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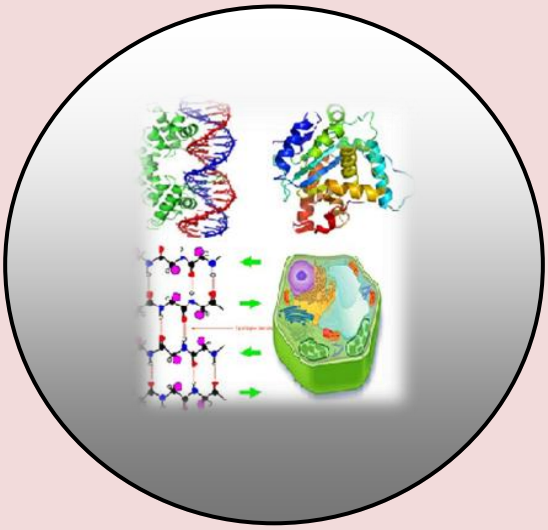

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RESEARCH PAPER

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Supplementation of *Moringa oleifera* Leaf Flour in Diet to Increase Growth Performance and Reduce Cholesterol Content in Meat of East Timor Local Pigs

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ABSTRACT

The objectives of this study was to evaluate the effects of adding differents level of Moringa oleifera leaf flour (MLF) in diet to increase growth performance and reduce the cholesterol content in meat of East Timor local pigs. A total of 40 local pigs male in growth phase were used and randomly allotted to four treatments in a completely randomized design. Pigs were allotted to four treatments. Each treatment consisted of five replications with two pigs per pen in a randomly complete block design. The experiments were carried out in the following way: T0: basal diet without the supplement of MLF; T1: supplemented with 3% of MLF; T2: supplemented with 6% of MLF and T3: supplemented with 9% of MLF. The results showed that the treated of pigs in Group T2 exhibited higher significantly different (P<0.05) on feed efficiencies than the Group T0, T1 and T3 of pigs. No significant differences (P>0.05) in the feed consumption was observed among the dietary treated groups. Dietary MLF significantly different (P<0.01) were increased cholesterol and beta-caroten content in meat of pigs. We concluded that use of 3-6% MLF in diets can reduce meat cholesterol contents, but increasing growth performance and meat beta-carotene content of local pork.
Keywords: Moringa oleifera, Cholesterol, Phytochemical, Beta-Caroten and Pigs.

INTRODUCTION

Timor-Leste's local pigs which called "*fahi timór*" are still traditionally maintained by 71.58% of the head of household, with a total local pig population of 419,169 pigs (Timor-Leste Population and Housing Census, 2015). Those, local pigs' species has enough potential to be developed, because it has been well adapted and is spread fairly evenly throughout the territory of Timor-Leste. Traditional extensive maintenance without regard to the procedure of feeding both quantity and quality and without handling reproduction of animals, this greatly affects the performance of pig production so that achieving ideal slaughtering weight takes about 2-3 years or even more. This causes high levels of local pork fat so it is less demanded by the community, especially pork consumers.

High cholesterol content in food is one of the consideration factors for consumers in consuming foods of animal products, especially pork and is considered a source of disease, including coronary heart disease (Maliandasari *et al.,* 2015), which is characterized by hardening of the arterial walls and levels high fat (hyperlipidemia) in the blood especially cholesterol (hypercholesterolemia) and pork fat contain cholesterol up to 80 mg /100 gr (Murray *et al.,* 2009). Consuming high and excessive cholesterol is one of the factors causing generative diseases (Oetoro, 2009).

