

Review

Phosphorus Bioavailability: A Key Aspect for Conserving this Critical Animal Feed Resource with Reference to Broiler Nutrition

Xiuhua Li *, Dagong Zhang, Tsung Y. Yang † and Wayne L. Bryden

Poultry Science Unit, School of Agriculture and Food Sciences, The University of Queensland, Gatton Qld 4343, Australia; d.zhang@uq.edu.au (D.Z.); t.yang2@uq.edu.au (T.Y.Y.); w.bryden@uq.edu.au (W.L.B.)

* Correspondence: x.li1@uq.edu.au

† Deceased.

Academic Editor: Les Copeland

Received: 8 March 2016; Accepted: 23 May 2016; Published: 27 May 2016

Abstract: Phosphorus (P) is an essential element, and the majority of animal feed phosphate is derived from phosphate rock that is a non-renewable resource. Current global P reserves may be depleted in 50–100 years. This poses the challenge of securing future P supply for the global animal feed industries. Currently, nutritionists formulate diets with substantial safety margins to guarantee that animals do not become P deficient. Excessive dietary P concentrations increase, not only the cost of diets, but also P excretion and pollution of the environment. We contend that understanding P bioavailability is central to the sustainable use of this mineral in animal agriculture. Poultry accounts for approximately 50% of animal feed phosphate consumption worldwide and for this reason we use the meat chicken or broiler as a case study to explore the nuances of P bioavailability. We conclude that, to tackle the challenge of dietary P bioavailability, cooperative research on a global scale is needed to standardise measurement procedures in order to produce a robust and reliable database which can be used by nutritionists to formulate diets to meet the bird's P requirements precisely. Achievement of this goal will assist endeavours to sustain the global supply of phosphorus.

Keywords: phosphorus; requirements; available P; bioavailability; poultry; animal nutrition; rock phosphate; global feed supply

1. Introduction

Phosphorus (P) is an essential element for all forms of life, from single to multicellular organisms. It is required for normal muscle growth and egg formation, is an important component of nucleic acids, the genetic code, and phospholipids, and is also a co-factor of many enzyme systems. Phosphorus plays a vital role in maintaining osmotic and acid-base balance, energy metabolism, amino acid metabolism and protein synthesis. Phosphorus was named “life's bottleneck” by chemist and science writer Isaac Asimov [1], who claims: “Life can multiply until all the phosphorus has gone, and then there is an inexorable halt which nothing can prevent,” also writing, “We may be able to substitute nuclear power for coal, and plastic for wood, and yeast for meat, and friendliness for isolation—but for P there is neither substitute nor replacement.”

The main ingredients of non-ruminant diets are cereal grains and their by-products. These diets usually require supplementation with inorganic P, because of the low concentrations, and low availability of total P in cereal feed ingredients. Meat and bone meal has relatively high bioavailable P content but is banned from use in animal feed in European countries. This policy increases the demand for inorganic feed phosphates [2]. The majority of feed phosphate is derived from phosphate rock that is a non-renewable resource and becoming increasingly scarce and expensive [3]. Current global P reserves may be depleted in 50–100 years [4,5]. This poses the challenge of securing future P supply

for the international and national animal feed industries. Poultry account for approximately 50% of animal feed phosphate consumption worldwide [6]. Feed phosphates make a significant contribution to the P content of any broiler diet; generally providing as much as 60% of the bird's P requirements [7]. There is limited information on biologically determined P values in feed ingredients for poultry [8–10]. Therefore, nutritionists have to formulate diets with substantial safety margins to guarantee that birds do not become P deficient. Greater dietary P concentrations increase the cost of poultry production, and P excretion into manure. Excessive amounts of P in manure contribute to the pollution of lakes, streams and wetlands leading to surface water eutrophication. Eutrophication of fresh water globally is accelerated by P accumulation [11,12].

All these concerns have attracted much global attention and stimulated the re-examination of the use of inorganic P in animal diets. This issue is pertinent to all non-ruminant animals that require dietary P supplementation, especially poultry (broilers, layers and breeders) and pigs. In this review, the focus is on meat chickens or broilers as much of the P used in animal nutrition is in poultry diets. In an effort to optimise P use by poultry, the P requirements of the modern broiler have been re-examined [13–18] and defining P bioavailability in feedstuffs has commenced [2,19]. Mention of bioavailability, which encompasses digestion, absorption and utilisation of P, evokes much debate and is the thrust of this review.

In many instances, the terms “availability” and “bioavailability” are interchangeable when referring to nutrients. However, to avoid confusion in this review, we use the term bioavailability to encompass the digestion, absorption and utilisation of P, as the term “available P” has a specific connotation in P nutrition as described below. We contend that bioavailability is central to the sustainable use of P in animal agriculture. A thorough understanding of P bioavailability will permit the development of strategies to enhance efficiency of P utilisation, reduce wastage of P resources and reduce environmental pollution by P.

2. Phosphorus Metabolism

It is important when discussing phosphorus requirements and bioavailability to appreciate the metabolism of phosphorus (Figure 1) and to remember the close association that phosphorus homeostasis has with the metabolism of two other nutrients; calcium (Ca) and vitamin D. The major features of these interactions are briefly reviewed here and the interested reader is referred to detailed reviews on the topic [20,21]. The major store of Ca and P is the skeleton where the cationic and anionic forms, respectively, of these minerals connect to form hydroxyapatite, which confers rigidity on the bone matrix [2]. Bone is continually turned over and to ensure that the biological demands for both Ca and P are met, metabolism of these essential minerals is tightly coordinated.

Utilisation of Ca and P is modulated by the relative amounts of each in the diet [2]. However, the quantities of each available for their respective metabolic functions is determined by efficiencies of intestinal absorption, glomerular filtration, renal tubular reabsorption, rates of transfer from blood to bone, and intestinal endogenous losses [20]. The efficiencies of these processes in the different tissues are modulated by several hormones, chiefly parathyroid hormone (PTH) and the hormonal form of vitamin D₃ (1,25 dihydroxycholecalciferol; calcitriol) [20,21]. The control of P metabolism differs from that of Ca, with less control in the gut and greater control in the kidneys. Moreover, there is increasing evidence that dietary protein and carbohydrate affect intestinal metabolism of Ca and P [21]. Together, the factors that determine intestinal absorption and kidney excretion along with endocrine regulation of absorption and reabsorption in the kidneys facilitate P homeostasis.

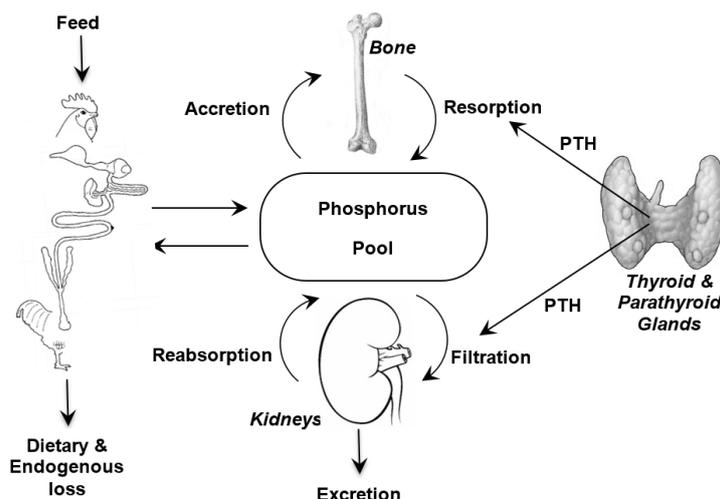


Figure 1. Phosphorus metabolism. Coordination of three organ systems, intestine, kidneys and bone, is central to Ca and P homeostasis. Reduced circulating concentrations of Ca causes increased secretion of parathyroid hormone (PTH) by the parathyroid glands. PTH increases bone resorption and renal Ca reabsorption but increased renal excretion of P. In the kidneys, PTH also stimulates formation of the hormonal form of vitamin D₃ which increases intestinal absorption of Ca and, to a lesser extent, the absorption of P. Elevated blood Ca concentrations as a result of these processes creates a negative feedback loop to ensure homeostasis. If hypercalcaemia occurs, calcitonin is secreted by the thyroid gland to inhibit bone resorption.

