American Journal of Animal and Veterinary Sciences

Original Research Paper

Antioxidizing Efficiency of Yogurt Probiotics Peptides Concerning Egg Yolk Nutrients

[1]Syifa Silviarahma Nurfadilah, [1]Lovita Adriani, [1]Deny Saefulhadjar, [1]Novi Mayasari, [2]Safri Ishmayana and [3]Mirnawati

[1]Department of Livestock Nutrition and Feed Technology, Faculty of Animal Science, Universitas Padjadjaran, Sumedang, West Java, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Padjadjaran, Sumedang, West Java, Indonesia
[3]Departement of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Padang, West Java, Indonesia

Article history
Received: 25-03-2024
Revised: 05-07-2024
Accepted: 20-10-2024

Corresponding Author:
Lovita Adriani
Department of Livestock Nutrition and Feed Technology, Faculty of Animal Science, Universitas Padjadjaran, Sumedang, West Java, Indonesia
Email: lovita@unpad.ac.d

Abstract: Yogurt contains bioactive peptides that serve as antioxidants. These peptides can inhibit the production of free radicals in laying chicken body cells, affecting egg quality. We aimed to evaluate the impact of the antioxidant potential of Water-Soluble Peptide Extract (WSPE) yogurt on the protein, lipid, and cholesterol levels in yolks from chicken eggs. We used a completely randomized design (CRD) technique. The study involved three treatments and twelve replicates. The treatments were P0: A basal diet; P1: A diet with 4% WSPE B1 (*Bifidobacterium* spp. + *Lactobacillus acidophilus*); and P2: A diet with 4% WSPE B2 (*B. bifidum* + *L. Bulgaricus* + *L. acidophilus* + *S. thermophilus*). The study used statistical analysis including the analysis of variance (ANOVA) and Duncan's multiple range test to examine the data. The result showed that WSPE yogurt significantly increased egg yolk protein and reduced egg yolk lipid levels. However, it increases the cholesterol levels.

Keywords: Antioxidant Peptide, Cholesterol, Lipid, Protein, Yogurt Probiotic

Introduction

Eggs are high-quality animal protein essential for the human body and contain various fatty acids, vitamins, and minerals (Sanlier and Üstün, 2021). The yolk contains most of the egg's lipid and protein, 17.5 and 32.5%, respectively. Most of the egg yolks' lipid and protein content is found in lipoproteins and offers several benefits, such as antioxidant, anti-inflammatory properties, and anti-microbial (Sarantidi *et al*., 2023; Cui *et al*., 2020). The cholesterol content of egg yolks is very high (275 mg). Cholesterol is essential for synthesizing sex hormones, vitamin D, and the proper functioning of nerves and the brain. Cholesterol is required to produce sex hormones, vitamin D, and nerve and brain function. However, excessive consumption might lead to hypercholesterolemia and death over time (Spence *et al*., 2010; Ohoilulin *et al*., 2023).

The health of the digestive tract and hepatocytes influences the chemical quality of egg yolk. The nutrients in eggs are absorbed and broken down by the digestive tract, particularly the intestines. The bloodstream transports these nutrients to the hepatocytes to produce egg yolk nutrients and precursors. The liver synthesizes egg yolk protein and cholesterol and is packaged as Vitellogenin (VTG). Simultaneously, the liver synthesizes lipids and cholesterol, which are then stored in follicles via endocytosis mediated by Very Low-Density Lipoprotein (VLDL) receptors. Substantial nutrients are transported to the oocyte through VLDL and VTG which plays an important role in follicular development within the ovary. (Gloux *et al*., 2019; Hu *et al*., 2014). Hence, the liver is essential in upholding the health and production of laying hens.

