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Study of the use of fermented *Moringa* leaves with probiotics to increase feed digestibility and reduce pathogenic bacteria in ducklings

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Abstract

Fermentation is an easy way to increase the nutritional value of feed and the results are palatable and act as probiotics.Fermentation products by probiotics and savonin compounds in herbal leaves can suppress pathogenic bacteria. This study aims to test the effect of giving fermented *Moringa oleifera* leaf flour (FML) by probiotics on the digestibility of nutrients and pathogenic bacteria in the digetive truct of duckling. Two hundred eighty eight healthy two-week-old male Bali ducklings with homogeneous average body weight were divided into four treatment groups, six replications and each replication used 72 ducklings. The four treatments, namely a group of ducks that were given rations without the addition of FML as control (A); ration with 2% FML (B); ration with 4% FML (C); and a ration with 6% FML (D), repectively. The results showed that the digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF) in duck groups B, C and D was higher ($P<0.05$) compared to Group A (control). On the other hand, the number of *Coliform* and *Eschericia coli* bacteria in duck groups B, C, and D, was significantly ($P<0.05$) lower than duck group A. It was concluded that the administration of 2-6% fermented *Moringa oleifera* leaf flour in feed can increase the digestibility of nutrients and can suppress the population of pathogenic bacteria in the duckling's digestive tract.

Keywords: Digestibility; Ducklings; Feed efficiency; *Moringa oleifera*; Probiotics

1. Introduction

The challenge faced by poultry farmers is exposure to disease-causing microorganisms and borne pathogens in feed in the environment. Therefore, probiotics combined with phytochemical compounds in herbal leaves can be added to poultry feed as an alternative to antibiotics. One effort to increase feed digestibility and reduce the population of pathogenic bacteria is to add various feed additives, because the use of antibiotics in livestock rations has been limited, because the residues have side effects on consumers [1].

Probiotic microbes used as fermentation inoculants will synergize with the phytochemical compounds of herbal leaves in the host's digestive tract, thereby improving the health and ability of the host animal to digest feed. Herbal leaves have anti-oxidant, anti-hypertensive, antidiabetic, antiinflammatory and anticarcinogenic compounds [2,3,4]. Since there was a ban on the use of antibiotics in poultry feed, several researchers have studied the use of products that have been proven to be viable alternatives to antibiotics, such as probiotics, prebiotics, synbiotics, herbs and essential oils [5,6,7,8,9].

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Providing probiotics through feed will have an impact on the microbial ecosystem in the duck's intestine, so that it can influence the population of pathogenic bacteria and increase feed digestibility, thereby increasing the efficiency of feed use and the health of the host animal [10,11]. The formation of balanced and stable microflora in the digestive tract will have a positive and very beneficial effect on nutrient absorption and chicken health.

Probiotic microbes can increase villus height and intestinal crypt depth, which has an impact on increasing the absorption of food substances, and can suppress the population of *Eschericia coli* and *C. perfringens* bacteria in the intestine, especially in the small intestine and cecum [12]. Probiotic supplementation with single or mixed strains increases the height of the villi in the duodenum, jejunum and ileum [13], so that nutrient absorption can be optimal.

Natural feed supplement *Moringa oleifera* leaves, contains phytochemical compounds, such as saponins, flavonoids and tannins [14,15]. According to [16,17], flavonoid compounds in herbal leaves have an estrogenic effect, which can stimulate growth and increase immunity.

This research aims to examine the effect of providing *Moringa oleifera* leaf flour fermented by probiotic microbes in the diet to increase digestibility and suppress pathogenic bacteria in the intestines of ducks.

2. Material and methods

2.1. Experimental design and Animal treatments

Two hundred eighty eight healthy two-week-old male Bali ducklings with homogeneous average body weight were divided into four treatment groups, six replications and each replication used 72 ducklings. The four treatments, namely a group of ducks that were given rations without the addition of FML as control (A); ration with 2% FML (B); ration with 4% FML (C); and a ration with 6% FML (D), repectively. The ration given is a standard ration according to the needs of the ducks (Table 1). The nutrient content of *Moringa* leaves used in calculating the composition of nutrients in the ration is according to [15].

