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Supplementation of a Calcium-fish oil mixture in the diet on egg quality in Lohmann Brown laying hens

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Abstract

The aim of this study was to examine the impact of dietary calcium-fish oil (Ca-fish oil) mixture supplementation on egg quality in Lohmann Brown laying hens aged 45-53 weeks. This study used 160 Lohmann Brown laying hens aged 53 which were randomized into 4 types of treatment and 4 replications and each replication used 40 hens with homogeneous body weights. The four treatments were levels of shellfish meal in fish oil, namely 0%, 1.2%, 2.2% and 3.2% for treatments P0, P1, P2, and P3, respectively. The results showed that supplementation of a mixture of shellfish flour with fish oil significantly ($P<0.05$) could increase egg weight, egg yolk color, egg white weight, egg yolk weight, eggshell weight, eggshell thickness, eggshell color, pH and haugh unit. But, significantly different ($P<0.05$) reduced fat and cholesterol levels in yolk. Meanwhile, the egg index and egg protein content were not significantly different ($P>0.05$). Based on the research results, it can be concluded that supplementation of a Calcium-fish oil mixture in feed at the level of 1.2-3.2% is able to improve the physical and chemical quality of eggs, and can reduce fat and cholesterol levels in yolk in Lohman Brown laying hens.

Keywords: Calcium; Egg quality; Fish oil; Lohman Brown laying hens

1. Introduction

Eggs are a food source that has a high animal protein content at an affordable price among the public. Eggs also have a relatively cheap price compared to other animal products. Eggs are a poultry product that has quite potential and is a food ingredient that has quite perfect nutritional content because it contains complete nutritional substances and is easy to digest [1]. Eggs contain complete essential amino acids, so eggs can be used as a benchmark in determining the protein quality of various food ingredients [2]. Apart from that, eggs are also very easy to get at a relatively cheaper price compared to other protein sources.

Egg quality is an indicator that refers to the standard of egg exterior quality and egg interior quality. The exterior quality of an egg consists of the proportions of the egg shape, the surface area of the egg, and the proportions of the egg shell. Egg interior quality includes albumin index, yolk proportion, yolk index, yolk to albumin ratio, and haugh units [3]. Indicators of the exterior of an egg if it is damaged can be seen from a decrease in egg weight and the appearance of spots on the egg shell [4], while indicators of the interior of an egg if it is damaged are characterized by evaporation, loss of carbon dioxide through the pores of the egg shell, and the entry of organisms through the pores of the egg shell [5].

Improving egg quality can be supported by providing nutrition according to livestock needs. Maximizing the production of laying hens is by meeting their energy needs, in addition to other nutritional elements such as protein, minerals and vitamins [6]. Shellfish flour has a CaCO3 element content of 99.4% and pure calcium reaching 39.5%, so it is very good

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as a supplement for livestock growth and development [7]. Fish oil comes from the oily tissue of certain fish. Some of the ingredients contained in fish oil include omega 3, omega 6, squalene, vitamin A, vitamin D, vitamin E, and vitamin K [8]. The saturated fatty acid content in yolk is low, while the unsaturated fatty acid content is high, especially long chain unsaturated fatty acids containing 20-22 C atoms or more. Some of these acids include EPA (eicosapentaenoic) and DHA (docosahexaenoic) [9].

Maulana et al. [10] stated that fish waste oil supplementation of 1-4% in the diet did not have a significant effect on egg weight, egg yolk and egg yolk color. Alik et al. [11] reported that administering lemuru fish oil at a level of 1-4% containing 0.002% L-carnitine could increase the color of quail egg yolks, but could not improve egg production and quality. Supplementation with 2-3% simping shell flour can improve egg quality in terms of egg yolk index and egg shell weight [7]. In contrast,[12] stated that giving rations with the addition of 1-3% shell flour to Isa brown laying hens had no effect on egg quality which included egg weight, egg index, shell weight, egg shell thickness, haugh units, pH and egg yolk color.

This research needs to be carried out to study further regarding the use of shellfish meal and fish oil on egg quality in Lohman Brown laying hens.

2. Material and methods

2.1. Animal treatments

This research was carried out at the Research Station of the Faculty of Animal Husbandry, Udayana University, located on Jalan Raya Sesetan, Gang Markisa, Denpasar, Bali and was approved by the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia. Sample analysis was carried out at the Nutritional Chemistry Laboratory, Poultry Laboratory and Food Microbiology Laboratory, Udayana University, Denpasar.