Various factors, such as free radicals within the body, can disturb the liver's normal functioning. They are generated in laying hens during their daily ovulation, which occurs when their production peaks. Oxidative stress occurs when the generation of endogenous antioxidants fails to counterbalance Reactive Oxygen Species (ROS) formation. Unregulated oxidative stress leads to protein oxidation, DNA damage, apoptosis, and inflammation (Adriani and Mushawwir, 2020;

Brouklogiannis *et al*., 2023). Oxidative stress induces mitochondrial malfunction, injuring hepatocytes (Abd El-Ghany and Babazadeh, 2022). This injury will adversely affect the process of egg yolk synthesis in laying hens. The generation of free radicals can diminish the nutritional content of eggs by amplifying lipid peroxidation in the egg yolk, decreasing egg quality (Hasbullah *et al*., 2020).

Feed additives may be incorporated into the diet to enhance the chemical composition of egg yolks. Feed additives enhance the well-being and efficiency of livestock and fulfill their nutritional requirements (Sulistyoningsih *et al*., 2014). The poultry industry can utilize probiotics as supplements in its feed. Probiotics are live microorganisms that enhance immunological function, decrease infections by harmful bacteria, enhance intestinal health and digestibility, increase enzyme activity, and improve intestinal morphology (Nabila *et al*., 2023).

Probiotics can be found in yogurt, which results from milk fermentation by Lactic Acid Bacteria (LAB), including *Lactobacillus*, *Bifidobacterium*, and *Streptococcus*. When the fermentation occurs, lactic acid bacteria will produce peptides, most of which are bioactive. This bioactive peptide serves multiple roles, including functioning as an antioxidant (Yuan *et al*., 2018). Numerous studies have indicated that antioxidants can reduce enzyme activities in producing ROS, prevent lipid peroxidation, and prevent free radicals from forming by contributing electrons to oxidant substances (Lobo *et al*., 2010; Hu *et al*., 2023). Our hypothesis suggested that antioxidant peptides can enhance the chemical composition of egg yolk. There are limited studies on the antioxidant peptide in the laying hens, particularly emphasizing chemical egg quality. Hence, this study assessed to determine the effect of antioxidant peptides derived from yogurt probiotics on the nutritive contents of egg yolk such as protein, fat, and cholesterol.

Materials and Methods

WSPE Yogurt Purification

The research will take place at the Health and Biomolecular Food Laboratory, Faculty of Mathematics and Natural Sciences, between June and November 2023. The purification process was conducted using gel filtration chromatography, as described by Gao *et al*. (2016), with minor adjustments. Gel filtration was used to divide the proteins according to their molecular weight or size using Sephadex G-25 as a matrix. The sample was added to 2% of the total volume of the matrix. It was later separated using a buffer of sodium acetate and natrium acetate with a pH of 4.5 (Ren *et al*., 2019). The final product was divided into fractions, each with a volume of 4 mL, and measured the absorbance using a spectrophotometer at 280 nm. The protein content of the fractions was measured using the biuret test, which allowed the identification of the fraction with the most significant peptide and protein profile. The detailed explanation of the gel-filtration chromatography mechanism is provided in the Fig. (1).

Antioxidant Activity Analysis

The antioxidant activity of WSPE yogurt and the peak of the purified fraction were evaluated with the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) method, following the method outlined by Jangnga *et al*. (2023), with minor adjustments. 20 mL of ethanol was used to dissolve the DPPH (1,5 mg) to make the solution. 1 mL of fraction obtained from column chromatography purification was combined with the DPPH solution. The mixture was then placed in a test tube and undisturbed for 30 min without light. The solution's absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. DPPH solution combined with distilled water was used as a control. The antioxidant activity was determined by the percentage of Radical Scavenging Activity (RSA) in the following equation (Valko *et al*., 2007):

$$RSA\ (\%) = \frac{(Absorbance\ of\ blank - Absorbance\ of\ sample)}{Absorbance\ of\ blank} \times 100$$

Experimental Design

ISA Brown strain laying hens aged 31 weeks were kept in a closed-house system for 42 days, including the preliminary period. The experiment trial was carried out according to the principles and safety protocols of the Animal Husbandry Faculty of Universitas Padjadjaran.

