Research Article

Effects of Combinations of Nano-Fountain Clerodendrum and Avocado Seed Flour on Growth Performance, Intestinal Profile, and Microbial Population of Lohmann Broiler Chickens

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Abstract | This study aims to determine the effect of combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology on growth performance, intestinal profile, and microbial population of Lohman chickens. A total 256 Lohmann chicken were used on the experimental diets. The Fountain clerodendrum and avocado seed flour were enhanced with nanotechnology 500 mm. The two-way nested Anova with eight treatments and four replication was employed, with each experimental unit consisting of eight Lohmann broiler chickens. The treatments consisted of B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% avocado seed flour, B_1T_2 = basal diet + 0.8% avocado seed flour, B_1T_3 = basal diet + 1.2% avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% avocado seed flour enhanced with nanotechnology, B_2T_2 = basal diet + 0.8% avocado seed flour enhanced with nanotechnology, B_2T_3 = basal diet + 1.2% avocado seed flour enhanced with nanotechnology. If there is significant effect ($p < 0.05$), the analysis continued with least significant differences (LSD). The result showed significantly difference ($p < 0.01$) on villus height and significant difference ($p < 0.05$) on microbial population. In contrast, the result showed no significantly difference ($p > 0.05$). In conclusion, combinations of nano-fountain clerodendrum and avocado seed flour help to increasing villus height and adverse microbial on Lohmann broiler chickens.

Keywords | Avocado, *Clerodendrum*, Growth Performance, Intestinal Profile, NanoTechnology

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INTRODUCTION

Broiler chickens play an important role in fulfilling animal protein in Indonesia. The national consumption reached 3,442,588 tons in last 2021 (Indonesia census data and statistic, 2020). The broiler chickens were favourable in Indonesia since it rapid harvested (4-5 weeks). In line, to support this condition the one factor to be key were optimalisation feed. Feed is an essential aspect that has a major impact to the broiler chickens' productions. Feed consisted nutrients as follows: carbohydrates, protein, vitamin, minerals, and including water content. To support the major nutrients there is feed additive that can adding approximately 1% from total formulation. In Indonesia Antibiotic is part of the feed additive on feed formulation for broiler chickens (Natsir et al., 2017).

Antibiotic using on broiler feeding in Indonesia were beginning to banned on Early January, 2018 (Ministry of Agriculture number 14/2017). Contrast findings with European Union were already implementing banning of antibiotics growth promoters (AGPs) since 1997 (Sjofjan et al., 2021). A lot of researchers taking alternative to anticipate the banning of the antibiotic growth promoters

by using bioactive substances from plant. The plant can be representative of the natural products that have played an important role for preventing the disease and enhance the health of the animals. For the last few centuries, Phyto biotics has been making rapid and quite popular among researcher. A lot of herbal products being questionnaire and lack information. One of the potential native plants that can be used as phyto biotics is Fountain clerodendrum (*Clerodendrum minahassae L.*). Fountain clerodendrum is a native plant that commonly used in Indonesia especially in Minahasa, North Sulawesi. Fountain clerodendrum consisted bioactive plants such as phenols, flavonoid, terpenoid, and steroid. Thus, bioactive compounds act as anti-microbial and defence system in response to protecting from adverse microorganism in intestinal of broiler chickens (Gheisar and Kim, 2018).

Another, potential phytobiotic that can be used as a feed additive is avocado. The production of avocado in Indonesia reached 410,094 tonnes last 2021. Moreover, avocado is grown in most area in Indonesia started from Sumatra, Java (West, and East region), Sulawesi, and Lesser Sunda Islands. In contrast, the avocado produced the agricultural by-product from it seeds. Reported from avocado seeds consisted 3,570 kcal/kg (metabolizable energy); 8.70% (crude protein); 6.11% (crude fibre); 0.70% (Ca); and 0.21% (P). Moreover, the avocado seed consisted carotenoid, minerals, phenolic, fatty acids, cyanogenic, and phenols substances. The bioactive substances both fountain clerodendrum and avocado seed can be potentially to reduce environmentally stress, reducing blood pressure, enhance the immune system, and increasing the growth performance and health of poultry (Valliammai et al., 2020). The size of the feed additive in nanoparticles has an influence on the ability of poultry to absorb the nutrients contained therein, which in turn affects the metabolic and physiological processes that occur in the animal's body, thus having an impact on increasing production performance (Gangadoo et al., 2016). This study aims to determine the effect of combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology on growth performance, intestinal profile, and microbial population of Lohman chickens.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the study was given by the Animal Care and Use Committee, University of Brawijaya, Number 979/UN10.F05/PN/2021.

