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Use of Cattle Rumen Fluid in Moringa Leaf Flour Fermentation (*Moringa oleifera* l.) to Improve the Performance of Laying Quail *(Coturnix coturnix japonica)*

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ABSTRACT
Moringa leaf flour is a plant that contains good nutrients and high antinutrients. One of the efforts to degrade crude fiber and detoxify antinutrients through fermentation with cattle rumen fluid. This research aims to determine the effect of fermented Moringa leaf flour with cattle rumen fluid on feed consumption, body weight gain, feed conversion female quail grower phase, and age at first egg-laying. This research was held at the Livestock laboratory of Animal Science Study Program, Faculty of Agriculture, Universitas Sumatera Utara, from February to March 2024. The design experiment used a completely randomized design with 4 treatments and 5 replications, where each plot contained 5 female quails. Treatment were consisted of T0 (100% commercial feed), T1 (96% commercial feed + 4% fermented moringa leaf flour), T2 (92% commercial feed + 8% fermented moringa leaf flour) and T3 (88% commercial feed + 12% fermented moringa leaf flour). The results showed that the effect of using fermented moringa flour with cattle rumen fluid mixed with commercial feed was able to affect the performance of laying quail, with the best treatment is T2 (92% commercial feed + 8% fermented moringa leaf flour).
Keyword: Moringa Leaf Flour, Cattle Rumen Fluid, Laying Quail, Feed Consumption, Body Weight Gain

1. Introduction

Quail is one of the most potential poultry to meet people's needs for animal protein, it is also in great demand to be developed because of faster maintenance, easy handling, and relatively small land and capital requirements. Based on quail population data from [1] in 2018 as many as 219,136 birds, then in 2019 it increased to 223,978 birds, and in 2020 it increased to 392,336 birds. The demand for quail meat and eggs is increasing every year and business opportunities for quail cultivation are very open [2].

Quail are usually fed commercial feed to meet their nutritional and production needs, but feed costs are relatively high as they account for 70-80% of total production costs. An attempt to reduce production costs without reducing quail production is to provide feed additives. One feed ingredient that has not been optimally utilized is moringa leaf meal, which has a high nutritional content, is effective and efficient in feed use.

Moringa (Moringa oleifera L.) is a leguminous plant that is easy to grow in various regions and is bred vegetatively and generatively. Moringa leaves contain antioxidants that can be used as medicine and contain primary metabolites such as protein, fat, carbohydrates, minerals, vitamins and amino acids that can be utilized as mixed feed ingredients for poultry [3]. However, this material is used in a limited way because it has a high content of antinutrients and crude fiber [4]. Research by [4] used the addition of moringa flour with ration levels of more than 3% can reduce the performance of quail aged 1-5 weeks.

Fermented feed is feed that is treated with microbial additives to break down complex physical, chemical and biological structures into simpler ones. According to [5], the fermentation process can reduce crude fiber, eliminate toxic compounds. Based on the results of the proximate test of moringa flour fermented with EM4 (Effective Microorganism-4), it produces changes in nutrient content, namely crude protein 11.87% to 24.71%, crude fiber 8.16% to 7.43%, crude fat 5.34% to 9.05%, and moisture content 11.87% to 14.96% [6].

Cattle rumen fluid obtained from slaughterhouses (RPH) can be utilized as fermentor material used in this study for fermentation of Moringa leaf flour because it contains microbes that degrade nutrients in feed. Utilization of rumen fluid has not been done optimally, but it has the potential to be processed into biomineral supplements. Research by [7], quail aged 1-6 weeks treated with probiotic rumen packaging can improve performance.

The fermentation experiment in this study is expected to reduce crude fiber and detoxify antinutrients in moringa flour. Fermented feed with cattle rumen fluid can help the process of nutrient absorption, improve intestinal microflora to improve poultry performance [8]. This research needs to be done to determine the effect of using cattle rumen fluid in moringa leaf flour as a feed additive to improve the performance of laying quail.

2. Materials and Methods

2.1. Place and Time

The research was conducted at the Animal Husbandry Study Program Stables and Research Laboratory of the Faculty of Agriculture, University of North Sumatra, for 1 month from February to March 2024.

2.2. Materials and Equipment

The materials used were quail cages, feed and water containers, digital scales with a capacity of 2 kg, tarpaulin, 5 watt incandescent lamp, thermos, filter, hygrometer, stationery, calculator, knife and cleaning tools.

