

# Barriers for Use of Di- and Tri-Calcium Phosphate of Animal Origin in Non-Ruminant Nutrition

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Advisory report from DCA – Danish Centre for Food and Agriculture

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## Data sheet

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## DESCRIPTION OF THE TASK

### *Aim:*

The purpose of this assignment is to clarify to which extent di- and tri-calcium phosphate of animal origin is currently used in feeding livestock and what the barriers are for using these sources of calcium and phosphate over mined monocalcium phosphate and calcium carbonate. If scientific studies applying di- and tri-calcium phosphate of animal origin in farm animal nutrition studies can be found, the aim is also to evaluate the digestibility in relation to other sources of calcium and phosphorus.

### BACKGROUND:

According to EU legislation it is allowed to use di- and tri-calcium phosphate of animal origin in feed for farmed animals except ruminants. However, it does not appear that the feed industry is applying di- and tri-calcium phosphate of animal origin in feeds since mined monocalcium phosphate and calcium carbonate still seem to be the most commonly used sources of calcium and phosphorus. Di- and tri-calcium phosphate of animal origin or perhaps monocalcium phosphate if possible to produce from animal origin, may be more sustainable sources of calcium and phosphorus in feed compared to mined sources.

In this assignment, we wish to achieve an overview of the potential barriers for using di- and tri-calcium phosphate of animal origin over mined monocalcium phosphate and calcium carbonate in farmed animals except ruminants. We also wish to have a description of the nutritional considerations (digestibility) of the animal sources, including an assessment of whether other forms of calcium phosphates of animal origin could be more appropriate to use.

It should also be stated whether forms other than di- and tri-calcium phosphate (fx. monocalcium phosphate) of animal origin can be/are produced. Furthermore, potential nutritional risks associated with the use of di- and tri-calcium phosphate and possibly other chemical forms of animal origin should be assessed.

Sustainability issues regarding use of mined sources of calcium and phosphorus versus di- and tri-calcium phosphate of animal origin should be assessed if possible.

## DANSK SAMMENDRAG

Fosfor (P) er et essentielt næringsstof med betydning for bl.a. knogledannelse, syre-base balance og energiomsætning i kroppen. Plantebaserede fodermidler til husdyr indeholder relativt store mængder P, men en stor andel af dette P er svært fordøjeligt/tilgængeligt for absorption i enmavede dyr. Derfor tilsættes fosfater med større fordøjelighed, såsom uorganisk mono, di- og tricalcium fosfat (hhv. MCP, DCP og TCP) til foder til enmavede dyr udvundet af råfosfat fra miner, for at opnå tilstrækkelig P forsyning til dyrene. En ca. 20 år gammel brancheaftale mellem parter i den danske foderstofindustri har medført, at kun MCP tilsættes til foderblandinger i Danmark. Overordnet er brug af råfosfat fra miner ikke en bæredygtig strategi, idet det ikke udgør en fornybar ressource. Di- og tricalcium fosfat af animalsk oprindelse, dvs. udvundet fra knogler af døde dyr eller fra æggeskaller, er en langt mere bæredygtig foderfosforkilde, som er godkendt i EU til brug i foder til enmavede dyr. Denne fosforkilde anvendes dog stort set ikke af foderstofbranchen i Danmark og det er uvist i hvilket omfang den finder anvendelse i andre europæiske lande. I denne rapport gennemgås resultater fra studier af den næringsmæssige værdi af mineralske calciumfosfater og af calcium-fosfater af animalsk oprindelse i enmavede, og potentielle barrierer for mere udbredt brug af calcium-fosfater af animalsk oprindelse identificeres.

Konventionel mineralsk DCP og MCP produceres ud fra råfosfat udvundet ved minedrift af hydroxyapatit i flere trin som bl.a. involverer reaktion med mineralsk syre. Processen hvorved både mineralsk MCP, DCP og TCP produceres er velbeskrevet og alle tre former produceres kommercielt. Calcium fosfater af animalsk oprindelse produceres ved at knogler, også indeholdende hydroxyapatit, knuses og affedtes, hvorefter de reagerer med syre og andre kemikalier således at DCP og TCP af foderkvalitet frembringes. Der er ikke beskrevet en metode til at frembringe MCP ud fra knogler. Både MCP, DCP og TCP kan desuden produceres ud fra æggeskaller.

