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Research Article
Effect of Supplementation of *Moringa oleifera* Leaf Powder on Reproductive Performance and Ovarian Morphometry of Pengging Ducks

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Abstract

Background and Objective: *Moringa oleifera* is one of the local food ingredients with adequate nutrient content to act as an antioxidant, hypocholesterolemia and cardiac stimulant. This research was designed to study the effect of supplementation of moringa leaf powder, on reproductive performance and ovarian morphometry of Pengging ducks. **Materials and Methods:** The study used a complete randomized design, with a total of eighty female Pengging ducks, 24 weeks old divided into five treatment groups and four replicates of each. The first group (control) was fed basal diet, without the supplement of moringa leaf powder, while the second to the fifth group was fed a basal diet with 2.5, 5, 7.5 and 10% Moringa leaf supplementation. **Results:** Age of sexual maturity was 2-3 days faster in the duck fed diet supplemented with 2.5-10% Moringa leaf powder. Concentrations of serum glucose, cholesterol and LDL were lower ($p<0.05$) in the group fed with moringa leaf supplement, although serum HDL and protein concentrations increased ($p<0.05$). MDA level of the liver ducks were significantly influenced ($p<0.05$) by moringa leaf supplement, as the diameter of F1 and F2 follicles, increased ($p<0.05$) in the groups fed diet supplemented with moringa leaf powder. **Conclusion:** The moringa leaf powder supplementation of 2.5-10%, improved the reproductive profile of the Pengging ducks which was supported by an increased in diameter of F1 and F2 follicles, without an increase in the serum cholesterol and MDA of the liver and ovary concentrations. Hence, moringa leaf powder was significant as a hepatoprotector and oviprotector, in sexually mature Pengging ducks.

Key words: Antioxidant, hepatoprotector, Indonesian local duck, low density lipoprotein, *Moringa oleifera* leaf powder, oviprotector, preovulatory follicle

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry eggs are the primary source of animal protein, widely consumed by Indonesians compared to meat and milk[1]. There has been continuous efforts to increase local Indonesian duck egg production. The increase in duck egg production however, cannot be separated from the improvement in the reproductive performance of the ducks[2]. Female ducks are declared sexually mature with their ability to produce eggs, indicated by their first lay (oviposition)[3]. Egg synthesis begins with the synthesis of yolk, which occurs in the liver for several days before reaching the age of sexual maturity. The liver actively metabolizes various nutrients, as the main substance in forming egg yolk precursor[4-5]. This egg yolk is the main source of protein, minerals, lipids and energy[6] for any poultry embryo development[7-8].

The main precursor of the egg yolk protein named vitellogenin (VTG), is synthesized in the liver under the control of estrogen, during the sexually mature period of birds. VTG is transported by the vascular system to the oocyte, where it is absorbed before deposition in the oocyte for enzymatical processing into the egg yolk protein. After the processing into the egg yolk protein, it is then stored in the form of yolk platelet or yolk globule, for the development of oocyte follicles in the ovary[9-12]. The success of female bird reproduction is greatly influenced by the functionality of reproductive organs, which plays a direct role in egg biosynthesis. Over time various species of herbal plants have been explored, in order to improve the functionality of the reproductive organs involved in egg biosynthesis. Notably, in Indonesia, moringa (*Moringa oleifera* Lam.) has been long known as a vegetable as an animal feed.

Moringa oleifera leaf possesses sufficient nutrient content, with significant antioxidant activities[13]. Numerous previous studies have shown moringa leaves, possess a wide range of biological activities such as radio-protection, analgesic, anti-pathogenic bacteria, antipyretic, antitumor, antiepileptic, anti-inflammatory, antiulcer, antihypertensive, diuretic, hypocholesterolemia, as well as cardiac and blood circulation stimulants[14]. Phytochemical analysis of *M. oleifera* leaves revealed its high deposition of potassium, phosphorous, zinc, iron, vitamin A and D, vitamin C and flavonoids[15]. The results also showed amino acid, aspartic acid, glutamic acid, alanine, valine, leucine, isoleucine, histidine, lysine, arginine, phenylalanine, tryptophan, cysteine and methionine[16]. *Moringa oleifera* leaves also contain antinutrient compounds, such as saponin, tannin, phenol, oxalate, phytate, lectin and gossypol[17-18]. One particular study that used *M. oleifera* leaves as feed supplement, showed an increase in feed intake, feed conversion, live body weight and carcass weight. The addition of up to 10% *M. oleifera* leaf powder into the basal feed by the researchers did not cause any negative effect on the performance of broiler production[19]. Similarly, *M. oleifera* leaf powder was used as a feed for laying hens, producing an increase in egg weight and egg yolk color score, although there was a notable decrease in the cholesterol content[20]. However, the use of *M. oleifera* leaf powder as a feed supplement in raising duck is still limited, hence the purpose of this research to study the overall effect of *M. oleifera* leaf powder as a feed supplement on reproductive performance and ovarian morphometry of Pengging ducks. This is indeed important, as the improved reproductive performance of Pengging ducks, in turn, can support increased production and quality of local Indonesian duck eggs.

