Evaluation of various Phytase enzymes for application in broiler feeding

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Dedication

This dissertation is dedicated to Grieve Chelwa. Thank you for the unwavering support and kindness.

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Declaration

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Summary

Feed costs form a major component of broiler production and the industry is always investigating ways of reducing feed costs. Phytase is an innovation that releases phosphorus from feed ingredients and as a consequence improves the nutritive value of broiler diets. A 34 day experiment was conducted to determine the effect of three different types of commercial phytase (HiPhos, OptiPhos and Quantum blue) at two levels (standard and three times the standard level) on production parameters, carcass characteristics and bone mineralization of broilers. Positive control (PC) diets were formulated based on the lower end of National Research Council (NRC) recommended values. Negative control (NC) diets were formulated on the PC diets less the matrix value for HiPhos 2000FTU (HP200). A positive control diet and negative control diet were compounded for three phases; starter, grower and finisher. Therefore eight diets were mixed: 1. Positive control (PC); 2. Negative control (NC); 3. NC + 2000FTU HiPhos (HP200); 4. NC + 1000FTU OptiPhos (OP400); 5. NC + 1000FTU Quantum blue (QB200); 6. NC + 6000FTU HiPhos (HP600); 7.NC + 3000FTU OptiPhos (OP1200) 8.NC + 3000FTU Quantum blue (QB600). The experiment involved 5120 day old broilers that were allocated to a completely randomised design with eight treatment diets and eight replications. The purpose of the study was, firstly to compare the effects of three different types of commercial phytase supplemented to maize-soya bean based diets on broiler performance outcomes. Secondly, to investigate the effects of each phytase at two inclusion levels. Thirdly, to evaluate the effects of three types of phytase on internal organs and intestinal morphology. At the end of the study, supplementation of phytase to NC diets improved live weight, average daily gains and cumulative gains. However, improvements were not comparable to those of the PC group. Breast colour, pH, and temperature, dressing percentage, internal organs, as well as thigh and wing portions were not affected by phytase inclusion. Cold carcass weight, breast and drumstick portions differed significantly between treatments. Gizzard weight expressed as a percentage of live weight differed significantly between treatments. Significant differences between treatments were also observed for intestinal morphometric observations. Phytase supplementation did not have an influence tibia length, tibia diameter, robusticity index, bone breaking strength, percent ash, percent phosphorus and percent calcium. Dry tibia weight, calcium to phosphorus ratio and the length of the tibia in relation to live weight differed between treatments. Computed tomography scans showed tibia structural abnormalities. Overall, phytase supplementation to negative control diets did not meet the phosphorus requirements for proper bone formation of broilers. In addition phytase supplementation at both inclusion levels did not pose a risk to the immune status of the broiler as internal organs were not negatively affected.

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Opsomming

Voerkoste is die hoof komponent van braaikuiken produksie en daarom is die industrie altyd op soek na nuwe innoverende maniere om hierdie koste te verminder. Die gebruik van fitase is so ŉ innovasie wat fosfor vanaf voerkomponente vrystel en so die voedingswaarde van die dieet verhoog. ŉ 34 dae eksperiment is uitgevoer om die invloed van drie tipes fitases (HiPhos, OptiPhos en Quantum blue) teen twee insluitingspeile (standaard en drie keer die standaard) op produksie parameters, karkas eienskappe en been mineralisering van braaikuikens te bepaal. Positiewe kontrole diëte (PK) was geformuleer gebaseer op die onderste vlakke van die Nasionale Navorsingsraad (NRC) se aanbevelingsvlakke. Negatiewe kontrole diëte (NC) is geformuleer op die PK diets minus die matriks waarde van HiPhos 2000 (HP200). ŉ Positiewe en negatiewe kontrole dieet is saamgestel vir drie fases nl. (i) aanvangs, (ii) groei en (iii) afronding. Gevolglik is daar agt eksperiementele diëte gemeng: 1. Positiewe kontrole (PK); 2. Negatiewe kontrole (NC); 3. NC + 2000FTU HiPhos (HP200); 4. NC + 1000FTU OptiPhos (OP400); 5. NC + 1000FTU Quantum blue (QB200); 6. NC + 6000FTU HiPhos (HP600); 7.NC + 3000FTU OptiPhos (OP1200) 8.NC + 3000FTU Quantum blue (QB600). Vir die eksperiment is 5 120 dagoud braaikuikens in ‘n totaal ewekansige uitleg toegedeel aan agt behandelings elk met agt herhalings. Die doel van die studie was, eerstens om die invloed van die behandelings op prestasie van braaikuikens te bepaal, tweedens om die invloed van die verskillende insluitinigspeile te bepaal en derdens om die invloed van die verskillende insluitingspeile op organe en die ingewandsmorfologie te bepaal. Aan die einde van die studie is gevind dat die aanvulling van fitases tot die NC dieet gelei het to verbeterde lewende massas, gemiddelde daaglikse toenames en kumulatiewe toenames. Hierdie verbetering was egter nie vergelykbaar met die PC nie. Kleur van die borsvleis, pH, temperatuur, uitslag persentasie, interne organe, dy en vlerk porsies was nie beïnvloed deur die behandeling nie. Gizzard massa uitgedruk as persentasie van lewende massa het egter betekenisvol verskil. Verskille is ook gevind t.o.v vir ingewandsmorfologie. Fitase aanvulling het geen invloed gehad op tibia lengte, deursnee, robuustheids indeks, been breek sterkte, persentasie as, persentasie fosfor of persentasie kalsium nie. Droë tibia massa, kalsium tot fosfor verhouding en die lengte van die tibia in verhouding tot lewende massa het verskil tussen behandelings. Gerekenariseerde tomografiese skanderings het strukturele tibia abnormaliteite aangedui. Oorhoofs kan aangeneem word dat die aanvulling van fitase ensieme teen peile tot drie keer die aanbevole insluiting nie gelei het tot dieselfde beskikbare fosforpeile van die PC dieet nie. Verder het die insluiting van fitase tot drie keer die aanbeveling geen negatiewe uitwerking op die immuunstatus van die braaikuikens gehad nie aangesien daar geen verskille in orgaan massas en verhoudings waargeneem is nie.

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Notes

The language and style used in this thesis are in accordance with the requirements of the South African Journal of Animal Science. This thesis represents a compilation of manuscripts were each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

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Abbreviations

ADG	Average daily gains
AME	Apparent metabolisable energy
ANOVA	Analysis of variance
Ca	Calcium
Co	Cobalt
CP	Crude protein
Cu	Copper
EPEF	European production efficiency factor
FCR	Feed conversion ratio
Fe	Iron
FTU	Phytase units
g	Gram
g/kg	Gram per kilogram
g/ton	Gram per ton
h	Hour
HCL	Hydrochloric acid
IP1	Myo-inositol monophosphate
IP2	Myo-inositol biphosphate
IP3	Myo-inositol trisphosphate
IP4	Myo-inositol tetrakisphosphate
IP5	Myo-inositol pentakisphosphate
IP6	Myo-inositol hexakisphosphate
KCl	Potassium chloride
Kg	Kilogram
L	Litres
m^2	Meter squared
min	Minute
mL	Millilitre
Mg	Magnesium
N	Newton
NIR	Near infrared spectroscopy
N/mm^2	Newtons per millimetre squared
NRC	National Research Council
P	Phosphorus
PER	Protein efficiency ratio
Phytate-P	Phytate bound phosphorus
pH_i	pH 15 minutes *post mortem* (initial pH)
pH_u	pH 24 hours *post mortem* (ultimate pH)

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Chapter 1

1.1 Introduction

Innovative feed formulation strategies such as the use of phytase in commercial broiler production systems can have an impact on the economy, the environment and society in general. A study done by Van Emmenes, (2014) showed that the production outcomes of broiler diets that were supplemented with a commercial phytase known as HiPhos were more profitable than those that were not supplemented with phytase. That means, for diets that contained dietary phytase, the cost per kilogram of broiler live weight reduced. Phosphorus supplementation is important in broiler diets so as to meet the demands for production (Wilkinson *et al.*, 2014). A reduction in the phosphorus intake of a growing bird has detrimental effects on growth and welfare of the bird (Driver *et al.*, 2006). Most of the phosphorus contained in a broiler diet is inorganic. Feed ingredients also contribute to the total phosphorus content of a diet. Phytase is an enzyme that releases bound phosphorus from feed ingredients (Nayini & Markakis, 1986). Therefore, the amount of inorganic phosphorus added to broiler diets can be reduced, which then leads to a reduction in feed costs.

In South Africa there is a shift towards the use of plant based feed ingredients from animal protein sources such as fish, meat and bone meal. Animal protein sources provide an additional benefit of mineral availability. However, plant sources such as maize and soya beans contain minerals that are not readily available for utilization as they are bound in the form of phytate (O'Dell *et al.*, 1972). Phytase hydrolyses the phytate complex and releases minerals that can be used for growth. Furthermore, with the introduction of dietary phytase the amount of excreted minerals in manure has reduced (Jongbloed & Lenis, 1992). This has addressed environmental concerns that are related to land and water pollution caused by excess phosphorus excretion in manure.

