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Pre-cecal Phosphorus Digestibility of Dicalcium Phosphate and Monocalcium **Phosphate in Broilers**

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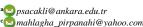
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ARTICLE INFO	ABSTRACT
Research Article	The aim of this study was to determine the precedule phosphorus (P) digestibility of dicalcium phosphate (DCP) and monocalcium phosphate (MCP) for broiler using the linear regression method. A total of 360-d-old male Ross 308 broilers were raised until 20 days of age with a
Received: 17/05/2020 Accepted: 10/06/2020	standard commercial starter diet (metabolizable energy, 3000 kcal/kg, crude protein, 22%, Ca 0.9%, P 0.6%). On 21 days of age all birds were weighed and assigned to 5 experimental treatment groups with 6 replicate cages each consisted of 12 birds. Each P source was included at two levels (to achieve increments of 0.075% and 0.15%) to the basal diet. Titanium dioxide was included at
Keywords: Dicalcium Phosphate Monocalcium Phosphate Prececal digestibility Phosphorus Broiler	0.5% in all diets as indigestible marker. On day 28, all birds were sacrificed and ileal digesta were collected. The prececal P digestibility of DCP and MCP were calculated by the linear regression to be 76.60% and 85.83%, respectively. The present results suggest that the P digestibility of the P sources should be considered when formulating broiler diets to achieve optimum dietary P concentrations without increasing diet cost and environmental pollution from P excretion.

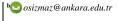
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Dikalsiyum Fosfat ve Monokalsiyum Fosfatın Broylerlerde Presekal Fosfor Sindirilebilirliğinin Belirlenmesi

MAKALE BİLGİSİ	ÖZ
Araştırma Makalesi	Bu çalışma dikalsiyum fosfat (DCP) ve monokalsiyum fosfatın (MCP) broylerde linear regresyon metoduyla presekal fosfor sindirile bilirliğini belirlemek amacıyla gerçekleştirilmiştir. Denemede toplam 360 adet bir günlük yaşta erkek broyler civciv (Ross 308) 21 günlük yaşa kadar standart
Geliş : 17/05/2020 Kabul : 10/06/2020	ticari broyler başlangıç yemi (Metabolize olabilir enerji 3000 kcal/kg, ham protein %22, Ca %0,9 P %0,6) ile beslenmiştir. Broylerler 21 günlük yaşta her biri 6 tekerrürden oluşan ve her tekerrürde 12 civciv bulunan 5 deneme grubuna ayrılmıştır. Fosfor sindirile bilirliği belirlenecek DCP ve MCP bazal rasyona P düzeyini %0,075 ve %0,15 düzeylerinde artıracak oranda ilave edilmiştir. Markır
Anahtar Kelimeler: Dikalsiyum fosfat Monokalsiyum fosfat Presekal sindirilebilirlik Fosfor Broyler	olarak titanyum dioksit %0,5 düzeyinde kullanılmıştır. Denemenin 28. gününde fosfor sindirile bilirliğini belirlemek için bütün hayvanlardan ileum içeriği toplanmıştır. Linear regresyon metoduyla DCP ve MCP'nin presekal fosfor sindirile bilirlikleri sırasıyla %76,60 ve %85,83 olarak hesaplanmıştır. Bu çalışma sonucunda broyler rasyonları formüle edilirken rasyon maliyetini ve çevre kirliliğini artırmadan, optimum fosfor düzeylerinin ayarlanması için fosfor kaynaklarının sindirile bilirliklerinin bilinmesinin önemi vurgulanmıştır.







Introduction

Phosphorus (P) is a biologically essential mineral for poultry nutrition due to its important roles in optimum bone mineralisation, skeletal health and cellular metabolism (Suttle, 2010.). Plant feed ingredients such as cereal grains or meals which are major components used in poultry nutrition are rich in phosphorus. However, availability of phosphorus in plant ingredients is poor because of presenting in the form of phytic acid. Monogastric animals are not able to use more than twothird of plant based phosphorus due to lack of/not enough endogenous phytase activity (Selle and Ravindran, 2007). Therefore, commercially available inorganic phosphate sources such as monocalcium phosphate (MCP), monodicalcium phosphate (MDCP) and dicalcium phosphate (DCP) are widely added to poultry diets to ensure P requirement (Sauvant et al., 2004; Viljoen, 2001). But, inorganic phosphorus sources (phosphate rock) is a non-renewable resource (Leske and Coon, 2002). As world population grow drastically, phosphorus demand will increase due to the need to produce more animal protein sources.

