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Original Article

Addition of *Bacillus subtilis* BN Strain Probiotic on Chicken Eggs

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ABSTRACT

The addition of probiotics is one effort to increase the chicken's appetite. There are many probiotics that can be mixed into feed to help improve the digestive and reproductive systems, including *Bacillus subtilis*, which has the function of increasing egg quality, including increasing shell thickness, increasing egg volume, increasing egg yolk and white index. This reseacrh aims to find out the effect of probiotic administration of *Bacillus subtilis BN Strain* on shell thickness, white index and chicken yolk index. This research used 38-week-old layer laying hens as many as 24 heads, randomized into four treatments with eight replays. Treatment consists of P0 without the addition of probiotics, P1 with the addition of *Bacillus subtilis BN Strain* as many as 0.005 grams / kg of feed, P2 with the addition of *Bacillus subtilis BN Strain* as many as 0.01 grams / kg of feed and P3 with the addition of *Bacillus subtilis BN Strain* as many as 0.02 grams / kg of feed. Based on the analysis of statistical data, the Analysis Of Variance (ANOVA) test showed no noticeable difference ($p>0.05$) to the thickness of the shell, white index or egg yolk index. The results showed that the addition of probiotic *Bacillus subtilis BN Strain* as many as 0.005 grams/kg of feed, 0.01 grams/kg of feed and 0.02 kg/kg of feed had no noticeable effect on the increase in egg shell thickness, egg white index and egg yolk index.

Keywords: Probiotics, *Bacillus subtilis BN Strain*, egg shell thickness, egg white index, egg yolk index

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Introduction

The need for consumption of chicken eggs in recent years has increased. This need is based on the economic value of protein from animal origin which is relatively cheap or more affordable when compared to chicken, fish or beef (Flachowsky *et al*., 2017). High consumption of protein needs must be accompanied by good processing of animal protein, because if it is not cooked at a temperature of 100-120°C it can cause residue consumption and will have an impact on increasing the risk of cancer (Endrinikapoulos *et al*., 2023). The large demand for consumption of chicken eggs requires delivery in large quantities without poor packaging and egg size selection. Vehicles carrying eggs pass through damaged roads, which causes many eggs to break during delivery, apart from the quality of the shell itself being less strong (Mertens *et al*., 2006).

Poor egg quality can be caused by the old age of the mother, resulting in the reproductive organs working less perfectly, so that the eggs produced have thin egg

shells, causing the eggs to crack and break easily so that the eggs are often contaminated with bacteria (Nasri *et al*., 2020). Recently, eggs have been widely processed and consumed as other products, eggs also have a delicious taste so they are popular with all levels of society, apart from that, the nutritional content of eggs is also quite complete, except for vitamin C (Réhault-Godbert *et al*., 2019). Eggs contain fat-soluble vitamins (A, D, E, and K) and also water-soluble vitamins (thiamin, vitamin B12, riboflavin, niacin, pantoneic acid and folic acid). Egg yolks have quite high cholesterol content (Spence *et al*., 2010). The protein content of eggs is found in the white and yolk.

According to Hoffman and Falvo (2004) one of the best sources of quality protein is eggs, and they contain high biological value and can be divided into egg yolk and egg white proteins. According to Kriseldi *et al*. (2018) who stated that reduced feed consumption results in reduced nutrition in the body and ultimately reduces egg production. The addition of probiotics is one effort to increase the chicken's appetite. Fesseha *et al*. (2021) stated that the effect of providing probiotics on ration consumption showed a significant difference, namely consumption of treated rations without probiotics was lower than consumption of treated rations with the addition of probiotics. The use of probiotics as a mixture of feed and drinking water has been widely used to increase livestock productivity. According to Lambo *et al*. (2021) the use of probiotics as a mixture of feed and drinking water has been widely used to increase livestock productivity.

