World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

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

Article DOI: https://doi.org/10.30574/wjbphs.2023.14.1.0199

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,

*Corresponding author: Ni Wayan Siti

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 andsuppresses 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_2SO_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+mecenteric 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, Denpasarby 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 (spryer) 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 andabdominal fat of ducks

Variables	FM leaf level in rations (%)				SEM2
	0	2	4	6	
Final body weight (g)	1372.29a3	1492.18b	1479.81b	1465.25b	32.071
Weight gain (g/bird)	1170.23a	1289.95b	1275.03b	1264.82b	29.826
Feed consumption (g/bird/56 days)	4692.62a	4605.12a	4628.36a	4566.00a	54.729
Feed conversion ratio	4.01a	3.57b	3.63b	3.61b	0.105
Abdominal-fat (% body weight)	3.19a	2.67b	2.59b	2.48b	0.145

Note: a,b Values with different letters in the same row are significantly different (P<0.05); SEM = standart 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

Variable	FM leaf level in rations (%)				Normal
	0	2	4	6	
Total *Coliform* (CFU/g)	$5.15 \times 10^6 \pm 1.31 \times 10^6$a	$4.35 \times 10^6 \pm 0.19 \times 10^6$b	$3.49 \times 10^6 \pm 0.28 \times 10^6$b	$3.91 \times 10^6 \pm 0.26 \times 10^6$b	4.0×10^6 – 9.4×10^6
Total *E. coli* (CFU/g)	$8.97 \times 10^5 \pm 1.05 \times 10^5$a	$6.76 \times 10^5 \pm 0.27 \times 10^5$b	$6.31 \times 10^5 \pm 0.12 \times 10^5$ b	$7.08 \times 10^5 \pm 0.29 \times 10^5$b	10^4 - 10^5

Note: MF = fermented Moringa leaves; [a,b]Values with different letters in the same row are significantly different (P<0.05); SEM = standart 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 *Sacharomyces spp*. in feed could increase growth and feed efficiency. Conversely, it can supress 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

[1] Ashok V, Gomashe PAG, Megha PJ, Neeta AD. Antimicrobial Activity of Indian Medicinal Plants: *Moringa oleifera* and *Saraca indica*. Int. J. Curr. Microbiol. App. Sci. 2014; 3(6): 161-169.

[2] Dalukdeniya DACK, DeSilva KLSR and Rathnayaka RMUSK. Antimicrobial activity of different extracts of leaves bark and roots of *Moringa oleifera* (Lam). Int. J. Curr. Microbiol. App. Sci. 2016; 5(7): 687-691.

[3] Godinez-Oviedo A, Guemes-Vera N and Acevedo-Sandoval OA. Nutritional and Phytochemical composition of *Moringa oleifera* Lam and its potential use as nutraceutical plant: A Review. Pakistan Journal of Nutrition. 2016; 15(4): 397-405

[4] Chukwuebuka E. *Moringa oleifera* "The Mother's Best Friend". International Journal of Nutrition and Food Sciences. 2015; 4(6): 624-630

[5] Gomez GS. Supplementation of Moringa leaf flour (*Moringa oleifera* Lam) in rations to increase the production and quality of local Timor-Leste pork. Dissertation, Doctor of Animal Husbandry Study Program, Faculty of Animal Husbandry, Udayana University, Denpasar. 2019.

[6] Alimyameen. Use of Probiotics and Prebiotics in Livestock. 2011; Available from: http://alimyameen. Blogspot. Com/2011/04/penggunaan-probiotik-danprebiotik-pada-13.html. cited: 25/02/2012

[7] Cervantes-Valencia ME, Yazmin Alcala-Canto, Abdelfattah ZM Salem, Ahmed E Kholif, Adriana Margarita Ducoing-Watty, María Josefa Bernad-Bernad and Carlos Gutiérrez-Olvera.Influence of Curcumin (*Curcuma longa*) as a natural anticoccidial alternative in adult rabbits: First Results, Italian J. of Anim. Sci. 2015; 14, 3838.

[8] YunizaA. and Yuherman.Content of phytochemical compound and antibacterial activity of cinnamon leaf (*Cinnamomum burmanii*) and Noni fruit and leaf (*Morinda citrifolia* L) mixture extract to replace antibiotics. Pakistan J. Nutr. 2015;14(8), 492-497. DOI: 10.3923/pjn.2015.492.497

[9] Zurmiati ME, Mahata MH, Abbas and Wizna. The aplication of probiotic on duck. Jurnal Peternakan Indonesia. 2014; 16(2): 134-144

[10] Hanczakowska E,Świątkiewicz M, Natonek-Wiśniewska M, and Okoń K. Effect of glutamine and/or probiotic (*Enterococcus faecium*) feed supplementation on piglet performance, intestines structure, and antibacterial activity. Czech J. Anim. Sci. 2017; 62(8): 313-322

[11] NRC. Nutrient Requirements of Poultry. 9th ed. Washington, DC, USA: National Academy Press; 1994.

[12] Sudatri NW. Identification of active compounds of turmeric rhizome (*Curcuma domestic* Val.) and tamarind fruit (*Tamarindus indica* L.) and their implementation to improve health performance and broiler production. Dissertation, Doctoral Program, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia. 2021.