This study used three treatments, with each replicated twelve times, calculated using a Completely Randomized Design (CRD). Each cage will house one chicken, resulting in 36 chickens. The birds were given three treatments: basal diet as control (T1), basal diet mixed with 4% WSPE B1 (T2), and basal diet mixed with 4% WSPE B2 (T3). The animals were given a mixture of feed and WSPE yogurt twice daily, a total of 120 g/head/day. Water was given ad libitum for drinking.

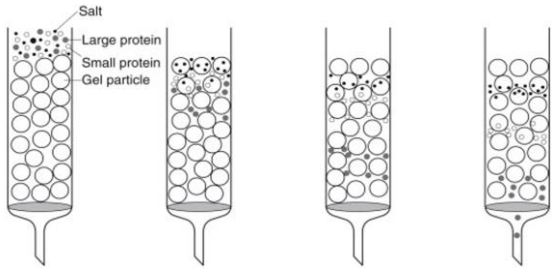

Fig. 1: The mechanism of gel-filtration chromatography (Prapulla and Karanth, 2014)

Data Collection

Eggs were collected during the second and fourth weeks, then separated and the yolk's nutrient content was examined at the Physiology and Biochemistry Laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran. The protein levels were examined using the Kjeldahl method, the lipid levels using the Soxhlet method, and lastly, the cholesterol levels using the Cholesterol *Oxidase–Peroxidase Aminoantipyrine Phenol* (CHOD-PAP) method.

Data Analysis

The Duncan's multiple range test was performed after analysis of variance (ANOVA) using IBM SPSS 22.0 to examine the data. The data were considered significantly different at $p>0.05$.

Results and Discussion

WSPE Yogurt Purification

This study has concluded that the fractions analyzed with the gel filtration chromatography and the biuret test yielded five peaks in each WSPE B1 and B2. This result is higher than that of Yukalo and Datsyshyn's (2019) findings, which only identified four peaks. It is due to the Sephadex G-100 gel matrix's higher cutoff of >10 kDa.

Peaks 1 and 2 represent proteins with large molecular weights. Peak 3 is a long peptide with a high aromatic ring concentration. Peak 4 is an unreactive peptide that cannot undergo a reaction with biuret, although it contains an aromatic ring. Peak 5 is an amino acid with an aromatic ring bond. The aromatic amino acids are tryptophan and tyrosine. On the other hand, phenylalanine also absorbs but is not visible at 280 nm (Simonian, 2022).

Antioxidant Activity Analysis

The antioxidant activity of WSPE yogurts B1 and B2 is shown in Fig. (2). The antioxidant activity increases after the purification process. According to Fig. (2), the highest antioxidant activity of B1 and B2 appeared at peak 3. Subsequently, the antioxidant activity of WSPE yogurt B2 was higher than B1. Compared to the previous studies, Aloğlu and Öner (2011) reported that peptides extracted from traditional and commercial yogurt had 17.36 and 18.67% antioxidant activity, respectively. Taha *et al.* (2017) have also reported that WSPE yogurt made from buffalo milk mixed with *Lb. Acidophilus* and *Lb. helveticus* has very high antioxidant activity.

