Efficacy of *Moringa oleifera* Lam. extracts and *Pediococcus pentosaceus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum* probiotic during starter period on growth performance of male broiler chicken [version 1; peer review: 1 approved with reservations]

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Abstract

**Background:** Probiotics and medicinal plants have been used to support human and livestock health. This research aimed to evaluate the potential of *Moringa oleifera* Lam. leaf extract on the growth of *Pediococcus pentosaceus*, *Lactobacillus acidophilus* and *L. plantarum* probiotic in vitro and to prove its potential as a feed additive, as an alternative to antibiotic growth promoters (AGP) to improve nutrient intake, body weight gain, feed conversion ratio and feed efficiency in broiler chicken starter phase.

**Methods:** This study consisted of three sub studies: (1) Screening test for phytochemical compounds; (2) evaluation of several doses of *Moringa* extract (0%, 0.1%, 0.2%, 0.3% ). At the end of treatment, each colony was measured using the total plate count; (3) evaluation of probiotics and *M. oleifera* in vivo to prove growth performance on starter-phase broiler chicken. All results were analyzed by analysis of variance (ANOVA) then followed by the Duncan test.

**Results:** (1) The phytochemical screening test *M. oleifera* extract...
contained flavonoids, saponins, tannins, triterpenoids and alkaloids; (2) *M. oleifera* extract at doses of 0.1%, 0.2%, 0.3% increased the growth of bacteria *Pediococcus pentosaceus*, *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* (*p* < 0.05); (3) The use of probiotics, *M. oleifera* extract and their combination showed that body weight, body weight gain, feed conversion rate (FCR) and feed efficiency significantly differed (*p* < 0.05) between the treatments, but there was no significant difference (*p* > 0.05) between the treatments on nutrient intake (dry matter, ash, crude protein, crude fiber and organic matter) on broiler chicken starter phase.

**Conclusions:** The use of *M. oleifera* extract at doses of 0.1%, 0.2% and 0.3% increased the growth of *P. pentosaceus*, *L. acidophilus* and *L. plantarum* bacteria *in vitro* and the use of probiotics, *M. oleifera* extract and their combination by *in vivo* improved the growth performance on starter phase of broilers chicken.

**Keywords**
Moringa oleifera extract, public health, probiotic, growth performance

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Introduction

Feed comprises ingredients provided to poultry to meet necessary dietary requirements for livestock growth, development, and reproduction. Breeders administer antibiotic growth promoters (AGP) to improve production, trigger growth, and act as an antibacterial. However, the use of these growth promoters in animal production is gradually being restricted and banned in some countries. One antibiotic substitution which can be used in feed is probiotics.

Phytotherapeutics are feed additives derived from pure plant materials. Phytotherapeutics are able to control micro-organisms in the digestive tract of poultry. Phytotherapeutics are able to increase metabolic activities in the body, so these phytotherapeutics can be used as feed additives in poultry. Phytotherapeutics have proven to have several functions, such as a growth-promoting effect, antimicrobial activity, anti-inflammation activity and improving performance.

Methods

This study was conducted in the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The trial design was a complete randomized design consisting of four treatments: P0, P1, P2, and P3, with five replications.

Moringa was extracted by mixing macerated moringa leaf with ethanol 70% and 96% as the solvent. Extraction with ethanol 70% can extract oligosaccharides. The phytochemical screening test was carried out in the form of the flavonoid test, tannin test, saponin test, triterpenoid test and alkaloid test.

Bacterial cell count measurement was conducted to measure the bacterial cell count of P. pentosaceus, L. acidophilus and L. plantarum before adding Moringa leaf extracts. A bacterial suspension comparison was performed using the McFarland 0.5 scale standard. After obtaining the same turbidity, a gradual dilution was conducted up to seven times to obtain a 10^1 bacterial concentration.

A sterile test tube was filled with Aquadest 9 mL mixed with Moringa leaf extracts with the following treatments: P0: Without M. oleifera extracts; P1: 0.1% M. oleifera extracts; P2: 0.2% M. oleifera extracts; P3: 0.3% M. oleifera extracts. Furthermore, each test tube was added with 1 mL of each isolate and incubated for 24 h at 37°C, after each isolate was extracted by mixing macerated moringa leaf with ethanol 70% and 96% as the solvent. Extraction with ethanol 70% can extract oligosaccharides.
bacteria was grown in the Agar MRS media using the pour plate method and incubated for 12 and 17 h. The growth of probiotic bacteria was calculated for the colonies.