Some elements of phytochemical compounds in *Moringa oleifera* which are antibacterial, and contain Beta-carotene which act as carbohydrate color active substances, and are in principle as one of the conditions for evaluating meat quality. Phytochemical compounds contained in *Moringa oleifera* leaf include: *flavonoids, saponins*, *tannins*, and several other *phenolic* compounds that have antimicrobial activity (Bukar *et al.,* 2010). Estrogen-like *flavonoids* have been shown to be able to slow down bone mass (*osteomalasia*), reduce blood cholesterol levels and increase HDL levels, whereas *saponins* are proven to be efficacious as anticancer, antimicrobial, and reduce blood cholesterol levels (Santoso *et al.,* 2002; Bidura *et al.,* 2017). Onthe other hend, Ahmed *et al.* (2017) reported that supplementation of herbal leaves in natural or fermented forms, turned out to have no effect on growth, but reduced feed consumption and increased feed efficiency compared to controls (without herbal leaf supplementation).

The use of *Moringa oleifera* leaf flour in this study was carried out as a form of feed supplement in local pig rations safely without endangering pig's health. Over the past 50 years, there has been increase in the use of antibiotics in livestock production as a growth promoter which at sub-therapeutic levels lead to the development of resistance among bacterial strains. These antibiotics leave residues in the dressed carcase which led to the banning of antibiotic growth promoter and the need for development of suitable alternatives. In Indonesia, using antibiotics as growth promoters in animal feed has been forbidden since 2018. Besides that, as information material regarding the use of active substances contained in *Moringa oleifera* leaf flour in an effort to improve production performance and quality, especially to stimulate the growth of local pigs so that they can get the ideal slaughter weight in accordance with the phase of life in a not too long time. Normal and rapid growth can produce high-quality carcasses and meats to meet the demands of animal protein needs for a consumer without concern for a level of food cholesterol which are considered to be a cause of generative diseases, and is expected to improve the level of income of local pig farmers. The purpose of this study was to determine the extent of the influence of *Moringa oleifera* leaf flour supplement in rations to increase growth performance n can reduce cholesterol content in meat of local pork.

MATERIAL AND METHODS

Animals and experimental design: A total of 40 local pigs male in growth phase were used, and their initial avarege weight 14.36±6.92 kg, and randomly allotted to four treatments in a completely randomised design, were used in a 90-d growth experiment. Pigs were allotted to four treatments. Each treatment consisted of five replications with two pigs per pen in a randomly complete block design. The experiments were carried out in the following way: Treatment T0: basal diet without the supplement of *Moringa oleifera* leaf flour; treatment T1: supplemented with 3% of *Moringa oleifera* leaf flour; treatment T2: supplemented with 6% of *Moringa oleifera* leaf flour; and treatment T3: supplemented with 9% of *Moringa oleifera* leaf flour. The animals were given water and feed *ad libitum* during the experimental period of 90 days.

Growth performance traits and diets: The compositions of the basal diets are shown in Table 1. The basal diet was formulated and manufactured before starting the trial, without the inclusion of any antibiotic growth promoters or antibiotic growth promoter alternatives. The experimental diets were formulate on the basis of yellow corn, rice bran and soybean meal, supplemented with *Moringa oleifera* leaf flour. To complement the mineral requirements has been added 2% of Mineral-mix. To get the ideal ration, it is formulated based on animals needs in accordance with NRC (1998) recommendations. Pigs had free access to experimental diets and drinking water. A combination of daylight and artificial light was used, with a 12-h light/dark cycle. The weight and feed disappearance were measured on day 0 and 90 post weaning for the calculation of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

Slaughter procedures: At the end of the experiment,the animals were fasted for 12 hours, drinking water is still provided so that the animals are not stress. The method of slaughtering is adapted to the instructions of slaughtering a pig, that is, the pig will be stunned by an electric current with a low voltage of about 80 volts. The electric current will go through the brain so that pigs will faint. Before being stunned, pigs are first doused with cold water to be clean. After the animals faint, the slaughtering process is cut using the technique of cutting the neck into large blood vessels and the heart near the anterior end of the sternum so that the blood process comes out as much as possible at the time of cutting. After collection of the samples of the *longissimus dorsi* (*baby picnic ribs*) on the left side of the carcasses was done for the meat cholesterol content and Beta-caroteneanalysis. The samples were carried refrigerated into the Laboratory in the Animal husbandry department. For the analysis of Cholesterol and Beta-carotene content were extracted according to the procedures described by Fardiaz (1992). Total cholesterol levels with commercial kits (Ezema and Eze, 2015). Cholesterol levels were analyzed according to the Lieberman-Burchard method (Lieberman and Burchard, 1980).