Apart from releasing phosphorus, phytase also releases calcium, magnesium, zinc and amino acids from phytate (Namkung & Leeson, 1999). In order to optimise these benefits, inclusion rates of phytase that are higher than recommended by the manufacturer can be considered.

The aims of the current study was firstly, to compare the effects of three different types of commercial phytase supplemented to maize-soya bean based diets on broiler performance outcomes. Performance outcomes were determined on production parameters and carcass characteristics. Secondly, to investigate the effects of each phytase at two inclusion levels namely the, standard recommended levels as prescribed

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by the respective manufacturers and an inclusion level of three times higher than the standard inclusion level. Qualitative response criteria that were used to investigate bioavailability of phosphorus included production parameters, bone mineralisation. Lastly, the effects phytase type and inclusion level on internal organs and intestinal morphology were evaluated.

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Chapter 2

Literature review

2.1 Introduction

Phytase is an enzyme that releases bound phosphorus from phytate in feed ingredients (Nayini & Markakis, 1986). The inclusion of phytase to diets has notably led to a reduction in the amount of inorganic phosphorus allocated to diets. Supplementing diets with phytase and concurrently reducing the amount of inorganic phosphorus leads to a reduction in feed cost.

Most commonly used constituents of plant-based broiler diets are maize and soya beans. A limitation of these ingredients is that they contain phytate (De Boland *et al.*, 1975; Selle *et al.*, 2003). Phytate is an anti-nutrient that binds phosphorus and several other cations making them unavailable for utilisation (Cosgrove & Irving, 1980). The bound cations include; magnesium, manganese, zinc, iron, calcium, potassium, copper and cobalt. Therefore, when diets do not meet the phosphorus requirements of broilers, bone defects and calcium related illnesses may occur. Consequently, the development and commercialisation of exogenous phytase has made phosphorous available for growth and proper bone development.

Despite the presence of intrinsic intestinal and seed phytase, phytate dephosphorlytion has not been sufficient to meet broiler demands. As a result diets have had to be supplemented with inorganic sources of phosphorus. Subsequently, phosphorus excreted in faecal matter contributes to nutrient surplus. A nutrient surplus in turn has negative environmental implications such as eutrophication and pollution. However, supplementation of phytase has led to a reduction in the amounts of phosphorus excreted in faecal matter (Jongbloed & Lenis, 1992).

Many types of phytase have been developed and are available commercially. Even more important, continuous innovation has led to the production of phytase with even higher efficacy (Cowieson & Adeola, 2005). However many factors play a role in the efficiency of phosphorus release. For instance, type of substrate added, amount of dietary calcium and phosphorus. In addition, various phytase characteristics such as temperature stability, pH range and proteolytic resistance play a major role in phytase efficacy. Furthermore, higher inclusion rates of phytase in diets are being considered as a strategy to reduce feed costs.

2.2 Phosphorus

The second most abundant mineral in an animal is phosphorus, whilst calcium is the most abundant mineral. Phosphorus and calcium are major constituents of bone. Over 90% of

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calcium and 80% of phosphorus found in the body is located in the skeletal structure (McDonald, 2002). Skeletal development is highly dependent on the amount of calcium and phosphorus allocated to diets, the phosphorus to calcium ratio, vitamin D, A and C.

Phosphorus is linked to nucleic acids to form adenosine triphosphate (ATP). The co enzyme ATP is important for; energy metabolism, acts as an ion pump, active transport of materials across the cell membrane, metabolic trapping and muscle contraction (Bender, 2014). In addition, phospholipids form part of the cell membrane fluidity and structure. During energy production, when ATP is sufficient, phosphocreatine is temporary stored in the muscle (McDonald, 2002).

Sources of dietary phosphorus include cereals, dicalcium phosphate, monocalcium phosphate, fish meal and meat and bone meal. However the phosphorus contained in cereals is not readily available. In dietary terms, total phosphorus includes all forms of phosphorus included in feed. However, available phosphorus refers to that which is absorbed from the diet. Digestible phosphorus is the amount of phosphorus fed in the diet after subtracting the amount of phosphorus in the ileum. Furthermore, retained phosphorus is that which is fed minus that which is excreted. Phosphorus that occurs bound in a seed is known as phytic acid or phytate, while that which is not bound is known as non phytate phosphorus. The difference between available and non phytate phosphorus is that available phosphorus includes both organic and inorganic phosphorus.

2.3 Phytate

Phytic acid is a hexa phosphorus acid ester of the 6-hydroxyl group cyclic alcohol myo-inositol. Chemically phytic acid is known as a myo-inositol 1 (IP1), 2 (IP2), 3 (IP3), 4 (IP4), 5 (IP5), 6 (IP6) and dihydrogen phosphate. The salts of phytic acid are described as phytate (Cosgrove & Irving, 1980). The molecular weight of phytate is 660g/mol and consists of a six-carbon myo-inositol ring ($C_6H_{18}O_{24}P_6$) (Bedford & Partridge, 2001). Specifically, a phytic acid salt of calcium and magnesium is known as phytin, while phytate is a mono to dodeca anion of phytic acid. In addition to forming complexes with phosphorus, phytic acid has the ability to chelate with cations such as calcium, zinc, iron and copper (Evans & Pierce, 1981). Chelates occur as a result of bond formation between cations and phosphate groups that have negatively charged anions. In the small intestines of monogastrics, the chelating potential of phytate poses a risk of nutritional deficiencies (Evans & Pierce, 1981; Harland & Oberleas, 1999).

2.3.1 Occurrence

Phytate acts as a reservoir for phosphorus, including other cations and is hydrolysed during germination (Greenwood & Batten, 1995). Also, phytate mediates phosphorus

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homeostasis in germinating seeds and growing seedlings (Matheson & Strother, 1969). Phytate is situated in the aleuronic layer for monocotyledons, whilst dicotyledonous phytate is stored as globoids in the kernel (Erdman, 1979; Lott, 1984). As a result, milling products such as wheat bran and gluten will contain more phytate than other portions of the seed as shown in Table 2.1. However, the amount of phytate in the seed will depend on the seed's age, variety, method of processing used and the location in which the plant was grown.

Table 2.1 Phytate and phytate phosphorus levels in different feed ingredients

Feed ingredient	Phytate g/kg	Phytate-P g/kg	Reference
Wheat	1.06		Kirby & Nelson, 1988
Wheat	0.78	2.20	Eeckhout & De Paepe, 1994
Wheat bran	3.14		Kirby & Nelson, 1988
Wheat bran		7.90	Steiner *et al.*, 2007
Maize	0.74		Kirby & Nelson, 1988
Maize		2.10	Selle *et al.*, 2003
Soya beans	1.39		Kirby & Nelson, 1988
Soya bean meal	1.93		Frank *et al.*, 2009
Soya bean meal		4.50	Selle *et al.*, 2003

Phytate-P – Phytate phosphorus

2.3.2 Nutritional importance

2.3.2.1 Phytate and mineral interaction

The amount of phosphorus contained in phytate complex is higher for cereals and wheat as compared to legumes and oil seed meal (Eeckhout & De Paepe, 1994). Phytate-phosphorus levels in different feed ingredients are shown in **Error! Reference source not ound.**. The amount of phosphorus in the phytic acid complex for most cereals and seeds is 282 g/kg. As has been reviewed by Ravindran, (1995), phosphorus content in broiler diets ranges from 2.5 to 4.0 g/kg feed. In addition, O'Dell *et al.*, (1972) reported that more than 80% of phosphorus in maize is bound to phytate. Phytic acid has six strongly acidic reactive sites with pK 1.5 to 2.0, two weakly acidic reactive sites with pK 6.0 and four very weakly acidic reactive sites with pK 9.0 to 11.0 (Erdman, 1979). Therefore, in the gastro intestinal tract (pH 5.5 - 8.0) phytic acid bears a strong negative charge making it possible to bind with other cations. Phytic acid forms insoluble complexes with cations at neutral pH (Oberleas, 1973). The influence of cation inhibition on phytate degradation has been reported to be in the order of Cu^{2+} >Zn^{2+} >Co^{2+} >Mn^{2+} >Fe^{3+} >Ca^{2+} (Vohra *et al.*, 1965) and similarly, Zn^{2+} >Fe^{2+} >Mn^{2+} >Fe^{3+} >Ca^{2+} >Mg^{2+} (Maenz *et al.*, 1999). Even though calcium has less inhibitory potential than most other cations, dietary concentrations are

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usually higher. Therefore phytate-calcium complex formation increases with incremental levels of phytic acid. Another study observed that zinc forms complexes with sodium phytate at physiological pH levels in chicks (Maddaiah *et al.*, 1964).