**Experimental design**

The study material consisted of probiotics from W.P. Lokapirnasari’s and A.B. Yulianto’s collections and *M. oleifera* extract in drinking water. The commercial feed contained dry matter (86.00%), crude lipid (8.00%), ash (7.00%), crude protein (20.00 %), crude fiber (5.00%) and organic matter (93.00%). This study used a completely randomized design, using 100 day-old chicks divided into five treatments and ten replications; each replication contained two chicken. The treatments in this study were as follows: T0 = control, without feed additive; T1 = 1% *P. pentosaceus*; T2 = 1% *L. acidophilus* and *L. plantarum*; T3 = 0.5% *P. pentosaceus* + 0.5% *L. acidophilus* and *L. plantarum*; T4 = 0.5% *P. pentosaceus* + 0.5% *L. acidophilus* and *L. plantarum* + 1% *M. oleifera* extracts. The observed variables included dry matter intake, organic matter intake, ash intake, crude protein intake, ether extract intake, crude fiber intake, organic matter intake, feed conversion ratio and feed efficiency. All variables were calculated with the following:

**Dry matter intake (g) = feed intake (g) × feed dry matter (%)**

**Organic matter intake (g) = feed intake (g) × feed organic matter (%) × feed dry matter (%)**

**Ash intake (g) = feed intake (g) × feed ash (%) × feed dry matter (%)**

**Crude protein intake (g) = feed intake (g) × feed crude protein (%) × feed dry matter (%)**

**Crude fat intake (g) = feed intake (g) × feed crude fat (%) × feed dry matter (%)**

**Crude fibre intake (g) = feed intake (g) × feed crude fibre (%) × feed dry matter (%)**

**Feed efficiency (%) = (body weight gain (kg)/feed intake (kg)) × 100.**

**Feed conversion ratio = feed intake (kg)/body weight gain (kg)**

**Ethical considerations**

This research has obtained approval from the Ethics Review Team Brawijaya University through a letter from the Head of the Ethics Review Team number 057-KEP-UB-2020. The treatment in this study used probiotics and herbal extracts as feed additives which are safe to use and are thought to have positive and beneficial properties and to not cause pain in experimental animals. Variable taking does not slaughter experimental animals.

**Table 1. Phytochemical screening of *M. oleifera* extract.**

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Result (ethanol 70%)</th>
<th>Result (ethanol 96%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 2. The growth of *P. pentosaceus*, *L. acidophilus* and *L. plantarum* on MRS Agar at medium temperature 37°C.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>P. pentosaceus</em> (Log CFU/mL)</th>
<th><em>L. acidophilus</em> (Log CFU/mL)</th>
<th><em>L. plantarum</em> (Log CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>0.44 ± 0.550</td>
<td>1.20 ± 7.07</td>
<td>0.86 ± 26.08</td>
</tr>
<tr>
<td>P1</td>
<td>1.34 ± 0.010</td>
<td>1.70 ± 18.71</td>
<td>1.56 ± 27.93</td>
</tr>
<tr>
<td>P2</td>
<td>2.93 ± 0.074</td>
<td>2.40 ± 39.37</td>
<td>1.98 ± 50.70</td>
</tr>
<tr>
<td>P3</td>
<td>2.52 ± 0.017</td>
<td>2.74 ± 23.02</td>
<td>2.10 ± 31.62</td>
</tr>
</tbody>
</table>

