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Myrmecodia pendans Supplementation in Broiler Drinking Water: Effects on Performance, Water Consumption, Carcass Yield, and Intestinal Microorganisms

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ABSTRACT

This study evaluated the efficacy of different levels of *Myrmecodia pendans* (MP) drinking water on the performance, water consumption, carcass yield, and intestinal microorganisms of broiler chickens during a 5-week experimental period. A total of 112 3-day-old Lohmann broilers (initial body weight 53.61 ± 0.77, CV 1.43%) were randomly assigned to 1 of 4 drinking water treatments: a control (without MP), 16 g MP, 32 g MP, and 48 g MP per litre of water. The results showed that MP drinking water significantly decreased total *Escherichia coli* and tended to decrease total intestinal microorganisms in a dose-dependent manner. Intestinal *Salmonella* was only found in the control treatment. Feed consumption significantly increased with MP administration, particularly at the 48 g dose, and a tendency for increased water consumption was observed. The administration of MP had no significant effect on body weight gain, feed conversion ratio, and percentage of carcass yield. In conclusion, the addition of MP at a minimum level of 16 g was able to inhibit the growth of total intestinal microorganisms, *Escherichia coli*, and *Salmonella*, and increased feed consumption, but had no significant effect on BWG, feed conversion ratio, and carcass yield. Further research is needed to investigate the specific mechanisms by which MP affects intestinal microorganisms.

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INTRODUCTION

Intestinal health is critical for broiler chicken growth, directly influencing nutrient digestion and absorption, and ultimately enhancing performance. A healthy intestine minimizes the presence of pathogenic bacteria like *Escherichia Coli* (*E. coli)* and *Salmonella*, which can disrupt poultry digestion (Lumbangaol, 2018). Salmonella can thrive in both aerobic and anaerobic conditions, causing gastrointestinal disorders.

Infections from *Escherichia* and *Salmonella* species significantly impair turkey production, leading to decreased egg output, reduced hatchability, and increased mortality rates (Kar et al., 2017). *E. coli* is a zoonotic commensal pathogen capable of causing infections in the digestive tract, respiratory system, and bloodstream in both humans and animals (Rahman et al., 2020). Meanwhile, *Salmonella* spp. is the etiological agent for salmonellosis in turkeys, notably manifesting as pullorum disease and fowl typhoid disease and fowl typhoid (Tawyabur et al., 2020). Given its zoonotic characteristic, *Salmonella* spp. can transmit to humans via the food chain, potentially triggering salmonellosis, gastroenteritis, enteric fever (Varga et al., 2019), and in some instances, posing life-threatening risk (Helms et al., 2002).

Traditionally, antibiotics treat bacterial infections in the avian digestive tract. However, their excessive use in poultry farms, particularly in developing nations, promotes the development of multi-drug resistant (MDR) bacteria (Alam et al., 2020; Dai et al., 2023). The spread of MDR bacteria to humans exacerbates the overall risk of antibiotic resistance, leading to a prohibition on antibiotic use. Consequently, herbal plants are increasingly explored as antibiotic alternatives in poultry (Dai et al., 2023; Sugiharto, 2021; Syaifudin & Sigit, 2019).

Myrmecodia pendans, is an epiphytic plant commonly found on large trees. Papuan communities have long used it in traditional medicine for various ailments, including cancer, tumors, gout, and rheumatism. *Myrmecodia pendans* contains phenolic compounds like flavonoids, saponins, tannins, glycosin, and phenols (Lisnanti & Fitriyah, 2017; Syaifudin & Sigit, 2019). Flavonoids and tannins inactivate viruses by inhibiting viral polymerases or binding to viral proteins, and they stimulate host cell immunity to block viral capsid formation, leading to viral particle destruction (Lisnanti & Fitriyah, 2017). In chickens, immunostimulants support proper cell function. A study on quail showed that 0.8% *Myrmecodia pendans* flour supplementation significantly reduced Total Plate Count but had no effect on intestinal *E. Coli* and *Salmonella* (Lumbangaol, 2018).

While previous research has investigated *Myrmecodia pendans* in other poultry species and through dietary supplementation, this study provides the first investigation into its application in drinking water for broilers in Manokwari, examining its impact on a comprehensive range of production and gut health parameters.

