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Effect of Fermented Moringa oleifera Leaf with Saccharomyces Sp. Extract in Drinking Water on Egg Production, Yolk Colour, and Egg Cholesterol Levels in Laying Chicken

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ABSTRACT

The present study was conducted to examine the effect of fermented Moringa leaf with Saccharomyces sp. (FMLS) extract in drinking water on egg production, yolk colour, and egg cholesterol levels in laying chicken. One hundred and twenty 40-wk-old hens were colony caged in an environmentally controlled house to evaluate the levels of FMLS extract in administration on hens. FMLS extract was prepared by macerating FMLS in distilled water (1:1, w/w). Hens were randomly divided into four equal groups: one served as a control and was administered with drinking water only. The other three groups were administered 2%; 4%; and 6% water extract of FMLS, respectively. Fermented Moringa leaf (FMLS) extract increased significantly (P<0.05) egg production and yolk colour, but not in egg weight (P>0.05). Fermented Moringa leaf extract administration results in lower (P<0.05) yolk cholesterol contents. Fermented Moringa leaf extract in drinking water increased egg production and yolk colour, but decreased yolk cholesterol contents of egg laying hens.

Keywords: Moringa leaf, Fermented, Cholesterol and Yolk Colour.

INTRODUCTION

Public awareness of the importance of animal protein fulfillment leads to high demand for chicken eggs. High market demand for chicken eggs makes this type of chicken much maintained by farmers. High cholesterol content in foodstuffs tends to be the main consideration of consumers in consuming food of animal origin, because it is a source of cholesterol for upper middle class people that can cause degenerative diseases such as coronary heart, characterized by hardening of walls arterial and high fat content (hyperlipidemia) in the blood especially cholesterol (hypercholesterolemia).

Moringa oleifera leaf is a traditional medicinal plant that has high nutrients, as an antibacterial, and contains beta-carotene as an egg yolk active substance. Phytochemical compounds enclosed there in are: flavonoids, saponins, tannins, and some other phenolic compounds that have antimicrobial activity (Bukar et al., 2010). Estrogen-like flavonoids can reduce bone loss (osteomalacia), can lower blood cholesterol levels, and increase HDL levels, while saponins are shown to be anticancer, antimicrobial, and lower serum cholesterol levels (Bidura et al., 2017). Producing quality egg products with high ration efficiency using the Moringa leaf extract (Sugiharto et al., 2017; Bidura et al., 2012) will provide knowledge to small-scale farmers to achieve food security, as well as enhance their business competitiveness to increase their income.
Fermentation of feed ingredients turned out to increase the nutritional value of feed, as reported by Santoso et al. (2016); Sugiharto et al. (2015); and Sugiharto et al. (2017) can significantly increase the nutrient content of feed, and can improve poultry performance compared with non fermentation. Beta-carotene can be increased in fermentation with fungus (Trichoderma harzianum), because these fungi are carotenogenic (producing beta-carotene) (Hirschberg; 2001; Hsieh and Yang, 2003; Ma et al., 2000). The ability of beta-carotene decreasing cholesterol associated with hydroxy methyl glutaril enzyme-CoA (Wang and Keasling, 2002). Fermentation of feed by using microbes that act as probiotics become interesting to study. Because probiotic microbes remain in fermented feed before and after consumed by poultry host. Previous studies have indicated that probiotics have beneficial effects on growth performances of poultry and blood parameters (Dlamini et al., 2012; Bidura et al., 2016; Hasan et al., 2016; Siti et al., 2014), improve nutrient quality of feed (Bidura et al., 2014; Bidura et al., 2015; Bidura and Siti, 2017; Candrawati et al., 2014), and can decrease egg cholesterol levels (Bidura et al., 2016). Besides being used separately, probiotic Saccharomyces sp and other herbs (Moringa oleifera) may be used simultaneously to improve the quality and quality of poultry products. Khmir S.cerevisiae in the digestive tract of poultry is expected to be able to synergize with poultry digestive microbe, so it will be able to synergize the beneficial effects together with active compounds on Moringa leaves to increase the quantity and quality of poultry production. Therefore, this study was conducted to examine the effect of FMLS in drinking water on on egg production, yolk colour, and egg cholesterol levels in laying chicken.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This study used 240 Lohmann Brown hens, 40 weeks of age, with a homogeneous body weight of 1758.25 ± 25.82 grams obtained from a commercial poultry farm. All chickens 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 phospor of 0.45%. Each cage equipped with food and drinking holder placed outside the cage. Sanitation of the equipment is done everyday by cleaning places for feeding and drinking. For the treatments, hens were placed into four groups each containing 10 hens: (A) hens were only given water as a drink, (B) hens were given 2 cc of FMLS extract in 100 cc of drinking water; (C) hens were given 4 cc of FMLS leaf extract in 100 cc of drinking water; and (D) hens were given 6 cc of FMLS leaf extract in 100 cc of drinking water. Each treatment was repeated 6 times for a total of 240 hens. Food and drinking water were given ad libitum. The individual hens were weighted weekly, food consumption and egg production was recorded daily.

