The Effect of Addition Fermented Dairy-Waste Water Sludge by *Aspergillus niger* in Ration on Growth Performance and The Caecal Microbial of Broiler Chickens

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**Abstract.** Dairy Wastewater Sludge (DWS) is sediment from milk processing with a nutritional content fits for feedstuff. This experimental research investigated the nutritional value of DWS on the growth of broilers, and its specific impact on the development of microflora on broiler digestion. Tapioca by-product was used as DWS binder while *Aspergillus niger* fermentation was applied to improve nutritional content. The rate of fermented DWS addition in the ration was evaluated by measuring broilers’ weight gain for 35 days and microflora qualities in the cecum by counting the number of non-pathogenic and pathogenic bacteria in the cecum at the end of the research. The research data were analyzed by ANOVA with Duncan’s multiple range test. The results showed that supplementing 20% fermented DWS in rations resulted in the highest body weight gain and could suppress the growth of pathogenic bacteria (*Salmonella* spp., *E. coli*, and *Enterobacteriaceae*) in caecal. The ratio of non-pathogenic and pathogenic bacteria increased proportionally to the addition of fermented DWS levels in the ration. Microorganism activity in the caecum was reflected in varied caecum weight of broilers treated with different levels of fermented DWS. The condition illustrates the good health status of livestock so as to optimize the growth of broilers.

**Keywords:** dairy wastewater sludge, broiler, microflora, cecum

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**Introduction**

Dairy wastewater solid (DWS) is a waste produced from milk processing industry after degradation process in the stabilization pond. DWS is a good source of single protein cells. Previous studies reported that the single cell proteins are potential animal feed ingredients (Zhao, 2012; Wang et al., 2013; Yunus et al., 2015). DWS has a high nutritional value as a source of protein such as 34.98% crude protein, 4.1% lactose, 9.77% crude fiber, 11.04% crude fat, 2.33% calcium, and 1.05% phosphorus based on dry matter (Marlina, 2010).

Lactose in DWS positively affects chicken gut microflora to improve production
performance. One contributing factor to the composition of chicken gastrointestinal microflora is feed ingredients (Pan and Yu., 2013; Pourabedin and Zhao., 2015; Kers et al., 2018). Growth of non-pathogenic bacteria, such as lactic acid bacteria in chicken’s gastrointestinal tract can reduce pathogenic bacteria through organic acids formation by controlling the pH in digestive tract. Chickens have a low ability to produce lactose enzymes; therefore, the lactose is not digested but turned into organic acids in cecum and colon which subsequently reduce pH in digestive tract and protect against pathogenic bacteria (Meimandipour et al. 2009, El-Banna et al., 2010; Alloui and Szczurek, 2017). The limiting factors in processing DWS include high moisture content (95%) and the pathogenic bacteria. Pathogenic bacteria commonly found in waste are Salmonella, Shigella, E. coli, Streptococcus, Pseudomonas aeruginosa, Mycobacterium, Gland lamblia (Romdhana et al., 2009; Tang, 2019). DWS is very susceptible to decay and therefore, reduce the quality of nutrients. One of the efforts to process DWS is to fermentation using Aspergillus niger – a fungus of Aspergillus genus that does not produce mycotoxins. Tapioca by-product (onggok) is added to bind water content in DWS and to provide carbohydrates source for Aspergillus niger.

We studied the effect of DWS fermented levels in the diet on broiler and caecal microbial population, and ratio of Lactobacillus spp., Enterobacteriaceae, Salmonella, and Escherichia coli.

Materials and Methods
Preparation of chicken and rations
This research used 120 day old chicks (DOC) final stock Cobb strain obtained from PT Charoen Pokphand Jaya Farm with <10% coefficient of variation were administered. The feed ingredients consisted of yellow corn, fine bran, soybean meal, coconut cake, fish meal, CaCO₃, coconut oil, top mix, and fermented DWS. Fermented DWS was made by mixing milk sludge with tapioca by-product (onggok) flour with a ratio of 70:30, then fermented with 6% A. niger and incubated for 3 days. The ration was prepared with 3000 kcal/kg metabolic energy and 22% crude protein (Daghir, 1995). The composition of the ration, feed substance and metabolizable energy of feed ingredients are presented in Table 1 and 2. The ration and vitamin-enriched drinking water were given ad libitum. Disease prevention was carried out by administering ND vaccines.

