ISSN: 0852-3681
E-ISSN: 2443-0765

Jurnal Ilmu-Ilmu Peternakan 32(2): 167 – 173
Available online at http://jiip.ub.ac.id

Egg production and blood cholesterol of layers fed after adding fragrant pandan leaf flour (*Pandanus amarylifolius* Roxb.)

Jein Rinny Leke*[1)], E. Wantasen[2)], J. Laihad[1)], E. Pudjihastuti[1)], A. Podung[1)] and R. Siahaan[3)]

[1)]Department of Animal Production, Faculty of Animal Husbandry, University of Sam Ratulangi, Manado, 95515. Indonesia.
[2)]Department of Social-Economic, Faculty of Animal Husbandry, University of Sam Ratulangi, Manado, 95515. Indonesia.
[3)]Biology Departement, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, Manado, 95515.Indonesia.

Submitted: 06 September 2021, Accepted: 08 August 2022

ABSTRACT: This study aimed to determine the performance of eggs and blood cholesterol of laying hens fed with fragrant pandan leaf flour (*Pandanus amarylifolius* Roxb). A total of 200 laying hens were used in the study, with 5 treatments and 5 replications, each filled with 8 chickens. The research design used was completely randomized (CRD), and further analysis was conducted using Duncan's Multiple Range Test. Treatments given fragrant pandan flour were 0%, 1%, 2%, 3%, and 4%. This research was conducted for 8 weeks. Parameters included: ration consumption (g/head), ration conversion, egg weight (g), Hen Day Production (%), blood cholesterol (mg/ml), blood HDL (mg/dl) and blood LDL (mg/dl). Results showed that the use of fragrant pandan leaf flour up to 4% gave a very significant effect ($P < 0.01$) on conversion, hen day egg weight production, blood cholesterol, blood HDL, and blood LDL, but not significantly different ($P > 0.05$) on ration consumption. The study concluded that using up to 4% of fragrant pandan leaf flour increases egg production and reduces ration conversion, blood cholesterol, blood HDL, and blood LDL of laying hens and ration consumption and does not cause negative effects on the laying hens.

Keywords: Egg performance; Blood cholesterol; Fragrant pandan flour

*Corresponding Author: rinileke@unsrat.ac.id

INTRODUCTION

Laying hens are one of the livestock that can produce eggs and meat. Healthy livestock conditions and maintaining high standards of animal welfare attribute to healthy livestock products. A healthy feed can be provided to the livestock through an approach in the field of animal feed nutrition technology to ensure the desired result. Health problems that occur in chickens are blood cholesterol, blood Low-Density Lipoprotein (LDL), and blood High-Density Lipoprotein (HDL), which are less or higher than normal limits in the blood (Badaruddin *et al*., 2021).

Feed attributes a substantial percentage of cost in poultry production. Feed costs account for 60-80% of total production costs. The cost incurred by farmers to provide commercial feed is greater than buying local feed (Permatahati *et al*., 2019). Conventional feed ingredients are always in short supply at high prices in some developing countries. The countries rely on feed ingredients imports which is a major cause of the increase in production costs because the feed costs contribute a large portion of livestock production costs. Some poultry expert researchers developed local products of non-conventional feed ingredients, such as additives, and local raw materials mixed in poultry feed. Local raw materials are used depending on the location, such as cottonseeds, sunflower seeds, and pumpkin seeds (Kasapidou, Sossidou, and Mitlianga 2015; Lira *et al.,* 2009; Tamiru *et al.,* 2021).

The use of antimicrobials, antibiotics, and antioxidants as growth promoters is officially prohibited (Alagbe, Omokore, and Tijani 2018). This calls for the usage of natural ingredients that contain natural antioxidants, such as pandan leaves. Pandan leaves can be used as a natural antioxidant (Margaretta *et al.,* 2011). Fragrant pandan leaves contain carbohydrates and amino acids. Carbohydrates in fragrant pandan include fructose and glucose can be used as an energy source and contain free amino acids. In addition, fragrant pandan leaves contain carotenoids which are elements that affect the color of egg yolk (Purba, Warnoto, and Zain, 2018). Carotenoids contained in pandan leaves consist of *β-carotene* and *lutein* which are found as the main carotenoids and several minor carotenoids identified in pandan leaves are namely *violaxanthin, neoxanthin, α-carotene, and zeaxanthin* (Silalahi, 2018). Fragrant pandan leaves can be used as both antioxidants and preservatives as well as antibacterial. The use of fragrant pandan leaf extract effectively inhibits the growth of *Escherichia coli* bacteria (Purnamasari, Rani Indriati, 2020).

