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Effects of Dietary Catechin *Uncaria gambir* Extract on Growth Performance, Carcass Characteristics, Plasma Lipids, Antioxidant Activity and Nutrient Digestibility in Broiler Chickens

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ABSTRACT

This study aimed to determine the effect of adding catechin gambir extract (*Uncaria gambir* Roxb) (CGE) on growth performance, carcass characteristics, blood plasma constituents, antioxidant activity, and nutrient digestibility in broiler chickens. 160 chicks were allocated to receive four treatment diets adding CGE was 0, 0.1, 0.2, and 0.3g/kg in basal diets with four replications. Bodyweight and feed intake were recorded to calculate growth performance. Digestibility measurements were carried out at the age of 31-40 days, and at 40 days, blood samples were taken to determine the total protein levels of cholesterol, lipoprotein cholesterol (LDL), high-density lipoprotein (HDL), and Malondialdehyde (MDA). Then these chickens are slaughtered to determine the percentage of viscera and muscles. Adding CGE to basal diets linearly ($P<0.05$) increased body weight gain and improved the feed conversion ratio of broiler grower, finisher, and the overall age of 1-40 days. The effect of GCE on carcass characteristics significantly ($P<0.05$) increased the percentage of thigh and drumstick and decreased abdominal fat. CGE linearly reduced total cholesterol and LDL while increased HDL and total protein in blood plasma. The MDA concentration linearly decreased in serum and broiler meat aged 40 days. In addition, CGE increased ($P<0.05$) the digestibility of crude protein and decreased fat digestibility in the diet. Adding 0.3g/kg CGE to the basal diet can be a feed additive to produce better performance and broiler meat.

Key words: Antioxidants, Broiler, Catechin, Digestibility, Malondialdehyde, Uncaria gambir

INTRODUCTION

The broiler industry plays an essential role in supplying meat as a source of animal protein for humans. Therefore, the meat produced must be healthy and free from harmful substances such as antibiotic residues and hormones, which concern using antibiotic growth promoters (AGP) (Lillehoj et al. 2018). Since the ban on AGP in many countries, medicinal plants as antibiotics and natural growth promoters in poultry diets have gained much attention (Solangi et al. 2020; El-Hack et al. 2020). In addition, medicinal plants can be used as alternatives to AGP as they exhibit antimicrobial properties and thus can become an integral part of poultry nutrition (Daramola 2019).

Flavonoids are compounds widely recommended as a substitute for AGP due to their ability as antibacterial, antioxidant, anti-inflammatory, and hepaprotective. Therefore, they provide better broiler performance in the form of increased body weight gain and feed efficiency (Giannenas et al. 2018) and they also improve meat quality (Liu et al. 2020) and reduce mortality (Prihambodo et al. 2021).

Catechin is a plant bioactive flavonoid compound that can be used as a broiler feed additive. There have been no reports of pure catechins in broiler diets. The inclusion of green tea (13.93% of catechins) as a feed additive in broilers can increase feed conversion ratio (FCR), decrease abdominal fat, and increase antioxidant status (El-Hack et al. 2020). Grape pomace (0.58mg/g of catechin) in a broiler diet can improve gut morphology and modify the cecal bacterial community and biochemical blood profiles without adverse effects on growth performance and meat quality (Erinle et al. 2022). Grapeseed (0.1g/g of catechin) is recommended as a synthetic antioxidant in broiler diets (Gungor et al. 2021).

The gambir is an extraction product of the gambir plant (*Uncaria gambir* Roxb) from the *Rubinace* family, a potential catechin producer from Indonesia. Gambir contains many monomeric flavonoids, almost totally

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catechins, and a trace of epicatechin (Widiyarti et al. 2020). Gambir catechin purification has been successfully carried out (Yeni et al. 2014; Ferdinal 2014) through 2-stage extraction. Gambir catechins are white to yellowish crystals and have a sweet taste (Ferdinal 2014). Therefore, we hypothesized that gambir catechins as a feed additive could cause positive effects on broiler nutrition. It is the first study to examine the effect of gambir catechin on growth performance, carcass characteristics, plasma lipids, antioxidant activity, and nutrient digestibility as an exploratory step for its use as AGP in broiler diets.