MATERIALS AND METHODS

Local ducks, raising management and research design: Eighty female Pengging ducks, at 24 weeks with the live weight of 1400-1600 g, were used as experimental animals. The experimental ducks were obtained from Breeding Farm, Bawak, Cawas Klaten, Central Java. The experimental ducks were placed into 20 plots of cage for one-week acclimatization, with each plot of cage containing four ducks. The research cage was in the form of a litter system with the base made of rice husk, with each plot of the cage dimension at $100 \times 150 \times 70$ cm^3. Each cage partitioned by bamboo slats, were equipped with a feeding container and an infused drinking water supply system.

The duck feed and drinking water were provided *ad libitum*, as the feed used during the study were wet mash formulated with *M. oleifera* leaf powder, adapted to the nutritional requirements of laying ducks (≥24 weeks). *M. oleifera* leaf powder was purchased from Flozindo Purbalingga, Central Java. The feedstock for each concentration of the *M. oleifera* leaf powder was made per week, by mixing *M. oleifera* leaf powder into the laying duck feed concentrate. After the homogenous mixture of *M. oleifera* leaf powder and concentrate, rice bran was added and then stirred again until the homogeneous feed was obtained. The composition of the feed ingredients was presented in Table 1. The feed supplementation of *M. oleifera* leaf powder were given twice a day, in the morning (07:00 a.m) and in the afternoon (15:00 p.m) for ten weeks, starting from age of 25-35 weeks.

The experimental design used in this study was a Complete Randomized Design (CRD) with five treatments,

Table 1: Composition of Pengging duck feed ingredients for laying period (24-35 weeks)

	Feed supplemented with *Moringa oleifera* leaf powder (%)				
Feed composition (%)	0	2.5	5	7.5	10
Rice bran	60.00	60.00	60.00	60.0	60.00
Concentrate*	40.00	37.50	35.00	32.5	30.00
Moringa oleifera leaf powder	0.00	2.50	5.00	7.5	10.00
Total	100.00	100.00	100.00	100.0	100.00
Nutrient contents from laboratory analysis					
Metabolic energy (kcal kg^{-1})	2630.50	2680.90	2790.57	2840.80	2880.45
Crude protein (%)	17.22	17.56	18.30	19.56	20.08
Fat (%)	6.16	5.40	5.25	4.25	4.16
Calcium (%)	1.82	2.05	2.56	2.90	3.04
Crude fiber (%)	3.07	3.25	3.57	4.09	4.21

*Laying duck feed concentrate were obtained from the feed manufacture containing 37% crude protein, 3.5% crude fat, 6% crude fiber, 13-14% calcium, 14-18% phosphor and 40% ash

consisting of ducks group on basal feed without the addition of *M. oleifera* leaf powder (control group); group of ducks fed with basal feed with the addition of 2.5% *M. oleifera* leaf powder; group of ducks fed with basal feed with the addition of 5% *M. oleifera* leaf powder; group of ducks fed with basal feed with the addition of 7.5% *M. oleifera* leaf powder and group of ducks fed with basal feed with the addition of 10% *M. oleifera* leaf powder. Each treatment consisted of four replicate, of female ducks. All experimental animals used in this study, were raised in accordance with the protocol determined by the Department of Biology, Faculty of Science and Mathematics, Diponegoro University.

