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Correspondence Address (Dergi İçin Yazışma Adresi):

Prof. Dr. Nedim KOŞUM
Journal of Animal Production Editor in Chief
Ege Universty, Faculty of Agriculture, Deperment of Animal Science
35100 Bornova, İzmir-TURKEY

Phone (Tel): +90 (232) 311 2718 (sekreter) **Fax:** +90 (232) 388 1867

E-posta (e-mail): nedim.koşum@ege.edu.tr, cagri.kandemir@ege.edu.tr

Ege Zootekni Derneği Yönetim Adresi:
Fevzipaşa Bulvarı No: 17 Azim Han K:4 D:408 Konak / İZMİR

Basım Yeri : Ege Üniversitesi Rektörlüğü Basımevi Müdürlüğü
No: 172/134 Kampüsiçi / Bornova, İzmir
Tel : 0232 311 18 19
e-mail : bsmmd@mail.ege.edu.tr

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Ahmet Önder ÜSTÜNDAĞ[1]
Mürsel ÖZDOĞAN[1]

Effects of Feed Additives Used As an Alternative to Antibiotics on Mineral Absorption and Bone Characteristics in Poultry: A Review

Antibiyotiklere Alternatif Olarak Kullanılan Yem Katkı Maddelerinin Kanatlılarda Mineral Emilimine ve Kemik Karakteristiklerine Etkileri:Derleme

[1] Adnan Menderes University Faculty of Agriculture Animal Science Department, South Campus Cakmar-Aydin / Türkiye

sorumlu yazar: austundag@adu.edu.tr

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ABSTRACT

Calcium and phosphorus are essential minerals for some important biochemical functions and bone integrity. Deficiency in rations or poor absorption of these minerals is an important problem in poultry nutrition, especially high producing laying hens and broilers, leading to economic losses such as deterioration of eggshell and bone characteristics as a result of mineral metabolism disorders. Therefore, maximization of absorption and bioavailability of these nutrients from the gut can be practical for preventing mineral metabolism disorders. Antibiotics widely used as growth promoters for many years in animal nutrition. After the prohibition of antibiotics, the research for alternatives to antibiotics has accelerated. Probiotics, prebiotics and organic acids are some of these alternatives. Most of the earlier studies showed that probiotics, prebiotics, and organic acids increased the mineral absorption and metabolism. In this review, effects of probiotics, prebiotics and organic acids on mineral utilization and bone characteristics in poultry are deliberated.

Anahtar Kelimeler:

Probiyotik, prebiyotik, organik asit, kanatlı, mineral kullanımı

ÖZ

Kalsiyum ve fosfor çeşitli biyokimyasal olaylar ve kemik bütünlüğü için esansiyel minerallerdir. Bu minerallerin rasyonlardaki yetersizliği veya düşük emilimi, kanatlı beslemede, özellikle de yüksek verim kapasitesine sahip yumurtacı tavuklar ve etlik piliçlerde mineral metabolizmasında meydana gelen aksaklıklar sonucunda yumurta kabuğu kalitesi ve kemik özelliklerinin bozulması gibi büyük ekonomik kayıplara neden olan önemli bir sorundur. Bu nedenle, bu minerallerin bağırsaklardan emiliminin ve biyoyararlılığının en üst düzeye çıkarılması, mineral metabolizması rahatsızlıklarının önlenmesinde pratik olabilir. Antibiyotikler hayvan beslemede uzun yıllardır büyüme destekleyici olarak yaygın şekilde kullanılmıştır. Antibiyotiklerin yasaklanmasından sonra, antibiyotiklere alternatif arayışları hız kazanmıştır. Probiyotikler, prebiyotikler ve organik asitler bu alternatiflerden bazılarıdır. Yapılan birçok çalışmada probiyotiklerin, prebiyotiklerin ve organik asitlerin kanatlılarda mineral emilimini ve metabolizmasını geliştirdiği belirtilmiştir. Bu derlemede, probiyotik, prebiyotik ve organik asitin kanatlılarda mineral emilimi ve kemik gelişimine olan etkileri üzerinde durulacaktır.

