

Background.

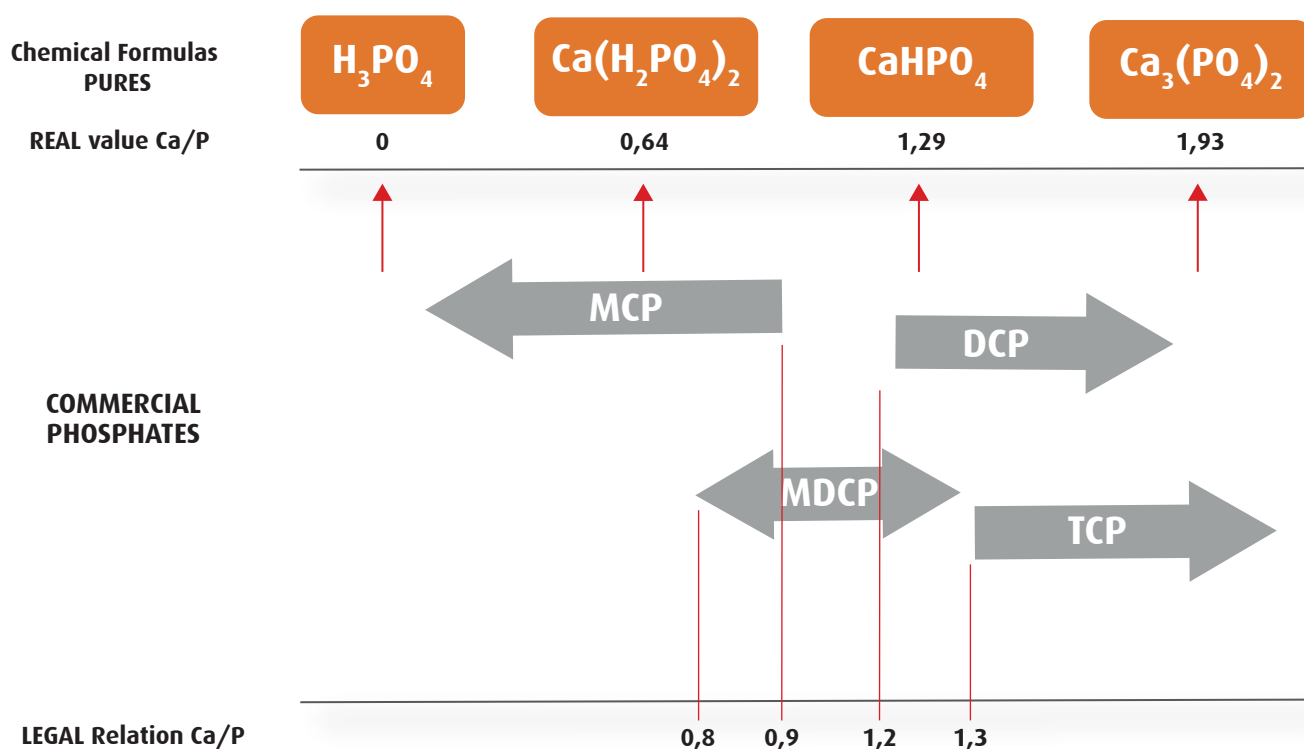
Phosphorus (P) is an essential mineral for the formation and maintenance of bone structures, for the synthesis of tissues such as muscle mass and for the formation of eggshells in birds. It participates in the metabolism of proteins, fats, carbohydrates, minerals and other nutrients in the body. It is a component of ATP, nucleic acids and phospholipids that integrate and give flexibility to the cell membranes (Georgyievskii et al., 1982).

The Regulation of the European Union allows the characterization of phosphates, even if they have different chemical composition, in the same legal concept according to the proportionality or relation between Ca/P (Regulation EC 1017/2017).

At present, commercial phosphates do not

constitute a defined chemical entity, but, due to the productive process, they are composed of different phosphate species resulting from undesired parallel reactions giving rise to final products with very variable parameters of water solubility, citric acid solubility, presence of other minerals and pH.