Different superscripts in the same column indicate a significant difference (p < 0.05).
Table 3. Feed intake and nutrient intake after giving probiotic, *M. oleifera* extract and a combination of probiotic and extract during the starter phase of broiler chicken.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g/chicken/day)</td>
<td>97.12 ± 11.60</td>
<td>104.63 ± 10.88</td>
<td>99.10 ± 12.73</td>
<td>103.59 ± 13.16</td>
<td>97.99 ± 8.32</td>
</tr>
<tr>
<td>Dry matter intake (g/chicken/day)</td>
<td>83.52 ± 9.97</td>
<td>89.98 ± 9.36</td>
<td>85.22 ± 10.94</td>
<td>89.08 ± 11.32</td>
<td>84.27 ± 7.15</td>
</tr>
<tr>
<td>Ash intake (g/chicken/day)</td>
<td>6.79 ± 0.81</td>
<td>7.32 ± 0.76</td>
<td>6.93 ± 0.89</td>
<td>7.25 ± 0.92</td>
<td>6.85 ± 0.58</td>
</tr>
<tr>
<td>Crude protein intake (g/chicken/day)</td>
<td>19.42 ± 2.31</td>
<td>20.92 ± 2.17</td>
<td>19.82 ± 2.54</td>
<td>20.72 ± 2.63</td>
<td>19.59 ± 1.66</td>
</tr>
<tr>
<td>Crude fat intake (g/chicken/day)</td>
<td>7.76 ± 0.92</td>
<td>8.37 ± 0.87</td>
<td>7.92 ± 1.01</td>
<td>8.28 ± 1.05</td>
<td>7.83 ± 0.66</td>
</tr>
<tr>
<td>Crude fiber intake (g/chicken/day)</td>
<td>4.85 ± 0.58</td>
<td>5.23 ± 0.54</td>
<td>4.95 ± 0.63</td>
<td>5.17 ± 0.66</td>
<td>4.89 ± 0.41</td>
</tr>
<tr>
<td>Organic matter intake (g/chicken/day)</td>
<td>90.31 ± 10.78</td>
<td>97.30 ± 10.12</td>
<td>92.16 ± 11.83</td>
<td>96.33 ± 12.24</td>
<td>91.12 ± 7.73</td>
</tr>
</tbody>
</table>

*a,b,c,d* different superscripts in the different columns indicate a significant difference (p < 0.05).

Table 4. Production performance after giving probiotic, extract *M. oleifera* and a combination of probiotic and extract during the starter phase of broiler chicken.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g/chicken)</td>
<td>801.20 ± 82.91</td>
<td>935.40 ± 96.37</td>
<td>949.10 ± 45.16</td>
<td>926.70 ± 76.96</td>
<td>899.60 ± 90.06</td>
</tr>
<tr>
<td>Average daily weight gain (g/chicken/day)</td>
<td>45.32 ± 7.62</td>
<td>65.72 ± 8.11</td>
<td>70.60 ± 9.35</td>
<td>62.52 ± 7.12</td>
<td>68.50 ± 13.33</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>2.16 ± 0.20</td>
<td>1.60 ± 0.15</td>
<td>1.41 ± 0.09</td>
<td>1.65 ± 0.04</td>
<td>1.48 ± 0.31</td>
</tr>
<tr>
<td>Feed efficiency (%)</td>
<td>46.57 ± 4.52</td>
<td>62.92 ± 5.60</td>
<td>71.30 ± 4.86</td>
<td>60.46 ± 1.40</td>
<td>70.26 ± 14.43</td>
</tr>
</tbody>
</table>

*a,b,c,d* different superscripts in the different columns indicate a significant difference (p < 0.05).
Statistical analysis
All data collected during this study were statistically analyzed under a completely randomized design. All data were tested for distribution normality and homogeneity statistics (analysis of variance). The data were analyzed using Statistical Design for Social Sciences (SPSS) v.22 (IBM Corp., NY, USA). Differences among means were detected using a one-way analysis of variance. The differences among means were determined using Duncan’s test (p < 0.05).

Results
Phytochemical screening of M. oleifera extract
*Moringa* leaf extract was tested for its chemical composition using a phytochemical screening test. At this stage, five kinds of tests were carried out: flavonoid test, tannin test, saponin test, triterpenoids test, and alkaloid test. The results of the phytochemical screening tests are presented in Table 1.

Evaluation of several doses of M. oleifera extract
The growth results of the probiotics *P. pentosaceus*, *L. acidophilus* and *L. plantarum* with several levels of *M. oleifera* extract showed significant differences between the treatments (p < 0.05) (Table 2, Figure 4).

Evaluation of probiotics, *M. oleifera* and combination of probiotics + *M. oleifera* in vivo
The result of feed intake and nutrient intake of dry matter, ash, crude protein, crude fat, crude fiber and organic matter listed in Table 3. There was no significant difference (p > 0.05) between the treatments of feed intake, dry matter intake, ash intake, crude protein intake, crude fat intake, crude fiber intake, and organic matter intake in this study.