Experimental design

A total 256 Lohmann chicken were used in a five-weeks trial. The one-way nested Anova with eight treatments and four replication was employed, with each experimental unit consisting of eight Lohmann broiler chickens. All Lohmann broiler chickens were allowed *ad libitum* access to water thought installed nipple drinkers. Moreover, All Lohmann broilers were housed in environmentally controlled rooms in 100 x 100 x 60 m^2 rice-hull-littered-pens. All Lohmann broilers chickens were vaccinated using Newcastle-Bronchitis vaccine (Lohmann Animal Health Int., Winslow, Maine 04901 USA) (one-day-old) and Gumboro vaccine (12-days-old). The treatments consisted of B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% fountain clerodendrum and avocado seed flour, B_1T_2 = basal diet + 0.8% fountain clerodendrum and avocado seed flour, B_1T_3 = basal diet + 1.2% fountain clerodendrum and avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% nano-fountain clerodendrum and avocado seed, B_2T_2 = basal diet + 0.8% nano-fountain clerodendrum and avocado seed, B_2T_3 = basal diet + 1.2% nano-fountain clerodendrum and avocado seed. The formulated feed consisted yellow maize, maize bran, soybean meal, bone meal, meat meal, soy oil, mineral premix, vitamin premix, anti-oxidant, and canthaxanthin. Representative of the formulated feed were analyzed for metabolizable energy (Kcal/kg), crude protein (CP), crude fibre (CF), calcium (Ca), and phosphor (P) according to established procedures described by (AOAC, 2000). The composition of formulated feed showed in the Table 1 and 2.

Preparation of the fountain clerodendrum and avocado seed

The preparation of the sampled were following Sjofjan and Adli, (2021). The materials used were fountain clerodendrum leaves from Manado, while avocado was taken from Malang area. The fountain clerodendrum leaves were chosen with diameter around 3-4 cm. In other hand, the avocado seed were chosed ranged between 250 mg. Second, both fountain clerodedendrum and avocado seed were sun dried for 24 hours and 4 hours in an oven at low temperature (60◦C) and grinded into a powder. In this experiment, the fountain clerodendrum and avocado seed flour were enhanced with nanotechnology 500 mm. In the end, the samples were put in the paper clip.

Growth performance

First, the Lohmann broiler chickens were individually weighed at the beginning and end of the trial. Second, feed intake was recorded as the differences between feed given and refusal feed. Feed intake was calculated after recording the mortalities of broiler chickens. Third, feed gain ration obtained from total feed consumed in certain period divided by the weight gain of broiler chickens. Mortalities were checked daily each pen.

Intestinal profile

In the end of experiment, representative Lohmann broiler

Table 1: Ingredient of Lohmann broiler chickens (0-21 days)

