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Effect of *Moringa oleifera* leaf powder in diets on feed digestibility and external egg quality characteristics in laying hens

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Abstract
The present study was designed to investigate the influence of *Moringa oleifera* leaf powder on feed digestibility and egg quality characteristics of laying hens up to 32 weeks old. One hundred and twenty 32 weeks of healthy laying hens with homogeneous body weight in a complete randomized design with four treatments and 6 replications. Laying hens were randomly divided into four groups: M0: basal diets without administration of *Moringa oleifera* leaves, M1: basal diets with 2% *Moringa oleifera* leaves; M2: basal diets with *Moringa oleifera* leaves 4%; and M3: basal diets with 6% *Moringa oleifera* leaves, repectively. Each treatment consisted of six replication cages with 5 hens randomly assigned to each cages. This study showed that administration of 4-6% the *Moringa* leaves powder were increased dry matter and organic matter digestibility, yolk and egg shell percentages, and shell thickness ($P< 0.05$), but not the albumen of eggs ($P>0.05$). The administration of 2-6% *Moringa* leaves powder in diets results in higher ($P< 0.05$) yolk colour of eggs. It was concluded that supplementation of 4-6% % *Moringa* leaves powder in diets, increased feed digestibility and external egg qualities of laying hens up to 32 weeks old.

Keywords: Feed digestibility, shell thickness, yolk color

Introduction
Eggs are products of refined poultry and loss of quality quickly during periods of collection and consumption. Thus, increasing and extending the shelf life of eggs is important for farmers and other poultry researchers. Various efforts have been made to increase the content of β-carotene and egg quality. The increase the yolk color will benefit the poultry industry and public health (Mahmoud *et al.*, 2010; Meliandasari *et al*., 2015) [25, 28]. In addition, lack of vitamin A causes decreased immune function, increasing the risk of infectious diseases in children (Tourniaire *et al*., 2009) [49]. Vitamin A deficiency can be caused by a lack of vitamin A intake itself or lack of availability of provitamin A. The survey results indicate that vitamin A deficiency is a major cause of pre-school child morbidity and mortality in developing countries (Shete and Quadro, 2013) [46]. It is also reported that more than 127 million children in the world experience a lack of vitamin A intake. Many efforts have been made to overcome vitamin A deficiency, including supplementation and fortification of vitamin A in food products, namely chicken eggs. Utilization of *Moringa oleifera* leaves as a source of natural provitamin A in making products can be used as an alternative to natural fortification efforts in an effort to overcome the problem of vitamin A deficiency, as well as natural provitamin A compounds that are far safer to consume than synthetic vitamin A.

In many countries various types of plant extracts have been used in traditional medical systems to treat microbial diseases. The phytochemical compounds contained in *Moringa* include: flavonoids, saponins, tannins, and several other phenolic compounds that have antimicrobial activity (Bukar *et al*., 2010) [10]. Phytochemicals in plants that have antimicrobial and antioxidant properties are the reason for this ability to use them in the treatment of diseases (Akinmoladun *et al.*, 2007; Oriabi, 2016; Prasad and Ganguly, 2012; Mbikay, 2012) [3, 36, 39, 26]. Antimicrobial activity from several phytochemicals present in these plants has been investigated and the possibility of using them to develop new antimicrobial drugs has also been studied (Dalukdeniya *et al.*, 2016; Elangovan *et al*., 2014; Goel, 2013) [13, 15, 17]. Several previous studies confirmed that extracts or compounds isolated from *M. oleifera* had antioxidant, anti-carcinogenic, anti-diabetic, anti-inflammatory, and anti-hypertensive

properties, as well as the ability to protect against liver damage (Ashok *et al.*, 2014; Chukwuebuka, 2015; Dalukdeniya *et al.*, 2016; Elangovan *et al.*, 2014; Godinez-Oviedo *et al*., 2016) [7, 15, 12, 13, 18].

The use of *Moringa* can be an alternative replace antibiotics, because of the rich and diverse phytochemical compounds, and efficacious as an antibacterial agent and can increase immunity (Godinez-Oviedo *et al.*, 2016; Dalukdeniya *et al*., 2016; Yuniza and Yuherman, 2015) [18, 13, 53]. It has been thought to control many diseases, as an endothelial vasodilatator and inhibits the activity of HMG Co-A (3-hydroxy-3-methyl-glutaryl Co-A), thereby inhibiting lipase lipoprotein which is responsible for plasma lipid hydrolysis and has health benefits in connection with their ability to change the fat profile and carcass yield of broilers (Adriani *et al.*, 2015; Ekayuni *et al*., 2017) [2, 14].

