The Phosphorus Availability of Feed Phosphates in Broilers

by

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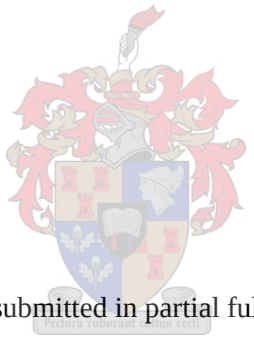

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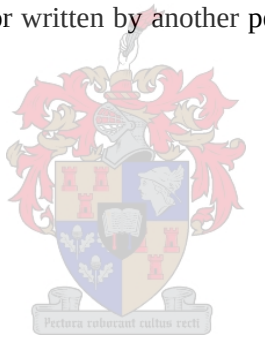
DECLARATION

I hereby declare that the research in this thesis is of my own investigation. Where use was made of the material previously published or written by another person it has been duly acknowledged in the text.



Steven Payne

Abstract

Broiler diets are supplemented with feed phosphates to ensure that adequate available phosphorus is provided in the diet to meet the bird's requirements. These feed phosphates make a considerable contribution to the total available phosphorus in the diet and small differences in their availability may have significant effects on whether the bird's requirements are met or not. The variation in availability of phosphorus between feed phosphates belonging to different classes and between feed phosphates of the same generic class is well documented. This variation can partially be attributed to differences in the physical and chemical composition of the relevant sources, but the reported differences may also be the result of differences in the evaluation method employed in the determination of these values. Three experiments were conducted to investigate variations to the balance technique in an attempt to develop a practical, standardised method for the determination of phosphorus availability. The advantage of wheat gluten as the protein source in the basal diet and higher available phosphorus inclusion levels in the test diets were investigated. The phosphorus availability of the feed phosphates tested was shown to be lower than previously reported and differed from experiment to experiment. The availability of phosphorus from a given source was shown to be not only an inherent property of the particular phosphate alone, but an experimentally determined value reflecting the absorption and utilization of the phosphorus under the conditions of the trial. This poses the challenge of developing an effective availability system that incorporates the dietary, physiological and environmental factors that may influence the potential availability of a particular phosphate source.

Samevatting

Braaikuiken rantsoene is met voerfosfate gesupplementeer om te verseker dat voldoende beskikbare fosfaat voorsien word om aan die behoeftes van die braaikuiken te voldoen. Hierdie voerfosfate maak ‘n groot bydrae tot die totale beskikbare fosfaat in die dieet. Klein verskille in die beskikbaarheid van die fosfor van hierdie fosfate kan ‘n noemenswaardige effek hê op die voldoening aan die braaikuiken se behoeftes, al dan nie. Die variasie wat bestaan in die fosforbeskikbaarheid van voerfosfate van verskillende klasse en tussen voerfosfate van dieselfde generiese klas is goed gedokumenteer. Hierdie variasie kan gedeeltelik aan die fisiese en chemiese verskille tussen die fosfate toegeskryf word, maar die gerapporteerde verskille mag ook die gevolg wees van die spesifieke ontledingsmetode wat gebruik is. Drie proewe is uitgevoer om verskille in die balansmetode te ondersoek in ’n poging om `n praktiese en gestandaardiseerde metode vir die bepaling van fosforbeskikbaarheid te ontwikkel. Die voordeel van koring gluten as die proteïenbron in die basale dieet en hoër beskikbare fosfor insluitingsvlakke in die proefrantsoene is ondersoek. Die fosforbeskikbaarheid van die voerfosfate wat getoets is was almal laer as wat voorheen gerapporteer is en het ook van eksperiment tot eksperiment verskil. Die resultate het getoon dat die fosforbeskikbaarheid van ’n voerfosfaat nie alleenlik ‘n produk van die fosfaat per se is nie, maar ook ‘n eksperimenteel bepaalde waarde is wat die absorpsie en benutting van die fosfor onder die spesifieke omstandighede van die proef reflekteer. Die uitdaging is dus om ‘n effektiewe beskikbaarheidsisteem te ontwikkel wat die voedings-, fisiologiese en omgewingsfaktore wat die potensiële beskikbaarheid van ‘n spesifieke fosfaatbron beïnvloed, inkorporeer.

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Contents

Chapter 1
General Introduction

The role of phosphorus (P) in broiler nutrition has taken on new significance in recent years. Previously, the major concern facing the poultry industry was how to ensure that the P requirements of the birds were met to ensure optimal growth and performance. Uncertainty regarding P availability resulted in a convention being widely adopted whereby dietary P was designated as either phytate P or non-phytate P; the assumption being made that poultry were only able to utilize the non-phytate P contribution. Since poultry diets consist primarily of feedstuffs of plant origin and a large, but variable, portion of the P in these products is found in the phytate form (Van der Klis & Versteegh, 1999), the majority of this P was considered unavailable to the birds and diets were supplemented with inorganic feed phosphates (Viljoen, 2001). The inaccuracies of the system and the detrimental consequences of failing to supply adequate P meant that nutritionists, as a rule, supplemented diets in excess of the birds' requirements as a safety measure (Waldroup, 1999).

Presently however, environmental pollution and the financial implications of excessive P in the excreta have become the greatest concerns. The disposal of poultry excreta has become a contentious issue. Previously, poultry excreta was employed successfully as an inexpensive source of fertilizer on agricultural lands, serving as a valuable source of P, nitrogen and potassium (Henuk & Dingle, 2003). However, P in poultry excreta, above the requirement of the plants, has been shown to seep down through the soil profile and contaminate the ground water or enter the surface water bodies through runoff, leading to eutrophication and encouraging the growth of algae (Sloan *et al.,* 1995). In countries such as the Netherlands, France and the United States of America there are already laws in place stipulating the maximum amount of P allowed in the litter (Simons *et al.,* 1990; Harter-Dennis, 2000).

These concerns have stimulated the re-evaluation of the broilers' existing P requirements (Leske & Coon, 2002; Dhandu & Angel, 2003) and the determination of the actual digestibility of P in all the commonly used feedstuffs incorporated in broiler diets (Van der Klis & Versteegh, 1999). A multi-faceted approach has been introduced in order to maximize the utilization of dietary P and thus achieve the underlying objective of reducing the amount of P in the excreta (Waldroup,

1999). Broilers are fed closer to their actual requirements, only highly digestible feed phosphates are incorporated into the diets and utilisation of previously unavailable P is enhanced.

The increased demand for supplements with a high P contribution has led to different phosphates being developed over the years and existing phosphates being continually improved. These include: monocalcium phosphate, dicalcium phosphate, mono-dicalcium phosphate, defluorinated rock phosphate and monosodium phosphate. Historically, broilers represent a very important market for feed phosphates, with poultry in general accounting for about 50% of the feed phosphates consumed annually worldwide (Devereux *et al.*, 1994). These feed phosphates make a significant contribution to the total available P content of any broiler diet; generally providing as much as 60% of the non-phytate P requirements of the bird (Waldroup, 1999). Change in any of the production parameters or the raw materials employed in their production may have an effect on the composition and/or quality of the feed phosphate produced and may ultimately affect the availability of the P in the respective product. Knowledge of the availability of P from different sources is essential to be able to compare the potential value of one phosphate against another and to screen sources of high availability for inclusion in the diets, since small differences in availability may have significant effects on the faecal P content. Research has shown distinct differences in the availability of P both between different feed phosphate sources (Van der Klis & Versteegh, 1999) and between feed phosphates within the same broadly defined generic classes (Waibel *et al.*, 1984). Use of an average value for the P content of any given product and assuming the availability of that P, based purely on the generic description of the product, may result in considerable over- or underestimation of the dietary available P level.

The main objective of this thesis was to evaluate different methods for determining the P availability of feed phosphates in broilers in an endeavour to develop a practical, reliable and repeatable standard method for the determination of P availability and the comparison of different P sources. A secondary objective was the evaluation of existing and emerging P sources to determine their respective availabilities.

Chapter 2

Literature review

2.1 Introduction

Phosphorus (P) is an essential element for all living organisms. Next to its major importance as a constituent of the skeleton, P is also an essential component of organic compounds and thereby involved in every aspect of metabolism (Soares, 1995). Phosphorus in broiler feeds originates mainly from plant feedstuffs, fish meal and inorganic feed phosphates. But, about 70% of the P found in plant feedstuffs is present as phytate P and was previously considered completely unavailable to poultry, while P from animal and inorganic origins was considered to be 100% available (Van der Klis & Versteegh, 1999). The poor availability of P in plant feedstuffs resulted in poultry diets been supplemented with inorganic feed phosphates in an attempt to supply sufficient P in a digestible form to support the requirements of the modern, rapidly growing birds, especially in the early stages of development (Viljoen, 2001).

It is common knowledge that the availability of P from these inorganic feed phosphates is not equal and the suitability of a feed phosphate for broiler diets is based primarily on the biological value of the particular phosphate. The biological value is an indication of the potential utilization of the P in that product (IFP, 2004). In the past, bone and growth response assays were utilised to determine these biological values relative to a reference phosphate source of high availability. However, in addition to these values being merely qualitative in nature, they exhibited considerable variability and were dependent on the response criteria used and the reference phosphate employed.

The prevailing uncertainty surrounding the availability of P from the different ingredients in the past and the consequences of feeding a P deficient diet resulted in phosphates being supplemented in excess of the birds' requirements, in an attempt to ensure an adequate supply of available P (Viljoen, 2001). This practice led to the reduced efficiency of P absorption from the gastrointestinal tract, increased P excretion via the kidneys and consequently higher concentrations of P being found in the faeces.

Environmental pollution problems in areas of intensive livestock production have prompted a renewed interest in the subject of P availability and highlighted the need for the development of more accurate systems of P evaluation. Balance assays were developed where the actual digestion and absorption of the P from all feed ingredients, including the feed phosphates, could be determined. These assays replaced the response assay and showed that in broilers, the digestibility of P of plant origin was between 16 and 72%, while the digestibility of P from inorganic feed phosphates varied between 55 and 92% (Van der Klis & Versteegh, 1999). Although the differences between phosphates encountered in the literature can be partially accounted for by differences in the physical and chemical compositions of the respective products, the results may also have been confounded by numerous other factors pertaining to the method of evaluation.

The objective of this chapter is to review the literature pertaining to the biological utilization of P in broilers, with the main emphasis on feed phosphates. The different methods utilized in the evaluation of feed phosphates and the determination of P availability as well as the numerous factors that may contribute to the wide range of P availability values reported will be discussed.

2.2 Phosphorus in poultry nutrition

2.2.1 Physiology and biochemistry

Phosphorus is an essential mineral for growing broilers and it is the second most abundant mineral in the body after calcium (Ca). Phosphorus has more known functions in the body than any other mineral and fulfils essential roles in both the structural as well as the metabolic functions (McDonald *et al.*, 1995; Soares, 1995), such as:

(1) Development and maintenance of the skeleton: the greatest proportion of P (80%) is devoted to maintaining and supporting the skeleton, where it is co-precipitated with Ca in the form of hydroxyapetite ($CaHPO_4$). The skeleton acts not only as a support system but as a reservoir of Ca and P (McDonald *et al.*, 1995; Soares, 1995),

(2) Integral component of the cell wall (phospholipids) (McDonald *et al.*, 1995; Soares, 1995),

(3) Energy transfer: Phosphorus plays a vital role in energy regulation. Certain phosphates, such as adenosine triphosphate (ATP) are universal accumulators

and donors of energy; they are present in all the body cells and ensure both the storage of energy and its utilization (McDonald *et al.*, 1995; Soares, 1995).

(4) Maintenance of osmotic balance (anion-cation balance) (Miles & Henry, 1997),

(5) Maintenance of pH balance ("acidogenic" effects in the digestive tract) (Miles & Henry, 1997),

(6) Amino acid and protein synthesis, transport of fatty acids: Phosphorus compounds are involved, directly or indirectly, in all major physiological functions. Phosphorylation is responsible for intestinal absorption, glycolysis and direct oxidation of carbohydrates, renal excretion, transport of lipids, exchange of amino acids, etc. Phosphorus is also a component of a large number of co-enzymes (McDonald *et al.*, 1995; Anselme, 2003),

(7) Growth and cell differentiation: Phosphorus forms part of the structure of nucleic acids, which are carriers of genetic material and regulate protein biosynthesis and immunity (McDonald *et al.*, 1995; Soares, 1995),

(8) Control of voluntary intake (appetite) (Bar & Hurwitz, 1984), and

(9) Efficiency of feed utilization (energy balance, protein synthesis and cell absorption).

The high chemical reactivity of P means that it only occurs in nature combined with oxygen or other elements in the form of phosphates (IFP, 2004). The single orthophosphate form of P, $(PO_4)^{3-}$, is well absorbed and utilized in the animal, but in the polymerized form (polyphosphates) or as phytate P, as found in plant feedstuffs, it is generally considered unavailable (Devereux, 1994). The NRC (1994) identifies dietary P as either being phytate P or non-phytate P (NPP) and calculates the available P in feedstuffs as total P minus phytate P, hence, the assumption that available P and NPP are synonymous.

In poultry, potentially available P solubilises in the gizzard where it becomes available for digestion in the form of orthophosphate. Absorption occurs in the duodenum and jejunum, due to the absorptive capacity of the intestinal mucosa and the prevailing pH in the intestine. However, absorption can continue at a decreasing rate further down the digestive tract because the passage rate of the chyme through the upper small intestine is often too rapid for complete absorption of the available P (IFP, 2004). The bird exhibits a limited amount of control over the absorption of available P from the gastrointestinal tract in comparison to other minerals such as Ca (Hegsted, 1973). The body content of P is primarily regulated by urinary excretion (Leske & Coon, 2002).

In practical terms, dietary Ca may be available but not be absorbed because of the Ca status of the bird, whereas a large proportion of the dietary P that is available will be absorbed but may be eliminated through the kidneys in the urine. Leske & Coon (2002) showed a marked increase in the total P excretion when the daily total P intake exceeded 197 mg/d, which is the amount required to support a steady physiological state within the bird.

The interaction of Ca and P is well documented. Adverse Ca: P ratios limit the utilization of P (McDonald *et al.*, 1995), while other mineral antagonists such as aluminium and magnesium may also influence P absorption (Soares, 1995). Harold *et al.* (1983) indicated that an excess of Ca may reduce the availability of P through the formation of insoluble calcium phosphates in the gastrointestinal tract. Van der Klis & Versteegh (1999) attributed the poor P utilization at sub-optimal Ca: available P ratios to, on one hand, the reduced P absorption from the small intestine at high levels of Ca (as a result of the afore mentioned calcium phosphates) and, on the other hand, the excretion of absorbed P if the Ca levels are too low. The authors showed that at low dietary Ca levels, P absorption from the small intestine was maximal but due to the lack of a proper counter ion it was deposited in the body with the lowest efficiency.

Under certain circumstances a P deficiency may occur; it can either be absolute, caused by insufficient supply of available P in the diet, or relative, due to reduced digestibility (Anselme, 2003). The later is caused by a too wide Ca: P ratio in the feed and the precipitation of unavailable calcium phosphate. The initial effect of a P deficiency is a fall in blood plasma phosphate levels. A severe deficiency can result in loss of appetite, weakness and death within a period of 10 to 12 days (Anselme, 2003). A less severe deficiency will result in a generally lower resistance to infection, a loss of appetite and a reduction in live weight gain due to an impaired feed efficiency (Bar & Hurwitz, 1984; Anselme, 2003; IFP, 2004). In broilers, specific deficiency symptoms include leg weakness and bone breakages, as well as tibial dyschondroplasia, osteomalacia and rickets (Waldroup, 1999; Anselme, 2003; IFP, 2004).

No mechanism is known for the active removal of P from the skeleton and the resultant reduction in bone ash, induced by a P deficiency. Reduced bone ash appears rather to be the result of inhibited bone formation during a deficiency (Bar & Hurwitz, 1984). It has been demonstrated, however, that during periods of starvation bone resorption does occur in an attempt to provide Ca for maintenance and to maintain Ca levels; at the same time P is released and is excreted (Anselme, 2003; Leske & Coon, 2003).

2.2.2 Broiler requirements

In poultry nutrition, the dietary P should meet the birds' requirements in the respective production phase (Van der Klis & Versteegh, 1999). Mineral requirements have generally been calculated by the factorial approach, taking into account the birds' requirements for maintenance and production, in addition to the genotype, particular age and level of performance of the bird (McDonald *et al.*, 1995). The requirements presented in Table 2.1 are based on the consumption of non-phytate P (NPP) and the incorrect assumption that NPP and available P are synonymous. They do not account for the fact that NPP may not be completely available and that phytate P may be partially used to fulfil the P requirements of the bird (Leske & Coon, 2002).

