Eulis Tanti Marlina et al/Animal Production. 21(1):43-48, 2019
Accredited by Kemenristek Dikti No 32a/E/KPT/2017. ISSN 1411-2027

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

Abstrak. Dairy Wastewater Sludge (DWS) adalah sedimen dari pengolahan susu. Kandungan nutrisi DWS dapat digunakan sebagai bahan pakan. Efek nutrisi dalam DWS diuji pada pertumbuhan broiler serta dampak spesifik pengembangan microflora pada pencernaan. Metode penelitian yang digunakan adalah eksperimental. Tapioca by-Product (onggok) digunakan sebagai DWS Binder sementara fermentasi dengan *Aspergillus niger* dilakukan untuk meningkatkan kandungan nutrisi. Tingkat penambahan DWS yang difermentasi dalam ransum dievaluasi melalui pengukuran kenaikan berat badan selama 35 hari dan kualitas mikroflora di sekum dengan menghitung jumlah bakteri non-patogen dan patogen pada akhir periode penelitian. Data penelitian dianalisis menggunakan ANOVA dan uji lanjut yang digunakan adalah tes Duncan. Hasil penelitian menunjukkan bahwa penambahan 20% DWS yang difermentasi dalam ransum menghasilkan kenaikan berat badan tertinggi dan dapat menekan pertumbuhan bakteri patogen (*Salmonella spp., E. koli dan Enterobacteriaceae*) di sekum. Rasio non patogen dan bakteri patogen meningkat secara proporsional untuk penambahan tingkat DWS yang difermentasi. Berat sekum dari Broiler dengan tingkat DWS yang difermentasi yang berbeda merupakan representasi aktivitas mikroorganisme di sekum. Kondisi ini bisa mengilustrasikan status kesehatan ternak yang baik sehingga dapat mengoptimalkan pertumbuhan broiler.

Kata kunci: dairy wastewater sludge, broiler, microflora, sekum

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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 *Aspergilus 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, $CaCO_3$, 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.

Table 1. Composition of research rations

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
$CaCO_3$	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
	....	....	....	....	%	....	....	....	Kcal/kg
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
$CaCO_3$	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 *Enterobacteriacae* 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 10^9 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).

Table 3. Feed substances and metabolizable energy

Nutrients	Level of fermented DWS in rations						
	0	5	10	15	20	25	Requirement*
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	
Calsium (%)	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		
		……………. g/head…………………..					
Body weight gain	R_0	1366.30	1426.40	1306.20	1388.00	5486.90	1371.73 ± 50.26
	R_1	1482.80	1547.40	1413.40	1260.00	5703.60	1425.90 ± 123.39
	R_2	1502.20	1476.00	1420.20	1467.40	5865.80	1466.45 ± 34.20
	R_3	1409.80	1497.80	1523.60	1522.00	5953.20	1488.30 ± 53.65
	R_4	1547.60	1541.00	1469.50	1406.20	5964.30	1491.08 ± 66.73
	R_5	1465.40	1522.60	1531.00	1436.40	5955.40	1488.85 ± 45.52
Cecum weight	R_0	3.54	3.82	3.57	2.91	13.84	3.46 ± 0.39^a
	R_1	3.91	4.84	4.96	5.91	19.62	4.91 ± 0.82^b
	R_2	5.22	5.02	4.69	4.11	19.04	4.76 ± 0.49^b
	R_3	5.76	5.35	4.90	5.54	21.55	5.39 ± 0.37^b
	R_4	7.03	6.67	6.17	5.44	25.31	6.33 ± 0.69^c
	R_5	6.43	5.45	6.71	6.96	25.55	6.39 ± 0.66^c

Note: 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, Different letter in each coloumn indicate difference between treatment, according Duncan's test (P<0,05)

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 *Enterobacteriacea 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 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
	……………………….$\log_{10}$ CFU/g………………………					
Lactobacillus spp.	9.00	9.04	9.20	9.23	9.38	9.40
Salmonella	2.64	2.23	nd	nd	nd	Nd
Escherichia coli	3.53	3.49	3.38	3.04	3.04	3.00
Enterobacteriaceae	3.82	3.62	3.60	3.43	3.36	3.00
L:S ratio	3.40	4.05	≈	≈	≈	≈
L:E ratio	2.55	2.59	2.72	3.03	3.08	3.13
L:En ratio	2.36	2.50	2.56	2.69	2.79	3.13

Note: nd = non detected, ≈ 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

cecum. 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 *E. coli, Salmonella*, and *Enterobacteriaceae*. The high number of *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 (*Lactobacillus sp.*) and inhibited the growth of pathogenic bacteria (*Salmonella sp., Echerichia coli* and *Enterobacteriaceae*) in the cecum of broiler chickens. It promoted health status which may optimize broilers' growth.

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