2.3.2.2 Phytate and pH

Solubility of phytate complex is dependent on pH, type of cation and cation concentration (Oberleas & Chan, 1997). For instance, increasing the amount of a cation may increase the likelihood of precipitates forming with other cations. Further, an *in vitro* study that was performed to mimic duodenal pH values observed that phytate complex formation is pH dependent (Nolan *et al.*, 1987). At higher pH values, phytate forms precipitates with cations such as calcium, magnesium and zinc making the complex insoluble (Kaufman & Kleinberg, 1971). However, zinc and copper complexes are also insoluble at low pH (Oberleas *et al.*, 1966). The pH of the gizzard and proventriculus lies within the range 2 to 2.1 (Farner, 1943). Consequently, formation of calcium-phytate complexes would be reduced.

2.3.2.3 Phytic acid, protein and proteolytic enzyme interaction

Phytate forms complexes with protein (Cosgrove, 1966), pepsin and trypsin (Vaintraub & Bulmaga, 1991) which would then have an influence on amino acid digestibility. At lower pH levels, proteins have a positive charge that form bonds with oppositely charged phytate through electrostatic charges. However, at higher pH, bonds are formed through salt bridges (Selle *et al.*, 2010). In the gastro intestinal tract of broilers, degradation of the phytate complex occurs in the gizzard. An *in vitro* investigation showed that phytic acid depressed gizzard extracted pepsinogen activity (Liu & Cowieson, 2000). Other reports have shown that *in vitro* phytate concentration influences trypsin inhibition (Singh & Krikorian, 1982; Caldwell, 1992). In addition, Deshpande & Damodaran, (1989) observed that at pH 3.0, phytate formed precipitates with trypsin and chymotrypsin. The author further explained that at low pH, secondary structural changes caused trypsin inhibition.

2.3.2.4 Phytate and starch

Starch forms hydrogen bonds with phytate or with the protein-phytate complex leading to a reduction in nutrients available for digestion (Rickard & Thompson, 1997). An *in vitro* study on starch digestibility with salivary amylase found that phytate significantly reduced starch degradation (Thompson & Yoon, 1984). Similarly, an *in vitro* experiment noted that at pH 4.15, phytate and myo-inositol-2-monophosphate reduced alpha amylase starch digestion by 8.5 and 78.3 %, respectively (Knuckles & Betschart, 1987). In another study, the addition of phytic acid had minimal effect on amylase, even though alpha amylase and maltase were affected by tannic acid (Björck & Nyman, 1987). The differences in the observed results from these studies might be a result of exposure of amylase to sodium

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before buffering to the desired pH levels. However, the effect of phytate on alpha amylase inhibition *in vivo* has not yet been established. Even though inclusion of alpha amylase to broiler diets positively affected corrected apparent metabolisable energy and apparent faecal digestibility of starch (Gracia *et al.*, 2003).

2.3.3 Environmental importance

Environmental impact of phytate results from a nutrient surplus that occurs in areas that produce large quantities of manure. Accumulation of poultry manure in a location leads to eutrophication of fresh water systems (Daniel *et al.*, 1998). Eco-system imbalances may occur as a result of pollution of fresh water deposits and may lead to loss in biodiversity. In addition, eutrophication of water bodies leads to the development of algae blooms that compete with aquatic species for oxygen. Inclusion of phytase to monogastric diets reduces phosphorus excretion in faecal matter which therefore reduces adverse effects on the environment (Jongbloed & Lenis, 1992).

2.4 Phytase

Phytase is defined as *meso*-inositol hexaphosphate phosphohydrolase, a type of phosphatase that has the ability to release bound phosphorus from phytate (Nayini & Markakis, 1986; Lasztity & Lasztity, 1990). Phytase is a phosphatase that activates the stepwise hydrolytic phosphate splitting of phytic acid (IP6) or phytate to lower inositol phosphate esters (IP1-IP5) and inorganic phosphate (Bedford & Partridge, 2001; Selle & Ravindran, 2007).

2.4.1 Classification of phytase

2.4.1.1 Site of activity

The International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB, Commission on Biochemical Nomenclature, 1978) describe groups based on the position of hydrolysis initiation. Firstly, those that cleave at the first carbon; secondly, those that cleave at third carbon of the inositol ring; thirdly, those that cleave at the sixth carbon position on the inositol ring.

Phytase of microbial origin usually cleave on the first carbon and the third carbon and are called 3-phytases (EC 3.1.3.8), while those of plant origin cleave on the sixth position, hence the name 6-phytase (EC 3.1.3.28) (Kornegay, 2001; Selle & Ravindran, 2007). Other studies have shown differences at the point of cleavage such as *Escherichia coli* which is a 6-phytase (Greiner *et al.*, 1993), *Peniophora lycii* and *Basidiomycete* fungi (Lassen *et al.*, 2001). Phytase hydrolysis will have a tendency to begin cleavage in a completely phosphorylated phytate (IP6), then a penta- ester, tetra- ester, tri- ester, di-ester and finally a mono-ester of inositol phosphate (Wyss *et al.*, 1999; Vats & Banerjee,

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2004). In most cases myo-inositol pentakisphosphate (IP5) is further hydrolysed to a reduced phosphorylated myo-inositol phosphate such as inostitol trikisphosphate IP3 (Kerovuo *et al.*, 2000; Quan *et al.*, 2004) or inositol phosphate (IP) (Wyss *et al.*, 1999; Casey; Walsh, 2004; Sajidan *et al.*, 2004).

2.4.1.2 Optimum pH

Phytases are classified as acid, neutral or alkaline depending on the pH range of activity (Konietzny & Greiner, 2002). However, two main groups are defined; firstly, acid phytases having maximum activity at pH 5.0 and secondly, alkaline phytases with maximum activity at pH 8.0. Since most phytases are derived from microbial origin and are developed to suit the acid to neutral conditions of the gastro intestinal tract, acid phosphatases are preferred. Table 2.2 shows sources and properties of microbial phytase that have been used for commercial production.

Table 2.2 Sources and properties of microbial phytase

Phytase	**pH range**	**Temperature (°C)**	**Site of Activity**	**Reference**
A. niger	5.0 - 5.5	55 - 58	3- phytase	Ullah & Gibson, 1987
A. fumigatus	5.0 - 6.0	60	3- phytase	Wyss *et al.*, 1999; Rodriguez *et al.*, 2000
A. oryzae	5.5	50	6- phytase	Shimizu, 1993
P. lycii	5.5	58	6- phytase	Lassen *et al.*, 2001; Ullah & Sethumadhavan, 2003
E. coli	4.5	55 - 60	6- phytase	Greiner *et al.*, 1993; Golovan *et al.*, 1999

2.4.1.3 Catalytic mechanism

Phytases are classified by site of activity and pH optimum as described in section 2.4.1.1 and 2.4.1.2 respectively. In addition, phytases are broadly classified by catalytic mechanisms. Phytases are structurally different and are grouped into histidine acid phytase, propeller phytase and purple acid phytase (Mullaney & Ullah, 2003). The phytases that are commonly used in commercial production are histidine acid phytases, which are further grouped into two classes (Bedford & Partridge, 2001). The first group with a low specific activity for phytic acid and a wide substrate specificity. The second group has a high specific activity for phytate and narrow substrate specificity.

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2.4.2 Measured activity

Phytase activity is measured in units. One unit of phytase activity is the amount of enzyme that liberates 1 μmol of inorganic phosphorus in 1min from a 5.1 mmol solution of sodium phytate at 37°C and pH 5.5 (Bedford & Partridge, 2001). Abbreviations used to denote phytase units include FTU, FTY, PU and U (Selle & Ravindran, 2007). Phosphorus equivalency value is defined as the amount of inorganic phosphorus that can be removed by a given amount of added or intrinsic phytase. For direct comparison, equivalency values must be adjusted by the estimated digestibility of the inorganic phosphorus sources that phytase replaces (Bedford & Partridge, 2001).

2.4.3 Occurrence

Phytase occurs widely in plant and animal species. Phytase has been found in the blood of young cattle (McCollum & Hart, 1908) in the root of maize (Hubel & Beck, 1996) and also in the seeds of germinating soybean (Hamada, 1996). Analysed feed ingredients from Belgium showed significant phytase activity in rye (5130 units/kg), triticale (1688 units/kg), wheat (1193 units/kg) and barley (583 units/kg) (Eeckhout & De Paepe, 1994). Other analysed ingredients such as maize, soya beans, peas and potato starch showed minimal to no phytase activity. In addition, after pelleting wheat bran, phytase activity more than halved. Even though phytase is intrinsic to the mucosa of monogastrics, several authors have noted minimal activity for complete de-phosphorylation (McCuaig *et al.*, 1972; Maenz & Classen, 1998; Applegate *et al.*, 2003). The presence of phytase in both the mucosa of monogastrics and the apparent presence in some grains have not been sufficient to hydrolyse phytate. On the other hand, abundant occurrence of phytase in microorganisms has enabled commercial production.