Measurement of Beta-carotene content: namely by entering as much as 0.10-0.50 into the centrifuge tube then adding 5 ml of acetone and 5 ml of pure petrollium ether (PE), then stirring evenly and centrifuging for 5 minutes at a speed of 3000 rpm. The supernatant was taken and stored in a test tube, while the sediment was added 5 ml of acetone and then centrifuged again until the supernatant was colorless (the supernatant becomes clear). The collected supernatant was then inserted into a separator tube and rinsed with 15 ml of distilled water and repeated three times. The rinsing water was then removed and the top of the tube (clear) was inserted into the test tube, then 1 g of $NaSO_4$ was added, then vortex. Then the clear part was taken and the PE solution was added until the volume becomes 10 ml and then read on the spectrometry absorbent (abs) at λ = 450 ηm. Total carotene (ug/100 g) = (total volume x abs x 100)/(0.2 x sample weight)

The composition of feed and nutrition of ration in accordance with the needs of pigs present on Table 1 below.

Table 1. Ingredients and calculated composition of the diets (percentage as-fed-basis).

Ingredients and nutrientes	Groups[1]			
	T0	T1	T2	T3
Yellow Corn	45.00	43.65	42.30	40.95
Rice Bran	33.00	31.95	30.90	29.85
Soybean	20.00	19.40	18.80	18.20
Moringa oleifera	*0.00*	*3.00*	*6.00*	*9.00*
Mineral-mix	2.00	2.00	2.00	2.00
Total	100	100	100	100
*Chemical composition **				
ME Kcal/kg (%DM)	2756.0	2757.2	2757.9	2758.7
CP (% DM)	15.43	15.97	16.51	17.05
CF (% DM)	5.86	6.55	7.24	7.93
EE (%DM)	9.65	9.83	10.01	10.19
Ca (%DM)	0.98	1.24	1.33	1.51
P-available (%DM)	0.38	0.56	0.61	0.72

ME: Metabolizable energy; DM: Dry Matter; CP: Crude Protein; CF: Crude fibre; EE: ether extract; Ca: Calcium; P: Phosphor.

Process of making flour *Moringa* leaves: *Moringa oleifera* leaves were dark green, thinly sliced and dried at room temperature for 1-2 days, then dried in an oven at 50°C for 24 hours. Then the *Moringa* leaves were pounded into fine powder. Laboratory analysis results show that *Moringa oleifera* leaves were contain: 89.52% DM; 33.68% CP; 29.10% CF; 15.92% EE; metabolizable energy: 2,815.40 kcal/kg; and beta-carotene: 25560 mg/100 g.

Statistical analysis: Data collected was subjected to Analysis of Variance and if significant different (P<0.05) among the treatment group was noted, they were then underwent further statistical analysis following Duncan's Multiple Range Test (Steel and Torrie, 1989).

RESULTS

The results were presented that the feed consumption, feed conversion ratio (feed consumption/ADG), ADG, meat cholesterol, and beta-caroten of meat in groups fed the experimental diets are shown in Table 2. The treated of pigs in Group T2 exhibited higher significantly different (P<0.05) on feed efficiencies than the Group T0, T1 and T3 of pigs. No significant differences (P>0.05) in the feed consumption was observed among the dietary treated groups.

