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Application of probiotics in commercial layer diets: a review

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Abstract

Probiotics of various commercial preparations are being considered worldwide for poultry as performance enhancers and suitable alternatives to antibiotics. Much of the previous efforts to explore beneficial effects of probiotics were directed for commercial broilers and scientific information for commercial layer nutrition is limited in contrast to that of broilers. To our knowledge, whatever information is available, no attempt has been made as yet to make a comprehensive review of the effects of probiotics on layer chicken covering different aspects of production. This review is prepared by consolidating and updating information available in the literature covering laying performance, serum chemistry, egg quality and its safety aspects. Data generated from a number of studies clearly indicated that apart from a few exceptions, probiotics are found to be effective for the improvement of laying performance; serum biochemical parameters, egg shell and albumen quality and they are suitable for the production of safe eggs. Effect of probiotics on layers is mediated by boosting up gut health thereby creating an environment for better nutrient assimilation and thus an improvement of production and egg quality is achieved. Factors affecting such results need to be assessed more precisely since they contribute to some contradictions in the literatures. Nevertheless, the feed industry can rely on probiotics as quite safe for the production of quality feed to support commercial layer nutrition.

Keywords: probiotics, laying performance, egg quality, serum chemistry, food safety

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Introduction

Application of biotechnological tools to boost up the production efficiency of poultry is a dynamic feed technology. Such technology needs to be well justified so that it does not create any harm to poultry as well as to the ultimate consumers of poultry products. With the advancement of biotechnology in poultry feeds and nutrition and banning of harmful growth promoters, antibiotics in particular, the use of probiotics is gaining momentum globally in feed formulation strategy. Antimicrobial resistance is now a worldwide anxiety (WHO, 2018) and alteration of immune response due to feeding of antibiotic growth promoters, has lead poultry nutritionists to find out suitable alternatives to antibiotics. Probiotics of various preparations are considered to be important tools in this regard (Kabir, 2007). It is now well established that newly hatched chicks' gut is sterile and establishment of microflora in the gut of chicken starts just after hatch from hatching tray and hatcher which is called "cloacal drinking", thereafter gradually with the introduction of feed and water. Microorganisms which colonize in the gut may be either beneficial or harmful based on their response to the host animal. Attachment of beneficial microflora is always desired but negative to it may also occur. The beneficial organisms maintain gut equilibrium, improve health of birds and enhance, or at least maintain production. On the contrary, harmful bacteria like *E. coli, Salmonella, Coliform* and others may alter the gut equilibrium to a negative direction. This problem can be elucidated by the supplementation of probiotic**s** as they are believed to have a positive impact on poultry. Until recently, a number of beneficial effects have been reported from research works with probiotics although there are some controversies. Supplementation of commercial preparations of probiotics through diet comprising either single or multi-strain positively affected laying performance and egg quality characteristics specifically shell thickness and Haugh unit of commercial layers (Ray, 2018). The factors on which exploration of beneficial effects of probiotics depend are the survival, stability and strain of organisms, type of host animal, manufacturing process, dose frequency, health and nutritional status of the birds, the age, physiological stress level, genetics of the host

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etc. (Chichlowski *et al.,* 2007; Aalaei *et el.,* 2018). Much of the research works with probiotics has been concentrated with broiler birds and information with layer birds, whatever available, is not yet reported systematically. In addition, variations in the result due to application of different probiotics have been reported due to factors as mentioned earlier. Although improvement in egg production (Ribeiro *et al.,* 2014), health of birds and egg quality (Lokhande *et al.,* 2013) have been reported, there are also inconsistencies in the literature. To authors' knowledge, no attempt has been taken yet to consolidate and update the results of feeding probiotics to layers in particular, which are available sporadically in the literatures. Therefore, this review is prepared in a synchronized form by consolidating and updating the results of probiotics' use in commercial layer nutrition covering productive performance, serum chemistry, egg quality and safety aspects.

