Review Article

Administration of Probiotics to Increase Egg Production and Extend the Productivity on Late-Phase Laying Hen: A Review

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Abstract | Eggs are a relatively inexpensive and easily obtained food. The average laying hens produce 240 – 260 eggs/bird/year. However, at the age of 80 weeks laying hens must be culled because their productivity has decreased below 50%. Many efforts have been made to extend the egg production cycle so that production increases before laying hens are culled. Probiotics supplementation is one method of increasing productivity in old age laying hens. This review explores the potential of probiotics that can increase egg production in laying hen late-phase, which discusses the reproductive system, including hormones and the reproductive tract, productivity, digestive system, and health of laying hens at an advanced age. This article attempts to provide a complete summary of the potential benefits of probiotics in prolonging the production cycle of late-phase laying hens based on existing literature. The research presented in this study offers pertinent insights and theoretical frameworks for enhancing productivity in aging hens, particularly for individuals engaged in small-scale farming.

Keywords | Late-phase laying hen, Probiotic, Reproductive, Digestion, Egg production, Egg quality, Health.

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INTRODUCTION

Over the last two decades, the worldwide manufacturing and utilisation of chicken eggs have demonstrated extraordinary, broad, and comprehensive dynamics (Zaheer, 2015). Eggs are an inexpensive protein source with high nutritional value, which is especially important for children's brain development (FAO, 2012; IEF, 2014), this explains why global egg consumption is so high. Indonesia's economy relies heavily on the poultry industry. In accordance with Association of Indonesian Animal Feed Producers, this industry can provide 65% of Indonesia's animal protein, employs 12 million individuals, and is worth over USD 34 billion (Wright and Darmawan, 2017). The evolution of egg production and consumption parallels that of milk, followed by the evolution of poultry meat. Compared to ruminants, the shorter life cycle of poultry allows production to adjust flexibly and develop towards fewer laying hens (FAO, 2018). Laying hens are one type of poultry that can produce a relatively high number of eggs, namely 240 – 260 eggs/bird/year. Some European countries, such as Belgium and the Netherlands, extend the production age of laying hens to above 90 weeks to reduce farm waste, maintain chicken welfare, and improve production efficiency. Apart from Europe, several countries in Asia, such as Japan, South Korea, Taiwan, and Thailand, also extend the production age of laying hens beyond 80 weeks. Extending the production age of laying hens beyond 80 weeks of age is still controversial and needs to be done with caution and consideration of the health and welfare of the hens. Therefore, strict supervision and compliance with each country's production and animal welfare regulations are required. In Indonesia, laying hens over 80 weeks of age must be culled under Directorate General of Livestock and Animal Health Circular No. 12141/SE/PK.230/F/11/2019 dated 10 November 2019. This instruction aims to balance supply and demand for egg consumption. High operational costs are the primary loss factor if it is late to do the culling. However, many small-scale farmers in Indonesia extend the rearing until the age of 110. By extending the productivity period of laying hens, more eggs will be produced so that the production cost per egg can be reduced due to the low feed intake of the late-phase laying hen.

Following the production peak, there is a decline in egg production quantity and quality (Liu et al., 2013). Laying hen productivity is affected by age, nutrition, housing systems, and health status (Vlckova et al., 2019). Some studies state the age of late-phase laying hens, but it is still unclear when it starts. Zhan et al. (2018) stated that the age of late-phase laying hens starts at 48 weeks, while Xu et al. (2023) is at age 58 (Xu et al., 2023). Depending on Hansen et al. (2003) and Wistedt et al. 2019, the age of old phase laying hens begins or starts at 70 weeks to 90 weeks or even more. In this study, the source of the cited studies is from the age range of 48 weeks to 90 weeks more. Production of the late phase of laying hens below 90% results from decreased function of physiological systems, primarily intestinal function. One of the efforts to increase the productivity of late-phase laying hens can be made by giving probiotics.

When ingested in sufficient quantities, probiotics confer health benefits. Probiotics change the abundance and activity of microbes, which ultimately regulate the balance of the flora ecosystem in the small intestine. This affects the host's metabolism and health (Adriani, 2005; Feng and Liu, 2022).

Probiotics reduce oxidative stress, promote gut health, and enhance livestock performance. In addition, probiotics promote the production of digestive enzymes and antibacterial substances that prevent the development of harmful bacteria (Kumalasari et al., 2020). Several researchers have administered probiotics with *Bacillus spp.*, *Lactobacillus spp.*, and *Bifidobacterium spp*. strains to late-phase laying hens. The results showed an improvement in egg production modulated from digestion and reproduction.

This review will examine the probiotic impact on the productivity of late-phase laying hens. It has significant economic consequences and can be used to develop better techniques for raising healthier late-phase laying hens. That makes it possible to extend the production life and future research directions. Finally, this article attempts to provide a complete summary of the potential benefits of probiotics in prolonging the production cycle of late-phase laying hens based on existing literature. The research presented in this study offers pertinent insights and theoretical frameworks for enhancing productivity in aging hens, particularly for individuals engaged in small-scale farming.

MATERIAL AND METHODS

This literature review includes research articles from Scopus, Proquest, Pubmed, and Science Direct. The utilization of four search engines so that the resulting data is a reputable international article. Articles published between 2012 and 2023 were retrieved and reviewed, as were relevant articles from the reference lists. Keywords using Boolean Search so that the articles searched can match the expected target, the keywords used were ("probiotic" OR "fermented feed") AND "late-phase laying hen" AND "reproductive"; ("probiotic" OR "fermented feed") AND "late-phase laying hen" AND "produti*"; ("probiotic" OR "fermented feed") AND "late-phase laying hen" AND ("health" OR "immune") were used to find the relevant article related to this research.

