Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **02 Year 2023** Page **318:324***

Analysis of the Physical Quality of Chicken Eggs Fed with Added Calcium Fish Oil by Spectroscopic Method

I Made Satriya Wibawa*[1,2], H. Suyanto[1], N.L. Watiniasih[2], And I.G. Mahardika [3]

[1,2]Physics Study Program, Faculty of Mathematics and Natural Sciences, Universitas Udayana – Bali, Indonesia

[3]Animal Husbandary Study Program, Faculty of Animal Husbandary, Universitas Udayana – Bali, Indonesia

*Corresponding author's E-mail: satriya_wibawa@unud.ac.id

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 22 Oct 2023

Abstract

This research was carried out with the aim of improving the physical quality, chicken eggs and the effect of optimum levels by adding fish oil calcium to the feed. The method was a completely randomized design with 4 treatments and 4 replications, namely P0 as control; P1 added fish oil 0.2% in feed; P2 added fish oil 0.4% in feed; P3 was added with 0.6% fish oil in the feed, each study unit used 10 chickens; the total chickens in the study were 160 chickens. After 30 days of the treatment, then the analysis was carried out every week for six weeks using an electric screw micrometer to measure the thickness of the eggshell, the LIBS method used to determine the hardness index, the SAA method used to measure the surface area and pore size. Barrett-Joiner_Halenda method used to determine the pore diameter of the eggshell, and the AAS method used to determine the concentration of calcium. The results showed that the addition of calcium from fish oil in general can improve physical quality of the eggshell compared to controls. The optimum level of fish oil calcium in the feed to produce the best physical quality is 0.4%.

Keywords: *Fish Oil Calcium, Physical Quality of Eggs, Spectroscopy*

1. Introduction

Eggs are a food that is widely consumed by the public (Zhang et al., 2020). In addition to being affordable, eggs also contain a lot of protein and substances needed by the human body compared to other protein sources (Idayanti et al., 2019; Miranda et al., 2015). Increasing egg production can be supported by providing nutrition according to livestock needs (Gustira et al., 2015; Omer et al., 2022). Maximizing the production of laying hens is to meet their energy needs as well as other nutritional elements such as protein, minerals and vitamins (Alhotan, 2021).

With the nutritional content in eggs, nutritionists recommend that eggs be consumed by growing children (Godbert et al., 2019). On the other hand the eggs produced do not last long under normal conditions, so breeders need to improve the quality of their eggs, particularly in terms of their durability and shelf life (McBride et al,, 2013; Nasri et al., 2020; 10. Poletti et al., 2021). Eggshell is the main factor in maintaining egg quality (Carrillo et al., 2021). Good shell quality will be able to maintain the quality of the eggs, especially the contents of the eggs (Carvalho et al., 2023). According to Jusuf et al. (2021), the standard for consumption of chicken eggs based on the level of physical quality of eggs is the condition of the shell, smooth, intact and clean. The pores per egg range from 7,000-17,000 which are used for gas exchange and are 0.01-0.07 μm in size and are scattered throughout the surface of the egg (Kaye et al., 2016; Dai et al., 2022; Ligen et al., 2022). Chicken eggshell thickness ranges from 0.330 - 0.350 mm. The shelf life of consumption chicken eggs at room temperature a maximum of 14 days after laying, or at temperatures between 40 C – 70 C maximum 30 days after laying (Feddern et al., 2017; Goliomytis et al., 2018).

Egg quality can be affected by shell quality such as composition, and eggshell thickness (Şekeroğlu & Duman, 2019; Saleh et al., 2019). The decline in egg quality, especially the quality of the contents is strongly influenced by the size of the composition of the constituent elements of the shell due to the interaction of the egg with air (Attia et al., 2021). The composition of the eggshell consists of 98.2%

calcium, 0.9% magnesium and 0.9% phosphorus (Alam & Geleel, 2018). Eggshell quality is influenced by internal and external factors including genotype, age, laying time, cage system, and balanced feeding with adequate Ca, P and mineral supplements (Ketta & Tůmová, 2016). Calcium supplementation is the key to eggshell quality, because each eggshell contains up to 3 g of Ca, so chicken feed must contain Ca in sufficient quantities and be used efficiently (Bartter et al., 2018; Rayan et al., 2022). The physical quality of the eggshell such as shell thickness, shell hardness index and shrinking the pores of the eggshell so as to protect the egg contents (Alig et al., 2023). This study aims to analyze the addition fish oil calcium in feed on physical quality (thickness, hardness and pore diameter of chicken egg shells), and the optimum level with the addition of fish oil calcium in chicken feed.