| Feed Ingredients        | Level of fermented DWS in ration (%) |
|-------------------------|-------------------------------------|
|                         | 0        | 5        | 10       | 15       | 20       | 25       |
| Fish meal               | 12.50    | 12.50    | 12.50    | 12.50    | 12.50    | 12.50    |
| Fermented DWS           | 0.00     | 5.00     | 10.00    | 15.00    | 20.00    | 25.00    |
| Soybean meal            | 19.50    | 18.00    | 16.00    | 14.25    | 12.50    | 11.00    |
| Coconut cake            | 4.00     | 4.00     | 4.00     | 3.75     | 3.00     | 2.50     |
| Yellow corn             | 58.50    | 56.00    | 53.00    | 50.00    | 46.50    | 44.00    |
| Fine bran               | 2.50     | 1.50     | 1.50     | 1.50     | 2.50     | 2.00     |
| Coconut oil             | 1.50     | 1.50     | 1.50     | 1.50     | 1.50     | 1.50     |
| CaCO₃                   | 0.50     | 0.50     | 0.50     | 0.50     | 0.50     | 0.50     |
| Premix                  | 1.00     | 1.00     | 1.00     | 1.00     | 1.00     | 1.00     |
| Total                   | 100.00   | 100.00   | 100.00   | 100.00   | 100.00   | 100.00   |

Note: DWS = dairy wastewater solid
Table 2. Feed substances and metabolizable energy of broiler feed ingredients.

| Feed Ingredients | Crude Protein | Crude Lipid | Crude Fiber | Ca | P | Lysine | Met. | Cystine | ME  |
|------------------|--------------|-------------|-------------|----|---|--------|------|---------|-----|
| Fish meal        | 58.00        | 9.00        | 1.00        | 7.70 | 3.90 | 6.50   | 1.80 | 0.94    | 2970 |
| Fermented DWS    | 21.76        | 3.42        | 6.64        | 0.96 | 0.76 | 0.81   | 0.19 | 0.30    | 3042 |
| Soybean          | 44.00        | 0.90        | 6.00        | 0.32 | 0.29 | 2.90   | 0.65 | 0.67    | 2240 |
| Meal             | 21.00        | 1.80        | 15.00       | 0.20 | 0.20 | 0.64   | 0.29 | 0.30    | 1540 |
| Coconut cake     | 8.60         | 3.90        | 2.00        | 0.02 | 0.10 | 0.20   | 0.18 | 0.18    | 3370 |
| Yellow corn      | 12.00        | 13.00       | 12.00       | 0.12 | 0.21 | 0.77   | 0.29 | 0.40    | 1630 |
| Fine bran        | 0.00         | 100.00      | 0.00        | 0.00 | 0.00 | 0.00   | 0.00 | 0.00    | 8600 |
| Coconut oil      | 0.00         | 0.00        | 0.00        | 40.00 | 0.00 | 0.00   | 0.00 | 0.00    | 0.00 |
| CaCO₃            | 0.00         | 0.00        | 0.00        | 24.00 | 12.00 | 0.00   | 0.00 | 0.00    | 0.00 |
| Bone meal        | 0.00         | 0.00        | 0.00        | 0.00 | 0.00 | 0.30   | 0.30 | 0.00    | 0.00 |
| Premix           |              |             |             |      |     |        |      |         |      |

Collections of sample and data

The broiler chickens were placed in 24 litter cages (5 each) measuring 1 x 0.5 x 0.75m. The cage was equipped with a feeder, drinker, thermometers, hygrometers, heating and lighting equipment, and sanitary equipment.

Body weight was measured weekly using a 5-kg analytical scale with 1g accuracy. Body weight gain was obtained from the discrepancy between final and initial body weight. The chickens were slaughtered at the end of the research. The cecum was cut, weighed, extracted in 1% physiological NaCl solution then frozen to be analyzed. The device for bacteria analysis were petri dishes, glass, osse, and micro objects.

Microbial quality measurement in cecum

Microbial quality in cecum was measured by calculating microflora population in cecum: Lactobacillus sp, Salmonella sp, E. coli, and Enterobacteriaceae bacteria using a total plate count method. Different media for analysis included Violet Red Bile Glucose Agar/VRBGA for Enterobacteriaceae, Mc Conkey Agar for Salmonella sp and E. coli, and MRS/de Man Rogosa Sharpe for Lactobacillus sp. Samples that had been diluted to 10⁹ were cultured in each medium, then incubated for 24 - 48 hours until colonies form. Total colonies calculated was total microbial cells per gram of sample (Kornacki et al., 2013; APHA, 2012). Broiler’s cecum was weighed during evisceration to determine microbial activity.