The result of body weight, daily body weight gain, feed conversion ratio, and feed efficiency are listed in Table 4. Body weight differed significantly (p < 0.05) between treatments. The control (T0) had the lowest body weight, which was different from all treatments. The average daily weight gain differed significantly (p < 0.05) between treatments. The control (T0), which differed from all treatments, had the lowest body weight gain. The FCR results revealed a significant difference (p < 0.05) between treatments. The feed efficiency results revealed a significant difference (p < 0.05) between treatments. T0 had the lowest feed efficiency value found.

Discussion
Phytochemical screening of *M. oleifera* extract
The phytochemical screening revealed flavonoids, tannins, saponins, terpenoids, and alkaloids which could serve as a natural source of antimicrobials. The results obtained from the flavonoid test were a change in color. There was a change in color to orange (Figure 1A). The orange to red color change is caused by the formation of flavilium salts. This indicates that the sample contained flavonoids.

In the tannin test, a positive result is indicated by a change in the color of the filtrate to green or blackish blue. In this test, the color of the filtrate changed to a dark green-black color (Figure 1B), so the sample was considered test-positive for

Figure 1. A: Flavonoid test, B: Tannin test, C: Saponin.
The test for the presence of saponin was indicated by the appearance of foam. The foam indicated the presence of glycosides which have the ability to form froth in water as they hydrolyze into glucose and other compounds. In the saponin test carried out, foam formed for approximately 10 minutes; with the addition of one drop of hydrochloric acid, the foam did not disappear and the sample tested positive for saponin (Figure 1C).

The addition of concentrated sulfuric acid causes water to hydrolyze, which reacts with acetyl derivatives to form a red ring. A positive test for the presence of triterpenoids is indicated by a color change to red or purplish red. In the test conducted, the color changed to red (Figure 2), therefore the sample tested positive for triterpenoid.

The alkaloid test in this study used three reagents: Mayer’s reagent, Bouchardat’s reagent and Wagner’s reagent. The test with Mayer’s reagent showed no white precipitate (Figure 3A), so the result is negative. With the Bouchardat reagent, a precipitate appeared and the color changed to orange (Figure 3B), therefore the result is positive. With Wagner’s reagent, a brown precipitate appeared (Figure 3C). The precipitate was indicated as a potassium-alkaloid. Of the three reagents used, two of them were positive, so it can be concluded that Moringa leaves contains alkaloids.

**Evaluation of several doses of M. oleifera extract**

The lowest growth of *P. pentosaceus, L. acidophilus* and *L. plantarum* was found for the treatment without *M. oleifera* extract (P0), which was different from all treatments. The fastest growth of *P. pentosaceus* was found with the addition of 0.2% *M. oleifera* extract (P2), followed by 0.3% *M. oleifera* extract (P3) and 0.1% *M. oleifera* extract (P1). The highest growth of *L. acidophilus* was shown with the addition of 0.3% *M. oleifera* extract (P3), followed by the addition of 0.2% *M. oleifera* extract (P2).
M. oleifera extract (P2) and 0.1% M. oleifera extract (P1). The highest growth of L. plantarum was shown in the 0.3% M. oleifera extract (P3) treatment, which was not different from 0.2% M. oleifera extract (P2), while P2 was not different from 0.1% M. oleifera extract (P1) (Table 2).

Bacterial growth of P. pentosaceus, L. acidophilus and L. plantarum was significantly improved after the addition of 0.1%, 0.2%, and 0.3% M. oleifera extract. This shows that the higher the M. oleifera extract concentration, the faster the bacterial growth of P. pentosaceus, L. acidophilus and L. plantarum. Microbial count difference was determined by the fermentation duration and nutrition availability. Primary nutrition required by P. pentosaceus, L. acidophilus and L. plantarum bacteria were carbon and nitrogen sources. Bacteria use carbon sources as the energy source and produced lactic acid while nitrogen is used to generate bacterial cell biomass.

M. oleifera extract contains the monosaccharides mannose, arabinose, xylose, and the oligosaccharides raffinose and stachyose. These oligosaccharides can be used as nutrition for bacterial growth. P. pentosaceus bacteria is an amylolytic bacterium that generates amylase and can hydrolyze carbohydrates into glucose. Glucose is a carbon source for bacteria as the energy fueling their growth and lactic acid formation. P. pentosaceus bacteria use oligosaccharides and monosaccharides in Moringa leaf as the energy source for fermentation.

