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Fermented Feed for Laying Hens: Effects on Egg Quality and the Number of Enteric Pathogenic Bacteria Present

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Abstract

Eggs are one of the most common food sources for human consumption because they are common, easy to obtain, and nutritious. Eggs contain abundant vitamins and minerals that the human body needs, are a good source of protein and fat and are rich in choline. Despite its high nutritional value, eggs are also one of the leading causes of food poisoning and foodborne diseases in various countries. This is due to contamination in eggs by pathogenic bacteria such as Salmonella, Shigella, Escherichia coli, and Campylobacter bacteria. Fermented chicken feed contains natural probiotics added to the feed, which improves gut health, better absorption of nutrients, inhibits the growth of pathogenic bacteria, enhances the immune response in chickens. This study is conducted to distinguish the diffrence in quality of eggs that can be physically observed in chicken eggs fed with fermented feed compared to chicken eggs fed regular feed, as well as to compare the number of total bacteria and enteric pathogenic bacteria contained in the contents of chicken eggs that includes *Escherichia coli* and *Salmonella* spp bacteria. The methods used in this research include observing egg quality comparisons, calculating and comparing the total number of bacteria, and testing for the presence of enteric pathogenic bacteria *Escherichia coli* and *Salmonella*, spp. using the plate count method. The resulting data was processed using the one-way ANOVA method with a confidence level of 95%. The results of this research show higher egg quality in fermented fed eggs which is characterized by the color of the yolk which tends to be darker yellow to orange and thicker egg shells. The total bacteria count showed that the bacteria was significantly lower in eggs from chickens fed fermented feed. Tests for the number of enteric bacteria showed positive results for E. coli in 1 out of 10 egg content samples from chicken fed with fermented feed, and positive results for *E. coli* in 2 out of 10 egg samples from chicken fed with regular feed, while test results for Salmonella spp. showed negative results in all samples.

Keywords: fermented feed; enteric pathogenic microbia; eggs; total bacteria count.

Abbreviations: LAB: Lactic Acid Bacteria; SS: Salmonella-Shigella; EMB: Eosin-Methylene Blue; PCA: Plate Count Agar; TPC: Total Plate Count

INTRODUCTION

Eggs are one of the most common food sources consumed by humans because they are a common, easy to obtain and nutritious food source. Eggs contain abundant vitamins and minerals that the human body needs, including calcium, iron, potassium, zinc, manganese, vitamin E, folate, and many more. Eggs are also a good source of protein and fat and rich in choline, which is an important nutrient for brain health. Even though they have high nutritional value, eggs are also one of the leading causes of food poisoning and foodborne diseases in various countries. This is caused by contamination that occurs in eggs by pathogenic bacteria during the production period. Food poisoning associated with egg-borne pathogens can cause high morbidity or mortality with symptoms such as diarrhea, vomiting, nausea and stomach cramps.

Enteric pathogenic bacteria are a group of bacteria that can cause intestinal infections that are transmitted through food, water, animals, or contact with infected individuals. (Arumugam et al., 2015). These bacteria can cause various gastrointestinal disorders, from mild to severe, which can be transmitted via the fecal-oral route. Some examples of enteric pathogens include Clostridioides difficile, Salmonella, Shigella, Escherichia coli, Campylobacter, Yersinia enterocolitica, and Vibrio cholerae. (Salihu et al., 2015).

To overcome pathogenic bacterial contamination in animal food products, especially eggs, in recent years many farms have added antibiotic supplements to animal feed. However, this gives rise to new problems, namely the emergence of more and more bacteria that have adapted to become resistant to various types of antibiotics. Research conducted by Jain and Yadav in 2017 found 73.33% of isolates of mutli-drug resistant

bacteria from egg samples with a resistance percentage of 86.66% to Cefixme antibiotics, 80% to Amoxicillin antibiotics, and 73.33% to Amoxyclave antibiotics. (Jain and Yadav., 2017). The emergence of bacteria that are resistant to antibiotics can be prevented by using other methods to combat pathogenic bacterial contamination in animals, namely by applying fermented feed. Fermented feed uses the basic principles of the growth of good microorganisms, one of which is Lactobacillus type bacteria which is capable of producing lactic acid which functions to fight the growth of pathogenic bacteria naturally and without the side effects that arise from the use of antibiotics in animal feed.