Criteria for Admission and Exclusion

The eligibility criteria were applied to the titles and abstracts. Articles that did not fit the guiding question and criteria were eliminated. Mendeley Desktop was used for this investigation to capture literature from metadata obtained from Scopus, Science Direct, Pubmed, and Proquest and track duplicate articles. The following data were tabulated in NVIVO 12 with various narrowing criteria, including author, research location, method, the unit of analysis (probiotic strain, dose of probiotic, chicken strain, and age of chicken), and variables (reproduction, production, egg quality, and health). This study includes original articles, review articles, proceedings articles, book chapters, and related websites. Articles published other than in English were not included in this study.

Characteristics of The Study

In the preliminary stage, 855 published article titles were perused, out of which 346 were deemed suitable for further evaluation. Subsequently, the articles were screened using Mendeley Desktop, selecting 342 articles after removing duplicates. Following the screening process, 271 papers were excluded based on their abstracts, and 71 articles were identified for review. Following a comprehensive screening process, 59 articles were deemed ineligible for inclusion based on predetermined criteria. The study incorporated the remaining 12 articles to conduct a thorough data analysis that ensured quality. The steps of the literature review are shown in Figure 1.

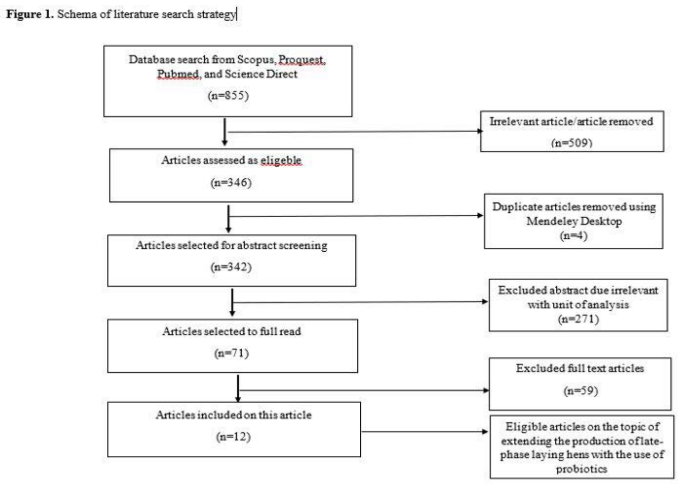

Figure 1: Scheme of literature search strategy

The Concept Of Probiotics

The digestive tract's microbial activity has a considerable effect on the metabolism of poultry. The host gut environment and feed affect gut microorganisms (Pan and Yu, 2014). The gut microbiome is primarily influenced by feed composition, including crucial nutrients, anti-nutritional factors, feed additives, and probiotics (Bajagal et al., 2016; Bryden et al., 2021). Probiotics are viable microorganisms that provide health benefits to the host when ingested in sufficient quantities (Hill et al., 2014). The definition needs to specify an adequate amount. However WHO regulations (2001) and National Standardization Agency (2009) require a minimum dose of 10^7 Colony Forming Units (CFU). Multiple research studies have indicated that an increased dosage of 10^9 colony-forming units per day administered orally is necessary for reestablishing and preserving bacterial equilibrium (Amaral et al., 2014; Zarate et al., 2006). Sufficient levels indicate a health benefit, but this does not necessarily imply that a more considerable number of dangerous bacteria is usually advantageous.

There are many benefits when adding probiotics to animal feed, including reducing pathogen colonization (competitive exclusion), acting as antibiotics, and inducing immune responses. Besides that, it can improve gut health, livestock performance, blood protein and albumin levels, and lower cholesterol and triglycerides. Giving probiotics in animal feed can produce enzymes, antibacterial substances, vitamins B and K, volatile fatty acids (acetic acid, butyric acid and propionic acid), and organic acids, improving livestock welfare (Gleeson et al., 2012; Shang et al., 2018; Villageliu and Lyte 2017; Kraimi et al., 2019; Kumalasari et al., 2020; Adriani et al., 2020; Adriani et al., 2023).

Production Of Probiotics

The selection of strains is crucial in producing probiotics because it affects the effectiveness and safety obtained. Considerations in selecting strains include the strains used must be (a) certified and not have adverse effects on health, (b) choose strains suitable for the purpose because some strains have higher probiotic activity than other strains and have the potential to help overcome health problems, (c) the selected strain must be able to develop and interact with the existing intestinal microflora to improve the balance of intestinal microflora, (d) the strain must be stable and resistant to environmental factors such as temperature, acids, and chemicals in order to function correctly in probiotic products. By considering these factors, selecting the right strain can be imperative to ascertain the quality and efficacy of the probiotic product.

The genera commonly used to manufacture probiotics include *Lactobacillus, Bacillus, Bifidobacteria,* and *Streptococcus. Lactobacillus* is a genus of bacteria that produce lactase and lactic acid. Lactobacillus bacteria are found naturally in the body. *Lactobacillus* strains often used in probiotics include *Lactobacillus bulgaricus, Lactobacillus reuteri,* and *Lactobacillus acidophilus. Lactobacillus bulgaricus* is a gram-positive and homofermentative bacterium. The primary outcome of glucose fermentation is limited to lactic acid production. The lactic acid produced acts as an inhibitor for pathogenic microbes so that fermented products with high lactic acid levels will last longer. *Lactobacillus reuteri* is a species commonly found in the intestines and mouth, reducing bacteria that cause tooth decay (Hecht, 2017). *Lactobacillus acidophilus* is a gram-positive bacterium that is a natural species found in the intestine. Due to its tolerance and resilience to gastric digestive enzymes (pH 1–5) and bile acids, *Lactobacillus acidophilus* can live throughout the stomach and upper small intestine. It is abundant in the small intestine and the rest of the digestive tract. This bacteria can adhere to the digestive tract's epithelial cells (Adriani, 2010).