P. pentosaceus is also classified into the proteolytic lactic acid bacterial group. Proteolytic bacteria are those capable of generating the protease enzyme to hydrolyze polypeptides in media into amino acids. Nitrogen is a constituting component of amino acids. Thus, bacteria can use amino acids as a nitrogen source for bacterial growth and DNA/RNA synthesis. Bacterial growth of P. pentosaceus on Agar MRS media ferments carbohydrates from Moringa leaf extracts into glucose and generate lactic acid. Metabolites of lactic acid are generated from glucose fermentation through glycolysis. P. pentosaceus metabolizes glucose into produce pyruvic acid through the Embden-Meyerhof pathway, which is then reduced into lactic acid by the lactase dehydrogenase enzyme and nicotinamide adenine dinucleotide phosphate (NADP).

High bacterial growth followed the high lactic acid concentration on the fermentation media. The pH reduction and total acid level increase on the fermentation media is beneficial since it hinders pathogenic microbes. The growth of P. pentosaceus bacteria produces pediocin, a bacteriocin that inhibits the growth of pathogenic bacteria. P. pentosaceus incubated with M. oleifera extract could be used to improve the nutritional quality of rice bran.
**M. oleifera** extract’s beneficial effects as phytobiotics are considered to be due to their antioxidant properties. Phytochemical compounds such as flavonoids, saponins and tannins, exhibit antimicrobial activity.³⁰ The phytochemical screening results in this study revealed flavonoids, tannins, saponins, triterpenoids, and alkaloids.

**Evaluation of probiotics, M. oleifera and combination of probiotics + M. oleifera in vivo**

The feed intake in this study showed that there was no significant difference (p > 0.05) between the treatments with values 97.12-104.63 (g/chicken/day). The dry matter intake showed that there was no significant difference (p > 0.05) between the treatments with values 83.52-89.98 (g/chicken/day). The results of ash intake in this study showed that there was no significant difference (p > 0.05) between treatments with values 6.79-7.32 (g/chicken/day). The results of crude protein intake showed no significant difference (p > 0.05) between treatments with values 19.42-20.92 (g/chicken/day). The results of crude fat intake showed no significant difference (p > 0.05) between treatments with values 7.76-8.37 (g/chicken/day). The results of crude fiber intake showed no significant difference (p > 0.05) between treatments with values 4.85-5.23 (g/chicken/day). The organic matter intake results in this study showed no significant difference (p > 0.05) between the T0, T1, T2, T3 and T4 treatments with values 90.31 – 97.30 (g/chicken/day) (Table 3).

Body weight was significantly different (p < 0.05) between the treatments. The lowest body weight was found in the control (T0), which was different from all treatments. For T1, T2, T3 and T4, there was no significant difference (p > 0.05), with values between 899.60-949.10 (g/chicken) in starter-phase broiler chickens.

Average daily weight gain was significantly different (p <0.05) between the treatments. The lowest body weight gain was found in the control (T0), which was different from all treatments. At T1, T2, T3 and T4, there was no significant difference (p > 0.05), with values between 89.9-96.4 (g/chicken/day).

The feed conversion ratio (FCR) results showed that there was a significant difference (p < 0.05) between the treatments. FCR values for T1, T2, T3 and T4 showed an improvement compared to the control (T0). The best FCR values were found for T2 (1.41) and T4 (1.48), which differed from all treatments. The FCR values at T1 and T3 showed no significant difference (p > 0.05), namely 1.60 and 1.65.

The feed efficiency results showed that there was a significant difference (p < 0.05) between the treatments. The lowest feed efficiency value was found for T0 (46.57%). The feed efficiency values for T1, T2, T3 and T4 showed an improvement compared to the control (T0). The best feed efficiency values were shown for T2 (71.30%) and T4 (70.45%) which were different from all treatments. The value of feed efficiency at T1 (62.92%) and T3 (60.45%) showed no significant difference (p > 0.05) (Table 4).