The concept of probiotics

The word 'probiotic' was used by Elie Metchnikoff in 1906, a Russian scientist and he was awarded Noble prize in medicine. He showed the beneficial effects of microbes replacing those which were harmful for treating intestinal illness. He is known as the father of probiotics (Rautray *et al.,* 2011). The probiotics are considered as "direct-fed microbial" and they affect the host positively by balancing the intestinal microbial populations (Fuller, 1989). Earlier, probiotics were referred to as substances which stimulate one another (Lilly and Stillwell, 1965). Currently, the term is used for animal feed supplement (Saleh and Hayashi, 2011) to explore beneficial effects. FAO and WHO jointly defined probiotics as beneficial organisms having beneficial effects on the body of host when administered in sufficient amount (FAO/WHO, 2001). This definition was adopted by the International Scientific Association for Probiotics and Prebiotics (Hill *et al.*, 2014). Probiotics may be classified as either bacteria (spore forming and non-spore forming), single or multi-strain, yeast, single species, multi-species, allochthonous and autochthonous (FAO, 2016). Microorganisms that are commonly used as probiotics are *Lactobacillus, Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium, Bifidobacterium* species and *E. coli* while yeast and fungus used as probiotics include *Saccharomyces cerevisiae* and *Aspergillus oryzae* (Fuller, 1989). Bacteria and yeasts are used as spores or as living microorganisms. Non-colonizing species are *Saccharomyces cerevisiae* and *Bacillus* spp. (spores) while colonizing species include *Lactobacillus* and *Enterococcus* spp. Among probiotic organisms, *Saccharomyces* are known to offer a source of good quality protein and B complex vitamins. Probiotics help in the digestion of food more efficiently, exert better metabolism, maintain the health status of the birds and cause faster weight gain in chicken (Park *et. al.,* 2016; Azemraw and Sewalem, 2017). Among probiotic bacteria, a small amount of anaerobic bacteria is found in crop, proventriculus and gizzard (Rastall, 2004) and large amount of facultative anaerobic bacteria are available in small intestine (Khan and Naz, 2013). Probiotic bacteria colonize in enterocyte, caecal and colonic epithelium, having the most colonized region, colon and caecum (10^{10}-10^{13} cfu/g) (Heczko *et al.,* 2000). Probiotics are supplied through powder, in encapsulated form or liquid form either in feed or water. Their use seems to be very much strain specific and no explicit recommendation has yet been developed for particular strains and thus has deviations in their feeding results. They deliberate beneficial effects by producing favorable conditions inside the gut of poultry to enhance performances.

Egg Production

Supplementation of probiotics in the diet of laying chicken favours egg production performance either by increasing egg number or maintaining production. Some probiotic preparations are also effective in adverse or stressful conditions and supposed to have no detrimental effects when used in appropriate amount. Research findings showed that use of probiotics in layer diets enhanced egg production (Ribeiro *et al.,* 2014; Peralta-Sánchez *et. al.,* 2019). Deviations to these observations also exist, but in many cases it affects the production positively, or at least maintains production as usual. Inclusion of *Lactobacillus acidophilus* (10^9 cfu/kg feed) significantly enhanced the number of eggs (Gallazi *et al.,* 2009). A combination of *Lactobacillus spp.* and *Bacillus spp.* as liquid probiotics mixed culture when fed to the ISA Brown layers showed an increase in egg production (Raka *et al.,* 2014). Abdelqader *et al.* (2013a) showed that feeding of *Bacillus subtilis* (maximum 2.3×10^8 cfu/g) had significantly better egg production compared to control at doses of 1g/kg feed and 0.5 g/kg feed for a duration of 10 weeks during late production period starting from 64 weeks. Results of a study by Khan *et al.* (2011) depicted that addition of probiotics (2×10^9 cfu/g) to the layer diet improved egg production. The probiotic organisms used in the trial were *L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus,* *Enterococcus faecium, Aspergillus oryzae and Candida pinpolopesi.* Similar positive effects on egg production in layer breeder aging 24 weeks also revealed by Panda *et al.* (2008) with the supplementation of 100 mg probiotics per kg diet (*L. sporogens,* 6x10^8 spore/g product). Increased egg production has been reported by probiotic feeding containing *Enterococci* based preparation (Arpasova *et al*., 2016); *Enterococcus faecium* (Zhang and Kim, 2013); heat-inactivated *L. salivarius* and *B. subtilis* (Zhang *et al*., 2012) and with a mixture of probiotics containing several organisms (Kalavathy *et al*., 2005). Mazanko *et al.* (2017) found positive improvement in egg production due to feeding of *B. subtilis* and *B. amyloliquefaciens*. Youruk *et al.* (2004) found linear increase in egg production when supplied with a number of organisms containing *Lactobacillus spp., Bifidobacterium bifidum, Streptococcus salivarius, E. faecium, A. oryza* and *C. pintolopesii*. Enhancement of egg laying was observed by Hassanein and Soliman (2010), upon feeding *Saccharomyces cerevisiae* at 0.4% and 0.8% as compared to control. The improvement in egg production due to low level of yeast inclusion is in agreement with the result of Shivani *et al.* (2003) and Shareef and Al-Dabbagh (2009) who observed higher percentage of egg production for hens fed yeast culture probiotics supplemented diets than the control hens. In contrast, no change in egg production was also reported by Balevi *et al.* (2001). Fathi *et al.* (2018) conducted experiment with three concentrations of dietary probiotic (0, 200 and 400 g/t feed) containing 4×10^9 cfu/g of *B. subtilis* involving three different breeds (White Leghorn, Saudi black and Saudi brown). They found statistically no significant difference in egg production performance among dietary treatments. Martinez *et al.* (2018) conducted an experiment using *S. cerevisiae* fermentation product at a dose of 1.25g/kg where they found no differences in egg production of Hy-Line commercial layer.

