1. INTRODUCTION

Historically there has been a move from the use of a total Ca (tCa) to total P (tP) ratio system (NRC, 1950) to a tCa to inorganic P (iP) ratio (NRC, 1954) to the use of tCa to available P (aP) that appeared in the 1984 NRC. In 1950 the requirements were 10 g/kg for tCa and 6 g/kg for tP (1.66 tCa:tP) (NRC, 1950). In the 1954 NRC the requirement was increased to 0.7 g/kg and a qualification was made to the tP values by specifying that of the 7 g/kg of tP required, 5.6 g/kg were needed from an inorganic source or animal protein source and plant P was given a 30% availability that could be part of the 5.6 g/kg requirement. The 1977 NRC still gave requirements in terms of tCa and tP with the proviso that part of the 7 g/kg of tP required from hatch to 8 weeks of age be supplied from inorganic sources of P and still using the calculation of 30% available P from plant sources. In the 1984 NRC, requirements for P were given as available P (aP) and aP values were also given for ingredients but no change was made to Ca values. The Ca:aP ratios recommended were 2.22 to 2.28 throughout growth (hatch to 8 weeks of age) in broilers. By 1994 (NRC, 1994) the term non phytin P (nPP) instead of aP was used but there were minimal if any changes to the ingredient values given or to the requirements. The tCa to nPP ratios recommended were 2.22 to 2.67 Ca depending on growth stage.
The low digestibility of P in plant sources (Van Der Klis and Versteegh, 1996; Coon and Leske, 1998; Angel et al., 2002; Tamim and Angel, 2003; Tamim et al., 2004) and the variable digestibility of P in animal and inorganic sources (Van Der Klis and Versteegh, 1996; Coon and Leske, 1998) prompted the change in the use of P, from total P to aP, nPP, dP or retainable P (rP) that better reflected availability of P in dietary sources. But there has has only recently been an interest in a digestible Ca system (dCa) for poultry. Because of the extensive use of phytases in poultry diets worldwide and the large excess in Ca usage in commercial poultry diets, the negative effect of Ca on phytate P and on phytase efficacy (Ballam et al., 1984; Scheideler and Sell, 1987; Mohammed et al., 1991; Mitchell and Edwards, 1996; Qian et al., 1997; Tamim and Angel, 2003; Li et al., 2015 a, b, c, d) there has been an emphasis on trying to get commercial nutritionists to use dCa in poultry diets. Because there is very little data on actual availability or digestibility, as opposed to relative availability, of calcium in feed ingredient sources nutritionists assume, in general, a 100% digestibility for Ca. Few actually will use availability values of 80 to 90% and these come primarily from research done determining relative availability. Relative availabilities clearly do not transfer directly to actual availabilities that can be used in formulation matrixes but can give an idea of availability rankings and relative differences. Based on reported Ca digestibilities in corn soy diets with no inorganic Ca or P sources added and the same diet with added limestone, one can calculate coefficient for the availability of Ca in the corn and SBM portion of the diet to be 0.2 to 0.3 (Tamim and Angel, 2003; Tamim et al., 2004) and for the Ca from limestone of between 0.6 and 0.7. In a corn and soy bean meal (SBM) the corn and SBM supply between 1.7 and 2.1 g/kg while other ingredients (limestone on phosphate source) supply the rest to achieve between 5 and 10 g of Ca/kg diet. We have up to now disregarded the low digestibility of this plant derived Ca because traditionally it represents a small (20%) part of a diet containing 10 g/kg of Ca typical of a broiler starter diet. But when we start seeing commercial withdrawal broiler diets with 6 g Ca/kg, then the digestibility of the Ca from the corn and SBM becomes important in that it is a greater proportion (33%) of the total Ca.

We will need to develop a dCa system to go with dP if we are to feed broilers at a requirement concentration for both P and Ca. That is, provide diets with concentrations of dCa and dP that meet the needs of the birds. For this we will need to develop methodologies to determine dCa in ingredients. Extensive work has been done on P availability methodology (Leske and Coon, 2002; Rodehutscord et al., 2002, 2003, 2012; Rodehutscord and Dieckmann, 2005; Shastak et al., 2012a and 2012b). A recent publication provides an extensive review on P availability determinations (Shastak and Rodehutscord, 2013) and another provides a consensus methodology for P availability in the presence or absence of phytase (Rodehutscord, 2013).
2.- PHYTIC ACID CHEMISTRY AS IT RELATES TO CATION CHELATIONS