Common yogurt bacteria include *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Streptococcus thermophilus* is a homofermentative organism that produces more than 85 percent lactic acid throughout its fermentation process. In addition to creating lactic acid, *Streptococcus thermophilus* also produces the enzyme lactase, which works to digest lactose in milk and deconstruct milk protein through the action of protease enzymes (Adriani, 2010). In line with Adriani (2005), however, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are not encompassed in the group of reliable probiotics since their numbers are reducing in the digestive tract, namely the large intestine. Combining multiple strains is more advantageous because it becomes more effective for health. It has been proven that consortium strains improve intestinal barrier function and growth performance (Sugiharto et al., 2018; Ouwehand et al., 2018).

As do *Lactobacillus* strains, the genus *Bifidobacterium* usually colonizes the human large intestine. *Bifidobacterium* bifidum is classified as gram-positive. In the fermentation process, it can break down glucose into acetic acid and lactic acid with a ratio of 3: 2. This bacterium belongs to the heterofermentative group. However, it does not produce CO_2 from glucose fermentation (Adriani, 2010). Consortium of *Lactobacillus* and *Bifidobacterium* as a yogurt starter in the right balance can increase lipase enzyme activity up to two times compared to yogurt with a mixture of bacteria in general (Lengkey and Adriani, 2009). These two bacteria are reliable because they can reach the large intestine. However, using Lactobacillus and Bifidobacterium is not exclusive due to probiotic microorganisms and the increasing number of probiotic foods available to consumers (Hecht, 2017).

The *Bacillus* genus is a spore-forming probiotic bacteria that allows almost unlimited storage until it is ready for consumption and can survive stomach acid in the bile and small intestine (Bruno, 2023). *Bacillus* is a stable and heat-resistant bacteria, so it is suitable for application as pelleted feed (Nicholson, 2002; Yang et al., 2019b). Several studies showed many benefits of added *B. subtilis* on feed livestock, including lowering the pH of the intestinal gut, increasing the bacterial composition of the intestinal microbiota, producing digestive enzymes, increasing nutrient absorption, improving FCR, and improving small intestine morphology (Mountzouris et al., 2010; Ma et al., 2012; Sen et al., 2012; Abdelqader et al., 2013; Sobczak and Koz1Owski, 2015; Zhang et al., 2016; Guo et al., 2017). *Bacillus subtilis* has the potential to enhance the thickness of eggshells by promoting the absorption of minerals and the deposition of calcium in bone tissue. *B. subtilis* is effective if added to the feed as much as $2x10^9$ CFU/kg (Abdelqader et al., 2013; Cui, 2018; Wang et al., 2021). The supplementation of *B. subtilis* has a comparatively advantageous impact compared to the supplementation of *B. licheniformis* (Yang et al., 2019b).

Clostridium butyricum is a gram-positive, endospore-forming bacterium that thrives in anaerobic conditions. Unlike *Lactobacillus* and *Bifidobacterium, C. butyricum* tolerates greater bile concentrations and temperatures (Kong et al., 2011; Zhan et al., 2018). In addition, *C. butyricum* can create Short-Chain Fatty Acids (SCFA), boost antioxidant enzyme activity in broiler chickens, and improve their immunological function (Cao et al., 2012; Zhao et al., 2013) This technology facilitates storage, transport, and avoiding contamination. Water, oxygen, heat, strong acids, bile, and environmental stresses, including pH, water activity, and oxygen in liquid preparations, might damage probiotics during storage and consumption (Wang et al., 2020a).

Probing probiotics in a powdered form can be produced through various technological approaches such as oven drying, vacuum oven, spray drying, drying with fluid flow, freeze-drying, and lyophilization. Each of these methodologies possesses a distinct array of benefits and drawbacks. Oven drying is considered to be one of the drying techniques. Reducing the number of microorganisms limits the efficacy of oven drying (Kumalasari et al., 2020). Kumalasari et al. research (2020) showed that lactic acid levels and the degree of acidity (pH) complied with the 2009 Indonesia Standard National. However, the total lactic acid bacteria decreased due to a decrease due to heating, but the results on broiler blood cholesterol were good. Drying using freeze-drying technology is often used for biological compounds, however, it is time-intensive, relatively more expensive, and has efficiency problems (Huang et al., 2017; Luangthongkam et al., 2021). The most widely used drying is spray drying because of significant results, which resulted in the survival of more lactic acid bacteria than freeze-drying. Nevertheless, preventing significant cell mortality during spray drying remains challenging (Alvarenga et al., 2018; Behboudi-Jobbehdar et al., 2013; Gotor-Vila et al., 2017; Liu et al., 2019; Wang et al., 2020c; Luangthongkam et al., 2021; Adriani et al., 2021).

Laying Hen

Lohman Brown was developed by a German company called Lohmann Tierzucht in the 1970s through crosses between several breeds of laying hens that were selectively selected. Lohmann Brown is known for its high egg productivity, good disease resistance, and easy adaptation to different environments. With a reasonable feed conversion rate, Lohmann Brown can produce around 300-320 eggs annually. Peak production on Lohmann Brown at 26-30 weeks after hatching. This chicken has a low mortality rate and good tolerance for extreme temperatures (Mishra and Mishra, 2016).

Hy-line was developed by the farming company Hy-Line International in the United States by combining several superior strains of laying hens. Hy-Line Brown is known for its high egg productivity, good resistance to disease and easy adaptation to different environments. Hy-Line Brown females can produce around 320-330 eggs per year. Peak production in these chickens usually occurs at 25-32 weeks of age. At that time, Hy-Line Brown can produce about 95% of the total maximum egg production that can be achieved in one year (Hy-line International, 2019).