The equipments used were 100 quails aged 2 weeks, quail commercial feed (P304C) from PT Charoen Pokphand Indonesia, feed additives namely moringa leaf flour and cattle rumen fluid obtained from the slaughterhouse, drinking water, brown sugar water, rodalon as a disinfectant and vitamins used, namely vitachick.

2.3. Research Methods.

This research method uses a descriptive Completely Randomized Design (CRD) of 4 treatments and 5 replicates, each replicate consisting of 5 heads. The treatments given were as follows:

T0 : 100% commercial feed (control)
T1 : 96% commercial feed + 4% fermented moringa leaf flour
T2 : 92% commercial feed + 8% fermented moringa leaf flour
T3 : 88% commercial feed + 12% fermented moringa leaf flour

Table 1. Nutritional content of feed ingredients

Feed Ingredients	Composition			
	CP (%)	Fat (%)	CF (%)	WC (%)
P304C*	19,5	3	7	13
FTDK**	24,83	10,73	6,54	14,78

Source: * PT Charoen Pokphand Indonesia
** Results of Proximate Analysis of Research Laboratory, Faculty of Agriculture, University of Sumatera Utara

Table 2. Treatment cage layout

T2R5	T2R3	T3R1	T3R2	T1R3
T2R4	T1R5	T0R1	T0R3	T0R5
T2R1	T3R5	T0R4	T1R2	T1R4
T3R3	T2R2	T1R1	T0R2	T3R4

2.4. Process of Making Moringa Leaf Flour Fermentation

2.4.1. Preparation of Cattle Rumen Fluid Starter

Put water in a thermos with a temperature of 37-41°C, then the water is discarded and replaced with 200 ml of rumen fluid at a constant temperature in the thermos. The cattle rumen fluid was filtered and homogenized with 50 ml of molasses, then stored for the fermentation process for 1 week. The rumen fluid starter is ready for use after being stored for 1 week [9].

2.4.2. Preparation of Moringa Leaf Flour

Fresh moringa leaves are taken and sorted from twigs and stems, then washed with clean water. The washed moringa leaves were dried in the sun at room temperature for 24 hours, then baked at 50°C for 24 hours, then ground with a blender until they became flour. Moringa leaf flour is sifted to make it smoother [10].

2.4.3. Making Moringa Leaf Flour Fermentation

Moringa leaf flour 1 kg was mixed with 250 ml rumen liquid starter, then packed in sterile plastic for fermentation process for 4 days anaerobically and stirred every 24 hours. After fermentation, the plastic packaging was opened to avoid mold. Moringa leaf meal fermentation product is ready to be given [11]).

2.5. Research parameters

2.5.1. Feed consumption (gram/head/week)

Feed consumption is obtained from the difference between the amount of feed given and the remaining feed measured every week divided by the number of livestock [12].

$$\text{FC} = \frac{Number\ of\ feedings - feed\ residue}{number\ of\ quails}$$

2.5.2. Body Weight Gain (gram/head/week)

Body weight gain is obtained from the difference between body weight at the end of the week and body weight in the initial week [12].

$$\text{BWG} = \text{End of week BW} - \text{Early of week BW}$$

2.5.3. Feed Conversion Ratio

Feed conversion ratio is obtained from dividing the amount of feed consumption by body weight gain in one week [12].

$$\text{FCR} = \frac{FC\ (gram/head/week)}{BWG\ (gram/head/week)}$$

2.5.4. Age at First Lay (days)

Observation of quail age in each cage unit that produces the first egg [12].

2.6. Data Analysis

The data obtained will be analyzed using Analysis of Variants (ANOVA). If the results are significantly different between treatments, they are further tested with the Duncan Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Feed Consumption

Feed consumption is the nutritional component of feed that enters the digestive tract that is utilized in the quail body [13]. Based on the results of the study, the fermentation of moringa leaf flour with cattle rumen fluid on feed consumption of female quail aged 3-6 weeks can be seen in Table 3.