2. Materials And Methods

Experimental Material:

The chickens used in this study were Lohmann Brown layers aged 54 weeks with average body weight (1756 gr ± 21.65 gr). The study was conducted in a completely randomized design (CRD) with 4 treatments and 4 repetitions with 10 individuals for each unit, so that the total number of chickens was 160. The four treatments were: feed without the addition of fish oil calcium as a control (P0) with a feed composition of 30% PL.241; 10% concentrate; 10% PB1; 30% corn; 10% rice bran; 9.5% fish meal and 0.5% minerals, feed composition (P1) is 30% PL.241; 10% concentrate; 10% PB1; 30% corn; 10% rice bran; 9.3% fishmeal; 0.5% minerals; and 0.2% calcium fish oil, feed composition (P2) is 30% PL.241; 10% concentrate; 10% PB1; 30% corn; 10% rice bran; 9.1% fishmeal; 0.5% minerals; and 0.4% calcium fish oil, feed composition (P3) is 30% PL.241; 10% concentrate; 10% PB1; 30% corn; 10% rice bran; 8.9% fishmeal; 0.5% minerals; and 0.6% calcium fish oil.

Method:

The feed has been mixed (using a mixing machine) according to the composition, then given to the chickens according to the treatment for 30 days. After 30 days the eggs were taken and the number of eggs taken and analyzed was 40 eggs for each treatment so that a total of 160 eggs were taken, then placed at room temperature. Several parameters were used to analyze the physical quality of the eggshell, including thickness, hardness, calcium concentration, and pore diameter of the eggshell. The thickness of the eggshell is measured using an electric screw micrometer. Egg shell hardness index was measured by Laser Induced Breakdown Spectroscopy (LIBS) method. For this purpose in the first week (the day after the eggs were taken) the steps taken were to take 1 egg at random for each treatment and replicate both control eggs P0 ((4 eggs) and eggs treated P1 (4 eggs); P2 (4 eggs); P3 (4 eggs), so the number of eggs taken for analysis in the first week was 16 eggs. This analysis was carried out every week with different eggs for 6 weeks. The pore diameter of the egg shell was measured using a Surface Area Analyzer (SAA) with the Barrett-Joiner_Halenda (BJH) method is a method for determining the diameter distribution of pore sizes.Calcium concentration in the shell was used with an Atomic Absorption Spectrophotometer (AAS) (Ajala et al., 2018). Before analysis, the eggshells were crushed with H_2SO_4 and HNO_3 which aims to break the calcium bonds of the compounds, so that formed calcium ions then analyzed with AAS.

3. Results and Discussion

The results of the analysis of the physical quality of eggshells are shown in Figure 1, Figure 3, Figure 4 and Table 1, which are the results of measurements of eggshell thickness, eggshell hardness, eggshell pore diameter, and eggshell calcium concentration respectively. Figure 1, is the result of measuring the thickness of the eggshell as measured using an electric screw micrometer. Measurements were made every week for 3 treatments and one control. Each measurement was carried out by 4 samples as repetitions whose results were averaged and shown in Figure1.

Table 1. Calcium concentration in eggshells

Calcium concentration in egg shells	
P	**Concentration (mg/L)**
P0	23.61
P1	25.49
P2	27.41
P3	25.42

Figure 1 shows the relationship between the length of egg storage and the thickness of the eggshell. Based on the data in Figure 1, chickens whose feed was not supplemented with fish oil calcium had the lowest thickness from the first to the sixth week of storage. In contrast, for chickens that were given feed with an additional fish oil calcium of 0.4% had the highest thickness of 0.36 mm until the fourth week is higher than SNI standard. The thickening of the eggshell is caused by the formation of layers of calcium carbonate ($CaCO_3$) crystals due to the addition of calcium from fish oil. The more addition of fish oil calcium, the more layers of $CaCO_3$ are formed, until an equilibrium crystal structure is reached. In this study, the crystal structure concentration equilibrium occurred when the feed was added with fish oil calcium of 0.4% (P2). The addition of more than 0.4% calcium fish oil will cause degradation of the $CaCO_3$ crystal layer, so that the thickness of the eggshell decreases as in P3. According to Le Chatelier's principle, if there is a change in an equilibrium, a reaction will occur against the change (Brown & MacKay, 1983).