Table 2.1 *Inorganic phosphorus (%) and calcium (%) requirements for broilers (NRC, 1994)*

Age (weeks)	Inorganic Phosphorus	Calcium
0-3	0.45	1.00
4-6	0.35	0.90
7-8	0.30	0.80

Considerable research has been done to support the current (NRC, 1994) recommended P requirements for broilers during the period from 0 to 3 weeks of age; however, these recommendations are based on research published from 1952 to 1983. Modern broiler strains are very different from older strains in terms of growth, feed conversion efficiency, nutrient utilization and bone structure characteristics (Dhandu & Angel, 2003), suggesting that these values may need to be revised.

Although it could be expected that the P demands of the modern rapidly growing broiler would be much greater than those of earlier genotypes (Waldroup, 1999), contemporary research reported by Van der Klis & Versteegh (1999) proposed the contrary. They suggested that the available P (aP) requirements are in the range of 2.35 to 3.68 g aP/kg, decreasing with age as the relative rate of bone growth declined. Similarly, Leske & Coon (2002) suggested that 3.9 g retainable P/kg would meet the requirement for young broilers.

The number of reports concerning the birds' requirements after 3 to 4 weeks is more limited, but they do suggest that the P needs are greatly reduced; Waldroup (1999) suggested that during the

later stages of production, when the birds have high levels of feed intake, there is little if any need for supplemental P in a corn-soybean diet. Current NRC (1994) recommendations for broilers between 32 and 42 days of age and between 42 and 49 days of age are 0.35 and 0.30 % NPP respectively. Dhandu & Angel (2003) determined a requirement of 0.20 and 0.16 % NPP for these periods respectively (based on a broken line analysis of the tibia ash data). The general consensus is that the actual P requirements are somewhat lower than the dietary allowances prescribed by the NRC (1994); possibly due to a better understanding of the subject and the removal of excessive safety margins.

Since both Ca and vitamin D are closely linked to the broiler's P requirements in many of its metabolic functions, it would be incorrect to consider it in isolation. For example accretion of P in the broiler's bones is affected by the presence or absence of both Ca and vitamin D. Consequently, in addition to adequate P levels, an acceptable Ca: P ratio, as well as suitable levels of vitamin D in the diet, are critical to ensure balanced nutrition (McDonald *et al.*, 1995).

It should be noted that the dietary P level required for maximum P retention is not necessarily the same as the P level required to maximize performance and bone strength (Leske & Coon, 2002). Therefore broiler producers should continually evaluate the advantage in weight gain, feed conversion, bone ash and bone strength obtained when feeding additional retainable P compared to feeding P levels that would maintain the steady state of the bird and maximize P retention. Any manipulations of the dietary P level should be done in an accurate manner, as the excessive reduction of dietary P could compromise productivity and animal welfare (Anselme, 2003).

2.3 Phosphorus sources

Phosphorus is supplied to the broiler from three main sources, namely: plant feedstuffs, animal feedstuffs and minerals (McDonald *et al.*, 1995). The feedstuffs from plant and animal origin make a significant contribution to the total P content of the broiler diet (Viljoen, 2003). However, the low availability of P in these sources is well known and inorganic P sources are normally used as a supplement to achieve the required dietary levels (Soares, 1995; Viljoen, 2003). In typical formulations for poultry, inorganic phosphates provide as much as 35% of the P requirement, while P of animal and plant origin contribute approximately 13 and 52% respectively (IFP, 2004).

2.3.1 Plant feedstuffs

Cereal grains, oil seeds and their respective by-products are the major constituents of broiler diets. The total P content (%) of typically used plant feedstuffs ranges from 0.9 to 17.2 % (Table 2.2). However, P present in these sources is well known for its poor availability (Van der Klis & Versteegh, 1999) and variable composition (Viljoen, 2003). A major portion of the P in plant feedstuffs is in the phytate bound form. Phytates are salts of phytic acid, an inositol with 1 to 6 phosphate groups giving inositol-1-phosphate to inositol-6-phosphate (Van der Klis & Versteegh, 1999). Phytic acid is particularly abundant in plant seeds; serving as the major storage form of P. The phytic molecule has a high P content (28.2 %) which plays an important role in the living cell (Cosgrove, 1980), and is liberated by an enzyme called phytase during germination (Viljoen, 2003). Early studies of plant feedstuffs commonly fed to broilers showed that approximately two-thirds of the total P content of grains and seeds was in the form of phytate P, but the values presented in Table 2.2 challenge this assumption. Van der Klis & Versteegh (1999) showed the phytate P in plant feedstuffs to vary between 28 and 82 % of the total P. A possible structure of the phytate molecule (myoinositol 1,2,3,4,5,6-hexakisphosphate) when combined with minerals and starch in acidic medium is presented in Figure 2.1.

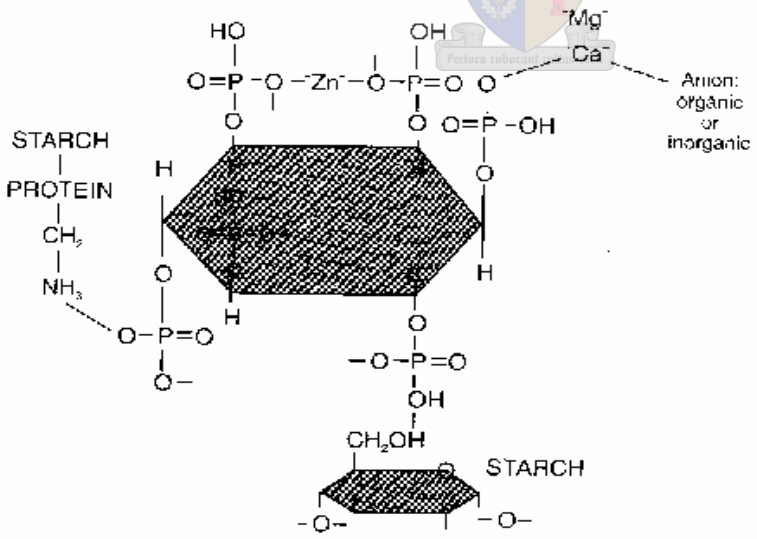

Figure 2.1 *Phytate-protein-starch complex molecule: a potential structure (Jongbloed et al., 2000)*

Table 2.2 *The phosphorus availability of some plant feedstuffs, measured in 3- week old broilers (Van der Klis & Versteegh, 1999)*

Feedstuff	Total P (g/kg)	Phytate-P (%)	Available P (% of total P)	Available P (g/kg)
Beans	4.9	74	52	2.5
Lupin	3.0	49	72	2.2
Maize	3.0	76	29	0.9
Maize gluten feed	9.0	45	52	4.7
Maize feed meal	5.1	47	50	2.6
Peas	4.1	63	41	1.7
Rape seed	10.9	65	33	3.6
Rice Bran	17.2	82	16	2.8
Soya bean (heat treated)	5.5	64	54	3.0
Soya bean meal (solvent extracted)	7.1	61	61	4.3
Sunflower seed (solvent extracted)	11.9	65	38	4.5
Tapioca	0.9	28	66	0.6
Wheat	3.4	74	48	1.6
Wheat middlings	10.8	74	36	3.9

In ruminants, phytate P can also be hydrolyzed to yield inorganic orthophosphates and inositol or hexose through the action of endogenous phytases. Poultry are however lacking or limited in endogenous phytase, meaning that phytate P is largely unavailable for absorption (Touchburn *et al.,* 1999). Phosphorus from phytic acid therefore assumes considerable nutritional significance since such a major portion of poultry diets consists of plant derived ingredients. Recent studies have shown that broilers are capable of using a portion of the phytate P (Touchburn *et al.,* 1999; Van der Klis & Versteegh, 1999), but no defined relationship could be established between phytate P and available P.

Comparisons with inorganic standards of high availability (sodium and potassium phosphates) indicated that cereals generally provide P with only 25 to 50 % of the availability (as a % of total P) of the mineral sources (Soares, 1995).

2.3.2 Animal feedstuffs

A variety of feed ingredients of animal origin are commonly used in balanced feeds because of their high quality protein and the significant amount of P that they contribute (Waldroup, 1999; Viljoen, 2003). Although their available P content (% of total P) is generally lower than that of inorganic P sources, the P in meat and fish meals is still considered highly available to monogastric animals when compared to standard phosphate sources (Soares, 1995). Phosphorus availability values for different animal sources commonly used in broiler diets are summarized in Table 2.3. Van der Klis & Versteegh (1999) found the available P in animal by-product feedstuffs to range between 59 and 74 % of total P. The animal sources tested also exhibited considerable variation (Van der Klis & Versteegh, 1999; Waldroup, 1999), suggesting the need for further, and continual, testing to ensure that the data employed in feed formulations are accurate.

Table 2.3 *The phosphorus availability of some animal feedstuffs, measured in 3-week old broilers (Van der Klis & Versteegh, 1999)*

Feedstuff	Total P (g/kg)	Available P (% of total P)	Available P (g/kg)
Bone meal	76	59	44.8
Fish meal	22	74	16.3
Meat meal	29	65	18.9
Meat and bone meal	60	66	39.6

2.3.3 Mineral sources

Formulation of typical plant-based diets for broilers demonstrates that it is impossible to meet the birds' P requirements with these materials alone. Additional P supplementation is therefore essential (Fernandes *et al.*, 1999). Up until the late 1940's, bone meal and soft rock phosphate were the main P supplements in animal feeds. The dramatic progress in broiler production with regard to growth and related bone development resulted in an increased demand for high P supplements and led to techniques being developed for manufacturing products with the highest possible content of P (Viljoen, 2003). These include: monocalcium phosphate (MCP), dicalcium phosphate (DCP), mono-dicalcium phosphate (MDCP), defluorinated rock phosphate (DFP) and monosodium phosphate (MSP).

Generally these feed phosphates are derived from natural rock phosphates, principally found in Africa, northern Europe, Asia, the Middle East and the USA (IFP, 2004). In their natural form these rock phosphates are unsuitable for direct use in animal feeds because the P they contain cannot be metabolized by the animal. They are therefore chemically treated so that the P is changed into the available orthophosphate $(PO_4)^{3-}$ form (IFP, 2004). Table 2.4 contains a list of the most commonly used feed phosphate sources and the respective compositions of these products.

The choice of P supplement depends primarily on its biological availability, chemical composition, cost, local accessibility, freedom from toxic impurities and physical handling properties. Other criteria to be considered are the age and productive stage of the birds being fed, electrolyte balance of the diet, acid–base balance, and "space saving" capacity of the product (Miles & Henry, 1997). The main inorganic phosphate sources used for animal production in South Africa are MDCP and DCP (Viljoen, 2003).

Table 2.4 *A list of commonly used inorganic feed phosphates and their respective phosphorus compositions (Van der Klis & Versteegh, 1999)*

	Formula	Total P (g/kg)	Average Total P (g/kg)	Available P (g/kg)
Calcium sodium phosphate (DFP)	$Ca_6Na_3P_5O_{20}$	175-195	180	106.2
Dicalcium phosphate (anhydrous)	CaH_2PO_4	175-215	197	108.4
Dicalcium phosphate (hydrous)	$CaH_2PO_4.2H_2O$	170-210	181	139.4
Monocalcium phosphate	$Ca(H_2PO_4)_2$	220-228	226	189.8
Mono-dicalcium phosphate (hydrous)	-	205-225	213	168.3
Monosodium phosphate	NaH_2PO_4	224	224	206.1

2.3.3.1 Manufacturing process

DCP, MCP and MDCP, generally named "calcium phosphates", are produced by reacting phosphoric acid with calcium salts (oxides, hydroxides and carbonates) to produce mixtures of MCP and DCP (Lima *et al.*, 1997; Waldroup, 1999; Viljoen, 2003). Although the terms mono- and dicalcium are commonly used in product descriptions, most commercial inorganic feed

phosphates in the above category are not pure products but mixtures of MCP and DCP (Lima *et al.*, 1997; Viljoen, 2003). For a phosphate to be classified as a MCP it must contain at least 80% MCP (Viljoen, 2001), while MDCP may contain MCP: DCP in the range of 50: 50 to 80: 20 (Viljoen, 2003).

By varying the relative proportions of the raw materials in the production process, the reaction conditions (heat, water and pressure) and the design conditions specific to a particular processing plant it is possible to produce a calcium phosphate having a predetermined composition in terms of total P content, total Ca content, Ca: P ratio, state of hydration and monobasic: dibasic phosphate ratio (J.N. Swart, personal communication; Viljoen, 2003). The composition is also dependent on the quality of raw materials employed; only high quality defluorinated phosphoric acid low in heavy metals and other impurities and good quality calcium salts should be used (Viljoen, 2003).

The second group, known as "defluorinated phosphates" (DFP), are produced by reacting phosphate rock with phosphoric acid and sodium carbonate and then calcining at 1,250ºC (Waldroup, 1999). It is considered more difficult to control the processes required to produce DFP than those used to produce calcium phosphates. In consequence, more variability in the composition of DFP tends to be found compared to that found in the composition of calcium phosphates (Waldroup, 1999).

2.4 Phosphorus availability

2.4.1 Methods of evaluation

The suitability of a feed phosphate for application in the broiler industry is based primarily on the biological value of the particular product. The biological value is an indication of the potential utilization of the P in the product and is expressed either in terms of availability, digestibility or retainability (IFP, 2004). Methods for evaluating the biological value can broadly be categorized into three types, namely: blood, bone and growth assays to determine the relative values of P availability, balance trials to determine the digestibility (absorption, retention) of P and *in vitro* or indirect tests to predict the P availability.

The different methods employed to estimate or determine the respective biological values have resulted in a wide array of terms and definitions emanating to describe the different concepts of P utilization. Essentially, the "bio"-availability of a nutrient is defined as the proportion (or percentage) of intake capable of being absorbed by the intestine and made available either for metabolic use or storage in animal tissue (Guèguen, 1994).

Studies on the available P content of feed phosphate sources date back to 1945. Since then there have been considerable developments with respect to the determination and comparison of the available P content of phosphate sources and a number of published studies have followed these early experiments, comparing the availability of both experimental and commercial phosphate sources available to the poultry industry (Gillis *et al.,* 1962; Nelson & Walker, 1964; Day *et al.*, 1973; Pensack, 1974; Huyghbaert *et al.*, 1980; Akpe *et al.,* 1987; Potchanakorn & Potter, 1987; Potter *et al.*, 1995; De Groote & Huyghebaert, 1997; Lima *et al.,* 1997; Van der Klis & Versteegh, 1999; Leske & Coon, 2002).

2.4.1.1 Blood, bone and growth assays

In the growing chick P is essentially transferred to the skeleton (80%) and the tissues (20%). The measurement of carcass P retention would seem to be the appropriate method for determining net P-utilisation (NPU) in different P sources (De Groote & Huyghebaert, 1997). However, determination of carcass P is cumbersome and Hurwitz (1964) found that a fairly constant ratio existed between carcass P and tibia P, indicating that tibia P may serve as a good estimate of carcass P. He demonstrated that the linear part of the response of tibia P on the total P intake, measured the NPU and the relative availability of P.

Although numerous different response criteria such as; bone ash (Gillis *et al.,* 1962; Hurwitz, 1964; Potter *et al.,* 1995; Ravindran *et al.,* 1995; Lima *et al.*, 1997), body weight (Pensack, 1974; Potter *et al.*, 1995; Ravindran *et al.*, 1995; Lima *et al.,* 1997), feed: gain ratio (Grimbergen *et al.*, 1985), P content of bone (Grimbergen *et al.,* 1985), bone strength (Lima *et al.,* 1997), bone densitometry (Akpe *et al.,* 1987), blood or plasma P (Boyd *at al,* 1983; Lima *et al.* 1997) and alkaline phosphatase (Boyd *at al,* 1983; Lima *et al.,* 1997) have been employed; the bone parameters have long been considered the most critical test for estimating the availability of P compounds (Ammerman, 1995). This seems appropriate since more than 80% of the P is

transferred to the skeleton and the composition and the percentage of P in the bone is rather constant (De Groote & Huyghebaert, 1997).

In the past, the conventional test of P supplements involved adding the phosphate sources to a P deficient diet to supply graded sub-optimum levels of P. These diets were then fed to young chicks and the response, generally expressed in terms of bone development or growth performance was then compared to that of a standard source, fed at equivalent total dietary P levels, to establish a relative biological value (RBV) for the test phosphate. The assumption being that the reference phosphate had an availability of 1.0 (Gillis *et al.*, 1962; Harms *et al.* 1967). Slope ratio of the dose-response lines appears to be the most appropriate method, although non-linearity and different intercepts may raise interpretation problems of the results (De Groote & Huyghebaert, 1997). Depending on the response criteria selected, it is possible to encounter both linear and non-linear responses to the addition of P over a wide range of P levels (Grimbergen *et al.*, 1985; Ravindran *et al.*, 1995; Potter *et al.*, 1995).