I fjerkræ er P tilgængeligheden målt som P fordøjelighed i DCP af animalsk oprindelse lig med eller større end P tilgængeligheden i mineralsk MCP, DCP og TCP. Desuden er P tilgængeligheden i TCP af animalsk oprindelse på samme niveau som i mineralsk DCP i fjerkræ. Fosfortilgængeligheden i kød- og benmel er lavere end i oprenset DCP og TCP fra knogler og lavere end i mineralsk MCP og DCP. Fosfor og calcium (Ca) tilgængeligheden i calcium-fosfater produceret fra æggeskaller er ikke undersøgt i fjerkræ. Tilgængeligheden af P og Ca i calcium-

fosfater af animalsk oprindelse er ikke undersøgt i grise, og der er således et stort behov for studier der belyser dette.

Brugen af calcium-fosfater af animalsk oprindelse i foder til enmavede er potentielt begrænset af følgende:

1) Foderstofindustriens ca. 20 år gamle brancheaftale, hvori man blev enige om, kun at anvende MCP i husdyrfoder fremfor DCP og TCP af økonomiske og miljømæssige hensyn. Eftersom formodentlig kun DCP og TCP af animalsk oprindelse er på markedet og næringsværdien (fordøjeligheden) af disse kilder i begrænset omfang er kendt i fjerkræ og slet ikke i grise, er der pt. intet incitament til anvendelse af disse.

2) Praktiske og udbudsmæssige forhold set fra foderproducenternes side. Produktion af foder til drøvtyggere og enmavede skal fysisk holdes adskilt, dvs. foregå på hver sin fabrik, hvis foderblandingerne til enmavede indeholder produkter som calcium fosfater af animalsk oprindelse. Dette kan være en praktisk og logistisk udfordring. Samtidig er der tilsyneladende kun ganske få producenter af animalske calcium fosfater på markedet og udbuddet er derfor formentligt også begrænset.

3) At det generelt fortsat betragtes som uetisk at tildele dyr foderbestanddele produceret ud fra dyret selv samt en general bekymring for spredning af sygdomme dyr imellem og måske dyr og mennesker imellem. Dette formodentlig på trods af de meget strikte godkendelsesprocedurer af produktet i EU systemet.

4) Uvidenhed blandt primærproducenter, foderstofindustri og aftagervirksomheder om at knogler og æggeskaller kan processeres til DCP og TCP (og måske MCP) som kan anvendes i foder til enmavede.

Det er potentielt muligt at producere MCP ud fra knogler og MCP, DCP og TCP ud fra æggeskaller. Der er dog et stort behov for at undersøge P tilgængeligheden i disse kilder i både fjerkræ og svin, for at kunne vurdere, om de helt eller delvist kan erstatte mineralske foderfosfater i foderblandinger og således formodentlig udgøre et mere bæredygtigt alternativ til disse.

## INTRODUCTION

Phosphorus (**P**) is an essential nutrient required for bone formation among other functions in the body. Plant feedstuffs, which constitute the bulk of feeds for non-ruminants such as pigs and poultry, have a high content of P (Woyengo and Nyachoti, 2011). However, most of P in plant feedstuffs is not available for utilization by non-ruminants (Woyengo and Nyachoti, 2013). Thus, rock phosphate-derived inorganic P sources such as mono-calcium phosphate (**MCP**), di-calcium phosphate (**DCP**), and tri-calcium phosphate (**TCP**) are added in diets for non-ruminants to meet P requirements (Selle and Ravindran, 2007). These rock phosphate-derived inorganic P sources are expensive. For instance, the average price of MCP for last 10 years is DKK 4.770 per ton (N. M. Sloth, personal communication, November 23, 2021). Also, the use of rock phosphate-derived inorganic P products as source of P in livestock feed is not sustainable because it will eventually lead to depletion of phosphate-rich rocks (Someus and Pugliese, 2018). Furthermore, the rock-derived calcium phosphates can contain hazardous compounds such as fluorine, cadmium, uranium, lead, mercury and arsenic (Lima et al., 1999). Thus, there is need for identification of P sources that are safe and whose use in formulation of feed for non-ruminants can be sustainable.