MATERIALS AND METHODS

Extraction and Purification of Gambir Catechins

Gambir was obtained from the gambir industry in Harau, Lima Puluh Kota District, West Sumatra, Indonesia. The catechin purification process was carried out by 2-stage extraction following the method suggested by Yeni et al. (2014) and Ferdinal (2014). In the first stage, gambir flour mashed by using a grinder machine with a 500-micron sieve, was macerated with ethanol for 3 days, then filtered, and the ethanol was evaporated using a rotary evaporator (Heidolph Laborota 4000) 280rpm rotation speed, temperature 50°C and pressure 460mbar, so that ethanol extract was produced. The second stage was extracted by adding 50% (w/v) distilled water by heating at 70°C with stirring for 20min. It was then filtered with a cotton cloth and then dried with a tunnel dryer at a temperature of 40°C to obtain catechin gambir extract (CGE), which was used as a feed additive.

Measurement of CGE Polyphenol Content

Measurement of CGE polyphenol content was carried out using the following method, and the measurement results are shown in Table 1.

The total phenolic content was determined by spectrophotometric methods (Chun et al. 2003). First, 1g of extract was added with 9mL of distilled water in a 25mL volumetric flask. Then 1mL of Folin-ciocalteu phenol reagent was added and allowed to stand for 5min. Then, 10mL of 7% Na_2CO_3 solution was added. Then the solution was diluted in a 25mL volumetric flask with distilled water and allowed to stand for 90min, and then the absorbance was measured at 750nm with a UV-Visible spectrophotometer (Shimadzu UV-1700) with the gallic acid standard.

The catechin content was determined following the method reported (Kassim et al. 2011) using HPLC. Samples were analyzed on a Shimadzu ADVP using a Chromolith SemiPrep RP-18 column (100-10 mm, Merck) at a flow rate of 0.5mL/min and detected at 280 nm using a UV detector. The chromatogram of a standard mixture of (+)-catechin hydrate, (-)-epicatechin, (-)-epicatechin gallate, (-)-gallocatechin, and (-)-epigallocatechin was obtained by the gradient elution as described by Kassim et al. (2011). Two different mobile phases were prepared accordingly: Solvent A, acetonitrile/water (50:50 v/v) with 0.1% acetic acid; solvent B, acetonitrile/water (5:95 v/v) with 0.1% acetic acid. All mobile phases were filtered through PTFE Millipore (Whatman) 0.45µm filter paper and degassed with a sonicator for 10 minutes.

Total Condensed tannin contents were calculated following a modified method (Kassim et al. 2011). 100mg of catechin extract was dissolved in 10mL of distilled water, then 2mL of 5M HCl and 2mL of formaldehyde (Merck) 37% were added. The mixture was heated under reflux for 60min, then filtered. The reddish precipitate was washed with 10mL of hot water five times. The precipitate was then dried in a silica gel desiccator and weighed. Yield is expressed as a percentage of the weight of the starting material.

The antioxidant activity test was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Yeni et al. 2014). First, the 4mL extract solution was added with 1mL of 0.001M DPPH solution, then shaken until homogeneous and allowed to stand for 30 minutes. Ethanol was used as a control. Next, the absorbance of the solution was measured with a UV-Visible spectrophotometer (Shimadzu UV-1700) at a wavelength (λ) of 517nm. Finally, the control absorbance value calculated the inhibition percentage (IC50).

Chicken and Treatment Diet

The experimental procedure was approved by the Center for Research and Community Service No. 195/PL25/PL.00.02/2020, following Government Regulation of the Republic of Indonesia No. 95 of 2012 concerning veterinary public health and animal welfare.

A total of 160 DOC broiler strains of Loughmann MB 202 males were reared in an open house cage. Chicks were allocated to 16 cage boxes containing ten birds to receive four treatment diets with four replicates for 40 days.

The diet used was commercially produced by Japfa Comfeed, Indonesia with the nutritional composition of the diet shown in Table 2. The treatment added CGE in basal diets of commercial diets in the starter and finisher phase of broiler, while the pre-starter phase was not added. The addition of CGE in the diet had 4 levels, namely 0, 0.1, 0.2 and 0.3g/kg. CGE flour was added to the floured diet and stirred with a horizontal type mixer (IW-200, Indonesia) until homogeneous. Diet was provided in flour, and drinking water was provided ad libitum.