This gives rise to the coexistence of commercial phosphates with different chemical composition within the same catalogue. These differences in composition generate a very different bioavailability in the animal. Bioavailability or degree of utilization is defined as the degree to which a nutrient ingested from a particular source is absorbed and available for use in animal metabolism (Forbes and Erdman, 1983). As shown in the attached table (Figure 1.), European



legislation establishes intervals based on the Ca/P ratio for the characterization and classification of each of the phosphates. As the Ca/P ratio is higher, the incorporation of dicalcium phosphate increases in the formulation of phosphate, until it reaches tricalcium phosphate.

In any existing table on digestibilities it is evident that dicalcium phosphate is less digestible than monocalcium. Therefore, increases in the presence of the latter would lead to an increase in digestibility. Since monocalcium phosphate is the only water-soluble calcium phosphate, increased water solubility should be related to increased digestibility.

For formulated phosphates, there is no evidence or scientific publication to date that demonstrates a linear correlation between the two parameters. The reason is that not only is there a great variability in the quality of non-water-soluble phosphates, but these can enter into very variable proportions in formulated phosphates. The chemical interactions in the mixtures are also a factor of variability that complicates a linearity between water solubility and digestibility.

Work bases.

Digestion is a process that occurs in a range of high to moderate acidity in poultry farming (Angel et al., 2010). Under these conditions, the stable phosphate anion would be mostly the monosubstituted one, which would be available for absorption both passively and actively. In the case of dicalcium phosphate, which is a disubstituted salt and insoluble in water, it would need a previous phase of acid attack and buffering to go into a monosubstituted state for absorption.

Hydrated compounds tend to have a faster solubilization than anhydrous ones, and it is for this reason that their digestibility is increased in all the in vivo tests that can be observed in the literature.

Pyrophosphate requires prior hydrolysis to pass into the form of orthophosphate, and to be buffered for absorption, so its digestibility is low.

Tricalcium phosphate is very stable, requiring a slower attack, so its digestibility is also impaired. Non-calcium metal phosphates have

high insolubility and very low digestibility. The use of hydrochloric, required in some phosphate production processes, leads to chloride levels up to five times higher than other more advanced techniques (Globalphos® Technology), and implies very negative charges in the digestion processes.

Design of predictive equations.

GlobalFeed has developed an analytical methodology based on numerous chemical analyses in different parameters, correlating its results with a multitude of biological tests carried out. As a result of this study, Predictive Equations for determining the digestibility of commercial phosphates have been obtained.

From this experience, and as it has been outlined previously, the in vitro solubility tests of phosphorus cannot be evaluated as the only reliable reference, since they are not a reliable measure of the estimation of nutritionally available phosphorus (Ammerman, 1995; Cauduro, 2009; Courtin, 1995; Day et al., 1973; Gueguen, 1995, 1999; Miles, 1997; Sullivan et al., 1992; Yoshida and Hoshii, 1979 and Waibel et al.) Up to date, these compositions have been able to be analyzed in a semiquantitative way with the diffraction of X Rays when the contents were higher than 5%, but its precision below these percentages, only allows to verify the presence of other compounds.

The methodology developed by GlobalFeed, allows the quantification of the parameters that appear reflected in the attached table that, by means of chemical balance, allow identifying the presence of these compounds that are part of the commercial phosphates.

Once the complete composition of the commercial phosphates is known, and based on in vivo tests carried out in the Polytechnic University of Valencia and on studies of the literature, a weighting of the digestibility of each one of these components has been carried out, which has served to construct a predictive equation, assigning a proportional objective value to the digestibility. In the in vivo trials carried out in GlobalFeed, phosphates previously evaluated in the Predictive Equations have been protocolized, confirming the reliability of the system implemented.