[13] Bukar A, Uba TI, and Oyeyi. Antimicrobical Profile of *Moringa oleifera* Lam Ekstracts against Some Food-Borne Microorganism. Bayero Journal of Pure and Applied Sciences, 2010; 3 (1): 43-48.

[14] Restiayanti L, Bidura IGNG, and Sumardani NLG. The effect of giving Moringa leaf extract (*Moringa oleifera* Lam) and garlic leaves (*Allium sativum*) through drinking water on the distribution of body fat and cholesterol levels in broilers aged 2-6 weeks. E-jurnal Peternakan Tropika, 2014; 2(3): 402-408.

[15] Hammershoj M, Kidmose U, Steenfeldt S. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture. 2010; 90: 1163–1171.

[16] Liu HN, Liu Y, Hu LL, Suo YL, Zhang L, Jin F, and Li Y. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poult. Sci. 2015; 93: 347-353.

[17] Oboh G and Elusiyan CA. Changes in the nutrient and antinutrient content of microfungi fermented cassava flour produced from low and medium cyanide variety of cassava tuber. African Journal of Biotechnology. 2007; 6(18): 2150-2157. https://doi.org/10.5897/AJB2007.000-2336

[18] Bidura IGNG, Siti NW, and Partama IBG. Effect of probiotics, *Saccharomyces spp*.Kb-5 and Kb-8, in diets on growth performance and cholesterol levels in ducks. South African Journal of Animal Science. 2019; 49(2): 219-226 http://dx.doi.org/10.4314/sajas.v49i2.2

[19] Prabowo LD, Mahfudz, and Atmomarsono U. Calcium and protein meat mass due to the use of waste product of Carrot powder in ration. Jurnal Sain Peternakan Indonesia. 2019; 14(2): 201-207. DOI: https://doi.org/10.31186/jspi.id.14.2.201-207

[20] Muzaki M.R, Mahfudz LD, and Muryani R. The effect of waste carrot product (*Daucus carrota* L) powder in the diet on broiler chickens performance. Jurnal Ilmu Ternak. 2017; 17(1): 14-20

[21] Siti NW, Wibawa AAPP, and Bidura IGNG. Identification of phytochemical compounds in *Morinda citrifolia* and implementation of *Morinda* leaf Juice via drinking water on carcass and abdominal fat in male Bali ducks (Anas sp.). WJPLS. 2021; 7(10): 167-172.

[22] Siti NW, and Bidura IGNG. Effects of carrot leaves on digestibility of feed, and cholesterol and β-carotene content of egg yolks. South African Journal of Animal Science. 2021;51(6): 786-792

[23] Mulia DS, Yulyanti E, Maryanto H and Purbomartono C. Quality improvement of Tofu waste as the raw material of fish feed with fermentation of *Rhizopus oligosporus*. Sainteks. 2015; 7(1): 10-20.

[24] Puspani E, Candrawati DPMA, and Bidura IGNG. Implementation of probiotics cellulolitic B-7 bacteria (isolation from buffalo rumen) into ration on the performance, abdominal fat and serum cholesterol of duck. Int. J. Curr. Microbiol. App. Sci. 2016; 5(11): 432-441.

[25] Roth N, Hofacre C, Zitz U, Greg Mathis F, Moder K, Doupovec B, Berghouse R, and Domig KJ. Prevalence of antibiotic-resistant *E. coli* in broilers challenged with a multi-resistant *E. coli* strain and received ampicillin, an organic acid-based feed additive or a synbiotic preparation. Poultry Science. 2019; 98(6): 2598-2607.

[26] Delannoy S, Schouler C, Souillard R, LénaYousfi, Devendec L, Lucas C, Kempf I. Diversity of *Escherichia coli* strains isolated from day-old broiler chicks, their environment and *colibacillosis* lesions in 80 flocks in France. Veterinary Microbiology. 2021; 252 https://doi.org/https://doi.org/10.1016/j.vetmic.2020.108923

[27] Bidura IGNB, Siti NW, Wibawa AAPP, Tirta Ariana IN, and Puspani E. The effect of Carot leaves meal fermented in diets on egg production, yolk cholesterol and beta-carotene in yolk of hens. Annals of the Romanian Society for Cell Biology. 2011; 25 (6): 18705-18711;

[28] Chen CY, Tsen HY, Lin CL, Yu B and Chen CS.Oral administration of a combination of select lactic acid bacteria strains to reduce the Salmonella invasion and inflammation of broiler chicks. Poultry Science. 2012; 91: 2139-2147. https://doi.org/10.3382/ps.2012-02237

[29] Wang Y, Zhou J, Wang G, Cai S, Zeng X and Qiao S. Advances in low-protein diets for swine. Journal of Animal Science and Biotechnology. 2018; 9: 60-60. https://doi.org/ 10.1186/s40104-018-0276-7.

[30] Chang CH, Teng PY, Lee TT and Yu B. The effects of the supplementation of multi-strain probiotics on intestinal microbiota, metabolites and inflammation of young SPF chickens challenged with *Salmonella enterica* subsp. enterica. Animal Science Journal. 2019; 90: 737-746. https://doi.org/10.1111/asj.13205

[31] Puspani E, Candrawati DPMA, and Bidura IGNG. Effect of yeast and multi vitamin-minerals mix in diet to stimulate the growth performance of ducks and suppress pathogenic bacteria. Annals of R.S.C.B. 2021; 25(6): 16698-1670