Chicken feed fermentation is a process that involves making a wet mixture from fermented wet food that is rich in probiotics for chickens. This process is believed to improve chicken health and egg quality. (Ibrahim et al., 2020). The type of microorganism selected for the fermentation process influences the substances produced after the fermentation process occurs. (Niba et al., 2009). Generally, the type of microorganism used for food fermentation is lactic acid bacteria (LAB) or Lactobacillus type bacteria because in the process it produces lactic acid which has many benefits, especially in the microecology of the digestive tract and has a good influence on the immune response. The microorganisms used in this research were Em4 solutions or effective microorganisms. The Em4 solution contains the main microorganisms in the form of phototrophic bacteria, lactic acid bacteria, yeast, actinomycetes and fermentation fungi. (Fajaruddin et al., 2013).

This research was done to determine the effect of fermented feed on egg quality that can be observed physically, including the color of the egg yolk and the thickness of the egg shell, to determine the difference in the number of enteric pathogenic bacteria contained in chicken eggs given fermented feed and chicken eggs fed regular feed, and to detect and differentiate the number of enteric pathogenic bacteria Escrherichia coli and Salmonella in chicken eggs fed fermented feed from chicken eggs fed regular feed.

MATERIALS AND METHODS

Study area

Samples were taken from two different chicken farms in the Condong Catur area of Yogyakarta city, which used different types of feed, which are fermented and regular feed. A total of 20 samples were taken, two from each farm, over five different periods.

Procedures

Preparation of Egg Samples and Egg Quality Observation

Each egg sample was soaked in 70% alcohol for 5 minutes to avoid contamination from bacteria on the egg shell. The sterile egg was cracked using a sterile spatula and poured into a sterilized jar. The color of the egg yolk was observed and documented for later comparison with other samples. Egg yolks and egg whites are homogenized by stirring using a sterile spatula until homogeneous. Serial dilutions were carried out for each sample up to a dilution of 10^{-5} by taking 1 ml of the sample and placing it in a test tube containing 9 ml of sterile distilled water.

Enumeration of Total Bacteria in Egg Samples

The results of the serial dilutions 10^{-3} to 10^{-5} were inoculated on 1 ml of PCA media using the spread plate method by taking 0.1 mL of suspension using a micropipette, all inoculated petri dishes then incubated in a 37°C incubator for 48 hours. The number of colonies was counted using the total plate count (TPC) method on each sample that had been incubated.

Identification and Enumeration of Enteric Pathogenic Bacteria Present

The same samples from the same suspensions were inoculated on EMB agar plate and SS agar plate using a pour plate method. All inoculated petri dishes then incubated in a 37°C incubator for 48 hours. The number of colonies were counted using total plate count (TPC) method on each sample that had been incubated.

Data analysis

All data that had been collected were analyzed using the one-way ANOVA method to determine the significance of the Difference between the two types of samples.

RESULTS AND DISCUSSION

Egg Quality Observation

The quality of the eggs tested in this research was done by comparing the color of the yolk from samples of chicken eggs fed fermented feed and samples of chicken eggs fed regular feed. Observation of the color of the egg yolk was carried out as an indicator of the carotenoid content contained in the chicken eggs samples, where a darker yellow to orange color indicated a higher carotenoid content in the egg.

Table 1. Average score value comparison of the yolk color of chicken eggs fed with fermented feed and chicken eggs fed with regular feed.

Types of Feed	Yolk Colour Score*
Fermented Feed	2,7
Regular Feed	1,7

*score 1 indicates lighter yellow, score 2 indicates yellow, score 3 indicates darker yellow, and score 4 indicates orange

Based on the results that had been obtained, eggs from chicken that are fed with fermented food tend to

have a darker yellow to orange color of yolk, which indicates a high content of carotenoids in the egg, while eggs from chicken that are fed with regular feed has a yolk color that tends to be yellow to lighter yellow which indicates a lower content of carotenoids. The carotenoid content in eggs is generally in the form of lutein and zeaxanthin, where these types of carotenoids cannot be synthesized in vitro so they must be obtained through the feed provided, therefore the profile of carotenoid content in eggs depends on the chicken's feeding. (Zaheer., 2017).

Based on the research conducted by Martínez-Sánchez and Pérez-Gálvez, 2023, feeding chickens with a wheat base such as regular chicken feed tends to result in eggs of lower quality, containing fewer carotenoids, resulting in egg yolks with a lighter color. Meanwhile, higher-quality pasteurized feed mixed with forages such as grass produces darker yellow to orange egg yolks due to the high carotenoid content in the eggs. (Martínez-Sánchez, and Pérez-Gálvez., 2023). Some of the ingredients of the fermented feed in this research are various types of leaves, grass and other forages, this then affects the carotenoid content in eggs, so chickens fed fermented feed have a higher carotenoid content in their eggs.

