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Effect of *Sauropus androgynus* leaf extract, fish oil and vitamin E on performance, egg quality and composition in laying hens

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ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi suplementasi ekstrak daun katuk (*Sauropus androgynus*), minyak ikan dan vitamin E terhadap performa, mutu telur dan komposisi nutrisi telur ayam. Empat puluh delapan ayam petelur (strain Dekalb Warren) umur 72 minggu dibagi menjadi enam kelompok perlakuan sebagai berikut: 1) Ayam petelur diberi pakan tanpa ekstrak daun katuk (EDK), minyak ikan dan vitamin E (P0) sebagai sebagai kontrol; 2) Ayam petelur diberi pakan dengan 27 g EDK/kg pakan (P1); 3) Ayam petelur diberi pakan dengan 27 g EDK/kg pakan dan 1,5% minyak ikan (P2); 4) Ayam petelur diberi pakan dengan 27 g EDK/ kg pakan, 1,5% minyak ikan dan 60 mg vitamin E/kg pakan (P3); 5) Ayam petelur diberi pakan dengan 27 g EDK/kg pakan dan 3% minyak ikan (P4); 6) Ayam petelur diberi pakan dengan 27 g EDK/kg pakan, 3% minyak ikan dan 60 mg vitamin E/kg diet (P5). Hasil penelitian menunjukkan bahwa perlakuan memberikan pengaruh yang berbeda nyata ($P < 0,05$) terhadap produksi telur, tebal kerabang telur, warna kuning, bau telur setelah dibelah ($P < 0,05$). Hasil penelitian menunjukkan bahwa perlakuan berpengaruh tidak nyata terhadap protein, lemak, β-karoten dari telur, tapi mempengaruhi kolesterol dan vitamin E telur. Kesimpulan, perlakuan meningkatkan kualitas dan vitamin E telur tetapi menurunkan kolesterol telur.

Kata kunci: esktrak daun katuk, minyak ikan, kolesterol, kualitas telur, komposisi telur

ABSTRACT

The objective of this study was to obtain enriched eggs with low cholesterol content. Forty-eight laying hens (strain Dekalb Warren) aged 72 weeks were distributed into six treatment groups as follows: 1) Laying hens fed diet without Sauropus androgynus leaf extracts (SALE), fish oil and vitamin E (P0) as the control; 2) Laying hens were fed diet with 27 g SALE/kg diet, but without fish oil (P1); 3) Laying hens were fed diet with 27 g SALE/kg diet, 1.5% fish oil (P2); 4) Laying hens were fed diet with 27 g SALE/kg diet, 1.5% fish oil and 60 mg vitamin E/kg diet (P3); 5) Laying hens were fed diet with 27 g SALE/kg diet, 3% fish oil (P4); 6) Laying hens were fed diet with 27 g SALE/kg 3% fish oil and 60 mg vitamin E/kg diet (P5). Experimental results showed that the treatments affected ($P < 0.05$) egg production, thick eggshell, egg white height, yolk color, the odor of eggs after the split ($P < 0.05$). It was shown that the treatments had no effect on the contents of protein, fat, β-carotene of eggs but affected the contents of cholesterol and vitamin E of eggs. In conclusion, the treatments improved egg quality and increased egg Vitamin E but reduced egg cholesterol.

Key words: Sauropus androgynus leaf extract, fish oil, vitamin E, egg quality, egg compositon

INTRODUCTION

Egg industries are faced with the demand of consumers who need the enriched eggs, but low in cholesterol content. The experimental results showed that there is a positive correlation between blood cholesterol concentration with an increase in the occurrence of atherosclerosis, coronary heart disease, stroke or other metabolic diseases (Willett, 2012). However, it is not easy to reduce cholesterol contents without reducing egg production and egg weight (Dikmen and Sahan, 2007).