The treated Group T2 exhibited higher significantly different (P<0.05) on ADG and feed efficinecies than the Group T0, T1 and T3 of pigs. Dietary *Moringa* leaf powder (*MLF*) increased beta-caroten in meat (P<0.05) of pigs. The beta-coroten content in meat of pigs in T3, T2 and T1 groups were increased: 24.35%; 30.65%; and 138.71%, repectively than group T0.

Table 2. Effect of *MLF* in diets on growth performance, cholesterol and beta-cororen of meat in local pigs.

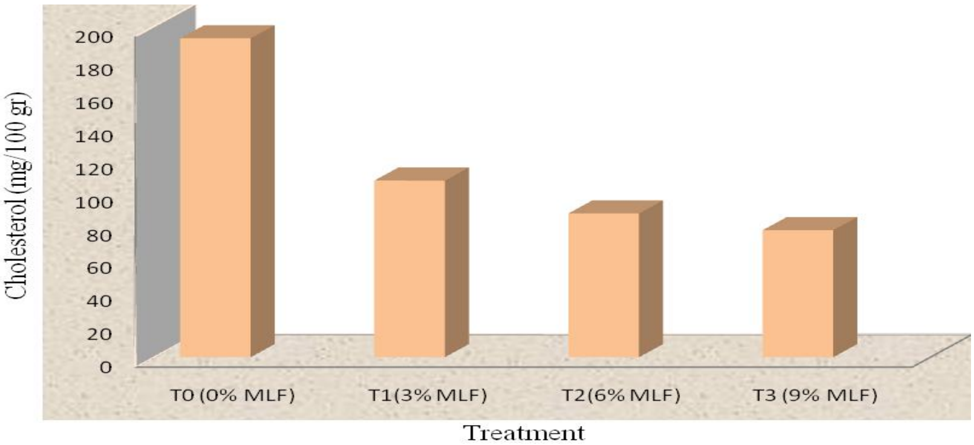
Variable	Treatments[1]			
	T0	T1	T2	T3
Feed consumption (g/d)	966.00a	858.00a	908.00a	854.00a
FCR (feed consumption/ADG)	5.47b[2]	4.86b	3.46a	4.80b
Avarage daily gains (g/d)	186.60b	172.80b	238.80a	159.20b
Meat cholesterol (mg/100g)	192.94±6.75a	106.86±6.32b	87.01±12.29c	76.85±4.96c
Beta-Caroten (ug/100g)	133.16±13.29a	165.59±5.38b	173.97±8.60b	317.87±15.91c

Note:

[1] T0: The basal diet without *MLF* (control); T1: The basal diet with 3% *MLF*; T2: The basal diet with 6% *MLF*; and T3: The basal diet with 9% *MLF*, respectively.

[2] Means with different superscripts within raw values are significantly different (*P*<0.05)

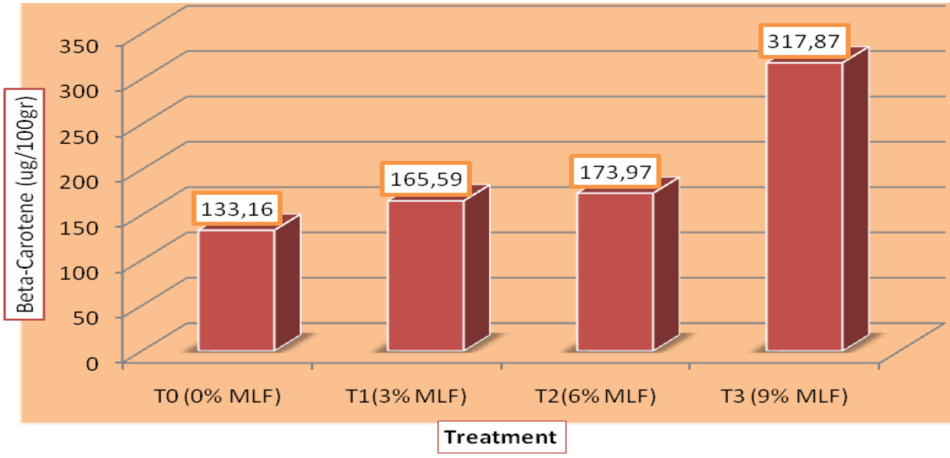
The results of the statistical analysis of local pork cholesterol content variables showed a high significantly difference (P<0.01) between the treatments observed in this study. Duncan's test for allocation of specific differences between the four treatments (Table 2), there was a difference (P<0.01) between the treatments T0 as a control with treatments T1, T2 and T3, each supplemented with *MLF* as much as 3%, 6% and 9%.