The content of beta-carotene in *Moringa* leaves is very high so it is very good for increasing color and beta-carotene contents in egg yolks. Previous research, after obtaining the total carotene content in *Moringa* leaves was 24735 ug/100 g. Beta-carotene supplementation was effective in increasing serum beta-carotene concentrations with little effect on serum retinol concentrations, reducing serum oxidative stress, and increasing biological antioxidant capacity (Otomaru *et al.*, 2018) [38]. β-carotene is provitamin A, the most abundant carotenoid in food and human tissue. This provides a number of beneficial functions in mammals, including humans, because of its ability to produce vitamin A as well as crucial signaling functions of its metabolites (Shete and Quadro, 2013) [46]. When added to the ration, bioactive compounds along with other phytochemicals can improve egg quality and have a positive effect on chicken health and performance. *Moringa* leaf extract can be useful to be used as an effective feed supplement for poultry to encourage results in relation to total weight gain and feed efficiency, reduce abdominal fat and cholesterol in broiler meat and egg of laying hens (Ekayuni *et al*., 2017) [14]. Herbs extract in drinking water increases egg production and can reduce cholesterol in serum and egg yolk in laying hens (Bidura *et al.*, 2017; Mahmoud *et al*., 2010; Yalcin *et al*., 2007) [8, 25, 52].

Based on this, the study was conducted to determine the effect of *Moringa* leaves in the diet on feed digestibility and egg quality of Lohmann Brown laying hens up to 32-week ages.

Material and Methods

Animals, treatments, and experimental design

This study was a feeding trial using onehundred and twenty 32 weeks of healthy laying hens with homogeneous body weight 1695.28 ± 22.93 grams obtained from commercial poultry farms in a complete randomized design with four treatments and 6 replications. All hens were given commercial feed specific for laying hens containing 2.750 kcal/kg of metabolizable energy (ME); 17% of CP; 3.5% of Ca; and available phosphor of 0.45% as a basal diets. Laying hens were randomly divided into four groups: M0: basal diets without administration of *Moringa oleifera* leaves, M1: basal diets with 2% *Moringa oleifera* leaves; M2: basal diets with 4% *Moringa oleifera* leaves; and M3: basal diets with 6% *Moringa oleifera* leaves, repectively. Each treatment consisted of six replication cages with 10 birds randomly assigned to each cage at 100×70×45 cm (length×width×height). Each experimental diet was in the form mash and birds have free access to feed and water during the experiment.

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.

Live performance

Continuous lighting and access to feeding and water were provided during the experiment. The birds were weighed at the start (age 32 weeks) and the end (age 44 weeks) of the experiment. Eggs were collected every day and egg production was expressed on a day-to-day basis (% of chicken days). Individual egg weights were recorded and then used to calculate the average egg weight for all trial periods. The total egg mass was calculated by multiplying the weight of the egg with egg production. Feed intake was measured based on cages (hens) every week. Daily feed intake per bird was calculated based on the total cage intake for the entire trial period and for the number of days in all periods. Feed conversion ratio (gram feed/gram egg mass) for all periods was calculated based on the cage of egg production, egg weight, and feed consumption. Egg quality parameters were measured using a multi-egg tester.

Quality of eggs and yolk minerals

Eggs are collected and labeled every day at 08.00 and 14.00 hours during the trial period. The percentage of egg production was calculated. Examination of egg and eggshell quality (shell weight, eggshell thickness, egg yolk weight and albumin, egg yolk color, albumen, and egg yolk height) were carried out at the end of the experiment. For this purpose, two eggs placed between 08.00 and 12.00 hours were taken randomly from each group on the 44th day of the week (a total of 12 eggs per group during the experiment). Eggs were weighed individually and the specific gravity of the egg, as the index of the thickness of the shell, was measured. After the egg was broken on the EQM measurement stand, the albumen and yolk height was measured. The intensity of the yolk color was evaluated and recorded according to Roche's egg yolk fan method. Albumen's weight was calculated by reducing the weight of the yolk and shells of the overall egg weight. To measure the weight of the shell, the eggshell was cleaned from the albumen which attaches to the membrane was removed; Egg shells were then dried at room temperature and expressed as a percentage of all eggs. Evaluation of egg quality was carried out on individual eggs, similar to the weight of the eggs measured.

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/100g) = (total volume x abs x 100)/(0.2 x sample weight).

Retention and excretion of nutrients

To determine nutrient digestibility values (dry matter and organic matter digestibility): The amount of food used was 100 g, this amount is based on a preliminary test with the consumption of laying rations. All birds are not fed for 24 hours to ensure that their digestive tract is empty of leftover feed. They were then force-fed with certain diets (all treatments). Stainless steel funnels with 40 cm stems wee used in forced feeding techniques (Bidura *et al.*, 2019) [9]. Water was available *ad libitum* during the trial period. Total dirt (excreta) were collected in plastic trays. The excreta samples were frozen, allowed to reach equilibrium with atmospheric humidity, weighed, and pounded through a 1 mm filter. Excreta samples and diets were carried out in an appropriate analysis to determine dry matter (DM) and organic matter (OM), respectively. Dry matter (DM) and organic matter (OM), and ash determination were carried out in accordance with the Official Analytical Chemistry Association (2005)[6]. All tests are carried out in triplicate.