Egg weight and egg mass output

A significant improvement in egg weight has been reported by Ramasamy *et al.* (2009) due to feeding *Lactobacillus* culture containing probiotic to laying hen throughout the laying cycle. The highest egg weight was chalked out by Raka *et al.* (2014) from the supplementation of liquid probiotics mixed culture (0.45% v/v) of 68.12g compared to control of 65.22g. Haddadin *et al.* (1996) reported that egg weight was significantly increased in *Lactobacillus* culture fed hens during 20-68 weeks of laying hen. Daneshyar *et al.* (2009) found that the addition of probiotics had significant effect on egg weight but not on egg mass. Egg mass was increased by supplementation of 0.4% and 0.8% of live yeast (*S. cerevisiae*) as stated by Hassanein and Soliman (2010). Average egg weight was not influenced significantly by adding yeast to diets (Nursoy *et al.,* 2004). Feeding viable *Lactobacillus* at 1100 mg per kg (4.4 ×10^7 cfu/kg) was found to increase egg size (Nahashon *et al.,* 1996). According to Mahdavi *et al.* (2005), diet containing *B. subtilis* and *B. licheniformis* did not increase egg weight significantly. Recently, Martinez *et al.* (2018) conducted an experiment using *S. cerevisiae* fermentation product at a dose of 1.25g/kg where they found no differences in egg weight and egg mass of Hy-Line layer. Fathi *et al.* (2018) found no significant differences when three different breeds were fed on three concentrations of dietary probiotics containing 4×10^9 cfu/g of *B. subtilis*. Abdelkader *et al.* (2013) reported that egg weight was gradually increased for *B. subtilis* supplemented groups. Khan and Naz (2013) conducted a trial where they used commercial multi-strain probiotic**s** with 2×10^9 cfu/g (*L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, E. faecium, A. oryzae and C. pinpolopesi*) and found significant increase in egg weight and egg mass compared to negative control. Panda *et al.* (2008) found no significant differences in egg weight when they fed *L. sporogenes* at 100mg (6×10^8 spore) per kg diet.

