



A NARRATIVE REVIEW ON THE BENEFICIAL IMPACTS OF PROBIOTICS ON POULTRY: AN UPDATED KNOWLEDGE

Aljohara M. Al-Otaibi¹, Mohamed E. Abd El-Hack^{2*}, Saif M. Dmour³, Noorah Alsowayeh⁴, Asmaa F. Khafaga⁵, Elwy A. Ashour¹,
Mohamed A. Nour-Eldeen¹, Sylwester Świątkiewicz^{6*}

¹Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia

²Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

³Department of Laboratory Medical Sciences, Princess Aisha Bent Al-Hussein Faculty of Nursing and Health Sciences, Al Hussein bin Talal University, Jordan

⁴Department of Biology, College of Education (Majmaah), Majmaah University, Al-Majmaah, 11952, Saudi Arabia

⁵Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Egypt

⁶National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

*Corresponding authors: dr.mohamed.e.abdalhaq@gmail.com; sylwester.swiatkiewicz@iz.edu.pl

Abstract

Probiotics are live microorganisms that can improve the host's health when given in sufficient doses. Due to growing consumer demand for poultry raised without antibiotics supplementation, the usage of probiotics has been rising gradually over time in poultry. This review aimed to summarize the impacts of probiotics on poultry's nutrient utilization, growth and carcass parameters, gut morphology, laying performance, biochemical parameters, immunity, and gut microbiota. The results revealed that probiotics enhanced the growth performance via improving digestion of protein, lipids, and carbohydrates, improving feed conversion ratio (FCR), increasing carcass and organs weights, improved liver and kidney function parameters, antioxidant effects, decreased serum lipids and cholesterol, improved poultry immunity via improving gut health and synthesis of antimicrobial peptides and prevention of microbial colonization, improved laying performance, fertility, hatchability and eggshell thickness. Therefore, probiotics have various benefits in poultry production compared to banned antibiotics. However, they are not always guaranteed to be growth promoters due to poorly understood modes of action, including their interaction with the host. So, further studies concerning their impacts on meat quality and histopathological changes are still required.

Key words: probiotics, poultry, growth, immunity, gut microbiota, laying performance

Due to their numerous positive effects, including those that support growth and productivity, immune system development, and health protection, feed additives and nutritional supplements are becoming more and more important in today's poultry industry and healthcare systems (Abd El-Hack et al., 2017). In general, feed supplements are tools that increase the potency of nutrients and have an impact on the performance of chickens (Ashour et al., 2020). Numerous feed additives, including probiotics, oligosaccharides, enzymes, and organic acids are utilized in chicken feed (Bin-Jumah et al., 2020). They are incorporated into poultry and animal diets in order to promote growth because of their tendency to increase feed consumption (Abd El-Hack et al., 2017).

Probiotics have been proposed as environmentally friendly feed additives and intriguingly unorthodox alternatives to chemotherapy in chicken. Moreover, to guarantee that it always has the right microbial balance, animal must maintain particular amounts of helpful microbiota in the digestive system (Abd El-Hack et al., 2018). While bifidin is effective against *S. aureus* and *M. flavus*, bifidocin B exhibits antibacterial activities towards various pathogens, for instance *Bacillus cereus*

and *Listeria monocytogenes* (Shah and Dave, 2002). *Bacillus toyonensis* (BT) is a well-known non-pathogenic bacterium utilized as a probiotic for feeding animals. It is aerobic, fermentative, gram-positive, and spore-forming (Roos et al., 2018). The supplements of *Lactobacillus* spp. to maize-barley hen diets enhanced FCR, egg mass, albumen quality, and egg production (Abd El-Hack et al., 2018).

Probiotics in chicken feed can increase performance, nutritional digestibility, and immune suppression in birds (Abd El-Hack et al., 2020; Abdel-Moneim et al., 2020 a; Saleh et al., 2021). Probiotics can also affect the host's health, performance, and sickness risk. They can correct dysbiosis and restore the gut microbial balance in healthy hosts by lowering harmful spores' growth and enhancing beneficial microbes' growth (Yadav and Jha, 2019). The genera *Lactobacillus*, *Bacillus*, *Streptococcus*, *Candida*, *Bifidobacterium*, *Aspergillus*, *Enterococcus*, and *Saccharomyces* are responsible for the majority of probiotic strains that are commonly used (Ahmed et al., 2014 a, b; Elbaz et al., 2021) and invest in particular health benefits for the host through the competitive eradication of dangerous bacteria and the gut's immune system re-

sion (Yadav and Jha, 2019). Numerous studies have discovered the impacts of probiotics on microbial fermentation, enzyme activity, and the gut microbiota in broiler birds' digestive tracts (Martínez et al., 2016; Wang et al., 2017 a). This review article aimed to highlight different beneficial effects of probiotics on poultry growth performance, production, reproduction and health status.

Impacts of probiotic supplementation on growth performance

The different impacts of probiotics on poultry are summarized in Table 1. Growth metrics are es-

sential for measuring economic performance and animal health, especially in chronic or moderate disorders with few obvious clinical indications (Kritas and Morrison, 2007). In a multispecies population, increased quail weight might be due to increased synthesis of specific vitamins, some active compounds, and digestive enzymes. This can reduce the pH of the intestine, improve digestion, and consequently increase the consumption of nutrients, which favorably influence body weight values and limit the growth of enteropathogens in the quail gut (Premavalli et al., 2018).

