

## **Using of Probiotics on Performance, Carcass Characteristic, Some Blood Parameters, and Intestine Morphology in Broiler Chickens**

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### **Abstract**

Today, poultry meat is one of the most important and rich sources of protein. With the increase of world population, the demand for this valuable food has also increased. In recent years, the production of meat has increased dramatically, but along this production increase the poultry industry faced with some problems such as disease, the emergence of a large variety of pathogens and bacteria which reduced the economical profit of the poultry industry and antibiotics were used to prevent this. Since the use of antibiotics in the poultry industry is limited due to harm to human health, one solution to eliminate antibiotics is to use probiotics. Probiotics are live microbial compounds that are added directly to livestock and poultry diets and have a very beneficial on their performance and health. This review explains the summary of previous researches about the use of probiotic's effects on performance, carcass features, internal organs weight, some blood parameters, and intestinal morphology in broiler chickens.

**Key Words:** Disease, Pathogens, Bacteria, Probiotics, Carcass, Morphology.

### **Etlik Piliçlerde Probiyotik Kullanımının Performans, Karkas Özellikleri, Bazı Kan Parametreleri ve Bağırsak Morfolojisi Üzerine Etkisi**

#### **Öz**

Günümüzde kanatlı eti en önemli ve zengin protein kaynaklarından biridir. Dünya nüfusunun artışıyla birlikte bu değerli yiyeceğe olan talep artmaktadır. Son yıllarda et üretimi fazla miktarda artmakta, ancak bu üretimin artışı ile birlikte kanatlı endüstrisi hastalıklar ve çok çeşitli patojenlerin ortaya çıkması gibi sorunlarla karşı karşıya kalarak ekonomik zararlara uğramaktadır. Kanatlı endüstrisinde bu sorunları engellemek için antibiyotikler kullanılmaktadır. Kanatlı endüstrisinde, antibiyotik kullanımı insan sağlığına verdiği zararlardan dolayı kısıtlanmakta ve antibiyotiklerin ortadan kaldırılmasının bir yolu probiyotik kullanımıdır. Probiyotikler, doğrudan canlı hayvan ve kümes hayvanları rasyonlarında eklenen, performansları ve sağlıkları üzerinde çok faydalı olan canlı mikrobiyal bileşiklerdir. Bu derleme, etlik piliçlerde performans, karkas özellikleri, iç organ ağırlığı, bazı kan parametreleri ve bağırsak morfolojisi üzerindeki probiyotik etkilerinin kullanımına ilişkin değerli araştırmaların özetini açıklamaktadır.

**Anahtar kelimeler:** Hastalık, Patojenler, Bakteri, Probiyotik, Morfoloji

## 1. Introduction

Nowadays, with the increase of population, access to healthy food sources has become one of the main concerns of human beings. Proteins play a very important role in daily nutrition. Meanwhile, cattle and poultry meat are important sources of protein in human nutrition. Due to the prevalence of various cardiovascular diseases and diabetes in today's society, which occurs following the excessive consumption of unhealthy foods (red meat), the tendency of consumers to poultry meat is increasing. Poultry meat has received more attention among white meat types due to its acceptability and easier availability. So, poultry meat is an important protein source and popular kind of human food. For this reason, poultry industry has progressed a lot in recent years. As is clear some of the problems such as disease have negative effects on poultry industry profit. Antibiotics are used for a long period of time to treat and prevent diseases and poultry health in the poultry industry (Butaye et al., 2003). But unfortunately it was reported that antibiotics residues in poultry production are dangerous to human health because antibiotics lead to the development of antibiotics resistance in human bodies (Shazali et al., 2014). In 2006, using of antibiotics in all livestock animals' nutrition was prohibited by the European Union and probiotics were used instead of antibiotics (Pochop et al., 2011). It was defined that probiotics are a live microbial feed supplement that beneficially effects on host animal body by improving the intestinal microbial balance (Fuller, 1989; Crawford, 1979). In many studies probiotics are used in animal diets in different shapes like essential oil (Badiri and Saber, 2016), milk kefir (Toghyani et al., 2015), yeast (Paryad and mahmoudi, 2008) and etc. Also, in many studies, probiotic effects have been reported on performance (Cho et al. 2013), carcass characteristic (Kandir and Yardimci, 2015), immunity (Silva et al., 2012), disease (Vanderhoof, 2001), blood parameters (Djouvinov et al., 2005), and intestinal morphology (Wang et al., 2015). The purpose of this study article was to review some of the studies which were conducted on broiler chickens using probiotics.

