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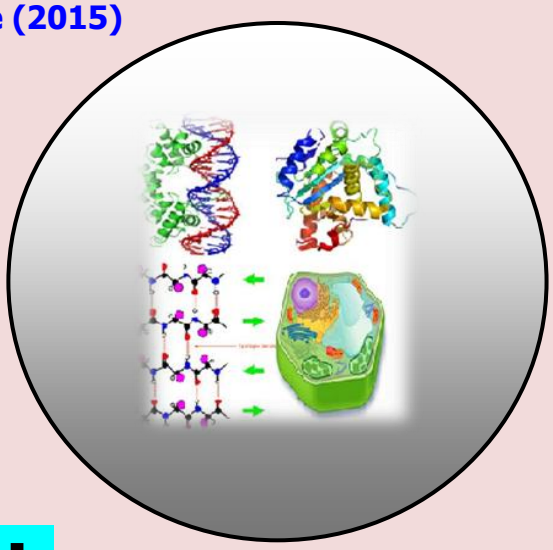
By

Ni Wayan Siti, I.G.N.G. Bidura and Ida Ayu Putri Utami

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 46.52 Poland, Europe (2015)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 34 (2) 2017 Pages No. 657-665



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 34, No. 2: 657-665, 2017
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 34/02/501/2017
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ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Ni Wayan Siti
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 17/10/2017 **Revised: 22/10/2017** **Accepted: 23/10/2017**

The Effect of Water Extract of Leaves *Moringa oleifera* on Egg Production and Yolk Cholesterol Levels in Egg Laying Hens

Ni Wayan Siti, I.G.N.G. Bidura and Ida Ayu Putri Utami

Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia, Jl. PB. Soedirman, Denpasar-Bali, Indonesia

ABSTRACT

The present study was conducted to determine the effect of Moringa oleifera leaves water extract on the egg production and yolk cholesterol level of 30-week old hens. This research was feeding trial using 120 laying chickens Lohmann Brown 30 weeks of age in a complete randomized design (RAL) with four treatments and 6 replications. The four treatments are the provision of drinking water without the extraction of Moringa leaf water as a control (A); drinking water, each added 2%, 4%, and 6% of Moringa leaf water extraction as treated B, C, and D, respectively. The results showed that the addition of Moringa oleifera leaf extract at a concentration of 2-6 cc/100 cc of drinking water was significant (P<0.05) to increase egg weight, egg count, hen-day production, feed efficiency, and egg yolk color of Lohmann Brown up to 30 weeks of ages. In contrast, significantly (P<0.05) reduced the fat content in eggs and egg cholesterol. It can be concluded that the addition of Moringa oleifera leaf extract at a concentration of 2-6 cc/100 cc of drinking water can increase egg production and decrease the cholesterol content of egg laying hens.
Key words: Moringa oleifera, Phytochemicals, Cholesterol and Eggs.

INTRODUCTION

High market demand for chicken eggs makes laying hens much cultivated by farmers. High cholesterol content in foodstuffs becomes the main consideration of consumers in consuming animal products, because it is a source of cholesterol for upper middle class people that can cause degenerative diseases, such as coronary heart (Meliandasari *et al.,* 2015), characterized by thickening of artery walls and levels cholesterol in the blood (hypercholesterolemia). The high cholesterol in animal products consumed can be a source of obesity and coronary heart disease. In Indonesia there is an increase in the number of illness and death of coronary heart disease caused by lifestyle changes, one of which is atherosclerotic lifestyle in the form of diet with fat intake >30%, saturated fatty acid >10% of total energy and cholesterol >300 mg per day (Sartika, 2008). Addition of *Moringa* fruit flour in diet of quail birds had beneficial effect in reducing the oxidative stress and

oxidative damage as reflected by reduced malondialdehyde levels and increased erythrocyte number and hemoglobin concentration (Adriani *et al.,*2017). Malondialdehyde (MDA) is the direct product of lipid peroxidation developed after radical attack and thus is an indicator of the extent of cell damage (Puvaca *et al*., 2015).