Data collection and parameter measurement: Measurements of the sexually mature duck live weight was carried out using digital weight scale (Avery weigh-tronix G220), the age of sexual maturity was determined by the number of days, for the first lay by the duck group in a plot of the cage[21]. Feed intake and feed conversion ratio (FCR: feed intake/body weight gain) were calculated every week, until the duck's onset of sexual maturity. After attainment of sexual maturity, their blood serum was collected for analysis of cholesterol, protein, glucose, LDL and HDL levels. The blood sample was taken through the brachial vein, using a 3 mL syringe (BD syringe), after which the blood-filled syringe was placed in a slanted position, for 1-2 h until serum was formed. The formed serum was then transferred into an Eppendorf tube (microtube), for centrifugation at a speed of 3000 rpm for 10 min. The individual serum formed were stored at a temperature of -20°C for protein analysis using the Biuret method[22], while cholesterol was measured using the CHOD-PAP method[23]. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were measured using the PEG method (CHOD-PAP), while the reading was carried out with a UV-Vis spectrophotometer[24],with glucose been measured using the GOD-PAP method[22].

Morphological observations on the ovaries were carried out after the experimental ducks attained sexual maturity. Each treatment group was represented by three ducks terminated by laceration of the jugular vein, esophagus and trachea. Weights of the ovary and liver were obtained by weighing, each organ using the digital scale (HWH Osaka series). The ovarian follicles were observed for the number of follicular hierarchies formed (F1-F5), F1-F3 weights, number of the small yellow follicles (SYF), number of large white follicles (LWF), F1 and F2 diameters (hierarchical follicles ≥10 mm in diameter), SYF diameter (5-10 mm), as well as LWF diameter (2-4 mm). The follicle diameter was measured by a digital caliper hardened (accuracy 0.01 mm). Three grams from each liver and ovary sample that had been weighed were used to measure the level of malondialdehyde (MDA). The liver and ovarian tissues were then chopped in cold conditions, after which the TBA method was then used to measure the level of MDA[25]. Five grams from each liver and ovary sample were also measured for total fat concentration. Afterwards, each liver and ovarian tissue was crushed before being heated using an oven at a temperature of 65-70°C for 24 h. The resulting dry tissue samples were weighed, before extraction using the Soxhlet method to obtain total liver and ovary fat concentration[26].

Statistical analysis: The data was analyzed using the one-way ANOVA, whilst the whole data analysis was done by the general linear model procedure in the SAS v9.0 program[27]. Every significant difference between treatment groups, was further analyzed by the Duncan's multiple range test. Significant difference was evaluated at the level of $p<0.05$, while the relationship between each parameter was determined from the value of the correlation coefficient (r).

RESULTS AND DISCUSSION

Reproductive performance of pengging ducks: The ducks that were supplementated with *M. oleifera* leaf powder, reached sexual mature age two to three days faster ($p<0.05$) than the control ducks (Table 2). However, live weight of the sexually matured liver and ovary, showed no significant difference ($p>0.05$), between the control duck group and duck group supplemented with *M. oleifera* leaf powder (Table 2). The feed intake in the duck group supplemented with *M. oleifera* leaf powder at concentrations of 7.5 and 10% decreased ($p<0.05$) by 2.95 and 11.66% respectively, than the control ducks. However, FCR did not show any significant ($p>0.05$) difference between the control ducks and the other duck groups supplemented with *M. oleifera* leaf powder (Table 2). Although, the FCR did not show a significant difference, supplementation of 10% *M. oleifera* leaf powder resulted in a lower FCR. This result indicated that diet containing *M. oleifera* leaf powder, can effectively be absorbed and metabolized, impacting on the increase of first laying live weight. Lower feed intake in the duck group supplemented with 10% *M. oleifera* leaf powder was thought to be related to adequate nutritional components, as regards high metabolic energy and protein contents (Table 1). Feed intake and FCR in this research, were however consistent with the results reported by Onu and Aniebo[28] and Sarker *et al.*[29]. Metabolic energy and protein in the feed was introduced daily as nutritional supplementations, to support reproductive processes, increase of live weight in order to hasten ideal sexual maturation of the live weight. This was targeted at achieving normal sexual maturity age eventually, as ducks often attain sexual maturity at specific live weight. Concomitant to these results, Hocking[30], Renema and Robinson[31] stated that live weight determines the sexual maturity age of laying birds. Similarly, the study conducted by Prasetyo and Susanti[32], also reported that live weight at first laying was recorded at 1663.5 and 1662,0 g for Mojosari and Tegal ducks respectively.