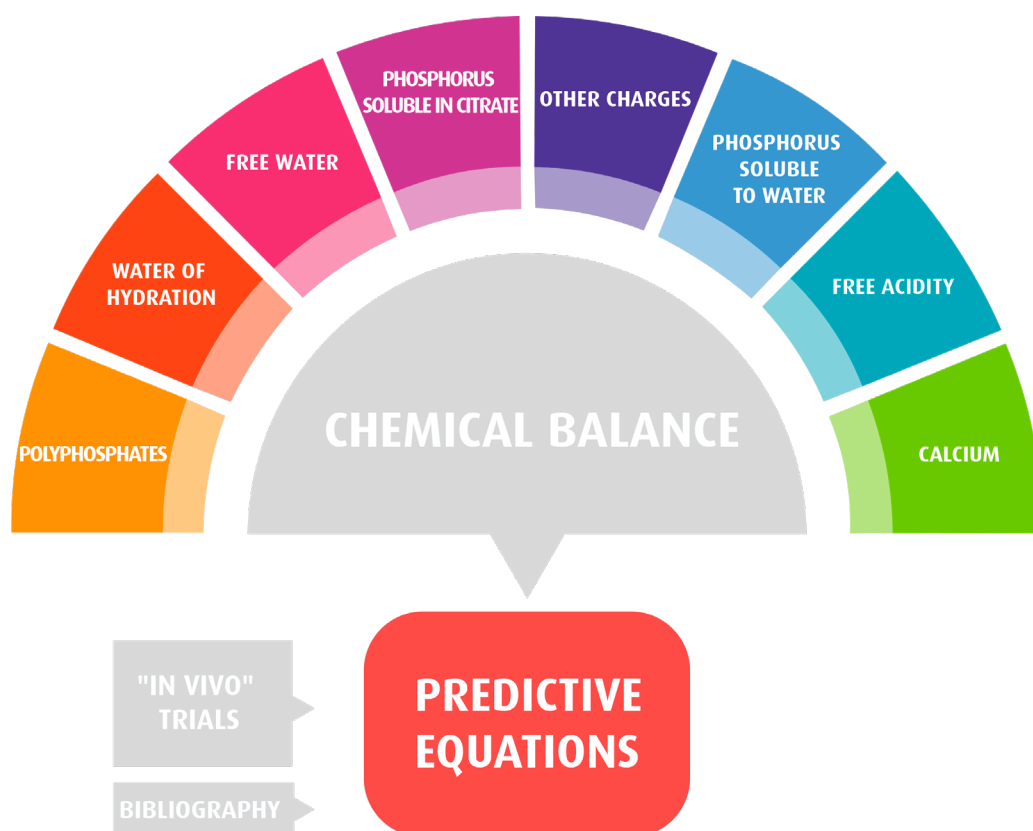


Figure 2. Descriptive diagram of the development of the predictive equations of phosphorus digestibility

Results in the predictive equations and *in vivo* tests.

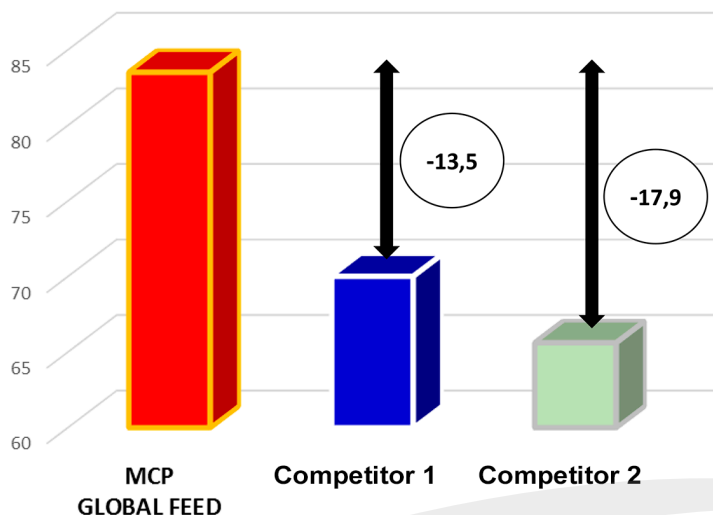
In the prediction of digestibility by means of the developed equations (EPCD), some first results were obtained that were contrasted in *in vivo* tests to evaluate their reliability. After the analytical evaluations described above, the obtained digestibility values were assigned. These same phosphates were used in farm *in vivo* trials.