Among of the different inorganic P sources, DCP and MCP are the most common used ingredients in poultry diets. The availability of P sources has been shown vary according to their source, molecular structure, age and species of the animals, diets composition such as Ca level, phytate in the plant ingredients etc. (Ammerman, 1995; Axe, 1998). In addition there is important differences with regard to P digestibility of ingredients according to various approach used to determine P digestibility. These differences may cause excessive or inadequate formulation of P level in the diets. Low P levels can depress growth performance of animals or high P levels can cause environmental pollution due to excessive P excreation (Lima, et al., 1997). Both economically and environmentally, optimum P formulation is very important for poultry diets. This requires adequate knowledge in the P digestibility and availability of different P ingredients.

In this study our aim was to determine preceal phosphorus (pcP) digestibility of P from two different inorganic P sources (DCP and MCP) according to currently considered as the preferable method which is developed the protocol by the (WPSA, 2013) for the determination of P digestibility.

Materials and Methods

Birds and Management: The experiment was conducted under the guidelines for animal care and use of Ankara University Ethic Commision (2019-19-179). A total of 360-d-old male Ross 308 broilers were obtained from a commercial hatchery (Beypiliç AŞ). The birds raised in floor pens (200×100 cm) with wood shavings. Until 20 days of age the birds received a commercial starter diet (metabolizable energy, 3000 kcal/kg, crude protein, 22%, Ca 0.9%, P 0.6%). Feed and water were provided ad libitum. On 21 days of age birds were weighed and 360 birds assigned to 5 treatment groups

with 6 replicate cages each consisted of 12 birds. Temperature was set at 34°C at d 1 and every week 3 or 4°C gradually reduced to 21°C at d 21 and after which it remained constant. Lighting schedule was 24 L:0D and controlled during the experimental period. Feed and water were provided ad libitum during the complete period of the experiment.

Experimental diets: Dicalcium phosphate (DCP) and Monocalcium phosphate (MCP) (Greenphos DCP and Greenphos MCP BAF Premiks ve Tarım Sanayi ve Dış Ticaret Ltd. Şti.- Turkey) were used in the study. Main ingredients of experimental diets consist of corn, soybean meal, corn starch and dry egg albumen to achieve a low P concentration and low intrinsic phytase activity.

DCP and MCP as the test ingredients were included at 2 levels (4.2 and 8.3 g/kg; 3.3 and 6.5 g/kg, respectively) into the basal diet to achieve of P content from test ingredients as 0.075 and 0.15% (Table 1).

Titanium dioxide (0.5%) was added as an indigestible marker. Experimental diets were pelleted with 3 mm diameter without steam. The study followed the protocol developed by the (WPSA, 2013) for the determination of P digestibility.

Data Collection

Body weight gain (BWG) and feed intake (FI) were measured at 21 and 28 days of age. On day 28, all birds were sacrified. The ileum part between Meckel's diverticulum and 2 cm prior to the ileo-ceca-colonic junction dissected. The terminal 2/3 of the section were obtained for digesta collection and this ileal contents were flushed with distilled water, pooled from all birds within a cage, immediately fozen at -20°C, and later digesta samples dried and ground.

Physical and Chemical Analysis

Samples of diet and ileal digesta were analysed for DM, Ca, total P and titanium. DM was determined by drying samples at 105°C, for 8 h in an oven (AOAC, 2012). Ash was determined at 550°C for 6 h in a furnace (AOAC, 2012). Ca and P concentrations were determined by spectrophotometric method. Titanium was determined by the colorimetric method as described by (Brandt and Allam, 1987).

Calculations

The prececal P digestibility of test ingredients were calculated according to the procedures described by (WPSA, 2013). The ileal P digestibility (Pd) was calculated for each diet and replicate cage using the following equation:

$$Pd(\%)=100-[100\times(TiO_{2 Diet} P_{Dig})/(TiO_{2 Dig}\times P_{Diet})]$$

Where, TiO₂ Diet, TiO₂ Digesta is the marker concentration in the diet and ileal digesta samples, PDigesta (P Dig), P Diet is the P concentration in the diet and ileal digesta samples. All analysed values were expressed as grams per kilogram of DM.

