3. RESULT & DISCUSSION

After 3 months of treatment, all the rats were sacrificed and blood serum, main organs (includes heart, liver, kidney, brain, and spleen), cecum, and colon were collected and their related assay was conducted. Throughout the studies experiment, all groups showed a normal increase in body weight and food intake which in line with the increase in body weight (data not shown). Both body weight and food intake were decreased during the first two days after DMH injection, but return to normal on the third day. A change in rat behaviour was observed, such as less active than usual and less responsive to a trigger, and it was speculated that the observed change may due to injection of DMH as a carcinogen.

There was no significant difference in blood biochemistry, organ weights and organ histopathological (all collected organ) between groups ($p>0.05$). Injecting DMH in the DMH-treated group induced ACF and tumors, but not in the normal group, indicating that DMH was the only factor causing ACF formation (data not shown). The specificity of subcutaneous DMH injection for colon cancer formation has been reported (Swenberg *et al.*, 1979). DMH will be metabolized in the liver to form methyldiazonium ion (MAM) that is a strong carcinogenic metabolite. This metabolite later will translocate to colon via bile acid or blood circulation (Perše & Cerar, 2011). The ACF form mostly on the distal colon followed by proximal colon on the cancer group (McGarrity *et al.*, 1988). With the increase of concentration in the three concentrations (low, medium and high) range, the ability of Jaboticaba treatment to inhibit the formation of ACF also increased (data not shown).

Within research associated with gut microbiota and SCFA, cecum was widely used due to high concentration of bacteria in the cecum ($\pm 10^8$ CFU/ml) compared to other site of large intestine site (Marteau *et al*., 2001). Other than that, the main fermentation of rat takes place in the cecum rather than colon due to the different anatomy to human colon (Nguyen *et al*., 2015). Thus, the cecum was used as the sample in most of the conducted assay in this research.

3.1. Effects of Jaboticaba extract on properties of cecum and feces

3.1.1. Microbiota

The effect of Jaboticaba on the composition of intestinal flora was analyzed to reveal the potential mechanism of Jaboticaba as a chemopreventing agent. The spread plate was used to evaluate *Bifidobacterium* spp in cecum samples, while 16S DNA sequencing was applied to group the microbiota in the cecum contents.

The viable cell count showed that population of *Bifidobacterium* spp. was slightly higher (no significant difference, $p>0.05$) in the high dose Jaboticaba treated group than that of DMH-induced group. Although not proven significantly different by statistic, this result indicates that Jaboticaba has the potential to stimulate the growth of beneficial bacteria in the intestine. Other researches had also reported pomegranate anthocyanin (Bialonska *et al*., 2010) and Jucara pulp activity in stimulating the growth of *Bifidobacterium* spp. (Guergoletto *et al*., 2016).

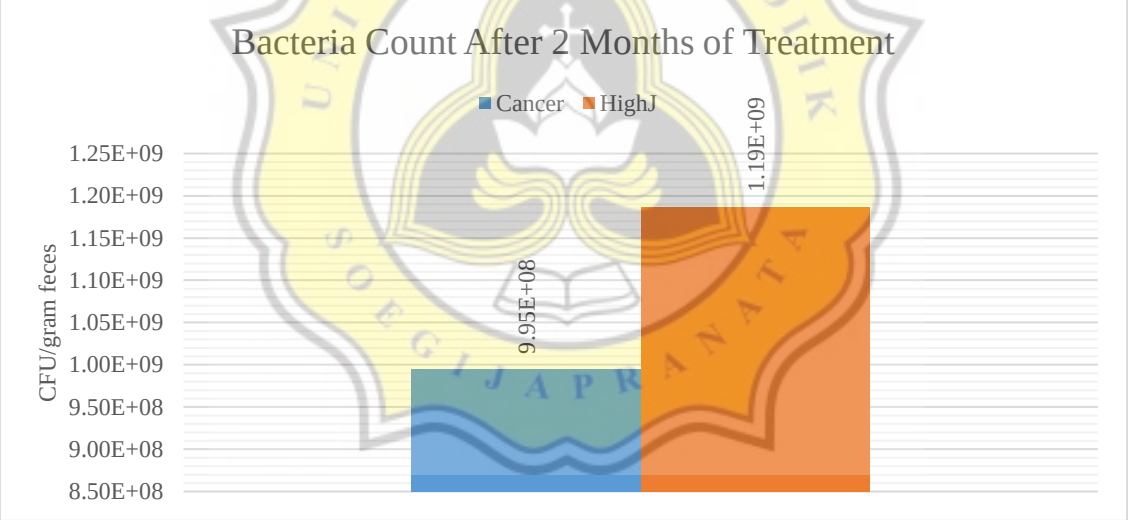


Figure 7. *Bifidobacterium* spp. count in feces in DMH-induced rats and high dose Jaboticaba-treated rats. Feces particles were collected after 2 months of treatment. DMH-induced rats: Sprague-Dawley rats were subcutaneous injection of DMH (30 mg/kg body weight) twice a week during the first 2 weeks to induce aberrant crypt formations. High dose Jaboticaba-treated rats: oral admission of 1.0 g/kg BW/day of Jaboticaba extract for continuous 2 months.