A RBV can be based on the response of a single criterion or it may be calculated from a combination of a number of response criteria. Sullivan (1966) based the biological value of P (BVP) on a three-response criterion: body weight (g/10) + bone ash (%) + 10 (gain: feed ratio). RBV were calculated as proportions of the BVP for each test phosphate and the BVP of the standard phosphate, expressed as percentage.

Both bone development and growth performance are, however, only suitably sensitive measures when applied to young rapidly growing animals (Ammerman, 1995). These response criteria have been shown to vary linearly with the quality and availability of the supplementary P source in young birds, but are less sensitive with older birds where the response in the respective parameters is inadequate (Dhandu & Angel, 2003; IFP, 2004).

Hitherto the majority of the data on the availability of P from P sources are relative values, derived from comparative assays. The purpose of these assays was to evaluate phosphate sources in a simple manner. These assays failed to determine the actual amount of P retained by the bird per unit of P source consumed (Leske & Coon, 2002) and merely provided comparative values of P utilization for the respective test phosphates relative to a standard reference material (Waibel *et al.*, 1984; Lima *et al.*, 1997).

2.4.1.2 Balance Method

Balance trials allow for the determination of quantitative values of P availability as opposed to merely qualitative values as in the methods discussed previously. Sibbald (1982) recognized the need for a simple, rapid assay for nutrient availability and proposed a bioassay to determine mineral availability of feed ingredients using a similar approach to the nutrient balance system used for true metabolizable energy (TME) evaluation. The major difference between the TME and mineral assays lies in the fact that excess minerals are excreted, whereas excess energy is stored as fat and has no effect on the excreta energy. In order to overcome this problem the maximum input of available P should be less than the P requirement.

The current balance method is based on feeding marginal dietary levels of P to minimize P excretion by the kidneys (De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Leske & Coon, 2002). It involves the quantitative measurement of the P intake (feed) and the P excreted (chyme or faeces) over a stipulated period of time. Grimbergen *et al.* (1985) and Van der Klis (1993) used a balance method where the apparent digestion was calculated by measuring the P concentration in the terminal ileum by means of an indigestible marker and the calculation of "ileal digestibility". The method had the advantage of avoiding the urinary excretion of P absorbed in excess of the birds' requirements. Excreta analysis is however simpler and because the assay can be carried out on large numbers without sacrificing the birds it was adopted by De Groote & Huyghebaert (1997), Van der Klis & Versteegh (1999) and Leske & Coon (2002). De Groote & Huyghebaert (1997) employed the European Reference Method (Bourdillon *et al.*, 1990), consisting of a 7 day period of adaptation to the respective experimental diet and a 4 day main balance period with restricted feeding and total excreta collection. Van der Klis & Versteegh (1999) employed a 10 day adaptation period followed by a 3 day (from 21 to 24 days of age) balance period and Leske & Coon (2002) employed a 3 day adaptation period followed by a 2 day balance period. Generally birds are fasted before and after the experimental period; ensuring that the P retained is derived from the P in the test diet and that the bird has ample opportunity to absorb and deposit all the available P. Phosphorus retention is then calculated as:

Phosphorus retention (%) = [(total phosphorus ingested – total phosphorus excreted)/ total phosphorus ingested] x 100.

Values determined using the aforementioned methodology are expressed as “apparent digestion” (Grimbergen *et al.*, 1985), “apparent absorption” (Ammerman, 1995; Van der Klis & Versteegh, 1999) and/or “P retention” (De Groote & Huyghebaert, 1997; Leske & Coon, 2002), essentially, all describing the same measure of availability. In this thesis the term available P has been adopted to describe the P that is absorbed from the gastrointestinal tract and retained within the body.

Whereas the methods described so far allow for the calculation of the apparent availability, they do not take into account endogenous losses and metabolic excretions. Balance trials, combined with radioactive markers to measure levels of endogenous P, enable true values of the availability to be determined. Ammerman (1995) describes the use of isotopes and double collections or comparative techniques for determining true absorption. True absorption corrects for that portion of the element, which has been absorbed into the body and subsequently excreted back into the gastrointestinal tract. This portion can be designated as “total endogenous faecal excretion” or “total metabolic faecal excretion”. The value for true absorption would be greater than for apparent absorption and is a more valid estimate of the amount of mineral element presented to body tissue for metabolic purposes. The value, where the endogenous P losses are considered, is expressed as a true absorption coefficient (TAC) (IFP, 2004).

2.4.1.3 *In vitro* tests or indirect tests

Determination of availability of phosphate sources by chick assay remain expensive, labour intensive and time consuming (Waldroup, 1999). A number of studies have explored the relationship of *in vitro* solubility tests of feed phosphates with their biological value as estimated by chick assays (Day *et al.*, 1973; Pensack, 1974). Conflicting results have been reported regarding the success of such tests in estimating the bioavailability of phosphates (Waldroup, 1999).

Day *et al.* (1973) assayed seven feed grade phosphates using tibia bone ash as criterion for availability and compared these values with the P solubility in 0.4% hydrochloric acid, 2% citric acid and ammonium citrate. The results suggested that P solubility in dilute acids could not be used to predict bioavailability. Despite these results, citric acid (2%) has been widely used since 1975 as an indication of P availability. The solubility in citric acid is higher than 85% for all

good, higher than 90% for all highly available and lower than 50% for all poorly available phosphates.

The solubility of P in water provides an indication of the ratio between MCP and DCP. MCP is completely soluble in water while DCP is completely insoluble (Viljoen, 2003). *In vivo* tests have demonstrated that, in general, MCP has a higher P availability than DCP (De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999) and data presented by Pensack (1974) demonstrated a correlation between biological availability and the content of MCP as measured by water solubility.

Ammonium citrate (Petermann method) can be used to distinguish between DCP and tricalcium phosphate (TCP) (including the hydroxyapetite of bone). *In vivo* tests show that MCP and DCP are both better absorbed by monogastric animals than the TCP form (Viljoen, 2003). However since several TCP, bone meal and DFP sources have known high P availabilities this method is also not an accurate indicator (J.N. Swart, personal communication).

2.5 Factors influencing phosphorus availability

2.5.1 Phosphate source

Contrary to previous beliefs, feed phosphates are no longer considered 100% available to poultry. Although it has been proven that most inorganic P sources have a high P availability, research does show that that there are distinct differences in availability between different generic sources (Viljoen, 2001). The P availability of all the commonly encountered feed phosphate sources has been determined and the differences between sources reported (Gillis *et al.* 1962; Nelson & Walker, 1964; Day *et al.*, 1973; Huyghbaert *et al.*, 1980; Waibel *et al.*, 1984; Grimbergen *et al.*, 1985; Potchanakorn & Potter, 1987; Soares, 1995; Potter *et al.*, 1995; Ravindran *et al.*. 1995; Lima *et al.*, 1997; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Leske & Coon, 2002).

Gillis *et al*. (1962) established that with regard to calcium phosphates, the primary calcium phosphate salt is the most available, followed by the secondary, with the tertiary salt being the least available. This relative ranking is supported by Grimbergen *et al.* (1985); they showed that the P availability of MCP was at least 20% better than hydrous DCP. Potchanakorn & Potter

(1987), using body weight and toe ash to estimate the relative bioavailability, reported an average availability of 92.6, 81.2 and 69.6% for MCP, DCP and DFP respectively, while De Groote & Huyghebaert (1997) reported values of 78.1 and 74.2% in one trial and 85.5 and 82.3% in a second trial for MCP and DCP respectively. Van der Klis & Versteegh (1999) found the available P in inorganic feed phosphates to range between 55 and 92% (Table 2.5).

Although exceptions do exist, one can generally accept that MSP has the highest availability, followed by MCP, MDCP, DCP, DFP and TCP respectively (Gillis *et al.* 1962; Nelson & Walker, 1964; Day *et al.*, 1973; Huyghbaert *et al.*, 1980; Waibel *et al.*, 1984; Grimbergen *et al.*, 1985; Potchanakorn & Potter, 1987; Soares, 1995; Potter *et al.*, 1995; Ravindran *et al.*. 1995; Lima *et al.*, 1997; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Waldroup, 1999; Leske & Coon, 2002). Since the production of feed phosphates has undergone continual improvement, the validity of this information would depend on when and how these studies were conducted. The most recent studies would provide more precise values (Waldroup, 1999).

Table 2.5 *The phosphorus availability of some commonly used feed phosphates (Van der Klis & Versteegh, 1999)*

Feed phosphate	**Available P (% of total P)**	**Available P (g/kg)**
Calcium sodium phosphate (DFP)	59	106.2
Dicalcium phosphate (anhydrous)	55	108.4
Dicalcium phosphate (hydrous)	77	139.4
Monocalcium phosphate	84	189.8
Mono-dicalcium phosphate (hydrous)	79	168.3
Monosodium phosphate	92	206.1

Differences in availability reported in the literature are not restricted to differences between generic sources alone. Waibel *et al.* (1984) investigated 20 commercial DCP sources and demonstrated differences in availability, relative to a highly available source, of as much as 30% between the different products within this broadly defined class (Table 2.6). A further variation of 32 and 18% was reported between the lowest and highest values (on a relative scale) for MDCP and MCP respectively. These differences may be attributed to differences and/or inconsistencies during the manufacturing of these products. Factors, such as the MCP: DCP ratio of the product,

whether the product is in a hydrated or anhydrous state, whether the product contains any impurities and the physical properties of the respective products, may all have an effect on their ultimate P availability.

Table 2.6 *The relative availability of phosphorus in commercially available dicalcium phosphate sources (Waibel et al., 1984)*

Source	Ca content (%)	P content (%)	Relative P availability
Reference[1]	18.1	20.6	100.0
1	21.8	18.8	100.7
2	20.6	19.0	87.6
3	21.4	19.0	77.2
4	20.4	19.1	85.7
5	24.0	18.5	78.9
6	22.2	18.4	75.1
7	23.0	18.6	87.4
8	23.0	19.0	76.3
9	21.2	18.9	106.3
10	20.8	18.9	98.6
11	20.0	19.0	94.1
12	20.6	18.7	93.1
13	22.2	18.0	104.8
14	22.8	17.7	104.0
15	18.1	18.8	96.0
16	18.8	20.1	95.1
17	20.4	19.0	81.8
18	21.4	18.8	77.7
19	20.6	19.1	91.7
20	20.8	18.9	95.6

[1]Compared to mono-dicalcium phosphate reference using tibia ash.

2.5.1.1 MCP: DCP ratio

The reaction between phosphoric acid and a calcium salt in the presence of water is used to produce calcium phosphates, as described earlier. The reaction can result in a range of products within the different generic classes, DCP, MDCP and MCP, each with its own MCP: DCP ratio. The superior availability of MCP in comparison to DCP has been reported (Van der Klis & Versteegh, 1999) and, consequently, the ratio of these two products in the end product may have an effect on the availability of the P. This relationship was previously examined by Pensack (1974), who reported that a calcium phosphate with a higher concentration of MCP relative to DCP had a higher biological availability and suggested a correlation between the MCP concentration in a calcium phosphate and its biological availability.

2.5.1.2 State of hydration

Depending on the manufacturing conditions and raw materials employed in the production of phosphates either a hydrous or anhydrous product can be formed. Although they both essentially represent the same broad generic product, the hydrous form has a substantially higher availability than the anhydrous form (Gillis *at al.* 1962; Rucker *et al.*, 1968; Grimbergen *et al.* 1985; Potter *et al.*, 1995; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Viljoen, 2003).

Rucker *et al.* (1968) found that hydrous DCP, $CaH_2PO_4.2H_2O$, dissolved more rapidly than anhydrous DCP, CaH_2PO_4, in an acid environment. This suggests that dissolution by gastric acid progresses more slowly for anhydrous DCP than for the hydrous DCP. In that report, the incorporation of hydrous DCP into bone was 25-50% greater than anhydrous DCP in 1 week old chicks. The difference in incorporation between the hydrous and anhydrous forms decreased gradually, so that by 5 weeks of age, equal amounts of P were incorporated into the femur when chicks were fed either form of the phosphate. Grimbergen *et al.* (1985) reported an apparent digestibility of 41.1 and 37.4% for hydrous DCP and anhydrous DCP respectively, and attributed the difference in availability to differences in the physical structure or the chemical properties of the materials. Potter *et al.* (1995) demonstrated that monohydrate MCP was more available than any other P source, including the standard dihydrate DCP.

De Groote & Huyghebaert (1997) reported values of 74.2 and 63.6% for hydrous and anhydrous DCP respectively, while Van der Klis & Versteegh (1999) reported a 22% difference in available P (% of total P) between hydrous and anhydrous DCP, with values of 77 and 55% available P respectively.

The results presented in Table 2.7 clearly indicate that the total P content could be misleading. Although anhydrous DCP has a higher total P content than dihydrate DCP (20 versus 18%), when digestibility is considered, the anhydrous DCP is shown to contain only 11% digestible P in comparison to 13.9% digestible P for the dihydrate DCP (Viljoen, 2003).

Table 2.7 *Comparative phosphorus values for poultry when digestibility is taken into consideration (Viljoen, 2003)*

Product[1]	**P Content (%)**	**Digestibility Coefficient (%)**	**Digestible P Content (%)**	**Relative Value**
MDCP	21	81	17.0	100
DCP dihydrate	18	77	13.9	81.5
DCP anhydrous	20	55	11.0	64.7
DFP	18	60	10.8	63.5
TCP	14	65	9.1	53.5

[1]MDCP = mono-dicalcium phosphate, DCP = dicalcium phosphate, DFP = defluorinated rock phosphate and TCP = tricalcium phosphate

2.5.1.3 Undesirable elements

Due to the nature of the raw materials employed in the manufacturing of feed phosphates there is the risk that the end product may contain undesirable elements. In addition to the concern that heavy metals derived from animal feed phosphates may accumulate in animal product, or in soil or crops following excretion (Viljoen, 2003), there is the consideration that these elements may be toxic to the animal and impair P availability. These elements include fluorine (F), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg). The maximum tolerable levels of these elements as determined for South Africa and the European Union are given in Table 2.8.

Table 2.8 *Maximum levels of undesirable elements according to the EU standards (EU Directive 74/63/EEC, adapted by 87/238/EEC & 96/25/EC)*

Undesirable Element	EU Standard
Fluorine (F)	Max 0.2%
Arsenic (As)	Max 10 mg/kg
Cadmium (Cd)	Max 10 mg/kg
Lead (Pb)	Max 30 mg/kg
Mercury (Hg)	Max 0.1mg/kg

2.5.1.4 Product uniformity

The physical uniformity and degree of fineness of the product may have an influence on the P availability. Lima *et al.* (1997) reported that products, classified as "coarse" or "irregular", were among those showing the highest availability. Larger particles were also reported to have a greater availability; this is possibly due to the fact that they are retained longer in the gizzard under more acidic conditions that may solubilize P more completely (Rucker *et al.,* 1968).

2.5.2 Trial differences

Hitherto the differences in availability reported have generally been attributed to differences in the physical and/or chemical properties of the particular sources. These reported availabilities are however, experimentally determined values which reflect the absorption and/or utilization of the P ingested under the conditions of the particular tests. The results presented by De Groote & Huyghebaert (1997) support the view that P utilisation data are very specific for a given set of experimental conditions.

Reported values are frequently expressed in percentage units. In certain studies, such as balance assays, the value represents the absolute proportion of the P that is absorbed by the bird and which is presented to the tissue for utilization. Availability values are often expressed, however, in relation to a response obtained with a standard reference material. Large discrepancies may exist between the values determined by the respective methods and a product exhibiting a high availability relative to a particular reference source may be poorly absorbed (Ammerman, 1995). Therefore the results from different trials must be carefully interpreted and are sometimes

questionable. Certain factors such as: reference source, P level, diet composition and form and response criteria, may have influenced the values determined in these studies.

2.5.2.1 Reference Source

Many of the assays reported give comparative or relative values of P availability where the reference phosphate is designated an availability of 100%. Numerous different reference standards have been used, including potassium and sodium phosphate (Harrold *et al.*, 1983), MCP (Akpe *et al.*, 1987), DCP (Potter *et al.*, 1995; Lima *et al.* 1997), TCP (Nelson & Peeler, 1961; Gillis *et al.*, 1962; Nelson & Walker, 1964) and phosphoric acid (Pensack, 1974). The choice of the reference material will have a direct influence on the magnitude and relative order of the reported values and researchers should attempt to employ the most efficiently utilized source with the least variability as reference. The different values listed in Table 2.9 for DCP may be partially attributed to the fact that the researchers did not utilize the same reference source in the respective studies.