Table 3. Average of feed consumption (grams/head/week)

Treatment	Replication					Mean ± SD
	1	2	3	4	5	
T0	94,47	95,93	93,00	96,20	98,13	95,55[a] ± 1,93

T1	93,27	100,73	104,47	103,40	98,93	$100,16^{b} \pm 4,43$
T2	99,93	102,93	97,33	98,27	102,47	$100,19^{b} \pm 2,48$
T3	102,27	95,87	105,33	102,40	104,00	$101,97^{b} \pm 3,64$

Description: Different superscripts indicate significant differences (P<0.05)

The highest average feed consumption of female quail during the study was found in the T3 at 101.97 grams/head/week and the lowest average was found in the T0 at 95.55 grams/head/week. The average feed consumption in Table 3 is still within the normal range and in accordance with the standard feed consumption of 3-5 weeks old quail recommended by [14] that feeding containing 20% crude protein can consume feed as much as 77.84-188.73 grams/head/week. It is suspected that fermentation of moringa flour with cattle rumen fluid can increase the palatability of quail feed. Lactic acid bacteria in the rumen, namely *Lactobacillus* sp. increase the enzyme β-glucanase in all segments of the digestive tract, inhibit the growth of pathogenic bacteria so as to increase feed consumption [15].

Based on the results of analysis of variance (Anova) showed that the treatment of moringa leaf flour fermentation had a significant effect ($P < 0.05$) on feed consumption of laying quails. It is suspected that the crude fiber content of fermented moringa flour decreases so that it can affect quail feed consumption. The results of this study are superior to the research of [4] which found that the provision of quail rations aged 1-5 weeks supplemented with moringa flour had a significantly negative effect on feed consumption. [16] stated that feed containing high crude fiber can reduce the digestibility of poultry feed. Some types of cellulotic rumen bacteria are able to produce cellulase to break down crude fiber components into glucose that can be used by poultry as an energy source [17].

Based on the results of the DMRT further test showed that T0 was significantly different ($P < 0.05$) with T1, T2 and T3. It is suspected that the starter component of cattle rumen fluid, molasses, can increase the palatability of quail feed. According to [18], molasses as a source of glucose can be digested by microbes for formation and growth and helps break down complex nutritional compounds into simpler ones. Molasses helps increase energy intake to carry out basic organ functions and process organic compounds in food through metabolic processes in the poultry body [19].

The treatment that affects the feed consumption of grower phase laying quail is T1 which is 100.16 grams/head/week. This is influenced by the starter component of cattle rumen fluid, namely molasses, which can increase the energy content of moringa leaf flour. The average feed consumption of the T1 treatment was higher than the research of [20] which found that male and female quails aged 1-5 weeks who were given basal feed with probiotic rumen packaging resulted in feed consumption of 79.45 grams/head/week. High energy availability is characterized by the use of feed ingredients containing low crude fiber so as to increase the amount of feed consumption [21].

3.2. Body Weight Gain

Body weight gain in units of time is a criterion for determining the growth rate of quail [13]. Based on the results of the study, the fermentation of moringa leaf flour with cattle rumen fluid on body weight gain of quail aged 3-6 weeks can be seen in Table 4.

Table 4. Average of body weight gain (grams/head/week)

Treatment	Replication					Mean ± SD
	1	2	3	4	5	
T0	24,33	26,67	23,67	24,00	23,00	$24,55^{A} \pm 1,93$
T1	25,00	30,00	28,00	30,00	25,33	$27,67^{AB} \pm 2,43$
T2	27,33	29,33	30,00	30,67	27,00	$28,87^{B} \pm 1,63$
T3	32,00	30,33	31,00	26,00	29,33	$29,73^{B} \pm 2,30$

Description: Different superscripts indicate very significant differences (P<0.01)

The highest average weight gain of female quail during the study was in T3 at 29.73 grams/head/week and the lowest average was in T0 at 24.55 grams/head/week. The body weight of the laying quail reached 141-156 grams/head at the age of 6 weeks in accordance with the standard quail body weight according to the statement of [6] which is 143 grams/head. It is suspected that rumen microbes can access the digestive tract to improve enzymatic performance in digestion. According to [18], *Lactobacillus* sp. bacteria synthesize proteins, essential fatty acids, phospholipids, minerals and vitamins B1, B2 and B12 in feed fermentation which function as probiotics to increase poultry body weight gain.