There are many probiotics that can be mixed into feed to help improve the digestive and reproductive systems, including *Bacillus subtilis*, which has the function of increasing egg quality, including increasing shell thickness, increasing egg volume, increasing egg yolk and white index. This is proven by the statement of Wang *et al*. (2021) in their research stated that *Bacillus subtilis* increased egg production, egg weight, egg mass and reduced feed conversion. Mun *et al*. (2021) stated that the probiotic *Bacillus subtilis* helps the process of digestion and absorption of food essences in the chicken's body, this has an impact on increasing the chicken's appetite so that more nutrients enter and the nutrients for egg formation are better, this will also have an impact on increasing the quality of chicken eggs.

The probiotic microorganism *Bacillus subtilis* has been studied as a potential feed additive because it is capable of producing extraordinary extracellular enzymes, including protease, amylase, cellulase and lipase (Chen *et al*., 2009). This enzyme can increase the digestibility of protein, carbohydrates and lipids in chickens which results in an increase in the white index, yellow index and egg shell thickness (Salim *et al*., 2013). Research on the use of probiotics needs to be carried out in order to obtain high production results and good egg quality, so this research was carried out to determine the effect of *Bacillus subtilis* probiotics on egg white index, egg yolk index and chicken egg shell thickness.

Materials and methods

Research design

This research was carried out at a laying hen farm located on Jalan KH. Chusnan Ali, Talun Village, Montong District, Tuban Regency during October 2019 – November 2019. This research used 24 layer phase laying hens aged 38 weeks, randomized into four treatments with six replications. Treatments for layer chickens were control, treatment with probiotics.

Feeding

Chickens are given food twice a day in the morning and evening. Feed that has been mixed with probiotics with a homogeneity level of 80-120% is given at ± 120 g/head/day. Drinking water is provided ad libitum. Probiotic *Bacillus subtilis BN Strain* is mixed with complete feed for New Hope 7183A laying hens with a homogeneity level of 80-120%. The cage is cleaned first before the cage is used, then sprayed with disinfectant. Feeding and drinking places must be cleaned and ensure the flow of drinking water is smooth.

Chickens were randomly assigned to 4 treatments. Each treatment contained 6 chickens with an average chicken weight of 1,320 grams. Laying hens start to be reared at 38 weeks and are adapted for 1 week until they are 39 weeks old. Treatment is carried out when the chickens are 39 - 43 weeks old (for 4 weeks). Laying hens are placed in battery cages and labeled according to the treatment division. Probiotics were mixed with feed as follows P0 (control): without adding probiotics; P1: 0.005 grams of probiotic *Bacillus subtilis BN Strain* /kg feed; P2: 0.01 grams of probiotic *Bacillus subtilis BN Strain* /kg feed; P3: 0.02 grams of probiotic *Bacillus subtilis BN Strain* /kg feed.

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Sample Collection

Fresh eggs were cracked every day in the last week. Eggs were broken one by one to collect data on shell thickness, egg white height, egg white diameter, egg yolk height and egg yolk diameter. The data was collected for measuring shell thickness, egg white index and egg yolk index which were measured using a spherometer and caliper.

The eggs are broken one by one, the egg shell is separated from the white and yolk, the thickness of the egg shell is measured using a caliper, the yolk and white of the egg are placed on a flat glass, the length and width of the egg white are measured using a caliper (white and yolk should not be separately), the height of the egg white was measured using a spherometer, the egg was separated from the egg white, then the length and width of the yolk were measured using a caliper, the height of the egg yolk was measured using a spherometer.

Data Analysis

The data that has been obtained will be statistically analyzed using Analysis of Variance (ANOVA) to determine the significance of differences in the averages of the treatments given. Results that were significantly different were then continued with Duncan's Multiple Range Test with a level of 5% to determine the best treatment results. Statistical analysis using the SPSS 23.0 for Windows program.