Noticeably with the data, age of sexual maturation was faster in all groups of ducks supplementated with *M. oleifera* leaf powder, indicating a faster rate of ovarian follicle development than the control ducks. Clearly, the increased rate of egg yolk precursor deposition from the liver into the ovarian follicles, also influenced the development of follicles forming the preovulatory hierarchy. The yolk precursor was formed in the liver cells through the process of vitellogenesis as demonstrated by Deeley *et al.*[9]. Although, vitellogenesis was massive in the hepatocytes, the weight of the liver and ovary in the experimental ducks did not increase. Hence, during the process of vitellogenesis, the function of existing liver cells was optimized, alongside reduction in the damage of the liver cells, by the bioactive antioxidant component of the *M. oleifera* leaf powder. The antioxidant role of *M. oleifera* leaf powder in this current study may also be related to the presence of β-carotene by 123.04 mg kg^{-1} of the dried leaf powder.

The increased yolk deposition into the developed ovarian follicles, contributed to the increased size of follicles, for the potential selection of white and yellow follicles to form the follicular hierarchy. The formation of the preovulatory hierarchy in turn, determined the time of ovulation. Furthermore, the increased number and size of blood vessels, also contributed to the ordered hierarchy of preovulatory follicles. As each follicle progressed through the final development, there was an accommodated delivery of progressively greater amounts of yolk as reported by Johnson[33]. The sexual maturity age of the Pengging ducks from this study was parallel with the onset of first laying of Tegal and Mojosari ducks[32].

Supplementation of the *Moringa oleifera* leaf powder into the feed significantly ($p<0.05$) reduced serum glucose, cholesterol and low-density lipoprotein (LDL) concentrations. However, there was a significant ($p<0.05$) increase in high-density lipoprotein (HDL) and protein concentrations (Table 3). The Serum glucose concentrations were lower by 5.88, 9.46, 12.51 and 19.59% in the duck group supplemented with

Table 2: Age of sexual maturity, live weight, liver and ovary weight, feed intake and FCR of sexually mature Pengging ducks supplemented with *Moringa oleifera* leaf powder

	Treatments of *Moringa oleifera* leaf powder (%)				
Items	0	2.5	5	7.5	10
Age of sexual maturity (days)	208.00±1.00[a]	205.00±0.58[b]	205.00±0.58[b]	205.00±0.58[b]	206.00±1.15[b]
Live weight of sexual maturity (g)	1558.00±10.05	1730.00±8.07	1625.00±9.12	1630.00±5.12	1667.00±6.04
Liver weight (%)	2.69±0.42	3.47±0.57	3.39±1.09	3.44±0,45	3.63±0.78
Ovarian weight (%)	2.18±0.48	3.66±1.55	3.08±1.63	3.36±1.68	2.96±1.34
Feed intake (g group^{-1} day^{-1})	543.00±3.46[a]	539.00±3.61[a]	538.66±1.15[a]	527.33±3.79[b]	479.67±5.23[c]
FCR (g g^{-1})	5.11±1.89	4.49±2.30	6.26±1.95	4.57±0.94	3.89±1.44

[a-c]Different superscripts on the same row shows significantly different ($p<0.05$). Values are presented as Mean±SD

Table 3: The chemical concentration of serum, total fat of liver and ovary, as well as MDA of liver and ovary of sexually mature female Pengging ducks supplemented with *Moringa oleifera* leaf powder

	Treatments of *Moringa oleifera* leaf powder (%)				
Items	0	2.5	5	7.5	10
Glucose (mg dL^{-1})	219.35±2.26^{a}	206.45±3.24^{b}	198.60±1.87^{bc}	191.90±0.41^{c}	176.39±1.01^{d}
Protein (mg dL^{-1})	2.72±0.14^{d}	2.92±0.12^{c}	3.04±0.07^{bc}	3.19±0.07^{ab}	3.33±0.08^{a}
Cholesterol (mg dL^{-1})	185.44±4.13^{a}	177.58±2.49^{b}	172.78±0.43^{c}	171.49±1.31^{c}	166.84±0.80^{d}
LDL (mg dL^{-1})	162.57±0.81^{a}	159.58±0.79^{b}	152.11±1.34^{c}	146.96±0.98^{d}	139.76±0.75^{e}
HDL (mg dL^{-1})	54.77±1.40^{d}	61.90±1.47^{c}	70.84±2.51^{b}	74.63±2.11^{a}	76.58±1.04^{a}
Total liver fat (%)	21.92±0.13^{a}	20.07±0.35^{b}	19.55±0.07^{c}	19.29±0.13^{c}	18.67±0.21^{d}
Total ovarian fat (%)	28.22±0.12^{a}	27.43±0.25^{b}	27.07±0.18^{bc}	26.92±0.35^{c}	26.27±0.35^{d}
Liver MDA (mg kg^{-1})	0.16±0.01^{a}	0.14±0.04^{b}	0.13±0.03^{b}	0.12±0.02^{c}	0.09±0.01^{d}
Ovarian MDA (mg kg^{-1})	0.11±0.01^{a}	0.09±0.01^{b}	0.09±0.03^{b}	0.08±0.01^{b}	0.06±0.02^{c}