Ingredients (% as is basis)	B_1T_0	B_1T_1	B_1T_2	B_1T_3	B_2T_0	B_2T_1	B_2T_2	B_2T_3
Yellow Maize	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Distillers Dried Grain Soluble	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Bone meal	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
Meat meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Custom Mineral premix*	1.00	1.60	1.20	0.80	1.00	1.60	1.20	0.80
Vitamin premix**	1.00	0.40	0.40	0.40	1.00	0.40	0.40	0.40
Nano-fountain clerodendrum and avocado seed	0.00	0.00	0.00	0.00	0.00	0.40	0.80	1.20
Fountain clerodendrum and avocado seed flour	0.00	0.40	0.80	1.20	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition								
ME (Kcal/kg)	2,868.93	2,858.90	2,763,40	2,774.48	2,868.93	2,858.90	2,763,40	2,774.48
Crude Protein (CP)	22.31	22.22	22.26	22.35	22.31	22.22	22.26	22.35
Crude fibre (CF)	3.10	3.09	3.09	3.10	3.10	3.09	3.09	3.10
Calcium (Ca)	1.88	1.87	1.87	1.88	1.88	1.87	1.87	1.88
Phosphorus (P)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Proximate composition (Wet chemical analysed)								
ME (Kcal/kg)	2,823.82	2,821,82	2,803,10	2.812.33	2,823.82	2,821,82	2,803,10	2.812.33
Crude Protein (CP)	23.78	23.22	23.10	23.02	23.78	23.22	23.10	23.02
Crude fibre (CF)	4.23	4.12	4.01	4.03	4.23	4.12	4.01	4.03
Calcium (Ca)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Phosphorus (P)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

**: Vitamin A, 6000IU, Vitamin D3, 1000IU, Vitamin E, 10mg, Vitamin K3, 1.5mg, Vitamin B1, 5mg, Vitamin B2, 2.5mg, Vitamin B6 0.5mg,
Vitamin B12, 2.0mg, niacin, 5.5mg, pantothenic acid, 0.2mg, betaine, 30mg.
*: Iron, 12.50mg, copper, 3mg, manganese, 37.5mg, zinc, 31.32mg, iodine, 5mg and selenium 0.0625mg

Table 2: Ingredient of Lohmann broiler chickens (22-35 days)

Ingredients (% as is basis)	B_1T_0	B_1T_1	B_1T_2	B_1T_3	B_2T_0	B_2T_1	B_2T_2	B_2T_3
Yellow Maize	46.00	46.00	46.00	46.00	46.00	46.00	46.00	46.00
Distillers Dried Grain Soluble	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Soybean	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Meat meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Soy oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Custom Mineral premix*	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix**	1.00	1.60	1.20	0.80	1.00	1.60	1.20	0.80
Nano-fountain clerodendrum and avocado seed	0.00	0.00	0.00	0.00	0.00	0.40	0.80	1.20
Avocado seed flour	0.00	0.40	0.80	1.20	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition								

ME (Kcal/kg)	3118.80	3,118.46	3,112.59	3,119.74	3118.80	3,118.46	3,112.59	3,119.74
Crude Protein (CP)	19.08	19.05	19.08	18.85	19.08	19.05	19.08	18.85
Crude fibre (CF)	3.77	3.74	3.77	3.78	3.77	3.74	3.77	3.78
Calcium (Ca)	0.76	0.75	0.76	0.76	0.76	0.75	0.76	0.76
Phosphorus (P)	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Proximate composition (Wet chemical analysed)								
ME (Kcal/kg)	3125.00	3130.00	3120.00	3125.00	3125.00	3125.00	3125.00	3125.00
Crude Protein (CP)	23.78	23.20	23.25	23.20	23.78	23.20	23.25	23.20
Crude fibre (CF)	4.23	4.23	4.23	4.23	4.23	4.23	4.23	4.23
Calcium (Ca)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Phosphorus (P)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

**: Vitamin A, 6000IU, Vitamin D3, 1000IU, Vitamin E, 10mg, Vitamin K3, 1.5mg, Vitamin B1, 5mg, Vitamin B2, 2.5mg, Vitamin B6 0.5mg,
Vitamin B12, 2.0mg, niacin, 5.5mg, pantothenic acid, 0.2mg, betaine, 30mg.
*: Iron, 12.50mg, copper, 3mg, manganese, 37.5mg, zinc, 31.32mg, iodine, 5mg and selenium 0.0625mg

Table 3: Effects of combinations of fountain clerodendrum. and avocado seed flour enhanced with nanotechnology on growth performance of Lohmann broiler chickens