Result

Egg shell thickness

Table 1. Eggshell thickness value

Treatment	Egg shell thickness value (cm)
P0	$0.038^{a} \pm 0.004$
P1	$0.040^{a} \pm 0.000$
P2	$0.040^{a} \pm 0.000$
P3	$0.041^{a} \pm 0.004$

Note: [a] The same superscript in the same column indicates no significant difference (p>0.05)

The average results and standard deviation of egg shell thickness values for each treatment are listed in table 1. The results of the average egg shell thickness values do not show a significant difference, which means that the average value of egg shell thickness is the same, namely P0 0.38 mm , P1 0.40 mm, P2 0.40 mm, and P3 0.41 mm.

Egg white index

Table 2. Egg white index value

Treatment	Egg white index value
P0	$0.126^{a} \pm 0.017$
P1	$0.136^{a} \pm 0.013$
P2	$0.138^{a} \pm 0.011$
P3	$0.139^{a} \pm 0.014$

Note: [a] The same superscript in the same column indicates no significant difference (p>0.05)

The average results and standard deviation of the egg white index values for each treatment are listed in table 2. The results of the average egg white index values do not show a significant difference, this means that the average egg white index values are the same.

Egg yolk index

The average and standard deviation of the egg yolk index values for each treatment are listed in table 3. The results of the average egg yolk index values do not show a significant difference, which means that the average egg yolk index values are the same.

Table 3. Egg yolk index value

Treatment	Egg yolk index value
P0	$0.403^{a} \pm 0.009$
P1	$0.405^{a} \pm 0.018$
P2	$0.410^{a} \pm 0.023$
P3	$0.411^{a} \pm 0.015$

Note: [a] The same superscript in the same column indicates no significant difference (p>0.05)

Discussion

Egg shell thickness

The average shell thickness values obtained were P0= 0.38 mm, P1= 0.40 mm, P2= 0.40 mm and P3= 0.41 mm. The results of the analysis using the ANOVA test showed that the average shell thickness showed no significant difference between the control P0 (without the addition of probiotics) and the treatment P1 given 0.005 grams of *Bacillus subtilis BN Strain* probiotics, P2 given 0.01 grams of *Bacillus subtilis BN Strain* probiotics and P3 was given the probiotic *Bacillus subtilis BN Strain* 0.02 grams. The lack of difference in shell thickness can be caused by a lack of accuracy in the measuring instrument, namely a vernier caliper with an accuracy of 0.1 mm, which should use an accuracy of 0.01 mm. Another disadvantage of the vernier caliper is that the small numbers make it difficult for nearsighted people to see

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them, so they need to be photographed and then enlarge the image to see them.

The absence of differences in shell thickness could also be caused by chickens consuming probiotics experiencing increased mucus in the small intestine. This fact is in accordance with Brümmer *et al.* (2010) that administration of cell wall products (mucus) from probiotics can stimulate goblet cells in the small intestine to produce mucus. This increase in mucus in the chicken's intestines is thought to be the cause of disrupted absorption of food substances so that the chicken will consume more rations to meet its nutritional needs. The difference was not significant because the feed given to chickens that were not given probiotics or were given probiotics met their needs. The thickness of laying hens' shells can be influenced by calcium and phosphorus consumption. Increasing or decreasing calcium consumption can affect the thickness of the egg shell. McClelland *et al.* (2021) stated that increasing the calcium content will result in thicker eggshell thickness.

Zhang *et al*. (2022) stated that shell thickness can be influenced by the nutrient content of the diet, shell layer components, age, physiological condition of the body and stress.

The calcium consumption of laying hens raised using or without probiotics as written on the feed label is 3.25-4.25%. The nutritional content is in accordance with the feed quality requirements for laying hens, namely 3.25-4.25%.

According to Ergun and Yamak (2017) shell thickness consists of thick, medium and thin. Krunt *et al.* (2021) stated that the average shell thickness of the Isa Brown strain is 0.33mm - 0.35 mm. Based on the results of this study, the average value of egg shell thickness can be classified into the thick category, namely in the control treatment and the provision of *Bacillus subtilis BN Strain* probiotics, namely with a value of more than 0.35 mm. Calcium consumption and use of calcium minerals followed by the addition of phosphorus minerals can influence shell thickness (Gutiérrez *et al*., 2020).