Maintenance pullets significantly impact subsequent egg production and the welfare of adult layers (Janczak and Riber, 2015; Jongman, 2021). Following the culmination of egg production and quality, encompassing egg-laying velocity, eggshell integrity, and nutritional value, the aging of laying hens will result in a decline in these factors. This is attributed to the accumulation of oxidative stress incurred through prolonged egg production (Liu et al., 2013; Liu et al., 2018).

The capacity of chicken to lay eggs typically begins to decline between the ages of 60-64 weeks. This decline is caused by the deterioration of morphological structures in the small intestine related to aging, such as the height of the villi, the depth of the crypt, and the composition of the bacteria. The morphological characteristics of the intestine structure and bacterial composition are crucial in optimizing nutrient utilization efficiency and enhancing product performance (National Academics of Science, 2017; Yang et al., 2019b).

The critical period occurs when the pullets attain the prescribed weight threshold between 14 to 16 weeks and exhibit the appropriate body composition to sustain egg-laying capacity beyond 90 weeks. Hence, it is imperative to adhere to a particular growth trajectory. The significance of this cannot be overstated in the context of laying hen production. The primary objective in Europe is to enhance egg production through selective breeding aimed at augmenting egg durability and stability of quality, thereby enabling commercial flocks to extend their egg-laying cycle to 90-100 weeks. Although there may be opposition to current agricultural practices, extending the egg-laying period of hens is a rational strategy for optimizing resource utilization, given the financial and environmental advantages (Bain and Dunn, 2016).

Egg Production

Asia accounts for the majority of global egg production, with a share of over 64%. China is the foremost egg producer globally, contributing to 38% of the total production, while the United States and India follow with a share of 7% each. In response to increasing demand, the production of eggs has been augmented from 15 to 93 million tons. Over the past 30 years, global egg output has surged by 150 percent. Asia's production has nearly quadrupled. About 80% of rural households in developing nations keep poultry (FAO, 2023).

Egg production is influenced by various factors, including feed consumption (both in terms of quality and quantity), water intake, the intensity and duration of light exposure, parasite infestation, disease, and various management and environmental factors. As for other causes, according to Jacob et al. (2014), noninfectious causes include age, improper nutrition, negligence of feed ingredients, and poisoning. Husbandry management errors include conditions, running out of feed and drinking water, inadequate day length, and high temperatures. In addition, ectoparasites and endoparasites significantly affect production. Ectoparasites are parasitic organisms that feed on the outside of the host body. The ectoparasites include lice, fowl mites, and fleas. Apart from parasites, various diseases also need to be watched out for during rearing.Egg production is usually based on the age at which the first egg is laid so that it is known how quickly the hen reaches sexual maturity. Egg production is measured through Hen Day Production (HDP), Hen House Production (HHP), and weekly production. HDP is a term used in the livestock industry to measure the number of eggs produced by a group of hens in one day. This term is obtained by calculating the egg production divided by the number of hens on that day, multiplied by 100%. HDP measurement is essential to help farmers monitor productivity and improve hen management and feed if necessary. HHP is a term used to measure the number of eggs produced by all the hens in a pen or chicken farm in one day. HHP is calculated by calculating the egg production produced divided by the number of hens at the beginning of the production period multiplied by 100%. HHP measurement helps farmers monitor the overall productivity of the chicken farm and forecast future egg production. From here, farmers can determine strategies and actions to increase the productivity of chicken eggs. Weekly egg production is a term used to measure the number of eggs produced by a group of hens in one week. Measuring weekly egg production is important to help farmers monitor hen productivity every week and make decisions regarding management, feed, and health. By measuring weekly egg production, farmers can evaluate the hen farm's performance over time and identify factors that affect hen productivity.

Potential Role Of Probiotics

Dietary composition, including fundamental nutrients, anti-nutritional factors, and feed additives, especially probiotics, can affect the gut microbiota of all animals (Bajagal et al., 2016). There have been various studies on probiotics administered to laying hens. However, study on probiotic administration to laying hens in late-phase is limited. Several studies on the administration of probiotics and their effect on laying hens late-phase are presented in Table 1 and Table 2. Late-phase laying hens in this study starts at 48 weeks and continue until 90 weeks or more cited. Providing probiotics to livestock can modulate the immune system by producing antimicrobial metabolites (antitoxic effect), competitive with pathogenic bacteria in the intestine (Ahasan et al., 2015), and preventing intestinal and reproductive diseases (Shini et al., 2013), resulting in improved performance, egg production, and blood quality

Table 1: Studies of Improvement Egg Production with Administered Probiotics in Late-Phase Laying Hens

Probiotic Species	Age Hen (weeks)	Strain of Laying Hen	Dosage	Dietary Treatment (% egg production)		Author	Study Location
				Control	Probiotic		
Lactobacillus acidophilus, Lactobacillus rhamosus, Lactobacillus bulgaricus, Bifidobacterium bifidum, Streptococcus thermophilus, E. faecium, A. oryzae, Clostridium pintolopesi, and *Lactobacillus plantarum*	70-90	White Leghorn	10^6 CFU/ml	58,51	64,95	Anwar and Rahman, 2016	Pakistan
Lactobacillus acidophilus, Lactobacillus bulgaricus, Streptococcus thermophilus, and *Bifidobacterium bifidum*	90	Lohman Brown	4%	9,75	21,3	Adriani et.al, 2021	Indonesia
Bacillus licheniformis and *Bacillus subtilis*	60-72	Hy-Line Brown	6,6 x 10^5 : 3,3 x 10^5 CFU/g	75,9	82,2	Yang et.al, 2019	China
Bacillus subtilis	64-75	Lohman White	2.3 × 10^8 CFU/g	70	74	Adelqader et.al, 2013	Jordan
	72 to 79	Hy-Line Brown	2 x 10^9 CFU/kg	88.88	89.50	Wang et.al, 2021	China
Clostridium butyricum	48	Jinghong-1 strain	5 × 10^4 CFU/g	85.4	91,4	Zhan et.al, 2018	China
	59	Hy-Line Brown	8,4 × 10^8 CFU/kg	83,4	87,5	Wang et. al, 2020	China
Lactobacillus salivarius	56-67	Hy-Line Brown	1 x 10^8 CFU/kg	88.35	86.92	Xu et. al, 2022	China
Rhodobacter capsulatus	54-62	Hy-Line Brown	0,15% of 10^8 CFU/kg	76,92	79,20	Lokhande et. al, 2013	Korea
Enterococcus faecalis	72	Hy-Line Brown	7,5 x 10^8 CFU/kg	79,4	84,1	Zhang et. al, 2019	China

