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The Effect of Aqueous Extract of Fermented Garlic Leaf (*Allium Sativum*) on the Egg Production and Yolk Cholesterol Contents of Layers

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ABSTRACT

Objective: *The present study was conducted to determine the effects of fermented Garlic (Allium sativum) leaf in aqueous extract on the egg production and yolk cholesterol level of 40-week old hens.* ***Material and Methods****: One hundred and twenty 40-wk-old hens were colony caged in an environmentally controlled house to evaluate the levels of fermented Garlic leaf in aqueous extract administration on hens. Fermented Garlic leaf extract was prepared by macerating fermented Garlic leaf 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 fermented Garlic leaf, respectively.* ***Results:*** *Fermented Garlic leaf extract increased egg production (P<0.05), but not the efficiency of feed consumption (P>0.05). Fermented Garlic leaf extract administration results in lower (P<0.05) serum and yolk cholesterol contents.* ***Conclusion:*** *Fermented Garlic leaf extract in drinking water increased egg production, but decreased serum and yolk cholesterol contents of egg laying hens.*

Keywords: *Allium sativum*, fermented, cholesterol, egg laying hens

INTRODUCTION

Egg chickens are livestock products that have more advantages. Eggs are a delicate food product and lose quality rapidly during the period between collection and consumption. However, because of the egg high in fat and cholesterol, it is suspected as a cause of stroke and coronary heart disease. Decreasing egg yolk cholesterol would be beneficial to the poultry industry and public health (Mahmoud *et al*., 2010). Thus, improving and extending egg shelf life is important to breeders and other poultry researchers. Numerous efforts have been made to lower the cholesterol content of eggs. One alternative feedstuff that can be used to reduce levels of fat and cholesterol is *Garlic* leaves (*Allium sativum*). The use of *Garlic* leaves depends on productivity and potential health benefits of *Garlic* as well as food consumption and for medicinal purposes, however, the potency for utilization *Garlic* leaves extract as poultry supplemented ingredients are also need to be studied.

In many countries different types of plant extracts have been used in traditional medical systems to treat for considering to control many diseases, particularly atherosclerosis and dyslipidemia and microbial disease. According of Akinmoladun *et al.* (2007), that phytochemicals present in those plants having antimicrobial and antioxidant properties are

the reason for this ability to use them in disease treatments. Antimicrobial activities of some phytochemicals present in such plants have been investigated and the possibility of using them to develop new antimicrobial drugs has also been studied (Dalukdeniya *et al., 2016).*

Garlic has been shown to have anti-thrombotic activity, blood lipids, blood pressure, and has a protective effect on the heart (Kasuga *et al.,* 2001), antibacterial properties, and potent food pathogen inhibitors (Lee *et al*., 2003). The mechanism of garlic has been shown to be an effective antioxidant and its ability to stimulate the immune response (Lim *et al*., 2006). *Garlic* shows efficacy as a broad-spectrum antibiotic against gram-positive and gram-negative bacteria. Other benefits of *Garlic* are reduced cholesterol levels (Bidura *et al*., 2017), blood pressure, cancer prevention, immune system, and infectious treatment, as well as antioxidants (Ao *et al*., 2010). *Garlic* contains sulfur compounds, such as allicin, diallyl disulfide (DADS), and diallyll trisulphida (DATS), which are responsible for most pharmacological properties of *Garlic*, while non-sulfur compounds from *Garlic* include allixin, flavonoids, saponins, and fructans (Silvam, 2001).

Fermentation of herbal leaves (*Sauropus androgynus*) were increased beta-carotene 173 ppm/254 ppm before fermentation and after fermentation (Syahruddin and Rita, 2012). According Hsieh and Yang (2003) and Hirschberg (2001), beta-carotene can be increased in fermentation with fungus *Trichoderma harzianum*, because these fungi are carotenogenic (producing beta-carotene). The ability of beta-carotene decreasing cholesterol associated with hydroxy methyl glutaril enzyme-CoA (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 mevalonic and derived from acetyl CoA.

This study was conducted to evaluate effect of aqueous extract of fermented Garlic leaf (*Allium sativum*) on the egg production and yolk cholesterol contents of layers up to fourty weeks of age.

MATERIALS AND METHODS

Animals, treatments, and experimental design: This study used 120 Lohmann Brown hens, 40 weeks of age, with a homogeneous body weight of 1725.38 ± 20.72 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 phosphor of 0.45%. For the treatments, hens were placed into four groups each containing 5 hens: (A) hens were only given water as a drink, (B) hens were given 2 cc of fermented Garlic leaf extract in 100 cc of drinking water, (C) hens were given 4 cc of fermented *Garlic* leaf extract in 100 cc of drinking water, and hens were given 6 cc of fermented *Garlic* leaf extract in 100 cc of drinking water (D), respectively. Each treatment was repeated 6 times for a total of 120 hens. Feed and drinking liquid were given *ad libitum*. The individual hens were weighted weekly, and feed consumption and egg production was recorded daily.