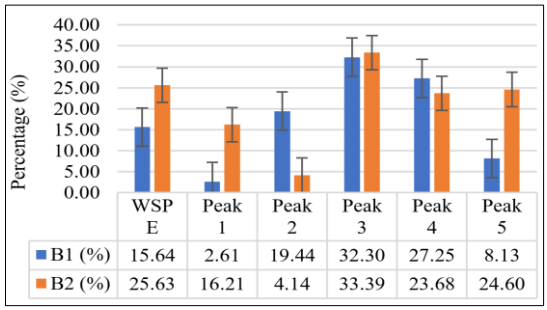

Fig. 2: DPPH inhibition percentage of WSPE yogurt B1 and B2

Peak 3 contains tryptophan, tyrosine, and phenylalanine, which act as antioxidants. This result was similar to Mann *et al.* (2017) findings, who concluded that tryptophan, tyrosine, and phenylalanine are hydrophobic and have aromatic residues capable of donating electrons to free radicals, converting them into non-radical compounds. Zou *et al.* (2016) added that these hydrophobic amino acids promote the transport of antioxidant peptides to the organs via hydrophobic interactions, preventing free radical damage.

Tryptophan, tyrosine, and phenylalanine are essential amino acids that help to synthesize the major neurotransmitters involved in numerous physiological activities, including serotonin, dopamine, and noradrenaline. Tryptophan is essential in chicken feed due to its involvement in the process of protein synthesis, which leads to improved performance and the production of hormone-like compounds. Studies indicated that these amino acids can decrease malondialdehyde levels, a compound resulting from lipid peroxidation that occurs during oxidative stress conditions (Macelline *et al.*, 2021; Dong and Zou, 2017).

Egg Yolk Nutrient Levels

Table (1) illustrates the effect of WSPE yogurt on the nutrient contents of egg yolk such as protein, lipid, and cholesterol. The results indicate that WSPE yogurt can enhance ($p<0.05$) egg yolk protein levels. The protein levels in T1 and T2 were higher compared to the control group. Meanwhile, no significant difference was observed in egg yolk lipid levels between the control and treatment groups. Although the change was not statistically significant, egg yolk lipid levels in treated chickens were lower compared to the control. No significant change was observed in egg yolk cholesterol levels between the control and treatment groups. However, the cholesterol levels were higher in treatments T1 and T2 than in the control group. Previous studies have indicated that probiotics enhance egg yolk protein and cholesterol levels while reducing lipid levels (Trani *et al.*, 2016; Situemang *et al.*, 2024). In contrast, other studies have reported that probiotics increased lipid levels and lowered cholesterol levels in egg yolk (Ramasamy *et al.*, 2009; Lokapirnasari *et al.*, 2020; Alaqil *et al.*, 2020).

Table 1: Protein, lipid and cholesterol levels in egg yolk

Parameters	Diets			p-value
	T1	T2	T3	
Protein (%)	16.41±0.15[a]	16.92±0.25[b]	16.77±0.31[b]	0.014
Lipid (%)	31.08±1.59[a]	28.92±2.1[a]	28.47±2.08[a]	0.11
Cholesterol (mg/dL)	255.58±17.08[a]	271.10±23.55[a]	260.28±28.24[a]	0.58

[a-b]Different superscripts indicate a significant difference ($p<0.05$)

The increased egg yolk protein level indicates that the antioxidants in WSPE yogurt can protect body cells from free radicals, ensuring that nutrition absorption from the digestive system to liver cells via the bloodstream flows effectively. This was in line with Mishra and Jha (2019), who stated that the antioxidant components in yogurt probiotics prevent free radicals from damaging intestinal cells and assist in maintaining intestinal mucosa health. The intestinal mucosa plays an important role in digestion and nutrient synthesis, whereas antioxidants sustain a variety of microbiota in epithelial cells. Maintaining gut health can improve nutrient absorption. Aside from preserving intestinal health, antioxidants can protect liver cells from oxidative stress. Allameh *et al.* (2023) stated that by neutralizing ROS and sustaining the redox state at physiological levels, antioxidants can protect liver cells against oxidative damage. Antioxidants of WSPE yogurt can improve the performance of antioxidant defense enzymes in liver cells, such as superoxide dismutase, glutathione peroxidase, and catalase. Maintaining liver cell health promotes protein synthesis, increasing egg yolk protein levels.