Di- and tricalcium phosphates of animal origin have been approved by the EU for use in non-ruminant feeds since 2003 (EC, 2003). The DCP and TCP of animal origin do not contain hazardous compounds, and their use for formulation of non-ruminant feeds can be sustainable because their use in formulation of non-ruminant feeds can result in reduced loss of P from nutrient cycle (Someus and Pugliese, 2018). Also, bone-derived calcium phosphates are generally by-products of gelatin production from bones, and hence they are cheaper than the rock phosphate-derived calcium phosphates. For instance, the current price of bone-derived MCP produced in France (euro 400 per ton; A. van den Bosch, personal communication, November 19, 2021) is 3 times lower than the current price of rock phosphate-derived MCP in Denmark (euro 1200 per ton; N. M. Sloth, personal communication, November 23, 2021). The nutritive value of DCP and TCP of animal origin for non-ruminants has been determined in some studies (e.g., Sullivan et al., 1994; van Harn et al., 2017; Trairatapiwan et al., 2018); however, this information has not been reviewed earlier. Also, the use of DCP and TCP of animal origin in formulation of non-ruminant feeds has been limited. The review of data on the nutritive value of DCP and TCP of animal origin for non-ruminants, and identification of barriers for the use of

DCP and TCP of animal origin in formulating non-ruminant feeds is important for increasing and optimizing the utilization of these P sources in formulating the non-ruminant feeds. The production and the nutritive value of the conventional rock-derived MCP, DCP and TCP for non-ruminants have been determined in several studies (e.g., Poulsen, 2007; Gonzalez-Vega et al., 2015; Kwon and Kim, 2017; Zhang and Adeola, 2018); the number of studies that have determined the production and the nutritive value of the rock-derived DCP and TCP for non-ruminants is greater than the number of studies that have determined the production and the nutritive value of the animal-derived DCP and TCP for non-ruminants. Thus, the review of recent data on production of MCP, DCP and TCP from rock phosphates and the nutritive value of rock-derived MCP, DCP and TCP for non-ruminants is important for gaining knowledge that can be used to optimize utilization of DCP and TCP of animal origin in formulating non-ruminant feeds. The objective of this study is to review results from studies on the nutritive value of the rock- and animal-derived calcium phosphates for non-ruminants, potential barriers for using animal-derived calcium phosphates over rock-derived calcium phosphates in formulation of feeds for non-ruminants. Also, areas that need further research with regard to production and the nutritive value of the rock- and animal-derived calcium phosphates for non-ruminants are suggested.

## OVERVIEW OF NON-RUMINANT P NUTRITION

Phosphorus is required by animals for bone, teeth, cell membrane, nucleic acid (RNA and DNA) and milk formations; production and storage of energy; activation of various enzymes, hormones and cell signaling molecules; and maintaining normal acid-base balance (Knochel, 2006). Insufficient P supply relative to the need at a given physiological stage of animal can result in reduced growth and production as well as in impaired bone development and mineralization (Misiura et al., 2020). Oversupply of P relative to the need at a given physiological stage of an animal is expensive for the farmer (Selle and Ravindran, 2007) and increase P excretion (Lopes et al., 2009). Excessive supply of P through manure to arable land is considered an environmental problem and therefore the influx of P to farm land is tightly regulated in countries like Denmark (Maguire et al., 2009).

There are two forms of P in diets for non-ruminants; phytate-bound P and non-phytate P (Selle et al., 2003; Steiner et al. 2007). Phytate is present in feedstuffs of plant origin; it serves as

storage form of P in plants (Selle and Ravindran, 2007). Non-phytate P includes organic and inorganic P. Phytate-bound P constitute at least 60% of total P in cereal grains and their co-products, and oilseed meals that are used to formulate non-ruminant feeds (Humer et al., 2015). When non-ruminant animals such as pigs and poultry ingest feed that contain phytase, some of the ingested phytate-bound P in feed is liberated in the stomach and upper part of small intestine by the phytase (Woyengo and Nyachoti, 2011). Most of the phytate-bound P is not liberated in the stomach and upper part of small intestine if the ingested feed does not contain phytase (Selle and Ravindran, 2007). Most of organic non-phytate P is solubilized (converted) to inorganic P, and it is inorganic P that is absorbed from the small intestine into the body (Care, 1994); phytate P is not absorbed (Rutherford et al., 2004). It should be noted that phytate becomes available for the action of phytase after its release from the feed matrix (Woyengo and Nyachoti, 2011). Also, it is expected that organic non-phytate P becomes available for solubilization into inorganic P after its release from the feed matrix. Finally, like any other mineral, it is “free” inorganic P (i.e., not P embedded in feed matrix or complexed with other dietary components) that can be absorbed (Care, 1994; González-Vega and Stein, 2014). Thus, the availability of dietary P for absorption is heavily dependent of degradation of other dietary components that surround the P. In addition to dietary P, non-ruminants release endogenous P into the gastrointestinal tract via salivary, gastric, biliary, exocrine pancreatic juices; and intestinal secretions (Fan et al., 2001), which may or may not be reabsorbed in the small intestine.