MATERIALS AND METHODS

Bird management

One hundred and twelve 3-d-old Lohmann broilers (straight run) were randomly assigned to 1 of 4 drinking water treatments (7 replicate pens; 4 birds per pen). The initial body weight of the birds was 53,61 ± 0,77 (CV = 1,43%). Birds were housed in cages, 50 cm (l) x 50 cm (w) x 40 cm (h). The floor was covered with 6 cm of wood shavings and equipped with 1 feeder and 1 drinker. During the experiment all broiler chickens were given commercial diet *ad libitum* in the same husbandry conditions. Drinking water was given according to the treatment applied. The cage was illuminated 24 hours.

Drinking water treatment

Drinking water treatments were applied with the pattern of 5 days ON and 2 days OFF. The drinking water pattern was applied based on (Lisnanti et al., 2018) who found a best result when *Myrmecodia pendans* (MP) was given at 5 days/week. Four drinking water treatments applied were: P0: 1 L drinking water (DW) + 0 g MP, P1: 1 L DW + 16 g MP, P2: 1 L DW + 32 g MP, P3: 1 L DW + 48 g MP. The determination of the quantity level of *Myrmecodia pendans* given refers to Supriadi (2010), namely 2 tablespoons of *Myrmecodia pendans* mixed with 3 cups of water.

Myrmecodia pendans drinking water

- The procedure in making *Myrmecodia pendans* drinking water is as followed:
- The *Myrmecodia pendans* powder obtained from the pharmacy was weighed according to the treatment, was then added 1 L of water and boiled.
- After boiling, the mixture was removed and cooled.
- After it was cool, the mixture was then filtered through a cloth.
- The *Myrmecodia pendans* pulp that is in the filter cloth is set aside and the *Myrmecodia pendans* drinking water was ready for use.
- To avoid damage, the *Myrmecodia pendans* drinking water was stored in the refrigerator before it was used.
- The *Myrmecodia pendans* drinking water was made every day during the experiment.

Variable measured

Variable measured in the experiment were Feed Consumption (FC)(g/b/d), Body Weight Gain (BWG) (g/b/d), Feed Conversion Ratio (FCR) (g/g), Water Consumption (WC) (ml/b/d), carcass yield (% of live weight), Total intestinal microorganism, Total intestinal *Salmonella*, and Total intestinal *Eschericia Coli* (*E. Coli*). Body weight and feed consumption of broiler chickens were weighed at the beginning of experiment, week 1, week 3, and week 5. Carcass yield and intestinal microorganism were determined at week 5.

Sample collection and analysis

At the end of the experiment (week 5), two birds from each of replication were randomly selected, weighed, and slaughtered to measure the variables below:

Carcass

The bird was slaughtered by cutting the jugular vein. The slaughtered bird was dipped into hot water (53-55ºC) about 1 minute, and the feathers were plucked. The bird was then eviscerated, and the internal digestive organ was pulled out. The bird was then cut off in the neck and the hocks and was weighed to get carcass weight. Carcass weight was represented as g/100 g live weight.

Microorganism analysis

The slaughtered bird was eviscerated, and the internal digestive organ was pulled out. The intestine was cut in the beginning part of duodenum until the ileo-ceco-colic junction. The intestinal digesta was squeezed and was collected in sterile tube and was then stored in the freezer for microorganism analysis. Approximately 1 g of intestinal digesta was placed in a closed sterile specimen tube. After the tube is weighed, 10 ml of Ringer's solution was added. The tube was rotated in a vortex and then diluted 3 times, each dilution of 1 ml of the first solution was added 9 ml of Ringer's solution. Each time dilution, the tube was rotated in a vortex. Petri dishes with agar media were used to measure total microbes and determine the types of microbes (*E. Coli* and *Salmonella*). *E. Coli* was analyzed using Eosin methylene blue agar (EMBA) media, while *Salmonella* sp was analyzed using *Salmonella Shigella Agar* (SSA) media. Total microbes were analyzed using Nutrient Agar media. Approximately 0.04 ml of the solution is dropped on the agar medium, the drop is spread on the agar. The petri dish containing the agar media which had been dripped with the solution was then incubated aerobically for a maximum of 48 hours at 37ºC. The total microbial count was done manually.