In this study, the lipid levels in egg yolks were reduced due to the probiotics altering the gut flora. By maintaining the health of the intestinal microbiota, probiotics can increase the efficiency of lipid metabolism and reduce the lipids presented to the egg yolk. This was in line with Alaqil *et al*. (2020), who stated that the gut microbiota is critical in the metabolism of feed lipids and the creation of lipid-derived signaling molecules, which can alter egg yolk composition. Moreover, this study showed that WSPE yogurt's antioxidants can prevent oxidative injury to lipids. Omri *et al*. (2019) indicated that lipid degradation in egg yolk may occur due to lipid oxidation and the formation of lipid hydroperoxides, both induced by free radicals. Antioxidants will neutralize free radicals, increasing the quality of egg yolk lipids.

In this study, WSPE yogurt did not reduce cholesterol levels in egg yolks. It is challenging to implement the mechanism for cholesterol reduction because laying hens at peak production will continue to produce endogenous cholesterol to fulfill their nutritional requirements. This was in line with Mushawwir *et al.* (2017), who stated that endogenous cholesterol synthesis can increase egg yolk cholesterol levels. Increased endogenous cholesterol directed to ovarian follicles raises egg yolk cholesterol levels. Faradillah *et al.* (2015) also mentioned that antioxidants can prevent lipid peroxidation while increasing egg yolk cholesterol levels. This is because antioxidants penetrate the membrane sac and convert into lipoproteins, facilitating embryonic growth. Despite the increase in egg yolk cholesterol levels, the egg yolk lipids remained decreased. This indicates that cholesterol has little effect on lipids, as Campos *et al*. (2016) stated that the lipid profile consists of 66% triglycerides, 28% phospholipids, and 6% cholesterol.

Conclusion

WSPE yogurts B1 and B2 produced five peaks upon purification, with peak 3 having the highest antioxidant activity. Further research on peptide purification using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method is required to precisely determine the peptide's size based on its molecular weight.

WSPE yogurt 4% is recommended for inclusion into Isa brown laying hens' diets, as it enhances egg yolk protein and lipid levels. However, 4% WSPE yogurt did not reduce egg yolk cholesterol levels. Therefore, administering yogurt probiotics to laying hens is recommended to enhance egg yolk protein levels while reducing lipid levels.

Acknowledgment

The authors would like to express their sincere gratitude to the Directorate of Research and Community Service (DRPM) of Universitas Padjadjaran for providing funding for this research through the Academic Leadership Grant (ALG) No.: 1549/un6.3.1/PT.00/2023. The health and biomolecular food laboratory, the faculty of mathematics and natural sciences and then the physiology and biochemistry laboratory the ruminant nutrition and animal feed chemistry laboratory, and the faculty of animal husbandry of Universitas Padjadjaran, are fully appreciated by the authors for the research facility support.

Funding Information

This research was funded by the Directorate of Research and Community Service (DRPM) of Universitas Padjadjaran through the Academic Leadership Grant (ALG) No.: 1549/un6.3.1/PT.00/2023.

Author's Contributions

Syifa Silviarahma Nurfadilah: Drafted the manuscript and collected the data for analysis.

Lovita Adriani: Designed the research and reviewed and edited the manuscript.

Safri Ishmayana: Developed the concept, designed and supervised the analysis and reviewed the manuscript.

Deny Saefulhadjar, Novi Mayasari and Mirnawati: Reviewed and edited the manuscript.

Ethics

The article is written fully by the authors and has never been published before. All authors have read, reviewed, and approved the manuscript. There are no ethical issues involved.