## 2. Probiotics and Their Mechanisms of Action

The term probiotics have been used in several different ways over the years. The term was first used by Lilly and Stillwell (1965), for substances secreted by microorganisms that stimulated the growth of other microorganisms. Since then, many scientists have turned their attention to probiotics and many definitions have been proposed. Parker (1974) stated that probiotics are animal feed supplements that have a beneficial effect on the host animal by affecting the intestinal flora. Fuller (1989), introduced probiotics as a live microbial food that has positive effects on the host by improving the microbial balance of the host intestines. According to the definition of Anonim (2001), probiotics are live microorganisms of nontoxic and nonpathogenic if used properly, they can cause positive symptoms in the host. Probiotics may contain one or more microbial strains and can be used in the form of powders, capsules, tablets, granules, or pastes also; they can be taken directly or with water and food. Probiotics are non-absorbable components in food that selectively stimulate the growth and activity of a limited number of intestinal bacteria (Gipson et al., 1995). The most commonly used prebiotics are oligosaccharides. These are indigestible carbohydrates that work in two ways:

1) they provide the necessary nutrients for beneficial microorganisms.

2) they create a space for pathogenic bacteria to attach and prevent them from attaching to the intestinal wall. Extensive research on human and animal models shows multiple roles of these materials. Some of the beneficial effects of probiotics are:

- 1) Modify intestinal microbiota,
- 2) Stimulate the immune system,
- 3) Reduce inflammatory reactions,
- 4) Prevent pathogen colonization,
- 5) Enhance animal performance,
- 6) Prevent cancer,

7) Lower serum cholesterol and triglyceride (Patterson and Burkholder, 2003). Probiotics used in poultry diets may be a type of bacteria or yeast. The types of bacteria that used as probiotics are: *Escherichia coli*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactococcus*, *Lactobacillus*, *Streptococcus*, and yeast mainly *saccharomyces cerevisiae*, and *saccharomyces bulardii* (Fuller,

1989; Patterson and Burkholder, 2003; Kabir et al., 2004; Mountzouris et al., 2007). *Bifidobacterium* and *Lactobacillus species* are mainly used in human food while *Bacillus*, *Enterococcus*, and yeast species are commonly used in animal feed (Simon et al., 2001). Probiotics reduce the incidence and duration of disease by directly inhibiting pathogens or increasing colonization resistance. Previous studies show the mode of action of probiotics on poultry:

- 1) improving feed intake and digestion (Awad et al., 2006),
- 2) stimulating the immune system (Nayebpor et al., 2007; Mathivanan and Kalaiarasi, 2007),
- 3) maintaining normal intestinal microflora by competitive exclusion and antagonism (Rantala and Nurmi, 1973; Kizerwetter-Swida and Binek, 2009),
- 4) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (Cole et al., 1987; Yoon et al., 2004).

### 3. Effects of Probiotics on Feed Intake, Body Weight Gain, and Feed Conversion Ratio:

Many studies were conducted about of effects of different shapes of probiotics on feed intake, body weight gain, and feed conversion ratio in poultry. For example, in the study was conducted with Chen et al., (2018) it was reported, supplementing broiler diets with *L. rhamnosus* and *E. faecium* have a significant effect on body weight and feed conversion ratio in broiler chickens so that the higher body weight gain and feed conversion ratio was related to the group which was fed a diet containing *L. rhamnosus*. Rehman et al., (2020) reported that, supplementation of broiler chickens diets with 0, 1, and 2 g/kg probiotics (Protexin) and 0, 1, and 1.5 g/kg of prebiotics (active MOS) does not have a significant effects on feed intake, but feed conversion ratio and body weight gain significantly affected by prebiotics. Supplementing of broiler chickens diet with 2g /100g of *Lactobacillus acidophilus* have beneficial effects on body weight, average daily gain, and feed conversion ratio compared to the control group (Forte et al., 2018). Broiler chickens fed dietary which was containing  $2 \times 10^{10}$  cfu/kg,  $3 \times 10^{10}$  cfu/kg,  $2 \times 10^{10}$  cfu/kg *Bacillus subtilis fmbj* (BS fmbj) for 42 days. The result obtained from this study showed that BS fmbj significantly improved the average daily gain,