Statistical analysis

All data were analyzed by ANOVA to determine the difference between treatments. If differences were found ($P< 0.05$), further analysis is carried out with Duncan's multiple range test.

Results

The results are presented that the final body weight, dry matter and organic matter digestibility, and external egg quality characteristics in groups fed the experimental diets are shown in Table 1. The treated laying hens exhibited higher significantly different ($P< 0.05$) on dry matter and organic matter digestibility than the control bird. No significant differences (P>0.05) in the final body weight were observed among the dietary treated groups.

The treated laying hens exhibited higher significantly different ($P< 0.05$) on egg shell, egg yolk, shell thickness, and yolk color than the control of laying hens. No significant differences (P>0.05) in the albumen, haugh unit, egg shape, and specific gravity were observed among the dietary treated groups. Laying hens in M2 and M3 groups responsed with a higher shell thickness and yolk color ($P< 0.05$) than those Group M0 and M1. Dietary *Moringa* leaf powder increased yellowness in yolk color ($P< 0.05$) in egg laying hens. The egg yolk color in M2 and M3 hen groups were increased 29.19% and 32.59% than group M0.

Table 1: Effect of *Moringa oleifera* leaves powder in diets on feed digestibility and external egg quality in laying hens up to 32 weeks old

Variables	Groups[1]				SEM[2]
	M0	M1	M2	M3	
Initial body weight (g)	1528.05a	1504.52a	1534.69a	1517.15a	22.528
Final body weight (g)	1649.37a	1618.05a	1659.23a	1636.81a	25.062
Dry matter digestibility (%)	79.02b[3]	79.14b	82.89a	82.92a	0.509
Organic matter digestibility (%)	81.27b	81.19b	85.27a	85.47a	0.513
Egg shell (% egg weight)	12.02b	12.15b	12.81a	12.88a	0.194
Egg yolk (% egg weight)	27.25b	27.32b	29.08a	29.17a	0.419
Egg albumen (% egg weight)	60.73a	60.53a	58.11a	57.95a	0.973
Haugh unit (white height: egg weight)	76.04a	76.19a	76.93a	76.64a	0.251
Egg shape (egg width/egg length) x 100%	76.39a	75.82a	76.17a	76.34a	0.182
Specific gravity (weight: volume)	1.042a	1.045a	1.039a	1.049a	0.029
Shell thickness (mm)	0.405b[3]	0.419b	0.485a	0.491a	0.013
Yolk colour (1-15)	6.75b	7.18b	8.72a	8.95a	0.408

[1] M0: The basal diet without *Moringa* powder (control); B: The basal diet with 2% *Moringa* powder; C: The basal diet with 4% *Moringa* powder; and D: The basal diet with 4% *Moringa* powder, respectively. [2] SEM: standard error of treatment means [3] Means with different superscripts within raw values are significantly different ($P< 0.05$)

In addition, the results show that an additional *Moringa* leaves powder (group M2 and M3) in diets resulted in a significant ($P< 0.05$) increase in shell thickness of birds were: 19.75% and 21.23%, repspectively higher than control.

Discussion

The use of *Moringa* in basal diets can increases feed digestibility in groups M2 and M3 rather than M0 and M1 groups. *Moringa* leaf extract can be useful to be used as an effective feed supplement in poultry to improve feed efficiency in poultry (Sanchez *et al.*, 2005) [43]. 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) [51, 19]. *Moringa oleifera* extract was found to be more effective in controlling gram negative bacteria tested than gram-positive bacteria (Dalukdeniya *et al*., 2016) [13].

Digestibility of dry matter and organic matter in groups M2 and M3 were improved by the addition of the Moringa 4-6% in diets than controls. These findings are in agreement with previous research of Hernandes *et al*. (2004) [21], that plant extract supplements can increase the digestibility of nutrients in the digestive tract of poultry. Herbal extracts (Garlic) can increase the activity of pancreatic enzymes and microenvironmental conditions for better utilization of nutrients in mice (Ramakrishna *et al*., 2003) [40]. Similarly, Ossebi (2010) [37] reported that *Moringa* leaves up to 24% in feed did not cause adverse effects on nutrient absorption and could significantly increase protein digestibility, energy, and mineral utilization. This causes higher mineral content of Ca and Mg in yolk and eggshell than controls. *Moringa oleifera* leaves are known to be very poor in anti-nutritional content and have been used in ruminant rations (Soliva *et al.*, 2005) [47] and in other poultry or monogastrics. This result is contrary to that reported by Teteh *et al*. (2013) [48] that the