Chicken eggs, a source of animal protein, occupy the first position as a source of animal protein. Eggs are animal products that can meet the needs of animal protein for humans because eggs have complete nutritional value such as 17.07% protein, 32.21% fat, and 2.03% minerals (Faitarone *et al*., 2013). Egg production, egg weight, ration conversion, and internal and external egg quality were determined by the amount of feed consumed (Leke *et al.,* 2020). During the COVID-19 pandemic, chicken eggs are useful as food that meet protein demand. In addition, chicken eggs are also used in the food industry as an ingredient in cakes and snacks, and other industries. The increasing industrial and societal needs for qualified chicken eggs encourage breeders to improve the quality of production from laying hens. The purpose of the study was to determine the egg production and blood cholesterol of laying hens fed with fragrant pandan leaf flour *(P. amarylifolius).* Based on this objective, the research is entitled "Egg production and blood cholesterol of layers fed after adding fragrant pandan leaf flour (*Pandanus amarylifolius* Roxb)*".*

MATERIALS AND METHODS

Materials

The pandan leaf flour was produced in the Tomohon City area, North Sulawesi Province. Pandan leaves were cleaned with

water, cut into 2-5 cm pieces, then dried in the sun for 2-3 days after drying and ground into flour. A quantity of 1 kg of pandan leaves produced 167 grams of fragrant pandan flour.

This study used 200 heads of laying hens aged 83 weeks. The cage used in this study was a battery cage consisting of 25 units, each of which has a size of 40 x 37 x 30 cm. Each cage unit was equipped with a place to eat and drink made of gutters and paralon pipes.

Each unit of the cage was occupied by 8 chickens, where there was a divider between chickens. The equipment used in the study included digital scales, calipers, Roche Yolk Color Fan, scissors, storage containers, sitting scales, milling, plastic bags, sacks, pliers, shovels, buckets, hose scoops, and egg trays.

The treatment rations were arranged based on the needs of layered laying hens with ±17% protein and 2650-2950 Kcal/kg energy with the addition of fragrant pandan leaf flour. The treatment ration used consisted of 45% corn, 10% rice bran, 42% Layer Chicken Concentrate (LCC), and 3% CaCo3. The treatment ration was given as much as 125 g/head/day and given 2 times a day, i.e., 50% of the feed at 6.00 am and 2.00 pm. Drinking water was provided *ad libitum*.

Table 1. Nutritional Content of Treatment Ration

Nutrition Composition	Treatment				
	R0	R1	R2	R3	R4
Crude Protein (%)	17.34	17.27	17.20	17.13	17.05
Crude Fiber (%)	5.04	5.32	5.61	5.89	6.17
Crude Fat (%)	7.26	7.22	7.18	7.14	7.10
Ca (%)	2.42	2.50	2.57	2.65	2.72
P (%)	1.05	1.13	1.21	1.28	1.36
Metabolism Energy (KCal/kg) *	2762.50	2738.09	2713.67	2689.26	2664.84

Note: *The calculation result of 70 % multiplied with GE Kcal/kg. (These are calculated values or analytical values)

Research Methods

The research method used a Completely Randomized Design (CRD) consisting of 5 treatments and 5 replicates resulting in 25 treatment units. The treatments were arranged based on the appropriate metabolic energy and protein, including Pandan Leaves Flour (PLF) with the treatments given as follows, P0: Basal Ration, P1: Basal Ration 99% + 1% PLF, P2: Basal Ration 98% + 2% PLF, P3: Basal Ration 97% + 3 % PLF, and P4: 96% Basal Ration + 4% PLF.