Parameters	B_1T_0	B_1T_1	B_1T_2	B_1T_3	B_2T_0	B_2T_1	B_2T_2	B_2T_3	SEM
BW (g)	2,284.83	2,296.79	2310,65	2,301.84	2,280.25	2,326.42	2,346.13	2306.8	13.52
Feed intake (g)	3,793.89	3,729.69	3762,45	3,687,81	3,799.05	3,717.75	3,642,13	3,623.00	115.7
BWG (g)	2,242.05	2,253.73	2267.40	2,258.81	2,237.16	2,284.51	2,303.18	2,265.29	14.60
FCR	1.70	1.66	1.66	1.64	1.63	1.63	1.58	1.60	0.05
IOFC (IDR/head)	11749,05	12453,68	12416,7	12769,83	11550,57	13127,69	14065,6	13475,39	3322

[a, b, c, d] Means with different superscripts in the row differ significantly ($p < 0.05$). B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% avocado seed flour, B_1T_2 = basal diet + 0.8% avocado seed flour, B_1T_3 = basal diet + 1.2% avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% avocado seed flour enhanced with nanotechnology, B_2T_2 = basal diet + 0.8% avocado seed flour enhanced with nanotechnology, B_2T_3 = basal diet + 1.2% avocado seed flour enhanced with nanotechnology. BW – body weight; BWG – body weight gain; FCR – feed conversion ratio; IOFC – income over feed cost.

Table 4: Effects of combinations of fountain clerodendrum. and avocado seed flour enhanced with nanotechnology on intestinal profile of Lohmann broiler chickens

Parameters	B_1T_0	B_1T_1	B_1T_2	B_1T_3	B_2T_0	B_2T_1	B_2T_2	B_2T_3	SEM
Villus height (µm)	368.69^a	453.92^b	499.10^c	379.44^a	361.22^a	511.29^b	627.48^c	609.01^c	18.28
Crypt depth (µm)	118.15^a	106.37^b	120.00^b	118.53^b	120.80^a	120.81^b	130.60^c	124.60^c	2.94
Total villi	43.12	52.25	53.00	57.25	45.62	52.12	58.50	57.00	1.43
VH/CD	3.12	4.26	4.15	3.20	2.99	4.23	4.80	4.88	0.21

[a, b, c, d] Means with different superscripts in the row differ significantly ($p < 0.05$). B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% avocado seed flour, B_1T_2 = basal diet + 0.8% avocado seed flour, B_1T_3 = basal diet + 1.2% avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% avocado seed flour enhanced with nanotechnology, B_2T_2 = basal diet + 0.8% avocado seed flour enhanced with nanotechnology, B_2T_3 = basal diet + 1.2% avocado seed flour enhanced with nanotechnology. VH – villus height ; CD – crypt depth

Table 5: Effects of combinations of fountain clerodendrum. and avocado seed flour enhanced with nanotechnology on microbial of Lohmann broiler chickens

Parameters	B_1T_0	B_1T_1	B_1T_2	B_1T_3	B_2T_0	B_2T_1	B_2T_2	B_2T_3	SEM
LAB (log cfu/ml)	8.80	7.93	7.57	7.57	7.66	8.30	9.31	8.72	0.17
Escherichia coli (log cfu/ml)	5.16	5.70	5.85	6.38	4.73	5.60	4.77	4.89	0.03
Salmonella sp (log cfu/ml)	3.93	4.08	3.98	4.31	4.03	3.77	3.68	3.76	0.18

[a, b, c, d] Means with different superscripts in the row differ significantly ($p < 0.05$). B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% avocado seed flour, B_1T_2 = basal diet + 0.8% avocado seed flour, B_1T_3 = basal diet + 1.2% avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% avocado seed flour enhanced with nanotechnology, B_2T_2 = basal diet + 0.8% avocado seed flour enhanced with nanotechnology, B_2T_3 = basal diet + 1.2% avocado seed flour enhanced with nanotechnology. LAB – lactic acid bacteria

chickens were slaughtered using systematic random sampling. First, selected intestinal profile specimens of 3-4cm near ileocecal junction were cut. Selected specimens were put into NaCL and 10% formaldehyde liquid. Second, the specific specimens were put into glass-slide using *Haemotoxylin-eosin* coloring following (Sjofjan and Adli, 2020) method.