Process of making flour *Garlic* leaves and fermentation: Before being fermented, *Garlic* 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 *Garlic* leaves were ground to fine powder form. Powdered *Garlic* leaves were then analyzed and prepared for fermentation using Khamir *Saccharomyces sp*, with inoculum dose of 9% incubated for 10 days with the

thickness of 2 cm substrate at pH 5.5 and temperature 30°C (Syahruddin *et al*., 2013). The leaves of Garlic fermented were blended and macerated overnight in distilled water (1:1, w/w) (Parwata *et al*., 2016). The blended of fermented leaves extract was then filtered using a cheese cloth. This fermented 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. Once every two weeks, the eggs from three consecutive days were used to measure egg weight and quality. Yolk color was determined by using a Roche color fan (1 to 15). Yolk cholesterol content was analyzed for two consecutive weeks. The blood samples were randomly collected from two (2) birds per replicate at the end of the study at 10th week and analyzed for the estimation of blood to determine the total serum cholesterol content. 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% fermented of Garlic (*Allium Sativum*) leaf extract in drinking water significantly increased (P<0.05) the average number of eggs produced, the average total egg weight 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 2.56/head (Table 1). This was significantly different (P<0.05) from hens in treatment groups B, C and D an average of 7.81%; 9.38% and 8.20%.

Table 1. The effect of fermented Garlic (*Allium Sativum*) leaf water extract added in drinking water and administered to 40-50 weeks aged of egg laying hens on the egg production, feed conversion ratio, serum and yolk cholesterol levels.

Variables	Groups[1)]				SEM[2)]
	A	B	C	D	
Feed Consumption (g/head/70d)	9940.37a	10025.28 a	10102.14 a	10082.51 a	65.69 2
Water consumption (l/head/70d)	29.671a	30.725a	31.029a	31.155a	1.528
Total egg weight (g/head/70 days)	3882.05b[3)]	4239.95a	4350.28a	4283.70a	72.09
Egg weight (g/head)	67.58a	67.96a	69.55a	68.80a	1.904
The number of eggs (egg/70 days)	57.44b	62.39a	62.55a	62.26a	1.305
Hen-day production (%)	82.05a	89.13b	89.36b	88.94b	2.037
Feed conversion ratio (feed	2.56a	2.36b	2.32b	2.35b	0.041

consumption: total egg weight)					
Ether extract of yolk (%)	25.51[a]	23.74[b]	22.83[b]	23.37[b]	0,307
Yolk color (1 to 15)	7.19 [a]	8.84[b]	8.59[b]	8.77[b]	0.206
Serum cholesterol (mg/dl)	167.05a	153.81b	150.72b	152.90b	2.271
Yolk cholesterol (mg/dl)	531.74a	487.81b	490.05b	495.63b	8.037

Notes:

1. (A) hens were only given water as a drink, (B) hens were given 2 cc of fermented *Garlic* leaf extract in 100 cc of drinking water, (C) hens were given 4 cc of fermented *Garlic* leaf extract in 100 cc of drinking water, and hens were given 6 cc of fermented *Garlic* leaf extract in 100 cc of drinking water (D), 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 2-6%% (2-6 cc/100 cc) of *Garlic* (*Allium Sativum*) leaf extract in drinking water resulted in a significant (P<0.05) decrease in levels of ether extract in the yolk of hens (Table 1). *Garlic* (*Allium Sativum*) leaf extract in drinking water had significant (p<0.05) reduction both in serum and yolk cholesterol level than the control (group A). The lowest mean serum cholesterol level was group C (150.72 mg/dl) or 9.78% lower than control. The serum cholesterol levels on group B, C and D had no a significantly (P>0.05) different, respectively.

Eggs yolk cholesterol levels from the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) also had significantly (p<0.05) lower cholesterol content compared to the control (Table 1). Eggs yolk cholesterol levels of the group B was 8.26%, followed by group C with yolk cholesterol levels was 7.84% and Group D was 6.79% were decreased rather than group A.

The results study shows that 2-6% fermented of *Garlic* (*Allium Sativum*) leaf extract in drinking water significantly increased (P<0.05) the average yolk color of eggs. Eggs yolk color of the group B, C, and D were 22.95%; 19.47%, and 21.97% higher rather than group A (Table 1).

DISCUSSION

We measured the effects of oral administration of the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) on egg production and yolk cholesterol levels in layer hens. The extract increased total egg weight and egg production, but there was no significant change in feed and water consumption. Serum and egg-yolk cholesterol content decreased significantly in the egg-laying hens administered the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc). The banning of the use of antibiotics as feed additives has accelerated and led to find nontraditional feed additives to be used in animal feeds. Herbal extracts are being used as feed additives to improve animal performance especially under the intensive management systems (William and Losa, 2001). Both health status and general performance can be supported by plant extracts (Gill *et al*., 2002).