Researchers on the enrichment of nutrients in eggs mostly worked only on one nutrient alone. If the feed additive only provides one type of nutrient enrichment, this requires a lot of feed additive which would be difficult in practice. Thus, it is necessary to find a more effective feed additive for enriching nutrients of eggs while lowering cholesterol without lowering production. The potential of feed additive for such purposes is the combination of *Sauropus androgynus* leaves extract (SALE) (Santoso, 2001; Santoso, 2014; Santoso *et al*., 2001; Samad *et al.*, 2013, Samad *et al.*, 2014), fish oil (Bovet *et al.*, 2007) or vitamin E (Traber and Atkinson, 2007). It has been established that SALE improves production and egg quality (Santoso *et al.*, 2005; Santoso *et al.*, 2010b), increase levels of vitamin A and β-carotene, reduce cholesterol, and alter the composition of the fatty acids and amino acids in the egg yolk (Santoso and Fenita, 2016). The inclusion of SALE may be more useful, if SALE is added fish oil as a source of polyunsaturated fatty acids (PUFAs) (Bovet *et al.,* 2007; Domingo *et al.*, 2007; Cherian, 2008). However, it has been established that fish oil is easily oxidized (Huber *et al.*, 2009; Rupasinghe and Yasmin, 2010) resulting in unacceptable color, odor, and flavor of eggs. The inclusion of antioxidants in the diet may overcome this problem. Natural antioxidant, which is very effective for *preventing* oxidation is vitamin E. Vitamin E supplementation of 60 mg/kg ration is very effective for preventing oxidation of PUFAs (Chen *et al.*, 1998).

Based on the description above, this study was designed to evaluate the effect of the supplementation of SALE and fish oil plus vitamin E on performance, egg quality, proteins, amino acids, fatty acids, as well as cholesterol in eggs.

MATERIALS AND METHODS

Sauropus androgynus Leaf Extraction

The extraction of *Sauropus androgynus* leaf was done by the method of Santoso (2001), and rough fish oil was purified by the method of Sari *et al.* (2015).

Maintenance of Laying Hens

This study used the laying hens (strain Dekalb Warren) aged 72 weeks. The level of SALE of 27 g/kg diet at previous study (Santoso and Fenita, 2016) was used in the present study. Forty-eight laying hens were distributed into six treatment groups as follows:

1. Laying hens were fed diet without supplementation (P0).
2. Laying hens were fed diet supplemented with 27 g SALE/kg diet (P1).
3. Laying hens were fed diet supplemented with 27 g SALE/kg diet and 1.5% fish oil (P2).
4. Laying hens were fed diet supplemented with 27 g SALE/kg diet, 1.5% fish oil and 60 mg vitamin E/kg diet (P3).
5. Laying hens were fed diet supplemented with 27 g SALE/kg diet, 3% fish oil (P4).
6. Laying hens were fed diet supplemented with 27 g SALE/kg diet, 3% fish oil and 60 mg vitamin E/kg diet (P5).

Each treatment group consisted of eight replicates with one laying hen each. The composition of experimental diets is listed in Table 1. Laying hens were fed during a 30-day trial. Diet was given as much as 100 g/day/bird. Feed intake, feed conversion ratio, and egg production were measured weekly.

Sampling and Chemical Analysis

At the end of the study, a total of five eggs in each group were collected and were then analyzed the contents of protein, fat, amino acids, fatty acids, cholesterol, β-carotene, vitamin E and fatty acid oxidation in egg yolk. The contents of protein and fat were measured by the method of AOAC (2012). The total lipids of eggs were extracted and methylated with 20% boron trifluoride methanol complex in methanol solution (Kowalski, 2007), and the fatty acid composition was then determined by gas chromatography. Amino acid composition was measured by the

Table 1. The Composition of Experimental Diets

Feedstuffs (g/kg)	P0	P1	P2	P3	P4	P5
Yellow corn	510.00	510.00	505.00	504.94	490.00	489.94
Soybean meal	140.00	140.00	140.00	140.00	140.00	140.00
Rice bran	200.00	200.00	200.00	200.00	200.00	200.00
Fish meal	70.00	70.00	70.00	70.00	70.00	70.00
Palm oil	10.00	10.00	0	0	0	0
Fish oil	0	0	15.00	15.00	30.00	30.00
SALE*	0	+	+	+	+	+
Vitamin E	0	0	0	0.06	0	0.06
Calcium carbonate	35.00	35.00	35.00	35.00	35.00	35.00
Mineral mixture	30.00	30.00	30.00	30.00	30.00	30.00
Premix	5.00	5.00	5.00	5.00	5.00	5.00
Nutrient values						
Protein, %	16.78	16.78	16.73	16,73	16,60	16,60
ME, kcal/kg	2,754.50	2,754.50	2,785.75	2,785.55	2,883.50	2,883.30

*SALE= *Sauropus androgynus* leaf extract was supplemented to diet at level of 27 g/kg diet

method as described by Henderson and Brooks (2010). Cholesterol content was determined by the method as reviewed by Dinh *et al.* (2011). β-carotene and vitamin E were determined by the methods of Grzelinska *et al.* (2007).