Bodyweight Measurement

For chickens aged 20 and 40 days, body weight and feed intake were measured after being fasted for 12 hours to determine average daily gain (ADG), average daily feed intake (FI), and feed conversion (FC) = FI/ADG.

Blood and Organ Sampling

At broiler aged 40 days, blood samples were taken from 8 cages randomly selected with two animals per cage. Blood samples were taken from the *cutanea ulnar vein* as much as 5mL. Blood sample preparation was carried out to obtain protein-free blood filtrate to determine serum glucose levels and total protein, cholesterol, LDL, HDL, and MDA levels using spectro-photometric techniques.

The Elitech group (2012) method calculated the total cholesterol and HDL of broiler blood serum. First, up to 10µL of broiler blood serum was pipetted and then poured into the test tube and 1000µL cholesterol reagent was added and mixed, then incubated for 10 minutes. Afterward, it was calculated by a photometer. Next, broiler blood serum was pipetted up to 250µL, the reagent of HDL up to 500µL

was added and centrifuged for 10 minutes at the speed of 2500rpm. Then, the supernatant was pipetted 100µL and added to the cholesterol reagent 1000µL, mixed and incubated for 10 minutes, then it was calculated by a photometer. Finally, the LDL levels were determined with formula, LDL = Total cholesterol-HDL-1/5 Triglyceride.

Then these chickens were slaughtered and bled. Then, the visceral organs (liver, spleen, thymus, and bursa), breast muscles, and thigh muscles were collected; and the percentage of offal was calculated.

Antioxidant Status

The right thigh muscles of slaughtered poultry were separated from the carcasses, washed in 0.9% NaCl solution, individually packaged, and immediately frozen at -20°C until analysis. Frozen samples were thawed, and 1g of each sample was diluted with 9mL of ice-cold phosphate-buffered saline (pH=7.2), homogenized in a homogenizer, and centrifuged at 7378g for 15min. Malondialdehyde levels in the meat tissue homogenate supernatant and serum were tested using a commercial test kit (E-BC-K025-S; Elabsciece).

Digestibility

Eight cages were randomly selected at 30 days of age, with two birds per cage transferred to individual cages for a period (31-40 days) for digestibility testing following the procedure (Solangi et al. 2020). The adaptation period was given for three days and continued with measurements of feed intake, water intake, and total feces expelled for six days (age 34-39 d). The feces collected in 24 hours were weighed, and samples were taken from each chicken and then dried at 60°C, followed by an analysis of crude protein, ether extract and crude fiber content (AOAC 2005). The same analysis was also carried out on the feed samples.

Statistics

The study data were tabulated and started with the normality test with SPSS (SPSS Inc., Chicago, Illinois) using the Shapiro-Wilk test. Then one-way ANOVA test was carried out to see the effect of the treatment. Orthogonal polynomial contrast analysis was used to analyze the linear and quadratic effect of the level of CGE addition in the treatment diets.

RESULTS

Growth Performance of Broiler

Table 3 shows the effect of adding CGE on broiler growth performance. The growth of broiler body weight in the starter period (age 1-20 d), finisher period (age 21-40 d), and overall period (age 1-40 d) increased linearly (P=0.022), (P=0.007), and (P=0.004) with an increase in the level of addition of CGE from 0, 0.1, 0.2 and 0.3g/kg in the diet. Feed intake was not affected by the addition of CGE in all measured phases. Feed conversion was not significant in the starter phase (age 1-20 d) but decreased linearly in the finisher and overall phases (P=0.026 and P=0.017) due to the addition of CGE in the diets.

Carcass Characteristics

The effect of the addition of CGE in broiler diets on carcass characteristics is shown in Table 4. The addition of CGE in the diet linearly increased the percentage of thigh and drumstick (P=0.03) and decreased abdominal fat (P=0.02) but did not affect the percentage of eviscerated yield, breast, liver, gizzard, pancreas, gallbladder, heart and spleen.