Results of 16S DNA sequencing showed Jaboticaba extract could regulate microbiota composition. In the phylum level, *Bacteroidetes* was enriched in the group that received Jaboticaba extract, in a dose-dependent manner (Figure 10). The DH group was found with the highest Bacteroides genus which indicated that the Jaboticaba extract had the

ability to change the CRC-microbiome environment since research reported that the *Bacteroidetes* populations were low in subjects (animal models and humans) with colorectal cancer (Shen *et al*., 2010; Zhu *et al*., 2014) and inflammatory bowel disease (Crohn's disease and ulcerative colitis) (Alam *et al*., 2020).

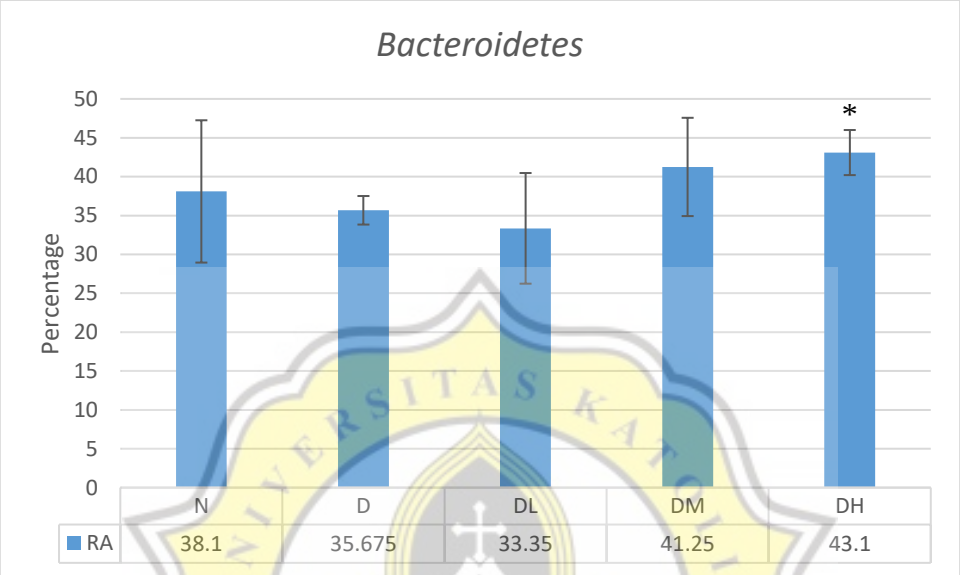


Figure 8. Relative abundance of *Bacteroidetes* on Sprague-Dawley cecum content. N: normal control group; D: DMH-induced group; DL: low dose of Jaboticaba treated group; DM: medium dose of Jaboticaba treated group; H: high dose of Jaboticaba treated group.

Based on the results of 16S DNA sequencing, the treatment resulted in the most significant changes in the genus level of the cecum content flora. Jaboticaba treatment caused a significant increase in the abundance of *Tannerelaceae*, *Ruminococcus 1*, *Lachnospiraceae NK4A136* and in the family of *Lachnospiraceae* (Figure 11). The abundance of *Tannerelaceae* increased with the increase of Jaboticaba concentration, while *Ruminococcus 1* and *Lachnospiraceae NK4A136* were significantly higher in the group receiving low dose of Jaboticaba extract. Higher concentrations (medium dose and high dose) of Jaboticaba extract had similar results on *Lachnospiraceae* NK4A136, but the effect of higher concentrations Jaboticaba extract on *Ruminococcus1* was not obvious.

The content of *Tannerelaceae* in the human intestine is usually about 1%, and its role is still unclear. Study reported that *Tannerelaceae* was less abundant in patients with inflammatory bowel disease. This disease was one of the condition from which it might develop into colorectal cancer (Beaugerie & Itzkowitz, 2015).

Ruminococcus 1 and *Lachnospiraceae* NK4A136 are both SCFA producing bacteria. In general, the *Lachnospiraceae* family consists of polysaccharide-fermenting bacteria that produce short-chain fatty acids as their metabolites, especially propionic acid (Ríos-Covián *et al*., 2016) and butyric acid (Venegas *et al*., 2018). *Ruminococcus* is considered to be a kind of beneficial bacteria, and *Ruminoccus blautia* is a well-known butyrate-producing bacteria. Research also reported mucin production activity of *R. blautia*, which is important for inhibiting pathogenic bacteria from invading the colonic wall barrier (Song *et al.*, 2018).