Medicinal plants and spices is one type of agricultural commodity that has a bright enough prospect to be developed. Plants that have the potential for medicines (herbs) are quite many types and have not been widely utilized. Traditional knowledge about the use of plants is very important because it will increase the diversity of vegetable resources and is the basis of economic botany and other applied botany. Therefore, the use of natural supplement feeds from medicinal plants and spices is an alternative that can be used as a substitute for commercial supplement feed in rations. One of the natural supplement feeds that can be used is *Moringa* leaf extract. In this case, the use of *Moringa citrifolia* can be an alternative to lowering fat and cholesterol content in eggs, and replacing antibiotics, due to its rich and diverse phytochemical compounds, and efficacious as an antibacterial agent and can boost immunity (Yuniza and Yuherman, 2015). Burham (2017) reported that *Moringa leaf* analysis contained dry matter 94.60%, ash content 2.13%, crude protein 58.32%, oil 11.3%, crude fiber 4.75% and total carbohydrate content is 23.5%. The phytochemicals analysis of *Moringa* seeds in this study are phytic acid (351.12 mg/100g), tannin (0.13 mg/100g) and total polyphenols (629.70 mg/100g). The minerals content are Ca (42.13 mg/100g), phosphorus (1311.20 mg/100g), and iron (55.98 mg/100g). Some articles and research studies have reported that the dry leaves of *M. oleifera* contain 7 times more vitamin C than orange, 10 times vitamin A than carrot, 17 times calcium than milk, 15 times potassium than bananas, 25 times iron than spinach and 9 times proteins than yogurt (Fuglie, 1999).

Moringa oleifera leaf (*Moringa oleifera*) is a traditional medicinal plant that has high nutrients, as an antibacterial, and contains beta carotene as yellow active substance. Phytochemical compounds contained include: flavonoids, saponins, tannins, and some other phenolic compounds that have antimicrobial activity (Bukar *et al*., 2010). Estrogen-like flavonoids are able to slow bone mass loss (osteomalacia), lower blood cholesterol levels and increase HDL levels, while saponins are proven efficacious as anticancer, antimicrobial, and lower blood cholesterol (Santoso *et al.,* 2002). *Moringa oleifera* was claimed to boost immune systems. Apart from the medicinal uses, *Moringa oleifera* was reported to be a good source of vitamins and amino acids. *M. oleifera* is a miracle tree with a great indigenous source of highly digestible proteins, Ca, Fe and Vitamin C. It contains all the essential nutritional elements that are essential for livestock and human beings (Fuglie, 1999).

Akhouri *et al*. (2013) suggests that the efficacy of *Moringa oleifera* leaf extract can be used advantageously as an effective feed supplement in poultry, because the results are very encouraging with respect to weight gain and feed efficiency in broiler chickens. It can also be used potentially before mass vaccination of chicks for immunomodulating properties such as levamisole.

In research conducted by Hestera (2008), that the use of *Moringa oleifera* leaf 10% in feed can decrease cholesterol content of chicken meat, and from research conducted Restiayanti *et al*. (2014) said that giving *Moringa oleifera* leaf extract as much 50 g/liter of drinking water given to broilers can lower abdominal fat and serum cholesterol levels in broiler. This study was conducted to know effect of water extract of *Moringa oleifera* leaves in drinking water on egg production and cholesterol level of egg laying chickens.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This research was feeding trial using 120 laying chickens Lohmann Brown 30 weeks of age in a complete randomized design (CRD) with four treatments and 6 replications. The four treatments are the provision of drinking water without the extraction of *Moringa* leaf water as a control (A); drinking water, each added 2%, 4%, and 6% of *Moringa leaf* water extraction as treated B, C, and D, respectively.

All chickens were given commercial feed specific for laying hens containing 2.750 kcal/kg of Metabolizable Energy (ME); 16% of CP; 3.5% of Ca; and available phosphor of 0.45%. For the treatments, hens were placed into four groups each containing 5 hens: (1) hens were only given water as a drink, (2) hens were given 2 cc of *Moringa* leaf extract in 100cc of drinking water, (3) hens were given 4 cc of *Moringa* leaf extract in 100 cc of drinking water, and (4) hens were given 6 cc of *Moringa* leaf extract in 100 cc of drinking water. Each treatment was repeated 6 times for a total of 120 hens. Food and drinking liquid were given *ad libitum*. The individual hens were weighted weekly, and feed consumption and egg production was recorded daily.

Preparation of *Moringa oleifera* leaf Extract: Fresh leaves of the *Moringa oleifera* were obtained from the local fresh food market. The leaves of both plants were blended and macerated overnight in distilled water (1:1, w/w) (Bidura *et al.,* 2017). The blended extract was then filtered using a cheese cloth. This extract was used for the treatment.