Graph. 1. East Timor Local Pork Cholesterol Content.

The results obtained during the 90 days of this study are presented in Graph 1 and Table 2 below. These results indicate that the supplementation of *MLF* in the ration can affect the level of cholesterol content in local pork. Significant decrease in pork cholesterol content occurred in treatments T2 and T3 when compared with treatment T0 and T1.

The results of statistical analysis of the Beta-carotene variable in this study showed that there was a difference in the total content of Beta-carotene in local pork, especially those that consumed ration with supplementation of *Moringa* leaf flour. These results indicate that the higher the level of supplementation of *Moringa* leaf flour in the ration can significantly increase the total beta-carotene in local pork (Table 2 and Graphic 2).



Graph 3. Beta-Carotene content in local pork that receives treatment with different levels of MLF supplementation in the basal ration.

DISCUSSION

In the current study, dietary inclusion of *MLF* to a greater ADG and feed efficiency without affecting feed consumption in growing-finishing pigs compared with control diet throughout the entire experiment, indicating that the *MLF* may had some positive effects on the animal, which is in agreement with Sanchez *et al. (*2005), that the main way of action of this active ingredient is the inhibition of microbial pathogens and endotoxins in the intestine and increased pancreatic activity, resulting in better metabolism and utilization of nutrients (Windisch *et al.,* 2008; Grashorn, 2010). According Dalukdeniya *et al*. (2016), *MLF* extract was found to be more effective in controlling gram negative bacteria tested than gram-positive bacteria. MLF are known to be very poor in anti-nutritional content and have been used in ruminant rations (Soliva *et al.,* 2005) and in other poultry or monogastrics. This result is contrary to that reported by Teteh *et al*. (2013) that the high use of MLF in feed can cause increased levels of saponin as an antinutrient which can reduce digestion and absorption of nutrients, especially lipids.

Tannins in *MLF* act as a protein denatured and prevent bacterial digestion, while *flavonoids* are water soluble compounds for antimicrobial and antiviral work (Naiborhu, 2002). Phytochemical compounds contained in *MLF* include: *flavonoids, saponins, tannins*, and several other *phenolic* compounds that have antimicrobial activity (Bukar *et al.,* 2010). Its mechanism of action in inhibiting bacteria is carried out by denaturing proteins and damaging bacterial cell membranes by dissolving fats found in cell walls (Naiborhu, 2002). It was further stated that the occurrence of cell membranes resulted in inhibited the activity and biosynthesis of specific enzymes needed in the metabolic process and this condition which ultimately caused death in bacteria. Ahmed *et al*. (2016) reported that *MLF* can also be used as a cover for wounds and laxatives and as an anti-anemia. *Moringa* leaves besides having a believed amino acid content balanced and high-efficacy secondary metabolites also contain several anti-nutritional compounds.

This means that supplementation of 6% to 9% *MLF* can cause a very good reduction in local pork cholesterol, so that it can improve the quality of pork for consumption without worrying about high and excessive cholesterol content which is one of the factors causing generative diseases (Ahmed *et al.,* 2016). Cholesterol is a fat that has the molecular formula $C_{27}H_{45}OH$. Cholesterol is not found in plant foods. Plant cells do not contain cholesterol-like ingredients, but contain lots of hytosterol ingredients (Linder, 1986).