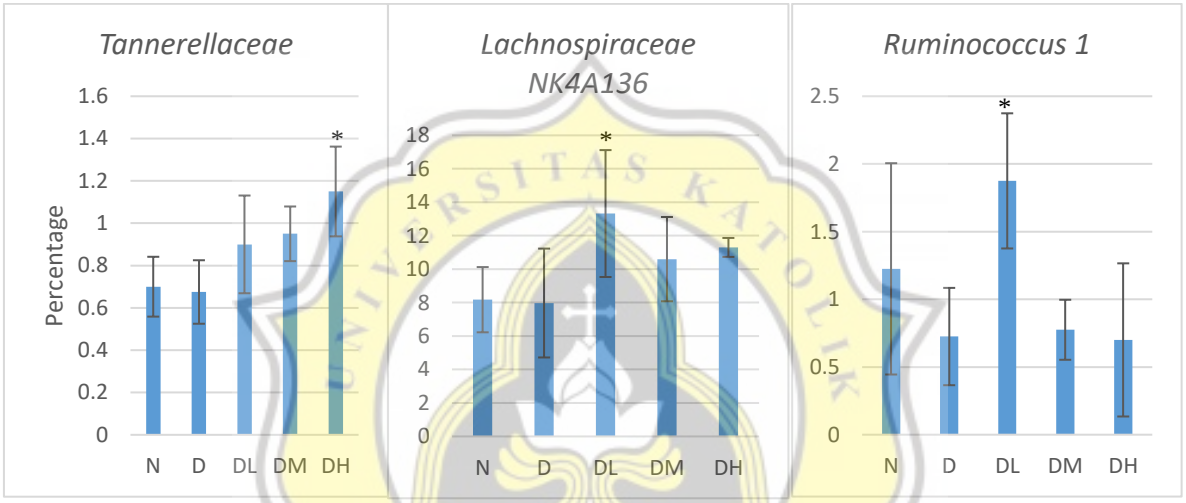


Figure 9. Relative abundance of *Tannerelaceae*, *Lachnospiraceae NK4A136* and *Ruminococcus 1* on Sprague-Dawley cecum content.

Therefore, due to the higher number of SCFA-producing strains in low-dose treatment group, it was expected that the concentration of propionic acid and butyric acid in feces and cecum would be higher than in other groups. This will further be explained in the section of short-chain fatty acid.

DMH-induced group had the highest amount of *Lachnoclostridium* than other groups (Figure 12). This genus was recently proposed as a biomarker for detection of colorectal cancer (Liang *et al*., 2019). The high population of *Lachnoclostridium* in the DMH induction group showed that DMH induced ACF in this study was sufficient to trigger the change in *Lachnoclostridium* population. On the other hand, the *Lachnoclostridium* population was suppressed by Jaboticaba treatment and even restored to normal levels. In a dose-dependent manner, medium and high concentrations of Jaboticaba extract had

more prominent results, inhibiting the growth of *Lachnoclostridium* to the point that it was lower than normal control group.

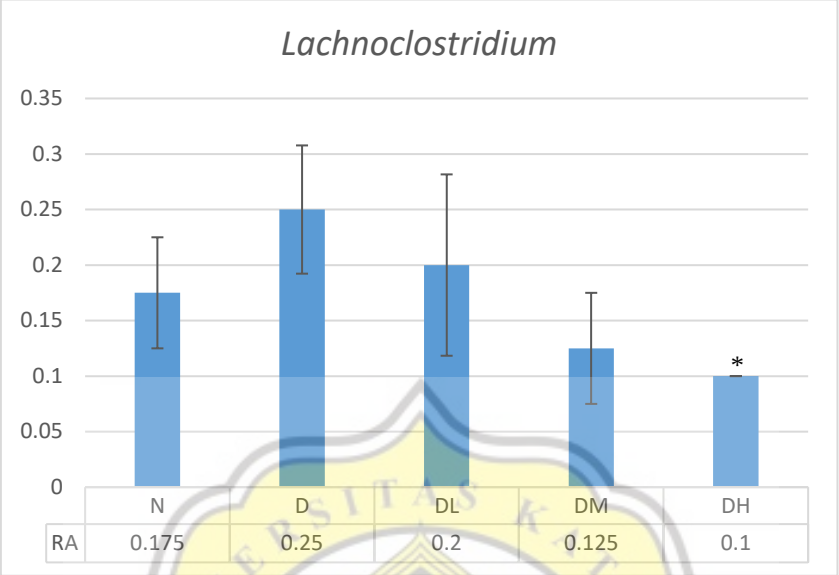
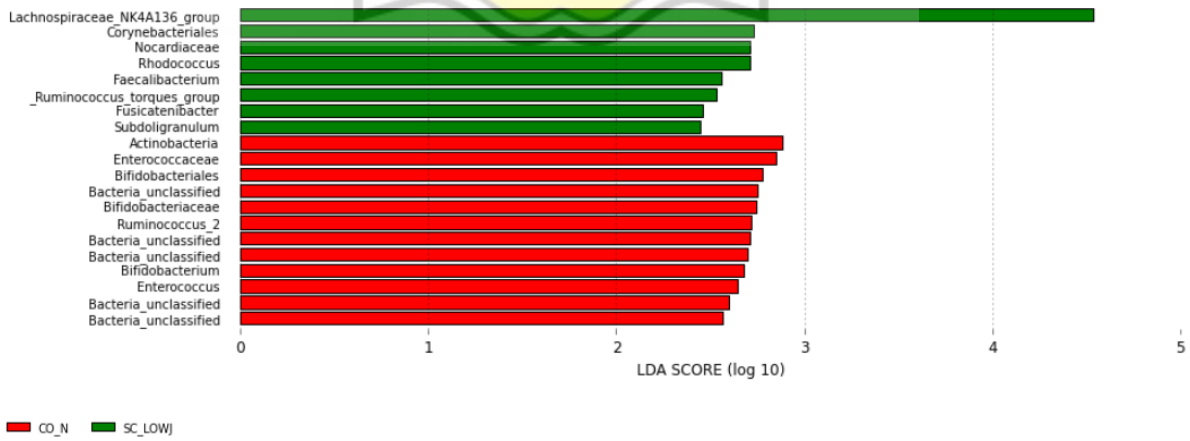


Figure 10. Relative abundance of *Lachnoclostridium* on Sprague-Dawley cecum content

Linear discriminant analysis effect size (LEFSe) was done to analyze a specific microbiota biomarker of the group by comparing relative abundance. The graph in Figure 13. shows result of Linear discriminant analysis (LDA), with transforming effect size t to log10 for quantitative measurement. Each color indicates which strain in the relevant groups is significantly rich and may be used as biomarkers.