Table 2: Studies of Improvement Egg Quality with Administered Probiotics in Late-Phase Laying Hens

Probiotic species	Age hen (weeks)	Strain of Laying Hen	Dosage	Treatment	Quality						Author	Study Location
					Egg weight (g)	Eggshell strength (kg/cm²)	Eggshell thickness (mm)	Albumen height (mm)	Yolk color	Haugh unit		
Bacillus subtilis	64-75	Lohman White	2.3 × 10[illegible] CFU/g	Control	61,0		0,33				Abdelqader et. al, 2013	Jordan
				Probiotic	64,8		0,36					
	72-79	Hy-Line Brown	2 x 10[illegible] CFU/kg	Control	65,85	3,76	0,431				Wang et. al, 2021	China
				Probiotic	66,10	3,85	0,435					
Clostridium butyricum	48	Jinghong-1 strain	5 × 10[illegible] CFU/g	Control	61,4	2,4	0,29	5,70	5,50	75,8	Zhan et. al, 2018	China
				Probiotic	62,5	3,55	0,32	6,67	5,92	81,5		
	59	Hy-Line Brown	8.4 × 10[illegible] CFU/kg	Control	69,78	34,02	0,44	6,85	6,70	78,46	Wang et. al, 2020	China
				Probiotic	70,13	36,48	0,43	7,04	7,52	80,55		
Bacillus licheniformis and *Bacillus subtilis*	60 to 72	Hy-Line Brown	6,6 x 10[illegible] : 3,3 x 10[illegible] CFU/g	Control	61,8	3,58	0,32	7,8	6,3	84,8	Yang et. al, 2019	China
				Probiotic	62,1	3,88	0,34	8,0	6,8	89,9		
Lactobacillus salivarius	56-67	Hyline Brown	1 x 10[illegible] CFU/kg	Control	63,10	3,43	0,32	6,67		78,84	Xu et. al, 2022	China
				Probiotic	63,74	3,68	0,34	6,94		82,08		
Rhodobacter capsulatus	54-62	Hy-Line Brown	10[illegible]CFU/kg 0,15%	Control	61,87	3,83	0,39	8,66	8			Korea
				Probiotic	63,83	4,16	0,41	9,03	9,4		Lokhande et. al, 2013	
Enterococcus faecalis	72	Hy-Line Brown	7,5 x 10[illegible] CFU/kg	Control	61,2		0,38	5,42		70,2	Zhang et. al, 2019	China
				Probiotic	61,7		0,39	5,40		70,7		
Bacillus subtilis, Lactic Acid Bacteria, and *Saccharomyces*	58	Tianfu Green Shell	1 × 10[illegible] CFU/g : 2 × 10[illegible] CFU/g : 1 × 10[illegible] CFU/g),	Control	55,61	3,46	0,31	7,13	13,40	85,49	Xu et. al, 2023	China
				Probiotic	56,36	4,15	0,34	7,68	12,00	88,63		

(Kalsum et al., 2012; Zhang et al., 2012). Additionally, laying hens reproductive health can be enhanced by probiotics by lowering reproductive system infections such as oviduct and reproductive tract infections (Yang et al., 2019b).

The Role Of Probiotics In Reproduction

The hypothalamus regulates reproduction in birds in response to environmental and endocrine cues. The pituitary gland is the body's main gland, consisting of adenohypophysis and neurohypophysis. The adenohypophysis secretes five primary hormones, and they are as follows: growth hormone (GH), prolactin (PRL), gonadotrophs (FSH and LH), and adrenocorticotropic hormone (ACTH) (Anwar et al., 2012). Egg production and quality decline rapidly as chicks age resulting in reduced activity of the hypothalamic cells that promote reproduction. The result is a decrease in the weight and function of the reproductive tract, resulting in a delayed phase (Gautron et al., 2021).

The ovary and oviduct constitute the reproductive system of laying hens (infundibulum, magnum, isthmus, uterus, and vagina). The ovary is a crucial foundation of the laying hen as changes or damage to ovarian pathology affects follicle number and growth, ultimately affecting the quantity and quality of eggs produced (Xu et al., 2023). Reproductive hormones, specifically the hormones Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) influence the development and growth of the reproductive tract. The process of follicular development and ovulation, governed by reproductive hormones, determines the ability to produce eggs. FSH is a hormone essential for follicle growth, development, dominance, maturation, and ovulation (Lv et al., 2019). In addition, LH and progesterone play important roles in sexual maturity, participating in ovulation and ovoposition, and egg production (Puebla-Osorio et al., 2002; Anwar and Rahman, 2016). The reproductive tract's growth and development must be optimal for follicle development and the preparation of material components to produce high-quality eggs.