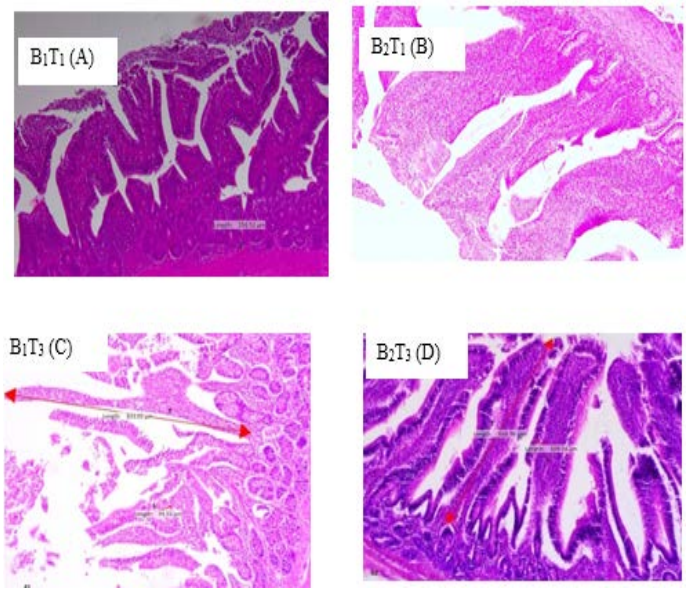

Figure 1-4: Intestinal profile of Lohmann broiler chickens with 100x scale of zooming. B_1T_0 = basal diet, B_1T_1 = basal diet + 0.4% avocado seed flour, B_1T_2 = basal diet + 0.8% avocado seed flour, B_1T_3 = basal diet + 1.2% avocado seed flour, B_2T_0 = basal diet, B_2T_1 = basal diet + 0.4% avocado seed flour enhanced with nanotechnology, B_2T_2 = basal diet + 0.8% avocado seed flour enhanced with nanotechnology, B_2T_3 = basal diet + 1.2% avocado seed flour enhanced with nanotechnology.

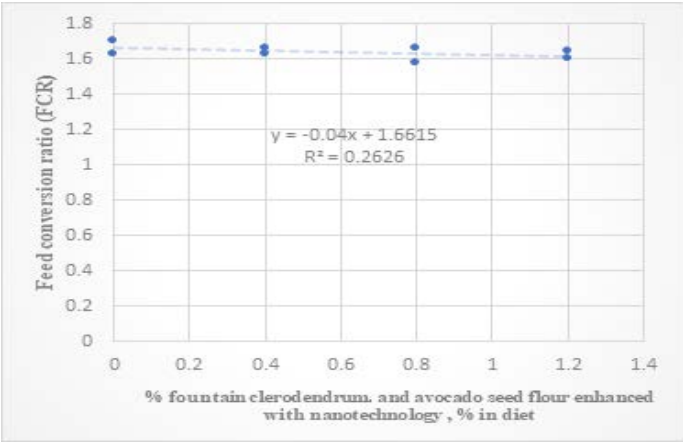

Figure 5: Feed conversion ratio after feed fountain clerodendrum. and avocado seed flour enhanced with nanotechnology

Microbial population

During the calculated of microbial population. Chyme was collected from ileocecal junction at the end of experiments (Sjofjan and Adli, 2020). The medium prepared was MacConkey agar plates for determining the coliform and lactobacilli. The chyme was diluted 10^{-4} to 10^{-6} and then cultivated into specific media. The media were incubated for 24 hours at 37^0C.