INTRODUCTION

Minerals achieve important biological functions in the animal organism. Among the minerals, calcium and phosphorus are very important for nutrition of laying hens and broilers (De Carvalho Mello et al., 2012). Calcium is the most prevalent mineral in the body and 99% is found in the skeleton. The preferential role of calcium provides structural strength of bones. It also plays critical roles in the body such as muscle and nerve conduction, blood coagulation, eggshell calcification and control of hormone secretions such as vitamin D3 and parathyroid hormone. The second most common mineral in the body is phosphorus and 80% is found in bones. It has many important roles such as bone formation, the structure of nucleic acids, phospholipids, cell membranes, acid-base balance, and energy transfer (De Matos, 2008; Wilkinson et al., 2011; De Carvalho Mello et al., 2012). Because of these critical roles of calcium and phosphorus, availability of these minerals is an important problem in poultry nutrition. As a result of deficiency in rations or poor absorption of these minerals, mineral metabolism disorders, leading to economic losses such as deterioration of eggshell and bone characteristics in especially high producing laying hens and broilers, can occur (Świątkiewicz and Arczewska-Wlosek, 2012a). In addition to mineral deficiency, in case of skeletal systems had not kept up with the increased performance that obtained as a result of genetic selection, some adverse effects in support system such as bones, joints, tendons and the connective tissue of the legs and feet can be observed (Dibner et al., 2007). The etiology of bone abnormalities is generally complex and it is not depended on a single factor. Factors affecting the intestinal epithelium, leading to the reduction of nutrient absorption, as well as anti-nutritional factors in the diet, can induce leg disorders caused by nutritional imbalance. Thus genetics, management, nutrition, hygiene, and diseases will influence the occurrence of leg problems under field or experimental conditions (Waldenstedt, 2006; Otutumi et al., 2012). Among these factors, nutrition plays an important role in preventing leg disorders and skeletal problems in the poultry industry. It has been reported that thirteen minerals, six amino acids, eight vitamins and also dietary protein and energy levels may be directly related to these problems. Therefore, maximization of absorption and bioavailability of these nutrients from the gut can be practical for preventing mineral metabolism disorders (Houshmand et al., 2011; Abdelqader et al., 2013). For this purpose, antibiotics have been widely used as growth promoters for more than 50 years in animal nutrition. But, the appearance of antibiotic resistant bacteria and residual antibiotics in meat caused to ban of antibiotics. Ban of antibiotics in 2006 and increasing demand of organic production have increased interest in searching for alternative to antibiotics (Üstündağ and Özdoğan, 2011; İpçak et al., 2017). Probiotics, prebiotics and organic acids, essential oils and plant extracts, bacteriocins, antimicrobial peptides, bacteriophages and feed enzymes take place among these alternatives (Alloui et al, 2013). Many studies have shown that feed additives used as an alternative to antibiotics (probiotics, prebiotics, and organic acids) improve mineral utilization and metabolism, and prevent problems observed in bone characteristics (Houshmand et al., 2011). The aim of this review is to explicate the effects of probiotics, prebiotics and organic acids on mineral utilization and bone characteristics in broiler and laying hens.

Mode of Action of Feed Additives

Probiotics

Probiotics have been defined as live microbial feed supplements that beneficially affect host animals by improving their intestinal microbial balance (Fuller, 1989; Patterson and Burkholder, 2003; Coeuret et al., 2004). Important species commonly used as probiotics are *L. bulgaricus, L. plantarum, L. acidophilus, L. helveticus, L. lactis, L. salivarius, L. casei, Bacillus subtilis, Enterococcus faecium, Streptococcus thermophilus, Enterococcus faecalis, Aspergillus oryzae, Saccharomyces cerevisiae, Bifidobacterium spp.* and *E. coli* (Kabir, 2009; Khan and Naz, 2013).

It has been explained some possible mechanisms of probiotics on mineral utilization and bone characteristics. Synthesis of vitamins like D, C, and K by probiotics is one of these mechanisms. These vitamins are involved in calcium metabolism. Also, probiotics produce short-chain fatty acids (SCFA) which reduce parathyroid hormone followed by an increase in mineral absorption via their solubilization. Another mechanism is producing phytase enzyme by probiotics which can release minerals depressed by phytate, resulting in increased availability of minerals (Parvaneh et al., 2014).

Prebiotics

Prebiotics are defined as non-digestible feedstuffs that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Prebiotics are small fragments of carbohydrates and are commercially available as oligosaccharides of galactose, fructose or mannose. The most commonly used prebiotic preparations are

mannanoligosaccharide (MOS), fructooligosaccharide (FOS), transgalactooligosaccharide (TOS), inulin, glucooligosaccharide, xylooligosaccharide, isomaltooligosaccharide, soybean oligosaccharide, polydextrose and lactosucrose (Ganguly, 2013).