3. Phosphorus Requirements

Research on the P requirement of broilers has been the subject of numerous investigations for many decades; however, the minimum requirement for this nutrient has still not been established with any certainty. The published results are variable as different breeds of birds, age of birds, feed ingredients (with or without phytase supplementation), Ca and P sources were used in different studies. In some instances, the differences observed may reflect true differences in requirements, not merely variation resulting from experimental conditions. To further complicate interpretation of results, different criteria, including body weight gain, feed conversion ratio (FCR), tibia breaking strength, tibia ash, or toe ash contents have been used to assess responses to P (Table 1).

Table 1. Summary of available P requirements (g/kg diet) of broilers.

Criteria of Assessment	AP (g/k) (Bird Age, Day)		Reference
	1–21	21–42	
Carcass P analysis	3.7 (day 0–10), 3.0 (day 10–30)	2.4 (day 30–40)	[2]
Bone breaking strength	3.9	3.0	[13]
FCR		1.63	[22]
FCR	3.3–3.9		[23]
Growth and tibia ash	3.2–2.8 (day 18–32)	2.4–1.9 (day 32–42), 1.1 (day 43–49)	[24,25]
Growth, bone ash and processing losses	4.5	4.0	[26]
Weight gain	3.5 (day 0–14)	3.0 (day 15–49)	[15,18]
Weight gain	3.2–3.4		[27]
Weight gain		1.86	[22]
Weight gain	3.2–3.5		[23]
Weight gain	2.8–2.9		[28]
Tibia or toe ash	4.5	3.5	[29]
Tibia ash		3.3	[22]
Tibia ash	3.5–3.9		[23]
Tibia ash	3.7–3.9	3.1 (day 49)	[30]

Evidence in the literature clearly demonstrates that the P requirement of broilers is much lower than NRC [29] recommendations and the values currently used by the industry [2,7,13,15,17,18,22–25,27,28,31]. The greatest challenge in applying a lower dietary P strategy in practice is to decide what dietary concentrations of P to use in a formulation. This is due to a lack of information on biologically determined P values in feed ingredients for poultry and an agreed P evaluation system [8–10].

4. Evaluation of Procedures that Determine Bioavailable Phosphorus

There are many approaches for determining P bioavailability of feed and feed ingredients. Some methods are biological and rely on *in vivo* measures including growth, bone strength, bone ash content and blood parameters. Chemical procedures have also been developed to estimate P bioavailability. Most published values using both biological and chemical procedures are qualitative rather than quantitative. Recently, there has been an international move to establish ileal digestible P as the method of choice for determining P bioavailability. An overview of the different approaches for the determination P bioavailability is given below.

4.1. Biologically Determined Values

Initial attempts to quantitate the availability of P for chicks from various phosphate sources used tibia bone ash as the response factor. The method was based on relative percentage of tibia bone ash of chicks fed a test supplement to that of chicks fed a reference standard, thus obtaining relative bioavailability P values [32]. A positive linear relationship was found between tibia bone ash percentage and dietary P intake. A similar relationship was found with toe ash percentages [33–35]. Hoshii and Yoshida [36] demonstrated that P bioavailability determined with a slope ratio assay using toe ash was closely associated with the values determined by carcass P retention, suggesting that changes in toe ash concentration reflect changes in net P utilisation.

In the 1980s and '90s, the bioavailability of P was routinely estimated by non-linear bioassays (asymptotic or sigmoidal curves) of body weight and tibia bone or toe ash, using a graded range of P supplements [37–41]. Others [42,43] determined P bioavailability using responses in bone ash content, bone breaking strength, body weight gain or blood inorganic P concentration of birds fed a test P source compared with the response of birds to a standard reference P source. Bioassays based on growth, blood parameters, tibia bone or toe ash percentage and tibia bone breaking strength can only provide a relative biological reference value or ranking [42,43] and do not measure true or apparent P bioavailability *per se*. The values do not define the quantity of bioavailable P and therefore have limited value when formulating diets [44].

Early attempts to determine true or apparent P retention by collecting excreta in balance studies with chicks were unsuccessful [45,46]. Later, Simons and Versteegh [47] were successful using a balance assay based on excreta collection, when birds were fed a basal diet, nearly free of P (5 g/kg Ca), to which test phosphates were added at low concentrations of approximately 2 g/kg AP. At this level, there is negligible urinary P excretion [47].

4.2. Chemically Determined Values

The most common P evaluation system (NRC, [29,48]) is based on the relationship of P with phytate; non-phytate P (NPP) and phytate P (PP). Phytate is the bound form in which P is found in plants; it is discussed in detail elsewhere (see [49–53]). The NPP system has the advantage of being based on the chemical analysis of feed ingredients. Non-phytate P is the P in the feed or ingredient that is not bound as part of phytic acid. It can be determined by subtracting analysed PP from analysed total P [14]. With this approach, it is assumed that NPP, together with P from non-plant sources are completely available to poultry and non-ruminant animals; assuming that PP is not available to non-ruminant animals. If PP in plant feedstuffs is not analysed, it is assumed that NPP is 30% of total plant P and the remaining 70% is PP. Non-phytate P has been referred to as “AP” or used

interchangeably with “AP.” These assumptions based on simple differentiation from chemical analysis have been adopted in diet formulation worldwide, since publication by the NRC in 1977.

The NPP system has limitations. Chemical analysis determines how much P is present, but does not indicate to what degree, if any, P is utilised by animals and birds. In other words, chemical analysis does not provide information on P bioavailability of the ingredient or diet and thus any changes in PP and P bioavailability of an ingredient will not be reflected in this system. Numerous studies have found that NPP is not 100% bioavailable and up to 80% of PP can be bioavailable to birds [8,54,55]. In fact, no elements are completely absorbed and utilised by poultry [56].

Phytate has been found in the excreta of the chicks fed a phytate-free (casein-gelatin) diet, whereas none was found in the excreta of germ-free chicks fed the same diet. It appears that phytate may be synthesised by the microflora in the avian digestive tract [57]. Therefore, it is possible that the net microbial hydrolysis of phytate occurring during transit of digesta through gastrointestinal tract may be partly offset by microbial synthesis [58]. This makes determination of dietary NPP requirement by total collection or indigestible marker and analysing NPP in excreta unreliable since some NPP may have been incorporated into phytate. Other chemical or *in vitro* approaches have attempted to relate the solubility of P supplements to P bioavailability determined *in vivo* and have been unsuccessful [59].

