Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers

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ABSTRACT A broiler study was performed to determine the pre-cecal phosphorus (P) digestibility of 5 P sources, 3 from animal (Delfos, Calfos, and porcine bone meal) and 2 of inorganic (monocalcium phosphate [MCP] and dicalcium phosphate [DCP]) origin. Delfos is processed from bones resulting in a dicalcium phosphate product, and Calfos is processed from bones in which part of the gelatin is removed but in which the hydroxy-apatite matrix is preserved. During the first 14 d, birds were housed in floor pens bedded with wood shavings and received a commercial starter diet. At d 14, broilers were randomly assigned to pens (0.9 m^2, 10 birds/pen) with a slatted floor. From d 14 onwards, one of the 6 experimental diets (a basal diet, and 5 diets containing the P sources) was provided. Test diets were replicated 6 times, and the basal diet 8 times. Electron microscopy images of test products were made in order to verify whether the spatial structure of the test products could be related to the pre-cecal P digestibility of the same products. Diets met or exceeded CVB (2011) requirements for all nutrients except for P and were formulated to contain a calcium to total P ratio of between 1.4 and 1.6 and a minimal amount of phytate P. Diets contained 5 g/kg titanium oxide as a marker to determine digestibility of P. At d 24 all birds were euthanized, after which the content of the terminal part of the ileum was sampled. The P digestibility was calculated by linear regression according to World's Poultry Science Association (WPSA) protocol for determination of pre-cecal P digestibility. Pre-cecal P digestibility of MCP, DCP, Delfos, Calfos, and porcine bone meal was 88.5, 82.4, 94.5, 86.9, and 78.2%, respectively. Based on visual inspection of electron microscopy images of test products, the spatial structure of the test products might be related to P digestibility. It is concluded that processing of bone meal increases the pre-cecal P digestibility in broilers.

Key words: broilers, phosphorus, bone meal, digestibility

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INTRODUCTION

Phosphorus (**P**) and calcium (**Ca**) are essential minerals for poultry and play key roles in cellular metabolism, cellular regulatory mechanisms, and in bone mineralization (Suttle, 2010). In most poultry diets, a source rich in P is included to fulfill the P requirement of the animals. Most often this happens by inclusion of rock phosphate sources, but inclusion of P from animal origin such as bone meal happens as well. In general, digestibility of P from animal origin such as bone meal, meat and bone meal, meat meal, and fish meal is lower than the digestibility of P coming from inorganic phosphates, and is more variable. However, chemical and thermo-physical processing of bones may increase the digestibility of P and Ca. In this study, the pre-cecal P digestibility of 3 commercial P and Ca containing products of animal origin, that differed from each other with respect to chemical and thermo-physical processing, were compared with the pre-cecal P digestibility of the inorganic P sources monohydrate monocalcium phosphate (**MCP**) and anhydrate dicalcium phosphate (**DCP**).

The P digestibility was determined by use of a pre-cecal method, largely following the World's Poultry Science Association (**WPSA**) protocol (Rodehutscord, 2013). A pre-cecal method for determining the P digestibility of the test products was chosen, as it is more closely related to the true digestibility of P compared to a method based on the difference in P ingested and P excreted that cannot distinguish between undigested dietary P in excreta and digested but unutilized P that is excreted via the urine (Rodehutscord et al., 2012).

Electron microscopy images of test products were made to check whether the spatial structure of the

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Table 1. Analyzed dry matter, ash, calcium and phosphorus, and calculated crude protein content (g/kg) of test products.

	Delfos[1]	Calfos[2]	Bone meal[3]	MCP[4]	DCP[5]
Dry matter	922	967	936	983	980
Ash	720	788	565	806	892
Ca	234	308	222	182	275
P	168	140	105	220	190
CP[6]	0	100	350	0	0

[1]Delfos is a dihydrate dicalciumphosphate and produced from bones that were crushed, degreased, and then soaked during 5 d in a hydrochloric solution to dissolve the gelatin and bone phosphate. All of the gelatin was separated and the remaining phosphate was precipitated by addition of calcium hydroxide.

[2]Calfos is a degelatinized bone meal and produced from porcine bones that were crushed, degreased, and afterwards pressure cooked to remove most of the gelatin and then finely ground. The phosphor is present in the form of hydroxy-apatite.