Note: CO_N : normal control group
SC_LOWJ : DL group (low Jaboticaba concentration)

Figure 11. Comparison of relative abundance by LDA score.

LEFSe only identified biomarkers from the control group and DL group. No significant biomarker can be detected from the cancer group which is in line with the statistic result done using ANOVA. The Control group was found to be enriched in *Actinobacteria, Enterococcaceae, Enterococcus, Bifidobacteriales* and *Ruminococcus 2* while DL group had a significant abundance of *Lachnospiraceae* NK4A136, *Corynebacteriales, Nocardiaceae, Rhodococcus, Faecalibacterium, Ruminococcus torquez* and *Fusicatenibacter*.

The Normal group had the highest abundance of *Bifidobacteriales*, and the order includes *Bifidobacterium*. However, the abundance of *Enterococcus* in normal group was also significantly dominant. *Enterococcus* is associated with many diseases, such as urinary tract infections (Shankar *et al*., 2001), meningitis (Pintado *et al*., 2003) and colorectal cancer (Zhou *et al*., 2016). In this genus, *Enterococcus faecalis* is one of the bacteria associated with colorectal cancer (Zhou *et al*., 2016). *Enterococcus faecalis* can trigger colonic cell mutations by damaging deoxyribonucleic acid. Nonetheless, this species is naturally abundant in the human intestine because it is one of the first bacteria to colonize in the human intestine and plays an important role in the intestinal immune system of newborn babies. Research reported around 10^5 to 10^7 CFU/g of *E. faecalis* in adult human fecal (De Almeida *et al*., 2019) and the difference in the relative abundance of healthy tissue with tumor tissue of colorectal cancer was 91% vs 93% (Zhou *et al*., 2016). Therefore, the high concentration of *Enterococcus* in the normal group is not related to the risk of colorectal cancer. In addition to that, those bacteria mentioned above was around 0.1-0.2% of total bacteria population and only presented in some sample of normal group and none in other groups. With no confirmation from ANOVA result, the conclusion of this data can't be taken.

DL group, on the other hand, showed a significant abundance of beneficial bacteria, such as *Lachnospiraceae* NK4A13*6* (detailed in the previous section), *Fusicatenibacter*, belong to the family of *Lachnospiraceae*, bacteria associated with increasing fat intake in the human intestine and reducing cholesterol and fecal secondary bile acid in the feces (Prieto *et al*., 2018) and *Faecalibacterium*, the genus of *Faecalibacterium prausnitzii* which known as the main producer of butyrate and able to affect the expression of tight

junction protein, mucus secretion and serotonin restoration. *Lachnospiraceae* NK4A136, *Fusicatenibacter*, and *Faecalibacterium prausnitzii* all play an important role in inhibiting the development of colorectal cancer (Lopez-Siles *et al.*, 2017).

Other identified bacteria in the DL group, including *Rhodococcus, Ruminococcus torques* and *Subdoligranulum* were reported as an opportunistic pathogen. *Subdoligranulum* is a member of *Ruminococcaceae* and can produce butyrate in the human gut. *Subdoligranulum* isolated from human feces, was found as SCFA producer when cultured in vitro (Holmstrøm *et al*., 2004). However, later studies have shown that the amount of *Subdoligranulum* in type 2 diabetes patients is very high, it seems that the abundance of *Subdoligranulum* is related to glucose intolerance (Zhang *et al*., 2013). It was also identified in a subject with obesity (Kim, Song, & Kim, 2014). The latest research reported a high content of *Subdoligranulum* in gastrointestinal neoplasms including stomach, colon, rectal tumors (Youssef *et al*., 2018). Furthermore, *Subdoligranulum* also correlates with food sensitivity in children (Chen *et al.*, 2016), chronic inflammation and poor metabolic control and also inhibits bifidogenic bacteria to ferment inulin which is beneficial in the prevention of colon cancer (Chumpitazi *et al*., 2014).

Little is known about *Rhodococcus* and its relationship with colorectal cancer. However, pathological studies have identified *Rhodococcus equi* as bacteria that infect subjects with impaired immunity, such as HIV patients (Sughayer *et al.*, 1997). *Ruminococcus torques* is a mucin-degrading bacteria (Hoskins, Kriaris, & Niedermeyer, 1985) that is associated with Crohn's disease (Martinez-Medina *et al*., 2006) because the loss of mucin increases the pathogen's ability to infect the colonic mucosa, resulting in inflammation and mutation of the relevant cells. However, the percentage of mentioned biomarker bacteria (except *Lachnospiraceae NK4A136*) in the DL group was lower (about 0.1-0.2% in some samples) and was not supported by ANOVA results, so these strains can be ignored.