The ration given to chickens is commercial ration 511 for laying hens in the second laying phase produced by PT. Charoen Pokphand, Indonesia. Feeding to chickens was done *ad libitum*, which means unlimited. Feeding was done in the morning and evening and was given ¾ of the feeder capacity to avoid food being spilled. Drinking water for chickens was given *ad libitum* and replaced every day to keep it clean and fit to drink.

2.2. Experimental design

This study used 160 Lohmann Brown laying hens aged 53 which were randomized into 4 types of treatment and 4 replications and each replication used 40 hens with homogeneous body weights. The four treatments were levels of shellfish meal in fish oil, namely 0%, 1.2%, 2.2% and 3.2% for treatments P0, P1, P2, and P3, respectively.

2.3. Shellfish flour and Fish oil

Shellfish flour as a source of calcium used in this research is local shellfish flour which was bought and sold at the Poultry Shop in Jemberana Regency, Bali Province, Indonesia. The fish oil used in this study is a commercial Scott's emulsion fish oil product and can be found in pharmacies and other drug stores.

2.4. Measurement of observed variables.

Egg weight was measured every day by weighing the eggs on a scale with an accuracy of 0.001g. Likewise with the weight of egg white, yolk and egg shell. Eggshell thickness is obtained by measuring the eggshell using a micrometer (mm), without removing the thin layer inside the eggshell. The color of the egg shell varies from pale white to dark brown. To determine the color score, use the Egg Shell Color Fan which consists of 15 color series. Egg yolks vary in color from pale yellow to dark reddish orange. Egg yolk color will be measured using the Egg Yolk Color Fan which consists of 15 color series [13].

Haugh units (HU) relate to egg weight and egg white height. The higher the haugh unit value indicates the higher the egg quality [14]. HU will be measured using a caliper. The egg index is obtained from measuring the diameter, width of the egg and length of the egg using a caliper. Egg pH can be obtained by mixing egg white and egg yolk until homogeneous and measured using a digital pH meter.

Protein levels can be determined using the Kjeldahl method. Cholesterol analysis used the Lieberman-Burchad method from [15]. Measurement of fat content uses the Soxhlet extraction method with the principle that fat can be extracted using ether or a fat solvent. If the solvent is evaporated, the fat will be left behind [16].

2.5. Statistical analysis

The data obtained was analyzed using variance. If between treatments there are significantly different results (P<0.05) then the analysis will continue with Duncan's multiple range test.

3. Results and discussion

3.1. Physical quality of eggs

The physical quality of eggs in Lohman Brown laying hens that were given Ca-fish oil in the ration was related to egg weight, egg yolk color, egg white weight, egg yolk weight, egg shell weight, egg shell thickness, egg shell color, egg pH, haugh units (HU), and egg index can be seen in Table 1.

Table 1 Effect of Ca-fish oil supplementation in the diet on the physical quality of eggs of Lohman Brown laying hens from 45-53 weeks of age

Variable	Ca-fish oil level in feed (%)				SEM
	0	1.2	2.2	3.2	
Egg weight, g	55.90[a]	62.41[bc]	65.32[c]	60.43[b]	1.00
Egg yolk color (1-15)	8.19[a]	8.69[a]	9.56[b]	9.50[b]	0.18
Egg wihite weight, g	32.81[a]	37.57[b]	40.00[b]	36.76[b]	0.82
Egg yolk weight, g	14.78[a]	15.93[bc]	16.41[c]	15.19[ab]	0.20
Weight of eggshell, g	7.53[a]	8.41[b]	8.21[b]	8.02[ab]	0.12
Thick egg shell (mm)	43.31[a]	44.94[b]	45.19[b]	44.38[ab]	0.24
Eggshell color(1-15)	9.44[a]	12.69[b]	13.19[b]	12.81[b]	0.40
pH of eggs	7.19[a]	7.25[b]	7.35[c]	7.37[c]	0.02
HaughUnit(HU)	92.36[a]	96.48[b]	95.40[b]	95.93[b]	0.60
Egg index(%)	76.57[a]	77.30[a]	76.28[a]	77.60[a]	0.35