Performance, egg quality metrics, and laboratory analysis: 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 colour was determined by using a Roche yolk colour fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. The yolk samples were randomly collected from two (2) birds per replicate at the end of the study at 10\(^\text{th}\) week and analyzed for the estimation of yolk to determine the total yolk cholesterol content.

Process of making flour Moringa leaves and fermentation: Fresh leaves of the Moringa leaf were obtained from the local fresh food market. Before being fermented, Moringa leaves that have dark green, thinly sliced and dried in room temperature for 1-2 days, then dried in oven at temperature 50°C for 24 hours. Furthermore Moringa leaves were ground to fine powder form. Powdered Moringa leaves were then analyzed and prepared for fermentation using khmir Saccharomyces spp. N-2 (isolated from yeast culture) (Bidura et al., 2012), with inoculum dose of 5% incubated for 4 days with the thickness of 2 cm substrate at pH 5.5 and temperature 30°C and than macerated overnight in distilled water (1:1, w/w) (Parwata et al., 2016). The blended extract was then filtered using a cheese cloth. This extract was used for the treatment.

Performance, egg quality metrics, and laboratory analysis: 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, feed conversion ratio (FCR, feed DM intake/total egg weight). Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk colour was determined by using a Roche colour fan (1 to 15). 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
The results study shows that 2-6% FMLS extract in drinking water significantly increased (P<0.05) the average total egg weight and the average number of eggs produced as well as the average hen-day production were noted among the treatments (Table 1). However, no significant differences (P>0.05) in the feed consumption, water consumption, and egg weight per head (g/head). The average value of FCR (feed consumption: total egg weight) over ten weeks of observation in the control group was 2.43/head (Table 1). This was significantly different (P<0.05) from hens in treatment groups B, C, and D an average of 7.41%; 4.94%; and 4.53% more than control (Group A), respectively.

Table 1. The effect of FMLS aqueous extract added in drinking water and administered to 40-50 weeks aged of egg laying hens to the egg production and feed efficiencies.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups†††</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Consumption (g/head/70days)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>SEM††</td>
</tr>
<tr>
<td></td>
<td>9290.38a</td>
<td>9307.45a</td>
<td>9348.92a</td>
<td>9295.36a</td>
<td>78.481</td>
</tr>
<tr>
<td>Water consumption (/head/70days)</td>
<td>27.361a</td>
<td>28.074a</td>
<td>28.048a</td>
<td>27.695a</td>
<td>1.052</td>
</tr>
<tr>
<td>Total egg weight (g/head/70 days)</td>
<td>3818.34b</td>
<td>4136.64a</td>
<td>4047.15a</td>
<td>4015.11a</td>
<td>52.925</td>
</tr>
<tr>
<td>Egg weight (g/head)</td>
<td>67.68a</td>
<td>66.68a</td>
<td>65.02a</td>
<td>64.51a</td>
<td>1.973</td>
</tr>
<tr>
<td>The number of eggs (egg/70 days)</td>
<td>56.42b</td>
<td>62.04a</td>
<td>62.24a</td>
<td>62.24a</td>
<td>1.402</td>
</tr>
<tr>
<td>Hen-day production (%)</td>
<td>80.06a</td>
<td>88.63b</td>
<td>88.92b</td>
<td>87.85b</td>
<td>1.375</td>
</tr>
<tr>
<td>Feed conversion ratio (feed consumption: total egg weight)</td>
<td>2.43a</td>
<td>2.25b</td>
<td>2.31b</td>
<td>2.32b</td>
<td>0.025</td>
</tr>
<tr>
<td>Yolk colour (1 to 15)</td>
<td>7.42a</td>
<td>8.61b</td>
<td>8.84b</td>
<td>8.75b</td>
<td>0.327</td>
</tr>
<tr>
<td>Yolk cholesterol (mg/dl)</td>
<td>527.31a</td>
<td>489.53b</td>
<td>492.96b</td>
<td>483.05b</td>
<td>5.813</td>
</tr>
</tbody>
</table>

Notes:
1. A: drinking water without FMLS extract as control; (B): drinking water with 2 cc/100 cc FMLS extract; and (C): drinking water with 4 cc/100 cc FMLS extract, and drinking water with 6 cc/100 cc FMLS extract, 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 FMLS extract (2-6 cc/100 cc) in drinking water resulted in a significant (P<0.05) increase in yolk colour of eggs (Table 1). Eggs yolk colour of the group C was 8.84 followed by group D was 8.75; 8.61 (B); and 7.42 (Group A), respectively.