Table 2.9 *The relative availability of dicalcium phosphate using different reference sources*

Source[1]	Reference Source[1]	Response Criteria	Relative Value	Reference
DCP	MSP	Bone Ash	97	Sullivan (1967)
DCP	MCP (monohydrate)	Bone Ash	88	Waibel *et al.* (1984)
DCP	DCP (monohydrate)	Bone Ash	71	Grimbergen *et al.* (1985)

[1]DCP = dicalcium phosphate; MCP = monocalcium phosphate; MSP = monosodium phosphate

2.5.2.2 Dietary level of phosphorus

Nelson & Peeler (1961) recognized that it was necessary to feed a P deficient basal diet in a bioassay if a difference in response was to be elicited between phosphate sources, and that the sources to be tested should be added to the basal diet at sub-optimal levels of P, such that they remain below the requirements of the bird. The importance of this concept is highlighted by more recent research conducted by Leske & Coon (2002) and presented in Table 2.10. Within the range of NPP provided by the MDCP sources, differences could be observed in the bone development. Bone strength was shown to increase linearly as the retainable P level increased from 0.2 to 0.34% and may have continued to increase at levels in excess of 0.34%, but at a reduced rate.

Table 2.10 *Effect of dietary retainable P levels fed broilers from 10 to 24 d of age on bone development in conjunction with a 5-d bioassay retention experiment (Leske & Coon, 2002)*

Source[1]	Diet total P (%)	Source NPP (%)	Source P retention[2] (%)	Breaking strength[2] (kg)	Bone ash[2] (%)	Bone ash [2] (g/tibia)
MCP	0.323	0.007	X	6.8 ± 2.3	41.1 ± 2.8	0.69± 0.09
MCP	0.328	0.012	X	6.1 ± 1.6	41.9 ± 2.5	0.64 ± 0.07
MCP	0.375	0.059	98.0 ± 2.7	9.4 ± 1.9	45.0 ± 2.8	0.78 ± 0.07
MCP	0.422	0.106	94.0 ± 3.3	13.3 ± 2.8	47.3 ± 1.2	0.90 ± 0.08
MCP	0.667	0.355	58.5 ± 4.4	15.7 ± 2.6	50.2 ± 1.0	1.08 ± 0.07
MCP	0.784	0.473	58.9 ± 2.6	17.2 ± 3.6	52.0 ± 1.8	1.18± 0.13
MCP	0.900	0.592	45.4 ± 3.1	16.7 ± 2.3	50.4 ± 1.8	1.12 ± 0.10
MCP	1.134	0.828	47.6 ± 5.5	18.1 ± 1.4	51.8 ± 1.2	1.22 ± 0.13
MDCP 1	0.326	0.010	X	7.0 ± 1.8	42.9 ± 2.5	0.70 ± 0.08
MDCP 1	0.346	0.030	87.9 ± 7.9	8.4 ± 2.3	42.4 ± 2.3	0.76 ± 0.08
MDCP 1	0.376	0.061	94.4 ± 3.1	9.0 ± 2.1	44.1 ± 2.2	0.76 ± 0.10
MDCP 1	0.396	0.081	76.6 ± 5.7	9.3 ± 1.7	46.3 ± 2.0	0.83 ± 0.10
MDCP 2	0.326	0.010	X	7.5 ± 1.8	42.0 ± 2.4	0.69 ± 0.07
MDCP 2	0.346	0.030	72.9 ± 25.6	7.9 ± 1.5	43.6 ± 2.7	0.73 ± 0.06
MDCP 2	0.376	0.060	82.0 ± 6.7	9.3 ± 1.4	46.1 ± 1.9	0.79 ± 0.08
MDCP 2	0.395	0.080	80.3 ± 6.9	9.8 ± 1.9	46.0 ± 2.6	0.81 ± 0.07
MDCP 3	0.327	0.011	X	7.6 ± 2.3	41.7 ± 3.2	0.67 ± 0.09
MDCP 3	0.348	0.032	88.3 ± 10.9	8.0 ± 2.2	43.3 ± 2.8	0.72 ± 0.05
MDCP 3	0.380	0.065	90.5 ± 14.1	10.4 ± 1.9	47.1 ± 1.3	0.81 ± 0.08
MDCP 3	0.402	0.086	81.2 ± 11.3	10.9 ± 2.7	46.6 ± 1.6	0.80 ± 0.13

[1] MCP = Monocalcium Phosphate; MDCP = Mono-dicalcium Phosphate [2] Means ± SD

The P range over which a response can be elicited is dependent on the particular response criterion utilized (Waldroup, 1999). Phosphorus supplementation beyond the particular "sensitivity" range will not reflect differences between sources, and excess P will be excreted. As mentioned earlier, the slope ratio method, with multiple inclusion levels, has negated the need to select an arbitrary P level for inclusion in response assays.

The balance method also relies on marginal dietary P levels, since P in excess of the birds' requirement is actively excreted. Van der Klis & Versteegh (1999) recommended that the test diets to be fed in such cases should be formulated to contain 1.8 g available P/kg, while Leske & Coon (2002) reported that a dietary NPP level between 1.6 and 2.1 g/kg resulted in the greatest retention of total P and NPP. The retention of P from the reagent-grade MCP significantly declined at higher levels of supplementation and the retention was shown to decrease from 94 to 58.5% when the NPP level was increased from 2.1 g/kg to the NRC (1994) suggested level of 4.5 g/kg (Leske & Coon, 2002). Van der Klis (1993) was however able to successfully use a much higher available P level of 3.0 g/kg in his ileal digestibility trial because under such circumstances urinary P could not confound the availability value.

Since the dietary P level required for maximum P retention is not necessarily the same as the P level required to maximize performance and bone strength (Leske & Coon, 2002), the decision on what P level to include is reliant on the type of assay being employed. It is important, however, where the total dietary concentration of the mineral is by necessity less than requirement, that the source of the element of interest represents the major portion of the total dietary concentration of that element (Ammerman, 1995). The author suggested that the wider the ratio between the test element and the basal diet element, the more sensitive the test is for measuring bioavailability. In the evaluation method described by Van der Klis & Versteegh (1999), the basal diet contained 0.2 g P/kg feed; this is the recommended maximum of 10% of the total available P content of the test diet (dietary Ca and aP level were 5.0 and 1.8 g/ kg feed respectively).

Although the reports in the literature suggest that the best results in terms of retention are achieved when the dietary P levels are sub-optimal, it is necessary to ensure good liveability of the birds in order to obtain credible results. Consideration should be given to the high mortality rate experienced on critically P deficient diets. Gillis *et al.* (1962) reported that a basal diet containing 0.7 g/kg P was insufficient to support chick life beyond 10 days, but diets containing 2.0 g/kg P were shown to support chick life satisfactorily (Nelson & Walker, 1964). Potter *et al.* (1995) reported a 38% mortality of chicks fed a basal diet containing 4.0 g/kg total P (1.6 g/kg NPP), decreasing to 14.5 and 4.5% when the amount of P added in the diet was increased from 0.5 to 0.8 and 1.2 g /kg respectively.

2.5.2.3 Dietary level of calcium and Ca: P ratio

Adverse Ca: P ratios may limit the utilization of P; especially at sub-optimal P levels such as are used in P assay diets (Harms *et al.,* 1967). Because of the potential influence the Ca level may have on the availability of P from the test phosphate, the debate has arisen of whether to hold the Ca level constant (Rucker *et al.*, 1968; Lima *et al.,* 1997) or whether to maintain a constant Ca: P ratio in assay diets. Generally a 2:1 ratio has been selected because it is characteristic of the actual ratio retained by chicks with near optimal nutrition and it meets the requirement, for any simple assay, of maintaining some constant Ca: P at all dosage levels (Nelson & Walker, 1964; Harms *et al.*, 1968). More recently, Leske & Coon (2002) confirmed that this was indeed the ideal ratio and reported maximum retention of dietary retainable P at a dietary inclusion of 4.8 g/kg Ca and 2.4 g/kg retainable P.

De Groote & Huyghebaert (1997) investigated the effect of Ca level on P retention and found that although average P retention at the 10.5g Ca/kg level was slightly lower than at the 9.1g Ca/kg level, this difference was not significant. The P retention values they obtained were within the range published previously by Simons *et al.* (1990), who used the same method but at a considerably lower level of 5.0g Ca/kg.

2.5.2.4 Phytic acid content of diet

The results of an experiment conducted by Harrold *et al.* (1983) indicate that the presence of phytic acid in the basal diet may reduce the availability of P added from a highly available source; while it stands to reason that excess available P in the basal diet may also reduce the absorption of P from the test source. Therefore the P composition of the basal diet and any factors which will improve the utilization of phytate P in the diet, and therefore contribute to available P and reduce phytate P, may confound the final results.

It is possible to modify the ability of the broiler to digest and utilize phytate P through the use of exogenous phytases. Commercial development of exogenous phytase enzymes can increase the ability of the chick to utilize a portion of the phytate P (Simons *et al.*, 1990; Touchburn *et al.*, 1999). In addition, new isomers of Vitamin D have been shown to enhance intestinal phytase or to act additively with microbial phytase to improve P utilization in chick diets (Edwards, 1993).

Phosphorus availability studies using test diets devoid of phytic acid may have overestimated the actual bioavailability under commercial conditions. Harrold *et al.* (1983) suggested that in experiments evaluating the availability of P in various ingredients for use in commercial diets, it may be desirable to utilize a practical (corn-soybean diet) basal diet.

2.5.2.5 Physico-chemical nature of diet

Van der Klis (1993) has shown that the physico-chemical nature of the diet may affect P availability. In that report, increasing the intestinal viscosity through cereal inclusion in the diet reduced the absorption of dietary P resulting in poorer broiler performance. The results showed an 8% reduction in absorption of P from MCP in broiler diets when 1% carboxy methylcellulose was included.

De Groote & Huyghebaert (1997) investigated the effect of feed form on P utilization. P retention for MCP and DCP with the crumbled diets was 7 to 8% points higher than with pelleted diets. The relative significant difference of 4% between the two P sources remained the same in both experiments and appeared to be unaffected by diet form. The difference was attributed to incomplete digestion of the diet in the pelleted form.

2.5.2.6 Electrolyte balance of diet

Maintaining the correct acid-base balance in animals is essential for them to express their full genetic potential for growth and bone development (Miles & Henry, 1997). Reagent-grade P is available in either the monobasic ($H_2PO_4^-$) or dibasic (HPO_4^{2-}) form, as MCP [$Ca(H_2PO_4)_2$] or DCP [$CaHPO_4$] respectively. The monobasic form is an extremely strong acidogenic anion compared to the dibasic form and supplemental feed phosphates where the majority of P is in the monobasic form might have an adverse effect on performance (Miles & Henry, 1997). Keshavarz (1994) showed that birds fed P in the monobasic form significantly decreased their feed intake within 24 h after been offered the feed. Average daily intake in hens fed 1.95% total P in the non-acidogenic (dibasic) form was 107 g/d, whereas for those receiving the acidogenic (monobasic) form, it was only 29 g/d. The effect of this characteristic on P availability might be expressed indirectly through an increased or decreased intake of P and a disruption of the Ca: P ratio.

2.5.2.7 Response criteria

The importance of selecting a response criterion that is suitable and sufficiently precise is paramount. A suitable criterion should have a consistent response to increasing P levels in the diet and exhibits minimal variation. Ideally, the results of these evaluations should produce P availability estimates with small standard errors and narrow confidence intervals. It is generally accepted that response criteria related to bone parameters are more sensitive to measuring and comparing P availability than either growth or performance parameters (Nelson & Walker, 1964; Pensack, 1974; Potter *et al.*, 1995). Growth and performance parameters exhibit greater variability than bone parameters, possibly as a result of them being more sensitive to a greater number of variables than calcification of bone. Potter *et al.* (1995) indicated that a reduction of 11.5% in relative bioavailability of a test phosphate compared with the standard was required for significance when body weight was used as a measure, while a difference of only 10.7% was required where toe ash was used. Noteworthy is the fact that combining the two methods of evaluation resulted in a difference of 7.7%, from the standard, being required for significance. This supports the use of a relative value index, such as the three-response criterion proposed by Sullivan (1966) and employed by Lima *et al.* (1997). Ravindran *et al.* (1995) evaluated the efficiency of various criteria in the determination of P availability and concluded that in addition to the RBV of P from a given source being different when determination was based on different response criteria, the relative ranking of the various sources may also differ.

Where the slope technique is used, biased results may occur because researchers have assumed a linear response in a curved region. Both Ravindran *et al.* (1995) and Potter *et al.* (1995) reported examples where non-linear (asymptotic and sigmoidal) regressions showed a better fit than linear regressions for the response of different criteria to dietary P.

The balance technique does not only provide a quantitative value of P availability, but it is also more sensitive to differences in availability between sources. Grimbergen *et al.* (1985) compared the availability of three phosphate sources against one another; MCP, hydrous and anhydrous DCP. Evaluation of growth response and feed conversion indicated that MCP and hydrous DCP were both significantly more available than anhydrous DCP, but only when apparent “ileal” digestibility was considered could a significant difference be established between the MCP and the hydrous DCP. Leske & Coon (2002) compared the results obtained in a retention bioassay to the results from a more traditional assay measuring bone parameters (Table 2.10). Even though

the source P retention (%) was considerably better for MDCP1 and MDCP3 than for MDCP2, this superiority was not clearly reflected in any of the bone parameters measured.

2.6 Discussion

Precision farming is no more evident than in the production of broiler chickens. Attention to the specific P requirements of the birds and the P contribution of the various feed ingredients incorporated in the diets has increased considerably as a result of public concern for the environment and the potential financial implications for the producers. The result has been the challenging of previously held assumptions regarding the availability of P and the revision of the previous systems for calculating P utilization. The importance of being able to ascribe a P availability value to an ingredient that describes the digestibility of the P and allows the calculation of the amount of P retained and excreted has been emphasised in this review. The replacement of bone and growth assays with the balance approach has gone a long way towards achieving this ideal.

The balance approach has not, however, improved the availability of previously poorly available ingredients. It has merely quantified this parameter and enabled the accurate supplementation of the diet with feed phosphates, of predetermined availability. The balance approach has further confirmed the assumed differences in digestibility between feed phosphates and shown that these differences extend to phosphate sources of the same generic class. Various reasons for these differences were suggested and potentially confounding factors were identified. The review of the literature also showed that the values determined for P availability by different researchers were dependent on the specific conditions employed in the respective trials, which suggests the need to standardise these experiments in order to be able to justifiably compare sources evaluated in separate experiments. It also suggested that the values determined under experimental conditions may not reflect the actual P availability of the respective sources under practical conditions.

Consequently, the objective of the following trials is to evaluate various modifications to the referenced methods in an attempt to develop a method which will yield practical values for different P sources which may find application in the formulation of broiler diets in the local southern African market.

Chapter 3

The evaluation of phosphorus availability in feed phosphates

3.1 Introduction

Increasing concern surrounding the management of intensive agricultural systems, such as broiler production, and their effects on the environment have resulted in these systems being re-evaluated and certain aspects of production being adapted. Recently, the role of phosphorus (P) in broiler nutrition has taken on new significance. Concerns about environmental pollution have led to the revision of the broilers' P requirements and the re-evaluation of the available P content of the most commonly used feedstuffs utilised in broiler diets in an attempt to minimize excessive P excretion in the faeces.

New legislation implemented in the European Union, banning the use of animal products for feeding livestock (including poultry), previously a significant contributor of P in the diet, has placed an even greater demand on the utilization of P from the remaining plant and supplementary phosphate sources. The need for supplements with the highest possible P content has resulted in the development and continual improvement of a variety of feed phosphates, including: monocalcium phosphate (MCP), dicalcium phosphate (DCP), mono-dicalcium phosphate (MDCP), defluorinated rock phosphate (DFP) and monosodium phosphate (MSP). Poultry represent the major market for these feed phosphate supplements, accounting for about 50% of the total feed phosphates consumed annually worldwide (Devereux *et al.*, 1994) and in any typical feed formulation these phosphates can provide as much as 60% of the non-phytate P requirement of the broiler (Waldroup, 1999). The composition of these phosphates varies as a result of the production parameters and raw materials employed in their manufacture and this has an influence on the availability of the P, both between different generic sources, as well as within broadly defined sources of the same description (Viljoen, 2001).

These feed phosphates contribute significantly towards the broilers' P requirement and small differences in availability may have significant effects on the amount of faecal P excreted (Waldroup, 1999). Therefore, existing and emerging products entering the southern African

market should be evaluated regularly in order to allow accurate diet formulations, under the local conditions, and the inclusion of adequate available P to sustain economical performance while reducing total dietary P and minimizing P excretion. The objective of this trial was to determine the P and Ca retention, expressed as availability, of five different phosphate sources by conducting a bioassay. The bioassay was based on the balance technique of Van der Klis & Versteegh (1999).

3.2 Materials and methods

3.2.1 Phosphate sources

Five phosphate sources were studied in this trial. Two MCP sources and two MDCP sources were compared with an analytical grade MSP source. The total P and Ca contents of the experimental sources are given in Table 3.1. The chemical analyses were performed by CAL LABS (Central Analytical Laboratories (Pty) Ltd). Samples were boiled with concentrated nitric and hydrochloric acid to get the P into solution. The P was then determined as orthophosphate by reaction with molybdovanadate solution. This reaction produces a coloured product in acidic media. This coloured product was measured photometrically. These sources were analysed after the test diets had been formulated and therefore did not allow for the products' particular specifications to be used in the diet formulation.