Phosphorus is absorbed in the small intestine by both passive (paracellular) and active (transcellular by trans-membrane proteins) transport mechanisms (Adedokun and Adeola, 2013). Active transport of P is the predominant mode of absorption when luminal P availability is low relative to the body requirement, whereas passive transport is the predominant mode of absorption when luminal P availability is high relative to the body requirement (Kari et al., 2010; Hill Gallant and Vorland, 2021; Yee et al., 2021). The active P absorption in the small intestine is by Na-dependent mechanisms, which involves active pumping of Na from the epithelial cells via basolateral membranes by the action of Na-K-ATPase enzyme. This active pumping of Na from the epithelial cells creates a Na gradient that drives the trans-membrane proteins that bind Na and P at the same time to transport P from the small intestinal lumen into the cells (Huber et al., 2002; Kari et al., 2010). Passive transport mechanisms involve movement of phosphate ions



through tight junctions (Yee et al., 2021). In general, the passive transport accounts for most of P that is absorbed in the small intestine (Hill Gallant and Vorland, 2021; Yee et al., 2021).

The P of both dietary and endogenous origin that is unabsorbed in the small intestine move into the large intestine. Insignificant amounts of P is absorbed or endogenously secreted in the large intestine. For instance, the standardized ileal digestibility (**SID**) of P value for field pea for pigs (55%) did not differ from standardized total tract digestibility (**STTD**) of P (51%) for the same feedstuff (Johnston et al., 2013). Thus, most of the P that is not absorbed in the small intestine is excreted via feces. The absorbed P is utilized for several functions in the body. The excess (un-utilized) P in the body is excreted via urine. Feces are the major routes of P excretion from the body. For instance, fecal P and urinary P, respectively, constituted 83 and 17% of total P excreted by growing pigs (Poulsen et al., 1999). The amount of P that is excreted via urine does not change with change in digestible P intake at digestible P intake below the dietary P requirement; however, the amount of P that is excreted via urine increases with increase in digestible P intake at digestible P intake above the dietary P requirement (Gutierrez et al., 2015).

## DETERMINATION OF P AVAILABILITY IN FEEDSTUFFS FOR NON-RUMINANTS

The availability of P in feedstuffs for non-ruminants can be determined using in vivo digestibility assays or relative bioavailability assays. However, the use of the relative bioavailability assays for estimating P availability of feedstuffs for non-ruminants has the following limitations (Working Group No 2: Nutrition of the European Federation of Branches of WPSA, 2013). First, the assay is relatively expensive because it involves feeding diets to a large number of animals for a long period of time. Second, the P availability values for test feedstuff obtained may vary with standard feedstuff used. Third, the relative bioavailability values cannot be used in formulation of feeds because they are not quantitative measures. Because of these reasons, the relative bioavailability assay can be used for ranking P sources within a study, but not for generating data on P availability in the P sources that can be used for accurate formulation of diets for non-ruminants (She et al., 2017). Digestibility assays, compared with relative bioavailability assays, are better methods of estimating P availability in feedstuffs as discussed in the following paragraph.

The digestibility of P can be measured at the ileal or total gastrointestinal (**GI**) tract level. As previously mentioned, some endogenous P is secreted in the GI tract, some of which may

escape re-absorption, leading to underestimation of the P digestibility. Also, apparent digestibility values of P for feedstuffs, like of any other nutrients, are dependent on the level of P in the feedstuffs or diet (Johnston et al., 2013), and are not additive (She et al., 2017). Hence, it is necessary to correct for the endogenous loss of P while estimating P digestibility values of feedstuffs for non-ruminants. It is not easy to measure total endogenous losses of P; also, the total endogenous losses of P vary among studies (NRC, 2012). However, it is easy to measure basal endogenous losses of P by feeding a P-free diet or by regression method (She et al., 2017), and the basal endogenous losses of P values do not vary among studies (NRC, 2012). Thus, practically, standardized digestibility assay is recommended for estimating P digestibility values of feedstuffs for non-ruminants such as pigs (NRC, 2012). As previously mentioned, insignificant amounts of P is absorbed or secreted in the large intestine. Thus, SID of P values of feedstuffs for non-ruminants are not different from the STTD values of the same feedstuffs. Since it is much easier to measure total tract digestibility than ileal digestibility of nutrients in pigs, STTD assay has been recommended for estimating P availability in feedstuffs for pigs (NRC, 2012). For poultry, it is not easy to measure total tract digestibility of nutrients because feces and urine are excreted together. Thus, SID assay is the recommended method for estimating P availability in feedstuffs for poultry (Shastak and Rodehutsord, 2013; Working Group No 2: Nutrition of the European Federation of Branches of WPSA, 2013). However, apparent total tract digestibility (**ATTD**) of P values are used for calculations of the nutrient standards for Danish pigs. Thus, if available, ATTD of P values in pigs for calcium phosphates from previous studies are discussed in this current study.

