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Fatty Acid Profile and Cholesterol Levels of Quail Eggs Fed with Kayambang Flour (*Salvinia molesta* D.S Mitchell) in Ration Based on Lemuru Fish Oil and Palm Oil Combination

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ABSTRACT

This study aimed to analyze the effect of adding a *Salvinia molesta* D.S Mitchell, *Sardinela longecips* oil, and palm oil to profile fatty acid compotition of quail egg. This study used a complete randomized design (CRD) with 4 treatments and 5 replications. The ration contained 21% crude protein and 3000 kcal/kg metabolic energy. The treatment rations used, consisted of control rations (without using Kayambang flour (P0) and treatment rations using 2%, 4% and 6% Kayambang flour on rations based on 4% lemuru fish oil and 2% palm oil (P2, P4 and P6) The results showed that the use of Kayambang leaf meal at levels of 2%, 4% and 6% in a ration containing 4% lemuru fish oil and 2% palm oil increased total n-3 fatty acids, EPA and DHA in egg yolks ($P<0.05$). Egg yolk cholesterol tended to decrease with the use of Kayambang flour. It can be concluded that the use of 2% and 4% Kayambang flour resulted in the ratio of n- 6 and n- 3 in egg yolks of 6.74:1 and 7.48: 1.

Key words: cholesterol, fatty acid, omega 3, *Salvinia molesta* D.S Mitchell, *Sardinela longecips*

INTRODUCTION

The development of functional food products to improve public health is growing with public understanding of healthy food ingredients. Healthy food ingredients have their market even though the price is relatively higher than commercial food ingredients. Efficient production of functional products is the goal of many researchers by trying to engineer feed (Sumiati et al. 2016; Melindasari et al. 2015; Darmawan et al. 2013; Liang et al. 2020), including functional products of meat or eggs that are low in cholesterol and high in n- 3 or a balanced product of n-6 and n-3. Indonesia has a source of palm oil high in n-6 and lemuru fish oil high in n-3.

Lemuru fish oil is a by-product of lemuru fish processing, is widely available in Banyuwangi, East Java and has not been widely used (Fanani et al. 2018).The use of lemuru fish oil and palm oil in poultry rations with various use levels has been investigated (Rusmana 2007; Fanani et al 2019; Sani et al 2015; Zaim 2012). The combination of Lemuru fish oil and palm oil used in rations with an antioxidant source of Kayambang flour has not been studied. Kayambang is a plant that local poultry farmers widely supply. Kayambang is easy to cultivate and is widely found on watery land (rice fields, lakes, fish ponds). Kayambang has the potential to be used as an alternative feed for poultry because it has sufficient nutrients, as a feed ingredient and a source of antioxidants. It can be seen from the protein content of Kayambang flour the protein content is 15.90 – 17.34% (Akhadiarto 2010; Sumiati et al. 1987). The linolenic acid content of Kayambang flour is 0.75% (Meliandasari et al. 2015). Kayambang has many phytochemical compounds including phenols, flavonoids, alkaloids, tannins and saponins (Nithya et al., 2016).

Kayambang contains flavonoids 5.6-10.89 mg QE/g, tannins 63.1-90.8 mg/g and saponins 24-42 mg/g. Bachaki et al. (2018) phytochemical compounds in Kayambang extract are tannins, saponins, steroids, titerpenoids. The solvent extract influences phytochemical levels in Kayambang (Bachaki et al. 2018: Nithya et al. 2016). The content of saturated fatty acids in palm oil is 49.9%, and unsaturated fatty acids are 49.7%. The highest type of fatty acid in palm oil was palmitic acid, the highest at 44.0%, then oleic acid, which was included in unsaturated fatty acids, at 39.2% (Mancini et al. 2015). Palm oil contains 44% palmitic acid, 5% stearic acid, 39% monounsaturated oleic acid, and 10% linoleic acid (n-6) (Mancini et al. 1985). Judging from its content, palm oil has a balance between the content of saturated and unsaturated fatty acids and can be used as a source of n-6. Lemuru fish oil contains 58.418 mg/g of polyunsaturated fatty acids n-3 series (Rusmana, 2008). Maulana et al. (2014) stated that lemuru fish oil contains 38.15% saturated fatty acids, 32.18% monounsaturated fatty acids, and 28.44%