Data collection methods for blood cholesterol, LDL, and HDL were carried out in week 8. Chickens fasted for ± 6 hours before blood samples were taken. Blood samples were taken as much as ± 1 ml for the EDTA tube and ± 2 ml for the non-EDTA tube by taking blood under the wing (brachial vein) using a syringe. Blood samples were put into two different tubes, namely test tubes containing anti-coagulant Ethylene Diamine Tetra Acetic Acid (EDTA) and non-EDTA test tubes.

Research Variable

The ration consumption was measured by the ration given minus the remaining ration (g); egg weight (g/egg) is the weight achieved by each egg (g), weighed with a digital scale from each producing parent egg; egg production in terms of Hen Day Production (HDP %) was calculated by the number of egg production divided by the number of chickens multiplied by 100%. The ration conversion was calculated as the ratio between ration consumption in a certain period with egg weight achieved at the same time.

Chicken blood cholesterol, LDL, and HDL were recorded by checking the total cholesterol by the Chod Pap *(Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol)* method.

Data Analysis

The data obtained were statistically analyzed with Complete Randomized Design and if there was a significant effect then further analysis would be carried out using Duncan Multiple Distance Test (Steel, and Torrie, 1995).

RESULT AND DISCUSSION

Data from observations and calculations of average ration consumption, Hen Day Production, ration conversion (RC), blood cholesterol, LDL dan HDL, from each treatment are listed in Table 2. Based on Analysis of Variance (ANOVA). The use of pandan leaf flour up to 4% showed a highly significant difference, i.e., ($P < 0.01$) on HDP, conversion, blood cholesterol, LDL, and HDL but showed no significant difference ($P > 0.05$) concerning ration consumption. It can be stated that the average ration consumption is 115.1 – 115.8 g/head, the average HDP is 87.63 – 93.53 %, and the average RC is 1.91 -2.03. This indicates that ration consumption and egg production are still in the same range.

Table 2. The effect of pandan leaf flour treatment on ration consumption, Hen Day Production, conversion, blood cholesterol, blood LDL, and blood HDL

Parameter	R0	R1	R2	R3	R4	Significane
Consumption Ration (g/head)	115,7± 0,65	115.7± 0,49	115.6± 1,22	115.8± 2,26	115.1± 0,65	ns
Hen Day Production (%)	87.63^{a}± 2,92	90.16^{b}± 1,29	91.78^{c}± 1,00	93.53^{d}± 1,87	94.81^{e}± 1,40	sig
Conversion	2.03^{c}±0,01	1.93^{b}±0,03	1.91^{b}±0,04	1.91^{b}±0,04	1.85^{a}± 0,02	sig
Blood Cholesterol (mg/dl)	$109,47^{d}$±4,99	$101,66^{c}$± 4,74	99.94^{b}±0,47	102.15^{c}±4,42	95.76^{a}± 5,99	sig
Blood HDL (mg/dl)	34.46^{d}±3.95	34.84^{e}±2.67	31.74^{b}±2.26	30.98^{a}± 1.11	32.12^{c}±2.13	sig
Blood LDL (mg/dl)	70.10^{a}±10.32	70.10^{a}±0,00	60.95^{c}±7.90	70.66^{a}±2.65	69.74^{b}±1.16	sig

^{a,b,c,d} Superscript was different in similar line showing significant effect ($P <0,01$)

The nutritional content of pandan leaves is in the form of carbohydrates, fats, proteins, and amino acids (Suryani *et al.*, 2020). Egg production was increased due to the consumption of rations increased with pandan leaf flour as a source of antioxidants. Feed ingredients contained antioxidants and antibacterial which given to laying hens can improve intestinal performance in the process of absorption of poultry feed nutrients to provide health, and can reduce the death rate of chickens so that egg production can increase (Laptev *et al.*, 2021). Pandan leaf flour level of 4% increases egg production and provides adequate feed conversion. Egg production is influenced by several factors such as chicken strain, ration given, mortality, culling, health and maintenance management, age at first laying eggs, peak egg production, and persistence of laying eggs, feed consumption will affect egg production (Farooq *et al.*, 2002). The absorption of food substances was enhanced by the fragrant pandan leaf flour that can suppress the microflora in the chicken intestine. Pandan leaves contain carotenoid compounds such as Violaxanthin, Neoxanthin, lutein, Zeaxanthin, Lutein epoxide, carotene, and beta-carotene (Ningrum and Schreiner, 2014). Pandan leaves also contain alkaloids, flavonoids, saponins, tannins, and polyphenols that function as antioxidants (Margaretta *et al*., 2011). Antioxidants in fragrant pandan can suppress microflora in chicken intestines (Oluwafemi, and Grace Funmi Reuben, 2020).