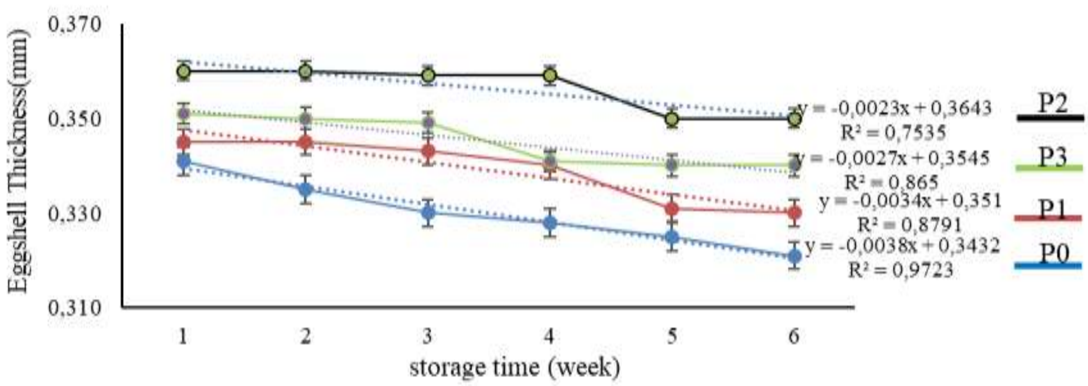
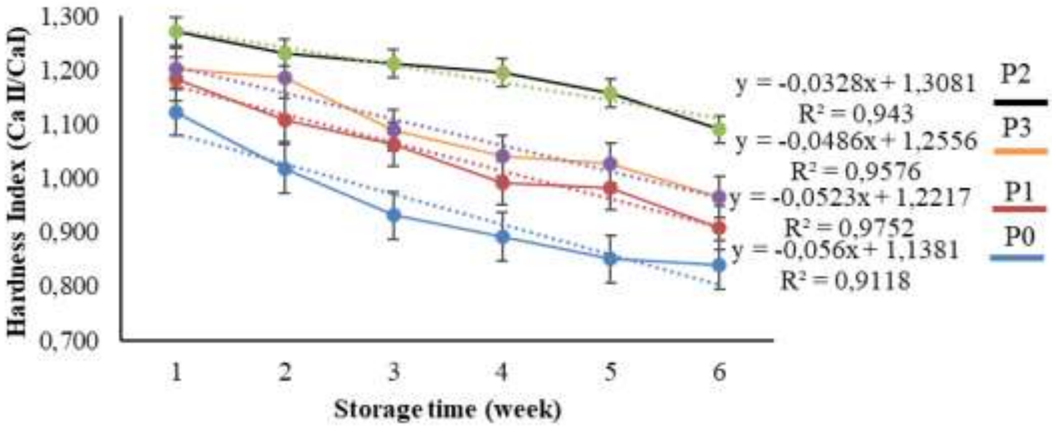


Figure (1): Graph of eggshell thickness function of storage time

Apart from that, Figure 1 shows that feed supplemented with fish oil calcium causes eggshell resistance to be longer than without addition, namely until the third week for P1 and P3, and until the fourth week for P2 and thereafter decreased. This thickness resistance is due to the fact that the $CaCO_3$ crystal layer that is formed has a stronger bond than without the $CaCO_3$ crystal layer and is even stronger if the crystal structure in the eggshell occurs at a concentration equilibrium such as in P2, while the decrease in eggshell thickness is due to conditions or circumstances. The contents of the egg undergo changes and result in a reaction between the inside of the egg shell and the liquid contents of the egg and erosion occurs.

To find out the elements contained in the eggshell and to find out the hardness index of the eggshell function of adding fish oil calcium oil, an analysis was carried out using Laser Induced Breakdown Spectroscopy (LIBS) and the results are shown in Figure 2.