Table 1. Different impacts of probiotics from different bacterial strains on poultry

Strain	Origin and characteristics	Impacts	Authors
<i>Bacillus subtilis</i>	Spore-forming bacteria that survive in the environment during harsh conditions. Resistant to alkali, acid, and heat. Soil bacteria are found in the gastrointestinal tract of humans and ruminants.	Enhances growth and laying performance, immune system and gut health.	Forte et al., 2016; Fathi et al., 2017; Alagawany et al., 2018; Jha et al., 2020
<i>Bacillus licheniformis</i>	Found in the soil. Found on bird feathers, especially back and chest feathers.	Improves intestinal health of sub-clinical necrotic enteritis. Enhance the growth and laying performance.	Cheng et al., 2017; Kan et al., 2021; Pan et al., 2022
<i>Bifidobacterium bifidum</i>	Normally live in human intestines, stomach, breast milk, and often in the vagina.	Reducing cellulitis in broiler chickens. had antimicrobial activity against some pathogenic bacteria. Enhance gut health and immune system.	Pender et al., 2017; Estrada et al., 2001; Coman et al., 2020
<i>Bifidobacterium animalis</i>	Found in the large intestines of most of mammals, including humans.	Reduced caecal coliforms and <i>E. coli</i> populations. Improved the immune system, gut physiology, and health via increasing villus length and absorption process.	Abou-Kassem et al., 2020; Abd El-Moneim et al., 2020
<i>Bacillus amyloliquefaciens</i>	Plant-root pathogen discovered in soil.	Improved growth performance, antioxidant status, immunity, and digestive enzyme activity of broilers. Enhances gut health.	Ahmed et al., 2014 a, b; Li et al., 2015; Mazanko et al., 2018; Sun et al., 2022
<i>Bacillus coagulans</i>	Is a lactic acid-forming bacterial species and it exhibits characteristics typical of both genera <i>Bacillus</i> and <i>Lactobacillus</i> .	Has a protective effect against <i>Salmonella enteritidis</i> . Improved body weight, average daily gain, antioxidant capacity, immunity function and gut health in broilers.	Wu et al., 2018; Zhen et al., 2018; Zhang et al., 2021
<i>Lactobacillus acidophilus</i>	Found in the human and animal gastrointestinal tract and mouth.	improve the growth performance, intestinal health, and survival of broilers.	Li et al., 2018; Wu et al., 2021
<i>Lactobacillus bif fermentans</i>	Found in animal gastrointestinal tract and human.	Enhances broiler growth performance and health of the digestive tract.	Timmerman et al., 2006
<i>Pediococcus acidilactici</i>	Bacteria are found in fermented dairy products, vegetables, and meat.	Improved performance; meat quality and intestinal health in broilers. Promoting broiler growth of body and visceral organs.	Satheesh et al., 2012
<i>Saccharomyces cerevisiae</i>	Originally isolated from the skin of grapes.	Maximize broiler productivity. Improve laying performance. Improve broiler gut health.	Hassanein et al., 2010; Wulandari and Syahniar, 2018; Ahiwe et al., 2021
<i>Streptococcus faecium</i>	Bacteria isolated from the gastrointestinal tracts of humans and other mammals.	Enhance broiler growth performance, improve intestinal morphology.	Cao et al., 2013; Alagawany et al., 2018

Probiotics are live bacteria, yeasts, or fungi that help maintain a healthy digestive system by supplementing the gastrointestinal flora, thereby promoting the growth performance and overall health of poultry (Jha et al., 2020). Khaksefidi and Ghoorchi (2006) indicated that adding probiotic *Bacillus subtilis* to broiler chicks enhanced broiler body weight and weight gain. Rehman et al. (2020) reported that the weight gain of broilers was increased by probiotics (Protexin) during the starter and finisher phases ($P = 0.028$ or 0.04 , respectively). Safalaoh (2006) revealed that diets supplemented with an effective microbial preparation had considerably ($P < 0.05$) greater body weight increases (2094 ± 11 g) than the control diet (2057 ± 15 g), resulting in a 2% increase. Vicente et al. (2007) indicated that the probiotic *Lactobacillus* sp. in broilers and turkeys significantly raised the body weight compared with the control (Torres-Rodriguez et al., 2007). When broilers were given diets containing *Aspergillus oryzae*, the body weight gain was increased (Rehman et al., 2007). The same findings were reported by Sallh and Al Husary (2009), who noticed that broilers fed on diets enriched with 1.5 g/kg of probiotic ingredients *A. oryzae* gained more body weight.

Zhang et al. (2021 a, b) fed Arbor Acres (AA) broilers chicks basal diets with 1% *Lactobacillus acidophilus*, 1% *L. casei*, and 1% *Bifidobacterium* in the water for 42 days and reported that the inclusion of probiotic significantly enhanced body weight, average daily feed intake (ADFI), and average daily weight gain of chicks

According to Zeweil et al. (2007), the supplementation of *Saccharomyces cerevisiae* improved the body weight and body weight increase of broilers ($P < 0.05$) over those of the control group. Furthermore, when *S. cerevisiae* was introduced at rates of 1, 1.5, and 2% compared to control and the other treatments, body weight gain was considerably ($P < 0.05$) enhanced.

Many researchers have used *B. subtilis* as a component of probiotics. According to Abaza et al. (2008), using *S. cerevisiae*, *B. subtilis*, and *B. licheniformis* in the diets improved body weight by approximately 2.10, 3.02, and 2.70%, respectively and body weight gain by approximately 2.69, 2.09, and 3.01%. Additionally, Fayed and Tony (2008) demonstrated that using the probiotic mixture (Biovet-YC®), including mixtures of *Lactobacillus* spp. and yeast cultures, increased body weight gain in broiler chicks. Another study reported that probiotic supplements exhibited a significant positive impact on body weight gain in broiler chicks since providing broilers' diets with probiotic microflora at levels of 10, 20, and 50 g/50 kg diets significantly increased the live body weight when compared to the control group (Taklimi et al., 2010). Furthermore, Nuyens et al. (2010) showed using *B. subtilis* PB6 in broiler diets enhanced the live weight at 42 days old, from 2.536 to 2.644 kg. Knap et al. (2011) also demonstrated that by day 42, the *B. subtilis* treated groups had a numerical advantage over the positive control group in terms of body weight growth (2.212 and 2.182 kg, respectively).

However, the difference was not significant. Furthermore, regardless of the meal's spore concentration, Molnár et al. (2011 a) demonstrated that broilers fed diets enhanced with *Bacillus subtilis* resulted in considerable improvement in body weight in the supplied groups versus the control ones. Similarly, birds (poult and chicks) fed meals supplemented with *B. subtilis* gained considerably more total body weight than the non-treated group (Shivaramaiah et al., 2011). Besides, *B. subtilis* enhanced quails' growth performance. They positively influenced body weight (BW) and body weight gain (BWG) throughout the brooding period due to their capability to produce highly active proteases, amylases, and lipases that break down feed components and increase the number of nutrients accessible for absorption (Li et al., 2014) (Figure 1).