Adding probiotics to feed can reflect an increase in hormonal status and productivity. This is because it can optimize the FSH hormone by increasing follicle growth which increases egg yolk weight, and the LH hormone, which increases the ovulation rate, which can be seen from the increase in the percentage of egg production (Khalid and Abdul-Rahman, 2011).

The administration of *Bacillus licheniformis* and *Bacillus amyloliquefaciens* to laying hens elevates serum follicle-stimulating hormone (FSH) and estradiol levels, leading to an increase in ovarian weight and the facilitation of follicular growth and maturation. (Lei et al., 2013; Zhou et al., 2020). The observed enhancements can be attributed to the regulatory effects of probiotics on ovarian development, which stimulate the secretion of follicle-stimulating hormone (FSH) and estradiol, thereby enhancing reproductive capacity and improving follicular quality (Xu et al., 2023). The study of Anwar and Rahman (2016) investigated the use of probiotics in laying hens aged 70 to 90 weeks on the post-molting cycle efficacy of laying hens, as seen from the dynamics of the pituitary gonadotrophs. The production increase compared to the control was 11.4%. Laying hens fed probiotics consisting of *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus rhamosus, Bifidobacterium bifidum, Candida pintolopesi, Enterococcus faecium, Streptococcus thermophilus, Aspergillus oryzae*, and *Lactobacillus plantarum* as much as 10^6 CFU/ml, showed an increase in production performance connected with an increase in gonadotroph cellular dynamics, increased diameter of the gonadotrophs of FSH and LH. This increase in diameter accounts for the increased production of these hormones and, ultimately, the higher egg production. Due to the enhanced pituitary response in LHRH release in post-molted chicks, increased LH release is reported in the plasma after the molt (Anwar and Rahman, 2016). Another study revealed that plasma LH concentrations decreased after molting and increased again after feeding.

Probiotics have been shown to improve follicular development in laying hens. Probiotics can regulate follicular development by increasing levels of reproductive hormones such as FSH, estradiol, and growth hormone while decreasing levels of adrenal cortical hormones. The correlation between health function and reproductive function has been observed, and it has been found that the administration of probiotics can enhance the regulation of host metabolism. The utilization of probiotics and their associated compounds has been observed to augment reproductive success by improving the efficacy of the epithelial barrier against pathogenic microorganisms (Feng and Liu, 2022).

The Role Of Probiotics In Gut

Due to physiological factors, egg production declines steadily with age. The physiological characteristics of old chickens include degeneration of the morphological structure and bacterial composition of the intestinal mucosa , which causes reduced absorption of nutrients (Karcher et al., 2015; Yang et al., 2019a). The role of the digestive tract and microbial ecology is vital in production (Ricke et al., 2022). The maintenance of physiological and behavioural equilibrium in chickens, as well as their optimal growth, reproduction, health, and well-being, depends on the balance of the gut microbiota (Clavijo and Flórez, 2018). The gut microbiota benefits the host through regulating digestion and immunity, as well as the maintenance of the integrity of the intestinal mucosa. The large intestine harbours various microbiotas essential for synthesising B vitamins and short-chain fatty acids. Supplementation of probiotic bacteria induces the proliferation of intestinal epithelial cells in avian species (Yang et al., 2019a). Healthy gut microbiota is rich in *Bifidobacterium spp., Faecalibacterium spp., Ruminococcus spp.* and *Prevotella spp.* (Jr et al., 2019; Villapol, 2020). As other species diminish, Firmicutes, Bacteroides, Desulfobacterota, and Actinobacteriota increase during late-phase egg-laying (Lv et al., 2019; Xu et al., 2023). Lee et al. (2019) stated that the number of microbial species such as *Megamonas, Bacteroides,* and *Oscillospira* increased in the oviducts as laying hens matured. *Pseudomonas* is the most abundant general genus in the cloaca,while *Lactobacillus* is the most abundant general genus in the colon and magnum. *Pseudomonas* is an aerobic bacterium that can quickly colonize the cloaca compared to the colon and magnum (Lemfack et al., 2020).The gradual rise in mucosal permeability resulting from harm to the intestinal mucosa paves the way for pathogen infection. The composition of the intestinal microflora undergoes alterations for the maturation process of chicks, leading to a heightened susceptibility of the intestinal mucosal system and a concomitant decline in its structural integrity. Therefore, feeding beneficial bacteria such as probiotics can restore gut integrity, improve gut health, and increase nutrient availability and absorption (Abdelqader et al., 2013).Lactic acid bacteria have been found to enhance enzyme activity and the production of lactic acid, short-chain fatty acids, and antimicrobial compounds such as bacteriocins, hydrogen peroxide, and diacetyl. These compounds have been shown to impede pathogenic microorganisms' growth and proliferation effectively. Reduced pathogenic bacteria activity increases enzyme activity and expands the intestinal villi's surface, improving nutrient absorption and digestion (Lengkey and Adriani, 2013). Poultry utilizes highly digestible feed to enhance its egg-laying capacity. The enhancement of nutrient absorption is directly proportional to the elongation of villi and the increase in their density in the small intestine. The utilization of multiple strains in probiotics is more efficacious compared to the use of single strains.. Strains in multi-species probiotics can serve a variety of functions. This consortium's strains have been demonstrated to improve gut barrier function (Ouwehand et al. 2018).

The Role Of Probiotics In Health

Studies have indicated that the administration of probiotics can benefit the immune system of animals (Deng et al., 2012; Bai et al., 2013). Probiotics may also modulate the immune system, produce antimicrobial metabolites, inhibit pathogen adhesion to the intestinal mucosal epithelium, and compete with pathogenic bacteria (Ahasan et al., 2015). Prior research has indicated that including probiotics in animal, diets affects the activity of antioxidant enzymes.