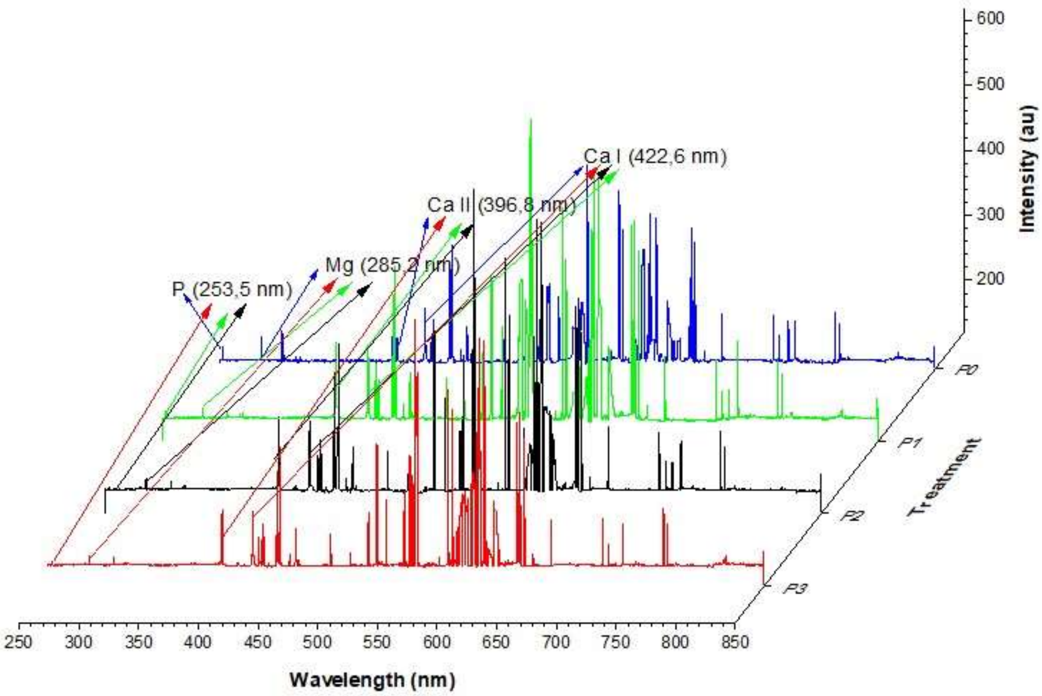


Figure (2): Spectral graph of eggshell function of adding fish oil calcium concentration detected in the first week

Figure 2 is the intensity spectra of the wavelength function of the eggshells of control P0 and those fed fish oil calcium (P1, P2, and P3) in the first week, which were analyzed by LIBS with laser energy (100 mJ). Based on the wavelength of the spectra, it was found that the egg shell contains elements including Ca (λ = 422.6 nm), P(λ = 253.5 nm), Mg (λ = 285.2 nm). To find out the hardness index of the egg shell function of adding fish oil calcium can be done by measuring the ratio of the intensity of ion calcium (Ca II; λ= 396.8 nm) to the intensity of neutral calcium (Ca I; λ= 422.6 nm) as shown in Figure 2 and Figure 3.

Figure (3): Graph of eggshell hardness from the first to the sixth week

Figure 3 shows that the addition of calcium fish oil to the feed causes the eggshell hardness to increase. This is due to the formation of calcium carbonate ($CaCO_3$) as an addition to the egg shell composition, so that the higher the concentration of fish oil calcium added to the feed, the thicker the resulting egg shell will be (Figure 1) and harder (Figure 3). Based on the research of Ajayan et al (2020) that the amount of calcium carbonate is always associated with the hardness and strength of the eggshell. This strength causes as shown in Figures 1 and 3, especially in P2, the stability of thickness and hardness until the fourth week. However, the amount of calcium carbonate ($CaCO_3$) formed in a compound only reaches a certain equilibrium point (Gautron et al., 2021). It is proven that giving fish oil calcium 0.6% will cause a decrease in the thickness of the eggshell (Figure 1) and also a decrease in the hardness index (Figure 3), which is due to the decay of the formation of calcium carbonate in the eggshell. So based on the results in Figures 1 and 3, equilibrium occurs when the feed is added with fish oil calcium of 0.4%.