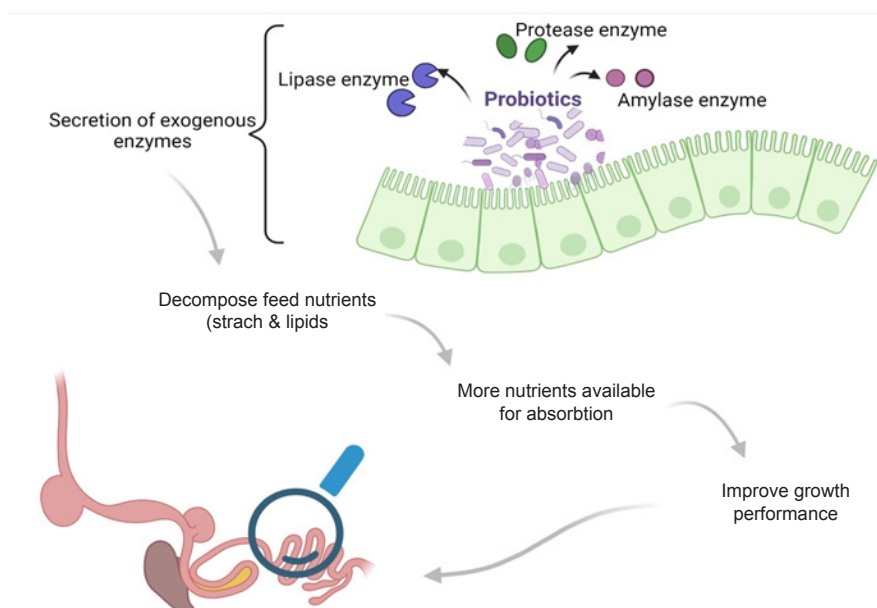


Figure 1. Effect of probiotics on growth performance

Interestingly, Abdel-Moneim et al. (2020 a) established that *B. subtilis* treatment increased live body weight linearly across all tested ages. Additionally, both the beginning phase (1–14 days old) and the overall one showed a linear rise in daily body weight gain (1–42 days old). According to Abou-Kassem et al. (2021), probiotics caused a substantial enhancement in quail weight at 21 and 42 days of age compared to the control. Additionally, results indicated that probiotic supplementation increased weight gain (WG) significantly throughout the grower and total trial periods compared to the control. The lowest single dosage of BT, where WG was lowered, was the only probiotic-treated group whose WG grew over the finisher period. BT's greatest dietary single level coincided with the maximum values of WG through the finisher and overall phases.

Mortality rate

Broiler chicken performance has been proven to be improved by probiotics in the poultry industry, which lowers the mortality rate of broiler chickens (Timmerman et al., 2006). Likewise, according to Vicente et al. (2007), probiotics were demonstrated to reduce chicken mortality. Using the probiotic mixture (Biovet-YC®), which contains mixtures of *Lactobacillus* spp. and yeast cultures, had favorable impacts on broiler behavior and can operate as an anti-stress element, which might have an impact on overall health conditions (Fayed and Tony, 2008). Also, after administering a probiotic mixture of bacteria (*L. plantarum*) and yeast (*S. cerevisiae*) through drinking water, the mortality rate of the supplemented chickens was lower than that of the non-treated ones (Thongsong et al., 2008). Likewise, Al-Homidan and Fahmy (2007) found that adding dry yeast to broiler chicks' basic food slightly enhanced their survival rate compared to the control.

Moreover, *Pediococcus acidilactici*-containing probiotics dramatically decreased death rates compared to controls (6 vs. 11.5%) (Ibrir et al., 2008). Alloui and Hadeif (2008) maintained that the experimental mortality average was 4.56% in the control chickens and 4.51% in those fed with a diet containing *P. acidilactici* (10^9 cfu/kg of feed), which may be regarded as being within the common range. Also, it was shown that broilers given probiotic microflora at 10, 20 and 50 g/50 kg diets exhibited a considerably decreased mortality rate compared with the non-mediated control ones. In contrast, Abdel-Samee and Abdel-Hakim (2002) demonstrated that adding the probiotic Bio-Top (0.1%) to Arbor Acres broiler chicks had no noticeable impact on mortality rates. Also, the mortality rate of the broilers was not affected by commercial probiotics in water or feed (O'Dea et al., 2006). Similarly, adding probiotic cultures to feed had no discernible effect on the mortality rates of broiler chicks (Al-Zenki et al., 2009; Abo-Mahara, 2010). Likewise, Brzóska et al. (2010) found that the mortality percentage had been considerably reduced ($P < 0.01$) upon adding the probiotic bacteria compared to the control ones.

Throughout the trial period, no mortality was noted in any of the experimental groups, according to Abdel-Moneim et al. (2020 a).

Feed consumption and conversion

One of the most biological approaches to increase feed consumption effectiveness is commercial probiotics as natural promoters for poultry nutrition. Probiotic culture added to the diet benefits the host animal by increasing appetite. In addition, it improved feed intake in broilers (Kim et al., 2003), feed conversion ratio of the host (Soliman et al., 2000; Cavit, 2003), and production of digestive enzymes (Saarela et al., 2000; Rehman et al., 2007), as well as it has positive effects on host health (Soomro et al., 2002). While, Yalçin et al. (2008) revealed that the feed efficiency average was quantitatively greater in the group treated with *S. cerevisiae*. Similar results were described by Kumari et al. (2001). Overall, *B. subtilis* can enhance quail growth performance due to its ability to release high-active proteases, lipases, and amylases that digest feed nutrients and raise the number of nutrients available for absorption. This beneficial effect of *B. subtilis* on FCR was demonstrated throughout the brooding period (Li et al., 2014). Furthermore, it was noted by Abdel-Moneim et al. (2019 b) that giving *B. subtilis* spores to quails led to a higher total FCR than the control group.