2.4.4 Nutritional importance of phytase

The development of microbial phytase has improved monogastric utilization of phytate phosphorus (Augspurger *et al.*, 2003). Phytase releases phosphorus from ingredients that are used in broiler diets. Therefore, the amount of inorganic phosphorus added to diets is less and feed costs are reduced. In an experiment performed under laboratory conditions, 1g of *Aspergillus ficuum* phytase released 950 milligrams of phosphorus from a calcium phytate complex (Nelson 1971). In addition, an increase in the amount of *A. ficuum* led to the complete dephosphorlytion of the phytate complex. Furthermore, a 21 day experiment with male broilers proved that phytase can replace 5.8g/kg phosphorus (Denbow *et al.*, 1995) and 1.0g/kg phosphorus (Mitchell & Edwards, 1996) from the phytate complex.

2.4.5 Factors determining effectiveness of phytase

Phytase have to be developed to suit the conditions in the gastro intestinal tract of broilers. Phytases are normally inhibited by their product (Greiner *et al.* 1993; Hu *et al.*

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1996; Greiner 2002; Lopez *et al.* 2000). Different types of phytase have specific pH and temperatures at which they function optimally as has been described in **Error! Reference ource not found.** . In addition, phytases are substrate specific (Wyss *et al.*, 1998) and are susceptible to proteolysis. *In vitro* studies have been used to determine pH values and temperature ranges for optimum phytase activity. Usually, *in vivo* experiments follow to evaluate phytase efficacy.

2.4.5.1 pH

The pH optimum of commercial phytase must be suitable for the chicken's gastro intestinal tract environment. By using protein engineering, the pH profile of an enzyme can be modified to suit the pH of the stomach (Kim *et al.*, 2006). Several studies have shown different ranges of pH optima for different phytase sources. Tamim *et al.*, (2004) reported that *Aspergillus ficuum*, a 3- phytase had a pH optimum lying between 4 and 4.5 and *Peniophora lycii,* a 6- phytase showed activity at pH 3 and increased activity at pH 5. Another study determined that *A. ficuum* had two pH optima at 2.5 and 5.5 (Simons *et al.*, 1990). Furthermore, phytase derived from *E. coli* had an optimal pH range of 2 to 4.5 (Adeola *et al.*, 2004). A laboratory prepared phytase derived from *A. niger* was observed to have optimum activity at pH 5 (Dvořáková *et al.*, 1997). Furthermore, a study that compared four commercial phytase and two laboratory prepared phytase reported that; two strains *of Aspergillus sp*. used in poultry diets had a pH optimum at 5.5. The other two commercial phytase; *Peniophora lycii* (6-phytase) and *A. awamori* (3-phytase) had a pH of 4.5 and 5.0 respectively. In addition, laboratory prepared *E. coli* and *Bacillus subtilis* had pH optima at 4.5 and 7.0 respectively (Igbasan *et al.*, 2000).

2.4.5.2 Temperature in vivo and thermo-stability during pelleting

A study reported temperature optima in for *A. ficuum* (from the strain *A. niger*), *A. ficuum* (from the strain *Brassica napus*), *Peniophora lycii* (6-phytase), *A. awamori* (3-phytase), *E. coli* (6-phytase) and *Bacillus subtilis* to be 50, 50, 50, 50, 60 and 60°C, respectively (Igbasan *et al.*, 2000). That means that the chicken's gastro-intestinal tract temperature of 37-40 °C is suitable for most strains of phytase. Even though pelleting temperatures range from 75 to 85°C, the *in vivo* activity of phytase indicated in the study by Igbasan *et al.*, (2000) would be optimum**.** In order to avoid denaturing of the protein structure of phytase during high pelleting temperatures, methods such as chemical coating are used. In addition, phytase can be sprayed onto feed post-pelleting. Biological modification can also be done to improve thermal stability. For instance, Wyss *et al.*, (1998) showed that phytase derived from *A. fumigatus* can retain active conformation at temperatures of up to 90°C.

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2.4.5.3 Proteolysis resistance

Trypsin and pepsin are enzymes that readily breakdown protein components during digestion. Phytase should be resistant to protease action so as to maintain an acceptable rate of activity in the digestive tract. Most commercial phytase are developed to be resistant to degradation. For example, Rodriguez *et al.*, (1999) observed that trypsin and pepsin resistant phytase is effective at releasing phosphorus from phytate. However, some laboratory prepared phytase derived from *A. ficuum* exhibited higher proteolytic stability in comparison to commercial phytase derived from *A. niger* and *A. oryzae* (Zhang *et al.*, 2010).

2.4.5.4 Phytase and mineral interactions

In vitro and *in vivo* studies evaluating the effect of calcium levels and *A. ficuum* (3-phytase) or *Peniophora lycii* (6- phytase) on the release of phosphorus from the phytate complex, found that including calcium led to a reduced solubility of the complex (Tamim *et al.*, 2004).

In another study, the activity of laboratory prepared *A. niger* phytase was inhibited by copper, zinc and inorganic monophosphate ions (Dvořáková *et al.*, 1997). However, *A. niger* was activated by calcium and magnesium ions. On the other hand, Zhang *et al.*, (2010) observed that phytase derived from *A. ficuum* was not affected by calcium, magnesium, manganese and zinc ions.

In a study where calcium to non-phytate phosphorus ratios were 4.1:1, 2.75:1, 2.1:1, 1.5:1 and 1.14:1; phosphorus played a more important role in production performance as compared to the amount of calcium (Wilkinson *et al.*, 2014). Similarly, another study indicated that as the calcium to phosphorus ratio increased, weight gain, and feed intake reduced (Amerah *et al.*, 2014).

The difference in results on the effect of minerals on phytase activity would seem to indicate that different species of phytase do not respond in a similar manner. This might be explained by the fact that dietary ingredients have different outcomes *in vivo*. For instance, reports indicate that EDTA forms chelates that hydrolyse phytic acid thereby improving phytase activity (Maenz *et al.*, 1999; Zhang *et al.*, 2010). On the other hand, some compounds form cations chelates that encourage insoluble complexes, such as those with zinc (Vohra *et al.*, 1965). In addition, other constituents of feed such as enzymes, vitamins and energy level of the diets may have an influence *in vivo* on the liberation of phosphorus.

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2.4.5.5 Effects of phytase on energy and amino acid levels of diets

The influence of phytase on apparent metabolisable energy (AME) and amino acid digestibility has not yet been established. However, studies have shown that supplementation of phytase improved feed intake (Kornegay *et al.*, 1996; Dilger *et al.*, 2004). An increase in feed consumed would then lead to an expected rise in daily AME. Furthermore, a 16 day experiment showed that inclusion rates greater than 100 FTU improved nutrient digestibility coefficients (Cowieson *et al.*, 2006). In this study, phytase inclusion rates of 150, 300, 600, 1200, 2400 and 24000 FTU were added to diets deficient in available phosphorus (3.0g/kg).

In a study done by Ravindran *et al.*, (2000), wheat-sorghum-soya meal diets formulated on three levels of phytic acid (10.4, 13.2 and 15.7 g/kg) indicated that amino acid digestibility was reduced in high phytic acid diets. However, supplementation of phytase at 400 FTU and 800 FTU improved amino acid digestibility and AME. Apparent metabolisable energy improvements were observed for adequate diets (4.5g/kg) as compared to diets that were deficient (2.3g/kg) in phosphorus. However, an experiment based on a wheat-soya diet with adequate phosphorus inclusion (4.5g/kg) found that AME was not improved by 500 FTU of phytase (Wu *et al.*, 2004)

However, phytase supplementation improved amino acid digestibility and AME of wheat-sorghum-soya meal based diets with adequate phosphorus but deficient in lysine (Ravindran *et al.*, 2001). In addition, weight gain and feed conversion ratio were improved from day 7 to 28.

Namkung & Leeson, (1999) reported that inclusion of 1000 FTU to diets containing low calcium (7.9g/kg) and low available phosphorus (3.5g/kg) increased corrected AME, digestibility of amino acids valine and isoleucine. In addition the authors observed improved feed conversion ratio and weight gains for male broilers.

2.4.6 Use of phytase matrix values in feed formulation

The use of matrix values to determine by how much a nutrient can be reduced is a strategy used for least cost formulation. The effects of phytase on live weight improvements have been credited to both the release of amino acids and an increase in AME (Shelton *et al.*, 2004). Therefore ingredient inclusion in diets can be reduced based on matrix values that are pooled from multiple experiments. In order to prove matrix values in trials, phosphorus levels should be at marginal levels. Meanwhile, phytase inclusion rates should be such that maximum doses do not result in performance which is equivalent to that of adequate diets.

2.4.7 Implications of the use of commercial phytase in broiler nutrition

The commercial phytases that are available in South Africa are shown in Table 3.3. Phytase inclusion rates in feed are based on matrix values and are recommended by the manufacturer. Furthermore, inclusion rates are not the same for all manufacturers making it difficult to compare the efficiency of each phytase. Qualitative response methods for the determination of the relative bioavailable phosphorus include bone criteria (bone mineralisation) growth and feed conversion (production parameters). Such response criteria are used to evaluate the efficacy of phytases.