## CONVENTIONAL INORGANIC P SOURCES IN DIETS FOR NON-RUMINANTS

The major types of rock phosphate-derived calcium phosphates that are added in conventional diets for non-ruminants include MCP, DCP, and TCP. Of these, MCP and DCP are the most widely used sources of inorganic P in the diets for non-ruminants worldwide. However, approximately 20 years ago, stakeholders in the Danish feedstuff industry agreed upon only applying MCP in monogastric feeds (Sehested et al., 2005) primarily due to MCP having the highest digestibility in monogastrics and therefore out of environmental concerns..

### *Production of Calcium Phosphates from Phosphate Ores.*

The conventional feed-grade MCP and DCP are produced from phosphate ores that contain a P and Ca rich compound known as hydroxyapatite (Someus and Pugliese, 2018). In addition to P and Ca, the phosphate ore can contain fluorine and other impurities such as cadmium (Lima et al., 1999; Someus and Pugliese, 2018). The general steps for production of DCP from rock phosphate include: (1) digestion of the rock phosphate with a mineral acid such as sulfuric acid or hydrochloric acid to yield phosphoric acid and calcium salts such as calcium sulfate or calcium chloride depending on the type of acid used to digest the rock phosphate; (2) filtration, purification (from heavy metals) and defluorination of phosphoric acid; and (3) reaction of the purified phosphoric acid with a calcium source such as limestone (calcium carbonate) or quick lime (calcium oxide) to form the feed-grade DCP (De Waal, 2003). The calcium salt generated in Step 1 can be hydrolyzed into calcium oxide or calcium hydroxide and mineral acid. The generated mineral acid can then be recycled (used in Step 1), whereas the calcium oxide or calcium hydroxide can be used as a calcium source in Step 3 (De Waal, 2003). The MCP can be produced from the feed-grade DCP by reacting the latter with phosphoric acid that has been purified and defluorinated (De Waal, 2003). The TCP can be produced from rock phosphate by reacting the rock phosphate with phosphoric acid in presence of soda ash and calcining the mixture to obtain defluorinated TCP (Azegami, 1969; Lima et al., 1999).

### *Content and availability of P and Ca in Calcium Phosphates from Phosphate Ores*

The DCP has a greater content of Ca and lower content of P than MCP, whereas TCP has a greater content of Ca and lower content of P than DCP. For instance, MCP, DCP and TCP contained 16.9% Ca and 21.5% P, 24.8% Ca and 18.8% P, and 34.2% Ca and 17.7% P, respectively (NRC, 2012). The greater content of P in MCP than of DCP is attributed to the greater amount of phosphoric acid that is reacted with a Ca source during the formation of MCP than during the formation of DCP as described previously in section on “Production of Calcium Phosphates from Phosphate Ores”. The lower content of P in TCP than in DCP is attributed to the fact that phosphoric acid is reacted with two sources of Ca (rock phosphate and soda ash) during the formation of the TCP, leading greater content of Ca in the TCP that dilute P in the same product.

The availability of P and Ca in MCP, DCP and TCP for non-ruminants has been determined in several studies. Poulsen (2007) reported a greater STTD of P value for MCP (64%) than for DCP (59%) in pigs. Petersen and Stein (2006) reported that the ATTD and STTD of P for DCP (81.5 and 88.4%, respectively) tended to be lower than those for MCP (88.0 and 94.9%, respectively) in pigs. Also, Kwon and Kim (2017) reported that the ATTD and STTD of P for DCP (78.4 and 87.0%, respectively) tended to be lower than those for MCP (85.9 and 93%, respectively), and that the ATTD and STTD of TCP (65.2 and 71.3%, respectively) was lower than that of MCP or DCP in pigs. In poultry, An et al. (2020) reported that the SID of P for MCP (89.8%) tended to be greater than that for DCP (79.5%), and that the SID of TCP (56.7%) was lower than that for MCP or DCP in broilers. Thus, it appears that in both pigs and poultry, the P digestibility of MCP is greater than that of DCP, which in turn, is greater than that for TCP. The P in MCP was more soluble in water than that in the DCP (Jamroz et al., 2013), implying that the former is more soluble than the latter at near neutral pH found in the small intestine, which is the major site of P absorption. The solubility of calcium phosphates is inversely related to the Ca to P ratio in the calcium phosphates (Mekmene et al., 2009), and hence the solubility of TCP is expected to be lower than that of DCP because of the greater Ca to P ratio in the former than in the latter. As previously mentioned in the section on “OVERVIEW OF NON-RUMINANT P NUTRITION”, it is only solubilized P that is absorbed in the gastrointestinal tract of animals, implying that the digestibility of P in inorganic P sources is positively correlated with the solubility of P in these P sources. Thus, the greater digestibility of P for MCP than for DCP, and the greater digestibility of P for DCP than for TCP for pigs and poultry could be attributed to the greater solubility of P in the MCP, followed by DCP and then TCP.