Understanding the chelating ability of phytic acid towards mineral cations is necessary if we are to understand the implications of these interactions. Phytic acid has 12 replaceable protons or reactive sites, six of these are strongly acidic with pKs that range from 1.5 to 2.0, two are weakly acidic with a pK of approximately 6.0 and four are very weakly acidic with a pK between 9.0 and 11.0 (Costello et al., 1976, Erdman, 1979). This means that at the pH values normally encountered in feeds, phytin will carry a strong negative charge and thus is capable of binding di- and trivalent cations such as Ca, Co, Cu, Fe, Mg, Mn, Ni and Zn in very stable complexes (Wise, 1983, Persson et al., 1998, Maenz et al., 1999) reducing the availability of these chelated minerals as well as the PP to the animal (Pallauf and Rimbach, 1997). The molar ratio of phytate to minerals as well as pH, influence the formation of these chelates (Wise, 1983, Pallauf and Rimbach, 1997). The pH of the solution influences the ionization potential of the phytic acid and of other molecules thus influencing binding capacity as well as the binding strength (Kaufman and Kleinberg, 1971). In his review, Wise (1983) suggested that a diet with a molar ratio of Ca:phytate greater than 6:1 leads to the formation of an insoluble Ca-phytate complex, which are inaccessible to phytase.

This is important, from a poultry nutrition standpoint, because the physical state or solubility of these chelates in the digesta is associated with the degree and strength of chelation. If the phytin-cation complex is precipitated, phytase added to the diet be unable to exert their hydrolytic effect. The increase in pH of the gastrointestinal tract (GIT) contents as it moves distally causes the PP molecule to be ionized and thus, more readily form complexes with divalent metal cations like Zn, Ca, Mg and Fe (Wise, 1983, Maenz et al., 1999). At higher pHs, this complexion results in decreased solubility (Kaufman and Kleinberg, 1971) of the complex and thus in decreased efficacy of phytase (Maenz et al., 1999).

Several researchers have reported the stability at specific pHs of IP6-mineral complexes. Stability of these complexes at a specific pH is related to the strength of the complexes. At a pH value of 7.4, Vohra et al. (1965) found that order of stability of Cu<sup>2+</sup> > Zn<sup>2+</sup> > Ni<sup>2+</sup> > Co<sup>2+</sup> > Mn<sup>2+</sup> > Fe<sup>2+</sup> > Ca<sup>2+</sup>. Maddaiah et al. (1964) reported that Zn<sup>2+</sup> had a greater stability than Cu<sup>2+</sup>. Both groups of researchers concluded that the readiness of the metal binding reaction to phytate as well as the solubility of the chelate was pH-dependent. Maenz et al. (1999) reported that the order of potency of minerals to inhibit PP hydrolysis at pH 7 was Zn<sup>2+</sup> > Fe<sup>2+</sup> > Mn<sup>2+</sup> > Fe<sup>3+</sup> > Ca<sup>2+</sup> > Mg<sup>2+</sup>. This ability of the different metal ions to inhibit PP hydrolysis would be related to the stability of the complex as well as to the pH of the solution and the phytin-mineral molar ratio. Despite these reports where...
the phytate-Ca\(^{2+}\) complex has lower stability than other metal ion-phytin complexes, in animal diets it may be Ca that plays the most critical role in minimizing the effectiveness of both exogenous as well of endogenous phytases. The level of Ca that is added to poultry diets is a magnitude of eight to 40 times higher than that for Zn and the impact of the much higher concentrations may be more powerful than the lower affinity. This would have an effect not only of the PP availability but also on Ca availability. Thus it is important to consider the impact of phytic acid concentrations in the diet on the availability of Ca from sources such as limestone.

Some of the discrepancies in the order of impact between the different metal ions may be due to differences in molar ratios, pH at which the work was done and overall characteristics of the solution. For example, stability of the metal-IP6 complexes as well as their solubility and their ability to inhibit PP hydrolysis increase as pH decreases but this decrease may be different for each cation (Persson et al., 1998). The impact of Ca may be exacerbated as secondary synergistic effects have been reported when two cations are present simultaneously; they increase the proportion of the metal-IP6 complex that precipitates (Simpson and Wise, 1990).

3.- VARIABILITY IN THE REPORTED Ca TO P RATIOS

Standard use of tCa to dP ratios of 2:1 have been implemented commercially for more than 30 years with the P being either available, nPP or digestible and with most formulation matrix values being of unknown source. In practical diets, nutritionists try to maintain a 1.8 to a 2.2 tCa to dP ratio regardless of cost. The question is why and does this constraint lead to diets that do not contain the correct dCa and dP needed by broilers and that are costly?