Statiscal Analysis

Data were analyzed using analysis of variance for Completely Randomized Design (SPSS 16.0., 2007). If the results of the F test were significant ($p<0.05$), then Duncan's test was used to determine differences among treatments. Treatments were stated to be significantly different at $p<0.05$. The difference between treatments with $0.05 \leq p \leq 0.10$ was considered as a tendency to be significant.

RESULTS

Feed Consumption (g/b/d)

Analysis of variance revealed that *Myrmecodia pendans* (MP) levels in drinking water significantly affected ($p<0.05$) feed consumption at Week 5 and across the overall mean (Weeks 1 to 5). The effect at Week 1 approached significance ($p=0.072$) (Table 1). Broilers receiving 48g MP tended to have higher consumption at Week 1. By Week 5 and for the overall mean, MP drinking water treatments significantly increased broiler feed consumption compared to the control. Feed consumption generally increased with higher MP levels, with 48 g MP showing the highest consumption, followed by 32 g MP, 16 g MP, and the control as the lowest.

Table 1. The Average of Feed Consumption, Body Weight, and Feed Conversion Ratio of Broiler Chickens Giving *Myrmecodia pendans* Drinking Water

	Treatment					
	P0	P1	P2	P3	SEM total	P value
Feed Consumption (g/b/d)						
Week 1	32.3	32.2	31.7	35.3	0.556	0.072^{ns}
Week 3	95.6	96.3	96.6	97.0	0.436	0.734^{ns}
Week 5	145.6^{a}	154.8^{b}	155.6^{b}	161.8^{b}	1.706	0.003*
Week 1 to 5	81.7^{a}	83.2^{ab}	83.7^{ab}	85.8^{b}	0.550	0.006*
Body Weight Gain (g/b/d)						
Week 1	27.14	27.23	26.46	26.60	0.187	0.379^{ns}
Week 3	74.67	73.23	76.69	74.53	1.054	0.733^{ns}
Week 5	73.07	79.17	73.40	74.99	3.435	0.928^{ns}
Week 1 to 5	62.03	63.00	62.91	63.17	0.743	0.955^{ns}
Feed Conversion Ratio (g/g)						
Week 1	1.190	1.181	1.200	1.331	0.023	0.057n^{s}
Week 3	1.282	1.209	1.264	1.308	0.023	0.506^{ns}
Week 5	2.390	2.126	2.151	2.297	0.163	0.940^{ns}
Week 1 to 5	1.326	1.325	1.334	1.366	0.018	0.857^{ns}

P0= 1 L DW + 0 g MP, P1= 1 L DW + 16 g MP, P2= 1 L DW + 32 g MP, P3= 1 L DW + 48 g MP. SEM= standard error mean, ns= non-significant ($p \geq 0.05$), *=significant ($p<0.05$).

^{a,b} Different superscripts in the same row show significantly different ($p<0.05$).

Body Weight Gain (g/b/d)

Body Weight Gain was not significantly influenced ($p>0.05$) by any treatment at Week 1, Week 3, Week 5, or the overall average (Table 1). Furthermore, body weight gain from Week 3 to Week 5 showed minimal increase, with some treatments even exhibiting a slight decrease (Table 1).

Feed Conversion Ratio (g/g)

The effect of MP levels on the Feed Conversion Ratio (FCR) is presented in Table 1. At Week 1, the effect approached significance ($p=0.057$). However, in subsequent weeks and for the overall average (Weeks 1 to 5), FCR was not significantly influenced by the treatments (p>0.05). At Week 1, broilers in the 48g MP group tended to exhibit a poorer FCR compared to other treatments, while 0 g MP, 16 g MP, and 32 g MP showed similar FCRs.

Water Consumption (ml/b/d)

ANOVA results for broiler drinking water consumption indicated a trend towards significance at Week 1 (p=0.088) and Week 5 (p=0.082), though the overall average (Weeks 1 to 5) was not significantly different (p>0.05) (Table 2). At Week 1, broilers receiving 48 g MP tended to consume less water than other treatments. Conversely, at Week 5, the MP treatment groups generally showed higher water consumption compared to the control.

