Effect of Moringa leaves fermented by probiotics (Saccharomyces spp.) on abdominal fat and pathogenic bacteria in the intestines of ducks

Ni Wayan Siti 1, *, I Gusti Nyoman Gde Bidura 1, Eny Puspani 1 and I Wayan Sukasana 2

1 Faculty of Animal Husbandry, Udayana University, Denpasar, Bali, Indonesia.
2 Agrotechnology Study Program, Tabanan University, Tabanan, Bali, Indonesia.

World Journal of Biology Pharmacy and Health Sciences, 2023, 14(01), 288–293

Publication history: Received on 18 March 2023; revised on 26 April 2023; accepted on 29 April 2023


Abstract

The use of probiotics in feed is expected to have a synergistic effect with phytochemicals in herbal leaves (Moringa leaves), so that it can improve the growth and health of ducks. The purpose of this study was to examine the effect of the inclusion of fermented Moringa oleifera leaf powder (FM) by probiotics, on growth and pathogenic bacteria in the jejunum of ducks. A total of 240 male ducks (Anas sp.) aged 2 weeks were randomized into 4 treatment groups with an FM-added diet, namely: 2% (B); 4% FM (C), and 6% FM (D), respectively. Feed without the addition of FM as a control (A). Each treatment was repeated 6 times with 40 ducks. The results, the final body weight (BW), live weight gain (LWG) and feed efficiency (FE) in group B, C, and D ducks were significantly (P≤0.05) higher than group A ducks. In contrast, abdominal fat, the number of Coliform bacteria and E. coli in group B, C, and D ducks, significantly (P≤0.05) lower than control (A). It was concluded that giving 2-6% FM in feed can increase growth and feed efficiency, and conversely, by reducing abdominal fat and pathogenic bacteria in the intestine of ducks.

Keywords: Moringa; Probiotics; Abdominal fat; Pathogenic bacteria

1. Introduction

One effort to reduce the amount of abdominal fat and the population of pathogenic bacteria in the intestine is by adding various feed additives, because the use of antibiotics in livestock rations has been limited because their residues have side effects on consumers. Interesting to study is the use of a combination of herbal leaves and probiotics. Herbal leaves contain compounds that act as anti-oxidant and anti-carcinogenic properties, as well as improve body health [1,2,3]. However, research results show different results. This is presumably due to the different doses, types of herbs, and ways of administration [4].

It is interesting to study the efficacy of Moringa leaves (Moringa oleifera L) as a natural feed supplement. Moringa leaves contain phytochemical compounds such as: saponins, flavonoids, and tannins [2, 4,5]. Moringa leaves can also function as a prebiotic in the digestive tract of poultry. According to [6], consuming prebiotics aims to stimulate the growth of certain organisms that have the potential to become probiotics in the digestive tract of poultry. Generally, normal flora of the digestive tract, but the amount is very limited, so it needs to be multiplied by regularly consuming feed containing probiotic microbes or prebiotics. The phytochemical compounds contained in Moringa leaves have good prospects for the development of new antimicrobial drugs [2,3]. As reported by [7,8] that the inclusion of herbal leaves (Moringa, curcumin, cinnamon, and Noni) in the diet can suppress pathogenic bacteria in the intestine and cholesterol levels in chicken meat.

Probiotics can change the digestive microbial ecosystem, and also produce natural antibiotics (bacitracin, hydrogen peroxide, acidolin), thereby affecting the health and performance of the host. Probiotics have a good effect on livestock.
including improving health and efficiency in feed use [9]. Villi height and depth of intestinal jejunal crypts in poultry increased by administration of probiotics, so that the absorption of nutrients can be optimal. In addition, probiotic microbes were able to suppress the population of *E. coli* and *Clostridium perfringens* in the jejunum and cecum of pigs [10].

Suppressing the population of pathogens in the intestine and producing animal products that are low in cholesterol through the use of fermented herbal leaves with probiotics, will increase the competitiveness of livestock products. Based on this, the purpose of this study was to examine the effect of FM in the diet to promotes growth and suppresses abdominal fat and pathogenic bacteria in ducks.

2. Material and methods

2.1. Material

Materials and tools used to analyze cholesterol levels were hexane alcohol with a ratio of 3:1, chloroform, acetic anhydrous and concentrated H$_2$SO$_4$. The tools used consist of analytical scales, vortex, waterbath, steam/reaction tube, centrifuge tube, Erlenmeyer flask, spectrophotometer and micropipette. For testing the Total Plate Count (TPC) were: BPW (Buffered Pepton Water), PCA (Plate Count Agar), and 70% alcohol. Equipment: autoclave, plastic gloves, spatula or spoon, sterile plastic, petri dishes, volume pipettes, test tubes, incubators, colony counters, digital scales, and laminar air flow. Materials for testing *Coliform* and *E.coli* were: BPW, Eosin Methylene Blue Agar (EMBA), and 70% alcohol. The equipment used was: autoclave, plastic gloves, spatula or spoon, sterile plastic, petri dishes, volume pipettes, test tubes, incubators, colony counters, digital scales, and laminar air flow.