polyunsaturated fatty acids. Lemuru fish oil is a source of polyunsaturated fatty acids (PUFA) with the main content of high n-3 fatty acids such as eicosapentaenoic acid (EPA) 14.36% and docosahexaenoic acid (DHA) 4.60% (Suseno et al. 2014). Using Kayambang in rations that use palm oil and lemuru fish oil to produce functional products in the form of quail eggs that have fatty acids, especially n-3 and n-6 in balance, will increase feed engineering innovation. Quail eggs are a cheap source of protein and marketing opportunities are still growing. The functional product of quail eggs with a good balance of omega 3 and omega 6 is a feed engineering innovation that is very beneficial for consumers. This study aimed to obtain quail eggs with a good balance of n-3 and n-6 by using different levels of Kayambang in rations using palm oil and lemuru fish oil.

MATERIALS AND METHODS

Animal unit (Quails), Cages, and Rations

In this study, 196 female *Coturnix coturnix japonica* quails aged 42 days were used that were reared until the age of 91 days. Each treatment had 5 replications and each replication consisted of 9-10 individuals. The initial bodyweight of the quail at the beginning of rearing was 121.25±4.96 grams.

Quails were kept in 20-story battery cages. The cage is equipped with a feed and drinking water container attached to the cage wall. The quail was given a layer ration containing 21% crude protein and metabolic energy of 3000 kcal/kg, in the form of flour (mash). The composition of the layer quail ration and its nutrient content are in Table 1, while the fatty acid content of the treatment ration is in Table 2.

Kayambang Flour Making

Kayambang used came from Situ Sawangan, Depok. Kayambang was cleaned and the roots were removed and then dried at 60^0C and then made into flour. The nutrient content of the Kayambang flour used in this study is listed in Table 3.

Table 1. Composition of layer quail treatment rations

Feed Ingredients	Use of Kayambang flour			
	0%(P0)	2%(P2)	4%(P4)	6%(P6)
Yellow corn	32	36	34	32
Rice bran	17.2	13.2	13.2	13.2
Soybean Meal	17	15	15	15
CGM	10	10	10	10
Fish meal	9	9	9	9
Kayambang flour (TDK)	0	2	4	6
Lemuru Fish Oil	0	4	4	4
Coconut oil	6	2	2	2
CaCO3	6	6	6	6
DCP	2	2	2	2
Premix	0.5	0.5	0.5	0.5
L-Lysin	0	0	0	0
DL-Methionine	0.3	0.3	0.3	0.3
Total	100	100	100	100
Nutrient content of the ration				
Metabolic Energy (kkal/ kg)	3,098.56	3,013.56	2,990.96	2,968.36
Crude protein (%)	21.51	21.01	21.18	21.35
Crude fat (%)	2.02	2.13	2.08	2.03
Crude fiber (%)	3.37	3.48	3.91	4.34
Calcium (%)	3.37	3.38	3.39	3.40
Phosphorus (%)	1.19	1.13	1.13	1.13
Phosphorus available (%)	0.84	0.83	0.83	0.82
Lysin (%)	1.21	1.16	1.16	1.16
Methionine(%)	0.83	0.83	0.84	0.85

Experiment Design and Data Analysis

Completely randomized design with 4 treatments and 5 replications were used in this study. Egg yolk fatty acid measurement variables were obtained from the composite every 2 replications. There are 2 samples used for the measurement of egg yolk fatty acids. The treatment of using Kayambang flour in the ration was its use as much as 2%, 4% and 6%.

The egg yolk fatty acid and cholesterol data were analyzed for variance, then continued with Duncan's test if there were significant differences in variance.