The results of analysis of variance (Anova) showed that the treatment had a very significant effect (P < 0.01) on body weight gain of laying quail. It is suspected that rumen microbes in the fermentation of moringa flour can reduce tannins, thus affecting quail body weight gain. The results of this study are better than the research of [4] using supplemented with moringa flour had a significantly negative effect on body weight gain of quail aged 1-5 weeks. According to [1], crude protein in moringa flour binds tannins which are protease inhibitors. Rumen microorganisms can methylate hydroxyl groups found in phenolic compounds, as well as produce extracellular polysaccharides, lipids or glycoproteins to prevent the toxic effects of tannins [19].

Based on the results of the DMRT further test showed that T0 was significantly different (P<0.01) with T2 and T3. This indicates that the treatment of moringa flour fermentation with rumen microbes is able to reduce crude fiber so as to increase quail body weight gain. According to [22], aerobic rumen cellulotic fungi, namely *Aspergillus terreus*, invade plant cell walls through lignin tissue which can stretch the bonds of lignocellulose and lignohemicellulose and then interact with rumen bacteria, namely *Acidothermus cellulolyticus* for the enzymatic degradation process in poultry digestibility. While T0 was not significantly different (P > 0.01) with T1. It is suspected that the fermented content of moringa flour is able to meet the energy needs of the quail body. The component of the fermentation process, molasses, contains energy from sucrose which allows protein substances to optimize poultry growth [19].

The treatment that affects the body weight gain of grower phase laying quail is T2 which is 28.87 grams/head/week. The average results of body weight gain in the T2 treatment were higher than the research of [7] that male and female quails aged 1-5 weeks who were given basal feed with the addition of probiotic rumen packaging resulted in body

weight gain of 19.11 grams/head/week. According to [15], *Lactobacillus* sp. bacteria can secrete the enzyme β-glucanase to stimulate the movement of mucin and microbial populations to improve intestinal microflora and increase body weight gain. Furthermore [23] stated that LAB microbes in the form of *Pediococcus pentosaceus* Strain N6 as probiotics added to feed caused an increase in feed absorption in the small intestine characterized by an increase in the length of the small intestine because these microbes have high antimicrobial activity against pathogenic bacteria.

3.3. Feed Convertion Ratio

The calculation of feed conversion is taken into consideration as an economic calculation [21]. Based on the results of the study, the fermentation of moringa flour with cattle rumen fluid on feed conversion of quail aged 3-6 weeks can be seen in Table 5.

Table 5. Average of feed convertion ratio

Treatment	Replication					Mean ± SD
	1	2	3	4	5	
T0	7,72	4,65	5,79	5,22	6,80	$6,03^{B}$ ± 1,23
T1	5,52	3,48	3,73	3,50	4,89	$4,22^{A}$ ± 0,93
T2	4,19	3,87	3,43	3,20	3,76	$3,69^{A}$ ± 0,39
T3	3,32	3,22	4,28	5,37	3,82	$4,00^{A}$ ± 0,88

Description: Different superscripts indicate very significant differences (P<0.01)

The highest average feed conversion of female quail during the study was found in T0 is 6.03 and the lowest average was found in T2 is 3.69. The average results of feed conversion in the moringa flour fermentation treatment are in accordance with the standard feed conversion of quail at the age of 3-5 weeks according to [6] statement which is 3.44-4.72. This is because rumen microbes can help the absorption of nutrients in the body and reduce excessive excreta. Rumen fluid also has a high fat and energy content that can improve feed efficiency which causes livestock to consume less feed but produce high body weight gain from fat synthesis [16].

The results of analysis of variance (Anova) showed that the treatment of the use of moringa leaf flour fermentation had a very significant effect (P < 0.01) on feed conversion of laying quails. It is suspected that rumen microbes increase enzymatic performance so that it can make efficient use of feed to reduce the value of quail feed conversion. The results of this study are superior to the research of [25] using a ration supplemented with Moringa leaf flour had a significantly negative effect on feed conversion of quail aged 1-5 weeks. According to [25], high crude fiber in moringa flour causes a decrease in nutrient absorption in the intestine so that excreta are wasted. Rumen fluid contains many cellulotic microbes that can digest the constituent components of the cell wall in the form of cellulose so that it is more easily digested by poultry [26].