Variations in environmental factors and gut flora may confuse the cause of the varying action of biological additions (Mahdavi et al., 2005). According to O'Dea et al. (2006), adding commercial probiotics in water, feed, or their combination had no impact on the broilers' feed conversion rate. Al-Homidan and Fahmy (2007) also noted that throughout the experiment, birds supplied with yeast culture consumed numerically more daily feed than the control. Furthermore, Abaza et al. (2008) noticed that throughout all experiment periods, there were no substantial changes in the conversion rate among groups fed diets mixed with *B. licheniformis*, *B. subtilis*, and *S. cerevisiae*, so far chicks fed diets provided with *S. cerevisiae* consumed significantly more feed than the control ones. Contrarily, according to Midilli et al. (2008), dietary supplements with probiotics had no noticeable impact on feed consumption compared to the non-supplemented control. According to Al-Zenki et al. (2009), supplementing feed with probiotic culture had no considerable impact on feed consumption. Brzóska et al. (2010) also included the probiotic bacterium *Pediococcus* spp. caused noticeable variations in the feed consumption and conversion ratio of broilers.

Carcass traits

Color characteristics in chicken breast and thigh muscle have also been assessed in a study by Haščík et al. (2014), where bee pollen extracts were incorporated into broiler feed. The parameters were measured 45 minutes after the death. As a result, several values differed significantly from those in this research. The thigh muscle

and breast values were 52.31–53.96 and 49.38–52.5, respectively. Also, thigh muscle and breast values were determined to be 4.53–7.38 and 0.98–2.05, respectively. Besides, it was 7.14–9.52 and 5.17–13.56 for breast and thigh muscles, respectively. Only statistically significant variations were found in the groups' liver, hearts, and abdominal fat weights. Both probiotic groups exhibited higher levels of belly fat. This may be attributed to the fact that both groups finished growing at the end of the fifth week and that the diet they had been consuming for the previous week was likely transferred to lipid deposits. Probiotic treatment in broilers did not impact the weight of the abdominal lipids (Alp et al., 1993).

Probiotics addition to broiler feed exhibited substantial advantages in dressing and carcass percentage at selling age (Soliman et al., 2003). Pelicia et al. (2004) demonstrated that using prebiotics of bacterial origin and combining them with prebiotics of yeast origin are viable options for free-range broiler chickens due to improved carcass production. Kumar et al. (2003) examined the impact of providing a probiotic, *Lactobacillus acidophilus*, to broiler feed at 1.0 g/kg on carcass parameters. The supplemented groups showed a substantial rise, compared to the control, in the average values of internal organs as a proportion of the body weight (kidneys and liver). Additionally, Kalavathy et al. (2003) investigated how a combination of twelve *Lactobacillus* spp. affected the development of belly fat in broiler chicks from 1 to 42 days old. They demonstrated that, compared to the control group, the probiotic treatment decreased the amount of belly fat deposited after 28 days. According to Vicente et al. (2007), using *L. reuteri* caused turkeys to have decreased relative intestinal weights and shorter, smaller intestines. Safalaoh (2006) found that supplementing the feed with microbial preparations significantly decreased the lipid content of the birds as determined by the abdominal fat content. According to Çelik et al. (2007), the probiotics and organic acids supplemented to diets raised the weights of the liver, large and small intestines, and the empty gizzard.

Additionally, Fayed and Tony (2008) demonstrated that broiler chickens' liver weights and dressing and carcass attributes were greatly enhanced by using the probiotic mixture (Biovet-YC), including mixtures of yeast cultures and *Lactobacillus* strains. Additionally, Abaza et al. (2008) revealed that the abdominal lipid % of chick's diets provided with *B. subtilis* and *B. licheniformis* were much lower than those in the non-supplemented group. Alloui and Hadeef (2008) confirmed that at day 56, abdominal fats and dressing contained one-day-old ISA 15 fed nearly two months on soybean-maize fed diets integrated with or without *Pediococcus acidilactici* (10^9 cfu/kg of feed). The carcass production of broilers and growth performance were positively enhanced by adding *P. acidilactici* (Chafai, 2006).

Probiotics had no discernible impact on absolute dressing weights, dressing percentage, heart, gizzard, or pancreas (Abo-Mahara et al., 2010). In contrast, their

findings showed an improvement in heart percentage and a considerable decline in liver percentage as a result of supplementing their meals with 0.1% probiotic Super Bio-Buds. The addition of *B. subtilis* (CHCC3810-DSM 17299) at a level of 2.08×10^8 cfu/g diet resulted in a considerable reduction in the absolute and liver percentage (Molnár et al., 2011 b).

According to Brzóska et al. (2010), feeding broilers on *Pediococcus* species enhanced the weight of both slaughter and carcass of hens considerably ($P < 0.01$). Contrarily, the research of Abd El-Gawad et al. (2004) gave two doses of crude protein, either with or without Zinc Bacitracin (Zinc B.) and/or one of three probiotics, including; Premalac, Biobadus, or Lacture. Their findings showed that crude protein or other studied feed ingredients did not significantly impact the carcass properties. However, the values of the effective microorganisms (EM) in supplied birds were substantially higher than those of the control group, with 69 ± 5 g and 67 ± 6 g, respectively, at 42 days of age (Safalaoh, 2006), even though there were no statistically differences in the dressing percentages among the microbial preparation groups and the control group.

In terms of abdominal fats at day 56, Alloui and Hadeef (2008) found no statistically significant variances between the control group and chickens that already had received a supplementary meal containing *P. acidilactici* with 10^9 cfu/kg of feed. Furthermore, Abaza et al. (2008) displayed that the chicks fed diets enriched with *B. subtilis*, *S. cerevisiae*, and *B. licheniformis* did not influence the percentages of giblets and dressing. Also, the dietary probiotic additions had no impact on the quantity of the abdominal fats/or fat yield, as well as the relative weight of the proventriculus, liver, and gizzard (Ibrir et al., 2008).