Oxidation was measured by analyzing malonaldehyde (MDA) as secondary oxidation products. The analysis was determined by the TBA method as described by Wada *et al.* (2011).

Egg quality measured included egg weight, eggshell thickness, albumen index, yolk index, air sac, yolk color and organoleptic tests. Ten trained sensory panelists were asked to compare the relative palatability of taste and a fishy odor. Yolk color was determined by comparing the color of the yolk with yolk color scale. Panelists were asked to rate the fishy odor and taste of a value of 1 to 5. The odor of eggs assessed based on the grade 1 (very fishy), grade 2 (fishy), grade 3 (slightly fishy), grade 4 (less fishy) and grade 5 (not fishy). Panelists were also asked to taste and rate the taste of bad eggs (score 1) to very good (5).

Statistical Analysis

All data were analyzed ANOVA (Toutenburg and Shalabh, 2009). If they were significantly different they were then tested further by Duncan's multiple range test.

RESULTS AND DISCUSSION

Egg Production

The effect of supplementation of *Sauropus androgynus* leaf extract (SALE), fish oil and vitamin E on egg production is listed in Table 2. The experimental results showed that the treatments affected egg production ($P<0.05$). DMRT test results showed that the P2, P3 and P5 had higher egg production than P0, whereas P4 had lower egg production than P0 ($P<0.05$).

This study differ from previous research that showed the SALE increased egg production in laying poultry (Ekstander *et al.*, 2013; Puspitasari *et al.*, 2012: Santoso *et al.*, 2005, Santoso *et al.*, 2010b). This indicates that the quality of the extracts varied with time and varieties. SALE quality difference is due to differences in the way of growing, harvesting, and the part of the leaves. Therefore, to maintain the stability of the quality of the extract, *Sauropus androgynus* needs to be cultivated commercially using a standard technique of growing and harvesting. In this way, *Sauropus androgynus* leaf could be expected to be

Table 2. The Effect of Supplementation of *Sauropus androgynus* Leaf Extract, Fish oil and Vitamin E on Egg Production (g/bird)

Treatments	Weeks				Total
	I	II	III	IV	
P0	305.00	176.85	185.75	267.48	935.07[b]
P1	203.54	139.90	263.65	263.60	870.69[ab]
P2	347.58	240.20	316.18	301.68	1,214.62[c]
P3	305.40	259.58	352.35	304.95	1,224.72[c]
P4	171.35	252.02	209.42	138.95	771.75[a]
P5	244.60	236.67	295.80	277.60	1,054.00[c]
SD	67.80	48.00	63.90	61.30	171.20

P0=Laying hens were fed diet without supplementation; P1=Laying hens were fed diet with *Sauropus androgynus* leaf extract (SALE); P2=Laying hens were fed diet with SALE, 1.5% fish oil; P3=Laying hens were fed diet with SALE, 1.5% fish oil and 60 mg vitamin E/kg diet; P4=Laying hens were fed diet with SALE, 3% fish oil; P5=Laying hens were fed diet with SALE, 3% fish oil and 60 mg vitamin E/kg diet.

The different superscript within the same colom show significantly different at $P<0.05$.

obtained with a relatively similar quality throughout the year.

The combination of 27 g/kg diet SALE and 1.5% fish oil increased egg production (P2 and P3). This means that substances present in SALE and fish oil contribute synergistically to increase egg production. Mariod *et al.* (2015) reported that the inclusion of fish oil increased hen day egg production. However, the combination of SALE, 1.5% fish oil and vitamin E did not increase egg production further. This result is contrary to the observation of Ziaei *et al.* (2013) who reported higher inclusion of vitamin E increased egg production in laying hens.