With regard to Ca digestibility, Gonzalez-Vega et al. (2015) reported that the STTD in pigs of Ca from limestone (60.4%) was lower than that for MCP (85.9%) or DCP (77.8%), which did not differ significantly in STTD of Ca. Kwon and Kim (2017) observed that the STTD of Ca in pigs for MCP (76.1%) did not differ from that for DCP (74.4%), but that for TCP (63.7%) tended to be lower than for MCP or DCP. In broilers, Anwar et al. (2018) observed greater SID of Ca for DCP than for MCP (38 vs. 32%). Also in broilers, the SID of Ca for limestone (63.7%) was lower than that for DCP (67.2%; Zhang and Adeola, 2018). From these studies, it is apparent that the digestibility of Ca in limestone for pigs and poultry is lower than that of MCP or DCP. The lower digestibility of Ca in limestone than in calcium phosphates can be attributed to the fact

that calcium phosphates are derived from both phosphoric acid and Ca source such as limestone, whereas limestone does not contain phosphoric acid, and that phosphoric acid is more soluble than limestone (Zhang and Adeola, 2018). The lower digestibility of Ca in TCP than in DCP or MCP could be attributed to the greater Ca to P ratio in the TCP than in the DCP or MCP.

## ANIMAL-DERIVED INORGANIC P SOURCES IN DIETS FOR NON-RUMINANTS

Bones contain hydroxyapatite that is similar to the one found in phosphate ores (Someus and Pugliese, 2018). Thus, inorganic P sources such as MCP, DCP and TCP can be produced from the bones derived from livestock (EC, 2003). Egg shells contain calcium carbonate that can also be used for production of calcium phosphates (Laohavisuti et al., 2021).

### *Production of Calcium Phosphates of Animal Origin*

The general steps for production of DCP from bones include: (1) crushing the bones; (2) degreasing the crushed bones using hot water; (3) digestion of the crushed and degreased bones with a mineral acid (specifically HCl) to yield phosphoric acid solution and solid material containing the osseine, which is the organic matter component of bones (it is mainly composed of collagen); and (4) reaction of the phosphoric acid with a calcium source such as limestone (calcium carbonate) or quick lime (calcium oxide) to form the feed-grade DCP (EC, 2003; van Harn et al., 2017). The solid material containing the osseine can be processed into gelatin, which is then marketed.

The general steps for production of TCP from bones include: (1) crushing the bones; (2) degreasing the crushed bones using hot water; (3) cooking crushed and degreased bones with steam; and (4) centrifugation of the steamed material to separate broth that contain protein from hydroxyapatite (TCP; EC, 2003). The TCP product produced is not pure and may in addition to hydroxyapatite, contain some gelatin and fat (EC, 2003). The method of producing MCP from bones has not been reported, and hence it is not clear if it is possible to produce MCP from bones. However, it may be possible to produce bone-derived MCP from bone-derived DCP using the same procedure that has been described for production of the conventional rock phosphate-derived MCP from the rock phosphate-derived DCP.

The DCP may be produced from eggshells by: (1) crushing eggshells that have been washed with household detergent; (2) calcining the crushed eggshells to produce calcium

carbonate powder; (3) reacting the calcium carbonate powder with phosphoric acid to produce DCP (Laohavisuti et al., 2021). The MCP may be produced from eggshells using the same method described for production of DCP from eggshells except that larger amount of phosphoric acid is reacted with calcium carbonate powder during the production of the MCP than during the production of the DCP (Laohavisuti et al., 2021). The TCP may be produced from eggshells by mixing the eggshell-derived MCP with eggshell-derived calcium carbonate powder followed by calcining the mixture to produce the TCP (Laohavisuti et al., 2021).