Egg white index

There was no significant difference in the average egg white index due to the less than optimal role of *Bacillus subtilis* in probiotics in secreting protease and lipase enzymes, so that it was less than optimal in the formation of amino acids as the main ingredients for the formation of egg whites (Su *et al*., 2020). Puglisi and Fernandez (2022) stated that the food nutrients that influence the egg white index are protein and amino acids in the ration. The absence of a real difference could also be due to rain in the outdoor coop which causes the feed to become wet, so that chicken feed consumption is reduced and the chickens become stressed. Feed consumption is influenced by environmental temperature, chicken health, housing, feed container, nutritional content in feed and stress (Hertamawati *et al*., 2022).

According to Quan and Benjakul (2019), albumen index values that are not significantly different are thought to occur because the average egg shape is oval. Eggs that are relatively long and narrow (oval or oval) at various sizes have a low egg index and eggs that are relatively short and wide (more towards round) have a high egg index. Each livestock produces a unique egg shape because egg shape is an inherited trait. Altuntaş and Şekeroğlu (2008) stated that oblong or oval shaped eggs have a low egg index, while eggs that are more round in shape have a large egg index in Merawang chicken eggs.

According to Quan and Benjakul (2019), albumen index values that are not significantly different are thought to occur because the function of the bacteria in probiotics from local microbes is not optimal. The addition of live microbes into the body will affect the host (poultry and ruminants) by improving the balance of microorganisms in the digestive tract (Pan and Yu, 2014). The results of the research indicate that nutrient absorption is hampered because the mucus produced covers the intestinal villi of the livestock. Conditions that occur in the digestive tract will have an impact on the eggs produced. Thus, the amount of nutrients such as protein absorbed by the body is lower, causing the formation of thick albumen to be hampered. Obstruction of the protein absorption process will result in low albumen index values.

Egg yolk index

The average value of the egg yolk index did not show a significant difference ($p>0.05$) between P0, P1, P2, and P3. This situation was influenced by the most important factors that influence the formation of the egg yolk index, namely protein and amino acids, because around 50% of the ingredients were Dried eggs contain protein so that the provision of amino acids in protein synthesis is very necessary to produce eggs (Wang *et al*., 2022). According to Aljumaah *et al*. (2020) *Bacillus subtilis* which plays a role in probiotics is less than optimal in secreting

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lipase and protease enzymes, so it is less than optimal in the formation of amino acids as the main ingredients for the formation of egg yolk. The feed consumption for laying hens was the same in the four chicken treatments so that the egg yolk index also had an insignificantly different effect. Feed is an important component in livestock business. According to Gao *et al.* (2021) feed has an important role in the process of egg formation, the body will absorb the nutrients produced from feed in the form of carbohydrates, proteins, fats, vitamins, calcium and minerals in molecular form and circulated throughout the body through the bloodstream as energy. Metabolic processes in cells will take place efficiently and effectively if the nutrients produced are optimal so as to ensure the availability of metabolic raw materials. The energy produced from this feed can be used for maintenance growth and egg production (Barzegar *et al*., 2019).

The adaptability of chickens to cage conditions and environments such as temperature and humidity is relatively low, so that the absorption of nutrients which have an impact on the yolk index of eggs produced by chickens with control treatment and probiotic administration is not significantly different. According to Barabas *et al.* (2022), environmental factors by using uniform cages and equipment so that all test animals receive the same lighting and temperature effects can make metabolism run well. Apart from that, it is thought to be caused by the relatively similar digestibility of the four groups, so that nutrient absorption for egg yolk index requirements is relatively the same (Liu *et al*., 2021).

Optimal egg production can be obtained if the animal's metabolic process runs well, good metabolic processes can be achieved with environmental and nutritional factors met (Chen *et al*., 2012). Protein is one of the factors that influences the formation of vitelline and khalaza membranes which function to maintain the strength of the yolk during the egg formation process so that a lack of protein supply will result in the yolk having a low level of strength (Zhai *et al*., 2022). This condition causes the index value which should increase to remain constant.