Table 3.3 Commercial name, manufacturer and source of phytase used in South Africa

Commercial name	**Manufacturer**	**Source**	**Origin**	**Reference**
OptiPhos	Huvepharma	*E. coli*	*Pichia pectoris*	EFSA, 2011
Quantum blue	AB Vista	*E. coli*	*Trichoderma reesei*	EFSA, 2013
HiPhos	DSM	*A. oryzae*	*Citrobacter braakii*	EFSA, 2012
Ronozyme	DSM	*A. oryzae*	*Peniophora lycii*	EFSA, 2012
Natuphos	BASF	*A. niger*	*A. ficuum*	(Bories *et al.*, 2007)

EFSA- European food safety authority

2.4.7.1 Production parameters

Production parameters are a response criteria used to establish how effective phytase is at replacing phosphorus. Parameters such as weight gain, feed intake and feed conversion ratio are obtained during a growth trial. The outcomes for these parameters should be the same for broilers fed deficient diets supplemented with phytase as well as for those fed adequate diets. However, production outcomes of phytase inclusion are varied and are affected by the type of phytase, calcium and phosphorus levels in the diet.

For instance, weight gain and feed intake were negatively affected at available phosphorus levels of 3.5g/kg and 2.5g/kg. The study found that 600FTU of Natuphos (phytase derived from *E. coli)* and adequate amounts of calcium at 9.5g/kg could not improve production efficiency at day 13 (Leeson *et al.*, 2000).

On the other hand, an experiment compared the effects of two commercial phytase (Natuphos and Ronozyme) that were supplemented to diets that contained 2.0g/kg non-phytate phosphorus and adequate calcium. An increase in phytase from 300FTU to

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750FTU led to an increase in average daily gains and average feed intake. In terms of production performance, Natuphos and Ronozyme did not differ (Payne *et al.*, 2005).

Similarly, a commercial diet was compared to an adjusted diet supplemented with 1200FTU per kg of Natuphos. The adjusted diet contained 4.0g/kg non-phytate phosphorus and 9.0g/kg calcium; 3.0g/kg non-phytate phosphorus and 8.0g/kg calcium; 2.0g/kg non-phytate phosphorus and 6.0g/kg calcium; for starter, grower and finisher respectively. Body weight and feed conversion ratio were not affected by marginal diets for all phases. The authors concluded that non-phytate phosphorus and calcium levels in the adjusted diets were adequate for the attainment of live weight at day 42 and 56 (Fritts & Waldroup, 2006).

Production performance outcomes differ depending on the type of phytase. For instance, addition of 500 FTU of commercial phytase to diets with inadequate available phosphorus (3.2g/kg and 2.8g/kg) for starter and finisher respectively led to better weight gain, feed intake and feed conversion ratio at week 5. However, a laboratory prepared phytase derived from *A. awamori* was not comparable to the commercial phytase (Lalpanmawia *et al.*, 2014).

2.4.7.2 Bone mineralisation

Proper bone development depends on the amount of calcium and phosphorus in the diet. Calcium plays an important role in ossification of the bone matrix. Phosphorus is needed for mineralisation and solidification of the organic bone matrix. An animal bone contains approximately 370g/kg of calcium and 170g/kg phosphorus (Maynard & Loosli, 1969). Therefore the bone maintains calcium to phosphorus ratio of 2:1. In developing bones calcium and phosphorus are deposited as amorphous tri calcium phosphate $(Ca_3PO_4)_2$ (Chiba, 2009). However, in mature bones a crystalline formation of hydroxyapatite exists $Ca_{10}(PO4)_6(OH)_2$. The bone is continuously resorbing and absorbing minerals with body fluids. A decrease in plasma phosphorus concentration will lead to mobilization of minerals from the bone matrix. An increase in absorbed phosphorus will lead to a higher deposition of phosphorus into the bone matrix.

Bone ash and strength are qualitative methods that are used to evaluate available phosphorus levels. The levels, as well as the digestive and absorptive efficiencies of dietary phosphorus, and calcium have an influence the bone ash and strength. In addition to weight gain, bone ash can be used as a measure to determine the efficiency of phytase. Furthermore, bone breaking strength is a rapid measure that is used to determine the relative biological value of phosphorus (Lima *et al.*, 1997).

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Pillai *et al.*, (2006) reported that a rise in dietary phosphorus and calcium resulted in a linear rise in tibia weight and ash. Similarly, Brenes *et al.*, (2003) reported that reduced levels of available phosphorus and zinc in the diet led to reduced tibia ash percentage, while the amount of magnesium in the tibia was not affected. In addition, Denbow *et al.*, (1998) reported that a rise in non phytate phosphorus led to a linear rise in toe ash and tibia shear force. Furthermore, Onyango *et al.*, (2005) noted that tibia ash was a better indicator of bone mineralisation since toe ash was not influenced by phytase supplementation.

Phytase addition to both deficient and adequate diets led to a rise in tibia phosphorus and calcium content (Waldroup *et al.*, 2000), although this was not observed for diets that contained adequate levels of non-phytate phosphorus (Yan & Waldroup, 2006). During the starter phase, the amount of calcium and phosphorus in the tibia was positively affected by phytase supplementation (Shelton & Southern, 2006). However this was not the same for the grower and finisher phase.

Furthermore, dietary phytase addition had a positive influence on the amount of tibia ash, calcium and phosphorus (Francesch & Geraert, 2009), but did not increase tibia magnesium (Brenes *et al.*, 2003). On the contrary, Viveros *et al.*, (2002) reported that phytase supplementation did not improve the amount of phosphorus and calcium in the tibia, but led to an increase in levels of zinc and magnesium. Similarly, the amount of copper, zinc, magnesium and manganese in the tibia ash was affected by the addition of 750FTU phytase (Zhou *et al.*, 2008). However, the amount of tibia calcium was higher at 500FTU than at 750FTU.

Liem *et al.*, (2008) reported that phytase supplementation led to a rise in tibia ash and reduced the occurrence of tibial dyschondroplasia and rickets. In a subsequent experiment, the tibia ash percent was not affected by supplementation of phytase and 1-α-hydroxycholecalciferol (α-OHD) included to the diet. However, bone ash increased with supplementation of both phytase and α-OHD. Furthermore, with an incremental inclusion of α-OHD and phytase, bone ash percent was negated (Liem *et al.*, 2009).

Bone breaking strength was influenced by growth period during the grower and finisher stage but not during the starter phase (Shelton & Southern, 2006). In addition, the authors proposed that the zinc content had an effect on the bone breaking strength but not manganese and copper content. Furthermore, Driver *et al.*, (2005) noted that starter and finisher diets deficient in calcium and phosphorus influenced bone integrity during slaughter and processing. The author concluded that tibia ash at day 18 could determine tibia and femur processing characteristics. Also, rickets observed at day 18 could determine 32 day long bone characteristics that are largely influenced by long-term

fluctuations. While, shorter bones such as the clavicle were largely influenced by short-term fluctuations of calcium and phosphorus.

Shaw *et al.*, (2010) validated the use of either the left or right side of the tibia for bone breaking strength (BBS) determination. However; sex had an effect on BBS. Furthermore, lower amounts of dietary non-phytate phosphorus (Hemme *et al.*, 2005; Santos *et al.*, 2008; Powell *et al.*, 2008; Shaw *et al.*, 2010a; Shaw *et al.*, 2010b) and calcium (Powell *et al.*, 2008; Létourneau-Montminy *et al.*, 2008) led to a reduction in the tibia breaking strength. Other studies have noted that phytase supplementation had a positive influence on BBS (Sohail & Roland, 1999; Ribeiro *et al.*, 2003; Santos *et al.*, 2008; Létourneau-Montminy *et al.*, 2008; Powell *et al.*, 2008; Han *et al.*, 2009; Shaw *et al.*, 2010a; Shaw *et al.*, 2010b). However, Powell *et al.*, (2008) noted that dietary calcium and phosphorus rather than phytase supplementation had an influence on BBS.

2.4.7.3 Carcass characteristics

Cufadar & Bahtiyarca, (2004) reported carcass weight improvements with the addition of Natuphos phytase for both male and female broilers fed diets containing low available phosphorus levels (2.5g/kg to 3.4g/kg) and varying amounts of zinc (40, 60 and 160 mg/kg diet). The authors suggested that phytase supplementation reduced zinc toxicity which improved meat quality. However, another study reported no improvements to “bloody” pectorals’ minor and major muscles when both 1-hydroxycholecalciferol and 1000 FTU of Natuphos phytase were added to deficient diets. Supposedly, bloody meat was caused by insufficient dietary calcium levels leading to loss of blood from bone fractures (Driver *et al.*, 2006).