Table 2. Fatty acid composition of treatment ration

Variable (%)	Use of Kayambang flour			
	0% (P0)	2% (P2)	4% (P4)	6% (P6)
Total fatty acids	70.25	60.43	61.41	61.9
SFA	30.6	30.91	35.81	35.96
MUFA	28.51	23.71	22.56	22.71
PUFA	11.13	5.28	3.04	3.21
n-3	0.3	0.31	0.19	0.12
n-6	10.55	5.2	2.4	2.42
n-9	0.14	0.15	0.22	0.46
EPA	0	0.04	0.04	0
DHA	0.04	0.12	0.06	0.03

Table 3 Nutrient content of Kayambang flour

Nutrient	Total
Dry matter (%)	87.04
Water content (%)	12.6
Crude protein (%)	11.19
Crude Fiber (%)	21.14
Crude Fat (%)	1.63
NFE (nitrogen free extract) (%)	43.23
Ash (%)	9.84
Calcium (%)	1.43
Phosphorus (%)	0.27
Gross energy (kcal/ kg)	4572

The results of the analysis of the ITP Laboratory, Faculty of Animal Husbandry, Bogor Agricultural University

Lemuru Fish Oil

The lemuru fish oil used in the ration was obtained from Muncar, Banyuwangi which has a fatty acid profile in Table 4.

Table 4 Fatty acid profile of lemuru fish oil

Fatty acid	Result (%)
Lauric acid C12:0	0.08
Tridecanoic acid C13:0	0.04
Myristic acid C14:0	8.82
Myristoleic acid C14:1	0.02
Pentadecanoic acid C15:0	0.49
Palmitic acid C16:0	21.88
Palmitoleic acid C16:1	5.99
Hepdecanoic acid C 17:0	0.61
Cis-10-heptadecanpat C17:1	0.08
Stearic Acid C 18:0	3.91
Elaidic acid C18:1n9t	0.06
Oleic acid C181n9c	8.60
Linolelaidic acid C18:2n9t	0.05
Linoleic acid C18:2n6c	2.95
Arachidonic acid C20:0	0.37
Y-linolenic acid C18:3n6	0.22
Cis-11-eicosenoic acid C20:1	0.72
Linolenic acid C18:3n3	0.69
Heneicosanoic acid C21:0	0.05
Cis-11,14-eicosedienoate C20:2	0.15
behenic acid C22:0	0.19
Cis-8,11,14-eicosetrienoate C20:3n6	0.20
Arachidonic acid C20:4n6	1.89
Tricosanoic acid C23:0	0.03
Cis-13,16-docosadienoic C 22:2	0.05
Lignoceric acid C24:0	0.12
Cis-5,8,11,14,17-eicosapentaenoate C20:5n3	11.22
Nervonic acid C 24:1	0.28
Cis-4,7,10,13,16,19-docosahexaenoate C22:6n3	10.53
Total fatty acids	80.30

Table 5. Profile of fatty acid types of lemuru fish oil compared to palm oil

Variable(%)	Lemuru Oil	Palm oil *
Total fatty acid	80.3	-
SFA	36.59	49.9
MUFA	15.69	39.2
PUFA	28.01	-
Omega 3	22.44	-
Omega 6	5.26	10.5
Omega 9	0.11	-
EPA	11.22	-
DHA	10.53	-

The results of the analysis of the Integrated Chemistry Laboratory, Bogor Agricultural University; *Research results of Mancini et al. (2015).

Variable Measurement

Egg sampling was carried out in the 7th week. Eggs were broken as many as 20 eggs per replication to take the yolk and then composited from two replications. Egg yolk samples were analyzed for cholesterol and fatty acid content.

Testing cholesterol levels using the Limberman-Burchard method (Kleiner and Dotti, 1958) while testing for fatty acids using the Association of Official Analytical Chemist method (AOAC, 1995).