Table 1: Polyphenol content of catechin gambir extract

Item	Value (%)
Catechin	95.87
Total Phenol	45,98
Condensed Tanin	1,98
Inhibition percentage (IC_{50}), (µg/mL)	93.92
Value are means, n = 4	

Table 2: Analyzed nutrient composition g/kg of commercial diets

Item	Pre-Starter (1-10 d)	Starter (11-20 d)	Finisher (21-40 d)
Dry matter, g/kg	884	879	878
Crude protein, g/kg	227.21	212.40	188.12
Lysine, g/kg	12	11	10
Methionine, g/kg	6	5	4
Ether extract, g/kg	25	38	41
Crude fiber, g/kg	28	30	43
Ash, g/kg	58	59	58
Calcium, g/kg	10	9	8
Phosphorus, g/kg	6	5	4
Apparent metabolizable energy, Kcal/kg	2.900	3.000	3.100

Plasma Constituents

The addition of CGE in the diet to blood plasma constituents of broilers (Table 5) shows that CGE linearly decreased total cholesterol (P=0.036), decreased low-density lipoprotein cholesterol (P=0.034), increased high-density lipoprotein cholesterol (P=0.017), and increased total protein (P=0.001) in the blood plasma of broilers aged 40 days. However, the content of Triglyceride and glucose in blood plasma was not affected by eating CGE.

Antioxidant Status

The effect of the addition of CGE in the diet on the antioxidant status of broilers aged 40 days is shown in Table 6. Antioxidant activity as measured by the concentration of MDA was found that increasing levels of CGE in broiler diets linearly decreased the concentration of MDA in serum (P=0.012) and tissue thigh meat (P=0.001) in broilers aged 40 days.

Nutrient Digestibility

Nutrient digestibility in broilers fed diets with the addition of CGE is shown in Table 6. The addition of CGE in diets linearly increased crude protein digestibility (P=0.037) and decreased ether extract digestibility (P=0.006). However, it did not affect crude fiber digestibility measured in broilers at 31-40 days of age.

DISCUSSION

The rapid growth of broilers with high feed efficiency is the goal of the broiler industry in producing healthy broiler meat. In this study, CGE was used as a source of catechins in broiler feed which acts as a herbal feed additive. In the pre-starter phase (age 1-10 d), no additional CGE was added to the diets to achieve average growth according to breeder standards, namely 320g with an FCR of 1.03 (JCI 2019).

Table 3: Effects of dietary CGE supplement on the growth performance of broiler

Item	CGE in basal diet, g/kg				SEM	P-value	
	0	0.1	0.2	0.3		Linear	Quadratic
Initial body weight, 10 d of age	239.8	238.7	241.9	243.5	2.43	-	-
Starter period, 11-20 d of age							
Weight gain, g	586.7	590.8	654.9	626.6	27.18	0.022	0.718
Feed intake, g	803.7	796.9	784.9	789.6	12.72	0.282	1.256
Feed conversion	1.37	1.35	1.21	1.26	0.05	0.053	0.534
Finisher period, 21-40 d of age							
Weight gain, g	1736	1692	1766	1854	24.48	0.007	0.035
Feed intake, g	3350	3322	3271	3162	74.22	0.075	0.860
Feed conversion	1.93	1.97	1.85	1.71	0.06	0.026	0.096
Overall, 1-40 d of age							
Weight gain, g	2562	2521	2663	2724	28.38	0.004	0.080
Feed intake, g	4434	4396	4330	4227	84.60	0.078	1.709
Feed conversion	1.73	1.74	1.63	1.55	0.04	0.017	0.199

SEM=Standard error mean; P=Probability of significant.

Table 4: Effect of catechin gambir extract on carcass characteristics at 40 day

Response parameter,	CGE in basal diet, kg/kg				SEM	P-value	
% of total weight	0	0.1	0.2	0.3		Linear	Quadratic
Eviscerated yield	70.23	69.97	69.93	69.85	0.14	0.077	0.621
Breast	22.31	24.00	23.78	23.62	0.72	5.240	0.064
Thigh and drumstick	17.98	18.30	18.76	21.50	0.89	0.030	0.130
Liver	2.03	2.10	1.99	1.92	0.06	0.085	0.187
Gizzard	1.87	1.89	1.90	1.72	0.05	0.058	0.054
Abdominal fat	1.08	0.99	0.96	0.91	0.02	0.020	0.095
Pancreas	0.15	0.17	0.15	0.18	0.01	0.285	1.741
Gallbladder	0.07	0.09	0.07	0.10	0.01	0.167	1.420
Heart	0.56	0.51	0.52	0.48	0.02	0.056	0.378
Lymph	0.14	0.16	0.14	0.16	0.01	0.259	1.677

SEM=Standard error mean; P=Probability of significant.