Available phosphorus levels had a positive influence on thigh and back yields (Teixeira *et al.*, 2013); the greatest portion yield response was for broilers that were fed diets that contained phytase at phosphorus inclusion rates of 3.0g/kg and 4.0g/kg. Angel *et al.*, (2006) reported that Ronozyme (derived from *A. oryzae*) had positive effects on the carcass weight for birds that were fed diets that contained low levels of phosphorus. Similarly, an experiment with diets containing low non-phytate phosphorus levels and varying levels of Rovaphos phytase had a positive effect on the carcass weight (Bingol *et al.*, 2009). In addition, the greatest carcass yield was obtained from broilers that were fed diets containing 1000 grams of phytase per ton of feed. In addition, exogenous enzymes have been reported to substantially influence carcass nutrient accumulation. For example, Olukosi *et al.*, (2008) noted that inclusion of phytase had an influence on carcass ash and calcium but did not influence carcass protein and fat content.

The effects of phytase supplementation on carcass portions has been noted in an experiment containing varying dietary levels of non-phytate phosphorus (4.5g/kg and

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3.0g/kg), and 500 FTU of Natuphos. Phytase supplementation had a positive effect on the carcass weight, leg quarter yields, breast and wing portions for female birds at seven weeks. The increased leg quarter yields correlated with a rise in the values of bone ash. Furthermore, an increase in tibia bone strength would have encouraged mobility leading to increased muscle development (Scheideler & Ferket, 2000). However, Rezaei *et al.*, (2007) found that diets which contained 500 FTU of Natuphos phytase at different nutrients equivalency values for phytase found no valid differences in carcass yield and breast portion for both sexes.

Another experiment done by Abudabos, (2012) consisted of feed with varying amounts of metabolisable energy (12.55 MJ/kg and 13.26 MJ/kg for finisher diets) and low levels of crude protein (170 and 180g/kg for finisher diets). Carcass and breast percentage were improved after supplementing a multi-enzyme Tomoko that included phytase at 10 FTU/gram. Furthermore, thigh and drumstick yields were not influenced by addition of the enzyme but were rather influenced by diet density. However, broilers fed an isoenergetic and isonitrogenous diet supplemented with Tomoko at 10 FTU/gram of phytase showed no differences in the carcass weights when compared to broilers that were not fed diets that contained multi-enzyme (Zakaria *et al.*, 2010)

2.5 Conclusion

Phytase releases bound minerals, amino acids and energy from the phytate complex of feed ingredients. The released nutrients can then be used for broiler growth. Phytase efficacy is influenced by calcium levels, phosphorus levels and apparent metabolisable energy of diets. Apart from temperature, other factors that affect phytase efficiency include pH, thermo stability during pelleting and the level of trace minerals in the diet. Therefore difference sources of phytase have different outcomes *in vivo.* In addition, inclusion rates of phytase affect the efficiency of phosphorus release. The studies that have been discussed have varying rates of inclusion of both phytase and phosphorus. In addition, the phytases discussed are from different sources and are produced by different companies in South Africa. It is therefore important to investigate the use of different types of commercial enzymes supplemented to diets that have low phosphorus levels on broiler production parameters under the same experimental conditions.

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Chapter 3

Comparison of production parameters of broiler chicks fed diets supplemented with phytase

Abstract

A 34 day experiment was conducted to determine the effect of three different types of phytase (HiPhos, OptiPhos and Quantum blue) on production parameters of unsexed Cobb500 broilers. Experimental diets were supplemented with phytase at standard levels and three times the standard level. Positive control (PC) diets were formulated based on the lower end of recommended NRC values. Whilst negative control (NC) diets were formulated on the PC diets less the matrix value for HiPhos 2000FTU (HP200). A positive control diet and negative control diet were compounded for three phases; starter, grower and finisher. Therefore, eight diets were mixed: 1. Positive control (PC); 2. Negative control (NC); 3. NC + 2000FTU HiPhos (HP200); 4. NC + 1000FTU OptiPhos (OP400); 5. NC + 1000FTU Quantum blue (QB200); 6. NC + 6000FTU HiPhos (HP600); 7.NC + 3000FTU OptiPhos (OP1200) 8.NC + 3000FTU Quantum blue (QB600). At the beginning of the trial 5120 day old broilers were allocated to a completely randomised design with eight treatment diets and eight replications. Weekly live weight and weekly feed intake were measured. Live weight, weekly gain, weekly feed intake, cumulative gain, cumulative feed intake, feed conversion ratio, average daily gains, liveability and European production efficiency factor were different ($p < 0.05$) at day 34 between dietary treatments. At the end of the trial, supplementation of phytase to NC diets improved live weight, average daily gains and cumulative gains. However improvements were not comparable to those of the PC group

Keywords: live weight, average daily gains, feed conversion ratio

3.1 Introduction

Although there has been an increasing demand for broiler meat from the consumer; a constraint for the producer has been an increase in the cost of feed ingredients. Therefore, producers have to utilize alternative methods that will lead to a reduction of feed ingredient costs without compromising broiler production performance (Selle & Ravindran, 2007). In addition, regulation on the levels of inorganic phosphorus inclusion in diets due to negative environmental factors limit feed formulation strategies (Correll, 1999; Maguire *et al.*, 2005).

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Macro and micronutrients play an important role in broiler growth. Some micronutrients are, however, not readily available in grains that are used as ingredients in diet formulation (Asada *et al.*, 1970). Feed ingredients for broiler diets contain various concentrations of phosphorus (Ravindran *et al.*, 1994; Viveros *et al.*, 2000; Selle *et al.*, 2003). The limitation of these ingredients is that they contain phytate which acts as an anti-nutrient (De Boland *et al.*, 1975; Davies & Reid, 1979). The anti-nutrient effects of phytate have negative implications for optimal phosphorus utilization and therefore limit broiler production performance (Sohail & Roland, 1999).

Phytate is a complex that binds to phosphorus (Cosgrove & Irving, 1980), calcium (Nelson *et al.*, 1968), zinc (O'Dell *et al.*, 1964; Savage *et al.*, 1964), selenium (Shan & Davis, 1994), amino acids (Cowieson *et al.*, 2004) and other trace elements, making them unavailable. Phytase, a non-nutritive feed additive, improves availability of phosphorus from the phytate complex of feed ingredients in the gastro-intestinal tract (Cosgrove, 1970; Konietzny & Greiner, 2002). The development of phytase has led to a reduction in the amount of inorganic phosphorus required in broiler diets (Cowieson & Adeola, 2005). Therefore, the utilization of the nutrients from the phytate complex has resulted in a reduction of feed costs.

Currently, production performance outcomes of commercial broilers are determined on standard doses of phytase that are recommended by the manufacturer. Standard doses are usually derived from calculated matrix values and vary depending on the type and source of phytase (Bedford, 2000). Therefore, higher doses are levels of phytase units that exceed the standard doses recommended by the manufacturer. Several research works on the effects of higher doses on broiler production performance have been reported (Shirley & Edwards, 2003; Augspurger & Baker, 2004; Cowieson *et al.*, 2011; Dos Santos *et al.*, 2013; Cowieson *et al.*, 2014). There is no consensus on whether or not higher doses of phytase can improve performance. Due to different types of phytase on the market and various manufacturer recommendations, matrix value outcomes become difficult to predict. Some studies are in agreement with high inclusion levels, while others have disagreed with this principle. Some reports suggest that doses of phytase included at levels higher than recommended led to an even higher response (Shirley & Edwards, 2003; Augspurger & Baker, 2004; Cowieson *et al.*, 2011; Dos Santos *et al.*, 2013; Cowieson *et al.*, 2014). However such a response has been observed to be log linear (Zhang *et al.*, 1996). Phytase activity is measured in phytase units (FTU). One FTU is defined as the amount of enzyme required to release 1 μmol of inorganic phosphorus per minute from sodium phytate at a pH of 5.5 and 37^{0}C.

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The possible use of higher doses of phytase in feed formulation may be beneficial in order to optimize broiler performance through improved body weight gains and lower feed conversion ratios. Several commercial phytases are available on the market and are derived from *E. coli* expressed in *Pichia pectoris* (OptiPhos), *E. coli* expressed in *Trichoderma reesei* (Quantum blue) and *A. oryzae* expressed in *Citrobacter braakii* (HiPhos).

The current study aimed to evaluate the efficacy of phytase supplemented to broiler diets. The objectives of the study were:

i. To investigate the effects of three different types of commercial phytase derived from either *A. oryzae* or *E. coli* on production performance of commercial broilers.

ii. To determine the effects of high doses of three different types of commercial phytase derived from either *A. oryzae* or *E. coli* on production performance.

3.2 Materials and methods

The description of treatment diets that were used for the entire duration of the trial is shown in Table 3.1. Ingredient composition was based on a maize soya meal diet as shown in Table 3.2. The PC diets were formulated based on the lower end of NRC recommended values. NC diets were formulated on the PC diets less the matrix value for HiPhos 2000FTU (HP200). The matrix value for HP200 was as recommended by the manufacturer (DSM Nutritional Products, Basel, Switzerland). The phytases that were used included: HiPhos 1000GT (DSM Nutritional Products, Basel, Switzerland), Quantum blue 5000 (AB Vista, Marlborough, United Kingdom) and OptiPhos 2500 (Huvepharma Sofia, Bulgaria). The commercial phytases used were derived from *E. coli* expressed in P. *pectoris* (OptiPhos), *E. coli* expressed in *T. reesei* (Quantum blue) and *E. coli* expressed in *A. oryzae* (HiPhos).