average daily feed intake, and feed conversion ratio of broilers (Kaiwen et al., 2017). Lan et al., (2016) demonstrated that supplementation of the diet with 0.05, 0.10, and 0.20% *Enterococcus faecium* (SLB 120) in broiler chickens diet caused linear increases in gain: feed ratio from 1-21 days of age and linear increase in body weight from 21-35 days of age. The effect of using 0.005%, 0.01%, 0.015%, and 0.02% probiotics in broiler chickens diets on their performance was investigated by Pourakbari et al., 2016. They were reported that feeding of 0.01% probiotic or higher levels of supplementation improved body weight gain (+12%) and feed conversion rate (-5%) when compared to the control group. Also, Bai et al., (2013) reported that received of different levels of probiotics by broiler chickens have a significant effect on feed intake, body weight gain, and feed conversion ratio at 21 days of age. Reports (Sharef and Dabbagh, 2009) have suggested that supplementation of diet with probiotics (1, 1.5, and 2%) improved body weight gain and feed conversion ratio, and also increased feed intake in broiler chickens. It is demonstrated that feeding of lower levels (2, 4, and  $6 \times 10^9$ ) of *Lactobacillus Bulgaricus* has more improving effects on the growth performance of broiler chickens than the high levels ( $8 \times 10^9$ ) of *Lactobacillus Bulgaricus* (Apata, 2008). Supplemented of broiler chickens diet with different levels of probiotic and microbes improved growth performance (Li et al., 2008). Also, Boratto et al. (2004) showed that there was a higher body weight gain in the group that was received diets including different levels of probiotics. Zulkifli et al. (2000) reported that 0.1% probiotic-supplemented diets improve body weight gain and feed conversion ratio from 1-21 days of age but this effect will be lost at 42 days of age. In constant of these result, Bitterncourt et al. (2011) indicated that supplementation of broiler diets with  $3.5 \times 10^{11}$  of the different spice of *Lactobacillus* improve body weight of broiler chickens during 1 to 35 days of on experiment but it does not have any effect on feed conversion ratio. In another study, it was demonstrated that feeding probiotics don't have a significant effect on broiler body weight gain. For example, Ramaro et al. (2004) explained that supplementing of broiler chickens diet with different levels of probiotics does not have any effect on broiler chicken body weight gain. However, in most studies, the positive effects of probiotics on poultry performance have been

reported. So that in the many studies it was reported that supplementation of probiotics in poultry diets can increase feed intake and most importantly increase nutrition digestibly by the increase of calcium, phosphor, crude protein, dry matter, and some amino acids digestibly (Gao et al., 2008; Li et al., 2008).

#### **4. Effects of Probiotics on Carcass and Organs Weight:**

Rashidi et al., (2020), used 0.5 mg/kg aflatoxin B, 3 and 6g/kg licorice extract, 0.5 g protexin/kg, 6g licorice extract+ 6g toxin binder/kg, 0.5mg aflatoxin B1+ 5g/kg poultry litter biochar in broiler chickens diet and reported that thymus, breast, and abdominal fat contents were differences between experimental groups but carcass percent, thigh, liver, bursa, and spleen was not affected by dietary probiotics. In the another study which was conducted by Biswas et al. (2021) it was reported that, using of MSP @10<sup>7</sup> CFU and MSP @10<sup>7</sup> CFU per g feed has a significant effect on thigh, neck, breast, drumstick, spleen, and thymus in broiler chickens. Poorghasemi et al., (2018), demonstrated that, breast and thigh percentages in broiler affected by a diet which was containing different dietary oil and probiotic, but carcass percentage and abdominal fat amounts were not affected by this ratio. On the other hand, Reis et al., (2017), investigated the effect of supplementation diet with 500 g Bacillus strain DSM17299 (1.6× 10<sup>9</sup> cfu/g) on broilers performance. They showed that duodenum weight was differences between experimental groups at 21 and 42 days of age but probiotic has no influence on the liver, spleen, Small intestine, ileum, and jejunum weights and lengths. In the recent study which was conducted by Sarangi et al., (2016), it stated that supplementation of broiler chickens diet with 50, 100, 400 and 500g/tonne probiotic have a significant effect on neck weight but, does not have any significant effect on liver, dressed, eviscerated, heart, liver, gizzard, wing, breast, back, thigh and drumstick weights Pourakbari et al (2016), indicated that adding a different percent (0.005%, 0.01%, 0.015%, and 0.02) of probiotics in broilers diet has a significant effect on wings, abdominal fat, left caecum, and thymus (but does not have any significant effect on breast, drumsticks, liver, and bile, spleen and bursa of fabricious Ashayerzadeh et al. (2009) indicated that supplementation broiler