The results of this study are in accordance with other studies that showed the administration of *Lactobacillus* probiotic can improve gut health and contributes to increased growth performance.³³ Probiotics play a role in modulating the balance of microbiota in the digestive tract and in developing intestinal health so that they affect feed consumption, digestibility, absorption of nutrients and improve feed conversion ratio and feed efficiency. The use of probiotics in poultry also plays a role in modulating the immune response, maintaining the health of the intestinal tract and influencing stress reduction.³²–³⁴

Other studies have shown that the use of *L. acidophilus* poses an obstacle to the invasion of pathogenic bacteria and modulates the immune response in vitro and in vivo. This is because *L. acidophilus* produces bacteriocin. Additionally, probiotics that are classified as lactic acid will produce acid thereby lowering the pH in the intestine. In poultry, the use of *L. acidophilus* can balance the intestinal microflora and reduce the proliferation of pathogenic bacteria through antagonism and competitive exclusion.³¹,³⁵ Protein availability can be increased by taking probiotics. Probiotics can improve the host’s health by increasing intestinal villi size and nutrient uptake.³⁶

The use of probiotics can modify the intestinal ecosystem by decreasing pH through acid production by lactic acid probiotic bacteria and modulating enzyme activation in the digestive tract.³⁷,³⁸ Several factors can affect the colonization of probiotic microbes in the intestine depending on the availability of fermented substrates (prebiotics), pH in the intestine, frequency and dosage of probiotics, genetics, age, health, stress factors and the nutritional status of the host.³⁹,⁴⁰ This relates to the ability of probiotic micro-organisms to secrete enzymes such as protease, lipase, and amylase to help digest proteins, fats, and starch so that they can increase the availability of nutrients and help increase the digestion of feed nutrients.³¹

**M. oleifera** extract contains phenolic compounds in insoluble and soluble bound forms. The bound phenolic compounds cannot be absorbed by the small intestine because they are bound by insoluble macromolecules such as cellulose,
hemicellulose, structural proteins and pectin; these compounds enter the large intestine (colon), a fermentation by the intestinal microbiota occurs, which releases bound phenolic compounds. The released phenolic compounds can decrease pH, inhibit the growth of pathogenic bacteria and modulate the development of fermentative microflora so that it can improve host health.\textsuperscript{42,43} \textit{Moringa} has no toxic effects and does not interfere with nutrient absorption.\textsuperscript{44}

In this study, giving probiotics, \textit{M. oleifera} extract and a combination of probiotics and \textit{M. oleifera} extract during the starter phase of broiler chicken increased body weight and average daily weight gain, increased feed efficiency and improved feed conversion compared with control. This is because the addition of probiotics to poultry can modulate the balance of microbiota in the intestine, increasing feed digestibility and nutrient absorption. This is also influenced by the viability requirements of probiotics, where probiotics must be able to survive and not be damaged during processing to storage. The addition of probiotics increases nutrient bioavailability and improves poultry feed conversion. Probiotics must be able to survive various stress factors during processing, storage and digestion. Several different probiotic strains can exhibit wide variations in their abilities, such as efficacy as probiotics, stability of process and functional properties at the targeted site.\textsuperscript{45}

The phytoconstituents present in \textit{M. oleifera} are flavonoids, niazirin, niazirin, proanthocyanidin, anthocyanins, kaempferol-3-O-(6\textsuperscript{”}-malonyl-glucoside), β-sitosterol, β-sitosterone, 4-hydroxymellein, octacosanic acid.\textsuperscript{46} \textit{M. oleifera} with ethanol extraction has a strong antioxidant activity.\textsuperscript{47} Hydro-alcoholic extract of Moringa leaves contain 21.1% polysaccharides (% of dry matter).\textsuperscript{44}

The addition of a combination of probiotics and \textit{M. oleifera} extract also resulted in better body weight gain, FCR and feed efficiency than the control. This is due to their proanthocyanidins content, which are condensed tannins (epicatechins and catechins) found in plants that provide protection against biotic and abiotic stresses, and act as antimicrobial, antioxidant, anti-inflammatory, vascular and cardiac activity.\textsuperscript{48–50}