Statistical Analysis

The performance data (all dietary treatments) and P digestibility of experimental diets (each test ingredient) were analysed using analysis of variation. Significant differences among treatment groups were compared using the Duncan's new multiple range test function of SPSS (2016).

Mortality rates were compared using a chisquared test. Statistical differences were considered significant at (P<0.05). P digestibility of each test ingredient was calculated as described by (WPSA, 2013), using the linear regression model.

Table 1. Ingredient and nutrient composition (g/kg as Fed Basis) of experimental diets

Ingredients	Basal diet	DCP 0.075%	5% DCP 0.15% MCP 0.075%		MCP 0.15%				
Corn	510	510	510	510	510				
Soybean meal	200	200	200	200	200				
Corn starch	142	142	141.2	142	141.5				
Dried egg albumen	100	100	100	100	100				
Soy oil	10	10	10	10	10				
Vitamin Mineralmix*	5.0	5.0	5.0	5.0	5.0				
Choline chloride	0.8	0.8	0.8	0.8	0.8				
Anticoccidial	0.5	0.5	0.5	0.5	0.5				
NaCl	3.5	3.5	3.5	3.5	3.5				
Lysine	3.4	3.4	3.4	3.4	3.4				
Methionine	1.8	1.8	1.8	1.8	1.8				
Threonine	1.0	1.0	1.0	1.0	1.0				
Titanium dioxide	5.0	5.0	5.0	5.0	5.0				
Limestone	10	9.5	9.5	9.5	11				
DCP	0	4.2	8.3	0	0				
MCP	0	0	0	3.3	6.5				
Silica	7	3.3	0	4.2	0				
Total	1000	1000	1000	1000	1000				
Nutrients calculated									
Crude protein, %	21.5	21.5	21.5	21.5	21.5				
ME,kcal/kg	3183	3183	3183	3183	3183				
Ca, %	0.36	0.44	0.54	0.40	0.53				
tP, %	0.27	0.35	0.42	0.34	0.42				

Content of vitamin and mineral premix provided per kg of diet: vitamin A, 15000 IU; vitamin D3, 5000 IU; vitamin, 50 mg; vitamin K3, 10 mg; vitamin B1, 4 mg; vitamin B2, 8 mg; vitamin B6, 5mg; vitamin B12, 0.025mg; niacin, 50 mg; pantothenic acid, 20 mg; folic acid, 20 mg; biotin, 0.25 mg; manganese, 100 mg; zinc, 150 mg; iron, 100 mg; cupper, 20 mg; iodine, 1.5 mg; cobalt, 0.5 mg; selenium, 0.2 mg; molybdenum, 1mg; magnesium, 50 mg.

Table 2. Growth performance and precedul phosphorus digestibility of greenphos dcp and mcp of birds fed experimental diets, 21-28 days of age (n=12 Broilers Per Treatment)

Parameters	Basal diet	DCP 0.075%	DCP 0.15%	MCP 0.075%	MCP 0.15%	SEM	P-value
Body weight, d 21 (g)	1040	1012	1038	1010	1011	18.85	0.974
Body weight, d 28 (g)	1371	1509	1576	1515	1566	24.95	0.057
Body weight gain, 21-28 d (g)	330 ^b	497^{a}	538 ^a	488a	555a	17.80	< 0.001
Feed intake 21-28 d (g)	480 ^b	665 ^a	728^{a}	680^{a}	756 ^a	22.55	< 0.001
FCR, Feed intake/weight gain	1.461	1.340	1.360	1.352	1.374	0.027	0.669
Mortality %	0	0	1.4	0	1.4		0.555
tP, g/kg DM	2.37	2.87	3.65	3.20	4.30		
P digestibility*, %	67.51	76.65	71.50	87.5	76.74	_	-
pcP digestibility**, g/kg	1.60	2.20	2.61	2.80	3.30	-	-