2.2. Methods

A total of 240 male ducks (*Anas sp.*) aged 2 weeks were randomized into 4 treatment groups with an FM-added diet, namely: 2% (B); 4% FM (C), and 6% FM (D), respectively. Feed without the addition of FM as a control (A). Each treatment was repeated 6 times with 40 ducks. All ducks were placed in battery colony cages made of wire and bamboo slats. The dimensions of each cage plot were: length 220 cm, width 150 cm and height 50 cm. Each plot contained 10 healthy male Bali ducks aged 2 weeks with homogeneous body weight. Places for food and drinking water are made of PVC pipes.

The ration given was a standard ration according to the needs of the ducks [11]. The nutrient content of *Moringa* leaves used in calculating the composition of nutrients in the ration is according to [5].

LWG in ducks was observed every week, namely: the difference between the final BW and the previous BW. Measuring FE every week, based on the value of the feed conversion ratio (feed intake: body weight gain). The lower the FCR value, the higher the feed efficiency. Abdominal fat was a combination of pad fat+mesenteric fat+ventricular fat. Abdominal fat percentage was the ratio between abdominal fat and body weight multiplied by 100%. Analysis of the content of *Coliform* and *E.coli* in the duck intestine were carried out at the Biology Laboratory, FMIPA, Udayana University, Denpasar by following the procedures carried out by [12].

2.3. Fermentation of Moringa Leaf Powder (*Moringa oleifera*).

*Moringa* leaves used were old *Moringa oleifera* leaves (green to yellow in color). *Moringa* leaves were dried in the sun. After drying, it was continued by grinding to become flour. *Moringa* leaf powder was then sprayed (sprayer) with a 10% sugar solution until the water content becomes 35% (balls not broken). Then mixed with the culture of *Saccharomyces spp.* as much as 1% of the total flour used. After stirring evenly, it was then put into a plastic bag which has previously been filled with small holes, then stored at room temperature for three days. After three days, the fermented *Moringa* leaf powder was ready to be mixed into the ration according to the level of treatment.

Data analysis uses one-way analysis of variance. If there is a significant difference (P<0.05) between treatments, continue with Duncan’s test.
3. Results and discussion

3.1. Growth performance and abdominal fat

Table 1 presents the results of research on the response of male Bali ducks to giving fermented Moringa leaf powder (FM) to feed. The inclusion of FM in the feed at the level of 2-6% significantly different (P≤0.05) increased the BW and LWG of ducks, as well as feed efficiency. The inclusion of FM in duck feed did not have a significant effect (P≥0.05) on feed consumption.

The average body weight of ducks in groups B, C and D were: 8.74%; 7.84%; and 6.77% significantly different (P≤0.05) higher than control (A). Likewise, the LWG of ducks of study in group B, C, and D, were: 10.23%; 8.96%; and 8.08% significantly (P≤0.05) higher than the group A. Feed efficiency for group B, C, and D ducks were: 10.97%; 9.48%; and 9.98% significantly (P≤0.05) higher than the FE in the duck A. Inclusion of 2-6% FM in the diet significantly (P≤0.05) reduced the percentage of abdominal fat in ducks. Percentage of abdominal fat in group B, C, and D ducks, were: 16.30%; 18.81%; and 22.26% significantly (P≤0.05) lower than abdominal fat in group A ducks.

Table 1 Effect of fermented Moringa leaves (FM) by Saccharomyces spp. in the ration on growth performance and abdominal fat of ducks

<table>
<thead>
<tr>
<th>Variables</th>
<th>FM leaf level in rations (%)</th>
<th>SEM2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>1372.29a3</td>
<td>1492.18b</td>
</tr>
<tr>
<td>Weight gain (g/bird)</td>
<td>1170.23a</td>
<td>1289.95b</td>
</tr>
<tr>
<td>Feed consumption (g/bird/56 days)</td>
<td>4692.62a</td>
<td>4605.12a</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>4.01a</td>
<td>3.57b</td>
</tr>
<tr>
<td>Abdominal-fat (% body weight)</td>
<td>3.19a</td>
<td>2.67b</td>
</tr>
</tbody>
</table>

Note: a,b Values with different letters in the same row are significantly different (P<0.05); SEM = standard error of the treatment means

Inclusion of 2-6% FM in the diet significantly (P≤0.05) reduced the percentage of abdominal fat in ducks. Percentage of abdominal fat in group B, C, and D ducks, were: 16.30%; 18.81%; and 22.26% significantly (P≤0.05) lower than abdominal fat in group A ducks. More details are presented in Table 1.

The results of the study found that giving 2-6% FM in the feed could increase the final body weight and weight gain of the ducks. The increase in BW and LWG of ducks was due to FM containing phytochemical compounds, such as saponins which have been shown to be efficacious in suppressing pathogenic bacteria in the intestines of ducks [13], and can increase body weight gain and feed efficiency [14]. FM apparently had no impact on feed consumption. In contrast to that reported by Hammershøj et al. [10], that feed consumption decreased with the inclusion of herbal leaves in the feed.