In addition, the results show that an additional of the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) resulted in a significant increased in hen egg production with a significant increase in total egg weight and the number of eggs. Similar by

Bidura *et al. (*2017) observed also reported that laying hens fed on *Allium sativum* and *Sauropus androgynus* showed higher egg production intensity and increases in egg mass. Supplemented of herb water extract was abled to increase eggs weight and egg yolks (Suarjana *et al*., 2018). *Moringa oleifera* leaves extract in drinking water resulted increased egg production and feed efficiencies (Siti *et al.*, 2017). Supplementing herbal extract showed potential to promote the improve the small intestine morphologi by different mechanisms (Karukarach *et al.*, 2016).

Herbs and their metabolites, known as bioactive compounds, play a key role because of their feed additive attributes (Grashorn, 2010). Some researcers (Windisch *et al*., 2008; Wallace *et al*., 2010; Ao *et al*., 2011; Embuscado, 2015) reported that these bioactive compounds, such as carotenoids, flavonoids, and essential oils, help to maintain animal health and productivity, and to produce safe and healthy chicken eggs. Ahmad *et al.* (2017), reported that herbs (*Moringa oleifera*) contains bioactive compounds such as beta-carotene, quercetin, and selenium. According Windisch *et al.* (2008), the primary mode of action of these active ingredients is inhibition of pathogenic microbes and endotoxins in the gut and enhanced pancreatic activity, resulting in better nutrient metabolism and utilization.

We found that the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) resulted significantly increased the efficiency of feed (feed consumption: total egg weight). This could be the a result of phytochemical contained in fermented *Garlic* leaf. Adibmoradi (2006) reported that administration of herbal extract (*Garlic*) can markedly increase villus height and crypt depth. Nusairat (2007) showed that the thickness of the epithelium and the number of goblet cells in the duodenum, jejunum, and ileum of poultry decreased and these features can increase nutrient absorption. They concluded that the morphological changes in the intestines of birds increases digestive capacity. Ramakrishna *et al. (2003)* reported that phytochemical compounds probably enhanced the activities of the pancreatic enzymes and provided micro-environment for better nutrient utilization in rats.

The fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) resulted significantly increased the yolk color of eggs. Acoording Loetscher *et al*. (2013), the color is an important quality trait of foods since it affects the consumers' perception of quality and intensity of aroma and flavor and their decision on purchase. Most consumers associate yolk color to age and health status of the animal and to the quality of eggs and egg products, but objectively this is not the case. Eggs yolk color from the fermented *Garlic* (*Allium Sativum*) leaf extract extract in drinking water (2 to 6 cc/100 cc) had significantly higher eggs yolk color compared to the control. Changes observed in yolk color are largely associated with the ingredients used in diets. Carotenoids play an important role in the development of different color scores and tones in egg yolk. Especially, lutein is active yolk colorant. Some researchers reported that supplementing herbal extract showed potential for increasing in egg yolk color. 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 color. That increasing the amount of fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water resulted in a linear increase in egg yolk color. Cayan and Erener (2015) reported that this increase in egg yolk color 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.

In addition, the results show that an additional of the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water (2 to 6 cc/100 cc) resulted in a significant decreased in ether extract of yolk, serum and yolk cholesterol of eggs. Patil *et al.* (2010) reported that the reduction of cholesterol and triglycerides by alkaloids were in part caused by the reduction of lipogenic enzymes activities and increased bile acid excretion in feces. The presence of beta-carotene in herbs leaf extract decreases the cholesterol level in the blood as it inhibits the action of HMG-CoA reductase enzyme that plays a role in the formation of mevalonate in cholesterol biosynthesis (Nuraini, 2006). These flavonoids have important role in scavenging of the free radicals. *Garlic* leaf contains phytochemical compounds that have important biological activities. Several previous studies confirmed that extracts or compounds isolated from *M.oleifera* have antioxidant, anti-carcinogenic, anti-diabetic, anti-imflammatory, and anti-hypertensive properties, as well as the ability to protect againts hepatic demage (Godinez-Oviedo *et al*., 2016). *Morinda citrifolia* juice supplementation in drinking water was significantly decreased the total cholesterol, triglyceride, and Low Density Lipoprotein (LDL) levels (Adriani *et al*., 2015). Nuraini (2006) reported tht increasing consumption beta-carotene greater than saturated fatty acid, it makes biosynthesis process by enzyme HMGCoA directed at beta-carotene, so that saturated fatty acids are not converted into cholesterol.

CONCLUSION

We conclude that an additional of the fermented *Garlic* (*Allium Sativum*) leaf extract in drinking water(2 to 6 cc/100 cc) improved egg production and may decrease both serum and yolk cholesterol in laying hens up to fourty weeks of age.

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