Principal component analysis was used to determine if there were any difference between microbiota between normal control group, cancer control group and Jaboticaba-treated group. Similar sample will have a closer distance with each other, forming a cluster, while a difference in sample will result in a bigger distance between those sample (Gloor &

Reid, 2016). As represented in Figure 14, the sample of 5 groups were represented in different colour. Normal control group and cancer control group were each clustered separately but in a close distance. This was in line with ANOVA result as there was no significant difference found between normal control and cancer control group. The cluster of DL was separated but also in a close distance with control group indicating it slight difference in microbiota composition to control group. On the other hand, DH group was separated from other cluster indicating a significant difference in gut microbiota composition. As no difference was detected in DH group on either ANOVA and LEFSe result, it was possible that the difference was not quite significant to be detected by ANOVA and LEFSe.

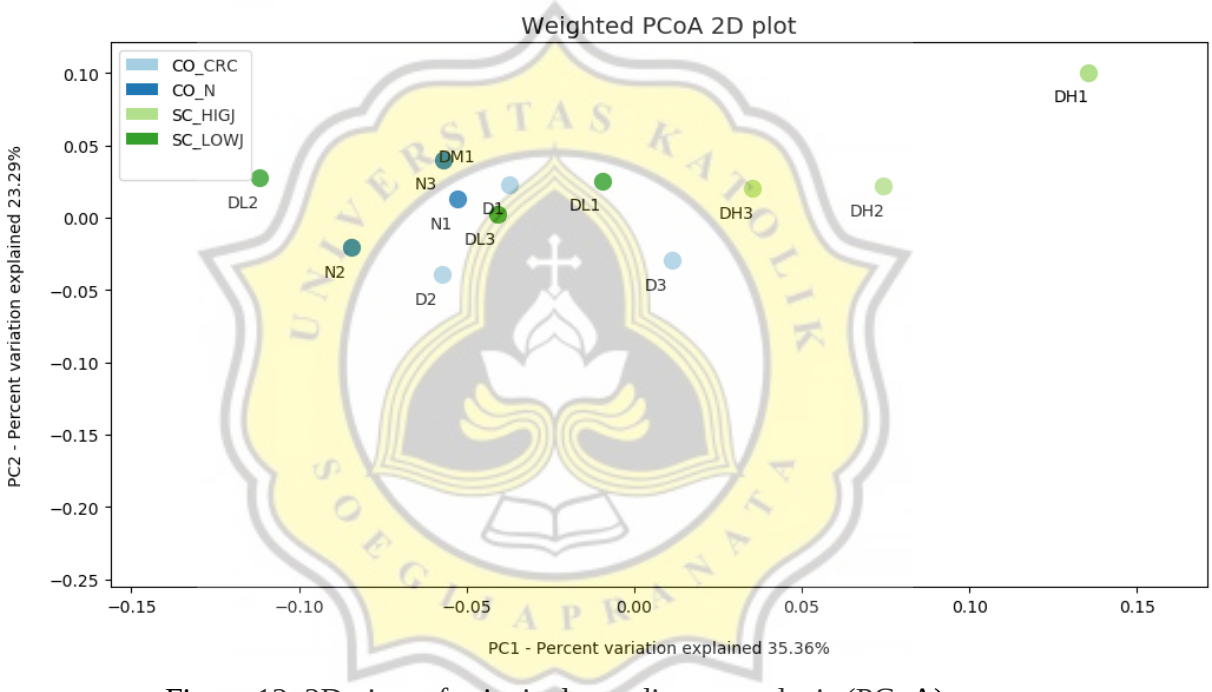


Figure 12. 2D view of principal coordinates analysis (PCoA)

3.1.2. pH

The pH of the cecum contents did not show differ significantly between the normal control and cancer control. The same result also reported by Hsu *et al*. (2004) for no differences in pH value between the healthy group and DMH induced group on Sprague-Dawley rats (Table 3). The pH values of cecum contents in both control (6.77±0.14) and DMH-induced group (6.87±0.07) were comparable to other studies, such as 6.58 for fasted Wistar rats (McConnell, Basit, & Murdan, 2008) and 6.8±0.1 for fasted Sprague-Dawley rats (Asano *et al.*, 2004). Regardless of dosage, Jaboticaba treatment caused a significant increase in the pH of the cecum contents ($p<0.05$).

Table 3. pH of the cecum contents[1]

	Control	Cancer Control	DL	DM	DH
Cecum pH	6.77±0.14[a]	6.87±0.07[a]	7.19±0.25[b]	7.09±0.08[b]	7.07±0.12[b]

[1]Values is means ± standard deviation. Control (n=4), cancer control (n=3), DL [DMH+low jaboticaba] (n=2), DM [DMH+medium jaboticaba] (n=4), DH [DMH+high jaboticaba] (n=4). Different letter indicate significant difference in the value. $P < 0.05$.

Regarding the pH of the feces, there was no significant difference between the groups within 8 weeks of the entire experiment (Table 4). The pH of feces averaged 6.14 ± 0.32, which was slightly lower than that of cecum content and other similar studies. Caderni *et al*. (1993) reported fecal pH was 7.15±0.09 in healthy SD rats, Leu *et al*. (2002) and Samelson *et al*. (1885) reported fecal pH were 6.9±0.06 and 7.3±0.1 in DMH-induced SD rats, respectively. However, the baseline was originally low and there was no significant difference in treatment.

