             |
|----------------|--------------------------|-----------------------------|
|                | pH                       | Viscosity<br>Digesta(dPa.s) |
| P <sub>0</sub> | 6.53 ± 0.07 <sup>a</sup> | 0.34 ± 0.02 <sup>a</sup>    |
| P <sub>1</sub> | 6.24 ± 0.04 <sup>a</sup> | 0.29 ± 0.02 <sup>b</sup>    |
| P <sub>2</sub> | 5.94 ± 0.06 <sup>b</sup> | 0.22 ± 0.02 <sup>c</sup>    |
| P <sub>3</sub> | 5.56 ± 0.07 <sup>c</sup> | 0.14 ± 0.01 <sup>d</sup>    |
| P <sub>4</sub> | 5.53 ± 0.13 <sup>d</sup> | 0.08 ± 0.01 <sup>e</sup>    |

**Description :** diffrent superscript in the same column indicated significant effect (P<0.01)

Pectin in green cincau leaves juice could be fermented by microflora to short chain fatty acid (VFA) and some spesific microflora produced latic acid so that pH decreased and inhibit patogen bacteria [14]. Type of fiber (selulosa, oat hulls and sugar beet pulp) gives significant variation on pH digesta which sugar beet pulp gives lowest pH because of pectin content [15].

### Digesta Viscosity

Decreased viscosity in treated bird than those given only basal diets indicated that pectin in green cincau leaves juice as soluble fibre was fermentable to support growth of anaerob bacteria. Increased viscosity was usually followed by increased population of anaerobic bacteria. The bacteria then increased SCFA (Short chain fatty acid) produced and affected in villous characteristic [16]. Supplementation of organic acid in the diet was capable to lowering digesta viscosity in laying hen [17]. Ratio of pectin dan selulosa affected differently on performance and phyco-chemical conditions of intestine [18].

## PERFORMANCE

### Feed intake

Green cincau leaves juice as additive in laying hens diets did not give significant effect on feed intake (Table 9). This indicated that fiber component in green cincau leaves juice can be tolerated by laying hens. The use of iso-protein and iso-energy in all diets used is also another factor that makes feed consumption remains unaffected. Basal diet formulated has crude protein of 18.02% and Metabolizable Energy of 2780 kcal.kg<sup>-1</sup> was in accordance to recommendation of NRC [8]. Soluble fiber and insoluble fiber in green cincau leaves juice might also give no changes toward digestion physiology particularly on passage rate, so that feed intake did not significantly affected (P>0.05) by the treatments.

### Egg weight

Treatments with various level of green cincau leaves juice did not significantly affect egg weight (Table 9). This may largely due to the addition of additive in the basal diet did not change protein and energy contents and therefore also their intake. Fiber component in green cincau leaves juice was able to reduce pH and intestinal viscosity but such effects could not able to change digestibility of nutrient so that no egg weight was observed in this study. Previous study observed that supplementation of 6% Hazel nut hulls in layer diets affected in yolk and egg weights [19].

**Tabel 9.** Effect of Green Cincau Leaves Juice on Laying Hens Performance

| Treatment      | Indicator  |                                       |                 |              |
|----------------|--|---------------------------------------|-----------------|--------------|
|                | Feed in take<br>(g.bird. <sup>-1</sup> day <sup>-1</sup> ) | Egg weight<br>(g.unit <sup>-1</sup> ) | Feed conversion | HDP (%)      |
| P <sub>0</sub> | 114.98 ± 3.48  | 67.43 ± 0.59                          | 2.12 ± 0.11     | 87.32 ± 1.64 |
| P <sub>1</sub> | 114.83 ± 1.81  | 67.04 ± 0.37                          | 2.01 ± 0.03     | 82.23 ± 2.53 |
| P <sub>2</sub> | 111.38 ± 3.11  | 67.71 ± 0.78                          | 2.08 ± 0.06     | 81.31 ± 6.24 |
| P <sub>3</sub> | 113.68 ± 2.96  | 67.33 ± 1.25                          | 2.02 ± 0.12     | 85.61 ± 5.34 |
| P <sub>4</sub> | 113.54 ± 0.75  | 66.28 ± 0.97                          | 2.04 ± 0.11     | 88.09 ± 2.45 |

### Feed Conversion

Green cincau leaves juice as additive in laying hens diets did not significantly affected to feed intake and egg weight, so that feed conversion ratio (Table 9). The potential effect green cincau leaves juice as prebiotics could not able to change the performances. A study observed that supple-mentation of prebiotics caused decreasing feed conversion [20]. Feed coversion decrease in broiler (1-14 day) diet with added of pectin than CMC (Carboxymethyl cellulose) was also reported [21]. This study found that adverse effect of pectin might be eliminated by selulose content in green cincau leaves juice, so that feed conversion did not change.

### Hen Day Production

On the basis of Hen day production no significant effect was observed (Table 9). Improvement GIT enviroment with lower pH and viscosity could not be able to increase nutrient digestion and absorption so that hen day production did not change.

### CONCLUSION

Green cincau leaves (*P. oblongifolia* merr) juice contained 0.62% soluble fiber majoring in pectin (0.55%). It effectively support growth of Lactic Acid Bacteria (LAB) with  $4.3 \times 10^2$  CFU.g<sup>-1</sup> sample but did not support the growth of *E. coli*. The used of green cincau leaves juice until 12.5 mL.bird.<sup>-1</sup>day<sup>-1</sup> in feed did not significantly affect (P>0.05) the laying hen performances.

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