Note: Values with different letters on the same row are significantly different (P<0.05); SEM=*Standard Error of the Treartment Mean*

The average weight of eggs in groups P1, P2 and P3 was 10.43%; 14.42%; and 7.50% significantly (P<0.05) higher than Hens P0 group. The color of yolk in groups P2 and P3 was: 16.73% and 16.0% lighter (P<0.05) yellower than the control group (P0). Ca-fish oil supplementation in feed significantly (P<0.05) increased white weight, yolk weight and eggshell weight. Egg shell thickness in the P3 chicken group was not significantly different (P>0.05) compared to the control. However, the thickness of the eggshells in the P1 and P2 chicken groups, namely 3.76% and 4.34%, was significantly (P<0.05) thicker than the control (P0).

The average color of eggshells in the P1, P2, and P3 hen groups were: 25.62%, 28.44%, and 26.34% significantly (P<0.05) higher compared to the P0 hen group, respectively. The average pH of eggs in the P1, P2, and P3 hen groups was 0.88%, 2.24%, and 2.45% significantly (P<0.05) higher than that of the P0 hen group, respectively. The Haugh unit (HU) values of Lohman Brown laying hens fed with Ca-fish oil supplementation, namely in the P1, P2 and P3 hen groups were 4.24%, 3.18% and 3.72% significant (P<0 .05) lower compared to the P0 hen group. More details are presented in Table 1. Supplementation of Ca-fish oil in the feed of Lohmann Brown laying hens had no impact (P>0.05) on the egg index.

Ca-fish oil supplementation in the ration can affect egg weight because fish oil contains fatty acids and vitamins where the body will process fatty acids and vitamins so that more protein will be synthesized to form albumin [17]. High levels of feed protein influence the protein synthesis of albumin and egg yolk, while albumin and egg yolk are the largest components in the formation of egg weight [18,19]. Sodak [20] stated that egg weight is influenced by several factors, namely the age of the chicken, environmental temperature, strain, nutritional content of the ration, body weight of the chicken, and age of egg laying.

The color of egg yolk increases with Ca-fish oil supplementation because fish oil contains carotene [21]. Muharlien [22] stated that the higher the score of the egg yolk color on the egg yolk color fan, the better the egg quality. Argo et al. [18,23] reported that the color of egg yolk is influenced by the nutritional content of the feed such as xanthophyll and β-carotene. Carotenoid pigments are one of the pigments that cannot be formed by the bird's body, so their availability must be assisted by providing feed that is high in carotene content [24].

Ca-fish oil supplementation significantly improves egg white quality. Febrianto et al.[17] stated that adding fat to the ration will improve the quality of egg whites. Yuwanta [5] states that egg yolk is composed mostly of water, while the solid part is composed of fat, protein, vitamins and minerals. A similar thing was reported by [25] that providing calcium up to 3% in feed can improve the quality of egg shells. The thickness of the egg shell is determined by the availability of calcium and phosphorus in the feed. This was stated by [26] that egg shells contain around 95% calcium in the form of calcium carbonate, and the rest is magnesium, phosphorus, sodium, potassium, zinc, iron, magnan and copper.

Yuwanta [5] states that the thickness of a good chicken eggshell ranges from 0.33-0.35 mm. Husna [27] explained that differences in egg shell thickness, apart from being caused by the calcium content in the feed, were also influenced by the type of livestock, strain and environmental temperature of the research location. The egg shell is the outermost part of the egg and it is important to pay attention to its quality, because the egg shell functions to protect the contents of the egg from the entry of bacteria that cause damage to the contents of the egg which can result in a decrease in the quality of the egg. Uniform shell color intensity is very important because many consumers judge egg quality by shell color [28].

Maimunah and Rokhman [29] stated that eggs with a dark brown shell color have stronger and thicker shells compared to eggs with a lighter brown color. Furthermore, [30] stated that eggs with a browner color had the lowest reduction in quality compared to eggs with a lighter brown color. According to [23], there is a significant correlation between eggshell color and shell strength, shell thickness, and shell weight. However, there is no clear correlation between egg shell color and egg weight, albumin, yolk, HU, yolk color, and Ca content in albumin and yolk.