Data analysis

Prior to statistical analysis, proc analysis of variance (ANOVA) using one-way ANOVA nested design was carried out using SAS OnDemand for Academics (ODA, Cary, NC,USA). The results were presented as standard error mean (SEM). Moreover, different between means were calculated using general linear models (GLM) least significant different testing. The following model was used:

$$Y_{ijk} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \epsilon ijk$$

Where Y_{ij} was parameters observed, β_0 was the overall mean, $\beta_1 x_1$ the effect level of fountain clerodendrum and avocado seed flour, $\beta_2 x_2$ fountain clerodendrum avocado seed flour enhanced with nanotechnology and ϵijk the amount of error number. The differ significant ($p < 0.05$), while no significant ($p > 0.05$). Moreover, probability vales were calculated using Duncan testing, if there differ significant ($p < 0.05$).

RESULTS AND DISCUSSION

Effects of combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology on intestinal profile of Lohman broiler chickens

The result of one-way ANOVA nested design showed that the combination of fountain clerodendrum and avocado seed flour had a significantly effect ($p < 0.01$) on villus height and crypt depth, but did not show a significant difference ($p > 0.05$) on total villus and villus height (VH) and crypt depth (CD) ratio (Table 4). This is because the flavonoid and phenol reducing the pathogen microorganism on the intestinal of broiler chickens. Table 4 shows that the treatment with nanotechnology present better than the treatments only using flour. The treatments basal diet + 0.8% nano-fountain clerodendrum and avocado seed presented highest, while basal diet + 1.2% fountain clerodendrum and avocado seed flour presented lower (Figure 1-4). In line, from George et al. (2021) avocado seed consisted bioactive phenol as a bioactive compound that help to reducing the negative bacteria, make stable environment, and as an anti-tumor. Another study found from Martial et al. (2021) showed that clerodendrum consisted antihyperlipidemic that help to reducing the amount of negative microorganism and help increasing number of villi in the intestinal.

Harahap et al. (2019) recommend the used of avocado seed

flour until 5% in formulated feed without any adverse effect. The used of nano-technology probably increase the absorption in the intestinal of broiler chickens. The smaller particle influenced the ability of villus to absorb the nutrients, which later converted into metabolic and physiological cycle in the broiler chicken's body. The increasing level of absorption may have synergistic increasing the growth performance of broiler chickens (Gangadoo et al., 2016). Previous research conducted, by Natsir et al. (2013) gave similar results where the treatment of adding photobiotic to the formulated feed showed a significantly in the number of villi compared to the control feed. Feng et al. (2009) said that minerals in nanoscale are more easily absorbed in the digestive tract, where in the body of livestock, nano minerals interact more effectively with organic and inorganic substances due to their larger surface area. The absorption of particles in the digestive tract depends on their diffusion and accessibility through the mucosa and contact with cells in the digestive tract. Smaller particle diameter is faster in the process of diffusion through the mucosa of the digestive tract to then reach the cells lining the intestine, then followed by absorption to enter the bloodstream. In addition, the anti-inflammatory and analgesic phenolic compounds have a protective mechanism. Flavonoids also protect cells through glutathione reductase activity and increase antioxidants that help in the cell repair process (Ekundina et al., 2015).

Effects of combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology on growth performance of Lohman broiler chickens.

The result of one-way ANOVA nested design showed that the combination of fountain clerodendrum and avocado seed flour didn't have a significant difference ($p > 0.05$) on growth performance (body weight, feed intake, body weight gain, feed conversion ratio, and income over feed cost) on lohman broiler chickens. Table 3 shows that the treatment with nanotechnology present better than the treatments only using flour. (Figure 5) the treatments basal diet + 0.8% nano-fountain clerodendrum and avocado seed presented highest, while basal diet + 1.2% fountain clerodendrum and avocado seed flour presented lower. reported that avocado seed flour can be used a maximum of 5% to increase body weight gain in broiler chickens. The increasing body weight gain until 5% level of treatment is uncertainly doubt. According to Van Ryssen et al. (2013) mentioned that the used of avocado seed powder until 29% were still safe without any toxicity symptom but reduced the growth performance of the broiler chickens. The most appropriate affected the reduced of growth performance were from anti nutritional factors. In contrast, Akinduro et al. (2021) mentioned the used of the avocado seed powder until 5.5% successfully enhanced feed conversion ratio, carcasses quality, and broiler chickens' prime cuts. Moreover, the used pf avocado seed flour until 5.5% level doesn't give adverse effect on the kidney of broiler chickens. In addition, Olabisi et al. (2021) the avocado seed meal stimulated the aspartate aminotransferase (AST) in the bloodstream. Second, the avocado seed meal inside into cycle to increasing the growth performance of broiler chickens (Olabisi et al., 2021).