Table 1 Composition of feed ingredients and nutrients in the diet of Bali ducks aged 2-10 weeks

Feed ingredients (%)	**FML level in feed (%)**				
	0	**2**	**4**	**6**	
Yellow corn	55.8	56.4	58	59.3	
Rice bran	15	15.2	12.8	10.3	
Coconut meal	9	6.5	6.5	6.5	
Soybean meal	9.5	9.5	8.3	8	
Fish meal	10.2	9.9	9.9	9.4	
Kelor	0	2	4	6	
Nacl	0.5	0.5	0.5	0.5	
Total	100	100	100	100	
Nutrient composition				NRC [16]	
Metabolizable energy, (Kcal/kg)	2900	2900	2900	2900	2900
Crude protein, (%)	18	18	18	18	18
Ether axtract, (%)	6.92	7.08	6.81	6.62	-
Crude fibre, (%)	4.84	4.75	4.68	4.64	-
Ca, (%)	0.86	0.88	0.93	0.94	0.60
P available, (%)	0.53	0.53	0.54	0.54	0.35
Arginine, (%)	1.41	1.36	1.33	1.32	1.00

Lysine, (%)	1.18	1.17	1.15	1.12	0.90
Methionine+Cystine, (%)	0.74	0.73	0.71	0.69	0.60
Triptofan, (%)	0.20	0.20	0.21	0.21	0.20

*)Based on calculation according to Scott *et al.* [17]

2.2. Equipment

Materials and tools used in total plate count (TPC) analysis 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 were autoclave, plastic gloves, spatula or spoon, sterile plastic, petridishes, volume pipettes, test tubes, incubators, colony counters, digital scales, and laminar air flow.

Analysis of pathogenics bacteria in duckling intestines was carried out at the Biology Laboratory, Udayana University, Denpasar by following the procedures carried out by [18].

2.3. Fermentation of *Moringa* Leaf Powder with Probiotic *Saccharomyces* spp.

Moringa leaves used are old *Moringa* 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 of incubation, the fermented *Moringa* leaf powder is ready to be used in feed.

2.4. Variable measurement

Nutrient digestibility using the force feeding technique [19] was used to determine the nutrient digestibility. Excreta and feed samples were collected and put into sterile tubes and then stored in a freezer for further analysis. Triplicate sample analysis was performed to determine DM, OM, CP, and EE [20]. At the end of the research, one duck from each experimental unit was taken to be slaughtered and the jejunum organs were taken to measure the height of the villi and crypt depth following the procedure [21].

3. Results and discussion

3.1. Nutrient digestibility

In Table 2, the response of male Bali ducks to being given fermented *Moringa* leaf flour (FML) is presented. Inclusion of FML in feed at the level of 2-6%, significantly ($P<0.05$) increased the digestibility of dry matter (DM), organic matter (OM), protein digestibility (CP), and crude fiber digestibility (CF) compared to the control.

Table 2 Effect of fermented Moringa leaves by *Saccharomyces spp.* (FML) in the ration on nutrient digestibility in male Bali ducks

Variables	**FML level in diets (%)**				**SE**
	0	**2**	**4**	**6**	
Digestibility (%)					
Dry matter	71.19a	74.81b	74.43b	73.92b	0.492
Organic matter	72.26a	76.19b	76.29b	75.41b	0.371
Crude protein	76.09a	79.52b	79.34b	78.83b	0.609
Ether extract	79.17a	77.04a	77.36a	77.15a	1.016
Crude fibre	37.92a	43.22b	44.61b	44.39b	1.402

Note: [a,b]Values with different letters in the same row are significantly different ($P<0.05$); FML = *Moringa* leaves fermented by *Saccharomyces* spp.

The use of fermented *Moringa* leaf flour (FML) in feed can increase (P<0.05) feed digestibility. Dry matter digestibility in duck groups B, C, and D was 5.08%; 4.55%; and higher (P<0.05) than the duckA group. Digestibility of organic matter in duck groups B, C, and D, namely 5.44%; 5.58%; and 4.40% higher (P<0.05) than group A.

The use of fermented *Moringa* leaf flour (FML) in feed can increase (P<0.05) feed digestibility. Duck groups B, C, and D had higher dry matter digestibility (P<0.05), namely 5.08%; 4.55%; and 3.83% higher (P<0.05) than duck group A. Likewise, organic matter digestibility in duck groups B, C, and D increased by 5.44%; 5.58%; and 4.40%, respectively significantly (P<0.05) higher compared to the control duck group.

The use of fermented *Moringa* leaf flour (FML) in the diet was increased (P<0.05) the CP digestibility of the diet. CP digestibility in duck groups B, C, and D were 4.51%; 4.27%; and 3.60% higher (P<0.05) than control group. Crude fiber digestibility in group B, C, and D ducks was significantly (P<0.05) higher, namely 13.98%; 17.64%; and 17.06% compared to the group of ducks fed without FML.

Nutrient digestibility increases with the presence of FML in the feed. According to [22], fermentation is an easy way to increase nutritional value and the results are palatable. Feed ingredients that have undergone fermentation will increase the content of amino acids, vitamins and nutritional digestibility. According to [23], during the fermentation process, the biosynthesis of vitamins, essential amino acids and protein occurs, which can increase the nutritional content of feed, resulting in increased protein quality and digestibility. Apart from that, according to [24], the enzyme activity produced by micro-organisms during the fermentation process causes chemical changes in the organic substrate. Mahfudz [25] stated that the role of *Saccharomyces* sp., apart from being a crude fiber degrader, can also be used as an inoculant source of probiotics.Saferi et al. [26] reported that enzymes produced by Saccharomyces cerevisiae can break down complex carbohydrates, such as cellulose, hemicellulose, and lignin, so that they are more easily digested by digestive enzymes. Probiotics in the chicken's digestive tract can increase nutrient digestibility, so that growth and feed efficiency can be optimal.