The air cavity in the egg is formed shortly after laying due to the difference in room temperature which is lower than the parent's body temperature, then the contents of the egg become colder and shrink, thus separating the inner and outer shell membranes, and this membrane separation usually occurs in the blunt part of the egg [30]. The change in pH value occurs due to the decomposition of the NaHCO3 compound into NaOH and CO2, so that the pH of chicken eggs is still categorized as fresh and evaporation has not occurred. Sihombing et al. [31] stated that storage time and storage room temperature affect the pH value, the longer the storage time, the higher the egg pH.

Haugh units are one of the criteria for determining egg quality by measuring albumen height or egg white height with egg weight. Mampioper et al. [32] stated that the Haugh unit value depends on the egg weight and albumen thickness. If egg weight decreases due to storage, there is a tendency for albumen thickness and haugh unit values to decrease. The higher the albumen value, the higher the haugh unit value produced. Factors that can influence the haugh unit value are albumin height, nutritional value of feed, protein intake, and weight of eggs produced [33].

3.2. Chemical qualities of eggs

The results of research on the chemical quality of eggs in Lohman Brown laying hens given Ca-fish oil in the ration on egg protein content, egg fat content and egg cholesterol content can be seen in table 2.

Table 2 Chemical quality of eggs in Lohman Brown laying hens given Ca-fish oil in the ration

Variable	Ca-fish oil level in feed (%)				SEM
	0	1.2	2.2	3.2	
Egg protein, %	12.27[a]	12.76[a]	12.81[a]	13.60[a]	0.25
Egg fat, %	53.91[b]	52.85[ab]	51.66[a]	52.77[ab]	0.30
Total cholesterol(ml/100g)	345.62[b]	262.51[a]	235.85[a]	246.50[a]	12.03

Note: Values with different letters on the same row are significantly different (P<0.05); SEM=*Standard Error of the Treartment Mean*

Supplementation of Ca-fish oil in the feed of Lohman Brown laying hens from 45-53 weeks of age did not have a significant impact on egg protein levels (Table 2). But, the crude fat and total cholesterol content in yolk showed significant differences (P<0.05). The lowest fat content in yolk was found in the P2 hen group, namely: 4.17%

significantly (P<0.05) lower than the control hen group (P0). The total cholesterol content in hen yolk groups P1, P2, and P3, respectively, is 24.05%; 31.76%; and 28.68% significantly lower compared to the P0 chicken group. More details are presented in Table 2.

Through a certain processing process, fish oil which is rich in nutrients contains omega 3, omega 6, vitamin A, protein, fat, antioxidants and glucose. Lestari [34] states that amino acids from feed that have been absorbed in the liver will then be formed into protein and distributed to the ovaries for the egg formation process. In cell membranes, calcium is strongly bound to phospholipids which play an important role in regulating cell membranes. In addition, calcium minerals can absorb vitamin B12 from the digestive tract as a result of microbial production and maintain the integrity of cell membranes and skeletal tissue [35].

Egg yolks containing fish oil have a lower free fatty acid content. Fat and cholesterol levels in eggs provide a positive correlation, because cholesterol is part of fat [36]. Cholesterol levels in yolk decreased with Ca-fish oil supplementation. The fat in fish oil contains many unsaturated fatty acids, so that in its metabolism it can reduce cholesterol [37] which ultimately results in lower cholesterol in egg yolks. It was also reported by [38] that monounsaturated fats (MUFA) are more effective in lowering blood cholesterol. The decrease in cholesterol content is caused by the Ca content which binds bile acids, where bile acids function to emulsify fat, making it easier to hydrolyze by the lipase enzyme. Nisa et al. [39] stated that cholesterol in the digestive tract is bound by the Ca-cellulose fraction whose capacity is four times the molecular weight of cellulase itself.

Cellulose in the digestive system has an impact on the rate of feed digestion so that the duration of food passing through the intestine becomes faster. This situation causes the feed not to be absorbed properly which can reduce the basic chemicals for the formation of cholesterol in the blood and tissues, as well as increasing the loss of bile salts in the duodenum. As a result, the liver needs more cholesterol to make bile salts which it obtains from tissue cholesterol stores [40].

4. Conclusion

It can be concluded that the physical quality of eggs in Lohman Brown laying hens increases with the supplementation of 1.2-3.2% Ca-fish oil in the feed. On the other hand, it can reduce fat and cholesterol levels in yolk.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The Animal Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia has approved this research.

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