Table 2. The Average of Water Consumption (ml/b/d) of Broiler Chickens Giving *Myrmecodia pendans* Drinking Water

Treatment	The average of water consumption (ml/b/d)			
	Week 1	Week 3	Week 5	Week 1 to 5
P0	83.6	227.8	369.4	249.2
P1	81.3	268.4	389.8	252.9
P2	82.8	266.8	377.5	250.0
P3	77.2	266.3	378.4	249.5
SEM total	0.981	9.404	2.862	0.786
P value	0.088^{ns}	0.364^{ns}	0.082^{ns}	0.319^{ns}

P0= 1 L DW + 0 g MP, P1= 1 L DW + 16 g MP, P2= 1 L DW + 32 g MP, P3= 1 L DW + 48 g MP. SEM= standard error mean, ns= non-significant (p>0.05).

Carcass Weight (%)

The levels of *Myrmecodia pendans* in drinking water did not significantly affect the percentage of broiler carcass weight at 5 weeks of age (p > 0.05) (Table 3). Carcass weight percentages ranged from 68.93% (16g MP) to 71.20% (control).

Table 3. Percentage of Carcass Weight and Total of Intestinal Microorganism, *Salmonella* and *Escherichia Coli* of Broiler Chickens Giving *Myrmecodia pendans* Drinking Water

Treatment	The percentage of carcass weight (%)	Total of Microorganism (10^{-7} cfu/ml)	Total of *Salmonella* (10^{-7} cfu/ml)	Total of *E.Coli* (10^{-7} cfu/ml)
P0	71.20	162.3	3.8	112.7^{a}
P1	68.93	74.3	0	2.7^{b}
P2	70.53	60.0	0	0^{b}
P3	69.50	29.0	0	0^{b}
SEM total	0.438	19.636	0	17.381
P value	0.274^{ns}	0.063^{ns}	0	0.016*

P0= 1 L DW + 0 g MP, P1= 1 L DW + 16 g MP, P2= 1 L DW + 32 g MP, P3= 1 L DW + 48 g MP. SEM= standard error mean, ns= non-significant (p>0.05), *= significant (p<0.05).

[a,b] Different superscripts in the same column show significantly different (p<0.05).

Total Intestinal Microorganism, Salmonella, and Escherichia Coli (cfu/ml)

Microorganism analysis of intestinal digesta from 5-week-old broilers showed that *Myrmecodia pendans* levels approached significance for total intestinal microorganisms (p = 0.063) but had a significant effect on total intestinal *E. coli* (p < 0.05) (Table 3). Notably, no *E. coli* was detected in the intestinal digesta at 32 g MP and 48 g MP levels. Both total intestinal microorganism and *E. coli* counts decreased with increasing *Myrmecodia pendans* levels. The highest total intestinal microorganism counts were observed in the 0 MP group, followed by 16 g MP, 32 g MP, and 48 g MP. For E. coli, counts were highest in the 0 g MP group (112.7×10^{-7} cfu/ml), significantly decreasing to 2.7×10^{-7} cfu/ml at 16 g MP, and becoming undetectable (0 cfu/ml) at 32 g MP and 48 g MP. Total intestinal *Salmonella* was detected only in the control group (3.8×10^{-7} cfu/ml), precluding ANOVA analysis for this variable. No *Salmonella* was detected in treatments receiving 16 g MP up to 48 g MP.

DISCUSSIONS

Feed consumption (g/b/d)

The insignificant feed consumption results in the first week were likely due to the suboptimal development of internal digestive organs and enzyme secretion in young chickens. However, as the broilers aged, feed consumption gradually increased. Notably, the 48g MP treatment consistently led to the highest feed consumption. This positive effect may be attributed to the flavonoid and saponin content in *Myrmecodia pendans* (Lisnanti & Fitriyah, 2017). Flavonoids, as natural antioxidants, reduce free radicals, thereby optimizing metabolism and increasing feed intake. Saponins, known to enhance cell wall permeability in the intestine, improve nutrient absorption (Dai et al., 2023), further stimulating feed consumption. These findings align with the significant increases observed in feed consumption at Week 5 and for the overall mean.

Body weight gain (g/b/d)

Body weight gain is primarily influenced by feed intake and conversion efficiency. Although feed consumption increased with age, this was not accompanied by an improved feed conversion rate, consequently, *Myrmecodia pendans* drinking water had no significant effect on body weight gain. This finding is consistent with previous research by Syaifudin and Sigit (2019) which also found no significant effect of Myrmecodia pendans on broiler weight gain.