COMPANIES	EPCD	
	MCP	DCP
GLOBAL FEED	91,0 %	81,0 %
COMP. BLUE	84,5 %	
COMP. ORANGE		68,6 %
COMP. GREEN	78,8 %	65,7 %

Figure 3. Predictive values of apparent digestibility (EPCD) from different commercial sources of monocalcium and dicalcium phosphate.



Effects of monocalcium phosphate source on the apparent phosphorus digestibility coefficient and bone mineralisation in broiler chickens



Apparent digestibility coefficient (%) +/- standard error of the mean P for different commercial P sources in broilers at 26 days of age

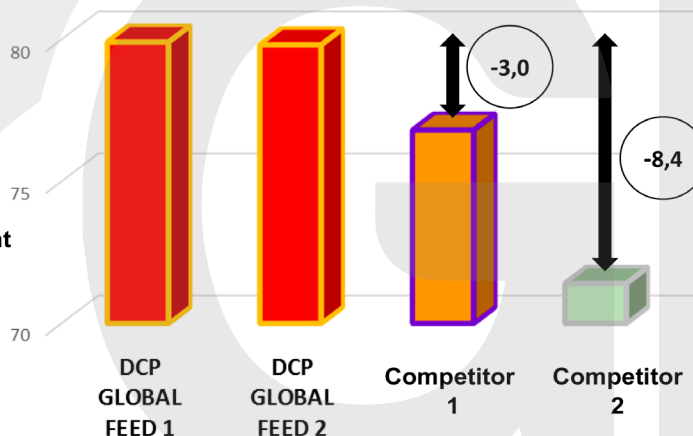
	MCP GBF	Competitor 1	Competitor 2
Apparent digestibility coefficient	83,5 ± 5,57	70,0 ± 5,10	65,6 ± 11,2
P-value compared to competition 2	0,093	0,661	-

MCP GBF: Basal feed + 1.5 gr/Kg total P from MCP GlobalFeed. Competitor 1: basal feed + 1.5 gr/Kg total P from Competitor 1. Competitor 2: basal feed + 1.5 gr/Kg total P from Competitor 2.

Figure 4. Apparent Digestibility Coefficient (ATTD) from different commercial sources of monocalcium phosphate.



Effects of dicalcium phosphate source on the apparent phosphorus digestibility coefficient and bone mineralisation in broiler chickens



Apparent digestibility coefficient (%) +/- standard error of the mean P for different commercial P sources in broilers at 26 days of age

	DCP GBF1	DCP GBF2	Competitor 1	Competitor 2
Apparent digestibility coefficient	79,9 ± 6,74	79,8 ± 3,61	76,8 ± 4,96	71,4 ± 5,00
P-value compared to competition 2	0,240	0,252	0,453	-

MCP GBF: Basal feed + 1.5 gr/Kg total P from MCP GlobalFeed1. DCP GBF2: Competitor 1: basal feed + 1.5 gr/Kg total P from Competitor 1. Competitor 2: basal feed + 1.5 gr/Kg total P from Competitor 2.

Figure 5. Apparent Digestibility Coefficient (ATTD) from different commercial sources of dicalcium phosphate.

Results of EPCD index against ATTD coefficient (*in vivo* digestibility).

In the two graphs obtained for each type of commercial phosphate (MCP and DCP), the correlation line between the calculated EPCD values and those obtained in the tests is reflected.