diets with 650 g t-1 antibiotic, 900 gt-1 probiotic, and 2000g t-1 prebiotic have a significant effect on the carcass, thigh, breast, and abdominal fat weights. Awad et al. (2009) demonstrated that supplementation of broiler diets with 0.5 and 1 kg/ton probiotics have a significant effect on proventriculus, liver, spleen, pancreas, cecum, and thymus weight but it does not have any effect on gizzard, small intestine, heart, colon, and bursa weight. It was reported that by Denli et al. (2003) received the diet containing 0.1, 0.2, and 0.15% probiotics can affect on average carcass weight, liver weight, and intestinal length but it does not have any effect on abdominal fat weight, and intestinal pH. In another study which was conducted by Awad et al. (2006), it was reported that received diets containing probiotics by broiler chickens can change gizzard, liver, jejunum, and cecum weight but, there wasn't significant effect on heart, duodenum, spleen, and pancreas weight. In another study Paryad and Mahmoudi (2008) suggested supplementation of broiler chicken diets with 0.5, 1.5, and 2% yeast have a significant effect on carcass, breast, legs, liver, heart, gizzard, and abdominal weights also these researches indicated that breast and leg meat dry matter, protein, and extract amount can be affected by diets probiotics. The other researchers (Dimcho et al., 2005; Penkov et al., 2004) founded more improvements in gizzard, liver, and heart weights of broiler chicken. In contrast with these reports In the previous study it was stated that supplementation of diets with 0.05, 0.10, and 0.20% Enterococcus faecium (SLB 120) does not have a significant effect on breast muscle, liver, bursa of fabricius, abdominal fat, spleen and gizzard weights of chickens (Lan et al., 2016). The feed of diets containing 1, 2, and 3 g/10 liters by broiler chickens unaltered breast, thigh, drumstick, wing liver, heart, gizzard, spleen, and bursa weight (Islam et al., 2004). The researchers have related the positive effect of probiotics on carcass quality to improving the uptake of nutrients and increasing nitrogen stability (Nahashon et al., 1996).

#### **5. Effects of Probiotics on Some Blood Parameters:**

Kaiwen et al., (2017) showed that using 2, 3 and 4 ×10<sup>10</sup> of BS fmjb probiotic in broiler chicken diet can cause significant differences in IgA and IgG, Glutathione, Glutathione reductase, Glutathione

peroxidase, Superoxide dismutase, and Methane dicarboxylic aldehyde but does not have any effect on IgM and Catalase values. In another study which was conducted by Pourakbari et al (2016), it was shown that; blood Albumin ( $\text{g dl}^{-1}$ ), Triglycerides ( $\text{mg dl}^{-1}$ ), total cholesterol ( $\text{mg dl}^{-1}$ ), VLDL, LDL, HDL, Alkaline phosphatase ( $\text{UL}^{-1}$ ), phosphorus ( $\text{mg dl}^{-1}$ ) can affect were affected by dietary probiotic, but there were not any significant differences in blood glucose ( $\text{mg dl}^{-1}$ ), total protein, uric acid ( $\text{mg dl}^{-1}$ ) and calcium ( $\text{mg dl}^{-1}$ ) values. Vahdatpour and Babazadeh (2016) suggested that adding 3, 6, 9, and 12% kefir in quail drinking water has a significant effect on aspartate aminotransferase and alkaline phosphatase but it does not have any effect on alanine transaminase and lactate dehydrogenase means of blood enzymes. Toghyani et al. (2015) investigated that adding different levels of kefir in broiler drinking water have a significant effect on serum total cholesterol, LDL-cholesterol, and HDL-cholesterol but the drinking of water containing kefir could not cause a significant effect on serum protein, albumin, and triglyceride between treatment groups. Wang et al. (2015) stated that there was a significant effect on albumin, alkaline phosphatase, total protein, but there wasn't significant effect on alanine aminotransferase, urea nitrogen, and growth hormone in broiler chickens that was received a diet which was supplemented with 0.07, 0.10, and 13% probiotic. It was demonstrated that fed a diet supplemented with 2.5 and 5 g/kg probiotics by broiler chickens have a significant effect on packed cell volume, hemoglobin, erythrocyte sedimentation rate, and also average content of serum red blood cell also serum glucose, LDL, HDL, and triglycerides was differences between treatment groups (Beski and Al-sardary, 2015). Toghyani et al. (2015) exposed that reduction of serum LDL and increasing HDL cholesterol by addition of molasses kefir might be due to the reduction of synthetic enzyme activities. Can et al. (2012) reported a significant increase in immunoglobulin M level of Çoruh trout which were supplemented with milk kefir. But some experiments have shown different results. For example, consuming different levels (0.5 mg, 3g, 6g, 1g, and 5g) of licorice extract, protein probiotic, toxin binder and poultry litter biochar in broiler chickens diet does not have any effect on blood glucose and HDL levels but values of total protein, triglyceride, cholesterol, LDL, albumin, uric acid, calcium, phosphorus, heterophil,