Pandan leaf flour treatment ranged from a conversion rate of 1.85 to 2.03. This shows that the provision of pandan leaf flour up to 4% can reduce the value of the

conversion so that the feed ingredients are more efficient and can increase egg production. This can be seen from the nutrition of pandan leaf flour as a source of antioxidants and antibacterial so that it can increase ration consumption providing egg production and conversion values are more efficient. The results of the conversion value of ISA Brown chicken aged 99 – 103 weeks on average were 2.78 – 3.02 (Febriyanti, Dewi, and Mahardika, 2020).

The average blood cholesterol in this study is 95.76 -109.47 mg/dl, blood LDL is 30.98 – 34.84 (mg/dl) and blood HDL is 60.95 – 70.10 (mg/dl). Research on laying hens fed with papaya pomace in dry form resulted in blood cholesterol of 103-123 (mg/dl), HDL 44-54 (mg/dl), LDL 91-119 (mg/dl) (Tamiru *et al.,* 2021). In a different study, the cholesterol concentration obtained ranged from 133.75-173.75 mg/dl (Badaruddin et al. 2021). Several studies suggested that normal chicken cholesterol is 129-297 mg/dl (Adewole *et al*. 2021). Research on the effect of substitution of wheat bran with cumin seed meal on the blood lipid profile of laying hens, namely: total blood cholesterol 178.7 – 210.7 mg/dl, blood HDL 31.33-42 mg/dl, blood LDL 123 -171.1 mg /dl, triglyceride 18.67 – 22.93 mg/dl (Saleh *et al.,* 2020).

Pandan leaf flour is a feed additive for herbal ingredients in feed and the rightful dosage should be used because if not, it can cause toxicity which will damage internal organs, especially the liver which is the center of metabolism. The occurrence of a decrease in blood cholesterol levels is because pandan leaf flour contains flavonoid compounds. The flavonoids contained to reduce the value of cholesterol levels by reducing blood viscosity and increasing the excretion of bile acids, thereby preventing the accumulation of fat in the blood vessels (Partama, and Trisnadewi, 2020).

Cholesterol is a type of fat that is produced in the liver. Cholesterol has the function to help cells produce hormones and digest fats. Excess cholesterol can cause blood vessels to narrow and can lead to heart disease. Cholesterol is associated with atherosclerosis, where there is an accumulation of cholesterol-containing materials on the walls of blood vessels that causes heart disease (Adewole *et al.,* 2021).

Cholesterol synthesis in the body begins with one acetyl CoA molecule and one Acetoacetyl-CoA molecule, hydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). The molecule is then reduced to mevalonate by the enzyme HMG-CoA reductase. Mevalonate is a basic material for the biosynthesis of various molecules, including cholesterol. Regulation of the rate-limiting of cholesterol synthesis can occur due to the action of statins which are competitive inhibitors of HMG-CoA reductase. The relative rate of cholesterol production varies according to cell type and organ function. All animal cells produce cholesterol; about 20–25% of the total daily cholesterol production is in the liver (Berg, Tymoczko, and Stryer, 2002). Low-density lipoprotein (LDL) and High-density lipoprotein (HDL) are a group of lipoproteins. People often refer to LDL as bad cholesterol because it can deposit fat on artery walls, attract macrophages, and cause atherosclerosis. People often mention HDL as good cholesterol or healthy cholesterol because it can separate fat molecules from macrophages in artery walls. Lipoproteins circulate fat throughout the body through the extracellular fluid and enter cells via receptor-mediated endocytosis (Dashti *et al.,* 2011). Cholesterol that has been synthesized will bind to LDL receptors and then be transported to liver cells, which are then used for bile acid synthesis. HDL transports fat particles through the HDL receptor, the scavenger receptor BI (SRBI), which mediates cholesterol withdrawal from HDL. The increase in cholesterol in cells occurs because the absorption of lipoproteins containing cholesterol by HDL receptors functions to transport excess cholesterol in the form of LDL, which is transported through the blood vessels. The process of blood cholesterol, blood HDL, and blood LDL has given pandan leaf flour

is thought to be due to the presence of antioxidants from fragrant pandan leaf flour (*P.amaryllifolius*) causing the conversion of cholesterol to bile acids in the body to increase. This resulted in the absorption of cholesterol in the jejunum slightly, so that blood cholesterol levels in chickens laying down.