The principal effect of prebiotics is associated with their metabolism of the microbiota. As a result of metabolism of the prebiotics, short chain fatty acids (SCFA) are generated (Gourbeyre et al., 2011; Slavin, 2013). According to Scholz-Ahrens et al. (2001, 2007) absorption surface of the intestinal mucosa is increased by the positive effect of SCFA on the enterocytes proliferation, the increased expression of Ca-binding proteins, the release of bone modulating factors, hydrolysis of phytate complexes. Also, prebiotics enhance the solubility of nutrients through increasing proteolytic enzymes activity and stimulating digestive enzymes activity (Hajati and Rezaei, 2010; Alloui et al., 2013; Dankowiakowska et al., 2013; Slavin, 2013).

Organic acids

Organic acids are considered to be any organic carboxylic acid of the general structure R-COOH (Dibner and Buttin, 2002; Papatsiros and Christodoulopoulos, 2011). Organic acids primarily include the saturated straight-chain monocarboxylic acids and their derivatives (unsaturated, hydroxylic, phenolic, and multi carboxylic versions) and are often generically referred to as fatty acids, volatile fatty acids or carboxylic acids (Ricke, 2003). Formic, propionic, acetic, sorbic, citric, fumaric, malonic and other acids, are widely used in animal nutrition as feed acidifiers (Świątkiewicz and Arczewska-Wlosek, 2012a).

Organic acids have bacteriostatic and bactericidal effects on Gram-negative bacteria, such as *Salmonella spp, E. coli, Clostridia spp, Listeria spp.* and some coliforms. But, only short-chain acids (C1–C7) have antimicrobial effect (Lückstädt and Mellor, 2011; Suryanarayana et al., 2012).

Another beneficial mechanism of organic acids besides of antimicrobial activity is to increase of digestive enzyme activity and solubility of minerals by means of decreasing intestinal pH (Świątkiewicz and Arczewska-Wlosek, 2012a). Also, organic acid anions can increase the digestion of calcium, phosphorus, magnesium, and zinc by forming of these minerals complexes and improve digestibility of protein (Yesilbag and Colpan, 2006; Adil et al., 2010, 2011; Suryanarayana et al., 2012).

Effect of Feed Additives on Mineral Absorption and Bone Characteristics in Laying Hens

A unique bone turnover synchronized with a daily egg-laying cycle in layers that rapid remodeling occurs in laying hen bones. Calcium requirement increases in laying hens during the egg production. Because eggshell contains average 2.5 g calcium. Due to rapid bone turnover and extensive calcium mobilization from bones for eggshell formation, eggshell damage and osteoporosis in laying hens during the egg production are very important problems and these problems have to be concerned (Lukić et al., 2009; Kim et al., 2012). Giving excess calcium to laying hens during egg production may inhibit the availability of other minerals such as phosphorus. Therefore, increasing calcium level in the diet to improve eggshell quality and bone characteristics might not be a practical solution. Alternatively, approaches through which calcium absorption and bioavailability can be maximized from the gut might be a more practical (Driver et al., 2005; Selle et al., 2009; Abdelqader et al., 2013). Świątkiewicz and Arczewska-Wlosek (2012a) reported that feed additives increasing the availability of calcium and other minerals may have a beneficial influence on eggshell and bone quality. Moreover, the results of a study conducted by Youssef et al. (2013) indicated that probiotics, prebiotics, and organic acids increased egg production, egg mass and some egg quality parameters such as shell thickness and yolk color. The results of this study have been shown in Table 1.

Several studies with layers indicated that supplementation of probiotics increased egg production, shell weight, shell thickness, shell breaking strength and serum calcium level (Panda et al., 2003, 2008; Mahdavi et al., 2005; Yousefi and Karkoudi, 2007; Zarei et al., 2011; Mikulski et al., 2012).

Abdelqader et al. (2013) reported that addition of *B.suptilis* at the level of 0.5 g/kg and 1 g/kg into diets of laying hens showed a significant increase in egg production, egg weight, egg mass, eggshell weight, eggshell thickness, eggshell density and some tibia traits such as tibia weight, tibia density, and tibia ash.