4.3. Ileal Digestibility as an Estimate of Phosphorus Bioavailability

In an attempt to overcome the difficulties associated with current approaches to P bioavailability determinations, the Nutrition Working Group of the European Federation of Branches of World’s Poultry Science Association (hereafter Rodehutschord and WPSA, [60]) has developed a protocol to determine ileal P digestibility by regression analysis. Two to three diets are formulated for each test P source. A low P basal diet is used, and a minimum of two levels of the P source under test are added to the test diet. At the highest level of inclusion, P supply must not exceed the requirement. With such conditions, the AP of the P source under test can be determined from the slopes of linear regression equations (see [60] for details). The protocol is currently being evaluated in an international collaborative ring test. Collinearity appears to exist between two variables used in the regression analysis and may lead to unreliable and unstable estimates of regression coefficients [61].

Finally, none of the methods outlined above measure P bioavailability *per se* but rather provide an estimate. Accurate measurements of bioavailability would require very expensive and laborious experiments, including the use of radioactive isotopes. What is required is a robust procedure that will provide reliable and repeatable estimates of P bioavailability. It would appear that precaecal P digestibility is the method of choice. A similar situation exists with amino acid availability where precaecal or ileal digestibility is used to estimate availability. Detailed discussions that describe amino acid digestibility assays are available [62].

5. Terms Used to Describe Bioavailable Phosphorus

Over the years, a number of terms have been suggested to define certain aspects of P utilisation as described by Rodehutschord and WPSA [60] and outlined below. These terms have largely arisen to reflect differing approaches to the determination of bioavailable P.

Digestible P is the portion of dietary total P that is not recovered in faeces. The determination of digestible P requires *in vivo* studies with quantitative determination of dietary P intake and P excretion in faeces. Alternatively, digestible P can be determined by using an indigestible marker. However, as bird excreta consists of faeces and urine, determination of digestible P requires colostomised birds to exclude urinary P content.

Retainable P (RP) indicates the proportion of dietary total P that is retained in the body and can be determined quantitatively following measurement of P intake and P excretion in faeces [60]. It is also called total tract P digestibility and is easy to measure. However, as urine is included in the determination of digestible P, this term is not an appropriate description of the measurement in poultry.

Importantly, if a P intake is lower than the requirement, the P content of the urine can be ignored. In these circumstances, the determination of RP should be similar to the determination of digestible P.

Available P (AP) is the quantity of P that is absorbed from the diet by the animal. This definition of AP is different from previous interpretations [29,60] where it has been equated to NPP and RP. In the currently acceptable definition of AP [60], it is determined by including an indigestible marker in the feed and is the proportion of total dietary P that is not recovered in the terminal ileum. It is also called precaecal digestible P or ileal P digestibility [60]. The major advantage of this method is that if P intake exceeds requirements, urinary excretion of P does not confound the results.

There is now general agreement that ileal digestible P is the preferred approach for determining AP as a quantitative estimate of P bioavailability. Rodehutsord and WPSA [60] recommend that retainable P values not be used in the future to avoid confusion. Retainable P reflects both P digestibility and post-absorptive utilisation since bird excreta includes faeces and urine. Dietary supply of P relative to requirement and body status affects total tract P utilisation [63]. In several studies of individual feed ingredients and mixed diets, the RP in total tract is substantially different from AP in the ileum (Table 2). Based on these and other publications that demonstrate a similar trend, both utilisation of P or Ca in feeds and requirement in birds should be expressed on an ileal digestible basis [63]. Furthermore, AP can be used to measure the marginal level of P supplied in animal diets to meet the P requirement of the animal [60].

Table 2. Comparisons of retainable P (RP) with available P (AP) in the ileum of poultry [63].

Ingredient	RP (%)	AP (%)	Reference
Low-phosphorus mixed diet	43	29	[64] ¹
Low-phytate soybean meal	77	94	[65] ²
Conventional soybean meal	60	94	[65] ²
Low-phosphorus mixed diet	59	52	[66] ¹
Low-phosphorus mixed diet	45	30	[67] ¹
Canola meal	39	66	[63] ²

¹ Expressed as apparent digestibility; ² Expressed as true digestibility derived from regression analysis.

6. Phosphorus Sources and Availability

Sources of P in poultry diets include feed ingredients of both plant and animal origin and also inorganic forms of the mineral. These sources differ in P bioavailability.

6.1. Plant Feedstuffs

Plant feedstuffs include cereal grains, oil seeds and the respective by-products. Phytate is the principal storage form of P in many plant tissues. As commercial broiler diets are primarily comprised of feedstuffs of plant origin, most of the P present in these ingredients is in the form of phytate P (PP) [68]. The concentration of PP within the same ingredient can vary considerably. Barrier-Guillot *et al.* [69] found that the concentration of PP ranged from 0.092% to 0.268% dry matter (DM) in wheat and depended on soil fertility, plant variety, and stage of plant maturity at harvest.

The bioavailability of PP for broilers ranges from 0% to 80% (Table 3) [2] depending on diets, bird age and metabolic adaptation. The degree to which PP is utilised by birds will depend, to a large extent, upon the hydrolysis of PP in the gastrointestinal tract. Moreover, the bioavailability of PP in different feed ingredients ranges from 0% to 60% [8,47,67], while the bioavailability of total P within ingredients varies considerably (15% to 75%; Li *et al.*, unpublished data [70]). There are many factors affecting PP utilisation: phytase activity of dietary ingredients, dietary concentrations of fibre, Ca, P and vitamin D₃, age of bird and genotype [58,71].

Table 3. Total P (TP) (g/kg), retainable P (RP) (% of TP), non-phytate P (NPP) (g/kg), phytate P (PP) (g/kg), PP (% of TP) and calculated PP degradability (%) of some of the feedstuffs in three-week-old broilers [2].

Feedstuffs	TP (g/kg)	RP (% TP) *	NPP (g/kg)	PP (g/kg)	PP (% TP)	PP Degradability (%) **
Beans	4.9	52	1.3	3.6	73.5	53
Lupin	3	72	1.5	1.5	50	80
Maize	3	29	0.7	2.3	76.7	16
Peas	4.1	41	1.5	2.6	63.4	23
Rapeseed	10.9	33	3.8	7.1	65.1	10
Rice bran	17.2	16	3.1	14.1	82	2
Soy bean (heat treated)	5.5	54	2	3.5	63.6	49
Soybean meal	7.1	61	2.8	4.3	60.6	61
Sunflower meal	11.9	38	4.2	7.7	64.7	19
Wheat	3.4	48	0.9	2.5	73.5	46
Wheat middlings	10.8	36	2.8	8	74.1	26

* Values were determined in broilers fed diets containing 1.8 g calculated retainable P and 5.0 g Ca per kg diet from 10 days of age onward. P retention was measured in a three-day balance period (from days 21 to 24) in which dietary P intake and excreta P output were measured quantitatively; ** Phytate P degradability was calculated as: $100 \times (AP/0.8 - NPP)/PP$ [2].

6.2. Animal Feedstuffs

There are a number of animal-sourced feedstuffs (fish meals, meat meals, meat and bone meals) used in poultry diets mainly as protein supplements. Among these, meat meal is also a potential P source depending on its bone content. Meat meals are prepared by rendering offal from abattoirs. There can be a wide variation between batches depending on the raw materials used and rendering conditions [72]. If the ash content is high, this indicates that it contains a higher amount of bone and is referred to as meat and bone meal. If the ash content is low it is referred to as meat meal.

Meat and bone meal is a variable source of protein for poultry diets, but can be an excellent source of Ca and P and other minerals. Therefore, it is commonly used as a P supplement in poultry diets [7]. However, there are few reports that have measured AP from animal-derived feedstuffs. Limited RP values of animal-derived feedstuffs are shown in Table 4.