Feed intake and feed conversion ratio

Results of feed consumption due to supplementation of probiotics in the diet of layers are not consistent. No significant effects on feed consumption have been reported in a number of studies (Mahdavi *et al.,* 2005; Ramasamy *et al.*, 2009; Fathi *et al.,* 2018). Some other authors (Yousefi and Karkoodi, 2007; Panda *et al.,* 2008; Abdelqader *et al.*, 2013a) also revealed no significant differences in the feed intake of layers due to inclusion of probiotics in the diet. However, decrease in feed intake in the probiotics treated groups was found in other studies (Yoruk *et al.*, 2004; Gallazzi *et al.*, 2009). In contrast, an increase in feed consumption was recorded in laying hens fed on probiotics (Raka *et al.,* 2014; Zhang and Kim, 2014). A study by Balevi *et al.* (2009) has shown improved FCR through the supplementation of a commercial probiotic**s** in the basal diet consisting *L. plantarum, Bifidobacteria bifidum, Streptococcus salivaries, E. faecium, A. oryza* and *C. pintolopesiie* in brown layers. A number of studies have shown that probiotics improved feed conversion ratios in layer chickens (Panda *et al.,* 2003; Yoruk *et al.,* 2004; Panda *et al.,* 2008; Gallazzi *et al.*, 2009; Abdelqader *et al.,* 2013a; Raka *et al.*, 2014). Inatomi *et al.* (2016) found that inclusion of probiotic**s** mixture in layer diet having *B. mesentericus, C. butyricum, and S. faecalis* significantly improved feed mass into egg mass. Contrary to this result, there was no significant difference in FCR between hens fed probiotic**s** supplemented diets and hens fed the control diet (Yousefi and Karkoodi, 2007; Fathi *et al*., 2018). Feed conversion was improved for hens fed the probiotics diet containing *S. cerevisiae* at 1.25g/kg (Martinez *et al.,* 2018).

Body weight, body weight gain and survivability

Probiotic**s** organisms are found to affect body weight positively. Sobczak and Kozłowski (2015) indicated that dietary *B. subtilis* led to a significant increase in the final body weights and weight gains of layer chickens (18-42 weeks). Neijat *et al.* (2019) found increased body weight through feeding probiotics. Amer and Khan (2012) showed that the supplementation of probiotics (*L. acidophilus, B. subtilis, S. cerevisiae and A. oryzae*) in *desi* chicken indicated significant increase in body weight gain after 6 weeks of experiment starting from 8 days of age feeding probiotics (1.5×10^{9}cfu per ml). Body weight did not significantly differ when *L. acidophilus* was fed in two experiments after 34 and 61 weeks of age (Gallazzi *et al.,* 2009). Panda *et al.* (2008) observed no differences in the weight gains (25-40 weeks) of hens fed diets supplemented with probiotic bacteria *L. sporogenes* with 6×10^{9} spores per gram. In a study by Abdelqader *et al.* (2013a), the body weights of laying hens were not affected by feed additives such as *B. subtilis*, inulin or synbiotics. Yousefi and Karkoodi (2007) found that body weight changes did not differ significantly among treatment groups of probiotics (*S. cerevisiae)* at doses of 0.05, 0.1 and 0.15% dietary supplementation for 63-75 weeks in commercial layers. Body weight of layer was not significantly affected by probiotics *(L. acidophilus, L. casei, B. bi¢dum, A. oryzae, S. faceium and Torulopsis sp*p) during 25-72 weeks (Panda *et al.,* 2003). A decrease in mortality was found by Yoruk *et al.* (2004) when probiotics was supplemented at a rate of 0.1% and 0.2% in the diet of late laying hens. The probiotic organisms were *Lactobacillus, Bifidobacterium, Streptococcus*, and *Enterococcus* spp.