[3]Bone meal is produced from porcine bones that are crushed, degreased, dried at 125°C, and then finely ground.

[4]Monohydrate monocalcium phosphate.

[5]Anhydrate dicalcium phosphate.

[6]Crude protein (CP) values as provided in the product description of supplier.

products could be related to the pre-cecal P digestibility of the corresponding products.

MATERIALS AND METHODS

The experiment was approved by the Ethical Commission of Wageningen UR, the Netherlands.

Test products, Diets

The first test product (Delfos; Rousselot, Isle-sur-la-Sorgue, France) was a dicalcium phosphate dihydrate produced from bones that were crushed, degreased, and soaked during 5 d in a hydro-chloric solution to dissolve the gelatin and bone phosphate. The gelatin was completely separated and the remaining phosphate precipitated by addition of calcium hydroxide (e.g., lime). The second test product (Calfos; Sonac Vuren BV, Vuren, the Netherlands) was a degelatinized bone meal, produced from porcine bones that were crushed, degreased, and afterwards pressure cooked to remove most of the gelatin, and then finely ground (with the P being present in the form of hydroxy-apatite). The third test product (pork bone meal, Sonac Vuren BV, Vuren, the Netherlands) was a bone meal produced from porcine bones that were crushed, degreased, dried at 125°C and then finely ground. The fourth and fifth test products were rock phosphates, namely, a monohydrate monocalcium phosphate (MCP; Aliphos, Tessenderlo Chemie S.A., Brussels, Belgium) and an anhydrate dicalcium phosphate (DCP; Windmill Dicalphos, Tessenderlo Chemie S.A., Brussels, Belgium). These test products were evaluated on pre-cecal digestibility of P (pcdP%; %). The analyzed composition of these test products with respect to dry matter, ash, Ca and P is shown in Table 1.

The composition of the test diets is reported in Table 2. Diets containing test products were formulated to contain less than 3 g/kg available P according to the CVB table (2011), and to contain a Ca:total P ratio of 1.4 (except for Calfos and bone meal that were calculated to have Ca: total P ratios of 1.58 and 1.49, respectively). Limestone content was used to obtain the desired Ca:total P ratio.

Birds and Management

For this study, 402 one-day-old male Ross 308 broilers were obtained from a commercial hatchery. Upon arrival the birds were vaccinated against IB and NCD, weighed, and group housed in pens of 5 m^2 on wood shavings (18 birds per m^2). During the first 14 d the birds received a commercial starter diet. At d 14 birds were individually weighed and distributed over 38 pens with flexible plastic slatted floors (Jansen Poultry Equipment, Barneveld, the Netherlands) in groups of 10 birds per pen resulting in 8 replicates for the basal diet and 6 replicates for the test diets. From d 14 until the end of the experiment at d 24, the birds received the experimental diets. Temperature was set at 34°C at d one, after which it was gradually reduced to 21°C at d 24. Lighting schedule was 24L:0D during d one and 2, 18L:6D between d 3 and 21, and 24L:0D between d 21 and 24 in order to ensure a steady-state situation with a homogenous distribution of feed intake during the last d of the experiment. Feed and water were provided ad libitum during the complete period of the experiment.

Sample Collection and Measurements

At d 14 and 24, animals were individually weighed and the total body weight per pen was determined. Cumulative feed intake was recorded during d 14 to 24, as the difference between the quantity of feed provided from d 14 until d 24 and corrected for the quantity of remaining feed at d 24. At d 24, birds were anesthetized with one mL/kg BW of a 5:3 mixture of Sedamun (Dechra Veterinary Products BV, Den Bosch, the Netherlands) and Ketamine (Alfasan Diergeneesmiddelen BV, Woerden, the Netherlands) via injection in the breast muscle and subsequently after 20 to 30 min euthanized with an injection of 0.5 mL of T61 (MSD Animal Health, Boxmeer, the Netherlands) in the wing artery. After euthanization, the birds were dissected and the posterior third (length approx. 10 to 12 cm) of the small intestinal section between Meckel's diverticulum and 2 cm before the junction to the ceca was removed and emptied by flushing the separated gut segment with distilled water. After collection, the samples were pooled per pen and immediately frozen and stored at −20 °C pending analysis.