Cholesterol is needed by the body as a precursor of bile acids, steroid hormones and is a component of plasma membrane and plasma lipoproteins (Martin *et al*., 1983). Cholesterol in the body comes from two sources, namely from food and biosynthesis. *Moringa* has long been used for, reducing cardiovascular disease, obesity and cholesterol, an important element in building and repairing cells in the body (Kurniasih, 2012). Piliang and Djojosoebagio (1990), cholesterol levels are influenced by the amount of fat in the feed and its metabolism. Whereas the ration used in this research was 9% (T3) higher in dietary fat supplemented with *MLF* which was 11.29% compared to control diet (T0) fat content was only about 10.08%. Cholesterol in pork that receives treatment T3 is lower than in pork that receive control treatment (T0). When viewed from the crude fiber content, the control ration (T0) was higher at 11.56%, while the treatment supplemented with *MLF* especially T3 with the crude fiber content was only 7.72%. According to Bidura *et al.* (2008) with the presence of crude fiber the flow rate of the ration will increase, absorption of fat in the intestine is inhibited by crude fiber so that it can be absorbed less in the body. Adibmoradi *et al*. (2016) reported that type of fibre influenced the performance and digestive traits of broilers with effects varying in accordance with the level of fibre. Rice hulls inclusion consistently improved growth performance and CP digestibility in broilers regardless of dietary inclusion level. However, barley hulls at 1.5% level improved crypt depth: villous height ratio and feed conversion ratio had an opposite effect on villous height.The results obtained in this study allegedly due to the presence of antioxidants in *MLF* causes cholesterol synthesis from the body through conversion by the liver to more bile acids, so that cholesterol absorption in the jejunum is less and meat cholesterol levels decrease. This is in accordance with the opinion of Kurniasih (2012) that Beta-sitosterol is a component in *Moringa* which can help overcome cholesterol problems. The results of laboratory analysis showed that *MLF* contain phytochemical compounds, such as flavonoids, fenolik, terpenoid, steroid, tannins, dan beta carotens (25560 mg/100 g). Phytochemical compounds of flavonoids, saponins, and tannins were able to slow down the reduction of bone mass, reduce blood cholesterol levels, and increase HDL levels, while saponins proved efficacious as anticancer, antimicrobial, and reduce blood cholesterol levels (Santoso *et al*., 2002; Bidura *et al.,* 2017). Hestera (2008), Cervantes-Valencia (2015) reported that the use of phytochemical properties in *MLF* and *Curcumin* leaves in feed can reduce cholesterol content of chicken meat. Xanthophils (such as lutein, zeaxanthin, meso-zeaxanthin, astaxanthin and canthaxanthin) are carotene oxygen derivatives (Von-Lintig, 2013). Beta carotene is a red-orange pigment found in plants and fruits, especially carrots and colorful vegetables. The name beta carotene comes from the Greek "beta" and Latin "carota" (carrot). It is the yellow/orange pigment that gives vegetables and fruits their rich colors (Arnum, 1998). These results indicate that *MLF* contains phytochemical compounds that can increase the beta-carotene of local pork, especially *terponoid* compounds which reflect that it is a derivative of *isoprene* units. Beta-carotene is an indicator of good quality meat. Beta-carotene can be trusted to reduce the risk of heart disease and cancer (Kosasih and Setiabudi, 2004). Consumption of beta-carotene as much as 50 mg per day in the diet can reduce the risk of heart disease. Repoted by Puspani *et al.* (2019) that rabbits fed with carrot leaves and supplemented with concentrate could improve performance and increased β-carotene content in rabbit meat compared than control.

CONCLUSION

The use of *Moringa oleifera* leaf flour in diets can reduce cholesterol contents in meat, but increasing growth performance and beta-carotene in meat which is one indicator for the quality of pork.

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CONFLICT OF INTEREST DECLARATION

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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