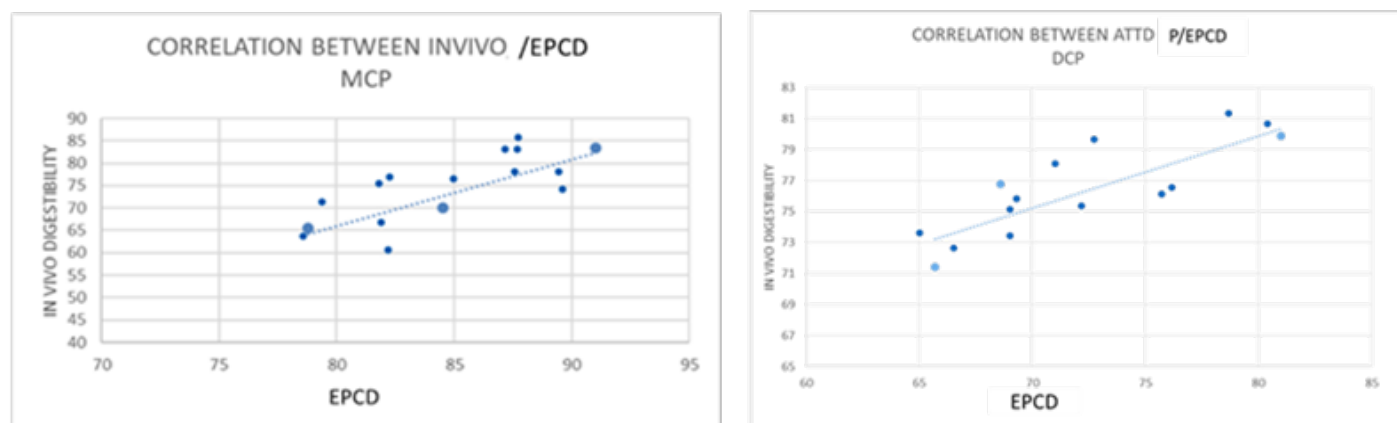


Figure 6. Correlation obtained between the apparent digestibility coefficient (ATTD) and the predictive values of digestibility (EPCD) from different commercial sources of monocalcium and dicalcium phosphate.

EPCD assessment in MCP.

The existing proportionality between the values calculated through the Predictive Equations (EPCD) and the results obtained in the *in vivo* tests, correspond to products with the same granulometry and under experimental farm conditions. This translates into small differences with field results, where the potential for expression of digestibility results is lower. For this reason, the practical application of the EPCD Index should be a tool for the adjustment of digestibility for nutritionists.

The starting basis is the digestibility data from different tables (FEDNA, NRC, CVB and INRA) that must correspond to an average value of phosphates in the market. A study has been made of phosphates called MCPs manufactured in Africa, Europe, Asia and South America, assigning each one its EPCD value. The average value has given 88. Based on the hypothesis that the value of the tables corresponds to the average value of the products on the market, it could be extrapolated that by formulating with a product of EPCD 91, an improvement in digestibility of $91/88 = 1,034$ would be introduced, so that the tables could be corrected with this factor and 83 % of tables could be replaced by $83 \% \times 1,034 = 85.8 \%$.

EPCD assessment in DCP.

As in the assessment for the MCP, the existing proportionality between the values

calculated through the Predictive Equations (EPCD) and the results obtained in the *in vivo* tests, correspond to products with the same granulometry (influential factor in the digestibility of the birds) and in experimental farm conditions (health, facilities and handling).

In this case, the correlation is less adjusted than in the MCP because it is necessary to improve the determination of the dicalcium anhydrate/dihydrate ratio. Due to the fact that the system used, besides measuring the hydration water, transforms the MCP into pyro, releasing reaction water that is counted as hydration.

Development of the EPCD index.

The availability of this index will allow a semi-quantitative comparison between different commercial phosphates. The application of this index can be used more reliably in poultry farming. In pigs it will be necessary to make an adjustment to the production phase.

The only factor not considered comparatively in the EPCD index is the variability in granulometry, so it would be necessary to apply it to products with the same granulometry. The correlation is less precise in its application on DCP, since it requires an improvement in the determination of hydration water. On the other hand, phosphorus homeostasis is intrinsically related to that of calcium, so the effect of intestinal absorption will also be.