Blood cholesterol

Recently, researchers have shown that dietary supplementation of probiotics decrease cholesterol and triglycerides in blood (Moataz *et al.,* 2018; Kanani *et al.,* 2018). Results of earlier studies were also consistent with cholesterol lowering effects of probiotics fed birds (Mohan *et al.,* 1995; Abdulrahim *et al.,* 1996). Reducing blood cholesterol level due to *B. subtilis* inclusion has also been reported in layers (Sobczak and Kozłowski, 2015). *Lactobacillus* culture containing probiotics supplementation reduced serum low-density lipoprotein (LDL) cholesterol (Kalavathy *et al*., 2003). A study by Zhang and Kim (2013) showed that serum total cholesterol concentration was decreased by feeding hens with probiotics. In another study of Zhang *et al.* (2012), a significant drop in total cholesterol content was evident. Amer and Khan (2012) showed that the supplementation of probiotics (*L. acidophilus, B. subtilis, S. cerevisiae* and *Aspergillus oryzae)* caused significant reduction in serum cholesterol concentration after 6 weeks. Similar reduction also stated by Chuke and Didacus (2015). Sohail *et al.* (2011) used a commercial multi-strain probiotic with a minimum presence of 2×10^{9} cfu/g (*L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, E. faecium, A. oryzae and C. pinpolopesi*). The study has shown a reduction in serum cholesterol but HDL-cholesterol was significantly increased. Mansoub (2010) reported that triglycerides and total cholesterol level of serum significantly decreased in groups supplemented with probiotics containing *L. acidophilus and L. casei* compared to control group. Shirley *et al.* (2017) found that probiotics supplementation decreased ($P<0.05$) serum total cholesterol at 36 weeks of age, and serum LDL cholesterol, alanine aminotransferase and alkaline phosphatase at 36 and 52 weeks of age. On the contrary, the dietary probiotics supplementation did not affect serum cholesterol or triglycerides in some other studies (Zarie *et al.*, 2011; Mohebbifar *et al.*, 2013).

Egg yolk cholesterol

Yolk cholesterol, triglycerides and very low density lipoprotein (VLDL) were significantly declined as a result of supplementation of *B. subtilis* and *B. licheniformis* compared to control or antibiotic-treated groups (Yang *et al.,* 2020). Some other findings are also in line with the cholesterol lowering effect of probiotics fed birds (Kanani *et al.*, 2018 and Ashayerizadeh *et al.,* 2011). The cholesterol content of eggs produced by probiotic (*Lactobacillus* culture) fed hens was significantly reduced by 15.3% and 10.4% when compared to those of the control hens at 24 and 28 weeks of age, respectively (Ramasamy *et al.,* 2009). Mikulski *et al.* (2012) reported that dietary supplementation of probiotics (*Pediococcus acidilactici*) decreased the egg cholesterol content and fatty acid composition in laying hens. Mousa *et al.* (2015) stated that egg yolk cholesterol was decreased markedly by probiotics supplementation. Several other authors also reported similar results (Panda *et al.,* 2003; Panda *et al.,* 2008; Ramasamy *et al.,* 2009) from their experiments where they found a reduction of cholesterol concentration in egg yolk due to use of probiotics. In a study of Kurtoglu *et al.* (2004), probiotics supplementation at 250, 500 and 750 mg/kg feed decreased the egg yolk cholesterol levels when compared with the control. Mahdavi *et al.* (2005) also expressed cholesterol lowering effects of probiotics. Sohail *et al.* (2011) conducted a study where they used a total of 180 Hy-Line W-98 hens, 40 weeks old and fed a commercial multi-strain probiotic. In that study, supplementation of a probiotic containing *L. plantarum, L. bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, E. faecium, A. oryzae* and *C. pinpolopesi* in laying hen diets has shown a reduction in egg yolk cholesterol concentration.

Egg shell quality

The dietary supplementation of probiotics improved shell quality and bone strength in laying hens (Yan *et al.,* 2019). Increase in shell thickness has been stated by researchers through probiotics supplementation (Panda *et. al.,* 2003; Panda *et al.,* 2008; Mohan *et al.,* 1995; Ray, 2018). Supplementation of probiotics tends to increase shell hardness and proportion positively. This beneficial effect may be attributed to a favorable environment in the gut (Panda *et al.,* 2008; Mikulski *et al.,* 2012) probably by calcium mobilization as a primary means to improve eggshell quality (Skrivan *et al.,* 2010). Li *et al.* (2006) found that dried *B. subtilis* cultures increased eggshell thickness. Balevi *et al.* (2009) reported that feeding with 0.5 g/kg multi strain probiotics diet caused statistically significant reduction on damaged egg ratio. Sobczak and Kozłowski (2015) found that probiotics for laying hens contributed to an improvement in eggshell quality. Similar results were reported by Abdelqader *et al.* (2013 a,b), where they stated that supplementation of 1g/kg feed and 0.5g/kg feed exhibited 8.4% and 7.5% increase, respectively, in eggshell thickness at the end of the study. According to Aghaei *et al.* (2010) and Mikulski *et al.* (2012), probiotics exerted a beneficial influence on eggshell thickness. Contrary to a number of positive reports on shell quality, Haddadin *et al.* (1996) found no significant effect (P>0.05) on shell hardness and shell thickness due to addition of a probiotic (*L. acidophilus)* in the diet. Fathi *et al.* (2018) found that *B. subtilis* at 200 and 400g/ton feed significantly increased shell weight.