Moreover, Midilli et al. (2008) looked at the effects of probiotics or prebiotic supplements on broiler growth performance. They found that dietary probiotics or prebiotic supplementation had no appreciable effects on carcass weight compared to the control. Awad et al. (2009) revealed a comparable, but not significant, decrease in carcass % in broilers fed a *B. subtilis*-supplemented diet compared to control broilers. The probiotic additions to the feed of broiler chicks had no appreciable impact on carcass quality (Khani and Hosseini, 2008; Mohamed and Bahnas, 2009; Taklimi et al., 2010). Also, adding *Pediococcus* species exhibited no substantial variations in percentage or the weight of legs and breast muscles (Brzóska et al., 2010).

Digestive enzymes

Probiotics were shown to improve the antioxidant resistance mechanism of laying Japanese quails. This reaction may be linked to probiotics' capability of inducing the production of certain components that trap reactive oxygen species, chelating free radicals, and limiting their cytotoxic effect (Abdel-Moneim et al., 2020 a). According to Wang et al. (2017 b), probiotics increase the activity of antioxidant enzymes, including GPx and SOD,

promoting the avian antioxidant defense system. Furthermore, the antioxidative enzyme system of probiotics may play a vital role in increasing the host's antioxidant state (Abdel-Moneim et al., 2020 b).

The recently improved performance in digestive enzymatic activities could be due to the cumulative impact of endogenous enzymes produced by the host and exogenous enzymes produced by probiotics. Wang and Gu (2010) noticed a significant rise in enzyme activity of amylase and protease activities upon supplementing the fed diets of Arbor Acres broilers with *B. coagulans* NJ0517. The growth enhancement could be explained by the enhanced digestion of fat and starch due to the elevated activities of amylolytic and lipolytic enzymes.

In contrast, probiotics did not influence the activity of the protease enzyme, as shown by Zhi-gang et al. (2014) and Zhang et al. (2016). Besides, there were no considerable changes in the enzymatic activities of lipolytic, proteolytic, and amylolytic enzymes upon probiotic treatment (Rodjan et al., 2018). The current increase in digestive enzyme activity may be caused by the contribution of endogenous enzymes generated by the host and probiotic bacteria's released enzymes (Saleh et al., 2014). The increased activity of protease, lipase and amylase enhanced the digestion of protein, lipids, and carbohydrates, which may provide some insight into the growth enhancement.

Blood biochemistry

The probiotic bacteria may boost the production of unconjugated bile acids, which might help to regulate serum cholesterol, a precursor to bile acids (De Smet et al., 1994). Reduced cholesterol levels are the end result of increased production of these acids. After consuming probiotics, a substantial decrease in cholesterol levels was observed in the blood serum and liver in cockerels and rats (Hosono, 2001). According to Abdel-Azeem et al. (2001), Japanese quail's blood parameters, including; globulin, albumin, GPT, total cholesterol, A/G ratio, and AST, were considerably affected by the microbial probiotic supplementation, while other parameters were unaffected. In a subsequent study, Abdel-Azeem (2002) stated that broilers fed with diets enriched with biological additives (*S. cerevisiae*) displayed greater globulin, albumin, and total plasma protein levels. Nevertheless, the total lipids, cholesterol, and A/G ratio were lower than those in the untreated control.

The addition of yeast resulted in a modest decrease in blood cholesterol compared to the non-supplemented control. However, the yeast did not negatively impact liver function regarding AST and ALT (Soliman et al., 2003). Broiler chicks were given a probiotic supplement (*L. acidophilus*) at a feeding rate of 1.0 g/kg, and the effects on blood components were investigated (Kumar et al., 2003). They revealed that, at 45 days of age, treated birds had lower mean AST and ALT rates, triglyceride levels, and low-density lipoprotein (LDL) than the

control ones. Yet, the serum's high-density lipoprotein (HDL) did not indicate a substantial change.

The probiotic dietary supplements positively affected the host animal's health by reducing the blood cholesterol levels in hens (Kim et al., 2003; Kurtoglu et al., 2004; Hajjaj et al., 2005). According to El-Yamny and Fadel (2004), serum total protein, A/G ratio, and albumin increased with yeast supplementation at dosages of 0.2, 0.4, and 0.6%; however, AST and ALT were unaffected. At the same time, cholesterol and lipid levels were considerably reduced. The probiotic strain *E. faecium* M74 and *L. acidophilus* have been shown to lower plasma cholesterol concentration (De Roos and Katan, 2000). *Lactobacillus* could digest the cholesterol molecules; however, the mechanism of *E. faecium* M74 activity is still unclear (Pereira and Gibson, 2002). Multiple probiotics comprising *Bacillus* spp., *Rhodobacter* spp., *Lactobacillus* spp., and *A. oryzae* were shown to reduce blood serum cholesterol in broiler chickens (Yoon et al., 2004; Capcarova et al., 2010). Based on Siam et al. (2004), laying hens fed a diet consisting of liquid cultures of *Bifidobacterium bifidum* (B) or *Lactobacillus acidophilus* (L), or a mixture of both, 50 g of L + 50 g of B/kg feed diet, decreased total lipid and cholesterol levels significantly after two-weeks post-treatment, and this trend continued throughout the experiment. Certain features decreased when supplemented with (B), (L), or (L + B) compared to the control group. Blood total lipids decreased by 5.2, 6.4, and 5.8%, respectively, while blood cholesterol decreased by 55.6, 58.3, and 50.0%.

According to Abd El-Gawad et al. (2004) reported that probiotics supplementation improved average values of albumin and total protein and decreased average values of cholesterol, ALT, AST, and total lipids. Also, supplementing broiler diets with probiotics significantly ($P < 0.05$) enhanced globulin, albumin, and total protein under both normal and high-temperature settings. Besides, total lipids and plasma cholesterol values decreased, though AST and ALT enzymes were not impacted (Tolba et al., 2004).