Table 3.1 *The total phosphorus and calcium contents of the five test phosphates*

P- source[1]	Total P (%)	Total Ca (%)
MSP	24.61	0.27
MCP (Test Phosphate A)	22.13	13.44
MDCP (Test Phosphate B)	20.45	14.70
MDCP (Test Phosphate C)	21.40	23.42
MCP (Test Phosphate D)	22.25	13.84

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

Water and citric acid soluble P were also determined. Samples were extracted with either water or 2% aqueous citric acid solution and then analysed as for total P.

3.2.2 Birds and housing

One hundred and twenty five one-day-old Ross 308 broiler chicks (as-hatched) were placed in wire-mesh cages. The birds were vaccinated as one-day-old chicks against Newcastle Disease and Infectious Bronchitis. The environmental temperature within the house was 31ºC for the first two days and was then decreased by 0.5ºC every second day to 20ºC. Continuous light was provided for the duration of the trial and birds were offered *ad libitum* access to feed and water. During the pre-experimental period, from day 1 to day 10, a commercial broiler starter diet was provided. On day 10, the number of birds was standardized at five birds per cage and the five test diets were allocated randomly amongst the cages, giving five replications per treatment.

3.2.3 Experimental diets

A purified basal diet was produced using P-free ingredients or feed components with very low P levels, as prescribed by Van der Klis (personal communication). This diet was formulated to meet the birds' nutrient requirements in all respects, except P, and resulted in a basal diet with an available P (aP) content of 1.3g aP/ kg diet. The composition of the basal diet is given in Table 3.2.

Table 3.2 *Composition on an as fed basis of the basal diet used in the experiment*

Ingredient	(g/kg)
Whey protein	143.4
L-arginine	11.8
L-isoleucine	1.4
L-phenylalanine	3.3
L-valine	2.0
DL-methionine	8.0
L-threonine	5.1
Vitamin and mineral premix	3.0
Sugar	100.0
Filler (Shavings)	169.6
Corn Starch	500.0
Limestone	11.9
Salt	0.5
Oil – soya	28.0

The test diets were formulated at an available P level of 3.5g aP/kg, a deviation from the method proposed by Van der Klis & Versteegh (1999) who utilised a level of 1.8g aP/kg. Phosphorus levels were calculated assuming availability values of 92% for the MSP, 84% for the MCPs and 79% for the MDCPs (Van der Klis & Versteegh, 1999). The feed phosphates were incorporated into the test diets by exchanging them for filler and the Ca contents were standardized using limestone. The available P and Ca contents of the diets as formulated, and the total P and Ca as analyzed are given in Table 3.3.

Table 3.3 *The available phosphorus and calcium contents on as fed basis (g/kg) of the experimental diets (calculated), and the total phosphorus and calcium on an as fed basis (g/kg) (analyzed)*

Diet	P- Source[1]	aP calculated	Ca calculated	Total P analyzed	Ca analyzed
1	MSP (Monohydrate)	3.6	5.1	3.6	5.6
2	MCP (Test Phosphate A)	3.4	4.7	3.4	5.8
3	MDCP (Test Phosphate B)	3.3	4.8	3.7	5.7
4	MDCP (Test Phosphate C)	3.4	5.9	3.8	6.6
5	MCP (Test Phosphate D)	3.4	4.8	2.6	5.4

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

3.2.4 Measurements and statistical analysis

3.2.4.1 Balance Trial

The birds were acclimated to the experimental diets for ten days (from day 10 to day 21) prior to initiating a three-day collection period. On day 21 the test diets were removed for a period of seven hours prior to the start of the experimental period to allow any feed previously consumed to be voided. The test diets were then re-allocated and individual excreta pans were placed under each cage for collection of faeces. Faeces were removed from the collection pans daily before being weighed and frozen. On day 24 the test diets were removed and the final faeces collection was performed seven hours after diet removal. Feed intake (g) was measured quantitatively for the period, day 21 to day 24. Feed remaining in the feeder on day 24 was subtracted from the total feed allocated on day 21. Mortality was recorded daily and the results were incorporated into the calculation of the feed consumption (g/bird/day).

Total P and Ca in the test diets and in the faeces were determined, as well as the moisture content of the diets and faeces. The analyses were conducted by CAL. Dry matter feed intake, dry matter faeces excretion and their respective P and Ca concentrations were calculated. The quantity of P and Ca retained were calculated using the following formula, and were expressed as available P and Ca (%).

Available Phosphorus (%) = [(total phosphorus ingested – total phosphorus excreted)/ total phosphorus ingested] x 100.

Where P excreted exceeded P consumed, yielding a negative P balance, values were rounded off to zero with regard to P availability (%) under the assumption that none of the P from the source was retained.

3.2.4.2 Experimental design and statistical analysis

The experiment had a completely randomised design with main effect of P source in the diet. Where data was expressed as a percentage, it was tested for normality using the Shapiro-Wilk's method. Normally distributed data were analysed by ANOVA using the GLM procedure of SAS 8.2 version (2000). Data that were not normally distributed were analysed by linear model analysis using the MIXED procedure of SAS 8.2 version (2000). Student's *t*-test was used to test differences between treatments when the treatment effect was significant. The probability level was set at 0.05%.

3.3 Results and discussion

3.3.1 *In vitro* tests

The five phosphate sources were analysed with respect to water and citric acid solubility. The results of these analyses are given in Tables 3.4. The *in vitro* solubility tests are considered a straightforward method for assessing product quality and predicting potential availability. Although conflicting results have been reported about the success of such tests in estimating the availability of phosphates (Waldroup, 1999), the solubility of a feed phosphate in 2% citric acid is commonly used to provide an indication of the level of P availability (IFP, 2004).

The solubility values presented in Table 3.4 suggest that the feed phosphates, with the exception of Test Phosphate C, should be readily available.

Table 3.4 *The citric acid solubility (%) and water solubility (%) of the five test phosphates*

P- source[1]	P citric acid solubility	P water solubility
MSP	99.35	98.29
MCP (Test Phosphate A)	94.35	81.34
MDCP (Test Phosphate B)	99.12	83.13
MDCP (Test Phosphate C)	81.64	40.89
MCP (Test Phosphate D)	99.69	89.39

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

Water solubility is an indicator of the MCP: DCP ratio of a product, since MCP is completely soluble while DCP is completely insoluble (Viljoen, 2001). The results confirm that Test Phosphates A and D were correctly classified as MCP. Test Phosphate B had a water solubility greater than 80% and could have been classified as MCP as well, while Test Phosphate C contained less than 50% MCP and should rightfully have been classified as a DCP.

3.3.2 Balance trial

The mean feed consumption (g/bird/day) and the dry matter (DM) digestibility of the respective diets, over the balance period are shown in Table 3.5. Phosphorus source had no effect on consumption or DM digestibility. The amount of feed consumed per bird per day was, however, less than expected. The consumption values were measured as an average over the balance trial period and, although this variable was not measured daily, consumption appeared to decline as the trial progressed. Average daily feed consumption for this period should have been between 95 and 113 g/day (Ross Breeders, 1998). This reduced feed intake could have resulted in the chickens being subjected to a severe deficiency, not only in terms of P but also all other nutrients.

The results of the P and Ca availability of the five test phosphates are shown in Table 3.6. Phosphorus source had an effect on the P availability of the experimental diets. The results suggest that the P availability of MDCP (Test Phosphate C) was significantly higher than the other sources and MCP (Test Phosphate D) was significantly lower. Although there were differences for P availability between the sources, the order of availability relative to the

reference source, MSP, was inconsistent with previously published data. De Groote & Huyghebaert (1997) reported that the apparent retention of P from MCP was 78.1% in one study and 85.5% in a second study, while Van der Klis & Versteegh (1999) reported values of 79, 84 and 92% for MDCP, MCP and MSP respectively. Similarly low values to those achieved in this experiment have however been reported previously. Grimbergen *et al*. (1985) determined apparent P digestion in turkeys utilizing MCP and reported values between 44.1 and 51.9%. The low P availability of Test Phosphate D may be attributed to the lower total P value in the test diet (2.6 g/kg diet vs. 3.5 g/kg diet).

Table 3.5 *Mean feed consumption (g/bird/day) and dry matter digestibility (%) of the five diets and their respective standard deviations for the period 21 to 24 days*

P-Source[1]	N	Consumption	Std Dev	DM Digestibility	Std Dev
MSP	5	30.1	3.48	73.3	1.73
MCP (Test Phosphate A)	5	33.5	2.34	72.7	0.95
MDCP (Test Phosphate B)	5	30.1	4.49	71.4	2.19
MDCP (Test Phosphate C	5	32.3	1.18	71.0	2.57
MCP (Test Phosphate D)	5	32.6	2.77	73.1	0.76
LSD[2]		3.48		2.35	

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]LSD = Least significant difference

Table 3.6 *The mean phosphorus (P) and calcium (Ca) availability (%) of the five diets and their respective standard deviations*

P- Source[1]	N	Available P [2]	Std Dev	Available Ca [2]	Std Dev
MSP	5	26.6^{b}	6.49	25.9^{c}	5.04
MCP (Test Phosphate A)	5	31.8^{b}	2.67	28.3^{cb}	2.54
MDCP (Test Phosphate B)	5	32.9^{b}	3.06	20.9^{c}	9.67
MDCP (Test Phosphate C)	5	41.2^{a}	6.32	35.9^{ab}	9.96
MCP (Test Phosphate D)	5	13.3^{c}	5.80	41.9^{a}	7.03
LSD[3]		6.78		9.76	

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P>0.05)
[3]LSD = Least significant difference

The low availability values could have been affected by the results observed in certain experimental units, i.e. in some replications allocated to diet 1 and diet 5, the net P balance

indicated that the birds excreted more P than they consumed. This active P excretion suggests that by the end of the 14 day experimental period the birds may have been breaking down body reserves to ensure adequate nutrient supply for maintenance, resulting in increased endogenous losses. It has been demonstrated that during periods of starvation bone resorption does occur in an attempt to provide Ca for maintenance and to maintain Ca levels; at the same time P is released and may be excreted (Anselme, 2003; Leske & Coon, 2002). In addition, potentially available P and Ca may not have been deposited in the body as a result of the physiological status of the bird and may have been excreted via the kidneys. This would influence the apparent availability of the minerals, as measured in this trial, and could partially account for the lower values determined.

Phosphorus source also appeared to have an effect on the Ca availability of the experimental diets. However these values were also inconsistent. The Ca retention values are not indicative of the Ca availability of the source per se, as the amount of Ca supplied by the $CaCO_3$ varied between the diets. Dilworth *et al.* (1964) suggested a correlation between the availability of P and Ca in feed phosphates. The data showing significant changes in P and Ca retention between MDCP (Test Phosphate B) and MDCP (Test Phosphate C) in this trial can be interpreted as showing the same relationship, especially since the $CaCO_3$ supplements were identical for these two diets.

The basal diet in this experiment contained 1.3g aP/kg according to the formulation program (Winfeed). This means that there were instances, diet 5 for example, where the basal diet contributed at least 50% of the total available P of the test diet. The source of P to be tested should represent the major portion of the total dietary concentration of P (Ammerman, 1995). The narrow ratio between the P contributed by the test sources and the basal dietary P in this experiment may have reduced the sensitivity to determine the absolute availability of the sources.

3.4 Conclusion

The major contribution of P from the basal diet may have been partially responsible for the lower values obtained in this trial, as well as for the inconsistencies with regards to the relative order of P availability between sources. The utilization of whey protein as the protein source made it difficult to maintain a suitably low P contribution in the basal diet while still catering for the protein requirements of the birds. Although whey protein has been widely used for this type of study, the suitability of different protein sources should be explored in the future when attempting

to produce a basal diet with a lower total P level, which should increase the sensitivity of the assay.

The lower availability values reported may also have been a consequence of the lower than expected feed intake over the trial period. Performance criteria such as growth and feed conversion efficiency have been criticised for not being sufficiently sensitive to differences in availability; however, these parameters in addition to certain bone parameters could be used to increase the sensibility of the test.

The results of this trial have provided a number of problems that need to be addressed, as well as potential solutions to increase the reliability of future results obtained, namely: the P source tested should make a more significant contribution to the total P content of the test diet, the test P source specifications should be used when formulating the test diet, hence P sources need to be analysed prior to the formulation of the diets and, finally, in an attempt to reduce variability further, birds utilised in such trials should be sexed.

Chapter 4

An evaluation of modifications to the reference method for the determination of available phosphorus in feed phosphates

4.1 Introduction

Nutritionists now realise the importance of supplying dietary phosphorus (P) that sustains economical performance while minimizing P excretion. Part of the multi-faceted approach proposed by Waldroup (1999) to achieve this objective is the use of phosphate sources with the highest biological value. Numerous different studies have been conducted over the years to evaluate the various feed phosphates available to the poultry industry (Gillis *et al.* 1962; Nelson & Walker, 1964; Day *et al.*, 1973; Huyghbaert *et al.*, 1980; Waibel *et al.,* 1984; Grimbergen *et al.*, 1985; Potchanakorn & Potter, 1987; Potter *et al.,* 1995; Ravindran *et al.,* 1995; Lima *et al.*, 1997; Van der Klis & Versteegh, 1999; Waldroup, 1999; Leske & Coon, 2002). The nature of these studies required that the test diets contain P predominantly from the test source and at a sub-optimal P level. This was achieved through the utilization of a basal diet containing extremely low P levels and the supplementation of this diet with the test P source, such that the test source constituted the major portion of the P in the diet (Ammerman, 1995).

Difficulty in formulating a basal diet with conventional ingredients that would meet the bird's requirements for all the other nutrients, but still be sufficiently low in P, resulted in the utilisation of purified basal diets. In the experiment conducted in the previous chapter the problem of producing a basal diet with a suitably low P content, using whey protein, was encountered. Various different protein sources: dried blood fibrin (Nelson & Walker, 1964; Pensack, 1974), casein (Harrold *et al.*, 1983), whey protein (Van der Klis & Versteegh, 1999), synthetic amino acids (Potter *et al.*, 1995; Van der Klis & Versteegh, 1999), soybean meal (Hurwitz, 1964; Harms *et al*., 1967; Pensack, 1974; Grimbergen *et al,* 1985; Potter *et al.*, 1995; Lima *et al.*, 1997) and egg white solids (Leske & Coon, 2002), have been employed in the past in an attempt to minimize the P in the basal diet while maintaining an acceptable protein level. In this experiment wheat gluten was evaluated as the protein source.

Phosphorus retention per se can be determined at any dietary P level, but balance trials employ marginal levels of P to enable the determination of the potential availability of the particular source. Available P in excess of the bird's requirement is excreted in the urine and would result in an underestimation of the availability. Van der Klis & Versteegh (1999) suggested that an available P (aP) level of 1.8 g aP/kg be employed in the test diets while Leske & Coon (2002) reported maximal P retention at dietary non-phytate P (NPP) levels between 1.6 and 2.1 g NPP/kg. Such severely deficient diets have however been reported to result in considerable mortality. Potter *et al.* (1995) experienced 38% mortality of birds fed a basal diet containing 1.6 g NPP/kg; this figure decreased from 38 to 14.5 and 4.5% as they increased the amount of P added to the basal diet from 0.5 to 0.8 and 1.2 g NPP/kg respectively. Recognising the importance of bird welfare, a higher available P level was investigated. The reference phosphate, monosodium phosphate (MSP), was included at two dietary available P levels 2.0 and 3.5 g aP/kg.

Two experiments were conducted. The objectives of the first experiment were to evaluate the modifications to the reference bioassay method and to determine the availability of a reference MSP (monohydrate), two monocalcium phosphates (MCP) and two mono-dicalcium phosphates (MDCP). A secondary objective was to compare the results of the bioassay with more traditional response criteria measuring bone breaking strength and bone ash. The objectives of the second experiment were to determine more accurately the feed intake of the test diets for the trial period and to re-evaluate the five phosphates tested in the first experiment to determine the repeatability of this assay. A further objective was to utilize the values determined in these experiments in establishing a relationship between P availability and citric acid and water solubility.

4.2 Materials and Methods

4.2.1 Phosphate sources

Five phosphate sources were studied in these experiments. Two MCP sources and two MDCP sources were compared with an analytical grade MSP (monohydrate) source. The phosphates were analysed for their total P and Ca contents (Central Analytical Laboratories (Pty) Ltd) as described in the previous chapter. The results of the chemical analyses were used to formulate the experimental diets. The total P and Ca contents of the test phosphates are given in Table 4.1.