Table 4. pH of the feces

	Week				
	Baseline	1	3	5	7
Control	5.97±0.14[a]	5.84±0.06[a]	6.12±0.17[a]	5.88±0.21[a]	6.51±0.62[a]
Cancer Control	5.89±0.07[a]	6.34±0.17[a]	5.91±0.25[a]	6.17±0.09[a]	6.21±0.18[a]
DL	6.07±0.14[a]	6.08±0.15[a]	6.12±0.16[a]	6.13±0.08[a]	6.45±0.39[a]
DM	6.12±0.42[a]	5.81±0.13[a]	6.11±0.21[a]	6.13±0.24[a]	6.30±0.19[a]
DH	6.20±0.08[a]	6.12±0.28[a]	6.16±0.16[a]	6.81±0.76[a]	6.31±0.07[a]

[2]Values is means ± standard deviation. DL [DMH+low jaboticaba], DM [DMH+medium jaboticaba], DH [DMH+high jaboticaba]. Different letter indicate significant difference in the value within each week. $P < 0.05$.

As for the pH value of feces was lower than the pH value of cecum, this was very different from the results of other existing studies, which might be related to the sample preparation of pH measurement. The watery cecum contents were easily homogenized in water, so it had better resolution and homogeneity. Moreover, the cecum contents were collected with a spatula immediately after opening the cecum with a scalpel, thus reducing the contamination of other substances.

Ideally, feces can be collected immediately after defecating from the rectum. However, because it was difficult to collect a sufficient amount of feces at a single time,

the stool particles in the tray were collected every hour until all the required feces were collected on the day. By using this method, the sample quality should be considered. In addition, fecal particles were in solid form, so fecal samples did not easy to homogenize with water. In addition, the water content of the cecum and fecal particles were different, even if they were homogenized at the same solvent ratio, it would affect the results.

Other possible reason was due to different circumstances of the rat upon sample collection. Research has reported higher pH value in intestinal content due to starvation. The pH of both cecum and distal colon of 24-hour starved Sprague-Dawley rat were 7.74 ± 0.12 and 7.37 ± 0.20, respectively while the pH of un-starved rat was 6.14 ± 0.07 and 6.87 ± 0.31 (Butler *et al*., 1990). Similar result was also reported for adult female Wistar rats with its cecum pH measured 5.9 ± 0.4 in normal condition and 6.58 ± 0.4 due to starvation. To get a better observation upon pH gradient in different colon site, the measurement of intestinal content between cecum, proximal and distal colon was done at the same time to limit variation (McConnell *et al*., 2008).

Due to the great differences in the research results, the correlation between pH and the development of colorectal cancer remains controversial. Many studies have found that the low pH of intestinal contents (including cecum and feces) indicated that it has a protective effect to prevent the occurrence of colorectal cancer. This is related to the production of SCFA during fermentation in the colon. SCFA has the acid properties and is well known for its protective effect on the colon (Lin & Visek, 1991). In the colon, SCFA is one of the components responsible for neutralizing ammonia produced during the degradation of urea and therefore neutralizing the pH of the colon (Lupton & Newmark, 1990).

However other research reported increased rate of cell proliferation due to low gastrointestinal pH. An acidified environment was stimulated by increasing the intake of fiber in Sprague-Dawley rat with DMH induction. The higher intake of fiber was later associated with higher tumor count in proximal colon (Jacobs & Lupton, 1986). No difference in pH value on different colon site (right, mid, left, whole colon) was also reported between healthy patient to those with colorectal cancer (Pye, Evans, Ledingham, & Hardcastle, 1990). Nevertheless, more research concluded the chemoprevention effect of lower colon pH due to SCFA acidic nature (Lin & Visek, 1991).

Lupton & Newmark (1990) reported that there was a correlation between the alkaline environment in the colon and the increased risk of colorectal cancer, because the alkaline environment contributed to the formation of secondary bile acid and ionized long-chain fatty acids (LCFA). Both of secondary bile acid and LCFA could irritate colonic epithelial cells, thus leaded to a higher risk of colorectal cancer (Lupton & Newmark, 1990). Colorectal cancer patient had been reported with higher pH value in their colon (Pye *et al*., 1990).

A study by Samelson *et al*. (1985) showed that in DMH-induced rats, the administration of dietary supplements reduced colon tumors, and the pH of both cecum content and feces also decreased. However, the administration of jaboticaba extract did reduce the ACF, but did not lower the pH of the cecum content or feces. It is not recommended to use pH or SCFA concentration alone to observe the state of the intestine, because the large intestine cells will absorb most of the SCFA and other metabolites and only excrete 5% into the feces (McNeil, Cummings, & James, 1978).

3.1.3. Short chain fatty acid

Short chain fatty acid (SCFA), such as acetic acid, propionic acid and butyric acid are the major bio-active SCFA produced by fermentation of gut microbiota (Cummings, 1981). Butyric acid is the most discussed because it can improve intestinal health (Hijova & Chmelarova, 2007).