While *Myrmecodia pendans* contains antimicrobial flavonoids that could theoretically enhance nutrient absorption and feed efficiency by inhibiting intestinal pathogens ((Lisnanti & Fitriyah, 2017; Syaifudin & Sigit, 2019), other factors likely influenced the observed stagnation or decrease in body weight gain from Week 3 to Week 5. High temperatures and humidity in Manokwari, resulting in a calculated Temperature Humidity Index (THI) of 26.7 (*unpublished data*)(an inconvenient condition for birds), are potential contributing factors. Heat stress is known to reduce villi length (Gogoi et al., 2021) which is crucial for efficient nutrient absorption.

Feed conversion ratio (g/g)

The Feed Conversion Ratio (FCR), representing the efficiency of feed conversion into body weight gain, was not significantly affected by *Myrmecodia pendans* levels in drinking water, except for a near-significant effect at Week 1 ($p = 0.057$). This aligns with findings from other studies on *Myrmecodia pendans* supplementation in broiler diets (Syaifudin and Sigit, 2019) and various herbal treatments via drinking water (Sugiharto, 2021). According to Sugiharto (2021), the nature and levels of the herbs as well as the experimental conditions may be responsible. The high THI in Manokwari likely contributed to this outcome.

Water consumption (ml/b/d)

The initially lower water consumption observed in birds receiving 48g MP might be due to its astringent taste, as poultry possess gustatory receptors to discern drink taste. However, the trend of higher drinking water consumption in MP-treated broilers compared to the control at Week 5 suggests that over time, broilers can tolerate MP levels in their drinking water. The efficacy of herbal supplements is highly dependent on palatability and subsequent intake (Sugiharto, 2021). If not adequately consumed, the potential benefits of the supplements may not be fully realized.

Carcass weight (%)

Myrmecodia pendans had no significant effect on the percentage of broiler carcass weight, consistent with research by Lisnanti et al. (2018). Although the percentage was not statistically significant, raw carcass weights (1781g for 0 MP, 1808g for 16g MP, 1883g for 32g MP, and 1903g for 48g MP - *unpublished data*) suggest that MP-treated birds were 1.5% to 6.9% heavier than controls. This indicates a positive, albeit perhaps sub-significant, effect of MP, potentially requiring a longer administration period to become statistically significant.

Total intestinal microorganism, *Salmonella*, and *E.Coli*

The observed dose-dependent decrease in total intestinal microorganisms and *E. coli* with increasing *Myrmecodia pendans* levels supports findings that active substances in *Myrmecodia pendans* possess antimicrobial effects capable of inhibiting pathogenic microbes (Syaifudin and Sigit, 2019). This aligns with (Lumbangaol, 2018), who reported that higher *Myrmecodia pendans* levels in quail feed increased the inhibitory effect on intestinal *E. coli* and *Salmonella*, though their results did not show zero counts.

In this study, the detection of zero *E. coli* and *Salmonella* in some MP-treated groups (especially for 32g MP and 48g MP for *E. coli*, and 16g MP onwards for *Salmonella*) might be influenced by the detection limits of the agar plate method (typically 10-100 CFU/sample). Factors like very low initial bacterial concentrations, limited bacterial transfer, or uneven distribution during inoculation could lead to undetectable colonies. Regardless, these "zero" values strongly indicate that *Myrmecodia pendans* at minimum levels of 16g effectively inhibited the growth of *E. coli* and *Salmonella*. This indirectly suggests an improvement in the intestinal health of broilers receiving MP-supplemented drinking water.

CONCLUSION

Based on the research findings and discussions, it can be concluded that supplementing broiler drinking water with *Myrmecodia pendans* at a minimum level of 16 g per litre effectively inhibited the development of total intestinal microorganisms, *E. coli*, and *Salmonella*, and increased feed consumption. A tendency for increased water consumption was also observed. However, no significant effects were observed on body weight gain (BWG), feed conversion ratio (FCR), or carcass weight percentage. Further research is warranted to elucidate the specific mechanisms through which *Myrmecodia pendans* influences intestinal microorganisms.

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