Table 4.1 *The total phosphorus and calcium contents (%) of the five test phosphates*

P- source[1]	Total P	Total Ca
MSP (Monohydrate)	22.44	<0.01
MCP (Test Phosphate A)	22.64	13.47
MDCP (Test Phosphate B)	21.11	15.77
MDCP (Test Phosphate C)	20.49	15.29
MCP (Test Phosphate D)	22.56	13.69

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

4.2.2 Birds and housing

Experiment 1. Fifty four day-old male and 54 day-old female Ross 308 broiler chicks were raised under standardized conditions as described in Chapter 3. On day 10 (248g mean body weight (BW) for males and 239g mean BW for females) the birds were placed in cages (three birds per cage) and the six test diets were allocated randomly amongst the cages giving six replications per diet and three replications per sex.

Experiment 2. Fifty four day-old male and 54 day-old female Ross 308 broiler chicks were raised under standardized conditions as described in Chapter 3. On day 10 (147g mean BW for males and 143g mean BW for females) the birds were placed in cages (three chicks per cage) and the six test diets were allocated randomly amongst the cages giving six replications per diet and three replications per sex. As a result of mortality in the initial 10-day period treatment 4, MDCP (Test Phosphate B), only received four replications per diet and two replications per sex.

4.2.3 Experimental diets

A purified basal diet was produced using P-free ingredients or feed components with very low P levels, which resulted in a basal diet with a total P content of 0.3 g/kg (as analysed). This is less than the maximum of 10% of the total available P content of the test diets as recommended by Van der Klis & Versteegh (1999). Wheat gluten (760 g CP/kg and 1.5 g P/kg) was used to replace whey protein as the major protein source in the basal diet. The basal diet was formulated to meet the birds' nutrient requirements in all respects, except P, and the composition and nutrient analysis are given in Tables 4.2 and Table 4.3 respectively.

Table 4.2 *Composition on an as fed basis of the basal diet used in the experiment*

Ingredient	(g/kg)
Vital wheat gluten	218.29
L-lysine HCl	12.00
L-arginine	7.50
L-isoleucine	2.50
L-phenylalanine	3.27
L-valine	2.50
DL-methionine	2.00
L-threonine	3.50
Vitamin and mineral premix [1]	3.00
Sugar	20.00
Filler (Shavings)	130.27
Corn Starch	535.79
Limestone	16.87
Salt	1.00
Sodium bicarbonate	6.00
Potassium hydroxide	5.50
Oil – soya	30.00

[1]Roche

Table 4.3 *Chemical composition of the basal diet used in the experiment (calculated)*

Nutrient	Content
AMEn	13.59MJ/kg
Crude Protein	194.0g/kg
Available Phosphorus	0.3g/kg
Calcium	6.3g/kg

The test diets were formulated at 7.0 g Ca/kg diet and 3.5 g aP/kg diet in a 2:1 ratio in order to ensure maximum retention of dietary P (Leske & Coon, 2002). The available P content of the test diets was calculated under the assumption of an available P value of 92% for the MSP (monohydrate), 84% for the MCPs and 79% for the MDCPs. The feed phosphates were incorporated into the test diets by exchanging them for filler and the Ca contents were standardised using limestone. The test diets were offered as mash. Diet 2 in *Experiment 1*, the reference MSP (2.0g aP/kg), was replaced with a commercial broiler starter in *Experiment 2*.

The available P and Ca contents of the diets, as formulated, are given in Table 4.4.

Table 4.4 *The available phosphorus and calcium contents on as fed basis (g/kg) of the experimental diets used (calculated)*

Diet	P- Source[1]	aP	Ca
1	MSP (Monohydrate)	3.5	7.0
2	MSP (Monohydrate)	2.0	7.0
3	MCP (Test Phosphate A)	3.5	7.0
4	MDCP (Test Phosphate B)	3.5	7.0
5	MDCP (Test Phosphate C)	3.5	7.0
6	MCP (Test Phosphate D)	3.5	7.0

[1] MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

4.2.4 Measurements and statistical analysis

4.2.4.1 Growth trial/ Production performance

Experiment 1 and 2. The birds were weighed per cage at the start and at the end of the experimental period (day 10 and day 21) after a seven hour fast. Mass gain (g) was calculated for each experimental unit (cage) by subtracting the initial bird mass from the final bird mass. Feed consumed (g) was measured quantitatively over the same period. The feed remaining in the feeders on day 21 was subtracted from the total feed allocated per experimental unit on day 10. Feed conversion ratio (FCR) (g feed/ g mass gained) was calculated by dividing the total mass gained per experimental unit by the total feed consumed for that unit. Mortalities were recorded daily and incorporated into the calculation of mass gained, feed consumption and FCR. *Experiment 2* differed from *Experiment 1* in that feed consumed (g) was measured daily. The feed remaining in the feeders was weighed each day, 24 hours after being allocated, and was subtracted from the total feed allocated per experimental unit for that day.

4.2.4.2 Balance trial

Experiment 1 and 2. The birds were acclimated to the test diets for 10 days (day 10 to day 21) prior to initiating a three day collection period from day 21 to day 24. On day 21 the test diets were removed for a period of seven hours prior to the collection period to allow any feed

previously consumed to be voided. The test diets were then re-allocated and individual excreta collection pans were placed under each cage. Birds had *ad libitum* access to the test diets and water during both the acclimation and collection periods. Faeces were removed from the collection pans daily before being weighed and frozen. On day 24 the test diets were removed and the final faeces collection was performed seven hours after diet removal. The birds were weighed per cage at the start and at the end of the experimental period (day 21 and day 24) after a seven hour fast. *Experiment 1.* Feed consumed (g) was measured quantitatively over the balance period. Feed remaining in the feeders on day 24 was weighed and subtracted from the total feed allocated per experimental unit on day 21. *Experiment 2*. Feed consumed (g) was measured quantitatively, daily. Feed remaining in the feeder was weighed on days 22, 23 and 24, exactly 24 hours after feeding, and was subtracted from the total feed allocated per experimental unit each day. *Experiment 1 and 2.* Mortality was recorded daily and incorporated into the calculation of feed consumption (g/bird/day). Total P and Ca in the test diets and excreta were measured, as well as the moisture content of the test diets and excreta to allow for the calculation of dry matter (DM) feed intake and DM faeces excretion and their respective P and Ca concentrations. The quantity of P and Ca retained were calculated using the following formula, and were expressed as available P and Ca.

Available phosphorus (%) = [(total phosphorus ingested – total phosphorus excreted)/ total phosphorus ingested] x 100.

Where P excreted exceeded P ingested, yielding a negative P balance, values were rounded off to zero with regard to P availability (%) under the assumption that none of the P from the source was retained.

4.2.4.3 Bone parameters

Experiment 1. On day 24 all surviving birds were killed by cervical dislocation and both the left and right tibias were removed and frozen for further analysis. Bone breaking strength was determined using the method prescribed by Flemming *et al.* (1998). The tibias were thawed and the meat was removed from the bone (the cartilage was left intact). A three-point destructive bending test was carried out using an Instron Universal materials testing machine. The marked centre point of each bone was placed between the two 10mm diameter retaining bars, set 30mm apart. The 10mm diameter crosshead probe approached the bone at 30mm min^{-1} until the tibia was

broken. The breaking strength (in N) was read as the peak of the curve produced by the connected plotter, following suitable calibration. The bones were then dried at 70ºC (until constant weight) and weighed. The dried bones were placed in a muffle furnace at 600ºC overnight. The tibia ash was weighed and the ash percentage was calculated as a percentage of the dry bone.

4.2.4.4 Experimental design and statistical analysis

Experiment 1 and 2. Both experiments had a factorial design, with main effects of P source in the diet and sex. Where data was expressed as a percentage, it was tested for normality using the Shapiro-Wilk's method. Normally distributed data were analysed by ANOVA using the GLM procedure of SAS 8.2 version (2000). Data that were not normally distributed or had unequal replications were analysed by linear model analysis using the MIXED procedure of SAS 8.2 version (2000). Student's *t*-test was used to test differences between the P sources and between the sexes when the respective effects were significant. The probability level was set at 0.05%.

4.3 Results and discussion

4.3.1 Experimental diets

The total P and Ca contents of the experimental diets, as analysed by CAL LABS (Central Analytical Laboratories (Pty) Ltd), are shown in Table 4.5.

Table 4.5 *The total phosphorus (P) and calcium (Ca) content of the test diets on an as fed basis (g/kg) (as analyzed)*

Diet	P- Source[1]	Total P	Ca
1	MSP (Monohydrate)	3.7	8.7
2[2]	MSP (Monohydrate)	2.3	8.1
2[3]	Commercial broiler starter	7.2	16.8
3	MCP (Test Phosphate A)	3.6	7.6
4	MDCP (Test Phosphate B)	3.7	7.1
5	MDCP (Test Phosphate C)	3.7	6.7
6	MCP (Test Phosphate D)	3.6	7.1

[1] MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]MSP (Monohydrate) (2.0g aP/kg) was included as diet 2 in *Experiment 1*
[3]A commercial broiler starter was included as diet 2 in *Experiment 2*

The total P level of the commercial starter was considerably higher than the corresponding value in the other test diets. Whereas the P contribution of the other test diets is mainly in the form of inorganic feed phosphates, P in the starter diet comes from both plant and mineral origin. Phosphorus from plant origin may contribute 52% of the total P in a typical broiler diet (IFP, 2004) and is generally considered only 70% available (Van der Klis & Versteegh, 1999). Although the test diets retained their Ca: aP ratio, the Ca: P ratio of the starter was 2.3: 1 implying a Ca: aP ratio of greater than 4.6: 1.

4.3.2 Growth trial/ Production performance

Experiment 1. The P source had a significant effect on total mass gained, total feed consumed and FCR. The results of the growth trial are shown in Table 4.6.

Table 4.6 *Mean mass gain (g), feed consumption (g) and feed conversion ratio (FCR) (g feed/ g mass gain) of the respective P sources for the period 10 to 21 days (Experiment 1)*

Diet	P- Source[1]	N	Mass gain[2]	Std Dev	Consumption[2]	Std Dev	FCR[2]	Std Dev
1	MSP (Monohydrate)	6	187[a]	18.0	808[b]	25.14	4.34[b]	0.35
2	MSP (Monohydrate) low	6	140[b]	34.7	747[c]	50.51	5.65[a]	1.72
3	MCP (Test Phosphate A)	6	193[a]	39.7	833[ab]	58.52	4.46[b]	0.86
4	MDCP (Test Phosphate B)	6	202[a]	21.3	878[a]	50.78	4.38[b]	0.27
5	MDCP (Test Phosphate C)	6	192[a]	26.7	834[ab]	47.98	4.43[b]	0.73
6	MCP (Test Phosphate D)	6	177[a]	19.3	808[b]	31.01	4.60[b]	0.44
LSD[3]			33.4		50.09		1.04	

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P > 0.05)
[3]LSD = Least significant difference

Although there were no significant differences between the P sources at the 3.5g aP/kg level for total mass gained or FCR, the MSP at the lower inclusion level of 2.0g aP/kg performed worse than all the sources at the 3.5 g aP/kg level with regards to all three parameters measured. Birds on the MDCP (Test Phosphate B) diet consumed more than birds on the MSP (3.5 g aP/kg) diet and MCP (Test Phosphate D).

Performance seemed to be improved with P supplementation. Improved responses to increasing dietary aP (3.5 g aP/kg vs. 2.0 g aP/kg) were observed for both mass gain (187 g vs. 140 g) and FCR (4.34 vs. 5.65). This is consistent with other studies where improvements for mass gain and FCR were reported for increasing dietary P, regardless of P source utilized (Lima *et al.*, 1997; Leske & Coon, 2002). The mortality within the group receiving MSP at the lower inclusion level was 66.7%; significantly higher than the treatments that employed a level of 3.5 g aP/kg. High mortality was previously reported by Potter *et al.* (1995) for diets with similarly low P levels; 38% mortality was reported when birds were fed a basal diet containing 1.6 g non-phytate P/kg.

Sex had no significant effect on mass gained or FCR, but significantly affected the total feed consumed. Males consumed more feed over the period 10 to 21days, as would be expected (Ross Breeders, 1998). The results are shown in Table 4.7.

Table 4.7 *Mean mass gain (g), feed consumption (g) and feed conversion ratio (g feed/ g mass gain) of the male and female replicates for the period 10 to 21 days (Experiment1)*

Sex	N	Mass gain[2]	Std Dev	Consumption[1]	Std Dev	FCR	Std Dev
Male	18	183	36.6	834[a]	45.7	4.77	1.20
Female	18	180	29.4	801[b]	65.5	4.51	0.56
LSD[2]		19.3		28.9		0.60	

[1]Means with the same letter are not significantly different (P > 0.05).
[2]LSD = Least significant difference

Experiment 2. The P source had a significant effect on the mass gained and the feed consumed during this period. The birds fed on the commercial starter performed, as expected, significantly better than the rest with respect to mass gained and feed consumed. The results are shown in Table 4.8. The mass gained for the treatments on the respective test diets varied between 66.7 and 89.8 g, while treatments on the commercial starter gained 1197.2g on average. The amount of feed consumed per treatment varied between 874 and 985 g for the test diets in comparison to 2605 g for the commercial starter.

Although consumption of the test diets between *Experiment 1* and *Experiment* 2 is comparable, there are obvious discrepancies for mass gained between the two experiments. Noteworthy, are the mean 10-day BW for the respective experiments, 147 and 143 g for the male and female chicks respectively in *Experiment 2* compared to 248 and 239 g in *Experiment 1*. Two different

broiler starters were used in the two experiments. The decreased performance in *Experiment 2* could have been the result of the high Ca concentration (16.8 g Ca/kg) of the commercial starter utilised in *Experiment 2*. Bryden & Balnave (1983) showed that increasing the dietary concentration of Ca in broilers beyond 14.0 g/kg substantially reduced their performance, depressing growth. The different starters used over the initial 10-day period did not seem to conclusively account for the considerable differences, but no further explanation is evident. Although the experiments were not run concurrently, the experimental conditions were controlled and therefore, as far as possible, identical. There were no significant differences between the sexes for either feed consumption or mass gained, unlike *Experiment 1* where males were shown to consume more than females (Table 4.9).

Table 4.8 *Mean mass gain (g) and feed consumption (g) of the respective P sources for the period 10 to 21 days (Experiment 2)*

Diet	P- Source[1]	N	Mass gain[2]	SEM[3]	Consumption[2]	SEM[3]
1	MSP (Monohydrate)	6	66.7[b]	16.0	919[a]	64.6
2	Commercial Broiler Starter	6	1197.2[a]	16.0	2 605[b]	64.6
3	MCP (Test Phosphate A)	6	74.5[b]	16.0	985[a]	64.6
4	MDCP (Test Phosphate B)	4	89.8[b]	19.6	984[a]	79.1
5	MDCP (Test Phosphate C)	6	80.0[b]	16.0	963[a]	64.6
6	MCP (Test Phosphate D)	6	82.2[b]	16.0	874[a]	64.6

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P > 0.0001)
[3]SEM = Standard error of the mean

Table 4.9 *Mean mass gain (g) and feed consumption (g) of male and female replicates for the period 10 to 21 days (Experiment 2)*

Sex	N	Mass gain[2]	Std Dev	Consumption[1]	Std Dev
Male	17	283	452	1250	653
Female	17	266	430	1221	687
LSD[1]		27.6		111.3	

[1]LSD = Least significant difference

The results of daily feed intake (Table 4.10) show that, in general consumption began to decrease after four days of being fed the test diets; the respective standard deviations indicate that the intake was extremely variable.