Table 5 and 6 shows the effect of Jaboticaca extract on the SCFA production. In cecum contents, the concentrations of acetic acid and propionic acid were not significantly different between the normal (healthy) group and the DMH induced group. DMH induced ACF formation but did not cause significant changes in the concentrations of acetic acid and propionic acid, while the concentration of butyric acid was significantly decreased. Jaboticaba treatment can restore the butyric acid reduction induced by DMH.

Table 5. Short Chain Fatty Acid Concentration of Rat Cecum[3]

Group	Acetic Acid (mmol/gram)	Propionic Acid (mmol/gram)	Butyric Acid (mmol/gram)
Normal	1.7 ± 0.08^{a}	0.74 ± 0.05^{a}	1.81 ± 0.03^{a}
Cancer	1.8 ± 0.17^{a}	0.73 ± 0.06^{a}	1.71 ± 0.05^{b}
DL	1.73 ± 0.09^{a}	0.69 ± 0.01^{a}	1.82 ± 0.04^{ab}
DM	1.76 ± 0.06^{a}	0.72 ± 0.05^{a}	1.82 ± 0.07^{ab}

DH	1.72±0.07[a]	0.72±0.03[a]	1.79±0.03[ab]

[3]Values is means ± standard deviation. Control (n=4), cancer control (n=3), DL [DMH+low jaboticaba] (n=4), DM [DMH+medium jaboticaba] (n=4), DH [DMH+high jaboticaba] (n=4). Different letter indicate significant difference in the value. *P < 0.05.* The concentration was expressed in micromolar/gram cecum (wet basis).

Table 6. Short Chain Fatty Acid Concentration of Rat Fecal[4]

	Week	Normal	Cancer	DL	DM	DH
Acetic Acid	Baseline	2.17±0.16[a]	2.26±0.39[a]	2.19±0.26[a]	2.37±0.37[a]	2.16±0.11[a]
	1	2.42±0.3[a]	2.12±0.25[a]	1.99±0.08[a]	2.19±0.23[a]	2.12±0.25[a]
	3	1.98±0.12[a]	2.1±0.47[a]	2.35±0.29[a]	2.28±0.42[a]	2.13±0.11[a]
	5	2.12±0.1[a]	2.35±0.24[a]	2.34±0.22[a]	2.2±0.28[a]	2.41±0.41[a]
	7	2.21±0.46[a]	2.49±01.2[a]	2.49±0.51[a]	2.7±0.96[a]	2.52±0.26[a]
Propionic Acid	Baseline	0.63±0.03[a]	0.6±0.01[a]	0.6±0.02[a]	0.66±0.08[a]	0.65±0.03[a]
	1	0.63±0.05[a]	0.58±0.02[a]	0.61±0.05[a]	0.59±0.03[a]	0.62±0.04[a]
	3	0.58±0.01[a]	0.6±0.06[a]	0.64±0.02[a]	0.62±0.07[a]	0.61±0.02[a]
	5	0.56±0.03[a]	0.61±0.03[a]	0.62±0.05[a]	0.62±0.03[a]	0.64±0.05[a]
	7	0.58±0.07[a]	0.5±0.35[a]	0.64±0.07[a]	0.63±0.01[a]	0.65±0.03[a]
Butyric Acid	Baseline	1.9±0.11[a]	1.94±0.03[a]	1.96±0.28[a]	2.14±0.32[a]	2.1±0.23[a]
	1	1.99±0.21[a]	1.92±0.16[a]	1.86±0.12[a]	1.88±0.09[a]	1.88±0.02[a]
	3	1.88±0.002[a]	1.87±0.09[a]	2.31±0.27[a]	1.87±0.13[a]	1.77±0.2[a]
	5	2.13±0.48[a]	2.09±0.22[a]	1.91±0.08[a]	1.87±0.05[a]	1.98±0.3[a]
	7	2.03±0.47[a]	1.86±0.13[a]	2.13±0.29[a]	1.79±0.21[a]	2.08±0.24[a]

[4]Values is means ± standard deviation. Control (n=4), cancer control (n=3), DL [DMH+low jaboticaba] (n=4), DM [DMH+medium jaboticaba] (n=4), DH [DMH+high jaboticaba] (n=4). Different letter indicate significant difference in the value. *P < 0.05*. The concentration was expressed in micromolar/gram (wet basis).

The fecal SCFA concentrations were not significantly different among groups (Table 6). Unlike in the cecum content, butyric acid in feces did not show an effect through treatment. As mentioned earlier, the difference between the cecum contents and fecal particles is that there is more fluid in the cecum, and the feces are solid. The lower moisture content in the fecal particles makes it more concentrated, resulting in a higher SCFA concentration. Other studies have overcome this problem by standardizing the moisture content of the samples by freeze-drying (Hsu *et al*., 2019). In this study, the samples were not freeze-dried due to insufficient sample size. Fecal particles may also be contaminated because samples are not collected immediately after defecation (Han *et al*., 2018).

3.2. Effect of Jaboticaba extract on antioxidant activity in rats

From diabetes to cancer, oxidative stress is increased during disease development (Alezandro *et al*., 2013). The increase in oxidative stress is due to the increase in free radicals and the inability of the antioxidant enzyme system to neutralize these free radicals. In the antioxidant system, catalase, glutathione peroxidase (GPx) and superoxide dismutase (SOD) are the main defences against radical species (Mates *et al*., 1999). Measuring activity of antioxidant enzymes in major organs is one of the methods for monitoring the oxidative stress level in the body.