Albumen quality

An increase in Haugh unit has been recorded by Gallazzi *et al.* (2009) with the inclusion of a probiotic containing *L. acidophilus* in the diet of laying hen at a dose of 10^9 cfu/kg of feed. Eggs laid by probiotics treated hens showed significantly higher albumen quality in terms of Haugh unit value. Chung *et al.* (2015) conducted an experiment with mixed probiotics containing five microbial species that included *A. oryzae*, *B. subtilis*, *S. cerevisiae*, *L. plantarum* and *Rhodopseudomonas capsulate*, and found that supplementation of 0.4% mixed probiotics showed greatest (P<0.05) Haugh unit. Similar results have been revealed by Zhang *et al*. (2012) and Sobczak and Kozłowski (2015). Improvement of protein quality in terms of albumen height and Haugh unit value due to dietary supplementation of *B. subtilis* containing probiotics has been noted in a recent study of Neijat *et al.* (2019). Khan *et al.* (2011) found that Haugh unit score was significantly increased (P<0.05) in the groups fed the multi-strain probiotics containing diets as compared to negative control group. In contrast, no significant enhancement of Haugh unit value was observed in several other studies (Mahdavi *et al.,* 2005; Panda *et al.,* 2008; Mikulski *et al.,* 2012; Fathi *et al.,* 2018).

Yolk quality

Improvement in the production of darker yolk color to get higher yolk color score has been reported in a number of experiments (Li *et al.,* 2006; Mikulski *et al.,* 2012). Sobczak and Kozłowski (2015) showed that eggs laid by probiotics treated group hens received significantly higher scores for yolk color. On the contrary, there was no significant difference in yolk color among treatments upon feeding mixed probiotics containing five microbial species (Chung *et al.,* 2015). Besides, Xu *et al.* (2006) and Zhang *et al.* (2012) did not note any change in yolk color in response to feeding probiotic bacteria. Yang *et al*. (2003) observed a quadratic effect of graded concentration of supplemental probiotics on yolk color. Fathi *et al.* (2018) reported no significant differences between the dietary groups with respect to yolk per cent and yolk color resulting from the supplementation of *B. subtilis* containing probiotic at different doses. No change in yolk color has been postulated in some other findings as well (Berrin 2011; Khan *et al.* 2011).

Safety aspects of probiotics

Safe food production is a global demand nowadays. Table egg, a cheapest source of animal protein for human consumption must be pathogen free to satisfy the safety aspects. Indiscriminate use of antibiotic growth promoters in poultry to improve their productivity causes antimicrobial resistance to health of people following consumption of foods (egg/meat) from chicken treated with that growth promoter (Park *et al*., 2016). Multi drug resistance organisms (MDRO) or "Superbug" has already been emerged both for animal and human due to continuous use of antibiotic growth promoters in the diet of poultry. However, Alfredson and Korolik, (2007) reported an increase in prevalence of infectious disease by *Campylobacter jejuni* or *Clostridium perfringens* in birds, if antibiotic growth promoters are excluded from diet. In this situation, probiotics could be an alternative for growth promotion as well as to ensure food safety (Chowdhury, 2018). Most concentrated source of *Salmonella spp.* is the ceca of GIT in poultry which subsequently contributed to the contamination of eggs. Feeding of probiotic**s** was found to be effective in reducing harmful bacterial population in the gut (Chichlowski *et al.,* 2007). Feeding probiotics containing *Lactobacillus* and *Bifidobacterium spp.* is related with the reduction of *Salmonella spp.* (Cox and pavic, 2009). Among the food pathogens, *Salmonella, E. coli* and *Campylobacter* are significantly responsible for contamination of food. A study from Hassanein and Soliman (2010) confirmed that feeding *S. cerevisiae* in live form at doses of 0.4% and 0.8% reduced these three organisms along with *Klebsiella spp., Staphylococcus spp*. and *Micrococcus spp.* Higgins *et al.* (2007) reported that probiotics significantly increased *Lactobacillus* and decreased *E. coli, Clostridium perfringens* and *Salmonella* count in the lower gut in poultry as well as in its excreta. Latorre *et al*. (2019) noted that supplementation of *Bacillus* spp. (*B. subtilis* and *B. amyloliquefaciens*) in poultry diet reduced undesirable bacteria in the duodenal content compared to control. Thus, use of probiotics is seemed to be quite appropriate for ensuring food safety during the course of husbandry practices.