To determine the physical condition of the pore of the eggshell, porosity is then analyzed, the results of which are shown in Figure 4, giving fish oil calcium as much as 0.4 % causes pore diameter number 7.2956 nm (0.0072 µm) smaller then SNI standard (0.01-0.07 µm), and the smallest when compared to the administration of fish oil calcium 0.2%; 0.6% and without fish oil calcium. The reduction in the pore diameter of the eggshell is due to the increased concentration of calcium in the eggshell which causes the distance between molecules to shrink. Apart from that the addition of calcium from fish oil forms calcium carbonate ($CaCO_3$) which forms regular long chain bonds (crystals) that resemble the shape of layers and are arranged in a zigzag manner between layers, so that the pores in one layer are covered with the next layer which causes a reduction in the number of pores in the layer. egg shell surface. So with the addition of fish oil calcium will reduce the pore size and pore density per unit area.

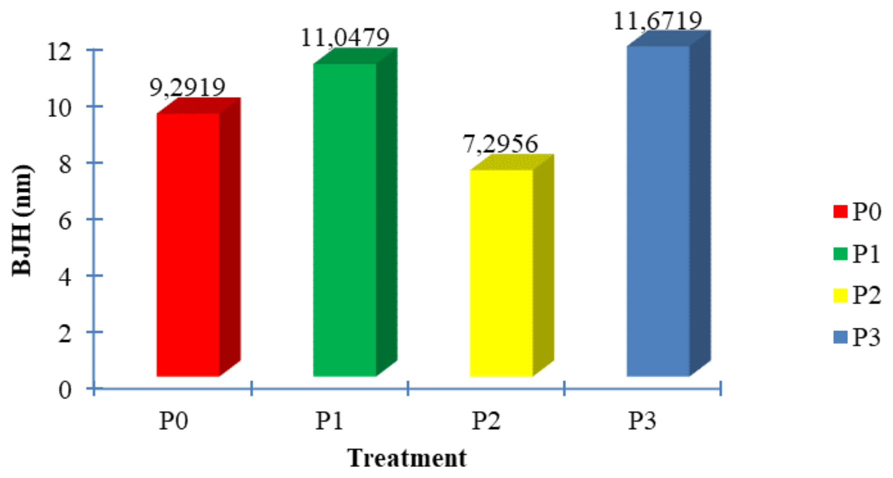


Figure (4): Graph of eggshell pore diameter

To prove the increased concentration of calcium in egg shells when chickens were given feed supplemented with fish oil calcium, a calcium test was carried out on egg shells using an Atomic Absorption Spectrophotometer (AAS) with the results as shown in Table 1.

In Table 1. Shows the effect of giving fish oil calcium to the concentration of calcium in egg shells. The administration of 0.4% fish oil calcium resulted in the highest calcium concentration, namely 27.41 mg/L, while an increase in 0.6% fish oil calcium in the feed caused a decrease in the calcium concentration in the eggshell. According to Gautron et al (2021) states, that egg shells with a certain (optimal) concentration of calcium carbonate in the form of calcite, which interacts with protein can form a hard shell structure, so as to protect the contents of the egg.

4. Conclusion

It Based on the research it can be concluded:

1. The addition of fish oil calcium to the feed can improve the physical quality (thickness, hardness and reduce the pore diameter of the skin.

2. The addition of 0.4% fish oil calcium to feed is the optimal level to get the best physical quality.

3. Improving the physical quality (thickness, hardness and pore diameter) of the eggshell.

Acknowledgements

The authors would like to thank the Faculty of Mathematics and Natural Sciences for the facilities in the form of AAS and LIBs that have been provided to support the implementation of this research. We also thank the Faculty of Animal Husbandry, Universitas Udayana for the chicken farming cage facilities that have been provided.

Conflict of interest:

The authors declare no conflict of interest in this writing.