$^{a-d}$Different superscripts on the same row shows significantly different ($p<0.05$). Values are presented as Mean±SD

M. oleifera leaf powder of 2.5, 5, 7.5 and 10% respectively, compared to the control ducks. The hypoglycemic action of *M. oleifera* leaf powder indicated that the presence of flavonoids can stimulate insulin secretion, similarly regulating glucose absorption and metabolism. Alternatively, glucose was used quickly as a source of energy in sexually mature ducks, to synthesize yolk and form follicular hierarchies. On the other hand, Abdull Razis *et al.*[34] suggested that the hypoglycemic activity of *M. oleifera* powder was caused by the presence of N-Benzyl thiocarbamate, N-benzyl carbamate and benzyl nitrile, which stimulates the release of insulin through β-pancreatic cells in rodents. Meanwhile, Ndong *et al.*[35] and Bienvenu *et al.*[36] also reported that *M. oleifera* leaf powder flavonoids were involved in controlling the activity of enzymes, that affect glucose metabolism in the liver.

The serum protein concentration in experimental ducks supplemented with *M. oleifera* leaf powder of 7.5 and 10% increased significantly ($p<0.05$) by 17.28 and 22.43%, respectively, compared to the control duck group. Similarly, there was an increase in protein concentration in the duck group supplemented with *M. oleifera* leaf powder, indicating that protein contained in the feed can be absorbed more effectively. The high serum protein concentration, was also a reflection of the increase protein content in the feed mixed with *M. oleifera* leaf powder. Protein from the feed was absorbed in the intestine, before its uptake by the liver, for its utilization as the egg precursor. Similarly, several studies have also shown that *M. oleifera* leaves are the ideal animal feed supplement, as they contain 56.14% digestible proteins[35], with essential amino acids, such as methionine, cysteine, tryptophan, lysine, arginine and histidine[37-38].

Cholesterol concentration in the experimental ducks supplemented with *M. oleifera* leaf powder of 5 and 10%, was lower significantly ($p<0.05$) by 6.83 and 10.03% respectively, than the control ducks. This low cholesterol concentration level is in line with the decrease in serum LDL concentration. Additionally, the total fat content in the liver and ovary (Table 3), was also lower in all the duck groups supplemented with *M. oleifera* leaf powder, when compared to the control ducks. This result indicated that the content of polyphenols in *M. oleifera* leaf powder can either inhibit lipid and cholesterol uptake by the gastrointestinal tract or increase cholesterol elimination by the liver. These results agrees with the research of Ghasi *et al.*[39] and Mehta *et al.*[40], who showed that hypocholesterolemic activity in *M. oleifera* leaf powder was significantly associated with increased fecal cholesterol excretion.

Furthermore, El-Gindy *et al.*[41] reported that the bioactive component of the *M. oleifera* leaf powder, inhibits the catalysis rate of HMG CoA reductase enzyme in cholesterol biosynthesis, reducing absorption of cholesterol contained in the feed. Meanwhile, Sarker *et al.*[29], Ndong *et al.*[35], Dei and De[42] and Sheikh *et al.*[43], stated that quercetin glycosides and other flavonoids contained in *M. oleifera* leaf powder, have the potential to be used as an hypocholesterolemic. This is also supported through the fiber in *M. oleifera* leaf powder, which could bind with the fat, acting as potential inhibitors of lipid absorption. Other research reports however showed that the high carotenoid content in *M. oleifera* leaves, can be converted to vitamin A in the liver and intestine, along with vitamins C and E, phytosterol and selenium. This conversion is vital to curbing lipoprotein oxidation especially LDL, resulting to lower serum LDL concentrations[39].