and lymphocyte can be significantly affected by dietary probiotics (Rashidi et al., 2020). Cho et al. (2013) showed that the average of white blood cell, red blood cell, lymphocyte, and IgG between experiment group those fed diets containing different levels of beta-glucan and kefir was not significant. In some studies it was reported that using different levels of probiotics in broiler chickens diet does not have a significant effect on white blood cell, red blood cell, and lymphocyte (Lan et al., 2017; Gheisar et al., 2016). In another experiment, Cenesiz et al. (2008), reported that different levels of kefir in broiler chickens drinking water could not cause any differences in blood aspartate aminotransferase and alanine transaminase enzymes. Sanders (2000) documented that probiotics have an inhibition effect on hepatic beta hydroxyl-beta-methylglutaryl coenzyme A reductase which is an intermediate of mevalonate during the synthesis of cholesterol from acetyl-Co A.

## 6. Effects of Probiotics on Some Intestinal Morphology:

Feeding of a diet containing 2g/100kg *Lactobacillus acidophilus* does not have a significant effect on muscular wall thickness and crypt depth but has a significant effect on villus height, mucosal layer height, and goblet pas<sup>+</sup> parameters (Forte et al., 2018). Chen et al. (2015) indicated that supplementation of broiler diets with different levels of probiotics has not any effect on villus height, crypt depth, and muscle depth but there was a statistically effect on villi: crypt ratio at the 35 days of the experiment. Wang et al. (2015) reported that there was a significant effect in the duodenum, jejunum, ileum lengths, and weights in the broiler chickens groups that received a diet containing 0.07, 0.10, and 13% probiotic. In the study, it was demonstrated that supplementation of different levels of probiotics can change chicken intestinal T cells, T helper lymphocytes, and cytotoxic T lymphocytes (Bai et al., 2013). Rezanezhad et al. (2013) showed that in the broiler chickens those are fed a diet supplemented with different levels and different probiotics there was a significant effect on villus height, crypt depth, and villus height. Lutfullah et al. (2011) reported that probiotic supplementation increased crypt cell proliferation but not any effect on intestinal weight

and length. In another study which was conducted by Awad et al. (2009), it was found that supplementation of the addition of probiotic to broiler chickens diet increased duodenum villus height and crypt depth ratio. According to Pelicano et al. (2005) feeding of diets that supplemented with probiotic has a significant effect on duodenum, jejunum, and ileum crypt depth but it does not have any effect on villus density. Barton (2000) and Niewold (2007) reported that probiotics play the role of growing promoter by inhibiting the production and excretion of catabolic mediators in intestinal inflammatory cells and subsequent a reduction in intestinal microflora. Also, it was reported that increasing villus height to crypt ratio is directly correlated with an increase of epithelial turnover (Fan et al., 1997) and longer villi are associated with activated cell mitosis (Samanay and Yamuchi, 2002). Also, some studies were conducted to investigating probiotic's effect on intestinal enzymes. For example, using *L. acidophilus* or a mixture of *Lactobacillus* culture in broiler chickens diet for 42 days caused a significant increase in amylase levels (Jin et al., 2000). Also, Collington et al., (1990), stated that using probiotics in piglet diets caused significantly higher carbohydrase enzyme activities in the small intestinal.

## 7. Conclusion

Probiotics have an important role and beneficial effects in poultry health and production. According to several studies, using probiotics in broiler diets improves feed intake, feed conversion ratio, final body weight, carcass parameters, blood parameters, and intestinal morphology. On the other hand, some studies showed that, probiotics do not have any significant effect on feed intake, growth performance. Although different probiotics with different levels have been used in the broiler diets, an accurate dosage of administration has not yet been determined. Therefore, it seems that probiotics can use with different levels in water and diet. In addition, both the positive results obtained and their economic status revealed that the use of certain probiotics will continue in the short and long term.

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