According to Kalavathy et al. (2003), broilers consumed diets including *Lactobacillus* at the sixth week of age had decreased cholesterol concentration in their carcasses and livers. A microbial preparation like effective microorganisms (EM) supplied birds had lower blood cholesterol levels at 42 days than the control (Safalaoh, 2006). Also, broilers consumed a diet supplemented with 50 mg per kg of multistrain probiotics, or 1 g of yeast per kg of feed, had lower blood cholesterol concentrations than the control group (Asli et al., 2007). Similarly, it was found that at 42 days old, the probiotic-supplemented chickens' total blood cholesterol concentration was considerably lower than the control chickens' (Thongsong et al., 2008). Organic acids, probiotics, and their mixtures dramatically enhanced total protein levels and decreased cholesterol concentration compared to the control groups (Abo-Mahara et al., 2010). Salim et al. (2011) found that adding probiotic strains (*L. acidophilus* and *B. bifidum*)

to the untreated aflatoxins treated group compared to the aflatoxin group improved the lowered levels of total protein. Albumin significantly improved ($P < 0.05$), demonstrating the probiotic bacteria's capacity to decrease the toxicity caused by aflatoxins. Contrarily, Santoso et al. (2001) concluded that broiler chicks that were fed with a commercial diet including fermented probiotic product from *B. subtilis* at 0.5, 1.0, and 2.0% for 21 days between 7 and 28 days of age did not influence the content of plasma cholesterol, phospholipids, and triglycerides.

Kurtoglu et al. (2004) also stated that the specificity of the probiotic impact on serum cholesterol did not appear until days 30 and 60. Broiler chicks, given diets with or without *S. cerevisiae* supplemented as 1 g/kg feed, exhibited no appreciable impact on the total protein, globulin, or albumin content (El-Ghamry et al., 2002). According to Abdel-Azeem (2002), broilers fed diets augmented with biological additives exhibited high blood total plasma protein, globulin, hemoglobin, and albumin levels.

However, the liver functions of ALT and AST were not significantly affected by *S. cerevisiae* (1 g/kg diet) for broiler chicks' diets. In contrast to the control group, reduced levels of total lipids, A/G ratio, and cholesterol (particularly in the yeast group) were found. ALT and AST measurements of liver function showed that yeast had no negative impacts, unlike the untreated control groups (Soliman et al., 2003). The supplementation with yeast at concentrations of 0.2, 0.4, or 0.6% enhanced blood total protein, A/G ratio, and albumin while not affecting ALT or AST (El-Yamny and Fadel, 2004; Tolba et al., 2004). According to Abaza et al. (2008), adding *S. cerevisiae*, *B. licheniformis* and *B. subtilis* to broiler feeds throughout growth did not significantly affect blood total protein, cholesterol levels, globulin, and albumin. Other researchers indicated that adding the probiotic Avian Plus feed supplements had no appreciable impact on blood plasma parameters, such as globulin, total protein, albumin, ALT, and AST levels (Sherif, 2009; Rabie et al., 2010). Adding organic acids, probiotics, and their mixtures in broiler chicks' feed did not substantially impact the concentration of AST and ALT (Abo-Mahara et al., 2010).

Remarkably, the *B. subtilis* supplementation increased blood concentrations of albumin and total protein blood concentrations while it also linearly decreased ALT, AST, urea-N, and creatinine levels. Though, *B. subtilis* levels in the diet did not impact blood levels of ALP, globulin, and uric acid in a quadratic or linear manner. Additionally, groups BS5, BS7, or BS9 showed a linear decline in the serum VLDL concentration while showing little or no change in the LDL concentration. Results showed that these treated groups displayed linear increases in HDL levels (Abdel-Moneim et al., 2020 a). In addition, they noted that all dietary supplements of *B. subtilis* linearly and quadratically decreased blood glucose levels compared with the un-supplemented group. At the same time, T3 and T4 activities showed a linear increase in supple-

mented groups compared to the control. The T3 and T4 levels were greatest and glucose levels were lowest in groups BS7 and BS9 (Abdel-Moneim et al., 2020 a). The antioxidant activities of growing Japanese quail's diets comprising *B. subtilis* were significantly improved. All investigated *B. subtilis* dietary levels decreased the serum MDA contents compared to the untreated control. At the same time, the CAT and GSH activities were linearly elevated in groups BS5 and BS (Abdel-Moneim et al., 2020 b).

Histomorphometry

Results of Ebeid et al. (2019) reported that increased villus height and better ileal development are indications for intestinal villi activity and are linked to improved digestive enzyme activities and nutrient absorption, which improve growth performance. The broiler chicks' nutritional absorption might be poor, and their performance could be inadequate due to excessive crypt depth (Xu et al., 2003; Abdel-Moneim et al., 2019 b). Although, it was noticed that there is a connection between the improved villi height in newly hatched chicks and the *in ovo* inoculation using *B. bifidum* and *B. longum* (Abd El-Moneim, 2017; Abdel-Moneim et al., 2019 a). Moreover, probiotic-supplemented groups showed a significantly higher villus length/crypt depth and villus length ratio than control groups, according to Olnood et al. (2015) and Abdel-Moneim et al. (2019 a).

Gut bacterial content

In the digestive systems of animals and humans, probiotic bacteria like *Bifidobacterium* spp. and *Lactobacillus* spp. are described as autochthonous microbiota. The adhesion of pathogenic bacteria can result in active growth and colonization because intestinal mucus and epithelial cells are the most sensitive. This microbial adherence is essential for the pathogen and epithelial cell connection (Ribet and Cossart, 2015). The food chains (egg, milk, meat, etc.) could be contaminated with unwanted pathogenic bacteria such as *E. coli*, *Shigella*, *Salmonella*, and *Listeria*, causing health problems (Food and Drug Administration, 2012). As a result, preventing bacterial attachment to the gastrointestinal mucosa is thought to be a successful strategy for reducing the risk of foodborne illness (Kim et al., 2015; Thöle et al., 2015). Serafini et al. (2013) used the epithelial intestinal cell monolayers (such as HT-29 and Caco-2) to examine the inhibitory effect of *B. bifidum* against the enteric adaption characteristics of harmful bacteria like *Cronobacter sakazakii* and *E. coli*. For ages, lactic acid bacteria have been employed in various useful meals. Probiotics provide the following main functional impacts: (i) synthesis of antimicrobial peptides such as bacteriocins (Martinez et al., 2013; Mandal et al., 2014), managing fat storage and digestion of diet fibers (Aronsson et al., 2010; DiBaise et al., 2012), mucosal immunity modulation (Hardy et al., 2013), and reduced pathogen colonization due to the management of gut flora through the competitive exclusion of harmful mi-

croorganisms (Yu et al., 2011; Kim et al., 2014; Li et al., 2014).