Table 4.10 *Average daily consumption (g/bird/day) and standard deviations for the period 10 to 20 days (Experiment 2)*

	Daily Consumption											
Day	**MSP[1]**		**Commercial Starter**		**MCP [1] Test Phosphate A**		**MDCP[1] Test Phosphate B**		**MDCP[1] Test Phosphate C**		**MCP[1] Test Phosphate D**	
	Mean	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**		**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**
10	39.6	9.1	58.7	14.6	40.7	11.4	43.8	10.9	38.5	4.9	37.2	9.5
11	34.4	5.9	66.1	18.4	33.6	6.4	41.8	17.3	40.4	10.2	29.2	9.8
12	39.7	9.3	93.6	18.0	47.4	8.7	51.6	7.6	49.0	14.4	39.4	3.7
13	49.6	7.5	116.8	17.1	54.0	13.3	58.3	6.4	50.1	14.5	47.9	14.9
14	44.9	12.7	102.1	11.5	56.6	17.8	45.2	16.5	58.6	25.4	42.6	14.0
15	27.8	8.2	89.3	6.3	29.6	2.2	25.1	7.4	31.7	16.1	26.0	3.7
16	15.2	4.9	68.1	2.4	17.6	3.0	16.6	1.9	20.3	5.8	15.7	6.3
17	10.0	3.2	53.2	3.6	9.3	1.9	11.8	3.3	11.9	2.6	12.2	1.6
18	17.3	3.9	70.2	6.0	16.3	2.3	19.0	1.2	14.5	4.0	12.9	1.7
19	13.9	3.4	73.6	6.2	14.3	2.8	17.1	2.2	18.1	3.7	14.4	4.1
20	15.1	2.7	76.9	9.0	14.9	2.3	18.6	2.7	16.2	2.7	17.3	4.5

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

As the growth trial progressed, the general health and condition of the birds on the test diets deteriorated and this appeared to further reduce the daily intake. Daily feed intakes per bird of between 55.6 and 68.0 g, for the period 10 to 23 days, were observed by Van der Klis (Unpublished data, 1998) for similar experimental diets. A possible explanation for the differences in feed intake could be that diets were pelleted in the case of Van der Klis (1998) but in the experiments conducted for this thesis the pelleting of the diets was not possible and the diets were offered as a mash. The consistency of the mash was extremely fine and this could have negatively affected the palatability of the diet. A further explanation for the poor intake, compared to normal feed intake, may be the appetite depressing effect of feeding a P deficient diet (Bar & Hurwitz, 1984). Although the purified test diets were formulated to satisfy the birds' nutrient requirements in all respects, except P, according to the NRC (1994), the low feed intake

on the test diets meant that these birds could have been marginalised with respect to all the nutrients in the diet.

4.3.3 Balance trial

Experiment 1. Neither P source nor sex had an effect on feed consumption over the balance trial period. Phosphorus source did however have a significant effect on the DM digestibility of the diets. Both MSP, at the higher inclusion level, and MCP (Test Phosphate D) had significantly higher values for DM digestibility than MDCP (Test Phosphate B). The results are shown in Table 4.11.

Table 4.11 *Mean feed consumption (g/ bird/ day) and dry matter (DM) digestibility (%) for male and female broilers fed six experimental diets for the period 21 to 24 days (Experiment 1)*

Diet	P-Source[1]	N	Consumption	SEM[2]	DM Digestibility[3]	SEM[2]
1	MSP (Monohydrate)	6	20.8	1.78	79.8[a]	2.33
2	MSP (Monohydrate) low	5	23.6	1.96	76.1[ab]	2.56
3	MCP (Test Phosphate A)	6	23.3	1.78	75.2[ab]	2.33
4	MDCP (Test Phosphate B)	5	21.7	1.96	74.1[b]	2.56
5	MDCP (Test Phosphate C)	5	25.3	1.96	75.0[ab]	2.56
6	MCP (Test Phosphate D)	6	24.6	1.78	78.4[a]	2.33
Sex	Male	17	23.8	1.14	75.9	1.39
	Female	16	22.6	1.03	75.6	1.34

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]SEM = Standard error of the mean
[3]Means with the same letter are not significantly different (P > 0.05)

The DM digestibility values obtained were consistent with the type of ingredients used to formulate the experimental diets. The lower DM digestibility reported for MDCP (Test Phosphate B) relative to MSP (3.5 g aP/kg) and MCP (Test Phosphate D) suggests that the physiological status of these birds may have been adversely affected by this treatment. The mean feed consumption (g/bird/day) measured over the balance period was 23.2 g, resulting in an intake of 81.3 mg aP/bird/day (with dietary inclusion level of 3.5 g aP/kg). This P intake is well below the level of 108 mg retainable P/day that is needed to maintain a steady physiological state within the bird (Leske & Coon, 2002). The birds on the 2.0 g aP/kg diet would have been more seriously

deprived and this may be partially responsible for the high mortality figure experienced for this group.

Phosphorus source significantly affected P availability, while sex had no significant effect on either P availability or Ca availability. The results are shown in Table 4.12. The P availability for both MSP treatments were significantly higher than that for MDCP (Test Phosphate B). Although not significant, the results suggest a tendency that the availability of P from MSP is also higher than from MDCP (Test Phosphate C) at the same P inclusion level (P = 0.0673).

Table 4.12 *The mean phosphorus (P) and calcium (Ca) availability (%) for male and female broilers fed six experimental diets (Experiment 1)*

Diet	P- Source[1]	N	Available P [2]	SEM[3]	Available Ca [2]	SEM[3]
1	MSP (Monohydrate)	6	47.2[a]	8.14	59.3[a]	5.22
2	MSP (Monohydrate)	5	41.2[a]	8.94	43.3[ab]	5.74
3	MCP (Test Phosphate A)	6	31.2[ab]	8.14	41.1[b]	5.22
4	MDCP (Test Phosphate B)	5	7.63[b]	8.94	23.1[c]	5.74
5	MDCP (Test Phosphate C)	5	23.0[ab]	8.94	32.3[bc]	5.74
6	MCP (Test Phosphate D)	6	32.3[ab]	8.14	37.3[bc]	5.22
Sex	Males	17	37.6	5.19	41.9	3.33
	Females	16	25.5	4.70	38.4	3.01

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P > 0.05)
[3]SEM = Standard error of the mean

There was no significant difference in P availability between MSP at the 2.0 g aP/kg and 3.5 g aP/kg inclusion levels. These results are in disagreement with Leske & Coon (2002) who reported an improvement in P retention from 58.5 to 94% when the NPP level was decreased from 0.45 to 0.21%. The relative ranking of the P availability, MSP higher than MDCP tends to be consistent with the order generally accepted in the literature (Soares, 1995; Van der Klis & Versteegh, 1999). The values obtained in this experiment are however lower than those previously reported for similar sources. Grimbergen *et al*. (1985) determined P digestion in turkeys utilizing MCP and DCP and reported similarly low values of 30.6 to 51.9%. De Groote & Huyghebaert (1997) reported that the apparent retention of P from MCP was 78.1% in one study and 85.5% in a second study. Van der Klis & Versteegh (1999) reported values of 79, 84 and 92% for MDCP, MCP and MSP respectively. The poor feed intake reported in Table 4.8 and its result on the

physiological condition of the birds in the experiment may be partially accountable for the lower values reported here.

Phosphorus source had a significant effect on Ca availability (Table 4.12). The superior P availability of MSP in comparison with MDCP (Test Phosphate B) obtained in this trial is supported by the significantly greater Ca availability for both the 2.0 g and 3.5 g aP/kg MSP treatments in comparison to the MDCP (Test Phosphate B) treatment. The Ca availability specific to the feed phosphates could not be calculated from the data, since the amount of $CaCO_3$ in the diet varied for the six test diets. Improved retention may be a consequence of the presence of a suitable counter ion (P) in the body to support bone deposition (Van der Klis & Versteegh, 1999). De Groote & Huyghebaert (1997) showed significant changes in Ca retention in the diets due to the inclusion of different feed phosphates; their data suggested a positive correlation between the availability of P and Ca in feed phosphates. The availability of Ca from $CaCO_3$ appears to be higher than from feed phosphates (Dilworth *et al.*, 1964), therefore the greater contribution of $CaCO_3$ to the total Ca in the MSP test diets may have contributed to the higher retention of Ca in these treatments.

Noteworthy is the fact that MDCP (Test Phosphate B) also had a lower DM digestibility than the MSP treatments; whether the lower digestibility is a consequence of the poorer mineral availability or vice versa is unsure and should be investigated further.

Experiment 2. Total feed consumption and average daily feed consumption (g/bird/day) for the different P sources are reported in Table 4.13 and Table 4.14 respectively. Phosphorus source had a significant effect on feed consumption but did not yield any differences between the test phosphates. As expected, the birds on the commercial diet consumed significantly more than the birds on the test diets. Sex, again, did not have an effect on consumption over the balance period (Table 4.13).

Neither P source nor sex had an effect on DM digestibility (Table 4.13). The DM digestibility in *Experiment 2* was generally lower than in *Experiment* 1, possibly as a consequence of the inferior general condition of the birds in *Experiment 2.* The poor initial growth, from day 0 to day 10, may have had a negative impact on the development of the gastrointestinal tract and this may be the cause of the reduced DM digestibility.

Table 4.13 *Total feed consumption (g) and dry matter digestibility (%) for male and female broilers fed six experimental diets for the period 21 to 24 days (Experiment 2)*

Diet	P- Source[1]	N	Consumed[2]	SEM[3]	DM Digestibility	SEM[3]
1	MSP (Monohydrate)	6	148[a]	17.8	71.3	4.06
2	Commercial Broiler Starter	6	852[b]	17.8	74.5	4.06
3	MCP (Test Phosphate A)	6	137[a]	17.8	69.7	4.06
4	MDCP (Test Phosphate B)	4	137[a]	21.8	71.2	4.97
5	MDCP (Test Phosphate C)	6	142[a]	17.8	71.4	4.06
6	MCP (Test Phosphate D)	6	151[a]	17.8	72.1	4.06
Sex	Male	17	262	10.6	70.4	2.42
	Female	17	259	10.6	73.0	2.42

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P > 0.05)
[3]SEM = Standard error of the mean

Table 4.14 *Average daily consumption (g/bird/day) and standard deviations for the period 21 to 24 days (Experiment 2)*

	Daily Consumption											
Day	**MSP[1]**		**Commercial Starter**		**MCP [1] Test Phosphate A**		**MDCP[1] Test Phosphate B**		**MDCP[1] Test Phosphate C**		**MCP[1] Test Phosphate D**	
	Mean	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**Mean**	**Std Dev**
21	19.3	2.9	98.9	6.6	20.5	4.4	24.4	9.0	23.4	3.3	22.0	6.1
22	15.6	1.5	86.4	5.5	16.3	4.1	17.5	3.9	15.6	3.1	15.5	6.1
23	13.6	2.9	73.4	5.9	10.9	3.2	14.9	3.0	15.7	2.1	15.1	4.2
24	5.0	1.5	25.4	7.5	8.1	3.5	6.5	6.4	8.2	1.0	4.9	3.6

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

There were instances, for replications on both diet 5 and diet 6, where the net P balance indicated that the birds excreted more P and Ca than they consumed. These instances were rounded off to zero with regard to P and Ca availability. The results of P and Ca availability for *Experiment 2* are shown in Table 4.15. Phosphorus availability was significantly affected by P source. Phosphorus from MSP was retained significantly more than P from MDCP (Test Phosphate B).

This is in agreement with the findings in *Experiment 1*. Although there were no further differences, a tendency (P = 0.0761) suggests that the availability of P from MCP (Test Phosphate A) was also higher than that of MDCP (Test Phosphate B). This would be in agreement with the relative order of availability proposed in the literature (Soares, 1995; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Leske & Coon, 2002). Noteworthy are the results of P retention for the birds fed the commercial starter. About 45% of the P consumed was retained. Similar findings were reported by Leske & Coon (2002), with a total P retention of 43.2% for a corn soybean broiler diet.

Phosphorus source also had an effect on Ca availability. The Ca retention for the commercial starter was the greatest and it was significantly better than the Ca retention for the birds on the MSP and MDCP (Test Phosphate B) treatments. The improved retention may be attributed to the fact that the birds on this treatment were actively growing and the resultant physiological demand for Ca for metabolism and bone deposition. Although birds receiving MDCP (Test Phosphate B) retained Ca the least, supporting the previous hypothesis that Ca and P availability were related and dependent on the presence of a suitable counter ion for retention, the result for MSP did not agree with those of the previous experiment.

Sex did not have an effect on P or Ca availability. This is in agreement with the results from *Experiment 1*.

Table 4.15 *The mean phosphorus (P) and calcium (Ca) availability (%) for male and female broilers fed six experimental diets (Experiment 2)*

Diet	P- Source[1]	N	Available P [2]	SEM[3]	Available Ca[2]	SEM[3]
1	MSP (Monohydrate)	6	47.0^{a}	3.96	22.9^{bc}	5.80
2	Commercial Broiler Starter	5	45.5^{ab}	4.40	46.6^{a}	6.45
3	MCP (Test Phosphate A)	5	44.4^{ab}	4.40	33.6^{abc}	6.45
4	MDCP (Test Phosphate B)	4	30.0^{b}	6.23	22.6^{c}	9.13
5	MDCP (Test Phosphate C)	6	41.2^{ab}	3.96	38.8^{abc}	5.80
6	MCP (Test Phosphate D)	5	37.6^{ab}	4.40	31.4^{abc}	6.45
Sex	Males	16	39.7	2.62	31.9	3.83
	Females	15	42.2	2.62	33.4	3.83

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]Means with the same letter are not significantly different (P > 0.05)
[3]SEM = Standard error of the mean

Ammerman (1995) suggested that the utilization of a nutrient may be enhanced if the intake of the respective nutrient is less than its requirement. In consequence, at the P intake levels recorded in this and the previous experiment, a maximum P retention could be theoretically expected. Contrary to this suggestion, the deficiency of P imposed in this experiment did not result in an increased utilization of the mineral. The chronic nature of the deficiency and the repercussions with regard to the bird health and overall physiological status meant that the birds could not compensate for the deficiency, resulting in retention values that were much lower than those previously reported in the literature (De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Leske & Coon, 2002).

4.3.4 Bone parameters

Experiment 1. Neither P source nor sex had an effect on bone breaking strength or bone ash. The results of the bone parameter tests are shown in Table 4.16. The P sources were added at a single inclusion level of 3.5 g aP/kg, with the exception of MSP. Generally, in an assay where bone parameters are the response criteria to be evaluated the P sources are included at graded sub-optimum levels and the response is expressed as a regression on P added. The slope of the regression line of the test phosphate is then compared to that of the reference phosphate.

Table 4.16 *The mean bone breaking strength (N) and bone ash (%) of 24 day old male and female broilers fed six experimental diets*

Diet	P- Source[1]	N	Breaking strength	SEM[2]	Bone Ash	SEM[2]
1	MSP (Monohydrate)	6	58.08	4.4756	14.31	0.6161
2	MSP (Monohydrate)	5	53.68	4.9169	12.45	0.6769
3	MCP (Test Phosphate A)	6	51.37	4.4756	12.78	0.6161
4	MDCP (Test Phosphate B)	5	55.71	4.4756	13.45	0.6161
5	MDCP (Test Phosphate C)	5	55.68	4.4756	12.59	0.6161
6	MCP (Test Phosphate D)	6	57.34	4.4756	12.63	0.6161
Sex	Males	17	55.89	2.6716	13.06	0.3678
	Females	16	54.85	2.584	13.04	0.3557

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate
[2]SEM = Standard error of the mean

The values reported were considerably lower than those previously reported in the literature (Lima *et al.*, 1997; Fernandes *et al.*, 1999; Leske and Coon, 2002). The poor bone strength and

lower bone ash may have been a consequence of inhibited bone formation as a result of the reduced P intake or as a result of bone resorption (Leske and Coon, 2002).

4.3.5 *In vitro* tests

The five phosphate sources were analysed with respect to heavy metal contaminants, water solubility and citric acid solubility. The results of these analyses are given in Tables 4.17 and Table 4.18. All phosphates were within the EU standards for the maximum tolerable levels for the respective heavy metals, with the exception of MCP (Test Phosphate A) that had a fluoride level marginally higher than the recommended maximum level of 0.2%.

Table 4.17 *The heavy metal content (mg/kg) of the five test phosphate sources*

P- source[1]	Fluoride	Cadmium	Lead	Mercury	Arsenic
MSP (Monohydrate)	<62.5	0.13	<0.01	0.05	1.8
MCP (Test Phosphate A)	2138	0.08	<0.01	0.08	1.4
MDCP (Test Phosphate B)	733	2.61	<0.01	0.03	4.7
MDCP (Test Phosphate C)	698	2.34	<0.01	0.04	<0.7
MCP (Test Phosphate D)	733	1.71	<0.01	0.07	6.6

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

Table 4.18 *The citric acid solubility (%) and water solubility (%) of the five test phosphates*

P- source[1]	P citric acid solubility	P water solubility
MSP (Monohydrate)	97.1	97.0
MCP (Test Phosphate A)	94.0	80.3
MDCP (Test Phosphate B)	94.6	64.1
MDCP (Test Phosphate C)	94.9	77.3
MCP (Test Phosphate D)	96.7	88.1

[1]MSP = monosodium phosphate; MCP = monocalcium phosphate; MDCP = mono-dicalcium phosphate

The *in vitro* solubility tests provide a rapid method for screening products to determine their suitability for inclusion in broiler diets. The principles behind the *in vitro* tests have been described in Chapter 2. Although not conclusive, the solubility values presented in Table 4.18 suggest that the P present in all the feed phosphates tested was readily available. MDCP (Test

Phosphate B) had the second lowest citric acid solubility value suggesting lower availability relative to the other sources tested; this source was the only source to exhibit a lower P availability than the reference MSP, both in *Experiment 1* and *Experiment 2*.