CONCLUSIONS

Fragrant pandan (*Pandanus amarylifolius* Roxb.) leaf flour contains antioxidants and flavonoids. This fragrant pandan leaf flour can be used to increase egg production and to reduce ration conversion, blood cholesterol, blood HDL, and blood LDL of laying hens and ration consumption. Fragrant pandan leaf flour can be used up to 4% level in laying hens' feed and does not cause negative effects on laying hens.

ACKNOWLEDGMENT

This research was funded by DIPA funds from Sam Ratulangi University in 2021. The authors would like to thank the Chancellor of Sam Ratulangi University and LPPM Unsrat. We are also grateful to CV Gunawan's Livestock Partners.

REFERENCES

Adewole, F. A., Egbeyale, L. T., Ekunseitan, D. A., Bello, K. O., Lala, O. A., & Famakinde, S. A. (2021). Effect of strain and sex on haematological and serum biochemical indices of tropical indigenous chickens. *Nigerian Journal of Animal Production*, *48*(2), 18–26. https://doi.org/10.51791/njap.v48i2.2924

Alagbe, J., Omokore, E., & Tijani. (2018). Effect on dietary supplementation of dried spondias mombin linn leaf on the performance and blood profile of broiler chicken. *Pacific International Journal*, *1*(4), 34–48.

Badaruddin, R., Aka, R., Ollong, A. R., & Tiya, N. A. D. (2021). Kadar kolesterol, asam urat dan glukosa darah ayam petelur yang diberi jus daun sirih (Piper betle Linn) pada level yang berbeda. *Jurnal Ilmu Peternakan Dan Veteriner Tropis (Journal of Tropical Animal and Veterinary Science)*, *11*(1), 76. https://doi.org/10.46549/jipvet.v11i1.172

Bayu Anggara, I. W., Partama, I. B. G., & Trisnadewi, A. A. A. S. (2020). Pengaruh pemberian sekam padi tanpa dan dengan fermentasi yang disuplementasi daun sirih dalam ransum terhadap bobot potong, non karkas eksternal, dan lemak abdominal itik bali betina. *Majalah Ilmiah Peternakan*, *23*(2), 78. https://doi.org/10.24843/MIP.2020.v23.i02.p06

Dashti, M., Kulik, W., Hoek, F., Veerman, E. C., Peppelenbosch, M. P., & Rezaee, F. (2011). A phospholipidomic analysis of all defined human plasma lipoproteins. *Scientific Reports*, *1*(1), 139. https://doi.org/10.1038/srep00139

Faitarone, A., Garcia, E., Roça, R. de O., Ricardo, H. de A., Andrade, E. N. de, Pelícia, K., & Vercese, F. (2013). Cholesterol levels and nutritional composition of commercial layers eggs fed diets with different vegetable oils. *Revista Brasileira de Ciência Avícola*, *15*(1), 31–37. https://doi.org/10.1590/S1516-635X2013000100006

Farooq, M., Mian, M. A., Durrani, F. R., & Syed, M. (2002). Egg production performance of commercial laying hens in Chakwal district, Pakistan. *Livestock Research for Rural Development*, *14*(2).

Kasapidou, E., Sossidou, E., & Mitlianga, P. (2015). Fruit and vegetable co-products as functional feed ingredients in farm animal nutrition for improved product quality. *Agriculture*, *5*(4), 1020–1034. https://doi.org/10.3390/agriculture5041020

Laptev, G. Y., Yildirim, E. A., Ilina, L. A., Filippova, V. A., Kochish, I. I., Gorfunkel, E. P., Dubrovin, A. V., Brazhnik, E. A., Narushin, V. G., Novikova, N. I., Novikova, O. B.,