Total Number of Bacteria

Fermented feed, apart from having the benefit of adding beneficial nutritional content for animal health, also produces substances that play a role in reducing pathogenic bacterial contamination in animals, especially in the digestive tract. Fermented feed uses the basic principles of the growth of good microorganisms, one of which is *Lactobacillus* type bacteria, which is capable of producing lactic acid, which functions to fight the growth of pathogenic bacteria naturally and without the side effects that arise from the use of antibiotics in animal feed. This research tests the total number of bacteria in the egg samples using the TPC method.

Table 2. Total Number of Bacteria Formed on PCA media of chicken eggs fed with fermented feed and chicken eggs fed with regular feed (CFU/mL).

Types of Feed	Total Number of Bacteria
Fermented Feed	$282{,}4 \times 10^4$
Regular Feed	$1170{,}3 \times 10^4$

The total number bacteria found in the egg contents from chickens fed with fermented feed are significantly less than those found in the egg contents from chickens fed with regular feed. This is because one of the microorganisms contained in the form of lactic acid bacteria (LAB) in the Em4 solution in fermented feed can suppress and inhibit the growth of pathogenic bacteria in the animal's digestive tract, thereby reducing the possibility of pathogenic bacterial contamination in vivo during the egg formation process.

Enteric Pathogenic Bacteria Present

Escherichia coli and *Salmonella spp.* are Enterobacter types of pathogenic bacteria that are commonly found in the contamination of livestock food, especially chicken meat and eggs. These two bacteria are generally found in the digestive tract of livestock animals, but sometimes in vivo contamination during fertilization and egg formation can also occur so that these two bacteria are found in the egg content. (Al-Bahry et al., 2012). Identification and enumeration of *E. coli* was carried out using EMBA selective media, where positive results were indicated by the formation of dark purple bacterial colonies with metallic green sheen. Meanwhile, the Identification and enumeration of *Salmonella spp*. contained in the sample was carried out using selective salmonella-shigella agar media where the results were positive for the content of salmonella spp. characterized by the formation of transparent colonies with black precipitate in the colony's center.

Table 3. Average total number of enteric pathogenic bacteria of *e.coli* and *salmonella spp.* found in egg contents.

Types of Feed	*E.coli*	*Salmonella spp.*
Fermented Feed	$0{,}46 \times 10^3 \pm 2{,}12$	0 ± 0*
Regular Feed	$11{,}73 \times 10^3 \pm 1292{,}33$	0 ± 0*

*no salmonella growth was identified, but the growth of other microbes was detected.

The results of testing the number of *E.coli* bacteria in EMBA media showed that the number of enteric *E.coli* bacteria observed in sample number 7 of the egg from chicken with fermented feed was 4.6×10^3 CFU/mL, while comparison samples 6 and 7 of the egg from chicken with regular feed amounted to 114×10^3 and 3.3×10^3 CFU/mL. Each *E. coli* positive colony that forms has a flat or smooth surface with a circular shape, convex elevation, and is dark purple in color with a metallic green sheen on the medium.

The results of the tests on SS agar medium showed no *salmonella spp.* bacteria were detected. In all samples, however, there was a color change in the medium to yellow, and the formation of dense, colorless bacterial colonies with entire margins, and flat elevations indicating the growth of other types of bacteria. SS agar medium has selective properties that only allow certain types of bacteria to grow on it, due to this fact, we know that the other types of microbia found on this medium are other types of *enterobacter* bacteria.

The test results of this research are in line with several previous studies where the contamination content of pathogenic bacteria *E.coli* and *Salmonella spp.* is more commonly found on the outside of egg shells than inside the egg's contents because both types of bacteria usually

grow in the digestive tract of animals. Several studies that stated similar results include research conducted by Fardous and Suzzaman in 2015, which stated results of 86.67 growth of pathogenic bacteria on the outside of egg shells, including 10 of 14 serotypes of *Salmonella* bacteria, but no contamination was found at all in the egg contents. (Fardous and Suzzaman., 2015). Research conducted by Chaemsanit et al. in 2015 showed relatively similar results where 118 anaerobic bacteria were isolated from 16 egg samples, of which 116 were found on the outer shell and only two from the egg's contents. The isolate obtained from the contents of eggs in this study was identified as a member of the Enterobacteriaceae bacteria. (Chaemsanit et al., 2015). Finally, research conducted by Adesiyun et al. in 2020 found that there were 48.7% E.coli type bacteria and 2% *Salmonella* type bacteria on the outside of egg shells, but only 5.1% contained *E.coli* type bacteria and no other types of bacteria. *Salmonella* in the eggs. (Adesiyun et al., 2020). The minimal content of *E.coli* and the absence of *Salmonella* content in the eggs are good results which prove that there is very minimal contamination of these two pathogenic bacteria during in vivo egg formation or external contamination during egg production.