Performance, egg quality metrics, and laboratoryanalysis: Eggs were collected and labeled on a daily basis at 08.00 h and 14.00 h throughout the experimental period. The percent egg production was calculated. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk cholesterol content was analyzed for two consecutive weeks. Cholesterol levels were analyzed following the Liberman-Burchard methods (Lieberman and Burchard, 1980).

Statistical analysis: All data were analyzed with ANOVA to determine the differences among treatments. If differences were found, then further analysis was performed with Duncan's multiple range test.

RESULTS AND DISCUSSION

The results are presented in Table 1. It is known that the addition of Moringa oleifera leaves extract of 2-6 cc/100 cc of drinking water has no significant effect (P> 0,05) on feed and drink consumption, and egg shell thickness. However, the extract of *Moringa* leaves in drinking water were significantly (P<0.05) increased total egg weight, egg weight, egg numbers, and hen-day productions. Efficiency of feed and yolk color in chickens consuming Moringa leaf extract at 2-6 cc/100 cc level of drinking water, significantly (P<0.05) increased compared with control. In contrast, the actual yolk fat and cholesterol levels (P<0.05) decreased with Moringa leaf extract in the drinking water administered.

Table 1. Effect of leaf water *Moringa oleifera* in drinking water on egg production and cholesterol levels in egg yolk in laying hens aged 30-40 weeks.

Variables	Treatments[1)]				SEM
	A	B	C	D	
Feed Consumption (g/head/days)	155,35a	154,82a	152,51a	155,03a	1,083
Water consumption (ml/head/days)	396,81a	401,62a	406,37a	403,75a	3,074
Total egg weight (g/head/70 days)	2840,17b	3107,32a	3123,11a	3116,72a	75,062
Egg weight (g/head)	57,54b	60,01a	60,28a	59,40a	0,485
The number of eggs (egg/70 days)	49,36b	51,78a	51,81a	52,47a	0,609
Hen-day production (%)	70,52a	73,97b	74,01b	74,95	1,028
FCR (eed consumption: egg weight)	2,70a	2,58b	2,53b	2,61b	0,017
Shell thickness (mm)	0,327a	0,322a	0,331a	0,329a	0,013
Yolk colour (1-15)	7,08b	8,27a	8,35a	8,43a	0,275
Egg fat (% DM)	26,74a	24,82b	24,62b	24,75b	0,517
Yolk cholesterol (mg/dl)	178,35a	165,62b	166,51b	164,72b	2,805

Added of *Moringa oleifera* leaf extract at the level of 2-6 cc/100 cc in drinking water was not significantly affect the consumption of feed and drinking water. This is because the feed energy content of the four treatments is the same. Chickens consume rations to meet energy needs. The results of this study are the same as reported by Bidura *et al*. (2017) who reported that 5 cc/100 cc of herbal extract (*Sauropus* and garlic leaves) in drinking water had no significant effect on feed and drink consumption. Wibawa *et al.* (2016) also reported that administration of herbal extracts of plants (garlic) in drinking water at concentrations 2.5-5% had no significant effect on feed and drink consumption. The same thing was reported by Syahruddin *et al*. (2013), that 14% of *Sauropus* leaves have no significant effect on feed consumption and feed efficiency. However, Ayssiwede *et al*. (2011) reported that the use of 24% of moringa leaves in rations did not have a negative impact on weight gain, feed efficiency, mortality, carcass, and internal organs compared to controls. However, there was a decrease in feed intake. Ekayuni *et al*. (2017) reported that giving 5% *Moringa* leaf water extract in drinking water was not significant effect on feed and drink consumption.

The increase in egg production and egg weight in chickens given *Moringa* leaf extract (Table 1) was due to the presence of phytochemical compounds on *Moringa* leaves, as reported by Akhouri *et al.* (2013) that the plant *Moringa oleifera* is one of the important crops often used as traditional medicine for the prevention of cataracts, cancer, peptic ulcers, skin diseases, low blood sugar, neurological conditions and diabetes. In addition, it is also efficacious to strengthen the liver, eyes, brain, bile, and the immune system. Moringa leaves are also used to kill intestinal worms, as well as a source of vitamin A, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron and α-tocopherol (Prasad and Ganguly, 2012). Sarag and Hobragade (2003) reported that broiler weight increased markedly after given *Tinospora cordifolia* leaf extract. The same thing was also reported by Thatte *et al.* (2001) and Kumari *et al.* (2012) where they recorded higher body weight gain in mice given *T. cordifolia* and *Asparagus racemosus*. The results of this study are the same as reported by Bidura *et al*. (2017) who reported that giving 5 cc/100 cc of herbal extract (*Sauropus* and garlic leaves) in drinking water increased egg production and total egg weight.