Research design and data analysis

The experimental study was conducted in a completely randomized design (CRD) with six ration treatments and four replicates. The treatments were the level of fermented DWS supplied into the ration, namely 0, 5, 10, 15, 20, and 25%. Data were subject to ANOVA, followed by Duncan’s test for discrepancies identified across treatments.

Results and Discussions

Broiler body weight gain is presented in Table 3. Statistical analysis result showed that the level of fermented products did not significantly affect broiler body weight gain. The effect of 25% fermented DWS on body weight gain was similar to that of control ration. This is due to the improvement of nutritional quality in fermented DWS; therefore, that the nutritional value in the ration can be increased (Marlina, 2010). Supplementing fermented DWS up to 25% could provide adequate sources of nutrition for broilers’ growth and performance. It is evident that broilers’ body weight gain in each treatment was between 1371.7 and 1491.1 g/head (Table 3).
The quality of cecum microflora was measured based on the ratio of the number of non-pathogenic bacteria (Lactobacillus spp) and pathogens (Salmonella sp, Escherichia coli and Enterobacteriaceae). The number of non-pathogenic bacteria reflected better health status (Patterson and Burkholder, 2003; Haque and Haque, 2017). The results showed discrepancies in the colonies and balance of lactic acid bacteria (Lactobacillus sp) against pathogenic bacteria such as Salmonella sp, Escherichia coli and Enterobacteriaceae sp. in each treatment (Table 4). Lactose fermentation in the cecum will produce lactic acid which reduces pH in the cecum. Pathogenic bacteria, as reflected in this study, are generally intolerant to low pH. Supplementing 25% fermented DWS resulted in a lower cecum bacteria population (E. coli, Salmonella, and Enterobacteriaceae) than the control (higher Lactobacillus population).

This results in a greater balance of non-pathogenic bacteria with pathogenic bacteria with increasing fermented DWS in the ration. Salmonella was non-existent in the cecum of broilers receiving 15% fermented DWS (R2) to 25% level (R5). Lactose in DWS positively affects chickens. As lactase enzymes is non-existent in chicken, lactose cannot be digested but passed to the cecum and colon to provide nutrient for lactic acid bacteria growth in

### Table 3. Feed substances and metabolizable energy

| Nutrients          | Level of fermented DWS in rations | Requirement* |
|--------------------|-----------------------------------|--------------|
|                    | 0       | 5       | 10      | 15      | 20      | 25      |            |
| Crude protein (%)  | 22.00   | 22.09   | 22.04   | 22.05   | 22.03   | 22.08   | 22.00      |
| Crude fat (%)      | 5.48    | 5.41    | 5.45    | 5.48    | 5.61    | 5.60    | 5.00-6.00  |
| Crude fiber (%)    | 3.37    | 3.44    | 3.59    | 3.72    | 3.88    | 3.94    | ≤ 8.00     |
| Lactose (%)        | -       | 1.04    | 1.16    | 1.25    | 1.42    | 1.85    |            |
| Calcium (%)        | 1.25    | 1.29    | 1.33    | 1.37    | 1.21    | 1.45    | 1.10—1.20  |
| Phosphor (%)       | 0.62    | 0.64    | 0.67    | 0.70    | 0.73    | 0.76    | 0.60-0.90  |
| Lysine (%)         | 1.54    | 1.53    | 1.50    | 1.48    | 1.47    | 1.46    | 0.90-1.10  |
| Methyonine (%)     | 0.48    | 0.47    | 0.46    | 0.45    | 0.45    | 0.44    | 0.40       |
| Methyonine + cystine (%) | 0.85 | 0.84    | 0.82    | 0.81    | 0.80    | 0.80    | 0.70-0.86  |
| ME (Kcal/kg)       | 2997    | 2980    | 2993    | 2995    | 3035    | 3054    | 3000       |

Note: *Daghir (1995)