CONCLUSIONS

The results of this research prove fermented feed increased the quality of chicken eggs, and furthermore also significantly reduces the number of total and *Enterobacter* type pathogenic bacteria in egg content. Moreover, this research also proves that giving fermented feed improves eggs' quality and nutritional content. So making and feeding fermented feed is another better option that can be developed further and applied more widely as an alternative to reduce the growth and contamination of pathogenic bacteria without using antibiotics.

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REFERENCES

Adesiyun, A., Offiah, N., Seepersadsingh, N., Rodrigo, S., Lashley, V. & Musai, L., 2006. Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs. *Food research international*, *39*(2), pp.212-219.

Al-Bahry, SN, Mahmoud, IY, Al-Mushrafi, SK, & Al-Ali, MA 2012. Penetration of Spoilage and Food Poisoning Bacteria into Fresh Chicken Egg: A Public Health Concern. Global Journal of Bio-Science & Biotechnology. 1 (1): 33-39. *Biotechnology*, *1*(1), pp.33-39.

Arumugam, K., Sudigdoadi, S. & Nugraha, G.I., 2015. Enteric Pathogen Bacteria in Non-Broiler Chicken Egg Shells from Traditional Market and Supermarket, Jatinangor Subdistrict, West Java. *Althea Medical Journal*, *2*(3), pp.414-417.

Chaemsanit, S., Akbar, A. & Anal, A.K., 2015. Isolation of total aerobic and pathogenic bacteria from table eggs and its contents. *Food and Applied Bioscience Journal*, *3*(1), pp.1-9.

Fajaruddin, Junus M dan Setyowati E 2013 Pengaruh lama fermentasi EM4 terhadap kandungan protein kasar padatan kering lumpur organik unit gas bio (the influences of lenght fermentation EM4 by containing crude dry solid protein of organic mud of gass bio) *Jurnal Ilmu-Ilmu Peternakan* 23(2) 14-18

Fardous, J. and Shamsuzzaman, S.M., 2015. Detection of potential pathogenic aerobic bacteria from egg shell and egg contents of hen collected from poultry. *Bangladesh Medical Research Council Bulletin*, *41*(2), pp.67-72.

Ibrahim, D., Abdelfattah-Hassan, A., Arisha, A.H., Abd El-Aziz, R.M., Sherief, W.R., Adli, S.H., El Sayed, R. & Metwally, A.E., 2020. Impact of feeding anaerobically fermented feed supplemented with acidifiers on its quality and growth performance, intestinal villi and enteric pathogens of mulard ducks. *Livestock Science*, *242*, p.104299.

Jain, A.K. and Yadav, R.A.J.E.S.H., 2017. Study of antibiotic resistance in bacteria isolated from table egg. *Int. J. Pharm. Bio Sci*, *8*(1), pp.668-674.

Kanhar, A.R., Phulpoto, I.A., Ur-Rehman, S., Qazi, M.A., Ghumro, W.A., Hussain, S.F., Kanhar, A.A., Ujjan, J.A. & Hussain, A., 2022. Isolation, molecular typing and antibiotic sensitivity profiling of enteric bacterial pathogen from chicken eggs.

Martínez-Sánchez, V. and Pérez-Gálvez, A., 2023. Microalgal carotenoids for food and feed applications. *Handbook of Food and Feed from Microalgae* (pp. 133-145). Academic Press.

Pijnacker, R., Dallman, T.J., Tijsma, A.S., Hawkins, G., Larkin, L., Kotila, S.M., Amore, G., Amato, E., Suzuki, P.M., Denayer, S. and Klamer, S., 2019. An international outbreak of Salmonella enterica serotype Enteritidis linked to eggs from Poland: a microbiological and epidemiological study. *The Lancet Infectious Diseases*, *19*(7), pp.778-786

Ranjitkar, S. and Engberg, R.M., 2016. The influence of feeding crimped kernel maize silage on growth performance and intestinal colonization with Campylobacter jejuni of broilers. *Avian Pathology*, *45*(2), pp.253-260.

Ranjitkar, S., Karlsson, A.H., Petersen, M.A., Bredie, W.L., Petersen, J.S. and Engberg, R.M., 2016. The influence of feeding crimped kernel maize silage on broiler production, nutrient digestibility and meat quality. *British poultry science*, *57*(1), pp.93-104.

Ranjitkar, S., Lawley, B., Tannock, G. and Engberg, R.M., 2016. Bacterial succession in the broiler gastrointestinal tract. *Applied and environmental microbiology*, *82*(8), pp.2399-2410.

Sugiharto, S. & Ranjitkar, S., 2019. Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review. Animal nutrition, 5(1), pp.1-10.

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