Table 4. Total P (TP) (g/kg), retainable P (RP) (% of TP) of animal-derived feedstuffs in three-week-old broilers [2].

Animal Feedstuffs	TP (g/kg)	RP (% TP) *
Bone meal	76	59
Fish meal	22	74
Meat meal	29	65
Meat and bone meal	60	66

* Values were determined in broilers fed diets containing 1.8 g calculated retainable P and 5.0 g Ca per kg diet from 10 days of age onward. P retention was measured in a three-day balance period (from days 21 to 24) in which dietary P intake and excreta P output were measured quantitatively.

6.3. Inorganic Phosphorus Sources

Inorganic P sources are commonly derived from natural rock phosphates. These natural phosphates are not readily utilised by poultry or other animals but must be chemically modified before inclusion in animal feed. Rock phosphates are changed into various orthophosphate forms: monocalcium phosphate, dicalcium phosphate, monodicalcium phosphate, defluorinated rock phosphate and monosodium phosphate [44]. Some commonly used mineral sources and their respective P composition and retention rate are listed in Table 5.

Most of these studies determined apparent retention of P from each of the inorganic sources within the deficiency range of P. Leske and Coon [13] showed dramatic reductions in P retention from monocalcium phosphate as dietary P concentration approached the requirement level (98% retention

at half of the P requirement to 59% retention at requirement level). Waldroup [73] reported that nearly 50% of excreted P is likely to be of inorganic origin.

Table 5. Inorganic feed phosphates and their respective total P (TP) (g/kg) and retainable P (RP) (% of TP) [2].

Inorganic P Source	TP (g/kg)	RP (% TP) *	Reference
Calcium sodium phosphate	180	59	[2] *
Dicalcium phosphate	183	83	[74] ^{1,2}
Dicalcium phosphate (anhydrous)	197	55	[2] *
Dicalcium phosphate (hydrous)	181	77	[2] *
Monocalcium phosphate	226	84	[2] *
Monodicalcium phosphate (hydrous)	213	79	[2] *
Monodicalcium phosphate	203	77	[13] ¹
Monodicalcium phosphate	200	80	[13] ¹
Monodicalcium phosphate	216	81	[13] ¹
Monosodium phosphate	224	92	[2] *
Defluorinated phosphate	182	86	[74] ^{1,2}
Defluorinated phosphate	179	76	[74] ^{1,2}

* Values were determined in broilers fed diets containing 1.8 g calculated retainable P and 5.0 g Ca per kg diet from 10 days of age onward. P retention was measured in a three-day balance period (from days 21 to 24) in which dietary P intake and excreta P output were measured quantitatively; ¹ Apparent utilisation of P from inorganic sources by broiler as determined under deficiency conditions; ^{1,2} Retainable P determined through broken line slope response.

Phosphorus must be in the phosphate form to be absorbed by birds. Improper processing and higher temperatures have a detrimental impact on the biological availability of calcium phosphates. As phosphates are heated, pyro- and meta- complexes are formed which greatly reduce the P bioavailability of inorganic sources. The availability of inorganic P sources is substantially affected by hydration, with hydrated phosphate sources generally more biologically available than anhydrous sources of the same type [75]. Feed phosphate with larger particle size has been shown to be more biologically available. Larger particle phosphate is retained longer in the gizzard allowing more complete digestion [76].

7. Factors Affecting Phosphorus Availability

Determination of the bioavailability of P in feedstuffs is a major challenge. There are many factors affecting P bioavailability in birds, including: experimental techniques; the chemical form of P; dietary concentrations of Ca, vitamin D₃, energy, protein, fat, and vitamin K; the availability and interaction of other nutrients in the diet, feed processing, and particle size; bird physiological and health factors, such as feed consumption, growth rate, sex and age; management factors, including ambient temperature and lighting program. Some of these factors have been investigated in detail [14] and the major factors are briefly described below.

7.1. Phosphorus and Phytate

High levels of dietary inorganic P reduce PP availability [49,77] due to either the inhibitory effect of P on phytase activity [78], a negative feedback mechanism by excessive amounts of dietary P, or inhibition by products of phytate hydrolysis [49,58].

Phytate is the principal storage form of P in plant feedstuffs and is considered to be an anti-nutritive factor [79]. Phytate carries a strong negative charge and is capable of binding di- and trivalent cations such as calcium, cobalt, copper, iron, magnesium, manganese, nickel and zinc in very stable complexes [58,80], reducing the availability of P as well as these minerals to the animal [2,54,55,81].

Phytate has been shown to inhibit activities of some digestive enzymes such as pepsin, α -amylase and trypsin. Phytate may inhibit proteolysis by changing the protein configuration of digestive enzymes [82]. Inhibition may also result from the chelation of Ca ions which are essential co-factors for the activity of trypsin and α -amylase leading to reduced protein and starch digestion [50,83–86].

It has also been reported that phytate can reduce fat digestibility by forming insoluble Ca-phytate complexes with fatty acids in the gut lumen [87]. In its chelated form, the phytate molecule is difficult to hydrolyse by phytase. The pH affects the solubility of phytate. Most phytate mineral complexes are soluble at low pHs (less than 3.5) with maximum insolubility occurring between 4 and 7 [88]. Champagne [89] found that Ca-phytate complexes precipitate at pH between 4 and 6, which is the approximate pH of the intestine where the Ca ions should be absorbed. Taylor [90] has suggested that the primary factor affecting PP utilisation is the Ca ion concentration in the small intestine where insoluble Ca-phytate complexes form. A precipitated phytate mineral complex would not be accessible for hydrolysis or absorption in the intestine.

7.2. Calcium

Calcium is an essential element for bone and egg shell formation, blood clotting, muscle contraction and transmission of nerve impulses. Calcium is also an important co-factor for many enzymes and hormones.

Increases in dietary P and Ca concentrations may affect apparent digestion of these nutrients. High levels of dietary Ca and low levels of P had detrimental effects on broiler performance [16,17,91,92]. Elevated dietary Ca levels increase pH in the gut and, as a result, P absorption [93] and retention [91] are decreased. High dietary Ca concentration can significantly increase gastrointestinal pH [94] and decrease not only phytate hydrolysis, but also pepsin activity in the proventriculus/gizzard and reduce apparent ileal protein digestibility [51]. High plasma P levels decrease Ca absorption from the gut [95]. The optimum dietary Ca-to-P ratio is of utmost importance for broiler production. With the Ca: P ratio, there will be a range of values that can meet metabolic demands for any production outcome. When dietary P concentration is high, birds can tolerate a wider range of Ca levels than when dietary P marginally meets the requirement (Li *et al.* unpublished data [96]).

Total Ca values are currently used for feed formulation since the bioavailability of Ca for feed ingredients have not been determined. Bioavailable Ca from plant raw materials is expected to be low. This is due to the high phytate content of these raw materials and the low contribution of Ca to the diet. Also, to some extent, phytate will negatively influence the availability of Ca from this dietary source by binding Ca in phytate-mineral complexes. Nevertheless, there is a need to determine the bioavailability of Ca in feed ingredients to further delineate the interaction of this nutrient with other nutrients and to improve the precision of feed formulation.

7.3. Vitamin D

Vitamin D₃ is essential for absorption of Ca and P utilization as depicted in Figure 1. Several reports have demonstrated that dietary addition of vitamin D₃ can significantly enhance the retention of P in birds [52]. However, excess vitamin D₃ and its metabolites have not been shown to have a further beneficial effect on bird productivity when birds are already consuming adequate vitamin D₃. Like many other minerals, understanding of the molecular mechanisms of P absorption, especially the role of the hormonal form of vitamin D₃, is still limited.