Discussion

Research reports reviewed for the preparation of this review showed a lot of positive effects of probiotics on laying chicken even though some controversies still exist. Supplementation of probiotics enhances performance of the birds by boosting gut health, better nutrient assimilation and thus improvement in production is achieved. The general mode of action of probiotics for getting advantageous effects may be direct antagonistic effects against particular organisms, resulting in decrease in number of harmful organisms or by an effect on their metabolism or by synthesis of some essential nutrients or by stimulation of immunity (Jadhav *et al.,* 2015). But it is possible that more than one mechanism may work simultaneously. Probiotics reduce viable count through the production of antibacterial substances (lactocidin, acidophillin, organic acids, bacteriocins) and hydrogen peroxide. Due to natural breakdown of probiotics and metabolism of nutrients in the gastrointestinal tract, the produced volatile fatty acids and organic acids lower the pH below required for the survival of pathogenic bacteria such as *E. coli* and *Salmonella spp* (Khan and Naz, 2013). As a result, the growth of harmful bacteria is inhibited. Another mechanism of action is "competitive exclusion" by which probiotic bacteria colonize in the adhesion sites on the intestinal epithelium and prevent colony formation of pathogenic bacteria (Chichlowski *et al.,* 2007). They improve digestion by increased activity of the digestive enzymes and better absorption of nutrients while decreased bacterial enzyme activity (glucoronidase, nitroreductase, azoreductase) which are produced by some pathogenic bacteria. In addition, probiotics reduce ammonia production. They stimulate immune system by higher production of immunoglobulins and increase activities of macrophages and lymphocytes and also by amplification of the production of γ-interferon (Yang and Choct, 2009). Moreover, probiotics improve the intestinal morphology through increasing the villus height, goblet cell number and decrease the crypt depth to create the environment favourable to the host. In precise, they have the potentiality to create favorable conditions in the host animal to boost up production. Out of many factors, the effect of probiotics on poultry performance may differ based on single or multi-strain composition and their doses (Ray, 2018). In case of single strain probiotics, it is easy to clarify the exact mechanism of action but for multi-strain, it has become quite difficult to state precisely which strain has actually contributed to what extent and by which mechanisms. But, for multi-strain probiotics, effectiveness is achieved when there is synergistic action among different strains and in such condition, it is supposed that multi-strain probiotics have more adhesive power than single strain (Timmerman *et al.,* 2004). Studies revealed that probiotics increase egg production. Such an increase in egg production might be due to elongated small and large intestinal lengths, suppressing effects of undesirable bacteria, stimulating effects on the growth of beneficial bacteria or enhanced activity of beneficial bacteria in the intestines, thereby triggering absorptive capacity of nutrients (Chen *et al.* 2005) that could be accounted their increased nutrients availability at the site of formation of egg components. But, as already mentioned, such results of improvement in egg laying capacity are not always consistent. It appears that application of probiotics either enhance production and egg quality or at least maintain such parameters without showing any detrimental effects. However, several researchers reported a minimum concentrations of probiotic**s** in each gram should be 10^6 cfu/g and for effectiveness in the body, an animal should have a daily intake of 10^8-10^9 organisms (Patterson and Burkholder, 2003; Toma and Pokrotnieks, 2006), but the type of organisms, their dose and length of feeding have not been explained as yet. In many cases, probiotics are performance enhancers as they improve efficiency in feed utilization and therefore improve feed conversion. The reduced feed intake in probiotic fed birds might be a reflection of efficient utilization of nutrients. Improving feed efficiency using nutritional approaches are common phenomena. Probiotics, being useful in efficient utilization