References

Abd El-Ghany, W. A., & Babazadeh, D. (2022). Betaine: A Potential Nutritional Metabolite in the Poultry Industry. *Animals*, *12*(19), 2624. https://doi.org/10.3390/ani12192624

Adriani, L., & Mushawwir, A. (2020). Correlation between Blood Parameters, Physiological and Liver Gene Expression Levels in Native Laying Hens Under Heat Stress. *IOP Conference Series: Earth and Environmental Science*, *466*(1), 012015. https://doi.org/10.1088/1755-1315/466/1/012015

Alaqil, A. A., Abbas, A. O., El-Beltagi, H. S., El-Atty, Hanaa. K. A., Mehaisen, G. M. K., & Moustafa, E. S. (2020). Dietary Supplementation of Probiotic Lactobacillus acidophilus Modulates Cholesterol Levels, Immune Response, and Productive Performance of Laying Hens. *Animals*, *10*(9), 1588. https://doi.org/10.3390/ani10091588

Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastiani, G., & Pantopoulos, K. (2023). Oxidative Stress in Liver Pathophysiology and Disease. *Antioxidants*, *12*(9), 1653. https://doi.org/10.3390/antiox12091653

Aloğlu, H. S., & Oner Z. (2011). Determination of antioxidant activity of bioactive peptide fractions obtained from yogurt. *Journal of Dairy Science*, *94*(11), 5305-5314. https://doi.org/10.3168/jds.2011-4285

Brouklogiannis, I. P., Anagnostopoulos, E. C., Griela, E., Paraskeuas, V. V., & Mountzouris, K. C. (2023). Dietary phytogenic inclusion level affects production performance and expression of ovarian cytoprotective genes in laying hens. *Poultry Science*, *102*(4), 102508. https://doi.org/10.1016/j.psj.2023.102508

Campos, A. M., Ricardo, F., Alves, E., Reis, A., Couto, D., Domingues, P., & Domingues, M. R. M. (2016). Lipidomic investigation of eggs' yolk: Changes in lipid profile of eggs from different conditions. *Food Research International*, *89*, 177–185. https://doi.org/10.1016/j.foodres.2016.07.006

Cui, Z., Amevor, F. K., Feng, Q., Kang, X., Song, W., Zhu, Q., Wang, Y., Li, D., & Zhao, X. (2020). Sexual Maturity Promotes Yolk Precursor Synthesis and Follicle Development in Hens via Liver-Blood-Ovary Signal Axis. *Animals*, *10*(12), 2348. https://doi.org/10.3390/ani10122348

Dong, X., & Zou, X. (2017). Effects of Excess Dietary Tryptophan on Laying Performance, Antioxidant Capacity and Immune Function of Laying Hens. *Amino Acid*. https://doi.org/10.5772/intechopen.68546

Faradillah, F., Mutia, R., & Abdullah, L. (2015). Substitution of Soybean Meal with Indigofera zollingeriana Top Leaf Meal on Egg Quality of Cortunix cortunix japonica. *Media Peternakan*, *38*(3), 192–197. https://doi.org/10.5398/medpet.2015.38.3.192

Gao, P., Li, J., Li, Z., Hao, J., & Zan, L. (2016). Establishment and application of milk fingerprint by gel filtration chromatography. *Journal of Dairy Science*, *99*(12), 9493–9501. https://doi.org/10.3168/jds.2015-10655

Gloux, A., Duclos, M. J., Brionne, A., Bourin, M., Nys, Y., & Réhault-Godbert, S. (2019). Integrative analysis of transcriptomic data related to the liver of laying hens: from physiological basics to newly identified functions. *BMC Genomics*, *20*(1), 821. https://doi.org/10.1186/s12864-019-6185-0

Hasbullah, I., Wulandari, Z., & Suci, D. M. (2020). Suplemen Jus Daun Afrika (Vernonia amygdalina) dalam Air Minum terhadap Komposisi Kimia dan Kadar Malondialdehid Telur Puyuh (Coturnix coturnix japonica). *Jurnal Ilmu Nutrisi Dan Teknologi Pakan*, *18*(2), 43–48. https://doi.org/10.29244/jintp.18.2.43-48