The present study showed that the HDL cholesterol concentration was significantly higher ($p<0.05$) by 36.26 and 39.82% in the ducks supplemented with *M. oleifera* leaf powder of 7.5 and 10% respectively, compared to control ducks (Table 3). This result indicates HDL facilitates the re-transport of cholesterol from extra hepatic tissues, to the liver for elimination or reuse by the body. HDL also plays a role in modifying LDL cholesterol, by making it difficult to oxidize. Although, the mechanism for increasing HDL levels was

unclear, there was a tendency that *M. oleifera* leaf powder flavonoids play an important role in HDL metabolism. Recently, Bienvenu *et al*.[36] in his study, demonstrated that *M. oleifera* leaf powder can increase HDL concentration, in the early stage of type 2 diabetes mellitus patients. Moreover, El-Gindy *et al*.[41] also reported similar results, stating that supplementation of *M. oleifera* leaves significantly stimulated, increase in HDL cholesterol of rabbits under moderate heat stress condition.

Furthermore, supplementation of *M. oleifera* leaf powder into the duck feed, significantly ($p<0.05$) affected the concentrations of the liver and ovarian MDA (Table 3). The addition of 10% *M. oleifera* leaf powder resulted in lower liver MDA concentrations by 43.75% compared to the control ducks. Meanwhile, the ovary MDA concentration was also lower by 45.45% in the 10% *M. oleifera* leaf powder supplement, compared to the control ducks. Similarly, the liver cells roles increased with the onset of the birds sexual maturation, as the liver is responsible for yolk synthesis. The resulting addition of the moringa leaf powder with the basal feed optimized, vitellogenesis in the hepatocyte cells, similar to the yolk deposition in the ovary, along with the resulting oxidative damage. Low MDA concentration in the liver and ovary was evidence, that β-carotene flavonoids or other bioactive components in *M. oleifera* leaf powder have the role of hepatoprotector in protecting hepatocytes and oviprotector in protecting ovary follicles. These results indicate the use of *M. oleifera* leaf powder as a feed supplement, which can prevent cell damage due to free radicals and reactive oxygen species (ROS), which are the mediators of oxidative process. The inability of cells to reduce ROS or counteract free radicals, can lead to oxidative stress or cell damage. These findings support the research conducted by Anwar *et al*.[16], El-Gindy *et al*.[41], Goyal *et al*.[44] and Lu *et al*.[45]. Moreover, Gopalakrishnan *et al*.[46] also suggested that moringa flavonoids such as quercetin and phenolic, contained in moringa can cleanse the ROS released by mitochondria. Similarly, Abdull Razis *et al*.[34] reported that the abundant content of g-tocopherol, vitamin E, vitamin C and β-carotene in moringa leaves, can prevent lipid peroxidation so that oxidative stress can be minimized.

Ovarian morphometry of sexually mature Pengging duck: The number of follicles and weight of F1 and F2 follicles in sexually mature Pengging ducks were not significantly ($p>0.0$) different among the groups (Table 4). However, diameters of F1 and F2 follicles were significantly ($p<0.05$) increased in groups with supplemented *M. oleifera* leaf powder, as compared to the control ducks. The increase in diameters of F1 and F2 was thought to be in line, with the rate of yolk precursor incorporation, to the growing ovarian follicles. The early follicular development spurred the LWF follicles, to develop into SYF, hence forming the preovulatory hierarchical follicles. The number of follicular hierarchy in this research was relatively constant, similar to the previous study by Johnson[33], that the number of preovulatory follicles remains constant from one laying sequence. The follicular hierarchy consists of 2 to approximately 6 preovulatory follicles, with the follicles not undergoing atresia after selection for final maturation and ovulation.