high use of *Moringa* leaves in feed can cause increased levels of saponin as an antinutrient which can reduce digestion and absorption of nutrients, especially lipids. According to Goel (2013) [17], the antimicrobial activity of plants is mainly caused by the presence of secondary metabolites. Plants are rich in various secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. These active compounds in the digestive tract of poultry will be able to help absorb nutrients. As reported by Adibmoradi *et al*. (2006) [1], that herbal active compounds (garlic) can increase villous height and crypto depth, and reduce epithelial thickness and the number of villous cells in the duodenum, jejunum, and poultry ileum. Increased height of villi, as well as the thickness of epithelium in the duodenum, jejunum and ileum will increase nutrient uptake (Nusairat, 2007) [32]. Results from Bidura *et al.* (2017) [8] found that the administration of *Sauropus* leaf extract in drinking water can significantly improve feed efficiency in laying hens.

Increased egg production and egg weight in hens given *Moringa* leaf is caused by the presence of phytochemical compounds on *Moringa* leaves, as reported by Prasad and Ganguly (2012) [39] that *Moringa* leaves are also a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and α-tocopherol. Supplementation of *Moringa* leaves affects egg productions and egg mass (Gakuya *et al*., 2014) [16] and egg yolk bioactive compounds. Antioxidants, flavonoids, carotenoids, amino acids, proteins, and energy levels that result in a decrease in egg water content can be the reason for increasing nutrient density in egg yolk.

According to Mabusela *et al*. (2018) [24], *Moringa oleifera* seed flour inclusions can improve the quality of external eggs, and increase fatty acid profiles. Some of the results of research on the effect of herbal extracts in poultry were carried out by Bidura *et al.* (2017) [8] that administration of *Allium sativum and Sauropus androginus* leaf can significantly increase body weight and feed efficiency in broiler chickens. Siti *et al.* (2017) [45] and Mohammed *et al.* (2012) [29] reported that *Moringa* leaves can increase egg production and egg quality, but decreasing yolk cholesterol in laying hens. Bidura *et al.* (2017) [8] who reported that administration of 5 cc/100 cc herbal extracts (*Sauropus* and *garlic* leaves) in drinking water increased egg production and total egg weight. The results of Yalcin *et al.* (2007) [52] reported that the addition of garlic powder at level 5 or 10 g/kg in feed, showed an increase in chicken egg production.

The yolk color in Groups M1, M2, and M3, has a significantly higher color compared to the control. The increase in egg yolk color in this study showed that *Moringa* leaves are rich in vitamin A or carotenoid pigments which are efficiently absorbed and utilized by chickens. The yellow color increase can be attributed to the carotenoid content of *Moringa* leaf powder. In this study, the carotene of *Moringa* was: 24735 ug/100 g. Besides that, *Moringa* pods are enriched with carotenoids and flavonoids, which are powerful natural antioxidants that can modify the levels of β-carotene and quercetin egg yolk (Gakuya *et al*., 2014) [16]. According to Amaglo (2010) [4]; Saini *et al.* (2014a) [41]; and Saini *et al.* (2014b) [42], β-carotene in *Moringa* pods ranges from 2.7 to 3.10 mg/100 g dried pods. When added to feed, this bioactive, along with phytochemicals, increases egg production and has a positive effect on chicken health. Carotenoids play an important role in the development of different color scores in egg yolk. Especially, lutein is an active yellow dye. According to Cayan and Erener (2015) [11] that increasing the amount of olive leaf powder in food results in a linear increase in the color of the yolk. This increase in the yolk color can be attributed to the carotenoid content of olive leaf powder (Nimalarante and Wu, 2015) [31]. Some researchers report that herbal extract supplements show the potential for an increase in the yolk color, as mulberry leaves, ginkgo (Lokaewmanee *et al*., 2009; Zhao *et al.*, 2013) [23, 55], *Allium sativum* and *Sauropus androgynus* (Bidura *et al*., 2017) [8], olive leaf powder (Zangeneh and Torki, 2011) [54], and carrots in feed (Okonkwo, 2009; Hammershoj *et al.*, 2010) [33, 20]. This observation is supported by the findings of Olugbemi *et al.* (2010a and 2010b) [34, 35] that the use of *Moringa oleifera* leaves 10-20% in broiler feed or laying can significantly increase the yellow color of the skin and egg yolk.

Conclusion

We conclude that supplementation in basal diets of 4-6% *Moringa oleifera* leaves powder were increased feed digestibility, yolk colour, yolk and egg shell percentages, and shell thickness in laying hens up to 32 weeks of ages.

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