The beta-carotene content in *Moringa* leaves is very high. Beta-carotene plays a role in the differentiation of intestinal epithelial cells, thereby increasing nutrient absorption. Increased protein absorption can increase protein synthesis and calcium mineral intake [27]. Muliani [28] reported that excessive concentrations of herbal extracts (*Curcumin*) in feed can actually reduce food absorption, due to the formation of lithocholic acid which causes damage to intestinal microvilli.

Fermentation is an easy way to increase nutritional value and the results are palatable [22]. According to [23], during the fermentation process, the biosynthesis of vitamins, essential amino acids and protein occurs which can increase the nutritional content, resulting in an increase in protein digestibility. Apart from that, according to [24], the enzyme activity produced by micro-organisms during the fermentation process causes chemical changes in the organic substrate. According to [26], enzymes produced by *Saccharomyces cerevisiae* can break down complex carbohydrates, such as cellulose, hemi sulolse, and lignin, making them easier to digest.

Probiotic *Saccharomyces spp.* used in the fermentation process of *Moringa* leaves can act as a probiotic in the digestive tract of chickens, thereby increasing enzymatic activity and absorption of food substances [10]. These results are proven in research [7] which reports that the use of probiotics in rations can increase the digestibility of dry matter and organic feed ingredients, as well as improve the nutritional quality of feed.

The reduced digestibility of ether extract, according to [29] is caused by a decrease in fat content caused by the breakdown of fat by the lipase enzyme from mold which is used as energy for its growth.

3.2. Pathogenic bacteria

The impact of using fermented *Moringa* leaves (FML) by *Saccharomyces* spp. in the ration on the population of *Coliform* and *Eschericia coli* bacteria in the intestines of duckling is presented in Table 3. The total population of *Coliform* bacteria in the jejunum experienced a significant decrease (P<0.05) in groups of ducks B, C, and D, namely 15.53%; 32.23%; and 24.08% significantly (P<0.05) lower compared to duck group A. Likewise, the population of Escherichia coli bacteria experienced a significant decrease (P<0.05) in duck groups B, C, and D, namely 24.64%; 29.65%; and 21.07% lower compared to group A. More details are presented in Table 3.

The presence of *Escherichia coli* bacteria in poultry manure is very high, so it can be an agent of disease transmission [30,31]. *Coliform* and *Eschericia coli* bacteria populations in duck intestines decreased with the presence of FML in the feed. *Moringa* leaves contain saponins, flavonoids and tannins which have antimicrobial activity [32,33]. Phenolic and

terpenoid compounds in herbal leaves can damage the cell walls of pathogenic bacteria. According to [34], phenolic compounds in herbal leaves can inhibit the growth of *S. aureus* bacteria. Terpenoid compounds can have antibacterial properties by damaging bacterial cell membranes. The inhibitory ability of herbal leaves against *Escherichia coli* and *Salmonella sp.* depends on the type of herb and the concentration of herbal extract (Yuniza and Yuherman, 2015).

Table 3 Impact of FML addition in feed on the population of *Coliform* and *Eschericia coli* bacteria in the jejunum of ducklings

Variable	FML level in diets (%)				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	$4.0 \times 10^6 - 9.4 \times 10^6$
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	$10^4 - 10^5$

[a,b] Values with different letters in the same row are significantly different ($P<0.05$); Cfu = colony forming units; FML = *Moringa* leaves fermented by *Saccharomyces spp.*

Fermented feed products using probiotic microbes have become one of several methods that have reduced *Salmonella* infections in chickens [35,36].Use of yeast *Saccharomyces sp.* as an inoculant, fermented *Moringa* leaves can act as a probiotic. The balance of microbes in the digestive tract of ducks can be maintained by probiotics, namely through a competitive exclusion mechanism between pathogenic bacteria and probiotic microbes [10], so that the presence of lactic acid bacteria in the intestine increases which can create an acidic atmosphere [37]. Probiotics can eliminate *Salmonella* colonization, improve intestinal immunity, and strengthen the intestinal barrier in chicken intestines [35,38]. Chang et al.[39] reported that feed supplementation with multi-strain probiotics improved chicken gut microbiota and induced different cytokine expression patterns in Salmonella infection.

4. Conclusion

It was concluded that supplementation of 2-6% fermented *Moringa oleifera* leaf meal with probiotics in feed can increase nutrient digestibility. On the other hand, it can significantly suppress the *Coliform* and *E. coli* population in the intestines of male Bali ducks.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All ducklings and research procedures in this study were approved by the Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

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