Chemical Analyses, Electron Microscopy, and Calculations

Test products were analyzed for P and Ca (ISO 11885, 1998b) and crude ash (ISO 5984, 2002). Diets were analyzed for DM (ISO 6496, 1998a), crude ash

Table 2. Content of ingredients and nutrients in diets (in g/kg as fed unless otherwise indicated). Concentrations of available P (aP; g/kg as fed) based on fecal digestibility experiments were obtained from CVB table (2011).

	Basal	Delfos	Calfos	Bone meal	MCP	DCP
Ingredients						
Maize starch, gelatinized	220	220	220	220	220	220
Maize starch, native	168	168	168	168	168	168
Soybean meal CF < 45, CP < 480	110	110	110	110	110	110
Egg white powder	100	100	100	100	100	100
Acid Casein	100	100	100	100	100	100
Sucrose	100	100	100	100	100	100
Oat hulls	70	70	70	70	70	70
Diamol[1]	70	57	58	55	58	59
Soya oil	40	40	40	40	40	40
Premix[2]	5.0	5.0	5.0	5.0	5.0	5.0
Titanium oxide	5.0	5.0	5.0	5.0	5.0	5.0
Limestone	3.3	2.8	0.0	0.0	6.2	2.6
Monocalcium phosphate	1.7	1.7	1.7	1.7	1.7	1.7
Potassium carbonate	3.6	3.6	3.6	3.4	3.6	3.6
Sodium bicarbonate	1.0	1.0	0.6	0.0	1.0	1.0
Magnesium oxide	1.0	1.0	0.8	0.9	1.0	1.0
Sodium chloride	0.2	0.1	0.2	0.0	0.1	0.2
L-Arginine	0.9	0.9	0.8	0.5	0.9	0.9
Delfos	0.0	12.7	0.0	0.0	0.0	0.0
Calfos	0.0	0.0	15.6	0.0	0.0	0.0
Bone meal	0.0	0.0	0.0	20.0	0.0	0.0
MCP	0.0	0.0	0.0	0.0	9.6	0.0
DCP	0.0	0.0	0.0	0.0	0.0	11.2
Nutrients calculated						
aP	1.00	2.23	2.23	2.22	2.70	2.56
Crude protein	228	228	230	234	228	228
Crude fiber	25	25	25	25	25	25
Crude fat	45	45	46	46	45	45
Phytate P	0.61	0.61	0.61	0.61	0.61	0.61
ME (MJ/kg)	12.7	12.7	12.8	12.8	12.7	12.7
Nutrients analyzed						
Dry matter	896	894	895	893	894	895
Ash	88	86	87	82	86	89
Crude protein	228	227	226	228	228	224
Ca	2.5	5.5	5.6	5.2	5.0	5.1
Phosphorus, total	2.3	4.8	4.5	4.3	4.5	4.5
Ca: P	1.1	1.1	1.3	1.2	1.1	1.1
Ti	2.7	3.0	3.1	2.9	3.0	3.1

[1]Diamol is a natural product made from diatoms (group of algae) and consists mainly of silica: 94 to 97% ash, 3 to 6% moisture, 74.0% SiO_2.

[2]Composition of premix provided per kg of diet: 12,000 IU vitamin A (source of vitamin A), 2,400 IU vitamin D3, 30 IU vitamin E (source of vitamin E), 1.5 mg vitamin K3, 2 mg vitamin B1, 7.5 mg vitamin B2, 10 mg d-pantothenic acid, 35 mg niacin amide, 200 μg biotin, 20 μg vitamin B12, 1 mg folic acid, 3.5 mg vitamin B6, 461 mg choline chloride, 80 mg Fe (as $FeSO_4 \bullet H_2O$), 12 mg Cu (as $CuSO_4 \bullet 5H_2O$), 60 mg Zn (as $ZnSO_4 \bullet H_2O$), 85 mg Mn (as MnO), 0.4 mg Co(as $CoSO_4 \bullet 7H_2O$), 0.8 mg I (as KI), 0.1 mg Se (as $Na_2SeO_3 \bullet 5H_2O$) and 50 mg anti-oxidant.