One of the five main functional properties of probiotics is the connection of bacteria to the mucous membrane of the digestive tract (Figure 2), which is thought to be crucial for the direct inhibition of pathogens and necessary for the efficient management of immune activities, providing a protective role towards intestinal pathogens (Van Tassell and Miller, 2011). Probiotics must adhere to colon cells at this stage to successfully colonize the host's digestive tract with microbes (Figure 3). Since adhesion is required to actively multiply and give resistance to elimination from the digestive tract as waste via peristalsis, it has been recognized as one of the important screening parameters for active probiotic strains (Duary et al., 2011). Bacterial attachment mechanisms to epithelial cells could be classified as (i) non-specific attachment when physicochemical attributes of the outer cell surfaces are considered; or (ii) specific attachment when specific molecules are expressed onto the bacterial cell membrane and straightly bind to the receptor sites of the outer mucosal surfaces (Duary et al., 2011; Van Tassell and Miller, 2011). This adhesion ability is determined by hydrophobic characteristics, ion concentration, pH, and physical morphology (Polak-Berecka et al., 2014). These parameters significantly impact bacterial adherence onto host intestinal tissues, indicating the intricacy of initial adhesion between bacteria and the mucosal surface.

The chemical structure of bacterial surfaces plays a critical role in the early contact with the intestinal tract's mucosal surface and epithelial cells. Investigation of cell surface hydrophobicity is commonly used to evaluate the physicochemical properties of the microbial outer surface (Krasowska and Sigler, 2014). It has been demonstrated that microbial strains with enhanced hydrophobicity adhere to colon cells more successfully than hydrophilic ones (Duary et al., 2011; Van Tassell and Miller, 2011). Hydrophobic microorganisms have significant adhesive action to inanimate surfaces such as hydrocarbon and non-polar liquids, whereas hydrophilic cells have limited adhesive action (Duary et al., 2011; Krasowska and Sigler, 2014).

Layers' productive performance

According to Kurtoglu et al. (2004), when 27-week-old layers were given a mixture of *B. licheniformis* (3.2×10^9 /g probiotic) and *B. subtilis* (3.2×10^9 /g probiotic) over the next 90 days, their egg production increased. Moreover, a significant enhancement in egg weight was found between 20 and 44 weeks of age, and *Lactobacillus*-enriched diets were administered to layers (Ramasamy et al., 2009). Additionally, the group of layers given a mixture of heat-inactivated *L. salivarius* and *B. subtilis* produced substantially more eggs per day than the non-supplemented control (Zhang et al., 2012).