Table 1. Effects of dietary treatments on performance and egg quality of laying hens (adapted from Youssef *et al.,* 2013).
Çizelge 1. *Diyetsel muamelelerin yumurtacı tavukların performansı ve yumurta kalitesine etkileri (Youssef ve ark., 2013 den alınmıştır)*

Dietary Treatment	Egg Production, % (eggs/hen/day)	Egg Weight, g	Egg Mass (g/hen/day)	Shell Thickness, mm	Yolk Color
Control	88.50^{b}	57.30	52.90^{c}	0.32^{c}	7.00^{b}
Probiotic (Protexin®)	91.90^{b}	62.00	56.20^{b}	0.38^{a}	7.40^{ab}
Prebiotic (Clostat®)	92.30^{b}	60.80	56.60^{b}	0.37^{ab}	8.00^{a}
Symbiotic (Dimound®)	91.80^{b}	59.60	56.40^{b}	0.37^{ab}	7.80^{a}
Organic Acids (Galliacid®)	97.30^{a}	62.50	60.40^{a}	0.36^{b}	7.80^{a}
Means	92.40	60.40	56.50	0.35	7.60
SE of means	±0.95	±0.85	±0.74	±0.01	±0.12
Significances	*	Ns	**	*	*

$^{a, b}$ Means within each column with no common superscripts are significantly different ($p<0.05$). Ns: Not significant, * $p<0.05$, ** $p<0.01$

Results of a study conducted by Świątkiewicz et al. (2014) showed that addition of 108 cfu/kg probiotic bacteria (L. salivarius) to layer diet containing 200 g/kg DDGS improved the bone breaking strength, yielding load and stiffness of tibia. But, it was reported that DDGS supplementation had no effect on biomechanical indices of the tibia.

There are many studies conducted to investigate the effects of prebiotics on laying performance, eggshell quality and mineral utilization in layers.

Chen et al. (2005) reported that supplementation of 10 g/kg inulin or oligofructose improved egg production and cumulative weekly egg weight. Świątkiewicz et al. (2010a,b) found that addition of prebiotic fructans into the diets with reduced and normal levels of Ca and P increased eggshell percent, density and breaking strength in hens at 46, 58 and 70 weeks of age. Also, they found a positive effect of prebiotic fructans on tibia and femur bone breaking strength in layers at 70 weeks of age. But, there was no interaction between Ca and P levels and prebiotic fructans. Hanafy (2010) reported that laying hens fed with diets including inulin at 1% and 1.5% had a higher egg production, egg weight, eggshell weight, shell thickness, shell Ca and P, serum Ca and P, tibia weight, tibia Ca and P than the control group.

Positive effects of organic acids on egg production, eggshell quality and mineral absorption have been determined by some researchers (Yesilbag and Colpan, 2006; Sengor et al., 2007; Sarı and Kaya, 2017). Soltan (2008) reported that supplementation of a commercial organic acid blend "ProviMax" containing calcium butyrate, calcium propionate, calcium lactate and fumaric acid improved eggshell thickness and serum Ca level.

Effect of Feed Additives on Mineral Absorption and Bone Characteristics in Broilers

Factors that reduce the mineral availability can lead to different bone abnormalities. Bone abnormalities and lameness are considerable problems especially in broilers and turkeys (Kestin et al., 2001; Waldenstedt, 2006; Dibner et al., 2007). These problems have been largely prevented by reducing growth rate. Reducing growth rate is achieved by several restriction techniques. However, reducing growth rate by decreasing feed intake and/or nutrient densities is not easily compatible with welfare demands (Waldenstedt, 2006). Therefore, except for the restriction in fast-growing broiler, leg problems have been tried to prevent by feed additives added to diets.

Ziaie et al. (2011) reported that probiotics, prebiotics, and organic acids had positive effects on morphometric parameters of tibia, Ca and P levels of tibia and serum. The results of this study have been shown in Table 2.

Several researchers reported that supplementation of probiotics in broiler diets positively affected the mineral utilization and bone parameters (Mutus et al., 2006; Abdulwahab and Horniaková, 2010; Hashemzadeh et al., 2013). Akhavan-Salamat et al. (2011) indicated that addition of yeast in diets increases bone calcium values which improves bone force in broilers. Similarly, Gutierrez-Fuentes et al. (2013) reported that broilers fed diet supplemented with a commercial lactic acid bacteria based probiotic FloraMax B-11 had higher bone breaking strength, tibia Ca and P than the control group.