7.4. Phytase

Numerous studies have demonstrated that dietary addition of phytase increases hydrolysis of PP and improves P utilisation in pigs and poultry [15–18,53]. Maximum utilisation of phytate by broilers fed diets with supplemental phytase was approximately 50% on average [97] following dietary P supplementation. There are many factors affecting the efficacy of microbial phytase such as phytase source and dose [98], physical factors of feedstuffs, feed particle size, Ca to P

ratio [99], Ca bioavailability and inorganic P supplements, vitamin D status [100], bird age [101] and body weight [102], gut pH, digesta retention time, and Ca and P status. Phytase was found to be most efficacious when added to diets containing low AP and adequate Ca concentrations (Li *et al.* unpublished data [96]).

Dietary Ca level affects the efficacy of phytase in broilers [103], but not to the same extent for all phytases [104]. The same source of phytase can produce different responses in different flocks [105,106]. Moreover, the efficacy of feed phytase may be improved by the simultaneous dietary addition of other exogenous enzymes. All these variables make predicting phytase responses very difficult and lead to variation in P availability.

7.5. Birds

The ability of poultry to absorb dietary P is known to be influenced by age and physiological state. Young chicks with a rapidly developing skeletal system are critically sensitive to P deficiency [107] and tend to use P more efficiently than do mature birds [108]. Older birds have a greater ability to use PP, possibly due to more phytase activity in their intestinal tract [56]. A concern often raised by commercial nutritionists is the relevance of digestibility values generated with birds of one age group to birds of another age or other species.

Applegate *et al.* [106] conducted two experiments in which chicks were fed the same diet and found that the apparent PP hydrolysis varied nearly two-fold between the first and second experiment. Similar results have been reported by Nelson and Kirby [109] and Tamim *et al.* [103]. Bird variations make it difficult to predict treatment responses of different flocks. The variation between experiments and between flocks arises from many sources, some of which are summarised in Table 6.

Table 6. Summary of variation in processes associated with feeding of phosphorus to broilers [10].

Source of Variation	Coefficient of Variation (%)
Sampling variation	5 to 10
Analytical variation	5
Mixer variation	5 to 10
Bird utilisation	16
Ingredient variation (corn and soybean meal)	8 to 13

8. Conclusions

Re-examination of the phosphorus requirement of modern broilers has demonstrated that it is lower than the NRC [29] recommendations and the values currently used by the chicken meat industry. However, there are practical limitations in applying this information to industry as there is limited data on the bioavailability and variability of P in feed ingredients; there are few reports that have measured P bioavailability of feedstuffs for poultry. Furthermore, the different methods used have made comparisons between the studies and application to practice extremely difficult. It is only when commercial poultry nutritionists have this information that they will be in a position to reduce the safety margins for P when formulating diets. To tackle these challenges, cooperative research on a global scale is needed to standardise measurement procedures in order to produce a robust and reliable database which can be used by nutritionists to formulate diets to meet the bird's P requirement precisely; this approach is appropriate as only a couple broiler strains dominate international markets. As a starting point, the universal adoption of ileal P digestibility [60] as the preferred procedure to determine AP would be a major step forward. Moreover, to make sense of the data derived and to allow comparison of the results, it will be necessary to report ingredient composition of experimental diets, dietary concentrations of Ca and non-phytate P, dietary electrolyte balance and mineral content, all of which influence P absorption. Achievement of these goals will assist endeavours to sustain the global supply of phosphorus.

Acknowledgments: The Australian Government Rural Industries Research and Development Corporation -Chicken Meat Program, Canberra, ACT, has supported the phosphorus research conducted by the authors.

Author Contributions: All the authors contributed equally to the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Asimov, I. *Asimov on Chemistry*; Doubleday: Garden City, NY, USA, 1974.
2. Van der Klis, J.D.; Versteegh, H.A.J. Phosphorus nutrition of poultry. In *Recent Developments in Poultry Nutrition 2*; Garnsworthy, P.C., Wiseman, J., Eds.; Nottingham University Press: Nottingham, UK, 1999; pp. 309–320.
3. Shastak, Y.; Witzig, M.; Hartung, K.; Rodehutschord, M. Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers. *Poult. Sci.* **2012**, *91*, 2201–2209. [[CrossRef](#)] [[PubMed](#)]
4. Cordel, D.; Drangert, J.; White, S. The story of phosphorus: Global food security and food for thought. *Glob. Environ. Chang.* **2009**, *19*, 292–305. [[CrossRef](#)]
5. Neset, T.S.; Cordell, D. Global phosphorus scarcity: Identifying synergies for a sustainable future. *J. Sci. Food Agric.* **2012**, *92*, 2–6. [[CrossRef](#)] [[PubMed](#)]
6. Devereux, C.; Smart, M.; Kalt, F.P.; Takei, N. Animal feeds: Phosphate supplements. In *CEH Marketing Research Report*; HIS Chemical: Englewood, CO, USA, 1994.
7. Waldroup, P.W. Nutritional approaches to reducing phosphorus excretion by poultry. *Poult. Sci.* **1999**, *78*, 683–691. [[CrossRef](#)] [[PubMed](#)]
8. Van der Klis, J.D.; Versteegh, H.A.J. Phosphorus nutrition in broilers. In *Recent Advances in Animal Nutrition*; Garnsworthy, P.C., Wiseman, J., Haresign, W., Eds.; Nottingham University press: Nottingham, UK, 1996; pp. 71–83.
9. Cossa, J.; Oloffs, K.; Kluge, H.; Jeroch, H. Investigation into the TP and PP content in different varieties of grain maize. In *Proceedings of the 11th European Symposium on Poultry Nutrition*; World's Poultry Science Association: Faaberg, Denmark, 1997; pp. 444–446.
10. Applegate, T.J.; Angel, R. Feasibility *versus* Practicality of Phosphorus Reduction in Poultry: Progress and Future Needs. USDA National Agricultural Library: West Lafayette, IN, USA. Available online: http://www.cals.ncsu.edu/waste_mgt/natlcenter/sanantonio/ApplegateandAngel.pdf (accessed on 8 December 2014).
11. Schindler, D.W. Evolution of phosphorus limitation in lakes. *Sciences* **1977**, *195*, 260–262. [[CrossRef](#)] [[PubMed](#)]
12. Sharples, A.N.; Chapra, S.C.; Wedepohl, R.; Sims, J.T.; Daniel, T.C.; Reddy, K.R. Managing agricultural phosphorus for protection of surface waters: Issues and options. *J. Environ. Qual.* **1994**, *23*, 437–451. [[CrossRef](#)]
13. Leske, K.; Coon, C.N. The development of feedstuff retainable phosphorus values for broilers. *Poult. Sci.* **2002**, *81*, 1681–1693. [[CrossRef](#)] [[PubMed](#)]
14. Angel, R. Calcium and phosphorus requirements in poultry. In *Proceedings of the 1st International Phytase Summit*, Washington, DC, USA, 28–30 September 2010; pp. 65–71.
15. Li, X.; Zhang, D.; Huang, K.H.; Bryden, W.L. Available phosphorus requirement of broilers fed sorghum based diet from day 22 to 49. In *Proceedings of the 19th European Symposium on Poultry Nutrition*, Potsdam, Germany, 26–29 August 2013; p. 200.
16. Li, X.; Zhang, D.; Huang, K.H.; Bryden, W.L. Dietary calcium levels and non-phytate phosphorus requirement of broilers from days 1 to 21. *Aust. Poult. Sci. Symp.* **2013**, *24*, 50.
17. Yang, T.Y.; Setiyawan, E.; Li, X.; Zhang, D.; Bryden, W.L. Dietary calcium levels and non-phytate phosphorus requirement of broilers from days 22–49. In *Proceedings of Recent Advances in Animal Nutrition in Australia 2013*; University of New England: Armidale, New South Wales, Australia, 2013.
18. Zhang, D.; Li, X.; Huang, K.H.; Bryden, W.L. Available phosphorus requirement of broilers fed sorghum based diet from day 1 to 21. In *Proceedings of the 19th European Symposium on Poultry Nutrition*, Potsdam, Germany, 26–29 August 2013; p. 196.
19. Li, X.; Zhang, D.; Bryden, W.L. Bioavailability of phosphorus in feedstuffs for broilers. In *Final Report for Rural Industries Research and Development Corporation*; Rural Industries Research and Development Corporation: Kingston, Australia, 2015.