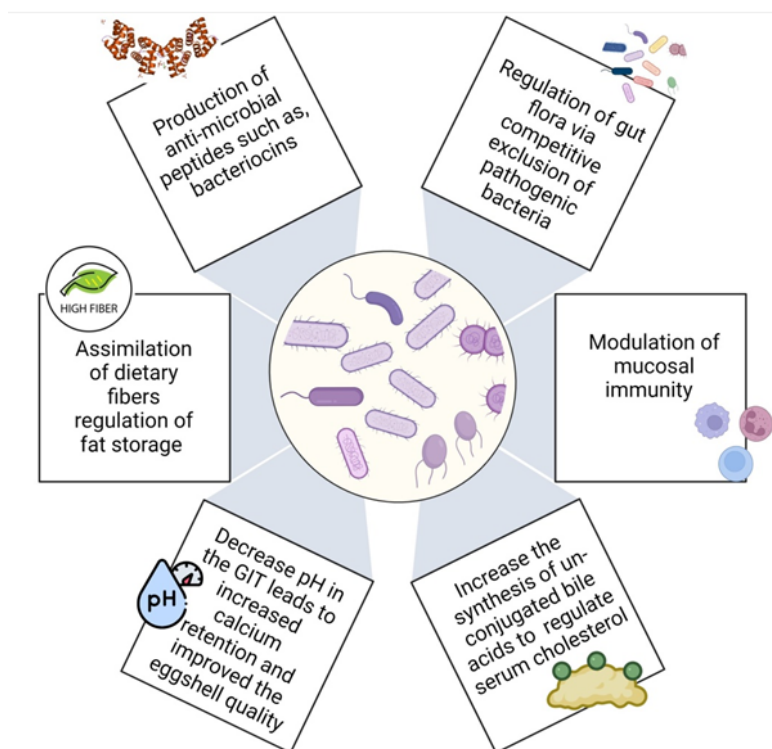


Figure 2. The key functional effects of probiotics

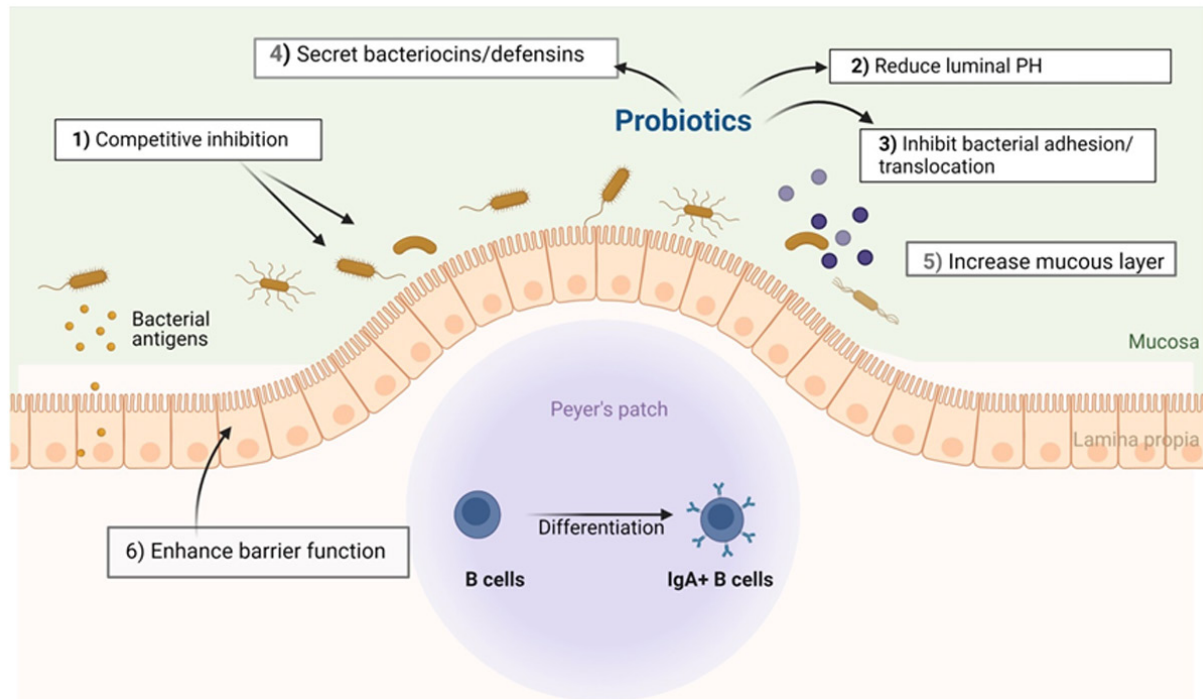


Figure 3. Effect of probiotics on gut bacterial content

The incorporation of probiotics into the diets of laying quail enhanced egg weight (EW) and egg numbers (EN) in comparison to the control group (Abd El-Moneim and Sabic, 2019 b). Interestingly, Jha et al. (2020) reported that probiotics, in addition to laying hens' diets, increase their daily feed consumption, enhance their retention of nitrogen and calcium, and shorten their intestinal length, increasing the laying rate. On the other hand, Balevi et al. (2001) showed no variations in EN or EW due to different bacterial strains, probiotic dosages and hens' ages. Also, adding the probiotic *B. subtilis* (0.10%) to laying quail meals for a two-week treatment had no significant effect on EM or EW compared to the untreated group (Manafi et al., 2016). Besides, the eight-week treatment of *Lactobacillus* spp. in the water supply had no impact on egg productivity (Naseem and King, 2020).

Recently, it was reported that supplementing broiler breeder diet with *B. subtilis*, PB6 (2×10^7 cfu/g) with 1 g/kg diet noticeably enhanced egg production under commercial farm conditions. However, the dietary treatments did not affect egg or body weight (Darsi and Zhaghari, 2021).

Fertility and hatchability

Many studies revealed that the hatchability (H%) and fertility (F%) of Japanese quail viable eggs ranged from 8 to 52 weeks old, from 40.00–70.34% to 48.00–94.00%, respectively (Seker et al., 2004). Probiotics were reported to positively influence egg hatchability and productivity rate in broiler breeders between the ages of 22- to 49-week-old. Yet, no positive benefits were shown for egg fertility (Bozkurt et al., 2011).

Additionally, Mojgani et al. (2020) found that quails given *B. megaterium* (10^8 cfu/ml) substantially decreased

embryonic mortality by around 10% and enhanced H% by approximately 12%. Also, Beck et al. (2019) noted a considerably lower percentage of pipped eggs than the control group. In contrast, Ayasan (2013) determined that diet supplementation with protexin, a commercial probiotic, at doses of 0.05 and 0.10%/kg fed had no effect on the H% and F% from the fertile eggs of Japanese quail layers when compared to the untreated group. According to Ballou et al. (2016), the *in ovo* addition of probiotics protects the chick against an encounter with harmful bacteria in the outside environment. In a recent study, Darsi and Zhaghari (2021) revealed that feeding broiler breeder hens with supplements containing *B. subtilis*, PB6 (2×10^7 cfu/g) with 1 g/kg fed diet significantly enhanced hatching rates and egg hatchability %.

Egg quality features

Japanese quails fed diets containing a probiotic had increased eggshell weight (Ayasan et al., 2006). The eggshell weight of birds were significantly increased, compared to the control, from 1.47 to 1.54 g, after providing a probiotic with 10 g/100 kg diets and 10 g/200 L drinking water, respectively (Lokapirnasari et al., 2019). However, adding *B. subtilis* to quail diets did not affect Haugh units or eggshells (Manafi et al., 2016).

Recently, commercial multi-strain probiotics were examined for their effects on egg quality traits and production efficiency. Compared to the control group, the results revealed that supplementation improved various egg production-related traits, including egg size and weight, yolk and albumin weight, and eggshell strength and thickness (Jha et al., 2020). However, Saksrithai et al. (2020) assumed that the egg quality characteristics of White Leghorn laying hens' egg quality parameters were

unaffected by a mixture of probiotics, including; *L. paracasei*, *L. rhamnosus*, and *L. plantarum* comprising 1×10^{12} cfu/kg fed diet. The idea that trace elements might enhance albumen quality via microbial supplements is supported by variations in mineral plasma levels in laying hens given microbial cultures. On the other hand, and Darsi Zhaghari (2021) concluded that adding *B. subtilis* PB6 to the broiler's diet with (2×10^7 cfu/g) as 1 g/kg of fed diet enhanced the egg's Haugh unit and considerably increased eggshell thickness under commercial farm conditions.

Conclusions

Probiotics are a broad category of living organisms that are used to treat a wide range of illnesses. Probiotics, also known as direct-fed microbes, are helpful bacteria that primarily function in the gastrointestinal system of the poultry to benefit the host. Including probiotics in the food can benefit animal performance and health by enhancing gut health and nutrient absorption. For instance, probiotic supplementation has been demonstrated to benefit poultry by enhancing their immune systems, changing their structural makeup, and increasing the production of cytokines, which defend the gut mucosal barrier against pathogens. All of these parameters positively reflect on poultry health, production and reproduction.

Conflicts of Interest

All authors declare that they have no conflicts of interest that could inappropriately influence this manuscript.

Author contribution

All authors contributed equally to writing this review article. All authors read and approved the final version of this manuscript.

Data Availability Statement

No data are available.

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