Fermentation is an easy way to increase nutritional value and the results are palatable [16]. According to [17] during the fermentation process, biosynthesis of vitamins and essential amino acids is formed which can increase the nutritional content, resulting in an increase in the quality and digestibility of protein. Probiotics in the digestive tract of ducks can increase nutrient digestibility, so growth and feed efficiency can be optimal. As reported by [18] that supplementing Saccharomyces spp. probiotics into feed can increase egg production and egg quality.

The inclusion of FM in the feed significantly increases the FE. The high content of beta-carotene in FM can cause differentiation of epithelial cells which can improve feed digestibility, and increase protein and calcium intake [19]. Supplementation of herbal extracts via drinking water significantly increases LWG and FE [14]. In contrast, [20] reported that the inclusion of 4% carrot leaf meal in the diet significantly reduced BW, but had no effect on feed consumption and FE in broilers.

Probiotic Saccharomyces spp. which is used in the Moringa leaf fermentation process can act as a probiotic in the digestive tract of chickens, thereby increasing enzymatic activity and absorption of nutrients [9]. These results were proven in research [18] which reported that the use of probiotics in rations can increase the digestibility of dry matter and organic matter of rations, as well as improve feed efficiency in ducks. Abdominal fat percentage decreased with the
presence of FM in the diet. Similar to the research by [21] who reported that the inclusion of herbal leaves (Morinda or Carrot) in drinking water significantly reduced abdominal fat content in ducks, and yolk cholesterol in hens [22]. Mulia et al. [23] who reported that there was a decrease in crude fat content caused by the breakdown of fat by the lipase mold enzyme which is used as energy for its growth. Puspani et al. [24] reported that probiotic supplementation in the diet significantly increased growth and reduced abdominal fat in ducks.

### 3.2. Coliform and *Eschericia coli* bacteria

Total Coliform bacteria in the duck’s jejunum decreased significantly (P<0.05) in group B, C, and D ducks, namely: 15.53%; 32.23%; and 24.08% significantly (P≤0.05) lower than group A ducks. Likewise, the number of *E. coli* bacteria experienced a significant decrease in group B, C, and D ducks, namely: 24.64%; 29.65%; and 21.07% significantly (P≤0.05) lower than the number of *E. coli* in the jejunum of control (A).

**Table 2** Effect of fermented *Moringa* leaves by *Saccharomyces spp.* in the ration on the number of *Coliform* and *E. coli* bacteria in the intestines of ducks

<table>
<thead>
<tr>
<th>Variable</th>
<th>FM leaf level in rations (%)</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total <em>Coliform</em></td>
<td>5.15 x 10⁶ ± 1.31 x 10⁶</td>
<td>4.35 x 10⁶ ± 0.19 x 10⁶</td>
</tr>
<tr>
<td>(CFU/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total <em>E. coli</em></td>
<td>8.97 x 10⁵ ± 1.05 x 10⁵</td>
<td>6.76 x 10⁵ ± 0.27 x 10⁵</td>
</tr>
<tr>
<td>(CFU/g)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: MF = fermented Moringa leaves; Values with different letters in the same row are significantly different (P<0.05); SEM = standard error of the treatment means; Cfu = cell forming unit.

The results of laboratory tests, it turns out that moringa leaf extract has strong antibacterial activity (*E. coli*), so that its use in feed can reduce the number of pathogenic bacteria. *Eschericia coli* bacteria are commensalism and can cause Colibasilosis in broilers and its presence in chicken feces is very high, so it becomes an agent of disease transmission [25,26]. Reduction of *E. coli* and *Salmonella sp.* is strongly influenced by the type and concentration of herbal extracts used [8].

The use of yeast *Saccharomyces sp.* as an inoculant, Moringa leaves can act as a probiotic in the digestive tract of ducks. According to [9], microbial balance in the digestive tract of poultry is due to the competitive exclusion mechanism between pathogenic bacteria and probiotics. As a result, the growth rate of pathogenic bacteria will be inhibited. Reported by [27] that the use of wet fermented feed products with probiotic microbes can lead to a significant increase in chicken production performance. Fermented feed products by probiotic microbes have been one of several methods that have reduced Salmonella infection in chickens [28,29]. Probiotics can eliminate *Salmonella* colonization, enhance intestinal immunity, strengthen the intestinal barrier, and suppress pathogenic bacteria in intestines [28,30,31].

### 4. Conclusion

It was concluded that the administration of 2-6% *Moringa oleifera* leaf meal which had been fermented by *Saccharomyces spp.* in feed could increase growth and feed efficiency. Conversely, it can suppress abdominal fat, coliform and *E. coli* bacteria in the intestines of ducks.

### Compliance with ethical standards

**Acknowledgments**

We would like to thank the Chancellor of Udayana University and Laboratory staff for supporting us in the sample analysis.

**Disclosure of conflict of interest**

There is no conflict of interest in this manuscript.
References