The inclusion of 3% fish oil decreased egg production, indicating that administration of fish oil is too high, which will reduce the production of eggs. Fish oil contains omega-3 fatty acid, which is easily oxidized. The more amount of omega-3 fatty acids are oxidized will cause disruption in the body's metabolism and activity of the chicken. The addition of vitamin E to the diet with 3% fish oil improved egg production (P5). Vitamin E is known as a natural antioxidant, and thus, supplementation of vitamin E would prevent oxidation of omega-3 fatty acids (Arab-Tehrany *et al.*, 2012).

Egg Quality

The effect of supplementation of *Sauropus androgynus* leaf extract, fish oil and vitamin E on egg quality is listed in Table 3. The results showed that SALE and fish oil plus vitamin E affected ($P<0.05$) eggshell thickness, and yolk color. DMRT test results indicated that eggshell thickness on the P0 was lower ($P<0.05$) than the P2, P3, and P4, but not different to P1 and P5. Yolk color at P0 was lower ($P<0.05$) than the P3 and P4.

The experimental results showed that although the SALE itself did not increase the eggshell thickness (P1), but its combination with fish oil and vitamin E increased eggshell thickness. As it is known that *Sauropus androgynus* leaf contains compounds that inhibit the metabolism of minerals, especially calcium. It appears that the addition of fish oil and/or vitamin E eliminates negative effects of *Sauropus androgynus* leaf. Saleh (2013) reported that fish oil inclusion at 1.25-2.5% increased eggshell thickness.

SALE by itself was not able to increase significantly the color of egg yolks, but its combination with fish oil increased significantly the color of egg yolks. Fish oil is rich in vitamin A, but this vitamin might not relate to an increase in yolk color since Yuan *et al.* (2014) reported that the inclusion of vitamin A decreased yolk color in broiler breeders. The present study shows although *Sauropus androgynus* leaf is rich in β-carotene (Santoso *et al.*, 2015), it did not increase

the content of β-carotene of yolk. Thus, it appears that other compounds may play a role in improving yolk color in laying hens fed diet supplemented with SALE, fish oil plus vitamin E. Yolk color is affected by several factors, such as different lipid profiles of diet, and the type and concentration of carotenoids (Olson *et al.*, 2008).

Organoleptic Tests

The effect of supplementation of *Sauropus androgynus* leaf extract, fish oil and vitamin E on the taste and odor of eggs is listed in Table 4. The results showed that the inclusion of SALE, fish oil plus vitamin E did not affect the taste, the odor of whole eggs and egg odor after peeling, but affected (P<0.05) egg odor after splitting. Further test results showed that P0 and P4 were more fishy odor (P<0.05) than other treatments.

This study is in contrary with our previous studies, which showed the SALE improved the taste of eggs (Santoso and Fenita, 2016). It was shown that SALE reduced fishy odor in the eggs. The ability of SALE on lowering the fishy odor was affected by the level of addition of fish oil to the diet. It has been established that fish oil increased fishy odor in the eggs (Lawlor *et al.*, 2010). This means SALE can reduce the odor caused by 1.5% fish oil inclusion. Madhu *et al.* (2014) reported that *Sauropus androgynus* leaf had high antioxidant activities, which may inhibit the oxidation of fatty acid resulting in a reduction in a fishy odor of eggs. It has been established

Table 3. The Effect of Supplementation of *Sauropus androgynus* Leaf Extract, Fish Oil and Vitamin E on Egg Quality

Variables	P0	P1	P2	P3	P4	P5	SD
Eggshell thickness (mm)	0.58^{a}	0.55^{a}	0.66^{b}	0.70 ^{b}	0.64^{b}	0.58^{a}*	0.06
Air sac (cm)	0.49	0.47	0.48	0.44	0.46	0.44^{ns}	0.02
Albumen index	0.07	0.07	0.06	0.08	0.06	0.07^{ns}	0.01
Yolk index	0.38	0.39	0.38	0.39	0.37	0.38^{ns}	0.01
Yolk color	6.73^{a}	7.30^{ab}	7.36^{ab}	7.51^{b}	8.16^{b}	7.38^{a}*	0.46
Yolk weight (g)	17.12	16.69	18.44	17.63	16.69	17.14^{ns}	0.67
Eggshell weight (g)	7.91	7.54	7.45	7.21	7.58	7.51^{ns}	0.23

P1, P2, P3, P4, P5 : see Table 2

The different superscript within the same row showed significantly different at P<0.05.