References:

Ajala, E.O., Eletta, O.O.A., Ajala, M.A and Oyeniyi, S.K, 2018. Characterization and evaluation of chicken eggshell for use as a bio-resource. *Arid Zone J. Eng., Tech. Env*., 14(1), p. 26-40. www.azojete.com.ng

Ajayan, N., Shahanamol, K.P., Arun, A.U and Soman, S, 2020. Quantitative variation in calcium carbonate content in shell of different chicken and duck varieties. *Adv. Zoo. and Bot*., 8(1), p.1-5. https://doi.org/10.13189 /azb.2020.080101

Alhotan, R, 2021. Commercial poultry feed formulation: current status, challanges, and future expectations. *World's Poult. Sci. J*., **77**(2), p.279 – 299. https://doi.org/10.1080/00439339.2021.1891400

Alig, B.N., Malheiros, R.D and Anderson, K.E, 2023. The effect of housing environment on physical egg quality of white egg layers. *Poultry*, 2, p. 222–234. https://doi.org/10.3390/poultry2020018

Allam, G and Geleel, O.A.E, 2018. Evaluating the mechanical properties, and calcium and fluoride release of glass-ionomer cement modified with chicken eggshell powder. *Dent. J*., 6(40). https://doi.org/10.3390/dj6030040

Attia, A.A., Al-Harthi, M.A and El-Maaty, M.A.H, 2020. Calcium and cholecalciferol levels in late-phase laying hens: effects on productive traits, egg quality, blood biochemistry, and immune responses. *Front. Vet. Sci*., **7**(389). https://doi.org/10.3389/fvets.2020.00389

Bartter, J., Diffey, H., Yeung, Y.H., O'Leary, F., Häsler, B., Maulaga, W and Alders, R, 2018. Use of chicken eggshell to improve dietary calcium intake in rural sub-Saharan Africa *Matern Child Nutr*., 14 (S3):e12649). https://doi.org/10.1111/mcn.12649

Brown, D.B and MacKay, J.A, 1983. Le Chatelier's principle, coupled equilibrium, and egg shells. *American Chem. Soc. Div. Chem. Ed*., 60(3). https://doi.org/10.1021/ed060p198

Carrillo, C.A., Reyes, C.B., Mozos, J.D.L., Gasca, N.D., Rodríguez, E.S., Ruiz, A.I.G and Navarro, A.B.R, 2021. Relationship between bone quality, egg production and eggshell quality in laying hens at the end of an extended production cycle (105 weeks). *Animals*, 11, p.623. https://doi.org/10.3390/ani11030623

Carvalho, D.C.O., Nunes, K.R.B., Gois, G.C., Moraes, E.A., Gervásio, R.C.R.G., Zuffo, M.C.R., Borges, Rodrigues, R.T.S and Brito, C.O., 2023. Quality of Japanese quail eggs according to different stirage periods and temperatures. *Acta Scientiarum Anim. Sci*., 45, p.e61040. https://doi.org/10.4025/actascianimsci. v45i1.61040

Dai, D., Qi, G., Wang, J., Zhang, H., Qiu, K and Wu, S.G., 2022. Intestinal microbiota of layer hens and its association with egg quality and safety. *Poultry Science*, 101**(**102008). https://doi.org/10.1016/j.psj.2022.102008

Feddern,V., De Prá, M.C., Mores, R., Nicoloso, R.S., Coldebella, A and Abreu, P.G., 2017. Egg quality assessment at different storage conditions, seasons and laying hen strains *Ciência e Agrotecnologia*, 41(3), p.322-333. http://dx.doi.org/10.1590/1413-70542017413002317

Gautron, J., Stapane, L., Le Roy, N., Nys, Y., Rodriguez-Navarro, A.B and Hincke, M.T, 2021. Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. *BMC Molecular and Cell Biology*, 22(11). https://doi.org/10.1186/s12860-021-00350-0

Godbert, S.R., Guyot, N and Nys, Y, 2019. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. *Nutrients*, 11, p.684. http://dx.doi.org/10.3390/nu11030684

Goliomytis, M., Kostaki, A., Avgoulas, G., Lantzouraki, D.Z., Siapi, E., Zoumpoulakis, P., Simitzis, P and Deligeorgis, S.G., 2018. Dietary supplementation with orange pulp (*Citrus sinensis*) improves egg yolk oxidative stability in laying hens. *Anim. Feed Sci. Tech*. https://doi.org/10.1016 /j.anifeedsci.2018.07.015

Gustira, D.E., Riyanti and Kurtini, T, 2015. Pengaruh kepadatan kandang terhadap performa produksi ayam petelur fase awal grower. *Jurnal Ilmiah Peternakan Terpadu*, 3(1), p.87-92. https://doi.org/10.23960 /jipt.v3i1.p%25p