Table 5: Effects of dietary CGE on plasma constituents of broiler chickens at 40 day

Item	CGE in basal diet, g/kg				SEM	P-value	
	0	0.1	0.2	0.3		Linear	Quadratic
Triglyceride, mg/dL	57.77	58.36	50.42	53.94	2.86	0.142	1.057
Total Cholesterol, mg/dL	150.22	144.13	124.42	125.17	8.01	0.036	1.411
LDL, mg/dL	77.53	78.49	71.89	62.38	4.28	0.034	0.171
HDL, mg/dL	46.80	48.55	48.88	65.16	3.23	0.017	0.050
Glucose, mg/dL	136.98	131.65	121.39	122.19	0.86	0.056	0.152
Total protein, mg/dL	2.57	3.83	3.68	3.51	0.12	0.001	0.012

SEM=Standard error mean; P=Probability of significant.

Table 6: Effects of dietary CGE on antioxidant status of broiler chickens at 40 day

Item	CGE in basal diet, g/kg				SEM	P-value	
	0	0.1	0.2	0.3		Linear	Quadratic
Malondialdehyde in blood serum							
nmol/mL	4.36	4.79	3.88	3.97	0.10	0.012	0.086
Malondialdehyde in thigh meat tissue							
nmol/g meat tissue	15.24	15.09	12.33	10.20	0.17	0.001	0.001
nmol/mg protein	1.65	1.63	1.33	1.10	0.02	0.001	0.001

SEM=Standard error mean; P=Probability of significant.

Table 7: Nutrient Digestibility

Item	CGE in basal diet, g/kg				SEM	P-value	
	0	0.1	0.2	0.3		Linear	Quadratic
Crude Protein	706.1	764.5	773.4	777.5	1.27	0.037	0.078
Ether Extract	733.4	717.4	695.0	692.5	1.01	0.006	0.037
Crude Fiber	327.5	295.0	330.0	322.5	0.82	0.862	0.113

SEM=Standard error mean; P=Probability of significant.

The addition of CGE 0.3g/kg in the basal diet from the starter to the finisher period (age 10-40 d) resulted in a 6.30% increase in body weight gain and a 1.61% improvement in FCR compared to the control. In line with this, a 10% increase in broiler growth and improved feed conversion was also reported with green tea catechins at the level of 0.3g/kg (El-Hack et al. 2020). Catechins are classified as flavonoid compounds that positively affect broiler performance at specific doses (Viveros et al. 2011). The results of a review of 78 research articles (Prihambodo et al. 2021) show that the addition of 0.8g/kg flavonoid compounds in the diet could increase broiler daily body weight gain (ADG) and improve FCR in the finisher phase but had no effect on the starter phase. In this study, the

concentration of catechin compounds in the diets of 0.287g/kg (treatment 0.3g/kg CGE) got a positive response to body weight gain and improvement in FCR. However, (Chamorro et al. 2013) reported a negative response of catechin compounds on broiler performance, starting to be seen at the catechin level of 0.42g/kg (5g/kg of Grape seed extracts).

Catechins act as antioxidant compounds. The antioxidant activity was seen from the IC_{50} value is 93.92g/mL (Table 1), indicating that CGE is classified as a potent antioxidant compound (Yeni et al. 2014). Antioxidant compounds have antibacterial activity against various microbial species in the digestive tract, both pathogenic (*Clostridium*) (Abolfathi et al. 2019) and other nuisance bacteria (Srividya et al. 2010). On the other hand, it can increase the population of *Lactobacillus* and *Enterococcus* probiotic bacteria in the intestine (Viveros et al. 2011). Therefore, adding catechins will result in healthier chicken intestines with increased digestibility and absorption capacity (Chamorro et al. 2019). This study also found that crude protein digestibility increased linearly with increasing levels of CGE in the diet (Table 6), so it was reflected in increased growth performance and improved feed conversion (Table 3).