### *Content and availability of P and Ca in Calcium Phosphates Animal Origin*

Like in rock phosphate-derived calcium phosphates, the animal-derived TCP has greater content of Ca and lower content of P than animal-derived DCP. For instance, bone-derived DCP and TCP contain 16.8% Ca and 23.4% P, and 30.8% Ca and 14.0% P, respectively (van Harn et al., 2017).

The availability of P and Ca in bone-derived DCP and TCP for non-ruminants has been determined. The relative bioavailability of P values in turkeys for 2 bone-derived DCP products (from 2 different companies) were 98.8 and 99.1% compared to the 100% for reference standard (calcium phosphate, dibasic dehydrate; Sullivan et al., 1994). In the same study of Sullivan et al. (1994), the relative bioavailability of the conventional rock phosphate-derived MCP was 96.4%, which was comparable to that of the 2 bone-derived DCP products. With regard to digestibility of P, the apparent ileal digestibility of P in broilers for bovine bone-derived DCP (80.1%) was similar to that (78.6%) for conventional rock phosphate-derived DCP (Barshan et al., 2019). The SID of P for bone-derived DCP (69.3%) was greater than that for conventional rock phosphate-derived MCP (64.6%) in broilers (Trairatapiwan et al., 2018). Similarly, SID of P in broilers for bone-derived DCP (94.5%) was greater than that for conventional rock phosphate-derived MCP (88.5%) or DCP (82.5%; van Harn et al., 2017). However, in the same study of van Harn et al. (2017), the SID of P value (86.5%) for a bone product (TCP) that was produced by degreasing and partially de-gelatinizing porcine bones but in which hydroxyapatite matrix was preserved, was lower than that for bone-derived DCP, but similar to that of conventional rock phosphate-derived MCP. Also, in the same study (van Harn et al., 2017), the SID of P value (78.2%) for porcine bone meal that was produced by crushing, degreasing and drying porcine bones at 125 °C was lower than that for bone-derived DCP, but similar to that for conventional rock

phosphate-derived DCP. Similarly, Barshan et al. (2019) reported that the apparent ileal digestibility of P value (57.2%) in broilers for bone meal that had been prepared by crushing and drying degreased bovine bones at 130°C was lower than that for bovine bone-derived DCP (80.1%) or conventional rock phosphate-derived DCP (78.6%).

The effects of dietary inclusion of bone-derived calcium phosphates on growth performance and bone mineralization of poultry have also been reported. Sullivan et al. (1994) determined the effects of including bone-derived DCP or 2 conventional rock phosphate-derived MCP products from 2 different sources in diets for turkey formulated to equal Ca content, and observed that the body weight gain (430 g) and tibia ash (37.2%) of turkeys at 21 days of age for bone-derived DCP were greater than the values (413 g body weight gain and 35.3% tibia ash) for one source of conventional rock phosphate-derived MCP, but similar to the values (443 g body weight gain and 38.0% tibia ash) of another source of conventional rock phosphate-derived MCP. Also, Barshan et al. (2019) did not observe differences in body weight gain (884 vs. 937 g) and tibia ash (66.4 vs. 67.7%) of broilers at 28 days of age due to replacement of the conventional rock phosphate-derived DCP with bovine bone-derived DCP in diets formulated to equal Ca content.

From these studies, it is apparent that P availability in bone-derived DCP is similar to or greater than that of conventional rock phosphate-derived DCP or MCP for poultry. Also, the P availability in bone-derived TCP is similar to that of conventional phosphate rock-derived DCP. However, the P availability in poultry for bone meal may be lower than that for bone-derived DCP or TCP, or lower than that for conventional phosphate rock-derived DCP or MCP. The relatively low availability of P in TCP and bone meal could be attributed to the presence of collagen/gelatin in these products due to incomplete bone matrix destruction. Collagen is complex protein that is poorly digested by endogenous enzymes in the small intestine of animals (Sousa et al., 2020) and can reduce the availability of P in the bone matrix for absorption (van Harn et al., 2017; Barshan et al., 2019). Thus, the inclusion of bone meal in diets for non-ruminants at the expense of conventional rock phosphate-derived MCP or DCP can result in increased excretion of P and N by the non-ruminants into the environment because of lower digestibility of P in the former compared to the latter, and because of the low digestibility of protein in collagen.