Effects of combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology on Microbial population of Lohmann broiler chickens

The result of one-way ANOVA nested design showed that the combination of fountain clerodendrum and avocado seed flour didn't have a significant difference ($p > 0.05$) microbial population (lactic acid bacteria, *Escherichia coli*, and *Salmonella sp.*) (Table 5). Even though, didn't significant difference on the whole microbial population, the *Escherichia coli* slightly decreasing when enhanced with nanotechnology. The microbial population began to decreasing when at basal diet + 0.4% nano-fountain clerodendrum and avocado seed (5.60); basal diet + 0.8% nano-fountain clerodendrum and avocado seed (4.77), basal diet + 1.2% nano-fountain clerodendrum and avocado seed (3.76) (Table 5). This condition happened because nano has a smaller size compared to flour, that make easily absorbed by the body of broiler chickens. Reported from Valliammai et al. (2020) the clerodendrum stabile to reduce against *Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cererus* and *Seratia marcescens*. In contrast, apparently reduce *Escherichia coli*, *Klebsiella pneumonia, Pseudomonas aerugonisa, Proteus vulgaris* and *Candida albicans* Valliammai et al. (2020). Reported from Leontopoulus et al. (2021) the antimicrobial properties that belongs to avocado seed were act as an enzyme inhibition, cell-mitochondria breakdawn, substrate derivation and bacterial colonization. Moreover, the flavonoids, phenols, steroids and terpenoids are active compounds that function as antibacterial contained in clerodendrum (Regar et al., 2022).

Active compounds such as flavonoids are synthesized by plants as a defense system and in response to infection by microorganisms so that these compounds are effective as antimicrobial compounds against a number of microorganisms on broiler (Parubak, 2013). Another reported that clerodendrum can inhibit the growth of *Escherichia coli* bacteria because the flavonoids contained can breakdown the membrane cells (Lomboan., 2015). In addition, Avocado seeds are rich in polyphenols which have strong antioxidant and antimicrobial abilities (Antasionasti, et al., 2017). In line, Liao et al. (2010) added that as a common mechanism, the smaller the particle size, the easier it will be to absorb and enter into the body system. Osuntokun

et al. (2017) reported that the phenolic compounds in avocado had an antibacterial effect against *Streptococcus sp.* Active compounds such as flavonoids are synthesized by plants as a defense system and in response to infection by microorganisms so that these compounds are effective as antimicrobial compounds against a number of microorganisms (Utami et al., 2017).

CONCLUSIONS

In conclusion, combinations of fountain clerodendrum and avocado seed flour enhanced with nanotechnology failed to increasing the growth of performance but in contrast help to increasing villus height and reduced adverse microbial on Lohmann broiler chickens.

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NOVELTY STATEMENT

There has been no research published about Fountain clerodendrum. and avocado seed flour enhanced with nanotechnology on broiler chickens as replacement of antibiotics growth promoters.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR'S CONTRIBUTION

MHN conceptualization, supervision, review the manuscript; OS conceptualization, supervision, and review the manuscript; YFN conceptualization, supervision, doing the research, DNA preparing manuscript, data analysis, revise grammatically, revise manuscript; LB collecting data, doing the *in-vivo* and *in-vitro* test; DFA collecting data, doing the *in-vivo* test and *in-vitro* test; WTU collecting data, doing the *in-vivo* test and *in-vitro* test.

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