(ISO 5984, 2002), crude protein (ISO 5983, 1997) Ca and P (ISO 11885, 1998b), and Ti (Van Bussel et al., 2010). Ileal digesta was analyzed for DM (ISO 6496, 1998a), crude ash (ISO 5984, 2002), Ca and P (ISO 11885, 1998b), and Ti (Van Bussel et al., 2010).

Scanning electron microscopy (**SEM**) images (Wageningen Electron Microscopy Center, the Netherlands) of test products were made at a magnifications of 35,000 times. The order of magnification was chosen such as to result in an optimal view of the spatial structure of all test products.

The pre-cecal digestibility of P, Ca, DM, ash, and organic matter was calculated as:

$$Y(\%) = 100 - [100 \times (Ti_{diet} \times Nutrient_{digesta}) / (Ti_{digesta} \times Nutrient_{diet})]$$

where Ti_{diet} and $Ti_{digesta}$ are the analyzed concentrations of Ti in the diet and digesta (g/kg DM), respectively, and where $Nutrient_{diet}$ and $Nutrient_{digesta}$ are the analyzed concentrations of nutrients in the diet and digesta, respectively.

Statistical Analysis

The pen was the experimental unit and statistical analyses were carried out using Genstat (17th edition, VSN International LTD, Hemel Hempstead, UK). Performance characteristics and digestibility values of diets were analyzed using ANOVA with row in the experimental facility included as the blocking factor next to diet as the explanatory variable. Differences between treatments were analyzed using Fisher's Least

Table 3. Performance of broilers during the experimental period d 14 until d 24.

	Basal diet	Delfos	Calfos	Bone meal	MCP	DCP	P	LSD
BW d 14 (kg)	0.471	0.472	0.472	0.471	0.468	0.475	0.660	0.009
BW d 24 (kg)	1.134[b]	1.284[a]	1.266[a]	1.292[a]	1.280[a]	1.261[a]	<.001	0.051
Feed intake (kg)	0.870[b]	1.085[a]	1.063[a]	1.075[a]	1.089[a]	1.063[a]	<.001	0.044
FCR	1.311	1.339	1.341	1.311	1.344	1.354	0.155	0.044
Mortality (%)	0.0	1.7	1.7	3.3	0.0	0.0	0.174	3.3

[a,b]Means within a row lacking a common superscript differ significantly ($P \leq 0.05$).

Table 4. Pre-cecal diet digestibility (%) of DM, CP, ash, Ca, and P of male broilers at d 24 of the experimental period as estimated by the difference method.

	Basal diet	Delfos	Calfos	Bone meal	MCP	DCP	P	LSD
DM	91.7	92.8	91.7	92.8	92.6	92.2	0.090	1.0
OM	94.2	94.8	94.0	94.7	94.6	94.5	0.346	0.8
Ash	68.1[b]	73.8[a]	70.3[b]	73.7[a]	73.6[a,b]	71.8[a,b]	0.007	3.4
P	75.2[d]	85.3[a]	80.8[b]	76.5[c,d]	81.7[b]	78.6[b–d]	<.001	3.2
Ca	70.0[b]	79.9[a]	70.3[b]	67.4[b]	69.7[b]	69.8[b]	0.002	5.6

[a–d]Means within a row lacking a common lowercase superscript differ significantly ($P \leq 0.05$).

Significant Difference (**LSD**) only in case the treatment effect was significant ($P \leq 0.05$). Digestibility of P for each test product was estimated using 2 methods. The first method was used as described in the WPSA protocol (Rodehutscord, 2013) using a multiple linear regression model including a common intercept value for all products and estimating regression coefficients of digestibility for each of the products tested simultaneously. In deviation of the WPSA-protocol, the estimation of the digestibility of P was based on the inclusion of one rather than 2 inclusion levels of each test product. The second method used to estimate digestibility of P of test products was based on the difference method as explained by Khan et al. (2003) based on the difference in P digestibility between basal diet and test diets, making it possible to statistically test for significances of differences in digestibility between test products.