The Ca availability of bone-derived phosphates has not been reported. Also, the P and Ca availabilities of calcium phosphates derived from egg shells for poultry have not been reported. Furthermore, the P and Ca availabilities in pigs of calcium phosphates derived from animal sources have not been reported. Hence, there is a need to fill these gaps in the knowledge. Based on data on the effects of replacement of the conventional rock phosphate-derived calcium phosphates with bone-derived calcium phosphates on growth performance and bone mineralization in poultry, it appears that the Ca availability in bone-derived calcium phosphates is either greater or similar to that in rock phosphate-derived calcium phosphates.

## POTENTIAL BARRIERS AND RISKS FOR USING CALCIUM PHOSPHATES OF ANIMAL ORIGIN IN FORMULATING FEEDS

The use of calcium phosphates of animal origin in formulating feeds for non-ruminants can potentially be limited by the following challenges:

First, there is limited information on the nutritive value of calcium phosphates of animal origin for non-ruminants. For instance, only a few studies have been conducted to determine P availability in calcium phosphates of animal origin for poultry. Furthermore, there is lack of information on P availability in calcium phosphates of animal origin for pigs. Thus, it is difficult for pig producers to include calcium phosphates of animal origin in pig feeds if they do not know the available P and Ca contents in these products.

Second, practical, supply-chain and structural considerations by the feed industry, may block for more widespread use of calcium phosphates of animal origin. If calcium phosphates of animal origin is included in diets for pigs and poultry, these diets need to be produced separately from ruminant feeds (on separate feed mills) to comply with EU legislation, which can be a practical challenge. Furthermore, the availability of calcium phosphates of animal origin may be scarce, since currently only few producers are on the market. Structural agreements between partners in the feedstuff industry, as in the Danish feedstuff sector on only using MCP over DCP and TCP in animal feeds could also be a barrier for using calcium phosphates of animal origin if only DCP and TCP animal products are manufactured. In Denmark, many farmers are mixing diets on-farm, and hence the need for separate feed mills for non-ruminants and ruminants should not be a problem.



Third, feeding animals calcium phosphates that are derived from the same animal species may be perceived as unethical, the feedstuff industry to deselect animal-derived calcium phosphates when formulating feed for non-ruminants. This unethical issue can, however, be avoided by producing the calcium phosphate products by animal species and making sure that the produced calcium phosphate products are not fed to animals of the same species, from which they have been derived from.

Fourth, despite the very well-documented approval process in the EU system, there may still be a general fear of the use of feedstuffs of animal origin in formulation of livestock feeds due to spread of diseases (such as bovine spongiform encephalopathy) from animals to other animals or perhaps from animals to human beings. However, this should not be a problem with regard to use of calcium phosphate of animal origin because they are treated with a lot of heat or heat plus acids during production, which should remove potential infectivity (EC, 2003).

Fifth, there is lack of awareness by livestock meat and egg processors, livestock feed manufacturers and livestock producers that food animal bones and egg shells can be processed into DCP and TCP products (and perhaps into MCP product) that can be included in livestock feeds as sources of P and Ca. Thus, livestock industry stakeholders should be made aware of advantages of using calcium phosphate of animal origin for formulating non-ruminant feeds in order to increase the utilization of these products as a source of P and Ca in diets for non-ruminants.

## CONCLUSIONS

It is concluded that it is legal to include calcium phosphates of animal origin in feeds for non-ruminants. The availability of P in bone-derived DCP is similar to or greater than that of rock phosphate-derived MCP, DCP or TCP in poultry. Also, P availability in bone-derived TCP is similar to that of rock phosphate-derived DCP in non-ruminants such as poultry. The P availability in animal-derived calcium phosphates in pigs are unknown. Because rock phosphate-derived calcium phosphates are non-renewable sources of P, whereas animal-derived calcium phosphates are, the use of bone-derived calcium phosphates such as DCP and TCP in formulation of non-ruminant feeds can contribute to sustainable production of non-ruminants products such as pork, poultry meat and eggs. Also, because the bone-derived calcium phosphates are by-products of gelatin production from bones, they can be cheaper than the rock

phosphate-derived calcium phosphates, leading to reduced cost of feed for non-ruminants. Bone-derived MCP and eggshell-derived MCP, DCP and TCP can potentially be produced.

Currently, the utilization of calcium phosphates of animal origin in formulating feeds for non-ruminants is limited by: (1) lack of adequate information on P availability in these products, (2) need for separation of feed mills for production of ruminant feeds from those for production of non-ruminant feeds, (3) belief that animals cannot be fed with products of animal origin, (4) image problem because of the belief that their use can lead to spread of zoonotic diseases, and (5) lack of awareness of the advantages (with regard to cost of feed and sustainable production of livestock products) of utilizing these products in formulation of livestock feeds.

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