The use of \textit{M. oleifera} extract improved FCR and increase feed efficiency due to the presence of secondary metabolites produced by the plant, which also play a role in giving flavor to feed, and are influenced by their ability to form complexes with macromolecules (polysaccharides and proteins) and metal ions.\textsuperscript{50,51} The use of probiotics and \textit{M. oleifera} extract for feed efficiency agrees with Nikpiran \textit{et al.} (2013)\textsuperscript{52} who found a better FCR with the addition of probiotics to broiler rations compared to controls. This is thought to be related to the availability of substrates and the presence of a better balance of microbiota in the gut, resulting in better feed efficiency.\textsuperscript{53,54} The finding of increased average daily gain and improvement of FCR in this study by administering \textit{M. oleifera} leaf extract to broiler chickens agrees with a previous study where the FCR value obtained was 1.41–1.65 and the average daily gain 62.52–70.60 g, while research by Akhouri (2013) using \textit{M. oleifera} extract in starter phase broilers produced an FCR of 1.77 and average daily gain of 52.57 g.\textsuperscript{55} The results of our study are also in line with Sjofjan's (2021) that the level of probiotics increased body weight gain, body weight, feed intake of broilers and improved FCR.\textsuperscript{56}

\textbf{Conclusions}

The phytochemical tests showed flavonoids, tannins, saponins, tripenoids, alkaloids. The use of \textit{M. oleifera} extract at doses of 0.1%, 0.2%, and 0.3% increased the growth of \textit{P. pentosaceus}, \textit{L. acidophilus} and \textit{L. plantarum} bacteria \textit{in vitro}. The use of probiotics, \textit{M. oleifera} extract and a combination of probiotics + \textit{M. oleifera} extract used \textit{in vivo} can improve the growth performance of starter-phase broilers chicken. Based on these results, the use of probiotics, \textit{M. oleifera} extract or combination of both could be used as alternative antibiotic growth promoters in the poultry industry.

\textbf{Data availability}

\textbf{Underlying data}

Figshare: Efficacy of Moringa oleifera Lam. extracts and Pediococcus pentosaceus, Lactobacillus acidophilus, Lactobacillus plantarum probiotic during starter period on growth performances of male broiler chicken, \url{https://doi.org/10.6084/m9.figshare.21802944.v3}.\textsuperscript{57}

This project contains the following underlying data:

- Widya PL excell.xlsx
- Untitled2 revised in english.sav Raw CFU.xlsx
- Raw growth isolate-revised in english.sav
Phytobiotics to improve health and production of chicken

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References


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This article addresses the role of Moringa oleifera Lam. leaf extract on the growth of Pediococcus pentosaceus, Lactobacillus acidophilus and L. plantarum probiotic as an alternative to antibiotic growth promoters (AGP) in livestock. The report of this article also proved the importance of probiotics in combating the current antimicrobial resistance organisms ravaging the livestock industrials today.

Abstract: The aim should capture the title; Summary of methods used need to be stated inside the abstract; Results should be written to flow with the methods used accordingly.

Introduction: Add Reference in last statement in paragraph three(3) and second to last paragraph; correct ‘ffectiveness’ to ‘effectiveness in last paragraph.

Method: Correct to; "Phytochemical screening test was carried out to determine the presence of Flavonoid, tannin..."

Result: Good; but you need to put all the results together; some part of your result interpretation was added to the discussion.

Discussion: Result interpretation (Fig 1A, 2, 3) should be part of results. Cut the result interpretations and add to the Results part. Try to comparing your findings with the findings of previous researchers.

Conclusion: Ok

I therefore recommend Approved with Reservations. The authors need to make the minor corrections pointed out on the reviewers report before indexing.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Microbiology/Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 26 Apr 2023

Widya Paramita Lokapirnasari

Revised from author
  - Abstract: The aim should capture the title
  - Revised: This research aimed to evaluate the efficacy of *Moringa oleifera* Lam. extracts and *Pediococcus pentosaceus, Lactobacillus acidophilus, Lactobacillus plantarum* probiotic during starter period on growth performances (nutrient intake, body weight gain, feed conversion ratio and feed efficiency) of male broiler chicken
  - Summary of methods used need to be stated inside the abstract.

Revised:
Methods: This study consisted of three sub studies: (1) Screening test for phytochemical compounds. The flavonoid test was conducted by Bate Smith-Metcalf method and Wilstatter method. The Tannin test with Denis’ reagent. The saponin test was performed by applying the Forth method. Triterpenoid tests were performed by applying the Liebermann-Bouchard method and the alkaloids test was conducted by implementing the method of Mayer, Bouchardat and Wagner. (2) in vitro evaluation of several doses of *Moringa* extract (0%, 0.1%, 0.2%, 0.3%), where each test tube was added with 1 mL of each isolate and incubated for 24 h at 37°C, after each isolate bacteria was grown in the Agar MRS media using the pour plate method and incubated for 12 h. The growth of probiotic bacteria was calculated for the colonies by using the total plate count; (3) evaluation of probiotics and *M.*
oleifera in vivo to prove growth performance (nutrient intake, body weight gain, feed conversion ratio and feed efficiency) on male broiler chicken. All results were analyzed by analysis of variance (ANOVA) then followed by the Duncan test.