Results from the study showed follicular growth tends to increase rapidly in follicles, that have occupied F2 and F1 positions, exhibited in the duck group supplemented with *M. oleifera* leaf powder. It was assumed that the bioactive component of *M. oleifera* leaf powder, was incorporated into the yolk contributing to protection of the yolk, from lipid peroxidation during its transport from the liver to developing follicles. Although, there was no significant ($p>0.05$) difference

Table 4: The number of follicles, follicle diameter and weight of follicle ovary of sexually mature female Pengging ducks supplemented with *Moringa oleifera* leaf powder

	Treatments of *Moringa oleifera* leaf powder (%)				
Items	0	2.5	5	7.5	10
No. of hierarchical follicles (n)	4.00±2.12	7.00±2.52	7.00±0s.71	6.00±2.08	6.00±0.00
No. of LWF (n)	13.00±6.11	17.00±6.66	16.00±4.51	14.00±2.65	16.00±1.53
No. of SYF (n)	5.00±3.21	9.00±2.08	6.00±2.58	9.00±2.64	8.00±1.52
Diameter of F1 follicle (mm)	21.91±6.03^{b}	30.72±5.38^{a}	33.46±5.93^{a}	34.81±2.50^{a}	33.42±5.15^{a}
Diameter of F2 follicle (mm)	18.30±6.54^{b}	29.33±3.14^{a}	28.87±5.48^{a}	26.47±2.26^{a}	27.24±1.65^{a}
Diameter of F3 follicle (mm)	22.25±0.95	21.93±6.13	25.20±2.21	22.82±0.98	22.93±1.11
Diameter of LWF (mm)	4.84±0.86	5.31±0.18	4.88±0.37	5.40±0.72	5.19±0.60
Diameter of SYF (mm)	7.77±1.71	7.62±1.72	7.72±1.98	7.66±0,63	7.33±1.43
Weight of F1 follicle (g)	11.95±3.66	16.63±3.50	16.80±7.50	16.55±2.56	16.13±5.09
Weight of F2 follicle (g)	7.2±3.86	13.03±4.59	13.25±6.70	13.90±1.84	12.00±5.86
Weight of F3 follicle (g)	4.84±2.93	9.33±4.89	9.05±6.43	9.10±6.40	10.45±0.67

$^{a-b}$Different superscripts on the same row shows significantly different ($p<0.05$). Values are presented as Mean±SD

amidst the duck groups, as regards SYF and LWF diameters and the weights of F1-F3, the weights of F1-F3 in these groups were higher than the control ducks. This increase in the weight of F1-F3, was contributory to the increase in sizes of F1-F3 diameters, confirmed by the positive correlation (r-value by 0.95, $p<0.01$) between follicular diameter and follicular weight.

The resulting increase in the size of ovarian follicle diameter and follicular weight, reflects an increase in the rate of yolk constituent deposition from the liver, into the developing ovarian follicles. The role of *M. oleifera* leaf flavonoid antioxidants, can prevent lipid peroxidation as the main ingredient in forming egg yolk constituents, further inhibiting ROS generation in follicular development. Similarly, the transport process of yolk constituents from the liver to the ovary was also protected by the antioxidant component of *M. oleifera* leaf powder. Conversely, excessive ROS generation inhibits follicular development, induces granular cell apoptosis and causes follicular atresia, resulting to ovarian function decline reported by Chang *et al.*[47]. With respect to the role of flavonoid antioxidants from *M. oleifera* leaves, Ndong *et al.*[48], Farooq *et al.*[49] and Singh *et al.*[50] revealed that quercetin and kaempferol, which are bioactive components of *M. oleifera* leaves, have high antioxidant activity with the greatest capacity as free radical cleaners, especially as antioxidants in hepatocyte growth factor (HGF). Furthermore, the role of the hepatoprotector of *M. oleifera* leaves was shown with a decrease in glutamic-oxaloacetic transaminase (aspartate aminotransferase)[51], glutamic-pyruvic transaminase[51-52] and alkaline phosphatase[53].

CONCLUSION

The overall conclusion of this study, was that the supplementation of *M. oleifera* leaf powder by 2.5-10% into Pengging duck feed could increase the reproductive profile of Pengging ducks, characterized by early attainment of sexual maturation. This is supported by an increase in the size of F1 and F2 follicles, without an increase in the concentrations of serum cholesterol, as well as the liver and ovarian MDA. Notably, *Moringa oleifera* leaf powder was vital as a hepatoprotector and oviprotector in sexually mature Pengging ducks.

SIGNIFICANCE STATEMENT

This study discovered that supplementation of *M. oleifera* leaf powder in feeds, can improve the reproductive profile of Pengging ducks. Consequently, this study will help researchers to uncover the critical area of precursor yolk deposited in follicle ovary, unexplored by researchers. Thus, a new study on the positive value of *M. oleifera* leaf powder for safe nutrition and evaluation of the long-term effect of its consumption, on performance production in the raising of local ducks may be arrived at.

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