Notes:

1. A: drinking water without *Moringa oleifera* leaves extract as control; (B): drinking water with 2 cc/100 cc *Moringa oleifera* water extract; (C): drinking water with 4 cc/100 cc *Moringa oleifera* leaves water extract, and (D): drinking water with 6 cc/100 cc *Moringa oleifera* leaves water extract, respectively.
2. SEM:Standard Error of Treatment Means
3. Means with different superscripts within raw values are significantly different ($P<0.05$)

The results of research on broiler chickens performed by Akhouri *et al*. (2013) that giving *Moringa oleifera* leaf powder at 250 mg/kg body weight can significantly increase weight gain and feed efficiency in broiler chickens. The results of Yalcin *et al*. (2006) reported that the addition of garlic powder at level 5 or 10 g/kg in feed, showed an increase in chicken egg production. The same thing was reported by Khan *et al.* (2007) that laying hens given garlic (2-8%) showed higher egg production intensity.

Moringa oleifera leaves in drinking water significantly increases feed efficiency (feed consumption : egg weight). This is due to the phytochemical properties of *Moringa oleifera* leaves. According to Goel (2013), plant antimicrobial activity is primarily due to 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 poultry digestive tract will be able to help the absorption of nutrients.

As reported by Adibmoradi *et al.* (2006), that the active compounds of garlic can increase villus height and cryptus depth, as well as decrease in epithelial thickness and number of villi cells in the duodenum, jejunum, and ileum of poultry. Increased villus height, as well as epithelial thickness and goblet in the duodenum, jejunum, and ileum will improve nutrient uptake (Nusairate, 2007). The results of Bidura *et al*. (2017) found that giving of *Sauropus* leaf extract of 5 cc/100 cc in drinking water significantly can improve feed efficiency in laying hens.

The results of the study using *Moringa oleifera* leaves, it has been reported that the leaves of *Moringa oleifera* are rich in nutrients, energy, and vitamins (Nuhu, 2010; Ayssiwede *et al.,* 2011; Mutayoba *et al.,* 2011). *Moringa oleifera* leaf is known to be very poor in its anti-nutritional content and has been used in both ruminant rations (Soliva *et al.,* 2005) as well as in other poultry or monogastric. The results depend largely on the nutritional value and level of use in the diet (Kakengi *et al.,* 2007; Nuhu, 2010; Olugbemi *et al*., 2010a and 2010b). Ossebi (2010) has found that giving *Moringa oleifera* leaves up to 24% in feed instead of peanut use did not cause adverse effects on the absorption of nutrients and can significantly improve protein digestibility, energy, and mineral utilization.

Reported also by Hernandes *et al*. (2004), that supplementation of plant extracts can improve nutrient digestibility in the poultry digestive tract. According to Adibmoradi *et al*. (2006), giving Garlic extracts can increase villus height and crypt depth, as well as decrease in epithelial thickness and goblet cell counts in the duodenum, jejunum, and poultry ileum. Similar results were reported by Ramakrishna *et al.* (2003), that Garlic supplementation in the diet can increase pancreatic enzyme activity and micro-environment conditions for better utilization of nutrients in mice.

Giving *Moringa oleifera* leaf extract at a concentration of 2-6 cc/100 cc in drinking water significantly decreased the fat and cholesterol content of the egg. Decreased cholesterol concentrations with supplementation of herbal extracts, due to decreased activity of the enzyme synthetase (Qureshi *et al.,* 1983). A significant decrease in the activity of 3-hydroxy-3-methylglutaryl-CoA reductase in the liver, cholesterol 7α-hydroxylase and fatty acid synthetase. It was reported by Sakine and Onbasilar (2006) that Garlic supplementation in feed can lower plasma cholesterol concentration when laying hens fed 0.50% Garlic flour and 1.0% in mice significantly lowered cholesterol and triglyceride (Eidi *et al.,* 2006). Wibawa *et al.* (2016) stated that the giving of Garlic extract in drinking water at a concentration of 2.5-5% significantly lowered the fat and cholesterol levels in blood serum in broiler chickens. The same is also reported by Bidura *et al.* (2017) that administration *Sauropus* leaf herbal extract as much as 5 cc/100 cc in drinking water significantly lowering cholesterol levels in serum and egg yolks.