Table 4. The Effect of Supplementation of *Sauropus androgynus* Leaf Extract, Fish Oil and Vitamin E on Egg Taste and Odor.

Variables	P0	P1	P2	P3	P4	P5	SD
Taste	2.75	3.38	3.50	2.94	2.44	2.81^{ns}	0.40
Whole egg odor	3.94	4.19	4.25	4.38	3.94	4.38 ^{ns}	0.20
Egg odor after peeling	3.19	2.81	3.88	3.13	3.38	3.56 ^{ns}	0.37
Egg odor after splitting	2.38^{a}	3.06^{b}	3.44^{b}	3.12^{b}	2.75^{a}	3.38^{b}	0.40

P1, P2, P3, P4, P5 : see Table 2

The different superscript within the same row showed significantly different at P<0.05. ns= non sigficant.

that *Sauropus androgynus* leaf is rich in β carotene, vitamin C, vitamin E and flavonoid (Andarwulan *et al.*, 2012), and other phenolic compounds (Nahak and Sahu, 2010), palmitic acid (Santoso, 2014) and chlorophyll (Samad *et al.*, 2014), protein (Madhu *et al*., 2014), which have antioxidant properties.

Nutritional Composition of Eggs

The effect of supplementation of *Sauropus androgynus* leaf extract, fish oil and vitamin E on β-carotene, protein, fat, cholesterol, vitamin E and TBA of eggs is listed in Table 5. The treatments had no effect on the contents of protein, fat, β-carotene of eggs and TBA (P>0.05), but they affected the contents of cholesterol and vitamin E of eggs (P<0.05). The P3, P4, and P5 had lower cholesterol contents than P0 and P1 (P<0.05), but not different from P2. The P2, P3, P4 and P5 had higher vitamin E than P0.

No change in β-carotene of an egg is in contrary with our previous study (Santoso and Fenita, 2016) who found that the inclusion of 27 g SALE/kg diet increased egg β-carotene content, whereas no change in protein and fat contents of the eggs were in agreement with Santoso and Fenita (2016) study.

SALE itself did not reduce egg cholesterol, which is in contrary to the previous observation (Kasmirah *et al.*, 2013; Santoso *et al*, 2005; Santoso and Fenita, 2016; Santoso and Suharyanto, 2011). The inclusion of fish oil plus SALE reduced egg cholesterol. Suripta and Astuti (2007) reported that fish oil inclusion reduced egg cholesterol in quails.

This study has proven that SALE itself, and in combination with fish oil increased the vitamin E content of eggs. *Sauropus androgynus* leaf contains vitamin E at 79.65 mg per 100 gram edible portion (dry weight basis) (Ching and Mohamed, 2001), whereas fish oil was also known to be rich in vitamin E. Santoso *et al.* (2010a) showed that combination of SALE and fish oil increased the content of vitamin E in broiler meats.

Fatty Acid Composition of Eggs

The effect of supplementation of *Sauropus androgynus* leaf extract, fish oil and vitamin E on fatty acid composition of eggs is listed in Table 6. The treatments had no effect on palmitic acid, stearic acid, oleic acid and linoleic acid (P>0.05), but they affected lauric acid and myristic acid (P<0.05). The P1, P2, P3, and P4 had lower myristic acid than P0 and P5 (P<0.05). The P0 had lower lauric acid than P1, P2, P3, P4, and P5 (P<0.05). The present study is agreement with the observation of Santoso and Fenita (2016) who reported that the inclusion of SALE at 27 g/kg reduced the content of egg miristic acid. In addition, Mariod *et al*. (2015) reported that the inclusion of fish oil also reduced miristic acid content.