Based on the results of the DMRT further test showed that the T0 treatment was significantly different (P <0.01) with T1, T2 and T3. This indicates that the treatment of fermented moringa flour with rumen microbes can reduce feed conversion by increasing feed consumption which affects body weight gain compared to the control treatment. According to [27], the low feed conversion is due to the rumen probiotic microbes successfully attached to the intestinal mucosal cells so that the absorption of nutrients in

the digestive system is perfect to produce meat. BAL in the rumen need carbohydrates as a source of energy to produce amylase enzymes and proteins as a source of nitrogen to produce protease enzymes to form cell biomass to help the absorption process of carbohydrates and amino acids in the small intestine [28].

The treatment that affected the feed conversion of grower phase laying quail was T2 with a value of 3.69. This indicates that the starter component of rumen fluid, namely molasses in the fermentation process of moringa flour, helps the efficient use of quail feed. The average results of feed conversion in the T2 treatment were lower than the research of [7] that quails aged 1-5 weeks who were given basal feed with the addition of rumen pack probiotics produced a feed conversion value of 4.09. According to [29], molasses can develop microbes to improve the balance of intestinal microflora and poultry digestive function. The presence of rumen microbes in feed containing fat, protein and carbohydrates can increase enzymatic digestive activity thereby improving intestinal health, absorption of nutrients to reduce excessive excreta [2].

3.4. Age at First Lay (days)

Sex maturity of laying quail is characterized by the age at which it initially produces its first egg [5]. Based on the results of the reserach of the fermentation of moringa leaf flour with cattle rumen fluid on the first age of quail laying eggs can be seen in Table 6.

Table 6. Average of age at first egg laying (days)

Treatment	Replication					Mean ± SD
	1	2	3	4	5	
T0	45	45	45	49	36	44,00^{ns} ± 4,29
T1	41	45	46	39	41	42,40^{ns} ± 2,97
T2	41	42	42	40	42	41,40^{ns} ± 0,89
T3	39	43	44	47	43	43,20^{ns} ± 2,86

Description: Different superscripts indicate no significant differences (P>0.05)

The fastest average age of first egg laying is found in T2 which is 41.40 days and the longest average is found in T0 is 44 days. The average results of the first age of laying eggs in the treatment of moringa leaf flour fermentation are in accordance with the standard age of the first quail laying eggs according to [13], which is 41-42 days old. This indicates that the protein content of moringa flour fermentation can help in the process of reproductive system of laying quail. In accordance with the statement of [29], feed containing protein has a direct impact on body growth and reproduction and complete amino acids affect the immune system components related to quail health. Then molasses can balance the poultry digestive microflora to produce better nutrient absorption and improve quail egg production performance.

The results of the analysis of variance (Anova) showed that the treatment had no significant effect (P>0.05) on the age of first quail egg laying. This is thought to be influenced by genes and moringa flour fermentation treatment compensates for the nutritional content of the control treatment. According to [13], as the age of quail increases, the growth rate is inhibited because protein synthesis helps to produce Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which plays a role in the formation of egg follicles. Rumen cellulotic bacteria, namely *Bacillus* spp. and *Actinomyces* spp. also play a role in the process of sexual maturity and egg production by producing cellulase enzymes

to degrade crude fiber into glucose in order to affect the level of feed consumption to assist in the reproductive development of poultry [30].

The results of the average age of first egg laying in Table 6 are in line with the research of [31] that rations supplemented with beer dregs fermented with probiotic bacterial concentrate consisting of lactic acid and propionic acid microbes produced an average age of first egg laying of 42-44 days. According to [32], Moringa leaf flour contains phytoestrogen compounds that are thought to trigger proliferation of magnum mucosal cells so that the mucosal folds look tight and compact which can help the role of endogenous estrogen in regulating hormone secretion and growth of reproductive organs. Rumen lactic acid bacteria is *Ruminococcus albus* increases systemic antioxidant ability by increasing jejenum and liver antioxidant proteins, and results in better egg production [33].

4. Conclusion

4.1. Conclusion

The results showed that the effect of using fermented moringa flour with cow rumen fluid mixed with commercial feed affected the performance of laying quail, with the best treatment found in T2 (commercial feed 92% + fermented moringa flour 8%).

4.2. Suggestion

In further research, it is better to use basal feed mixed with moringa flour fermented by rumen microbes and conduct proximate tests. The use of moringa flour fermented with rumen microbes as much as 8% in order to produce a lower feed conversion value so that it is more economical.

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