RESULTS AND DISCUSSION

Quail Egg Yolk Cholesterol

The use of 2%, 4%, and 6% Kayambang flour on egg cholesterol levels is shown in Table 6. Using 4% and 6% Kayambang flour can reduce egg yolk cholesterol by 7%-17% compared to controls. The decrease in cholesterol levels was not large in this study because the crude fiber content of the ration was still within normal limits, namely, 3.37% to 4.34% (Table 1), and the fat content of the ration was 2% (Table 1). Treatment rations using 4% lemuru oil have not significantly reduced cholesterol levels. According to Fanani et al. (2018), giving 4 and 6% fish oil in free-range chicken rations reduces blood cholesterol levels native chickens.

The role of beta-carotene pigment in lowering cholesterol and fat in Kayambang has not been seen as stated by Meliandasari et al. (2015) that Kayambang flour in the ration can reduce cholesterol levels. Decreased cholesterol can occur because of the role of beta-carotene to inhibit the formation of cholesterol by the enzyme HMG-CoA reductase. In addition, Kayambang flour also contains vitamin C of 3.30 mg per 30 g (Kurniawan et al. 2010). The role of vitamin C in Kayambang has not been seen because a high dose of vitamin C of around 150-200 mg/kg is required to show its effect on egg cholesterol levels (Sanda and Suleiman, 2015).

Table 6 Cholesterol and fat content of quail egg yolks

Variable	Use of Kayambang flour			
	0% (P0)	2% (P2)	4% (P4)	6% (P6)
Cholesterol (mg/ g)	19.61±0.56	21.00±1.14	16.21±0.77	18.08±2.34
Fat (%)	30.44±0,24	30.75±0.23	30.51±0.79	30.93±0.4

The disadvantage of producing eggs by using feed ingredients high in unsaturated fatty acids is that they are easily oxidized, so feed ingredients high in antioxidants are needed or you can use Vitamin E supplements. In this study, the role of antioxidants in Kayambang flour was still low, so it did not show its effect on egg cholesterol. Several studies on lemuru fish oil need to be supplemented with vitamin E as an antioxidant (Kouba and Mourot, 2011; Grune et al., 2001; Rymer and Lan Givens., 2010). EPA and DHA increased, the n6:n3 ratio decreased (Koreleski and Swiatkiwiecz, 2005).

Quail Egg Yolk Fatty Acid Profile

The use of Kayambang flour in layer quail rations of 2%, 4% and 6% had no significant effect on the value of total fatty acids, SFA, MUFA, n-9, and PUFA, but was significantly different ($P<0.05$) at the levels of n-3, EPA, DHA and n-6 quail egg yolk (Table 7). The content of n-3 in eggs can be increased by increasing the ration rich in n-3 fatty acids (Fraeye et al., 2012). Still, if not given a source of high n-3 fatty acids in the ration, the levels of n-3 in eggs are low, ranging from 0,13 - 0,14% (Suci et al., 2020) besides that the n-3 level is also influenced by the type of poultry (Suci et al. 2017). In particular, eggs enriched with n-3 PUFAs were more susceptible to lipid oxidation due to the higher proportion of PUFAs (Fraeye et al., 2012). In rations using 2-6% Kayambang flour, rations using 4% lemuru fish oil and 2% coconut oil (P2, P4 and P6) increased total fatty acids n-3, EPA and DHA compared to control rations (without Kayambang). and using 6% coconut oil) (PO). The percentage increase in total n-3 was 77%-190%, the highest increase in EPA was 500% (P2) and DHA the highest was 900%.