In vitro studies have shown that Jaboticaba has excellent antioxidant activity due to its rich phenolic compounds (Alezandro *et al*., 2013; Wu *et al*., 2013). In this study, *in vivo* study was conducted to assess whether the antioxidant properties of Jaboticaba could neutralize the oxidative stress induced by DMH injection.

Most antioxidant-related studies measure the activity of antioxidant enzymes in the liver, heart, and brain, of which the liver and heart are most commonly measured. Liver is the specific site with the highest catalase concentration, which is related to liver's main role in toxin and xenobiotic detoxification (Aebi, 1974). The heart is very commonly used in the study of antioxidant enzymes, while the antioxidant activity of the brain is lower than the heart and liver. Another alternative of organ is the kidney, whose enzyme levels range between the heart and liver (Cand & Verdetti, 1989).

In this study, the levels of catalase, GPx and SOD also varied from organ to organ (Table 7). Similar to other studies, the liver has the highest enzyme activity, followed by the heart and brain (Cand & Verdetti, 1989). The result showed no significant difference between 3 tested groups on all tested parameters ($p<0.05$). Nevertheless, the organs in the DMH-induced group had higher catalase and SOD activities compared to normal control, indicating that oxidative stress due to DMH induction was elevated. The GPx levels were reversed, the activity in the DMH-induced group was lower. Jaboticaba treatment was found to stimulate GPx activity in both heart and brain but decrease the activity in the liver. Compared with Lenquiste *et al*. (2015), the catalase values detected in this study are much higher, while SOD and GPx are much lower. The GPx value in the brain of the normal group was comparable to the result of Haleagrahara & Ponnusamy (2010). They reported that GPx activity in the striatum of the brain was 21.65 ± 1.17 nmol / min / mg.

Table 7. Enzyme Activity of Liver, Heart and Brain of Control, Cancer Control and DH group[5]

		Normal	Cancer	DH
Liver	Catalase (nmol/min/mg)	663.32±141.24[a]	3159.71±1822.17[a]	2776.12±1968.54[a]
	SOD (U/mg protein)	0.306±0.35[a]	1.682±2.30[a]	0.039±0.005[a]
	SOD (U/g organ)	7.3±0.19[a]	7.4±0.72[a]	5.8±0.79[a]
	GPx (nmol/min/mg)	0.99±1.4[a]	0.24±0.34[a]	0.01±0.02[a]
	GSH	399.3±16.78[a]	564.55±207.23[a]	345.23±170.95[a]
	GSSG	8.43	0.13	4.65±6.14
Heart	Catalase (nmol/min/mg)	15.01±5.77	144.5±119.55[a]	159.48±32.68[a]
	SOD (U/mg protein)	0.131±0.13[a]	0.138±0.058[a]	3.468±3.871[a]
	SOD (U/g organ)	254.9±34.84[a]	8.6±0.29[a]	133.2±14.55[a]
	GPx (nmol/min/mg)	161.21±222.89[a]	101.24±135.91[a]	273.42±15.29[a]
	GSH	173.35±6.1[a]	185.03±70.87[a]	205.44±91.26[a]
	GSSG	ND	ND	ND
Brain	Catalase (nmol/min/mg)	7.88±4.22[a]	36.51±13.76[a]	18.47±5.32[a]
	SOD (U/mg protein)	0.005±0.14[a]	0.144±0.05[a]	0.612±0.06[a]
	SOD (U/g organ)	5.2±0.14[a]	8.9±0.37[a]	23±0.51[a]
	GPx (nmol/min/mg)	20.19±27.78[a]	15.82±21.8[a]	29.25±0.39[a]
	GSH	114.46±1.24[a]	135.17±47.35[a]	120±95.6[a]
	GSSG	0.67	4.4	1.06±0.57

[5]Values is means ± standard deviation (n=2) per group. DH [DMH+high jaboticaba]. Different letter indicate significant difference in the value. $P < 0.05$.

Glutathione (GSH) and its oxidized form, glutathione disulfide (GSSG) are closely related to GPx activity, because two molecules of glutathione is needed to neutralize a molecule of hydrogen peroxide into water. The GSH in the exchange will be oxidized to GSSG (Wu *et al*., 2004). It is reported that the increase of glutathione levels in cells is positively correlated with cell proliferation, and therefore is related to the development of cancer (Carretero *et al*., 1999). Cancer cells had been found to trigger a high production of GSH to increase its sensitivity to anticancer agents (Calvert *et al.*, 1998). In this study, we also observed an increase in GSH levels in the DMH-induced group, especially in the liver, from which a significant increase could be observed. Jaboticaba treatment could restore GSH levels to normal levels. The GSH levels in the normal and DH groups of this study were similar to those reported in healthy Wistar rats (Lenquiste *et al*., 2015). GSSG was not detected in the heart tissue of all six test samples and in some of liver and brain samples. Low detection of GSSG was due to the nature of the fast and efficient reaction of GSH regeneration. GSH regeneration basically was done by glutathione reductase (GR) and NADPH which convert GSSG back to GSH. The fast nature of this reaction is related

to low substrate requirement to achieve highest enzyme reaction. Therefore, GSSG usually was not detected in cells except in the case of oxidative stress (Dringen, 2009).