- Results should be written to flow with the methods used accordingly.

Revised:

Results: The results showed (1) the phytochemical screening test M. oleifera extract contained positive of flavonoids, tannins, saponins, triterpenoids and alkaloids; (2) in vitro evaluation of M. oleifera extract at doses of 0.1%, 0.2%, 0.3% increased the growth of bacteria P. pentosaceus, L. acidophilus and L. plantarum (p<0.05); (3) The use of probiotics in vivo evaluation, M. oleifera extract and their combination showed improve growth performance. The result of body weight, body weight gain, FCR and feed efficiency significantly differed (p <0.05) between the treatments, but there was no significant difference (p > 0.05) between the treatments on nutrient intake (dry matter, ash, crude protein, crude fiber and organic matter) on broiler chicken starter phase.

- Introduction: Add Reference in last statement in paragraph three(3) and second to last paragraph;

Pediocin is a bacteriocin generated by P. pentosaceus, which, in a sufficient amount, can eliminate pathogenic bacteria such as Listeria monocytogens, Staphylococcus aureus, Escherichia coli, Vibrio alginolyticus, Pseudomonas stutzeri, and Aeromonas. P. pentosaceus bacterial growth requires nutrition with carbon, nitrogen, and mineral sources16,17,18


They allows to control micro-organism growth in the digestive tract of poultry by increasing the metabolic activities in the body, making them potential feed additives in poultry6,7.


Nutrition consumed by poultry is used for maintenance and production. Feed efficiency in broilers is influenced by the level of feed consumption and body weight gain. High feed efficiency is produced due to low feed intake followed by a high rate of body weight gain. A high level of feed efficiency can reduce livestock production costs6,7,26,27.

6. Yadav S, Jha R: Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. Journal of animal science and
Revised:
The study aimed to analyze the phytochemical properties of *Moringa* leaf extract and the effectiveness of *M. oleifera* extract with different dosages and incubation times on *in vitro* bacterial growth of *P. pentosaceus*, *L. acidophilus* and *L. plantarum* probiotic, as well as their effect on growth performance on broiler chicken starter phase *in vivo*.

- Method: Correct to; "Phytochemical screening test was carried out to determine the presence of flavonoid, tannin..."

Revised: The phytochemical screening test was carried out to determine the presence of the flavonoid, tannin, saponin, triterpenoid and alkaloid.

- Result: Good; but you need to put all the results together; some part of your result interpretation was added to the discussion.

Revised:
The results of positive flavonoid test indicated by change in color, there was a change in color to orange. The interpretation of positive tannin, indicated by the color of the filtrate changed to a dark green-black color and the presence of saponin indicated by the foam. The interpretation of flavonoid, tannin, saponin were indicate in Figure 1A, B, C. The Figure 2 showed the result of positive triterpenoid test and the figure 3 showed the result of alkaloid Test [Mayer reagent (-), Bouchardat reagent (+) and Wagner reagent (+)].

- Discussion: Result interpretation (Fig 1A, 2, 3) should be part of results. Cut the result interpretations and add to the Results part. Try to comparing your findings with the findings of previous researchers.

Revised:
These results are in accordance with the research of Kandeepan et al which showed that the results of phytochemical analysis of *M.oleifera* revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenoids. *M. oleifera* leaf extracts contain a substantial amount of phenolic chemicals, which are principally responsible for antioxidant activities. Secondary metabolites with bioactive properties. *M. oleifera* leaves continue to be excellent sources of micronutrients and phytochemicals for the creation of nutraceuticals and functional foods. Secondary metabolites extracted from *M. oleifera* leaves using chloroform, ethyl acetate, and ethanol contain bioactive substances such as such as steroids, saponins, tannins, flavonoids, terpenoids and phlobatannins.


**Competing Interests:** No competing interests were disclosed