20. Klasing, K.C. *Comparative Avian Nutrition*; CAB International: Wallingford, UK, 1998.
21. Vitti, D.M.S.S.; Kebreab, E., Eds.; *Phosphorus and Calcium Utilization and Requirements in Farm Animals*; CAB International: Wallingford, UK, 2010.
22. Yan, F.; Kersey, J.H.; Waldroup, P.W. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. *Poult. Sci.* **2001**, *80*, 455–459. [[CrossRef](#)] [[PubMed](#)]
23. Persia, M.E.; Saylor, W.W. Effects of broiler strain, dietary nonphytate phosphorus, and phytase supplementation on chick performance and tibia ash. *J. Appl. Poult. Res.* **2006**, *15*, 72–81. [[CrossRef](#)]
24. Angel, R.; Applegate, T.J.; Christman, M. Effect of dietary non-phytate phosphorus (nPP) on performance and bone measurements in broilers fed on a four-phase feeding system. *Poult. Sci.* **2000**, *79* (Suppl. 1), 21–22.
25. Angel, R.; Applegate, T.J.; Christman, M.; Mitchell, A.D. Effect of dietary non-phytate phosphorus (nPP) level on broiler performance and bone measurements in the starter and grower phase. *Poult. Sci.* **2000**, *79* (Suppl. 1), 22.
26. Moran, E.T., Jr.; Todd, M.C. Continuous submarginal phosphorus with broilers and the effect of preslaughter transportation: Carcass defects, further-processing yields, and tibia-femur integrity. *Poult. Sci.* **1994**, *73*, 1448–1457. [[CrossRef](#)] [[PubMed](#)]
27. Waldroup, P.W.; Kersey, J.H.; Saleh, E.A.; Fritts, C.A.; Yan, F.; Stilborn, H.L.; Crum, R.C., Jr.; Raboy, V. Non-phytate phosphorus requirement and phosphorus excretion of broilers fed diets composed of normal or high available phosphate corn with and without microbial phytase. *Poult. Sci.* **2000**, *79*, 1451–1459. [[CrossRef](#)] [[PubMed](#)]
28. Yan, F.; Waldroup, P.W. Non-phytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed normal or high available phosphate corn as influenced by phytase supplementation and vitamin D source. *Int. J. Poult. Sci.* **2006**, *5*, 219–228.
29. NRC. *Nutrient Requirements of Poultry*, 9th ed.; National Academy Press: Washington, DC, USA, 1994.
30. Yan, F.; Kersey, J.H.; Fritts, C.A.; Waldroup, P.W. Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. *Poult. Sci.* **2003**, *83*, 294–300. [[CrossRef](#)]
31. Dhandu, A.S.; Angel, R. Broiler non-phytin phosphorus requirement in the finisher and withdrawal phases of a commercial four-phase feeding system. *Poult. Sci.* **2003**, *82*, 1257–1265. [[CrossRef](#)] [[PubMed](#)]
32. Gillis, M.B.; Norris, L.C.; Heuser, G.F. Studies on the biological value of inorganic phosphates. *J. Nutr.* **1954**, *52*, 115–125. [[PubMed](#)]
33. Grau, C.R.; Zweigart, P.A. Phosphatic clay as a phosphorus source for chicks. *Poult. Sci.* **1953**, *32*, 500–503. [[CrossRef](#)]
34. Fritz, J.C.; Roberts, T.; Bochner, J.W.; Hove, E.L. Factors affecting the chick's requirement for phosphorus. *Poult. Sci.* **1969**, *48*, 307–320. [[CrossRef](#)] [[PubMed](#)]
35. Yoshida, M.; Hoshii, H. Improvement of biological assay to determine available phosphorus with growing chicks. *J. Poult. Sci.* **1977**, *14*, 33–43. [[CrossRef](#)]
36. Hoshii, H.; Yoshida, M. Comparison of phosphorus availabilities estimated biologically based on toe ash content and carcass phosphorus retention. *J. Poult. Sci.* **1977**, *14*, 279–283. [[CrossRef](#)]
37. Potchanacom, M.; Potter, L.M. Biological values of phosphorus from various sources for young turkeys. *Poult. Sci.* **1987**, *66*, 505–513.
38. Potter, L.M. Bio-availability of phosphorus from various phosphates based on body weight and toe ash measurements. *Poult. Sci.* **1988**, *67*, 96–102. [[CrossRef](#)] [[PubMed](#)]
39. Potter, L.M.; Potchanacom, M.; Ravindran, V.; Kornegay, E.T. Bio-availability of phosphorus in various phosphate sources using body weight and toe ash as response criteria. *Poult. Sci.* **1995**, *74*, 813–820. [[CrossRef](#)] [[PubMed](#)]
40. Ketels, E.; De Groote, G. The relative bio-availability and the ileal digestibility of phosphorus in mineral and animal sources. In Proceedings of the 18th World's Poultry Congress, Nagoya, Japan, 4–9 September 1988; pp. 873–875.
41. De Groote, G.; Ketels, P.; Seynaeve, P. Relative bio-availability of phosphorus in meat meals as influenced by particle size. In Proceedings of the International Poultry Congress, Istanbul, Turkey, 6–10 September 1991; pp. 42–52.
42. De Groote, G. Biological availability of phosphorus in feed phosphates for broilers. In Proceedings of the 4th European Symposium on Poultry Nutrition, Tours, France, 17–20 October 1983; Larbier, M., Ed.; pp. 91–102.