Feed mixing and pelleting was done at the Mariendahl Experimental Farm, Stellenbosch University, after purchase of feed ingredients from a commercial miller (Profile Feeds Limited). The feed pelleting temperature was 70°C. The application rate for the phytase was as follows: HiPhos at 200 grams per ton of feed (HP200), Quantum blue at 200 grams per ton of feed (QB200), OptiPhos at 400 grams per ton of feed (OP400), HiPhos at 600 grams per ton of feed (HP600), Quantum blue at 600 grams per ton of feed (QB600) and OptiPhos at 1200 grams per ton of feed (QB1200). Phytase was mixed with other ingredients before pelleting.

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Table 3.1 Description of diets that were used for the entire duration of the trial

Treatment	Description
PC	Formulated on the lower end of NRC recommended values
NC	Formulated on the lower end of NRC recommended value minus matrix value for HP200
HP200	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 2000FTU of HiPhos
QB200	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 1000FTU of Quantum blue
OP400	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 1000FTU of OptiPhos
HP600	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 6000FTU of HiPhos
QB600	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 3000FTU of Quantum blue
OP1200	Formulated on the lower end of NRC recommended value minus matrix value for HP200 plus 3000FTU of OptiPhos

3.2.1 Experimental diets

Diets were formulated according to manufacturer recommendations (DSM Nutritional Products, South Africa) as shown in Table 3.2. Format International software was used for feed formulation. The diets that were formulated consisted of a positive control (PC) and a negative control (NC) and calculated nutritional values of diet are as shown in Table 3.3. Feed allocation was such that each broiler received 900g starter, 1200g grower and 1200g finisher.

Table 3.2 Formulated ingredient composition (%) of starter, grower and finisher diets

	Starter		Grower		Finisher	
	Positive Control	Negative Control	Positive Control	Negative Control	Positive Control	Negative Control
Ingredients						
Maize	60.08	57.84	63.58	57.56	66.88	62.99
Soya bean oil cake 48%	24.98	22.99	16.29	18.00	13.15	9.73
Sunflower oil cake	6.00	9.00	6.59	4.04	5.79	9.00
Gluten 60	2.66	1.00	8.44	6.53	9.39	8.43
Wheat bran	2.00	7.00	1.00	11.35	1.00	7.64
Mono-calcium phosphate	1.01	0.12	0.96	0.05	0.89	…
Sunflower oil	1.00	0.07	1.00	0.62	1.00	0.50
Limestone 80%	0.81	0.58	0.85	0.61	0.82	0.61
Salt	0.56	0.46	0.55	0.50	0.44	0.40
Lysine	0.36	0.35	0.39	0.34	0.36	0.38
Methionine	0.23	0.25	0.10	0.12	0.03	0.04
Threonine	0.06	0.07	…	0.02	…	0.02
Premix	0.25	0.25	0.25	0.25	0.25	0.25

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Table 3.3 Mean calculated nutritional value of starter, grower and finisher diets

		Starter		Grower		Finisher	
		Positive Control	Negative Control	Positive Control	Negative Control	Positive Control	Negative Control
Calculated nutritional value							
Dry matter	g/kg	892.5	892.1	893.6	892.0	892.8	893.0
Moisture	g/kg	107.5	107.9	106.4	107.9	107.2	106.9
Crude Protein	g/kg	200.0	198.4	198.0	196.1	188.0	186.4
Crude Fibre	g/kg	42.6	50.7	40.2	45.7	38.2	47.3
Crude Fat	g/kg	40.2	31.9	40.5	38.3	41.1	3.7
Calcium	g/kg	7.0	4.7	6.6	4.3	6.2	3.9
Total phosphorus	g/kg	6.3	4.9	5.9	4.6	5.6	4.3
Available phosphorus	g/kg	3.3	1.7	3.2	1.5	3.0	1.3
AME	MJ/kg	12.6	12.2	12.9	12.6	13.2	12.8
Arginine	g/kg	11.8	12.1	10.4	10.5	9.5	9.5
Lysine	g/kg	11.3	11.2	10.0	9.9	9.0	8.9
Methionine	g/kg	5.4	5.4	4.5	4.5	3.9	3.9
TSAA	g/kg	8.3	8.3	7.6	7.5	6.8	6.8
Tryptophan	g/kg	2.0	2.0	1.7	1.8	1.6	1.6
Glycine + serine	g/kg	16.7	16.5	16.4	16.1	15.5	15.2
Histidine	g/kg	4.8	4.8	4.7	4.6	4.4	4.3
Isoleucine	g/kg	7.9	7.7	7.7	7.4	7.2	6.9
Valine	g/kg	8.4	8.3	8.4	8.1	7.9	7.8
Leucine	g/kg	17.1	15.7	20.5	18.9	20.5	19.3
Threonine	g/kg	7.3	7.2	6.6	6.6	6.2	6.2
Phenylalanine + tyrosine	g/kg	15.6	14.8	16.7	15.8	16.2	15.4
Cysteine	g/kg	2.9	2.8	3.1	3.0	3.0	3.0
Sodium	g/kg	2.2	1.8	2.2	2.0	1.8	1.6
Potassium	g/kg	8.0	8.6	6.3	7.3	5.7	6.2
Ash	g/kg	35.8	36.0	30.5	31.9	28.0	28.0

AME - Apparent metabolisable energy

TSAA - Total sulphur containing amino acid

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3.2.2 Feed analysis

Labelling and sampling of feed for chemical further analysis was done after bagging. Samples for feed analysis were collected at random. Samples were placed in airtight plastic bags and stored at 4°C until further analysis were performed. The two methods used for nutritional value determination were proximate analysis (wet chemistry) and near infrared spectroscopy (NIR). The parameters that were determined included moisture content, crude protein, crude fat and ash, as well as mineral content of feed.

3.2.2.1 Wet Chemistry

Analytical procedures for wet chemistry were done in the Department of Animal Sciences, Stellenbosch University, with the exception of mineral analyses. Mineral analysis determination was done at the Western Cape Department of Agriculture, Elsenburg.

3.2.2.1.1 Moisture determination

The moisture percentage of the feed samples was determined according to the Association of Official Analytical Chemists International (AOAC, 2002), Official method 934.01. Sub-sample feed analysis was done in duplicate by taking a clean empty porcelain crucible and placing it in the oven for 2h at 100°C to dry. The crucible for each sample was then placed in a desiccator and allowed to cool. The weight of the empty crucible was then taken and the scale was tarred to measure out 2g of feed sample. The crucible was then placed in an oven at 100°C for 24h until constant weight. The weight of the moisture free sample was obtained after placing the crucible in a desiccator to cool off. The moisture percentage was then calculated as shown in Equation 3.1.

Equation 3.1

$$\text{Moisture percentage} = \frac{(\text{Weight of crucible and moisture free test sample} - \text{Weight of empty and dry crucible B}) \times 100}{\text{Weight of air dried test sample}}$$

3.2.2.1.2 Ash determination

The method of ash determination was according to the Association of Official Analytical Chemists International (AOAC, 2002), Official method 942.05. After moisture determination, feed samples were then placed in a furnace at 600°C for 6h to ash. The weight of the ash was obtained after placing the crucible in a desiccator to cool off. The ash percentage was then calculated as shown in Equation 3.2.

Equation 3.2

$$\text{Ash percentage} = \frac{(\text{Weight of crucible and ash} - \text{Weight of empty and dry crucible}) \times 100}{\text{Weight of oven dried test sample}}$$

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3.2.2.1.3 Mineral Analysis for the determination of calcium and phosphorus

Ash samples were then used for mineral analysis following procedures as outlined by the Agricultural Laboratory Association of Southern Africa (ALASA) method 6.1.1 for feeds and plants. Ashed samples were taken and 5mL of 6M HCl was added individually. The ash sample was then placed in an oven for 30min at 50°C. Subsequently, 35mL distilled water was added and the solution was filtered into a brown bottle and made up to a final volume of 50mL with distilled water. Elements were measured on an Inductive Coupled Plasma Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 auto sampler. Element concentrations were calculated using iTEVA Analyst software.

3.2.2.1.4 Crude fat determination

The Diethyl Ether Reagent method using the Tecator Soxtec System HT 1043 Extraction Unit according to Association of Official Analytical Chemists International (2002), Official Method 920.39 was used to perform an ether extract for crude fat determination. Sub-sample analysis was done in duplicate by weighing 2g of feed sample in a soxlet thimble that was then placed in 50mL of diethyl ether measured into a fat extraction beaker. After fat extraction, the beakers were placed in an oven for drying at 100°C for 2h prior to weighing and calculating the fat percentage. The crude fat percentage was then calculated as shown in Equation 3.3.