Amino Acid Composition of Eggs

The effect of supplementation of SALE, fish oil and vitamin E on amino acid composition of eggs is presented in Table 7. It was shown that the treatments had no effect on aspartic acid, glutamic acid, glycine, histidine, alanine, proline, valine,

Table 5. The Effect of Supplementation of *Sauropus androgynus* Leaf Extract, Fish Oil and Vitamin E on β-Carotene, Protein, Fat, Cholesterol, Vitamin E and TBA of Eggs

Variables	P0	P1	P2	P3	P4	P5	SD
β-carotene (μg/100 g)	667.10	615.91	646.67	634.04	507.38	573.08	58.43^{ns}
Protein (%)	16.47	16.28	16.60	17.11	17.00	16.36	0.34^{ns}
Fat (%)	32.04	31.70	31.45	30.90	30.89	30.65	0.54^{ns}
Cholesterol (mg%)	3.10^{b}	2.94^{b}	2.58^{ab}	2.15^{a}	2.12^{a}	2.07^{a}	0.45^{ns}
TBA(mg/kg)	0.0076	0.0081	0.0073	0.0072	0.0073	0.0071	0.0004^{ns}
Vitamin E (mg/100 g)	9.08^{a}	10.90^{ab}	11.71^{b}	13.76^{cd}	12.85^{c}	14.60^{d}	2.01

P1, P2, P3, P4, P5 : see Table 2

The different superscript within the same row showed significantly different at P<0.05.

Tabel 6. The Effect of Supplementation of *Sauropus androgynus* Leaf Extract, Fish Oil and Vitamin E on Fatty Acid Composition of Eggs

Fatty acids (%)	P0	P1	P2	P3	P4	P5	SD
Lauric acid	0.003^{a}	0.019^{b}	0.060^{c}	0.019^{b}	0.011^{b}	0.293^{d}	0.112
Miristic acid	0.550^{b}	0.318^{a}	0.360^{a}	0.342^{a}	0.381^{a}	0.599^{b}	0.126
Palmitic acid	24.327	24.460	24.364	28.117	24.582	23.977^{ns}	1.554
Stearic acid	0.676	0.634	0.640	0.515	1.008	0.415^{ns}	0.201
Oleic acid	56.152	61.242	62.221	57.757	58.071	57.968^{ns}	2.321
Linoleic acid	8.724	8.042	8.691	7.237	7.266	6.388^{ns}	0.924

P1, P2, P3, P4, P5 : see Table 2

The different superscript within the same row showed significantly different at P<0.05.

Table 7. The Effect of Supplementation of Sauropus androgynus Leaf Extract, Fish Oil and Vitamin E on Amino Acid Composition of Eggs

Amino Acids (%)	P0	P1	P2	P3	P4	P5	SD
Aspartic acid	0.447	0.436	0.344	0.422	0.481	0.367^{ns}	0.051
Glutamic acid	0.613	0.711	0.542	0.694	0.828	0.692^{ns}	0.097
Serine	0.220^{a}	0.350^{b}	0.262^{b}	0.340^{b}	0.385^{b}	0.268^{a*}	0.063
Glycine	0.065	0.099	0.080	0.114	0.126	0.090^{ns}	0.022
Histidine	0.070	0.106	0.069	0.107	0.110	0.097^{ns}	0.019
Arginine	0.240^{a}	0.379^{b}	0.351^{b}	0.404^{c}	0.496^{c}	0.493^{b*}	0.096
Threonine	0.524^{b}	0.161^{a}	0.119^{a}	0.145^{a}	0.192^{a}	0.401^{b*}	0.165
Alanine	0.288	0.391	0.316	0.355	0.356	0.313^{ns}	0.037
Proline	0.105	0.189	0.130	0.165	0.191	0.108^{ns}	0.039
Tyrosine	0.239^{b}	0.180^{b}	0.143^{a}	0.139^{a}	0.198^{b}	0.121^{a*}	0.044
Valine	0.604	0.803	0.701	0.738	0.881	0.702^{ns}	0.095
Methionine	0.128^{a}	0.125^{a}	0.127^{a}	0.153^{b}	0.162^{b}	0.099^{a*}	0.022
Cystine	0.069^{a}	0.089^{a}	0.091^{ab}	0.116^{b}	0.132^{b}	0.087^{a*}	0.023
Isoleucine	0.136^{a}	0.182^{b}	0.158^{ab}	0.242^{b}	0.209^{b}	0.137^{a*}	0.042
Leucine	1.146	1.350	1.080	1.332	1.236	1.232^{ns}	0.104
Phenylalanine	0.137^{a}	0.173^{ab}	0.132^{a}	0.206^{b}	0.209^{b}	0.134^{a*}	0.036
Lysine	0.186	0.223	0.220	0.271	0.216	0.229^{ns}	0.027
Total amino acid	5.217	5.947	4.865	5.943	6.408	5.570	0.558