Conclusion

Based on the research that has been carried out, it can be concluded that additional doses of 0.005 grams, 0.01 grams and 0.02 grams/kg of feed cannot increase the value of egg shell thickness, egg white index and egg yolk index.

References

Aljumaah MR, Alkhulaifi MM, Abudabos AM, Aljumaah RS, Alsaleh AN, Stanley D. *Bacillus subtilis* PB6 based probiotic supplementation plays a role in the recovery after the necrotic enteritis challenge. PLoS One. 2020; 15(6): e0232781.

Altuntaş E, Şekeroğlu A. Effect of egg shape index on mechanical properties of chicken eggs. J Food Eng. 2008; 85(4): 606–612.

Barabas AJ, Darbyshire AK, Schlegel SL, Gaskill BN. Evaluation of Ambient Sound, Vibration, and Light in Rodent Housing Rooms. J Am Assoc Lab Anim Sci. 2022; 61(6): 660–671.

Barzegar S, Wu SB, Noblet J, Choct M, Swick RA. Energy efficiency and net energy prediction of feed in laying hens. Poult Sci. 2019; 98(11): 5746-5758.

Brümmer M, van Rensburg CJ, Moran CA. Saccharomyces cerevisiae cell wall products: The effects on gut morphology and performance of broiler chickens. S Afr J Anim Sci. 2010; 40(1): 14–21.

Chen KL, Kho WL, You SH, Yeh RH, Tang SW, Hsieh CW. Effects of *Bacillus subtilis* var. natto and Saccharomyces cerevisiae mixed fermented feed on the enhanced growth performance of broilers. Poult Sci. 2009; 88(2): 309–315.

Chen Y, Michalak M, Agellon LB. Importance of Nutrients and Nutrient Metabolism on Human Health. Yale J Biol Med. 2018; 91(2): 95-103.

Endrinikapoulos A, Afifah DN, Mexitalia M, Andoyo R, Hatimah I, Nuryanto N. Study of the importance of protein needs for catch-up growth in Indonesian stunted children: a narrative review. SAGE Open Med. 2023; 11: 20503121231165562.

Ergun OF, Yamak US. The effect of eggshell thickness on hatchability of quail eggs. Vet World. 2017; 10(9): 1114–1117.

Fesseha H, Demlie T, Mathewos M, Eshetu E. Effect of Lactobacillus Species Probiotics on Growth Performance of Dual-Purpose Chicken. Vet Med (Auckl). 2021; 12: 75–83.

Flachowsky G, Meyer U, Südekum KH. Land Use for Edible Protein of Animal Origin-A Review. Animals (Basel). 2017; 7(3): 25.

Gao Z, Zhang J, Li F, Zheng J, Xu G. Effect of Oils in Feed on the Production Performance and Egg Quality of Laying Hens. Animals (Basel). 2021; 11(12): 3482.

Gutiérrez OM, Porter AK, Viggeswarapu M, Roberts JL, Beck GR Jr. Effects of phosphorus and calcium to phosphorus consumption ratio on mineral metabolism and cardiometabolic health. J Nutr Biochem. 2020; 80: 108374.

How to Cite:
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Hertamawati RT, Prasetyo B, Suryadi U. Early production performance of crossing chickens raised on indoor or with outdoor access. IOP Conf Ser: Earth Environ Sci. 2022; 980: 012018.

Hoffman JR, Falvo MJ. Protein - Which is Best? J Sports Sci Med. 2004; 3(3): 118–130.

Kriseldi R, Tillman PB, Jiang Z, Dozier WA 3rd. Effects of feeding reduced crude protein diets on growth performance, nitrogen excretion, and plasma uric acid concentration of broiler chicks during the starter period. Poult Sci. 2018; 97(5): 1614–1626.

Krunt O, Zita L, Kraus A, Okrouhlá M, Chodová D, Stupka R. Guinea fowl (*Numida meleagris*) eggs and free-range housing: a convenient alternative to laying hens' eggs in terms of food safety? Poult Sci. 2021; 100(4): 101006.