### Table 4. Body weight gain and cecum weight in broiler with addition of dairy waste water sludge and tapioca by-product (onggok) flour mixture

| Parameter          | Treatment | Replication | Total Mean |
|--------------------|-----------|-------------|------------|
|                    |           | 1           | 2           | 3           | 4           |
| Body weight gain   | R0        | 1366.30     | 1426.40     | 1306.20     | 1388.00     | 5486.90     | 1371.73 ± 50.26 |
|                    | R1        | 1482.80     | 1547.40     | 1413.40     | 1260.00     | 5703.60     | 1425.90 ± 123.39 |
|                    | R2        | 1502.20     | 1476.00     | 1420.20     | 1467.40     | 5865.80     | 1466.45 ± 34.20 |
|                    | R3        | 1409.80     | 1497.80     | 1523.60     | 1522.00     | 5953.20     | 1488.30 ± 53.65 |
|                    | R4        | 1547.60     | 1541.00     | 1469.50     | 1406.20     | 5964.30     | 1491.08 ± 66.73 |
|                    | R5        | 1465.40     | 1522.60     | 1531.00     | 1436.40     | 5955.40     | 1488.85 ± 45.52 |
| Cecum weight       | R0        | 3.54        | 3.82        | 3.57        | 2.91        | 13.84       | 3.46 ± 0.39a |
|                    | R1        | 3.91        | 4.84        | 4.96        | 5.91        | 19.62       | 4.91 ± 0.82b |
|                    | R2        | 5.22        | 5.02        | 4.69        | 4.11        | 19.04       | 4.76 ± 0.49b |
|                    | R3        | 5.76        | 5.35        | 4.90        | 5.54        | 21.55       | 5.39 ± 0.37b |
|                    | R4        | 7.03        | 6.67        | 6.17        | 5.44        | 25.31       | 6.33 ± 0.69c |
|                    | R5        | 6.43        | 5.45        | 6.71        | 6.96        | 25.55       | 6.39 ± 0.66c |

Note: R0 = Ration with 0% fermented DWS; R1 = Ration with 5% fermented DWS; R2 = Ration with 10% fermented DWS; R3 = Ration with 15% fermented DWS; R4 = Ration with 20% fermented DWS; R5 = Ration with 25% fermented DWS, Different letter in each column indicate difference between treatment, according Duncan’s test (P<0.05)
Table 5. Cecum microflora with addition of dairy waste water sludge and tapioca by-product (onggok) flour in rations

| Bacteria          | Treatment | \( R_0 \) | \( R_1 \) | \( R_2 \) | \( R_3 \) | \( R_4 \) | \( R_5 \) |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Lactobacillus spp.| \( \ldots \) | 9.00      | 9.04      | 9.20      | 9.23      | 9.38      | 9.40      |
| Salmonella        | \( \ldots \) | 2.64      | 2.23      | nd        | nd        | nd        | Nd        |
| Escherichia coli  | \( \ldots \) | 3.53      | 3.49      | 3.38      | 3.04      | 3.04      | 3.00      |
| Enterobacteriaiae | \( \ldots \) | 3.82      | 3.62      | 3.60      | 3.43      | 3.36      | 3.00      |
| L:S ratio         | \( \ldots \) | 3.40      | 4.05      | =         | =         | =         | =         |
| L:E ratio         | \( \ldots \) | 2.55      | 2.59      | 2.72      | 3.03      | 3.08      | 3.13      |
| L:En ratio        | \( \ldots \) | 2.36      | 2.50      | 2.56      | 2.69      | 2.79      | 3.13      |

Note: \( \text{nd} \) = non detected, \( \approx \) = infinite, \( R_0 \) = Ration with 0% fermented DWS; \( R_1 \) = Ration with 5% fermented DWS; \( R_2 \) = Ration with 10% fermented DWS; \( R_3 \) = Ration with 15% fermented DWS; \( R_4 \) = Ration with 20% fermented DWS; \( R_5 \) = Ration with 25% fermented DWS.

Ccemin. Similarly, prebiotics is indigestible food ingredient but beneficial for their hosts by selectively stimulating the growth and activity of one or more bacteria in the large intestine (Dankowiakowska et al., 2013; Wilson Tang et al., 2019). Lactose as a probiotic for broilers is reflected in the growth of lactic acid bacteria in the cecum and the elimination of pathogenic bacteria such as \textit{E. coli}, \textit{Salmonella}, and \textit{Enterobacteriaiae}. The high number of \textit{Lactobacillus} sp in the cecum can produce a pH that is not conducive for the growth of pathogenic bacteria (Table 5).

**Conclusion**

Fermented DWS up to 25% in the ration resulted in a favorable condition for the growth of non-pathogenic microflora (\textit{Lactobacillus sp.}) and inhibited the growth of pathogenic bacteria (\textit{Salmonella sp.}, \textit{Escherichia coli} and \textit{Enterobacteriaiae}) in the cecum of broiler chickens. It promoted health status which may optimize broilers’ growth.

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