Dunyashev, T. P., Smolensky, V. I., Surai, P. F., Griffin, D. K., & Romanov, M. N. (2021). Effects of essential oils-based supplement and salmonella infection on gene expression, blood parameters, cecal microbiome, and egg production in laying hens. *Animals*, *11*(2), 360. https://doi.org/10.3390/ani11020360

Leke, J. R., Sompie, F. N., Wantasen, E., & Tallei, T. E. (2019). Nutritional characteristics and quality of eggs from laying hens fed on a dried papaya peel meal diet. *ANIMAL PRODUCTION*, *20*(3), 147. https://doi.org/10.20884/1.jap.2018.20.3.704

Lira, R. C., Rabello, C. B.-V., Ferreira, P. V., Lana, G. R. Q., Lüdke, J. V., & Dutra Junior, W. M. (2009). Inclusion of guava wastes in feed for broiler chickens. *Revista Brasileira de Zootecnia*, *38*(12), 2401–2407. https://doi.org/10.1590/S1516-35982009001200016

Magaretta, S., Handayani, S. dewi, Indraswati, N., & Hindarso, H. (2011). Ekstraksi senyawa phenolic pandanus amaryllifolius. *Widya Teknik*, *10*(1), 20–30.

Ningrum, A., & Schreiner, M. (2014). Carotenoid-cleavage activities of crude enzymes from pandanous amryllifolius. *Chemistry & Biodiversity*, *11*(11), 1871–1881. https://doi.org/10.1002/cbdv.201400029

Oluwafemi, R., & Reuben, G. (2020). Effect of feeding polylathia longifolia leaf meal as partial replacement of wheat offal. *European Journal of Agricultural and Rural Education*, *1*(1), 1–7.

Permatahati, D., Mutia, R., & Astuti, D. A. (2019). Effect of cricket meal (Gryllus bimaculatus) on production and physical quality of japanese quail egg. *Tropical Animal Science Journal*, *42*(1), 53–58. https://doi.org/10.5398/tasj.2019.42.1.53

Purba, I. E., Warnoto, W., & Zain, B. (2019). Penggunaan tepung daun kelor (Moringa oleifera) dalam Ransum terhadap Kualitas Telur Ayam Ras Petelur dari Umur 20 Bulan. *Jurnal Sain Peternakan Indonesia*, *13*(4), 377–387. https://doi.org/10.31186/jspi.id.13.4.377-387

Purnamasari, & Indriati, R. (2020). *Uji Efektivitas Ekstrak Daun Pandan Wangi (Pandanus Amaryllifolius Roxb.) Sebagai Antibakteri Terhadap Pertumbuhan Bakteri Escherichia Coli Penyebab Penyakit Diare*. Universitas Pasundan.

Saleh, A. A., Zaki, A., El- Awady, A., Amber, K., Badwi, N., Eid, Y., & Ebeid, T. A. (2020). The effect of substituting wheat bran with cumin seed meal on laying performance, egg quality characteristics and fatty acid profile in laying hens. *Veterinarski Arhiv*, *90*(1), 47–56. https://doi.org/10.24099/vet.arhiv.0500

Silalahi. (2018). Pandanus amaryllifolius Roxb (Pemanfaatan dan Potensinya Sebagai Pengawet Makanan). *Jurnal Pro Life*, *5*(3), 626–636.

Suryani, C. L., Wahyuningsih, T. D., Supriyadi, S., & Santoso, U. (2020). The potential of mature pandan leaves as a source of chlorophyll for natural food colorants. *Jurnal Teknologi Dan Industri Pangan*, *31*(2), 127–137. https://doi.org/10.6066/jtip.2020.31.2.127

Tamiru, B., Alkhtib, A., Tamiru, M., Demeke, S., Burton, E., Tolemariam, T., Debela, L., & Janssens, G. P. J. (2021). Evaluation of dried papaya pomace meal in laying hen diets. *Veterinary Medicine and Science*, *7*(5), 1914–1920. https://doi.org/10.1002/vms3.516

Widyantara, P. R., Wiyana, A. I., & Sarini, N. P. (2013). Performa ayam isa brown umur 99-103 minggu yang diberi ransum komersial dengan suplementasi tepung kulit kerang. *Jurnal Peternakan Tropika*, *1*(1), 545–548.