43. Sauveur, B. Bio-availability to poultry of plant-origin phosphorus-Methodological criticisms and results. In Proceedings of the 4th European Symposium on Poultry Nutrition, Tours, France, 17–20 October 1983; Larbier, M., Ed.; pp. 103–113.
44. Payne, S.G. The Phosphorus Availability of Feed Phosphates in Broilers. MS Thesis, University of Stellenbosh, Stellenbosh, South Africa, 2005.
45. Edwards, H.M., Jr.; Gillis, M.B. A chromic oxide balance method for determining phosphate availability. *Poult. Sci.* **1959**, *38*, 569–574. [[CrossRef](#)]
46. Sibbald, I.R. Measurement of mineral bio-availability: Extension of true metabolisable energy methodology. *Poult. Sci.* **1982**, *61*, 485–487. [[CrossRef](#)]
47. Simons, P.C.M.; Versteegh, H.A.J.; Jongbloed, A.W.; Kemme, P.A.; Slump, P. Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br. J. Nutr.* **1990**, *64*, 525–540. [[CrossRef](#)] [[PubMed](#)]
48. NRC. *Nutrient Requirements of Poultry*, 7th ed.; National Academy Press: Washington, DC, USA, 1977.
49. Ankra-Badu, G.A.; Aggrey, S.E.; Pesti, G.M.; Bakalli, R.I.; Edwards, H.M., Jr. Modelling of parameters affecting phytate phosphorus bioavailability in growing birds. *Poult. Sci.* **2004**, *83*, 1083–1088. [[CrossRef](#)] [[PubMed](#)]
50. Thompson, L.U.; Yoon, H. Starch digestibility as affected by poly phenols and phytic acid. *J. Food Sci.* **1984**, *49*, 1228–1229. [[CrossRef](#)]
51. Walk, C.L.; Bedford, M.R.; McElroy, A.P. Influence of limestone and phytase on broiler performance, gastrointestinal pH, and apparent ileal nutrient digestibility. *Poult. Sci.* **2011**, *91*, 1371–1378. [[CrossRef](#)] [[PubMed](#)]
52. Ravindran, V.; Bryden, W.L.; Kornegay, E.T. Phytates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* **1995**, *6*, 125–143.
53. Selle, P.H.; Ravindran, V. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Technol.* **2007**, *135*, 1–41. [[CrossRef](#)]
54. Angel, R.; Saylor, W.W.; Mitchell, A.D.; Powers, W.; Applegate, T.J. Effect of dietary phosphorus, phytase, and 25-hydroxycholecalciferol on broiler chicken bone mineralization, litter phosphorus, and processing yields. *Poult. Sci.* **2006**, *85*, 1200–1211. [[CrossRef](#)] [[PubMed](#)]
55. Rodehutsord, M.; Dieckmann, A.; Witzig, M.; Shastak, Y. A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. *Poult. Sci.* **2012**, *91*, 965–971. [[CrossRef](#)] [[PubMed](#)]
56. Axe, D. Phosphorus value in ingredient sources examined. *Feedstuffs* **1998**, *70*, 17–28.
57. Savage, J.E.; Yohe, J.M.; Pickett, E.E.; O'Dell, B.L. Zinc metabolism in the growing chick. Tissue concentration and effect of phytate on absorption. *Poult. Sci.* **1964**, *43*, 420–426. [[CrossRef](#)]
58. Wise, A. Dietary factors determining the biological activities of phytase. *Nutr. Abs. Rev.* **1983**, *53*, 791–806.
59. Shastak, Y.; Rodehutsord, M. Determination and estimation of phosphorus availability in growing poultry and their historical development. *World's Poult. Sci. J.* **2013**, *69*, 569–586. [[CrossRef](#)]
60. Rodehutsord, M.; WPSA (Working Group No. 2: Nutrition of the European Federation of Branches of World Poultry Science Association). Determination of phosphorus availability in poultry. *World's Poult. Sci. J.* **2013**, *69*, 687–698.
61. Allison, P. When Can You Safely Ignore Multicollinearity? 2012. Available online: <http://statisticalhorizons.com/multicollinearity> (accessed on 4 May 2015).
62. Bryden, W.L.; Li, X. Amino acid digestibility and poultry feed formulation: Expression, limitations and application. *Rev. Bras. Zootec.* **2010**, *39* (Suppl.), 279–287. [[CrossRef](#)]
63. Adeola, O.; Applegate, T.J. Phosphorus and calcium equivalency of phytase. In Proceedings of the 1st International Phytase Summit, Washington, DC, USA, 28–30 September 2010; pp. 45–64.
64. Adedokun, S.A.; Sands, J.S.; Adeola, O. Determining the equivalent phosphorus released by an *Escherichia coli* derived phytase in broiler chicks. *Can. J. Anim. Sci.* **2004**, *84*, 437–444. [[CrossRef](#)]
65. Dilger, R.N.; Adeola, O. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. *Poult. Sci.* **2006**, *85*, 661–668. [[CrossRef](#)] [[PubMed](#)]
66. Jendza, J.A.; Dilger, R.N.; Sands, J.S.; Adeola, O. Efficacy and equivalency of an *Escherichia coli*-derived phytase for replacing inorganic phosphorus in the diets of broiler chickens and young pigs. *J. Anim. Sci.* **2006**, *84*, 3364–3374. [[CrossRef](#)] [[PubMed](#)]

67. Nyannor, E.K.D.; Adeola, O. Corn expressing an *Escherichia coli*-derived phytase gene: Comparative evaluation study in broiler chicks. *Poult. Sci.* **2008**, *87*, 2015–2022. [[CrossRef](#)] [[PubMed](#)]
68. Nelson, T.S. The hydrolysis of phytate phosphorus by chicks and laying hens. *Poult. Sci.* **1976**, *55*, 2262–2264. [[CrossRef](#)] [[PubMed](#)]
69. Barrier-Guillot, B.; Casado, P.; Maupetit, P.; Jondreville, C.; Gatel, F. Wheat phosphorus availability: 1-*In vitro* study; Factors affecting endogenous phytasic activity and phytic phosphorus content. *J. Sci. Food Agric.* **1996**, *70*, 62–68. [[CrossRef](#)]
70. Li, X.; Zhang, D.; Bryden, W.L. University of Queensland: Gatton, Australia Unpublished Work. , 2015. University of Queensland: Gatton, Australia Unpublished Work. , 2015. University of Queensland: Gatton, Australia Unpublished Work. , 2015.
71. De Groote, G.; Huyghebaert, G. The bio-availability of phosphorus from feed phosphates for broilers as influenced by bio-assay method, dietary Ca-level and feed form. *Anim. Feed Sci. Technol.* **1997**, *69*, 329–340. [[CrossRef](#)]
72. Skurray, G.R. The nutritional evaluation of meat meals for poultry. *World's Poult. Sci. J.* **1974**, *30*, 129–136. [[CrossRef](#)]
73. Waldroup, P.W. Phosphorus, phytase, and the environment—A retrospective look. *Proc. MD Nutr. Conf. Feed Manuf.* **2002**, *49*, 195–202.
74. Coon, C.N.; Seo, S.; Manangi, M.K. The determination of retainable phosphorus, relative biological availability, and relative biological value of phosphorus sources for broiler. *Poult. Sci.* **2007**, *86*, 857–868. [[CrossRef](#)] [[PubMed](#)]
75. Gillis, M.B.; Edwards, H.M., Jr.; Young, R.J. Studies on the availability of calcium orthophosphates for chickens and turkeys. *J. Nutr.* **1962**, *78*, 155–162. [[PubMed](#)]
76. Lima, F.R.; Mendonca, C.X., Jr.; Alvarez, J.C.; Garzillo, J.M.F.; Ghion, E.; Leal, P.M. Biological evaluations of commercial dicalcium phosphates as sources of available phosphorus for broiler chicks. *Poult. Sci.* **1997**, *76*, 1707–1713. [[CrossRef](#)] [[PubMed](#)]
77. Sanders, A.M.; Edwards, H.M., Jr.; Roland, G.N. Calcium and phosphorus requirements of the chick as determined response surface analysis. *Br. J. Nutr.* **1992**, *67*, 21. [[CrossRef](#)]
78. Ravindran, V.; Cabahug, S.; Ravindran, G.; Selle, P.H.; Bryden, W.L. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. *Br. Poult. Sci.* **2000**, *41*, 193–200. [[CrossRef](#)] [[PubMed](#)]
79. Bryden, W.L.; Selle, P.H.; Ravindran, V.; Acamovic, T. Phytate: An anti-nutrient factor in animal diets. In *Poisonous Plants: Global Research and Solutions*; Panter, K.E., Wierenga, T.L., Pfister, J.A., Eds.; CABI Publishing: Wallingford, UK, 2007; pp. 279–284.
80. Maenz, D.D.; Engle-Schaan, C.M.; Newkirk, R.W.; Classen, H.L. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase susceptible forms of phytic acid in solution and in a slurry of canola meal. *Anim. Feed Sci. Technol.* **1999**, *81*, 177–192. [[CrossRef](#)]
81. Pallauf, J.; Rimbach, G.; Pippig, S.; Schindler, B.; Most, E. Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the bioavailability of dietary phosphorus, calcium, magnesium, zinc and protein in piglets. *Agribiol. Res.* **1994**, *47*, 39–48.
82. Singh, M.; Krikorian, A.D. Inhibition of trypsin activity *in vitro* by phytase. *J. Agric. Food Chem.* **1982**, *30*, 799–800. [[CrossRef](#)]
83. Deshpande, S.S.; Cheryan, M. Effects of phytate, divalent cations and their implications on alpha-amylase activity. *J. Food Sci.* **1984**, *49*, 516–519. [[CrossRef](#)]
84. Liener, I.E. Anti-nutritional factors in legume seeds: State of art. In *Recent Advances of Research on Anti-Nutritional Factors in Legume Seeds*; Huismen, J., Van Der Poel, T.F.B., Liener, I.E., Eds.; Pudoc: Wageningen, The Netherlands, 1989; pp. 6–13.
85. Knuckles, B.E.; Betschart, A.A. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. *J. Food Sci.* **1987**, *52*, 719–721. [[CrossRef](#)]
86. Caldwell, R.A. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. *J. Agric. Food Chem.* **1992**, *40*, 43–46. [[CrossRef](#)]
87. Leeson, S.; Summers, J.D.; Caston, L. Response of brown egg strain layers to dietary calcium or phosphorus. *Poult. Sci.* **1993**, *72*, 1510–1514. [[CrossRef](#)]