In the poultry industry, the performance and quality of the final phase of laying hens usually decrease, followed by an increase in mortality (Liu et al., 2018). Many microbial species colonize the intestinal tract of chicks from an early age, and they are crucial to various host physiological functions in the digestive tract and other body systems. The oviduct plays a crucial role in both egg formation and immune defence against pathogens in laying hens. The function above holds significant importance in ensuring the avian's general well-being and the secure generation of oviparous products (Shini et al., 2013).

Laying chickens egg production can be adversely affected by intestinal inflammation caused by mucosal barrier dysfunction. Understanding how mucosal barrier function affects egg production is crucial to increasing egg production (Nii, 2022). Oviducts are vulnerable to infection from bacterial and viral pathogenic microorganisms. The oviduct may be susceptible to infection by *Salmonella, E. coli,* and *Mycoplasma*, which can lead to functional disorders in egg formation and contamination of the eggs (Ozaki and Murase, 2009). *Salmonella enteritidis* is internalised by macrophages in the intestinal tract, which can migrate to the ovaries and fallopian tubes through the circulatory system (De Buck et al., 2004). Vaginal pathogens can reach the fallopian tubes from the cloaca. To prevent pathogen infection and maintain hygienic egg production, chicken oviduct mucosal barrier function must be improved (Nii et al., 2022).

In a study by Yoruk et al. (2004), probiotics at the final phase showed an increase in feed conversion with a decrease in mortality. Dietary prebiotics, probiotics, and synbiotics are potentially safe alternatives for enhancing poultry performance and health (Liu et al., 2019; Lv et al., 2019).

Zhan et al. (2018) reported that the administration of *C. butyricum* $5x10^4$ cfu/g can enhance the egg-laying performance and quality of laying hens. This is achieved through promoting immune function, augmentation of antioxidant capacity, and improvement of laying hen cecal microbiota during final production. Furthermore, the mechanisms of probiotic effect may include immune system modulation, antitoxic effects, pathogen adhesion to the intestinal mucosal epithelium, pro-reduction of antimicrobial metabolites, and competitive exclusion of probiotics and pathogenic bacteria (Ahasan et al., 2015) Numerous studies have demonstrated that consuming *C. butyricum* by animals yields advantageous outcomes on immunoglobulins. Various research studies have demonstrated that consuming *C. butyricum* by animals yields advantageous outcomes on immunoglobulins. This is due to probiotic's ability to produce large amounts of Short Chain Fatty Acid (SCFA), compete with pathogens on the intestinal surface, and inhibit pathogen adhesion via lipoteichoic acid (Gao et al., 2011; Mookiah et al., 2014).

The Impact Of Probiotics On Egg Production In Laying Hens

Several studies have shown an increase in production in late-phase laying hens after the administration of probiotics (Abdelqader et al., 2013; Lohkande et al., 2013; Anwar and Rahman, 2016; Yang et al., 2019b; Adriani et al., 2021; Wang et al., 2021). Research by Abdelqader et al. (2013) showed that giving probiotics with *B. subtilis* at 64 weeks increased egg production by 4% compared to the control. Lohkande et al. (2013) found that administering *R. capsulatus* to laying chickens aged 54 to 62 weeks increased egg production by 4%. Anwar and Rahman (2016) showed that using probiotics and symbiotic probiotics with vitamins improved egg production in laying hens in the post-molting phase (20 weeks) with an increase of 66.11% and 62.78% compared to the control 59.71%. This increase in egg production is closely related to the size of the pituitary gland FSH and LH gonadotrophs (Anwar et al., 2015). Research Yang et al. (2019b) administration of dried probiotics with a combination of *B. licheniformis* and *B. subtilis* ($6.6 \times 10^5 : 3.3 \times 10^5$ BL: BS) in old Hy-Line Brown chickens aged 60 weeks showed a laying rate of 82% after 16 weeks reared, 8% higher than in control chickens. This is related to changes in the histomorphology of the intestinal mucosa. The administration of probiotics significantly improved the height of villi, depth of crypts, and V/C values in the duodenum, jejunum, and ileum. Adriani et al. (2021) reported in a study that giving probiotic powder at 4% with the starter *L. acidophilus, L. bulgaricus, S. thermophilus*, and *B. bifidum* to late-phase laying hens Lohman Brown at 90 weeks resulted in a 118% increase in production. This is due to the presence of lactic acid bacteria, which increases nutrient digestibility and absorption. Lactic acid bacteria increase enzyme activity, lactic acid, SCFA, and antimicrobials (bacteriocins, hydrogen peroxide, diacetyl) effectively inhibit the development of pathogenic microbes. Symbiotic administration between probiotic *B. subtilis* 10^9 and Ca 3.6% reached 89.86% compared to the control treatment of 87.88%, although not significantly different (Wang et.al, 2021).In contrast to the findings of Xu et. al, (2022) the administration of *L. salivarius* probiotics to laying hens aged 56-67 weeks decreased compared to controls by 1.64%, although not statistically significantly different.

The Impact Of Probiotics On Egg Quality In Laying Hens

The performance and quality of eggs in laying hens will decrease with increasing age and sometimes followed by an increase in mortality (Liu et al., 2018). The quality of eggshells is a crucial factor that impacts the economic viability of egg production and the ability of eggs to hatch. The strength of the eggshell serves as a protective mechanism against physical harm and subsequent infiltration of pathogenic microorganisms. Increases in egg size can explain decreased eggshell quality during the final stages of production or late-phase laying hen, reductions in the metabolism of nutrients, especially Ca, and reproductive hormones, especially estrogens. The phenomenon of egg cracking could surpass 20% towards the conclusion of the laying cycle due to the comparatively reduced capacity of aged chickens to assimilate calcium effectively (Abdelqader et al., 2013; Attia et. al, 2020).