The cholesterol content in cholesterol content in egg yolk in chickens decreased significantly (P<0.05) in the presence of FMLS extract in drinking water. Increased level of FMLS in drinking water was significantly (P<0.05) lowered yolk cholesterol levels. Yolk cholesterol content of the group D was 8.39 followed by group B was 7.16%; and 6.51% (C) were lowered significantly different (P<0.05) than control (A), respectively.

DISCUSSION
The results of the first year study showed that phytochemical compounds contained in *Moringa* leaves are: flavonoids, saponins, tannins, and some other phenolic compounds that have antimicrobial activity. Supplementation of *Moringa oleifera* leaf extract at a concentration of 2-6 cc/100 cc on drinking water were increase egg production and lower cholesterol content in chicken eggs (Siti et al., 2017). Supplementation of FMLS extract in drinking water was found to have the same effect with first year research result, that is the increase of total egg weight and number of eggs compared with control, and no effect on feed consumption. Oka et al. (2016) reported that the antioxidant properties of the leaves of *Moringa* are very high, also high tannin content, that is equal to 831.92 mg/100 ml. Phytochemical compounds contained in Moringa leaves grown in Bali are alkaloids, flavonoids, phenolics, triterpenoids/ steroids, and tannins (Putra et al., 2016). This is due to the active compounds in fermented *Moringa* leaf extract, such as flavonoids, saponins, tannins, and beta-corotene.
The main workings of these active ingredients are by inhibition of pathogenic microbes and endotoxins in the intestines, increasing pancreatic activity, increasing metabolism, and absorption of nutrients in the gastrointestinal tract of chicken (Windisch et al., 2008; Grashorn, 2010). *Moringa* can be a phytogenic compound in feed based on its bioactive compounds, which can improve egg quality and have a positive impact on the health and performance of poultry (Yang et al., 2006; Portugaliza and Fernandez, 2011; Zanu et al., 2012; Ola-Fadunsin and Ademola, 2013). The content of β-carotene and quercetin compounds in *Moringa oleifera* leaves, ranging from 2.7–3.10 mg/100 g and 80–150 mg/100 g, respectively (Amaglo, 2010; Saini et al., 2014a; Saini et al., 2014b). When added to the ration, the bioactive compound along with other phytochemical compounds can improve egg quality and have a positive effect on the health and performance of the chicken. The fermentation process on *Moringa* leaves by probiotic microbes (FMLS) will be able to double function. First, before it is given to livestock, probiotic microbes will be able to help break down complex compounds into simple compounds that are easily digested by digestive enzymes. In addition, the fermentation process will be able to remove antinutritious compounds in feed ingredients, such as tannins. The second advantage of fermentation process by using probiotic microbe is when given to chicken, hence probiotic microbe will be able to assist metabolism process of nutrient in gastrointestinal tract of poultry. The main concept of fermentation of feed with probiotics is to increase the activity of probiotic microbes or provide a suitable condition to increase the number of bacteria involved in probiotics. Basically, fermentation is a chemical transformation of organic matter into simple compounds by active enzymes, ie complex organic catalyst produced by microorganisms, such as bacteria, yeast, or fungi. The use of wet fermented feed with probiotics can improve the performance of chickens (Hasan et al., 2016; Lokaewmanee et al., 2012; Sugiharto, 2016).