Hu, S., Liu, H., Pan, Z., Xia, L., Dong, X., Li, L., Xu, F., He, H., & Wang, J. (2014). Molecular cloning, expression profile and transcriptional modulation of two splice variants of very low density lipoprotein receptor during ovarian follicle development in geese (Anser cygnoide). *Animal Reproduction Science*, *149*(3–4), 281–296. https://doi.org/10.1016/j.anireprosci.2014.06.024

Hu, Y., Zhao, Y., Jia, X., Liu, D., Huang, X., Wang, C., Zhu, Y., Yue, C., Deng, S., & Lyu, Y. (2023). Lactic acid bacteria with a strong antioxidant function isolated from "Jiangshui," pickles, and feces. *Frontiers in Microbiology*, *14*, 1163662. https://doi.org/10.3389/fmicb.2023.1163662

Jangnga, I. P., Haskito, A. E. P., Sari, C., & Adrenalin, S. L. (2023). Total Lactic Acid Bacteria (LAB) and Antioxidant Activity of Goat's Milk Yoghurt with the Addition of Red Bran during Refrigeration Storage. *Journal of Applied Veterinary Science and Technology*, *4*(2), 61–66. https://doi.org/10.20473/javest.v4.i2.2023.61-66

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. In *Pharmacognosy Reviews* (Vol. 4, Issue 8, p. 118). https://doi.org/10.4103/0973-7847.70902

Lokapirnasari, W. P., Sahidu, A. M., Maslachah, L., Yulianto, A. B., & Najwan, R. (2020). The effect of combination Bifidobacterium sp and Lactobacillus acidophilus probiotic on egg yolk cholesterol, HDL, and LDL. *IOP Conference Series: Earth and Environmental Science*, *441*(1), 012049. https://doi.org/10.1088/1755-1315/441/1/012049

Macelline, S. P., Toghyani, M., Chrystal, P. V., Selle, P. H., & Liu, S. Y. (2021). Amino acid requirements for laying hens: a comprehensive review. *Poultry Science*, *100*(5), 101036. https://doi.org/10.1016/j.psj.2021.101036

Mann, B., Athira, S., Sharma, R., & Bajaj, R. (2017). Chapter 24 - Bioactive Peptides in Yogurt. *Yogurt in Health and Disease Prevention*, 411–426. https://doi.org/10.1016/b978-0-12-805134-4.00024-9

Mishra, B., & Jha, R. (2019). Oxidative Stress in the Poultry Gut: Potential Challenges and Interventions. *Frontiers in Veterinary Science*, *6*, 60. https://doi.org/10.3389/fvets.2019.00060

Mushawwir, A., Tanuwiria, U. H., Kamil, K. A., Adriani, L., & Wiradimadj, R. (2017). Effects of Volatile Oil of Garlic on Feed Utilization, Blood Biochemistry and Performance of Heat-stressed Japanese Quail. *Asian Journal of Poultry Science*, *11*(2), 83–89. https://doi.org/10.3923/ajpsaj.2017.83.89

Nabila, T. I., Adriani, L., Latipudin, D., Mushawwir, A., & Mayasari, N. (2023). Egg quality and production by the addition of dried consortium probiotics on late-phase laying hens. *Livestock and Animal Research*, *21*(2), 103. https://doi.org/10.20961/lar.v21i2.71956

Ohoilulin, Y. Y., Ralahalu, T. N., & Malle, D. (2023). Kadar Kolesterol Kuning Telur Ayam Ras pada Peternakan Ayam yang Berbeda. *Jurnal Ilmu Peternakan Dan Veteriner Tropis (Journal of Tropical Animal and Veterinary Science)*, *13*(2), 92–99. https://doi.org/10.46549/jipvet.v13i2.379

Omri, B., Alloui, N., Durazzo, A., Lucarini, M., Aiello, A., Romano, R., Santini, A., & Abdouli, H. (2019). Egg Yolk Antioxidants Profiles: Effect of Diet Supplementation with Linseeds and Tomato-Red Pepper Mixture before and after Storage. *Foods*, *8*(8), 320. https://doi.org/10.3390/foods8080320