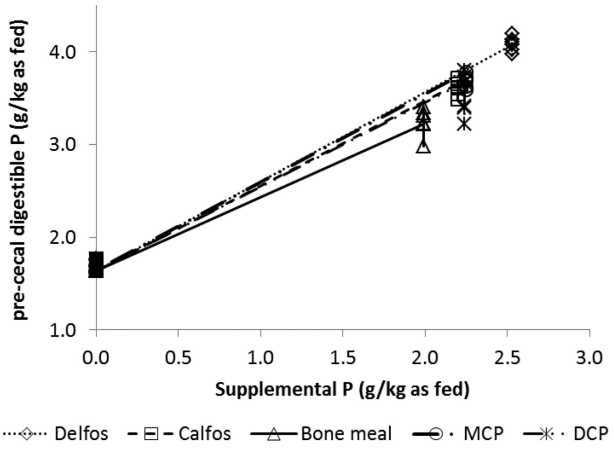

Figure 1. Relationship between pre-cecal digestible P and supplemental P as provided by the various test products above the basal ration using the WPSA method. Pre-cecal digestible P = 1.69 ± 0.047 + 0.945 ± 0.0272 × Delfos + 0.869 ± 0.0313 × Calfos + 0.782 ± 0.0346 × bone meal + 0.885 ± 0.0306 × MCP + 0.824 ± 0.0308 × DCP.

RESULTS

The measured Ca: total P ratios of all test diets ranged from 1.1 to 1.3 and was lower than the planned ratio of 1.4. Performance results of birds is reported in Table 3. The BW at d 24 and total feed intake from d 14 until 24 was significantly lower for birds receiving the basal diet compared to the other diets. Except for the basal diet, no significant differences in performance parameters between diets were observed.

In Table 4, the pre-cecal digestibility values of DM, CP, ash, P, and Ca of the test diets are given. The pre-cecal digestibility of ash for the Delfos and bone meal diet was significantly higher than for the basal diet and the Calfos diet. The pcdP% was significantly higher for the Delfos diet compared to all other diets. The pcdP% of the MCP and Calfos diets was significantly higher than the bone meal diet and the basal diet. The pre-cecal digestibility of Ca (pcdCa%; %) was significantly higher for the Delfos diet compared to all other diets, whereas there were no significant differences in pcdCa% between the other diets.

In Figure 1 the relationship between pre-cecal digestible P and supplemental P is given as provided by the various test products above the basal ration. The estimated pre-cecal digestibility coefficients are shown in the text under Figure 1 and were estimated following the WPSA 2013 protocol.

In Table 5, the pre-cecal digestibility of P of the test products are reported as calculated from the difference in digestibility between the basal diet and the test diets. The pcdP% of Delfos was significantly higher than that of Calfos, DCP, and bone meal, whereas there was no significant difference in pcdP% between Delfos and MCP. The pcdP% of Calfos and MCP was significantly higher than that of bone meal, whereas there was no significant difference between DCP and bone meal.

Table 5. Pre-cecal P digestibility (%) of the test products in male broilers at d 24 of the experimental period as estimated by the difference method.

	Delfos	Calfos	Bone meal	MCP	DCP	P	LSD
P	94.5[a]	86.9[b]	78.2[c]	88.5[a,b]	82.4[b,c]	<.001	6.9

[a–c]Means within a row lacking a common superscript differ significantly ($P \leq 0.05$).

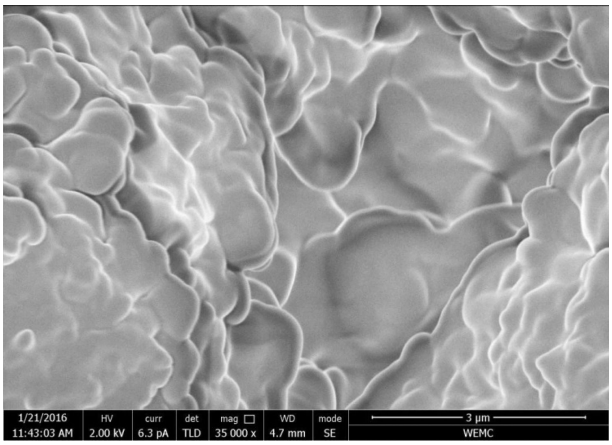

Figure 2. Scanning electron microscopy image of the test product bone meal at a magnification of 35,000 times.