Table 7 Fatty acid profile in quail egg yolks

Variable	Use of Kayambang leaf flour			
	0% (P0)	2% (P2)	4% (P4)	6% (P6)
Total fatty acids	84.45±1.99	83.79±2.46	82.09±0.93	84.08±0.19
SFA (%)	29.55±0.8	29.185±1.52	29.99±0.24	30.15±0.04
MUFA (%)	39.4±1.21	37.53±1.55	38.5±1.46	40.6±1.14
PUFA (%)	15.48±1.07	17.13±0.38	13.59±1.16	13.28±0.34
n- 3 (%)	0.75±0.16[a]	2.18±0.17[c]	1.56±0.36[c]	1.33±0.31[b]
n- 6 (%)	12.64±0.7[a]	14.72±0.36[b]	11.70±0.7[a]	12.11±0.74[a]
n- 9 (%)	0.15±0.02	0.15±0.04	0.15±0.04	0.16±0.04
EPA (%)	0.02±0.02[a]	0.12±0.01[b]	0.08±0.02[b]	0.065±0.02[b]
DHA (%)	0.66±0.3[a]	1.86±0.18[c]	1.32±0.33[c]	1.095±0.27[b]
Ratio n-6 : n-3	16.75 : 1	6.74 : 1	7.48 : 1	9.11 : 1
Ratio SFA : UFA	1 : 1.86	1 : 1.87	1 : 1.74	1 : 1.79

The results of the analysis of the Integrated Chemistry Laboratory, Bogor Agricultural University

In this study, the highest levels of n-3 eggs were found in the use of 2% Kayambang compared to other treatments. The increase in n-3 causes increased levels of EPA and DHA because n-3 (Alpha Linoneic Acid) can be converted to EPA and DHA. n-3 functions to help prevent blood clots, develop brain cells, reduce the risk of heart attacks, and be an anti-inflammatory in the body. Murray et al. (1995) stated that n-3 biosynthesis would inhibit n-6 biosynthesis. Fatty acids n-6 and n-3 compete as precursors or raw materials for eicosanoids, eicosanoids are produced from EPA at n-3 and Arachidonic Acid (AA) at n-6. If the amount of n-3 increases, the availability of eicosanoids is met from EPA, so it will suppress the number of n-6. The results showed that omega-6 decreased from control (P0) 12.64% to 11.705% at (P4). Grigorova et al. (2014) stated that the egg yolk of Coturnix coturnix japonica contains n-6 of 12.85%. Fatty acids including the most n-6 is linoleic acid. EPA and DHA are not essential fatty acids because EPA and DHA can be synthesized in the human body and animals, including fish. EPA and DHA in the body can be synthesized from linolenic acid and linoleic acid by consuming foods that contain high levels of linolenic acid and linoleic acid, which will increase the content of n-3 fatty acids EPA and DHA. EPA in the body is useful for forming blood vessels and the heart while DHA is a precursor of rhodopsin, a vital compound in the sensing and nervous system (Atmasier S, 2006). The increase in EPA and DHA was in line with the increase in n-3 levels in quail egg yolks. Linoleic acid and linolenic acid are precursors of EPA and DHA. The highest levels of EPA and DHA were in the treatment of giving 2% Kayambang (P2) under the highest n-3 levels at P2.

In this study, the administration of Kayambang flour, a combination of lemuru fish oil and palm oil in quail rations reduced the omega-6: omega-3 fatty acid balance in egg yolks from 16.75:1 to 6.74:1; 7.48 : 1; and 9.11 : 1. This balance decreases due to the increase in omega-3 fatty acids in egg yolks. According to Kouba and Mourot (2011), the product's ratio of omega 6 and omega 3 ranges from 10:1-15:1. The ideal ratio of omega-6 and omega-3 is if it can be close to 5:1 (Farrel, 1996). Health and Walfare Canada (1990) and the National Research Council (1989) recommend that the balance of omega-6 fatty acids compared to total omega-3 for consumption is 4:1 to 10:1. In this study, the ideal omega 6: omega 3 ratio was obtained using 2% rich (P2). The imbalance in the ratio of omega-6 and omega-3 to the human body can cause coronary heart disease and atherosclerosis due to competition for the formation of reactive eicosanoids in omega-6 and omega-3.

CONCLUSION

Using 2%-6% Kayambang flour in rations based on 4% lemuru fish oil and 2% coconut oil resulted in higher n-3 fatty acid content (linolenic, EPA and DHA) than rations without Kayambang flour and 6% coconut oil. The ideal ratio of n-6 and n-3, namely 6.74:1, was obtained using 2% Kayambang flour.

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