of feed by making the favorable environmental conditions inside the gut, improve feed conversion. Moreover, it added extra profit to the farm owners. In laying hen, growth is rarely affected during laying period as the feed is utilized mostly for egg production rather than live weight gain. Consequently, improvement in body weight gain is noticeable in earlier phases of layers but maintenance of good health and standard body weight in laying periods are always important. Although a variety of factors are responsible for having favourable effect of probiotics on bird, they are supposed to affect the host positively. Their introduction to poultry feed improve egg quality, particularly shell thickness and therefore, shell quality. The increase in shell thickness is associated with the production of short chain fatty acids due to fermentation which subsequently reduces the luminal pH (Scholz-Ahrens *et al.,* 2007). Reduced pH of intestinal lumen increases calcium solubility for absorption (Van den Heuvel *et al.,* 1999) whereas epithelial cell proliferation and villus height are stimulated by the production of short chain fatty acids (Garcia *et al.,* 2007) to increase the absorption rate (Scholz-Ahrens *et al.,* 2007), thus improve the egg shell quality. Such improvement in shell quality is more important for transportation and marketing to reduce egg breakage and number of abnormal shaped eggs. This attribute is much more helpful in aged hens where quality of shell is a major concern. Stress conditions are harmful for birds' performance in which probiotics are good performers for maintaining production by balancing gut microflora. Good quality and safe eggs are possible to obtain in probiotic fed birds. An improvement in yolk color has been stated in a very few studies. A reasonable explanation for an improvement in yolk color might be due to the fact that carotenoids from the diet is well absorbed and transferred into the egg yolk more efficiently, although exact mechanism of such action is not definitely known. The survivability of birds is always satisfactory because probiotics boost up immunity and thus beneficial in the maintenance of birds' health. Although considerable evidences have been growing in the recent years that egg yolk cholesterol has no influence on the cardiovascular problems (Abdollahi *et al.,* 2019; Rong *et al.,* 2013; Shin *et al.,* 2013), rather is useful in preventing heart diseases, researchers all over the world are making efforts to reduce cholesterol in eggs by genetic (Elkin, 2007) and dietary manipulations (Chowdhury *et al*., 2002; Chowdhury *et al.,* 2005; Dey *et al*., 2012; Mattioli *et al.,* 2016). Use of probiotics to reduce cholesterol and other components (triglycerides, very low density lipoprotein) in eggs is a new dimension side by side with other dietary means of manipulation. According to Corcoran *el al.* (2005), when digestion of fat is concerned, it depends on the level of gallbladder acids in digestion latex which subsequently contribute to the lipid content. Probiotic organisms have the competency to reduce fat content in blood. As the fat content is reduced in blood, it is generally believed that transportation to egg yolk would be less. Due to recent trends in exclusion of antibiotics in feed industry, probiotics appeared as a viable alternative not only as performance enhancers but also it plays an important role in ensuring the production of safe eggs for human race. Unlike some other non-nutritive growth promoters, probiotics are always considered to be safe as they leave no residues in egg for human consumption.

Conclusion

Dietary inclusion of probiotics for commercial layers may improve laying performances, and egg quality in terms of albumen and shell quality. Application of probiotics improves serum biochemical parameters by reducing total cholesterol, LDL-cholesterol and triglycerides, and increasing HDL-cholesterol. Factors affecting such results need to be assessed more precisely since they contribute to some contradictions in the literature. Nevertheless, supplementation of probiotics in the diet of laying chicken is safe and therefore may play a vital role globally not only as performance enhancers but also as a means of producing safe eggs for human.

Conflict of interest

The authors have no conflict of interest to declare.

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