88. Selle, P.H.; Ravindran, V.; Caldwell, R.A.; Bryden, W.L. Phytate and phytase: Consequences for protein utilisation. *Nutr. Res. Rev.* **2000**, *13*, 255–278. [[CrossRef](#)] [[PubMed](#)]
89. Champagne, E.T. Effects of pH on mineral-phytate, protein-mineral-phytate and mineral-fiber interactions. Possible consequences of atrophic gastritis on mineral bioavailability from high fiber foods. *J. Am. Coll. Nutr.* **1988**, *38*, 199–215. [[CrossRef](#)]
90. Taylor, T.G. Dietary phosphorus and egg shell thickness in the domestic fowl. *Br. Poult. Sci.* **1965**, *6*, 79–87. [[CrossRef](#)] [[PubMed](#)]
91. Keshavarz, K. The effect of dietary levels of calcium and phosphorus on performance and retention of these nutrients by laying hens. *Poult. Sci.* **1986**, *65*, 114–121. [[CrossRef](#)]
92. Keshavarz, K. Phosphorus requirement of laying hens with and without phytase. In Proceedings of the Cornell Nutrition Conference for Feed Manufacturers, Syracuse, NY, USA, 19–21 October 1999; pp. 81–92.
93. Hurwitz, S.; Bar, A. Absorption of calcium and phosphorus along the intestinal tract of the laying fowl as influenced by dietary calcium and eggshell formation. *J. Nutr.* **1965**, *86*, 433–438. [[PubMed](#)]
94. Guinotte, F.; Gautron, J.; Nys, Y.; Soumarmon, A. Calcium solubilisation and retention in the gastrointestinal tract in chicks (*Gallus domesticus*) as a function of gastric acid secretion inhibition and of calcium carbonate particle size. *Br. J. Nutr.* **1995**, *73*, 125–139. [[CrossRef](#)] [[PubMed](#)]
95. Keshavarz, K.; Austic, R.E. Effects of dietary minerals on acid–base balance and eggshell quality in chickens. *J. Nutr.* **1990**, *120*, 1360–1369. [[PubMed](#)]
96. Li, X.; Zhang, D.; Bryden, W.L.; Huang, K.H. University of Queensland: Gatton, Australia. Unpublished Work, 2013.
97. Selle, P.H.; Ravindran, V.; Bryden, W.L.; Scott, T. Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: A review. *J. Poult. Sci.* **2006**, *43*, 89–103. [[CrossRef](#)]
98. Shirley, R.B.; Edwards, H.M., Jr. Graded levels of phytase past industry standards improves broiler performance. *Poult. Sci.* **2003**, *82*, 671–680. [[CrossRef](#)] [[PubMed](#)]
99. Manangi, M.K.; Coon, C. Phytate phosphorus hydrolysis in broilers in response to dietary phytase, calcium, and phosphorus concentrations. *Poult. Sci.* **2008**, *87*, 1577–1586. [[CrossRef](#)] [[PubMed](#)]
100. Mohammed, A.; Gibney, M.J.; Taylor, T.G. The effects of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestion of phytate phosphorus by the chick. *Br. Poult. Sci.* **1991**, *66*, 251–259.
101. Kornegay, E.T. Nutritional, environmental and economic considerations for using phytase in pig and poultry diets. In *Nutrient Management of Food Animals to Enhance and Protect the Environment*; Kornegay, E.T., Ed.; Lewis Publishers: New York, NY, USA; CRC Press: Boca Raton, FL, USA, 1996; pp. 277–302.
102. Punna, S.; Roland, D.A., Sr. Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus-deficient diets from day one of age. *Poult. Sci.* **1999**, *78*, 1407–1411. [[CrossRef](#)] [[PubMed](#)]
103. Tamim, N.M.; Angel, R.; Christman, M. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. *Poult. Sci.* **2004**, *83*, 1358–1367. [[CrossRef](#)] [[PubMed](#)]
104. Augspurger, N.R.; Baker, D.H. Phytase improves dietary calcium utilisation in chicks, and oyster shell, carbonate, citrate, and citrate-malate forms of calcium are equally bioavailable. *Nutr. Res.* **2004**, *24*, 293–301. [[CrossRef](#)]
105. Angel, R.; Dhandu, A.S.; Applegate, T.J. Phosphorus requirements of broilers and the impact of exogenous phytases. In Proceedings of the Arkansas Poultry Nutrition Conference; University of Arkansas: Fayetteville, AR, USA, 2002; pp. 1–22.
106. Applegate, T.J.; Angel, R.; Classen, H.L. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. *Poult. Sci.* **2003**, *82*, 1140–1148. [[CrossRef](#)] [[PubMed](#)]
107. Nelson, T.S.; Peeler, H.T. The availability of phosphates from single and combined phosphates to chicks. *Poult. Sci.* **1961**, *40*, 1321–1327. [[CrossRef](#)]
108. Waldroup, P.W. Bioassays remain necessary to estimate phosphorus, calcium bioavailability. *Feedstuffs* **1996**, *68*, 13–23.
109. Nelson, T.S.; Kirby, L.K. The calcium binding properties of natural phytate in chick diets. *Nutr. Rep. Int.* **1987**, *35*, 949–956.