Table 2. Effects of dietary treatments on morphometric parameters of tibia and blood calcium and phosphorus levels of broilers (adapted from Ziaie *et al*., 2011).
Çizelge 2. *Diyetsel muamelelerin broilerlerin tibia morfometrik parametreleri ve kan kalsiyum ve fosfor seviyelerine etkileri (Ziaie ve ark., 2011 den alınmıştır)*

Parameters		Dietary Treatment[1]						
		T1	T2	T3	T4	T5	T6	SE of means
Tibia Weight, g		4.42^{b}	4.10^{a}	5.23^{a}	5.14^{a}	5.09^{a}	5.10^{a}	0.15
Tibia Length, cm		88.19^{b}	91.53^{a}	91.72^{a}	91.02^{a}	91.86^{a}	91.28^{a}	0.98
Tibiotarsi wt/ length index, mg/cm		50.11^{b}	55.76^{a}	56.30^{a}	56.46^{a}	55.47^{a}	55.97^{a}	1.49
Diaphysis diameter, mm		8.05	8.64	8.54	8.46	8.46	8.31	0.24
Thickness of the medial wall, mm		1.41^{b}	1.72^{a}	1.73^{a}	1.71^{a}	1.73^{a}	1.68^{a}	0.084
Thickness of lateral wall, mm		2.53^{b}	2.88^{a}	3.10^{a}	2.91^{a}	2.96^{a}	2.89^{a}	0.084
Medullary canal diameter, mm		5.33^{a}	4.58^{b}	4.57^{b}	4.54^{b}	4.56^{b}	4.62^{b}	0.072
Tibiotarsal index		33.38^{b}	46.72^{a}	46.22^{a}	46.07^{a}	45.92^{a}	44.12^{a}	1.78
	Time (d)							
Blood Ca, mg/dl	21	12.89^{b}	15.37^{a}	12.45^{b}	12.37^{b}	12.55^{b}	13.00^{b}	0.63
	28	13.31^{d}	15.12^{a}	16.00^{a}	13.87^{cd}	13.37^{d}	16.62^{c}	0.38
	35	12.50^{b}	17.00^{a}	16.87^{a}	16.52^{a}	16.62^{a}	16.32^{a}	0.81
	42	13.82^{c}	16.11^{b}	17.63^{a}	16.50^{a}	16.50^{ab}	16.50^{ab}	0.44
Blood P, mg/dl	21	3.46^{b}	5.11^{a}	4.94^{a}	5.00^{a}	4.50^{ab}	4.17^{ab}	0.41
	28	3.17^{b}	4.70^{a}	5.07^{a}	3.12^{b}	3.41^{b}	4.43^{a}	0.24
	35	2.57^{b}	4.12^{a}	3.63^{a}	3.81^{a}	3.83^{a}	3.82^{a}	0.35
	42	2.32^{b}	4.69^{a}	5.15^{a}	4.84^{a}	4.84^{a}	4.43^{a}	0.34

$^{a, b}$ Means with different superscripts within the same row differ significantly (P<0.05).
[1]T1= control, T2= control+15 ppm of Virginiamycin, T3= control+100 mg probiotic Protexin/kg diet, T4= control+100 mg prebiotic Immnuwall/kg diet, T5= control+450 mg commercial herbal blend (Digestrom)/kg diet, T6= control+400 mg of organic acid (Formycine) /kg diet.

A study carried out by Ortiz et al. (2009) showed that inclusion of different levels of inulin positively affected the relative apparent retention coefficients of Ca, Zn and Cu with tibia ash and Ca contents in broilers. However, another study conducted by Świątkiewicz et al. (2011) showed that prebiotics had not any beneficial effects on bone quality. Abdel-Fattah et al. (2008) reported that broilers fed diet containing organic acids had a significantly higher serum Ca and P levels. Adil et al. (2010) noted that lactic, butyric and fumaric acids increased serum Ca and P levels in broilers. It has been pointed out that supplementation of organic acids increases Ca retention in broiler (Świątkiewicz and Arczewska-Wlosek, 2012b). Liem et al. (2008) found that addition of citric, malic or fumaric acid increased tibia mineralization in broilers fed P deficient diet. Similarly, Houshmand et al. (2011) reported that organic acids had a positive effect on tibia characteristics and helped the broiler fed diet containing low Ca to overcome the leg problems related to Ca deficiency.

CONCLUSION

Target is to achieve high and quality yield in broiler or laying hen industries. But, bone abnormalities related to some metabolic disorders can lead to decrease in yields of poultry. In conclusion, results of various studies conducted by researchers showed that feed additives such as probiotics, prebiotics, and organic acids may be effectively used as alternative to antibiotics to increase mineral utilization in bones and to eliminate metabolic disorders in poultry.

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