P1, P2, P3, P4, P5 : see Table 2

The different superscript within the same row showed significantly different at P<0.05.

leucine and lysine (P>0.05), but significantly affected serine, arginine, threonine, tyrosine, methionine, cystine, isoleucine and phenylalanine (P<0.05). Serine was significantly higher in P1, P2, P3, and P4 than P0 and P5 (P<0.05). DMRT test results indicate that a arginine on the P1, P2, P3, P4, and P5 was significantly (P <0.05) higher than P0. Threonine was significantly higher in P0 and P5 than other treatments (P<0.05). Tyrosine was significantly lower in P2, P3, and P5 than in P0, P1, and P4 (P<0.05). Methionine was significantly higher in P3 and P4 than other treatments (P<0.05). Cystine was higher in P3 and P4 than P0, P1 and P5. Isoleusine was higher in P1, P3, and P4 than P0 and P5. Phenylalanine was higher in P3 and P4 than in P0, P2, and P5.

The mechanism of change in amino acid of eggs is still not understood. It was assumed that methyl-pyroglutamate was converted into glutamic acid in the digestive tract. Furthermore, it has been established that *Sauropus androgynus* leaf is rich in glutamic acid (Santoso, 2014; Santoso *et al.*, 2015). Furthermore, glutamic acid might affect the synthesis of other amino acids.

Sauropus androgynus leaf extract increased the number of *Bacillus subtilis* and *Lactobacillus sp.* in gastrointestinal tract (Santoso *et al.*, 2001). *Lactobacillus species* could enhance health effects in the gastrointestinal tract through many mechanisms, which include maintaining normal intestinal microflora, changing metabolism by increasing the activity of digestive enzymes, and decreasing the activity of bacterial enzyme and ammonia production, stimulating the immune system, and improving digestion (Kabir, 2009). Diets with 0.4% *Bacillus subtilis* natto enhanced the absorption of protein, stimulated hormone secretion, suppressed harmful microflora, and improved the duodenal structure and immune functions of Muscovy ducks (Sheng-Qiu *et al.*, 2013). Al-Fataftah *et al.* (2013) reported that an increase in *Lactobacillus sp* enhanced the contents of lysine, methionine and cystine, and vitamin B12 and vitamin B6 in broiler meats. They also reported that the microbial strains had potential for enhancing biosynthesis of vitamin B12, vitamin B6, lysine, methionine, and cystine.

Thus, the change in microorganism balance of gastrointestinal tract may influence amino acid metabolism in poultry. An increase in glutamic acid might stimulate arginine synthesis by bacteria in gastrointestinal tract since Xu *et al.* (2007) reported that glutamic acid has an important role in arginine synthesis in bacteria. This assumption may explain why the content of glutamic acid was not significantly increased.

The conversion of threonine to isoleucine by bacteria in the gastrointestinal tract may explain the occurrence of lower threonine accompanied by higher isoleucine. Methionine synthesis by bacteria starts at homoserine, which is the common precursor for isoleucine, threonine, and methionine. Creek (1968) reported that phenylalanine could be converted to tyrosine in poultry, whereas methionine could be converted to cystine. It appears that the conversion of phenylalanine to tyrosine was inhibited resuting higher phenylalanine dan lower tyrosine. Lower tyrosine may also be caused by the conversion of tyrosine into dopamine, norepinephrine, and epinephrine. There was a considerable synthesis of serine from the α-carbon of glycine in the liver of poultry (Vohra *et al.*, 1956).

CONCLUSION

The results showed that the combination SALE (27 g/kg diet) and 1.5% fish oil increased egg production. The combination of SALE and fish oil at a certain level improved yolk color and eggshell thickness. SALE reduced fishy odor in the eggs. The inclusion of SALE, fish oil, and vitamin E changed the amino acid balance and reduced cholesterol content in the eggs.

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