Prapulla, S. G., & Karanth, N. G. (2014). Fermentation (Industrial) | Recovery of Metabolites. *Encyclopedia of Food Microbiology*, 822–833. https://doi.org/10.1016/b978-0-12-384730-0.00109-9

Ramasamy, K., Abdullah, N., Jalaludin, S., Wong, M., & Ho, Y. W. (2009). Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. *Journal of the Science of Food and Agriculture*, *89*(3), 482–486. https://doi.org/10.1002/jsfa.3477

Ren, J., He, W., Li, C., He, S., & Niu, D. (2019). Purification and identification of a novel antifungal protein from Bacillus subtilis XB-1. *World Journal of Microbiology and Biotechnology*, *35*(10), 150. https://doi.org/10.1007/s11274-019-2726-6

Sanlier, N., & Üstün, D. (2021). Egg consumption and health effects: A narrative review. *Journal of Food Science*, *86*(10), 4250–4261. https://doi.org/10.1111/1750-3841.15892

Sarantidi, E., Ainatzoglou, A., Papadimitriou, C., Stamoula, E., Maghiorou, K., Miflidi, A., Trichopoulou, A., Mountzouris, K. C., & Anagnostopoulos, A. K. (2023). Egg White and Yolk Protein Atlas: New Protein Insights of a Global Landmark Food. *Foods*, *12*(18), 3470. https://doi.org/10.3390/foods12183470

Simonian, M. H. (2022). Spectrophotometric determination of protein concentration. *Current Protocols in Cell Biology*, *15*(1), A-3B. https://doi.org/10.1002/0471143030.cba03bs15

Spence, J. D., Jenkins, D. J., & Davignon, J. (2010). Dietary cholesterol and egg yolks: Not for patients at risk of vascular disease. *Canadian Journal of Cardiology*, *26*(9), e336–e339. https://doi.org/10.1016/s0828-282x(10)70456-6

Sulistyoningsih, M., Dzakiy, M. A., & Nurwahyunani, A. (2014). Optimization of feed additive on body weight, abdominal fat and blood glucose levels broiler chicken. *Bioma*, *3*(2), 5–13.

Taha, S., El Abd, M., De Gobba, C., Abdel-Hamid, M., Khalil, E., & Hassan, D. (2017). Antioxidant and antibacterial activities of bioactive peptides in buffalo's yoghurt fermented with different starter cultures. *Food Science and Biotechnology*, *26*(5), 1325–1332. https://doi.org/10.1007/s10068-017-0160-9

Trani, A., Loizzo, P., Calvano, C. D., Cassone, A., Gambacorta, G., Zambrini, A. V., Zambonin, C. G., & Faccia, M. (2016). Effects of a Lactobacillus acidophilus D2 enriched diet on yolk protein in hen eggs. *European Poultry Science (EPS)*, *80*. https://doi.org/10.1399/eps.2016.124

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*(1), 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

Yuan, H., Lv, J., Gong, J., Xiao, G., Zhu, R., Li, L., & Qiu, J. (2018). Secondary structures and their effects on antioxidant capacity of antioxidant peptides in yogurt. *International Journal of Food Properties*, *21*(1), 2167–2180. https://doi.org/10.1080/10942912.2018.1501700

Yukalo, V., & Datsyshyn, K. (2019). Gel Filtration of Cow Milk Whey Proteins. *Food Science and Technology*, *12*(4), 72–78. https://doi.org/10.15673/fst.v12i4.1183

Zou, T.-B., He, T.-P., Li, H.-B., Tang, H.-W., & Xia, E.-Q. (2016). The Structure-Activity Relationship of the Antioxidant Peptides from Natural Proteins. *Molecules*, *21*(1), 72. https://doi.org/10.3390/molecules21010072