Several studies have shown that probiotics in late-phase laying hens can improve egg quality compared to controls (Abdelqader et. al, 2013; Lokhande et. al, 2013; Zhan et. al, 2018; Yang et. al, 2019b; Wang et. al, 2020b; Wang et. al, 2021; Xu et. al, 2021). The enhancement of eggshell durability is linked to the capacity of probiotics to generate significant quantities of Short Chain Fatty Acid (SCFA) and reduce the pH, thereby fostering the assimilation and exploitation of essential micronutrients, such as calcium (Zhang et al., 2011). Increasing bone calcification in old laying hens can help reduce eggshell damage and other issues related to egg production's high calcium needs. Increased mineral uptake and enhanced calcium incorporation into skeletal tissue lead to the development of a thicker eggshell and an increase in egg weight (Chowdhury and Smith, 2001). The intestinal microflora ferments oligosaccharides without hydrolyzed. This results in enhanced calcium absorption. The reduction in luminal pH is a result of microbial fermentation, which aids in the conversion of water-insoluble Ca into ionic forms, thereby enhancing its solubility. Fermentation of undigested carbohydrates results in a higher concentration of short-chain carboxylic acids, which increase the absorption of minerals, especially Ca^{2+} (Roberfroid, 2000; Abdelqader et al., 2013).

Yolk color is influenced by intestinal absorption of fatty acids, which favor the absorption of fat-soluble constituents such as xanthophylls resulting in an elevation in precipitate into the yolk with a subsequent increase in yolk color (Wang et. al, 2020b).

Safety Aspects Of Probiotics

The importance of food safety to consumers, the food industry, and the economy cannot be disputed.. So is the ban on antibiotic growth promoters (AGPs) in livestock because they leave residues that are feared to harm consumers. Probiotics have been suggested as a potential substitute for antibiotic growth promoters (AGPs) to enhance the quality of meat and eggs. This phenomenon is necessitated by the concurrent rise in population and the demand for meat and eggs. The safety aspect of probiotics in laying hens is a crucial consideration for poultry producers. Ensuring that any supplement or additive used in poultry feed does not harm the bird's health or egg quality is vital.

The administration of probiotics has been shown to have potential benefits in mitigating oxidative stress, promoting gastrointestinal well-being, and augmenting livestock productivity. The benefits of probiotics depend on appropriate dosing, generally ranging from 10^6-10^7 CFU/g per day. Numerous academic studies have demonstrated that using probiotics yields beneficial outcomes for livestock performance, specifically for production and health. Especially using probiotics in old laying hens can improve production, egg quality, and health (Adriani et al., 2023; Adriani et al., 2021; Zhang et al., 2019). Probiotics produce lactic acid, organic acids, antimicrobial compounds (bacteriocins, hydrogen peroxide, and diacetyl), short-chain fatty acids (SCFA), several vitamins and minerals, antioxidants, and inhibition zones of disease-causing bacteria (Adriani et al, 2020; Kumalasari et al, 2020). The administration of probiotics to poultry has been found to facilitate the production of digestive enzymes and antibacterial compounds that can effectively inhibit the growth of pathogenic microorganisms. Moreover, poultry's immune system and metabolism are stimulated, leading to vitamin B and vitamin K synthesis, which serve as precursors to antioxidants (Gleeson et al., 2012).

Probiotics are generally considered safe for laying hens if given at the recommended dose and duration. The effectiveness of probiotics is contingent upon various factors, including the quantity and duration of probiotic consumption, the specific probiotic strain utilized, and the state and composition of the gut microbiota (Al-Fatah, 2020). The efficacy of probiotic preparations is contingent upon the interplay between various strains of probiotic bacteria in feed additives and the microflora of the gastrointestinal tract. This is a crucial factor to consider when selecting probiotics. A limitation associated with probiotics pertains to the necessity of comprehensively grasping the concept of synergism among diverse bacterial strains. Although probiotics are beneficial in promoting poultry health, their effectiveness can vary depending on the specific type of probiotic bacteria and the dose administered (Methner, 2000; Krysiak et al., 2021). Some probiotic bacteria are susceptible to specific environmental conditions, such as temperature and humidity, which can affect their stability and durability. This can reduce the effectiveness of probiotics if they cannot survive during shipping or storage.

Lactobacilli and *bifidobacteria* were initially considered suitable probiotic candidates due to their robust nature and ability to be isolated through technological means and produced on an industrial scale. Numerous investigations concerning next-generation probiotics (NGP) or direct therapeutic products (LBP) may pertain to microbial genera and species that have not been previously employed in the food sector. There is a search for potential candidates among gut bacteria associated with health, specifically those belonging to the genera *Bacteroides, Clostridium, Faecalibacterium,* and *Akkermansia.* Additionally, genetically modified strains are also being considered (Saarela, 2018).

CONCLUSIONS AND RECOMMENDATIONS

effective Laying hens in the late phase can increase their productivity in terms of economy and benefits. Probiotics are one way that can be done to increase the productivity of the late-phase. Probiotics play a role in the reproductive system by modulating FSH and LH hormones, blocking pathogenic bacteria in the reproductive tract, increasing feed absorption, and improving livestock health, resulting in increased production and egg quality. However, from several studies, the use of probiotic strains, probiotic dosage, and duration of administration must be considered for the effective maintenance of advanced laying hens.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

NOVELTY STATEMENT

Probiotics in the laying hen diet have been thoroughly researched and shown to provide significant benefits. On the other hand, using probiotics in late-phase laying hens is a novelty.

According to research, there are benefits to administering probiotics at the proper dosage and at the right time to extend the life of production and boost productivity. The data will aid researchers in conducting their research and determining the results.

AUTHOR CONTRIBUTION

All authors contributed to this manuscript.

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