Lambo MT, Chang X, Liu D. The Recent Trend in the Use of Multistrain Probiotics in Livestock Production: An Overview. Animals (Basel). 2021; 11(10): 2805.

Liu H, Ding P, Tong Y, He X, Yin Y, Zhang H, Song Z. Metabolomic analysis of the egg yolk during the embryonic development of broilers. Poult Sci. 2021; 100(4): 101014.

McClelland SC, Cassey P, Maurer G, Hauber ME, Portugal SJ. How much calcium to shell out? Eggshell calcium carbonate content is greater in birds with thinner shells, larger clutches and longer lifespans. J R Soc Interface. 2021; 18(182): 20210502.

Mertens K, Bamelis F, Kemps B, Kamers B, Verhoelst E, De Ketelaere B, Bain M, Decuypere E, De Baerdemaeker J. Monitoring of eggshell breakage and eggshell strength in different production chains of consumption eggs. Poult Sci. 2006; 85(9): 1670–1677.

Mun D, Kyoung H, Kong M, Ryu S, Jang KB, Baek J, Park KI, Song M, Kim Y. Effects of Bacillus-based probiotics on growth performance, nutrient digestibility, and intestinal health of weaned pigs. J Anim Sci Technol. 2021; 63(6): 1314–1327.

Nasri H, van den Brand H, Najar T, Bouzouaia M. Interactions between Egg Storage Duration and Breeder Age on Selected Egg Quality, Hatching Results, and Chicken Quality. Animals. 2020; 10(10): 1719.

Pan D, Yu Z. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 2014; 5(1): 108–119.

Puglisi MJ, Fernandez ML. The Health Benefits of Egg Protein. Nutrients. 2022; 14(14): 2904.

Quan TH, Benjakul S. Duck egg albumen: physicochemical and functional properties as affected by storage and processing. J Food Sci Technol. 2019; 56(3): 1104–1115.

Réhault-Godbert S, Guyot N, Nys Y. The Golden Egg: Nutritional Value, Bioactivities, and Emerging Benefits for Human Health. Nutrients. 2019; 11(3): 684.

Salim HM, Kang HK, Akter N, Kim DW, Kim JH, Kim MJ, Na JC, Jong HB, Choi HC, Suh OS, Kim WK. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poult Sci. 2013; 92(8): 2084–2090.

Spence JD, Jenkins DJ, Davignon J. Dietary cholesterol and egg yolks: not for patients at risk of vascular disease. Can J Cardiol. 2010; 26(9): e336-9.

Su Y, Liu C, Fang H, Zhang D. *Bacillus subtilis*: a universal cell factory for industry, agriculture, biomaterials and medicine. Microb Cell Fact. 2020; 19(1): 173.

Wang J, Wang WW, Qi GH, Cui CF, Wu SG, Zhang HJ, Xu L, Wang J. Effects of dietary *Bacillus subtilis* supplementation and calcium levels on performance and eggshell quality of laying hens in the late phase of production. Poult Sci. 2021; 100(3): 100970.

Wang Y, Wang J, Shi Y, Ye H, Luo W, Geng F. Quantitative proteomic analyses during formation of chicken egg yolk. Food Chem. 2022; 374: 131828.

Zhai YL, Dong SJ, Zou MM, Qin YD, Liu LL, Cao MH, Huang MQ, Vasseur L, You MS, Peng L. Vitelline Membrane Protein 26 Mutagenesis, Using CRISPR/Cas9, Results in Egg Collapse in *Plutella xylostella*. Int J Mol Sci. 2022; 23(17): 9538.

Zhang Y, Deng Y, Jin Y, Zhuang Z, Huang X, Li K, Wang S, Xia W, Ruan D, Wang S, Zheng C, Chen W. Dietary zinc supplementation affects eggshell quality and ultrastructure in commercial laying ducks by influencing calcium metabolism. Poult Sci. 2022; 101(1): 101539.

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