Reports abound in the literature where the tCa to dP ratios at “optimal” performance or bone mineralization are not 2:1. For example, Driver et al., (2005a) reported that a 1:1 tCa to tP ratio maximized body weight gain (BWG) and feed to gain ratios from 0 to 16 d of age in broilers. They fed a corn-SBM diet with graded increases in Ca and P from limestone, monocalcium phosphate and dicalcium phosphate. But, of note, they also reported similar BWG with tCa:tP ratios of between 0.94 to 1.25. Tibia ash was maximized at tCa to tP ratios of 1.07 to 1.35. Interestingly, in diets where tibia ash was maximized these authors reported a significant incidence of Ca rickets (10.4 g/kg tCa to 7.9 g/kg tP) and of tibial dischondroplasia (TD) (8.5 g/kg tCa to 7.9 g/kg tP). Incidence of TD was lowest when diets with a 1.29 to 2.17 tCa to tP ratio were fed. In diets with tCa to tP ratios close to 1 and below 1 the incidence of TD was higher especially when Ca
concentrations were below 6.4 g/kg. In a second study also to 16 days of age (Driver et al., 2005a) that included phytase (657 FTU/kg) shows that tCa to tP for maximal BWG was seen between 0.68 and 1.28 while that for maximal tibia ash was between 0.93 and 1.29. This shows that the inclusion of phytase allowed for lower tCa to tP ratios to be fed. These data suggest that availability improvements due to phytase are lower for Ca than for P. Based on this, nutritionists should at least question the 1 to 1 or even a 1.3 to 1 Ca and P matrix values for commercial phytases.

In another study where Ca requirements between 1 and 16 d of age were determined (Driver et al., 2005b) using broken line analysis and 1 concentration of tP (6.3 g/kg) and calculated nPP of 4.5 g/kg, the requirements reported were at tCa to tP ratios of 0.77, 0.99, 1.14 for BWG, feed to gain ratio and tibia ash, respectively. If one puts these values in terms of tCa to nPP ratios, the ratios would be 1.08, 1.39, and 1.60, respectively. Clearly the ratios reported by Driver et al., (2005a, b) differ from our standard industry use of 2:1 for tCa to aP ratios and opens the door for diets with broader or tighter ratios.

Care must be taken with implementation of wide or narrow tCa:aP or dP (Phillips et al., 2012; Jimenez-Moreno et al., 2013 a, b, c, d). When looking at using narrow ratios (below 1.3:1), it is important to not implement these ratios when at Ca below 7 g/kg. At low Ca concentrations the danger is that P availability will increase (Tamin and Angel, 2003) resulting in potentially inverse dCa to dP ratios and these ratios lead to rapid homeostatic driven mineral mobilization from bone and decreasing bone mineralization. When least cost formulation results in broad ratios (up to 4 tCa:1 aP) it is essential to make sure the P needs of the birds are met.

In recent Ca and P requirement work done at the University of Maryland (Jimenez-Moreno et al., 2013a) tCa to nPP ratio for maximal bone ash was found at 10.5 and 6.0 g/kg Ca and nPP, respectively or 1.61 tCa to nPP ratio from hatch to 10 days of age. The dCa and dP requirement was at 6.6 g/kg dCa and 5.0 g/kg dP (Jimenez_Moreno et al., 2013c). This lower apparent need for Ca in comparison to P in the prestarter phase may be related to the moderately high concentrations of Ca as compared to P in the residual yolk (Richards and Packard, 1996; Yair and Uni, 2011). Having a non dietary source, the residual yolk, as a source of Ca will result in narrow when only diet sources are considered as supplying the Ca. Residual yolk weights have been reported to decrease rapidly post hatch, in arbor acre chicks, until day 4 of age at which point they are only 25% of the weight of the residual yolk at hatch (Huang et al, 2008).
4. MAKING THE CASE FOR THE NEED FOR A DIGESTIBLE CALCIUM SYSTEM IN THE CONTEXT OF CALCIUM TO PHOSPHORUS RATIOS AND IMPLEMENTATION OF PHYTASE Ca MATRIX VALUES

We have known for some time that the digestibility of P is low and variable in seed based ingredients (Nelson 1976) and not 100% in animal or inorganic based sources (Van der Klis and Versteeg, 1996; Coon and Leske, 1998). The use of 100% availability of P in inorganic sources clearly is not warranted. Data on digestible Ca values ingredients is lacking as most of the data available is relative and not absolute.