The addition of CGE in the diets increased thigh and drumstick and a tendency to increase breast weight. These results align with some previous reports (Kamboh and Zhu 2013). This increase occurred due to the reduced fat content in broiler meat due to adding phenolic compounds to the diet (Giannenas et al. 2018). The addition of phenolic compounds has an antioxidant effect that can reduce the ratio of n-6/n-3 fatty acids in broiler thigh meat, positively affecting consumer health, especially warding off cardiovascular disease (Saleh et al. 2018).

The increased CGE level in diets linearly decreased the abdominal fat of the broiler. Indeed phenolic compounds generally cause a decrease in abdominal fat (Saraee et al. 2014). Regression analysis of 55 articles on the effect of flavonoids in broiler diets on abdominal fat shows an intercept of 1.65 with a solve of -0.93 (Prihambodo et al. 2021). This decrease in abdominal fat levels is closely related to the effect of catechins in inhibiting fat anabolism and stimulating lipid catabolism (Huang et al. 2015).

Cholesterol is a substance that is produced naturally from the process of fat metabolism in the body, which often binds to protein to form lipoproteins (High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL). This study found that the addition of CGE in broiler diets reduced total cholesterol and LDL. On the other hand, it increased HDL and total protein (Table 5). The same results were also reported by previous researchers (Saraei et al. 2016) with green tea powder as a source of catechin compounds. These results are closely related to the ability of catechin compounds to reduce enzyme activity to synthesize fatty acids in the liver (Fatty acid synthase and acetyl CoA carboxylase) (Huang et al. 2015).

MDA, a metabolite derived from lipid peroxidation, has been widely used to indicate oxidative damage (Chen et al. 2021). The lower the MDA concentration, the higher the capacity of broiler chickens to clean oxygen free radicals, namely reactive oxygen species (ROS) (Daramola, 2019). Decreased MDA levels in serum and thigh meat tissue may be related to (i) catechin scavenging activity of superoxide and hydroxyl radicals; (ii) chelation with metal ions and formation of inactive complexes; (iii) stimulation of the synthesis of endogenous antioxidant enzymes in cells (El-Hack et al. 2020).

Adding CGE to the diet can increase the digestibility of crude protein in broilers, which also occurs in the addition of other flavonoid compounds, as reported by Abolfathi et al. (2019); Solangi et al. (2020) and Liu et al. (2020). The content of flavonoids in broiler diets can increase the VH: CD (Villus height: Crypt depth) ratio in the duodenum, jejunum, and ileum (Prihambodo et al. 2021), which indicates a healthier chicken small intestine and higher absorption capacity (Abolfathi et al. 2019). Furthermore, as natural antioxidant compounds, catechins can reduce intestinal oxidative injury in broilers by increasing antioxidant capacity and inhibiting the intestine's inflammatory response, thereby improving intestinal function in digestion and absorption (Song et al. 2019). At a higher dose of catechins at 0.42g/kg (5g/kg grape seed extract), the digestibility of crude protein and essential amino acids (Arginine, Histidine, and Phenylalanine) and non-essential amino acids (Cystine, Glutamic Acid, Glycine, and Proline) began to decrease. Due to the interaction of reactive hydroxyl groups of polyphenols with protein carbonyl groups forming bonds (Chamorro et al. 2013). The decrease in fat digestibility obtained in this study further strengthens that catechins and flavonoids harm fat digestion (Solangi et al. 2020). *In vitro* studies have shown that epigallocatechin gallate interferes with the emulsification, digestion, and dissolution of lipid micelles, which causes a decrease in the absorption of dietary fat in the intestine (Koo and Noh 2007).

Conclusion

Based on these results, it can be concluded that CGE can be used as an herbal feed additive for broilers. The addition of 0.3g/kg CGE gave the best results for increasing growth performance, carcass characteristics, blood plasma constituents, antioxidant status, and nutrient digestibility in broilers. Catechins are classified as flavonoids with potent antioxidants activity to provide increased growth performance followed by an increase in carcass characteristics, a decrease in total cholesterol and LDL, an increase in HDL, and antioxidant status as a reflection of an increase in protein digestibility and a decrease in fat digestibility in broilers.

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Author's Contribution

Ramaiyulis supervised the experiment and wrote the original manuscript. Salvia, N. Fati, and T. Malvin conducted the experiment and data analysis. Mairizal prepared tables and finalized draft. The final version of the manuscript was read and approved by all authors.

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