Idayanti., Darmawati, S and Nurullita, U, 2009. Perbedaan variasi lama simpan telur ayam pada penyimpanan suhu almari es dengan suhu kamar terhadap total mikroba. *Jurnal Kesehatan*, 2, p.19-26. https://jurnal.unimus.ac.id/index.php /Analis/article/view/224

Jusuf, N., Yusuf, A and Salim, A.I.B., 2021. Total of microbial contaminations in rice chicken eggs save in room temperature. *J. Health, Tech. Sci***.,** 2(1), p. 23-29. https://doi.org/10.47918/jhts.v2i2.156

Kaye, J., Akpa, G.N., Alphonsus, C., Kabir, M., Zahraddeen, D and Shehu, D.M., 2016. Responsed to genetic improvement and heritability of egg production and egg quality traits in japanese quail (*Coturnix coturnix japonica*). *American Sci. Res. J. Eng., Tech., Sci*., 16(1), p. 277-292. http://asrjetsjournal.org/

Ketta, M and Tůmová, E, 2016. Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. *Czech J. Anim. Sci*., 61(7), p.299–309. https://doi.org/10.17221/46/2015-CJAS

Ligen, Z., Qian, W., Liping, W., Tenghao, W., Jing, Q., Junbo, L., Huiyan, J and Yuanfeng, W., 2022. Quality evaluation and lipidomics analysis of salted duck egg yolk under low-salt pickling process. *Food Chemistry*, **X**(16), p.100502. https://doi.org/10.1016/j.fochx.2022.100502

McBride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A., Wuenschel, M.J., Fernandez, A.A and Basilone, G., 2013. Energy acquisition and allocation to egg production in relation tofish reproductive strategies. *Fish and Fisheries*. https://doi.org/10.1111/faf.12043

Miranda, J. M, Anton, X., Valbuena, C.R., Saavedra, P.R., Rodriguez, J.A. Lamas, A., Franco, C.M and Cepeda, A, 2015. Egg and egg-derived foods: effects on human health and use as functional foods. *Nutrients*, **7**. p.706-729. http://dx.doi.org/10.3390/nu7010706

Nasri, H., Brand, H.V.D., Najjar, T and Bouzouaia, M, 2020. Egg storage and breeder age impact on egg quality and embryo development. *J Anim Physiol Anim Nutr*., 104, p. 257–268. https://dx.doi.org/10.1111/jpn.13240

Omer, A., Hailu, D and Whiting, S.J., 2022. Effect of a child-owned poultry intervention providing eggs on nutrition status and motor skills of young children in southern ethiopia: a cluster randomized and controlled community trial. *Int. J. Environ. Res. Public Health*., 19, p.15305. https://doi.org/10.3390/ijerph192215305

Poletti, B and Vieira, M.M., 2021. Shelf life of brown eggs from laying hens of different ages in organic production system. *Brazilian J. Anim. Envir. Res*., 4(1), p. 2-15. https://doi.org/10.34188/bjaerv4n1-001

Rayan, G.N., Galal, A., Fathi, M.M., Alawaid, S., Shehata, W and El-Attar, A.H., 2022. Assessing Differences in the Quality Properties and Ultrastructure of Eggshell as Affected by Chicken Strain and Flock Age During Incubation Period. *Brazilian J. Poult. Sci*., 25(1), p.001-010. http://dx.doi.org/10.1590/1806-9061-2023-1647

Saleh, G., Darra, N.E., Kharroubi, S and Farran, M.T., 2019. Influence of storage conditions on quality and safety of eggs collected from Lebanese farms. *Food Control*, 111, p.107058. https://doi.org/10.1016/j.foodcont.2019.107058

Şekeroğlu, A., Gök, H and Duman, M., 2016, Effects of egg shell color and storage duration on the external and internal egg quality traits of ATAK-S layer hybrids. *Cien. Inv. Agr*., 43(2), p.327-335. https://doi.org/10.4067/ s0718-16202016000200015

Zhang, X., Lv, M., Luo, X. Estill, J., Wang, L., Ren, M., Liu, Y., Feng, Z., Wang, J., Wang, X and Y. Chen, 2020. Egg consumption and health outcomes: a global evidence mapping based on an overview of systematic reviews. *Ann Transl Med*., 8(21), p. 1343. http://dx.doi.org/10.21037/atm-20-4243