As reported by Bidura et al. (2012), supplementation of probiotics as fermented feeding inculants is very important because it can increase the rate of growth, weight gain, feed use efficiency, and poultry performance. In addition, fermentation products affect the ecology of bacteria in the gastrointestinal tract and reduce the level of *Enterobacteriaceae* in various parts of the gastrointestinal tract of broiler chickens (Heres et al., 2003). The administration of fermented products by probiotics can lead to the reduction of pathogenic bacteria, including *Salmonella* and *Campylobacter* in the livestock digestive tract (Yamamoto et al., 2004; 2007). The same thing was reported by Hasal et al. (2016) that the use of wet fermented feed products with probiotic microbes can improve chicken performance. Lomkan et al. (2015) reported that the use of dried fermented products with probiotics had a very significant improvement on chicken performance parameters. It is assumed that fermented feeds generally improve the bacterial ecology of the gastrointestinal tract and the immune response in chicks. Therefore, it becomes an interesting study to improve the efficacy of *Moringa oleifera* leaf herbs through fermentation with probiotic microbes before being used as feed supplements in chickens, as well as to control chicken disease. This is interesting because the efficacy of herbs products fermented by microbial probiotics is double efficacious when compared with the original product (Bidura et al., 2014; Santoso et al., 2015; Wibawa et al., 2016). In addition to the goal of improving health, probiotics *Saccharomyces sp.* has long been used to improve the digestion and growth of poultry performance (Bidura et al., 2012; 2014; 2016). Besides being used separately, probiotics *Saccharomyces sp.* and other herbs additives can be used simultaneously to increase weight gain and feed efficiency of poultry. Provision of probiotics is associated with improved chemical, nutritional, and quality of broiler meat (Liu et al., 2012). Supplementation of the FMLS extract in drinking water resulted significantly increased the yolk colour of eggs. Probiotics can improve egg weight, feed efficiency, yolk colour, and egg shell quality (Mallo et al., 2010; Mountzouris et al., 2010). The colour is an important quality trait of foods since it affects the consumers’ perception of quality and intensity of aroma and flavour and their decision on purchase (Loetscher et al., 2013). Eggs yolk colour from the FMLS extract in drinking water had significantly higher eggs yolk colour compared to the control. Changes observed in yolk colour are largely associated with the ingredients used in diets. Carotenoids play an important role in the development of different colour scores of egg yolk, especially, lutein is active yolk colourant. Some researchers reported that supplementing herbal extract showed potential for increasing in egg yolk colour. It was the same observed by Lokaewmanee et al. (2009); Zhao et al. (2013) and Bidura et al. (2017) on the effects of herbs leaf increase in egg yolk colour. That the increase of the amount of FMLS extract in drinking water resulted in a linear increase in egg yolk colour. Cayan and Erenel (2015) reported that this increase in egg yolk colour can be attributed to the carotenoid contents of olive leaf powder. Beta-carotene and vitamin E are natural antioxidants, and antioxidants have an important role in inhibiting and scavenging free radicals. Mabusela et al. (2018) reported that *Moringa oleifera* meal can improved yolk colour, maintained external egg quality, and improved the fatty acid profile.
Decreasing of cholesterol levels in egg yolks in chickens supplemented *Moringa* fermented leaf extract. This is caused by bioactive compound flavonoids (quercetin) and carotenoids (β-Carotene) positively affected and reduced the levels of creatinine, glucose and cholesterol levels in the serum, which showed improved liver performance, whereas lowered creatinine levels indicated better kidney functionality in FMLS supplemented groups (Melesse et al., 2013; Elkloub et al., 2015). Lowered cholesterol levels in the yolk show the hypocholesterolemic effect of *Moringa*, which might be attributed to β-sitosterol-rich plant material, which has same structure as cholesterol and lowers uptake from the intestine (Ghasi et al., 2000). These antioxidants (β-Carotene and quercetin) and phytosterols (β-Sitosterol) affect the functionality of liver, kidneys and heart, resulting in improved metabolism, as indicated in biochemical parameters and antibody titers (Ghasi et al., 2000). *Katuk* (*Sauropus androgynus*) leaf can lower cholesterol levels because of the content of beta-carotene (Wardiny, 2006). Beta-carotene can be increased in fermentation with fungus *Trichoderma harzianum*, because these fungi are carotenogenics (producing beta-carotene) (Ma et al., 2000; Hirschberg, 2001; Hsieh and Yang, 2003). The ability of beta-carotene decreasing cholesterol associated with hydroxy methyl glutaril enzyme-CoA (HMG) (Wang and Keasling, 2002). This enzyme plays a role in the formation of mevalonic in the biosynthesis of cholesterol. Cholesterol synthesis and synthesis of beta-carotene are together through mevalonial and derived from acetyl CoA. If increasing consumption beta-carotene greater than saturated fatty acid, it makes biosynthesis process by enzyme HMG-CoA directed at beta-carotene, so that saturated fatty acids are not converted into cholesterol (McGilvery and Goldstein, 1996; Nuraini, 2006). The decrease in egg cholesterol levels is also due to the presence of probiotic microbes (*Saccharomyces spp.*) used as inoculants in *Moringa* leaf fermentation. Probiotics can improve egg weight, feed efficiency, yolk colour, egg shell quality, pathogen inhibition (Mallo et al., 2010; Mountzouris et al., 2010) and have the ability to lower serum cholesterol levels (Kusumawati et al., 2003). The lowest cholesterol level was obtained by feeding the chickens with diets containing 14% fermented katuk leaf (Syahruluddin et al., 2013). Santoso et al. (2015) reported that *Saccharomyces cerevisiae* fermented *Sauropus androgynus* leaves inclusion resulted in the best broiler meat quality as indicated by lower fat and cholesterol. Ekayuni et al. (2017) reported that supplementation of *Moringa oleifera* extract of 50 ml/liter of drinking water can reduce abdominal fat and cholesterol levels in meat in broiler chickens.

**CONCLUSION**

We conclude that an additional 2-6 cc/100 cc of FMLS extract in drinking water were increased egg production and yolk colour, but may decrease yolk cholesterol in laying hens up to forty weeks of age.

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