DCP: Dicalcium phosphate, MCP: Monocalcium phosphate, FCR: Feed conversion ratio, tP: Total phosphorus, pcP: Precedal phosphorus digestibility, *Phosphorus digestibility values were obtained from regression analysis (Figure 1 and 2) and **pcdP values of the diet were calculated according to this P digestibility values

Results and Discussion

Nowadays, economical and ecofriendly livestock production issues become more important than in the past. Phosphorus is the ingredient focused on in this issue. Therefore, ensuring optimum P level in broiler diets is big challenge for the industry. Many studies (Leske and Coon, 2002; Waldroup, 1999; Yan and Waldroup, 2006) demostrates that (NRC, 1994) recommendations for P requirement of broilers is much higher than actual need of the animal. The greatest challenge in optimizing P level is

the digestibility and bioavailability of different inorganic P sources. There are some measurements of bioavailability of P such as weight gain, blood parameters, tibia breaking strength, tibia and toe ash. All these parameters have been used for bioassays to measure the in vivo bioavailability of this mineral.

The results of the present study showed that the body weight gain and total feed intake during 21-28 days were significantly higher for birds fed with the test diets

compared to the basal diet. Body weight and FCR were improved by the addition of inorganic P sources but, there is no statistical difference among the groups (Table 2). During the experimental period only 2 birds died one of from DCP and one of from MCP groups. As seen in Table 2 the total P concentration had a significant (P<0.001) effect on BWG and FI during 21-28d of age. Birds fed with the diet having the lowest precedal digestible P (pcdP; g/kg) had lower BWG at d 28 and lower FI compared to birds fed with pcdP concentrations ranging from 1.60 to 3.30 g/kg. No differences in BW and FCR were observed between the diets with different pcdP concentrations. Smilarly, (Leske and Coon, 2002; Yan and Waldroup; 2006; Mello et al., 2012; Bikker et al., 2016) found that negative effect of the diets containing low P levels on growth performance. There was no differences between P sources (either DCP or MCP). Main effect resulted from P concentration. However, pcP digestibility of MCP was higher than that of DCP. Chemical composition of the P sources caused this difference.

In our study pcP digestibilities of DCP and MCP were found as 76.60% and 85.83%, respectively. These results higher than those of the some literatures (Shastak et al., 2012; Van der Klis and Versteegh, 1992) for DCP and MCP (Rodehutscord et al., 2012).

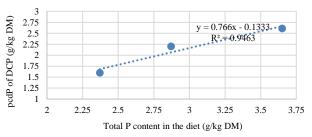


Figure 1. Pre-cecal Phosphorus Digestibility of DCP

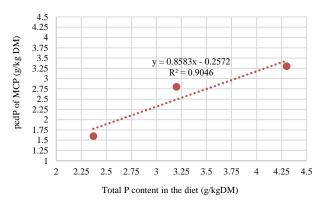


Figure 2. Pre-cecal Phosphorus Digestibility of MC

Ketels and De Groote, (1988) reported pcP digestibility of anhydrose DCP and DCP + H2O to be 67% and 73%, respectively. DCP used in our experiment was hydrate DCP and the P digestibility was similar. (Van der Klis and Versteegh, 1996) showed P retention levels of 92% for MCP + H2O and 55% for anhydrose DCP. These values were lower than our result for DCP and higher for MCP. There are highly differences on P

digestibility of MCP in the literature with 45% to 88% (Bikker et al., 2016; Rodehutscord et al., 2012; Van Harn et al; 2017) and DCP with 25% to 82.4% (Bikker et al., 2016; Shastak et al., 2012) These discrepancies between digestibility values might be resulted from differences in methodologies, animal age, basal diet, source of P, diet Ca, P level and Ca:P ratio. It is known that P digestibility from hydrose sources of DCP or MCP higher than that of anhydrose forms (De Groote and Lippens, 2002).

Another factor that affect P digestibility is particle size of phosphate source. Phosphate sources with larger particle size have more biologic availability because of longer retaining in the gizzard to be complete digestion.

Conclusion

As a conclusion, P level and bioavailability have important effect on growth performance, diet cost and environmental pollution. There are big differences in P digestibility values. While comparing phosphorus sources and including to the broiler diets digestibility values and the methods that used for assessment should be considered.

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