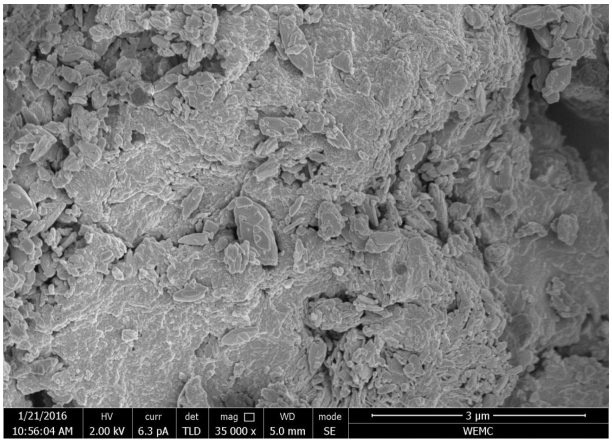

Figure 3. Scanning electron microscopy image of the test product DCP at a magnification of 35,000 times.

In Figures 2 through 6 the SEM images of the test products are shown in increasing order of pcdP%. Visual inspection of the images suggest that the surface area of bone meal was lowest compared to the other test products, whereas the test product Delfos seems to have the highest surface area of all test products.

DISCUSSION

As expected, the performance results of birds receiving the basal treatment were less than the other treatments due to a deficiency of digestible P. Although there were differences in the percentages and quantities of absorbed P for the 5 test diets (basal diet not included), there was no relationship between the quantity of absorbed P and growth or feed conversion,

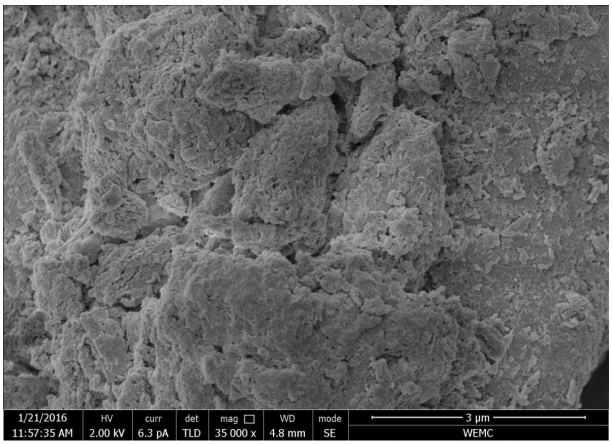

Figure 4. Scanning electron microscopy image of the test product Calfos at a magnification of 35,000 times.

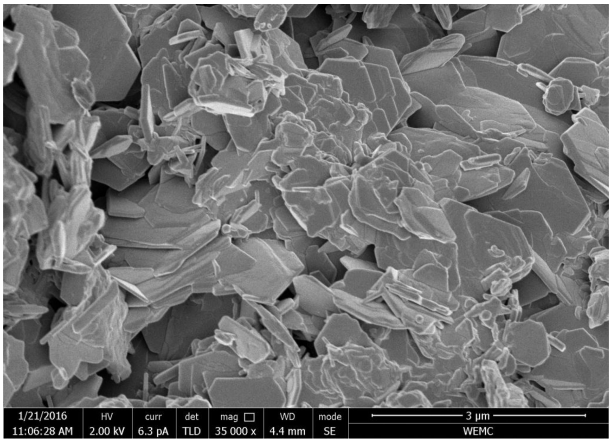

Figure 5. Scanning electron microscopy image of the test product MCP at a magnification of 35,000 times.

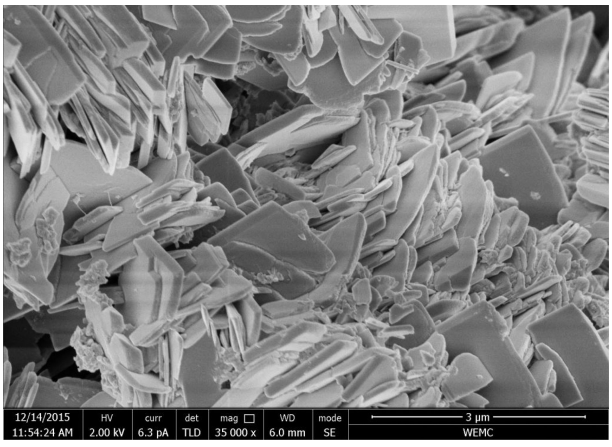

Figure 6. Scanning electron microscopy image of the test product Delfos at a magnification of 35,000 times.

indicating that the level of pre-cecal absorbable P varying from (3.3 g/kg for bone meal to 4.1 g/kg for Delfos) was not limiting growth of the birds fed one of the 5 test diets.