In recent work at the University of Maryland, true digestibilities of Ca and P from SBM, CaCO$_3$ and mono calcium phosphate were determined (Proszkowiec-Weglarz et al., 2013 a, b; Proszkowiec-Weglarz and Angel., 2013) and where the specific ingredients were the only source of the P or Ca in purified diets, true digestibility coefficient for Ca in limestone or mono calcium phosphate were 0.341 and 0.679, respectively in 25 d old broilers. Of importance is the low Ca digestibility of a feed grade limestone as compared to that in mono calcium phosphate when the absolute Ca concentration was similar. In a second study the calculated coefficient of digestibility of Ca in the same CaCO$_3$ (0.363) used in the first study was very close to that determined in the first study (Angel et al., 2013).

If indeed the digestibility of Ca from mono calcium phosphate is 2 times higher than that of Ca from a commercial source limestone, then what are the impacts on Ca to P ratios when phytases are used? When we use phytases in broiler diets, usually inorganic phosphates are decreased or removed and Ca from limestone increases and replaces that removed with the phosphate sources. If we use an example of a corn-SBM diet with and without phytase where we give phytase similar matrixes for Ca and P, the amount of mono calcium phosphate in the diet would be reduced by 60% and of limestone by 12%. What are the implications from a digestible ratio perspective? If we assume a dCa to dP ratio in the diet without phytase of 1.6 to 1 then the ratio in the diet with phytase would be close to a 1 to 1 ratio. This change brought about by the large decrease in the amount of mono calcium phosphate with the much higher dCa.

What Ca to P ratios to use become even more difficult to determine in diets with animal Ca and P sources, where sometimes under commercial conditions, we find ourselves adding inorganic P sources to maintain Ca to aP ratios. Having ingredient dCa as well as dP values is essential and to date little if any dCa ingredient data is available. Requirement work, in the future should provide data as dCa and dP requirements for optimization of performance and bone measures. We also must strive to define ranges of
dCa to dP that optimize performance and mineralization and minimize welfare issues as well as minimizing TD problems.

5.- CONCLUSIONS

As we try to formulate closer to the Ca and P requirements of the bird and use phytases with ever improving efficacies, it becomes more and more important to move to a dCa and a dP system that allows nutritionists to formulate diets that meet the actual needs of the bird rather than just lead the system with a chemical quantity of Ca of unknown availability. We must accept that dCa in inorganic sources varies widely within and between sources. Furthermore, when we use phytase we change Ca source from a calcium phosphate that usually has higher dCa, to a limestone that usually has a lower dCa and a more variable dCa within source.

The current system of tCa to a P value that partially reflects P digestibility no longer serves our needs for formulating diets where we are trying to minimize P concentrations, reduce costs, use alternate ingredients and feed additives that change both the digestibilities of Ca and P.

6.- SUMMARY

As we learn more about the negative impacts of calcium (Ca) on the availability of phosphorus (P) directly or through interactions with phytate P, as well as on the efficacy of phytase, it highlights how little we know about Ca, Ca to P ratios and points to areas where our knowledge is poor at best. Historically the ratio of Ca to P has been defined as total Ca and total P in the diet. References to total Ca and total P still appear in the literature. As the impact of phytate present in seed based ingredients and how variable and low phytate P availability was better understood, a change was made to a total Ca to available P ratio. Unfortunately, available P was defined crudely as the sum of total P in inorganic based sources plus total P in animal based sources and 30% of the P in plant based sources. This definition of available P has been refined and changed over time to reflect a better understanding of phytate P availability but only recently have we challenged the use of total Ca for formulating diets. Ultimately, for the animal, what is important is the amounts of Ca and P that are available for the animal to use for deposition in body tissues as well as for metabolic pathways and these are the values that we need to define with some accuracy. What name is given to these values, continues to be debated but for the purpose of this paper, digestible will be used. Digestible Ca or P will be defined as the amounts of
these nutrients in the diet that have disappeared from the digesta by the distal ileum and will be clearly identified as apparent (not corrected for endogenous losses) or true. The goal would then be to define a range of optimal digestible Ca (dCa) to digestible P (dP) ratios for diet formulation. In order for this to be feasible we would need to have ingredient dCa and dP values that we can include in formulation matrices. We also need a better understanding of the impact of other factors beyond the actual digestibility of the Ca and P in ingredients, that affect the ability of the animal to digest and/or absorb Ca and P. For example the selection of a phytase, phytase concentration, Ca concentration and particle size and solubility, P source and concentration, inclusion and concentrations of vitamin D or its metabolites, other feed additives, age, and diet ingredient selection will all impact Ca and P digestibilities. We also need to re explore the impact of dCa on dP and dP on dCa. The focus then of this short paper is to highlight the importance of moving towards a better system for Ca and P that reflects what the animal actually can use and not just a total content in a diet, how this affects Ca and P ratios, how we implement phytase matrices, as well as to highlight how little we know on this subject.

7.- REFERENCES