The ability of beta-carotene in lowering cholesterol associated with the enzyme hydroxy methyl glutaril-CoA (Wang and Keasling, 2002). This enzyme plays a role in mevalonic formation in cholesterol biosynthesis. The synthesis of cholesterol and beta-carotene together through mevalonik and derived from acetyl CoA. If the consumption of beta-carotene is greater than saturated fatty acids, then the biosynthetic process by HMG-CoA enzyme will be directed to beta-carotene synthesis, so that saturated fatty acids are not converted to cholesterol (McGilvery and Goldstein, 1996). The lowest cholesterol level is obtained when chickens are fed with 14% leaves of fermented *Sauropus* (Syahruddin *et al.*, 2013).

High consumption of beta-carotene can lower cholesterol levels in egg yolks, because beta-carotene can inhibit HMG-CoA reductase (Hydroxy metyl glutaryl-CoA) enzyme that plays a role in mevalonic formation. Mevalonic is needed in the process of cholesterol synthesis by inhibiting the enzyme, thereby inhibiting cholesterol formation (Syahruddin *et al.,* 2013). In addition, *Moringa oleifera* leaf contains a high enough antioxidant that is very instrumental in lowering fat and cholesterol. Oka *et al*. (2016) reported that the *Moringa oleifera* leaf antioxidant content is quite high, but still lower than that of *Sauropus* leaf. However, its vitamin C content is higher than that of *Saorupus* leaves.

Fermentation of herbal extracts (*Sauropus androgynus)* produces the best quality of broiler meat, shown by decreased fat and cholesterol, and high levels of vitamin A, beta-carotene, protein, iron, amino acids, and better fatty acids (Santoso *et al.* , 2015). *Sauropus androgynus* also reduces cholesterol content in broiler carcasses (Santoso and Sartini, 2001; Subekti, 2003) and in eggs (Santoso *et al*., 2005). Wibawa *et al.* (2016) stated that giving Garlic extract in drinking water at a concentration of 2.5-5% significantly decreased serum blood fat and cholesterol levels.

The results of Ekayuni *et al.* (2017) reported that administration of 5% *Moringa oleifera* leaf water extract in drinking water significantly decreased the amount of abdominal fat and cholesterol levels in broiler meat. The same thing was also reported by Widnyana *et al.* (2017) that giving 5% *Moringa oleifera* leaf extract in drinking water significantly lowered fat and serum cholesterol levels in broiler chickens.

The increased egg yolk in this study showed that *Moringa oleifera* leaves are rich in vitamin A or carotenoid pigments that are efficiently absorbed and utilized by chickens. This observation is supported by the findings of Olugbemi *et al*. (2010a and 2010b) which states that the use of *Moringa oleifera* leaves up to 10-20% in laying chicken or broiler rations can significantly increase the yellow color of the skin, abdominal-fat, and egg yolks. Similar results were reported by Onibi *et al.* (2008) that the use of 6-20% *Leuceana* flour or cassava in laying chicken feed and broiler chicken. Beta-carotene and vitamin E are natural antioxidants. It is known that antioxidants have an important role in inhibiting and stalling free radicals. In addition, flavonoids and vitamin C (Andarwulan, 2012) and other phenolic compounds (Nahak and Sahu, 2010) can also act as natural antioxidants.

CONCLUSION

We conclude that the addition of *Moringa oleifera* leaf extract at the level of 2-6 c/100 cc in drinking water can increase egg weight, egg numbers, hen-day production, feed efficiency, and egg yolk color. But, may decrease both fat and yolk cholesterol in laying hens up to thirty weeks of age.

ACKNOWLEDGEMENTS

The authors would like to thank to staff of laboratory attendants at the Nutrition Laboratory, Faculty of Animal Science, Udayana University for their assistance in chemical analysis of the samples. We also would like to thank the Head of Research and Public Service Department and Rector of Udayana University for their support during this study including research funding.

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Corresponding author: Ni Wayan Siti, Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia, Jl. PB. Soedirman, Denpasar-Bali, Indonesia
Email: *wayansiti@unud.ac.id* bidura_unud@yahoo.com