In general, high digestibility values of P were obtained compared to other studies. The reference product MCP had a pcdP% of 88.5 and was higher than results obtained by Simons et al. (1991) who observed total tract P digestibility values of MCP varying from 86 to 80% measured at a low average available P level of 1.8 g/kg and much higher than the pcdP% of MCP of 42 to 48% measured by Rodehutscord et al. (2012). The pcdP% of the reference product anhydrate DCP of 82.4% was substantially higher than results obtained by Van der Klis and Versteegh (1992) and Van der Klis and Versteegh (1993) who measured total tract P digestibility values of anhydrate DCP of 53.0 and 56.9%, respectively, using low average available P levels of less than 1.8 /kg. As well, lower anhydrate DCP pcdP% levels were observed by Shastak et al. (2012) of 25 to 30%. The pcdP% of bone meal of 78.2% observed in this study was comparable to the total tract P digestibility of bone meal of 79.3% observed in the study of Simons et al. (1991). However, it is substantially higher than the total tract P digestibility of bone meal observed in the study of Van der Klis and Versteegh (1992) of 63.5%. The pcdP% of bone meal observed in this study is also substantially higher compared to the determined P digestibility values of meat and bone meal in the study of Mutucumarana et al. (2014). These authors observed an average pcdP% digestibility of 57%. Van der Klis and Versteegh (1992) observed a total tract P digestibility of 68.5 and Simons et al. (1991) observed a total tract P digestibility of 49.8%. An explanation for the high pcdP% values observed in the current study might be the low average Ca:P ratio of aprox.1.2 compared to the other studies mentioned, which applied Ca:P ratios of around 2. Results from Mutucumarana et al. (2014) and Wilkinson et al. (2014) revealed a negative association between small intestinal digestibility of P and the dietary concentration of Ca. Differences in processing conditions of bones into bone meal might be another explanation for the large differences in observed pcdP% of bone meal between this study and the study of Mutucumarana et al. (2014).

The chemical and thermo-physical processes carried out on bones resulting in the commercial products Delfos and Calfos might explain the high pcdP% values, especially for Delfos. This product is the result of dissolving the bone matrix in hydrochloric acid, followed by precipitating the released P with calcium hydroxide, resulting in a pcdP%, which is comparable to products such as MCP and monosodium phosphate. The pcdP% of Calfos was significantly lower than that of Delfos, and this might be ascribed to a lower degree of bone matrix destruction compared to Delfos. The pcdP% of Calfos was significantly higher than that of bone meal, but comparable to the pcdP% of MCP and DCP, which could be explained by the fact that the heat and pressure treatment and removal of gelatin made part of the P captured in the bone matrix available for absorption in the small intestine. To the knowledge of the authors, no other studies have been published that have studied the effect of degelatinization of bone meal on digestibility of P. Based on a visual interpretation of the SEM images, it seems that the spatial structure of the test products as shown by the SEM images (Figures 2 to 6) can be related to the observed pcdP%. It seems that, based on visual inspection of the SEM images, the larger the surface area and the higher the degree of crystallinity, the higher the pcdP%. An explanation for this relation is that the larger surface area may enhance solubilization of P in the small intestinal tract and subsequently improve intestinal absorption of P.

It is concluded that 1) the pcdP% of Delfos is at least comparable or better than MCP, 2) the pcdP% of Calfos is comparable to MCP, and 3) that the pcdP% of bone meal is comparable to anhydrate DCP. The use of processed bone meal phosphates as a P source in broiler diets contributes to a sustainable animal husbandry as it 1) reduces the use of limited rock phosphate sources and 2) it may reduce the excretion of P into the environment due to the increased P digestibility of processed bone meal.

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