The water solubility values obtained for the five phosphate sources tested in this experiment confirm the classification of the respective products as described in Chapter 2. The two MCP sources have water solubility values greater than 80% while MDCP (Test Phosphate B) and MDCP (Test Phosphate C) have values of 64.08 and 77.26% respectively. It is generally recognised that MCP has a superior availability to DCP (De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Viljoen, 2001) and data presented by Pensack (1974) demonstrated a correlation between biological availability and the content of MCP as measured by water solubility. The water solubility value for MDCP (Test Phosphate B) indicated a MCP: DCP ratio of about 64: 46; a significantly higher DCP contribution in comparison to the other calcium phosphates analysed. Again, this source was the only source to perform significantly worse than the reference MSP, possibly as a result of the higher DCP content of the product. In *Experiment 2*, MCP (Test Phosphate A) with a water solubility of 80.26% tended to have a higher availability than MDCP (Test Phosphate B) ($P = 0.0761$), suggesting that there may be validity under certain circumstances to use water solubility as an indicator of relative availability.

4.4 Conclusion

The inclusion of wheat gluten as the primary protein source in the diet, replacing whey protein, had the desired effect of lowering the P concentration in the basal diet. The fact that the basal diet's contribution to the total P of the test diets was reduced to less than 10% may be partially responsible for the improved sensitivity with respect to P availability and the achievement of results that were more consistent with those reported in Chapter 2. Although the trial was not designed to measure a response in terms of bone strength and ash, these parameters were measured in order to confirm the trends observed in the balance assay. The results in terms of relative availability were inconclusive, but they achieved the objective of adding credibility to the balance trial by confirming marginalised bone development in comparison to other studies, suggesting that the lower availability values determined in these experiments were correct under the prevailing conditions.

The results of *Experiment* 1 and *Experiment 2* were in agreement with regard to the magnitude and relative availability of MSP, thus the objective of determining repeatability was partially achieved. Ca availability was however not consistent between the two experiments. The results of the *in vitro* tests suggest that both citric acid solubility and water solubility may be utilized as a rapid method for predicting the potential relative availability of a phosphate source under certain conditions.

Although the diets were balanced, the feed intake levels measured in both experiments were low. The results of *Experiment 2* confirmed the hypothesis that the daily intake was decreasing as the trial progressed. The inclusion of the commercial starter highlighted the severity of the situation. The poor feed intake and the confounding effect that it may have on the determination of the potential P availability of the respective sources require further investigation. If the issue of poor intake on the current diets cannot be resolved; perhaps the design of the experiment should be revised. One potential solution would be to improve the general condition of the birds at the start of the balance period by reducing the duration of the acclimation period. If the duration of the pre-trial period could be successfully decreased without impairing the results of the balance trial, the researcher may avoid the problems encountered as a result of the poor intake during this period.

Chapter 5
General discussion

The importance of incorporating a highly available product into broiler diets has not escaped the attention of feed phosphate manufacturers and new products are being developed and existing products continually improved. Different raw materials and manufacturing conditions employed in their production may lead to subtle changes in their chemical and physical composition and result in an altered phosphorus (P) availability. Since nutritionists are formulating diets closer to the actual requirements of the birds, small changes in availability can easily result in either a P deficiency in the birds or an excess of P in the faeces.

The objective of the first experiment was to evaluate five different phosphate sources for P and calcium (Ca) availability in an effort to monitor phosphate sources currently existing and entering the South African market and compare them to monosodium phosphate, an established reference source. The results of this experiment did not agree with reliable results previously published in the literature. Two possible shortcomings experienced in this experiment were the low feed intake of the birds fed the test diets and the high available P level of the basal diet.

A possible solution to these problems was to use a different protein source in the basal diet. The second and third experiments were conducted to evaluate the suitability of wheat gluten as an alternative protein source. The inclusion of wheat gluten had the desired effect of reducing the available P (aP) content in the basal diet from 1.3 g aP/kg to 0.3 g aP/kg. This allowed the formulation of test diets where the P from the test phosphate contributed the major portion of the total P. In these trials, five different phosphate sources were evaluated and although the availability results were lower than anticipated the relative order of availability for the respective sources was more in keeping with the accepted norm.

In addition, the P level in the test diets was investigated to ascertain whether a higher aP level could be utilised and still elicit credible results while reducing mortality. The results showed that although the birds on the 2.0 g aP/kg exhibited a significantly greater mortality rate, there was no difference in P or Ca utilization between the 2.0 and 3.5 g aP/kg treatments.

The replacement of whey protein with wheat gluten did not improve feed intake and the potential confounding effect of the low feed intake on the P availability was not substantiated. It appeared to substantially reduce the average availability of the sources, but whether there is an interaction between such a marginalised diet and the efficiency of P utilization between different sources is a subject for further investigation. Two possible solutions to improve the feed intake over the balance period are to pellet the experimental diets and ensure better bird health and condition entering the balance period by reducing the acclimation period.

However, noteworthy is the fact that even though birds under marginalised conditions performed significantly worse and exhibited poorer utilization of the available P provided than anticipated, the relative order of availability appeared to remain unaltered by the experimental conditions. The most highly available feed phosphate remained the best feed phosphate, even under the most adverse of conditions.

The results obtained in the course of this study highlighted an invaluable lesson. Regardless of the potential availability of the P in a feed phosphate, dietary factors and bird condition may result in a dramatically lower effective utilization of the P. It became evident that the availability of P was not an inherent property, characteristic of the material being assayed alone, but an experimentally determined value which reflects the absorption and utilization of the P ingested under the conditions of the test. The availability of a given P source may differ from one experiment to another and will almost definitely differ under practical conditions. This poses the challenge of developing an effective availability system that incorporates the dietary, environmental and physiological factors that may alter the potential availability of dietary P.

References

Al-Masri, M.R. (1995) Absorption and endogenous excretion of phosphorus in growing broiler chicks as influenced by calcium and phosphorus ratios in feed. *British Journal of Nutrition*, **74**: 407-415.

Akpe, M.P., P.E. Waibel, K. Larntz, A.L. Metz, S.L. Noll and M. M. Walser. (1987) Phosphorus availability bioassay using bone ash and bone densitometry as response criteria. *Poultry Science*, **66**: 713-720.

Anselme, P. (2003) Phosphorus in poultry nutrition. Key elements for performance and animal health. *Feed Magazine*. 01 March: 1-4.

Ammerman, C.B. (1995) Methods for estimation of mineral bioavailability. In: Ammerman, C.B., D.H. Baker, and A.J. Lewis (Eds). Bioavailability of Nutrients for Animals. Academic Press, New York, pp. 83-94.

Bar, A. and S. Hurwitz. (1984) Egg shell quality, medullary bone ash, intestinal calcium and phosphorus absorption and calcium-binding protein in phosphorus deficient hens. *Poultry Science*, **63**: 1975-1979.

Boyd, R.D., D. Hall and J.F. Wu. (1983) Plasma alkaline phosphatase as a criterion for determining biological availability of phosphorus for swine. *Journal of Animal Science* **57**: 396-402.

Bourdillon, A., B. Carré, L. Conan, J. Duperray, G. Huyghebaert, B. Leclercq, M. Lessire, J. McNab and J. Wiseman. (1990) European reference method for the in vivo determination of metabolisable energy with adult cockerels: reproducibility, effect of food intake and comparison with individual laboratory methods. *British Poultry Science*, **31**: 557-565.

Bryden, W.L. and D. Balnave. (1983) Dietary calcium and biotin status in poultry. In: *Proceedings of Technical Sessions, WPSA-Far East and South Pacific Federation*, Adelaide, Australia, 22nd and 23rd September.

Cosgrove, D.J. (1980) Inositol phosphates. Their chemistry, biochemistry and physiology. Elsevier Science Publishers, Amsterdam, pp.175.

Dhandu, A.S. and R. Angel. (2003) Broiler nonphytin phosphorus requirements in the finisher and withdrawal phases of a commercial four-phase feeding system. *Poultry Science*, **82**: 1257-1265.

Day, E.J., J. Mc Naughton and B.C. Dilworth. (1973) Chemical versus chick bioassay for phosphorus bioavailability of feed grade sources. *Poultry Science*, **52**: 393.

De Groote, G. and G. Huyghebaert. (1997) The bio-availability of phosphorus from feed phosphates for broilers as influenced by bio-assay method, dietary Ca-level, and feed form. *Animal Feed Science and Technology*, **69:** 329-340.

Devereux, C., M. Smart, F.P. Kalt and N. Takei. (1994) Animal feeds: Phosphate Supplements. CEH Marketing Research Report.

Dilworth, B.C. and E.J. Day. (1964) Phosphorus availability studies with feed grade phosphates. *Poultry Science*, **43**: 1039.

Edwards, H.M. (1993) Dietary 1,25-dihydroxycholecalciferol supplementation increases natural phytate phosphorus utilization in chickens. *Journal of Nutrition,* **123**: 567-577.

Fernandes, J.I.M., F.R. Lima, C.X. Mendoca Jr., I. Mabe, R. Albuquerque and P.M. Leal. (1999) Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. *Poultry Science*, **78**: 1729-1736.

Flemming, R.H., H.A. McCormack, L. McTier and C.C. Whitehead. (1998) Medullary bone and humeral breaking strength in laying hens. *Research in Veterinary Science*, **64**: 63-67.

Gillis, M.B., H.M. Edwards Jr., and R. J. Young. (1962) Studies on the availability of calcium orthophosphates to chickens and turkeys. *Journal of Nutrition*, **78**: 155-161.

Grimbergen, A.H.M., J.P. Cornelissen and H.P. Stappers. (1985) The relative availability of phosphorus in inorganic feed phosphates for young turkeys and pigs. *Animal Feed Science and Technology,* **13**: 117-131.

Guèguen, L. (1994) Determination of availability. Feed Mix: Special issue on Phosphates. Misset International, pp.12-15.

Harms, R.H., P.W. Waldroup and B.L. Damron. (1967) A comparison of phosphorus assay techniques with chicks. 2. Development of a calcium standard curve for monosodium phosphate. *Poultry Science*, **46**: 981-985.

Harrold, R.L., W.D. Slanger, C. N. Haugse and R.L. Johnson. (1983) Phosphorus bioavailability in the chick: Effects of protein source and calcium level. *Journal of Animal Science,* **57**: 1173-1181.

Harter- Dennis, J. (2000) Phytase application variations in broiler diets and legislative update. In: Lyons, T.P. and K.A. Jacques (Eds). Biotechnology in the feed industry. *Proceedings of Alltech's 16th Annual Symposium*. Nottingham University Press, pp.163-174.

Hegsted, D.M. (1973) Calcium and phosphorus. In: Goodhart, R.S. and M.E. Shils (Eds). Modern Nutrition in Health and Disease. Lea and Febiger, Philadelphia, pp.268-286.

Henuk, Y.L. and J.G. Dingle. (2003) Poultry manure: source of fertilizer, fuel and feed. *World's Poultry Science Journal,* **59**: 350-360.

Hurwitz, S. (1964) Estimation of net phosphorus utilization by the "slope" method. *Journal of Nutrition*, **84**: 83-91.

Huyghbaert, G., G. De Groote and L. Keppens. (1980) The relative biological availability of phosphorus in feed phosphates for broilers. *Ann. Zootech.* **19**: 245-263.

IFP (2004). Inorganic Feed Phosphates: http://www.feedphosphates.org

Jongbloed, A.W., P.A. Kemme, Z. Mroz and H. Van Diepen. (2000) Efficacy, use and application of microbial phytase in pig production: a review. In: Lyons, T.P. and K.A. Jacques (Eds). Biotechnology in the feed industry. *Proceedings of Alltech's 16th Annual Symposium*. Nottingham University Press, pp.111-129.

Keshavarz, K. (1994) Laying hens respond differently to high dietary levels of phosphorus in monobasic and dibasic calcium phosphate. *Poultry Science*, **73**: 687.

Lima, F.R., C.X. Mendonca Jr., J.C. Alvarez, J.M.F. Garzillo, E. Ghion and P.M. Leal. (1997) Biological evaluation of commercial dicalcium phosphates as sources of available phosphorus for broiler chicks. *Poultry Science*, **76**: 1707-1713.

Leske, K. and C. Coon. (2002) The development of feedstuff retainable phosphorus values for broilers. *Poultry Science*, **81**: 681-1693.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan. Minerals. In: McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan (Eds). Animal Nutrition 5th ed., Longman Press. pp 101-105.

Miles, R.D. and P.R. Henry. (1997) Defluorinated phosphates may provide advantages. *Feedstuffs*. 6 January: 12-15.

Nelson, T.S. and H.T. Peeler. (1961) The availability of phosphates from single and combined phosphates to chicks. *Poultry Science*, **40**: 1321-1327.

Nelson, T.S. and A.C. Walker. (1964) The biological evaluation of phosphorus compounds. *Poultry Science*, **43**: 94-98.

National Research Council (NRC). (1994). "Nutrient Requirements of Poultry" 9th ed. Nat. Acad. Press, Washington, DC.

Pensack, J.M. (1974) Biological availability of commercial feed phosphates. *Poultry Science*, **53**: 143-148.

Potter, L.M., M. Potchanakorn, V. Ravindran and E.T. Kornegay. (1995) Bioavailability of phosphorus in various phosphate sources using body weight and toe ash as criteria. *Poultry Science*, **74**: 813-820.

Potchanakorn, M. and L.M. Potter. (1987) Biological values of phosphorus in various sources for young turkeys. *Poultry Science*, **66**: 505-513.

Ravindran, V., E.T. Kornegay, L.M. Potter, B.O. Ogunabameru, M.K. Welten, J.H. Wilson and M. Potchanakorn. (1995) An evaluation of various response criteria in assessing biological availability of phosphorus for broilers. *Poultry Science*, **74**: 1820-1830.

Ross Breeders Ltd. (1998) 308 parent stock management manual. Ross Breeders LTD. Newbridge, Midlothian. Scotland. UK.

Rucker, R.B., H.E. Parker and J.G. Rogler. (1968) Utilization of calcium and phosphorus from hydrous and anhydrous calcium phosphates. *Journal of Nutrition*, **96**: 513-516.

Scheidler, S.E and J.L. Sell. (1987) Utilization of phytate phosphorus in laying hens as influenced by dietary phosphorus and calcium. *Nutr. Rep. Int.* **35**: 1073.

Sibbald, I. R. (1982) Measurement of mineral bioavailability: Extension of true metabolizable energy methodology. *Poultry Science*, **61**: 485-487.

Simons, P.C.M., H.A.J. Versteegh, A.W. Jongbloed, P.A. Kemme, P. Slump, K.D. Bos, M.G.E. Walters, R.F. Beudeker and G.J. Verschoar. (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *British Journal of Nutrition*, **64**: 525-540.

Sloan, D.K., R.H. Harms, D. Barnard and R. Nodstedt. (1995) Effects of diet on faeces composition and the implications on environmental quality. *Journal of Applied Poultry Research*, **4:** 379-383.

Soares, J.H. (1995) Phosphorus bioavailability. In: Ammerman, C.B., D.H. Baker, and A.J. Lewis (Eds). Bioavailability of nutrients for animals. Academic Press, New York, pp. 257-294.

Sullivan, T.W. (1966) A triple response method for determining biological value of phosphorus sources with young turkeys. *Poultry Science*, **45**: 1236-1245.

Sullivan, T.W. (1967) Relative biological value of ammonium polyphosphate and two phosphate supplements. *Poultry Science*, **45**: 1324-1330.

Sullivan, T. W and J. H. Douglas. (1990) Phosphorus bioassays – Developments in five decades. In: Proceedings of Pitman-Moore Nutrition for the Nineties Conference. Bloomington, MN, pp. 18-37.

Touchburn, S.P., S. Sebastian and E.R. Chavez. (1999) Phytase in poultry nutrition. In: Recent Developments in Poultry Nutrition, J. Wiseman and P.C. Garnswothy (Eds). Nottingham University Press (UK), pp. 147-163.

Van der Klis, J.D. (1993) Physico-chemical chyme conditions and mineral absorption in broilers. PhD thesis, Agricultural University, Wageningen, The Netherlands.

Van der Klis, J.D. and H.A.J. Versteegh. (1999) Phosphorus nutrition of poultry. In: Recent Developments in Poultry Nutrition 2, J. Wiseman and P.C. Garnswothy (Eds). Nottingham University Press (UK), pp. 309-320

Viljoen, J. (2001) Utilisation of feed phosphates: Fact or confusion? *Afma matrix*. December: pp. 24–27.

Viljoen, J. (2003) Quality of feed phosphate supplements for animal nutrition. South African Society of Animal Science: http://www.sasas.co.za/Popular/Popular.html

Waibel, P.E., N.A. Nahorniak, H.E. Dziuk, N.M. Walser and W.G. Olsen. (1984) Bioavailability of phosphorus in commercial feed phosphate supplements for turkeys. *Poultry Science*, **63**:730-737.

Waldroup, P.W. (1999) Nutritional approaches to reducing phosphorus excretion by poultry. *Poultry Science*, **78**: 683-691.