Equation 3.3

$$\text{Crude fat percentage} = \frac{\text{(Mass of soxlet beaker + fat) g} - \text{(Mass of soxlet beaker) g}}{\text{Mass of sample g}}$$

3.2.2.1.5 Crude protein determination

The crude protein determination was done by analysing the amount of nitrogen in the sample then multiplying that by a factor of 6.25 according to the Association of Official Analytical Chemists International (AOAC, 2002), Official Method 4.2.07, using a LECO FP528 apparatus. Sub-sample feed analysis was done in duplicate by weighing 0.1g feed sample in an aluminium foil which was then placed into the LECO FP528. The crude protein percentage was then calculated as shown in Equation 3.4.

Equation 3.4

$$\text{Crude protein percentage} = \text{Nitrogen percentage} \times 6.25$$

3.2.2.1.6 Near infrared spectroscopy (NIR)

Feed samples were collected at random post-pelleting from each treatment group. Near infrared spectroscopy was conducted at Profile Feeds (Western Cape, South Africa). The Perten DA7200 (Hagersten, Sweden) was used to perform the analysis.

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3.2.2.2 Phytase activity analysis

Samples were analysed in triplicate to conduct an evaluation of the phytase recovery rate. Feed samples were collected at random post-pelleting from each treatment so as to determine phytase activity. Laboratory analysis of phytase activity was performed according to manufacturer recommendations (DSM Biopract Berlin, Germany) as described by Gizzi *et al.*, 2008. Also, the International Organization for Standardization (Standard, I. 30024: 2009) was used as a reference method.

3.2.3 Animals and housing system

Cobb 500 Day old as hatched vaccinated broilers were randomly selected from a commercial hatchery and transported in standard cardboard boxes to the Mariendahl Experimental Farm, Stellenbosch University. Upon arrival, birds were randomly sorted into groups of eighty and weighed. Each group of 80 was randomly allocated to a pen which was equipped with an infrared light, a 4L chick fount and a tube feeder. The experimental facility consisted of two identical commercial type environmentally controlled poultry houses each divided into 32 pens. Five thousand one hundred and twenty day old broilers were allocated in a completely randomized design with eight treatments, eighty broilers per pen and eight replicates per treatment (n=640 birds/treatment). The total floor space for each pen was $4.01m^2$. Each pen had two tubular feeders ($0.119\ m^2$) and a bell drinker ($0.102\ m^2$). Change over from one feeding phase to another was according to feed allocation described in 3.2.1. Management practices were as outlined by Cobb International 2012. The experimental protocol was approved by the Animal Ethics Committee of Stellenbosch SU-ACUM13-00006.

3.2.4 Data collection

Live weight was determined at day old placement (day 0) and weekly thereafter until slaughter at day 34. Live weights were measured per pen and individual weights were calculated as an average. Weekly feed intake was determined by weighing the initial amount of feed offered to the birds and feed added during the period; then corrected for the feed that remained in the feeders at the end of the period. The feed intake per bird was calculated as an average. Live bird weight and feed intake were measured using a Mettler scale (Model ICS 429, Switzerland). Mortalities and morbidities were recorded twice daily, with all dead bird weights and necropsies noted. At the end of the trial percentage liveability was calculated as the number of birds that survived until day 34. Other collected data were then used to calculate the feed conversion ratio (FCR), European production efficiency factor (EPEF) and the protein efficiency ratio (PER) as shown in Equation 3.5, Equation 3.6 and Equation 3.7, respectively.

Equation 3.5

$$\text{Feed conversion ratio} = \frac{\text{Cumulative feed intake per bird (g)}}{\text{Average live weight gain per bird (g)}}$$

Equation 3.6

$$\text{European production efficiency factor} = \frac{\text{Liveability} \times \text{live weight gain (g)}}{\text{Age (days)} \times \text{FCR}}$$

Equation 3.7

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{(\text{Weekly feed intake (g)} \times \text{protein \% of diet}) / 100}$$

3.2.5 Statistical analysis

Average daily gains (ADG) were estimated by fitting a linear model to the live weight data. The slope of the model represented the rate of change and thus average daily gain. Parameters were tested for normality and homoscedasticity. One-way Analysis of Variance (ANOVA) was performed using the general linear model procedure of the SAS enterprise guide 5.1. Treatment means were compared by least significant difference ($p < 0.05$). A *post hoc* test of least significant difference was performed on treatment means using Bonferoni's test.

3.3 Results and discussion

3.3.1 Diets for starter, grower and finisher phase

Proximate analysis (expressed as percentage of dry matter) and NIR results for starter, grower and finisher phase are shown in Table 3.4. The numerical differences observed in the range of values for the crude protein, crude fat, phosphorus and calcium may be as a result of sampling procedures and variation in analytical methods. In addition experimental error or differences in calibration values may have a led to results that are not as exact as the calculated values. Furthermore, it should be noted that in the current study determination of the nutrient composition of ingredients was not performed prior to the formulation of diets. Calculated nutrient values of diets were based on the standard nutrient content values of the feed ingredients. Similar to the current study, other authors have reported a variation between the nutritional calculated values and the proximate analysed values (Ravindran *et al.*, 2000; Aureli *et al.*, 2011).

Table 3.4 Mean proximate analysis and near infrared spectroscopy results for starter, grower and finisher broiler diets expressed as percentage of dry matter (g/kg)

Phytase	-				HP200		OP400		QB200		HP600		OP1200		QB600	
Diet	Positive control		Negative Control		Negative Control		Negative Control		Negative Control		Negative Control		Negative Control		Negative Control	
	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]	NIR[1]	Proxi[2]
Starter																
Moisture	9.69	11.19	9.44	10.61	9.43	10.93	9.42	11.24	9.47	10.98	9.34	11.66	9.40	11.26	9.05	10.71
Protein	19.45	24.03	18.83	24.02	18.65	24.04	19.02	24.44	18.52	23.62	18.88	23.17	18.77	23.60	18.97	24.11
Fat	3.70	3.96	2.87	3.19	2.90	3.13	2.73	3.14	2.77	3.04	2.80	2.97	2.91	2.91	2.90	2.86
Ash	6.74	5.25	6.62	4.28	6.90	4.34	6.96	4.72	6.71	4.61	6.38	4.65	6.59	4.11	6.15	4.52
Calcium	0.99	0.54	1.04	0.34	1.03	0.34	1.06	0.38	0.99	0.40	1.18	0.37	0.99	0.35	1.02	0.37
Phosphorus	0.90	0.74	0.95	0.53	0.97	0.54	0.99	0.57	0.96	0.63	0.95	0.60	0.94	0.57	0.95	0.59
Grower																
Moisture	10.17	11.09	10.33	11.13	10.61	11.58	10.55	11.38	10.64	11.67	10.20	11.22	9.99	10.96	10.37	11.12
Protein	22.06	24.05	21.23	23.45	21.71	25.03	21.33	23.60	21.16	23.64	22.07	22.88	21.87	23.87	21.59	23.56
Fat	3.72	3.72	3.94	3.49	3.84	3.49	3.94	3.41	3.99	3.58	3.97	3.56	3.93	3.50	3.95	3.47
Ash	6.64	4.71	6.36	4.08	6.53	4.06	6.70	4.12	6.38	4.22	6.50	4.17	6.14	4.48	6.67	4.20
Calcium	0.94	0.61	0.91	0.37	0.97	0.36	0.93	0.36	0.92	0.34	0.90	0.39	0.92	0.45	0.94	0.35
Phosphorus	0.88	0.74	0.84	0.56	0.85	0.54	0.86	0.56	0.84	0.54	0.87	0.55	0.86	0.61	0.86	0.55
Finisher																
Moisture	9.98	10.42	10.55	11.12	10.75	10.90	10.09	10.50	10.69	10.82	10.43	10.56	10.33	10.90	10.49	10.87
Protein	21.62	23.10	20.73	23.24	20.61	21.95	21.12	22.59	20.53	22.85	20.86	23.13	21.07	23.22	21.04	22.93
Fat	3.80	3.77	3.43	2.61	3.31	3.67	3.41	3.64	3.41	3.45	3.46	3.69	3.26	3.38	3.31	3.49
Ash	6.59	4.34	6.43	3.88	6.14	3.97	5.78	4.10	6.16	3.80	6.46	3.99	6.16	3.80	6.50	3.75
Calcium	0.95	0.56	1.01	0.36	0.96	0.39	0.99	0.38	1.00	0.38	1.02	0.39	0.97	0.35	1.02	0.35
Phosphorus	0.83	0.70	0.87	0.59	0.88	0.58	0.88	0.59	0.87	0.58	0.90	0.57	0.89	0.58	0.89	0.58

OP400 & OP1200 – OptiPhos; QB200 & QB600 – Quantum blue; HP200 & HP600 – HiPhos

[1]NIR- Near infrared spectroscopy:[2] Proxi- Proximate analysis

3.3.2 Phytase analysis

Analysed phytase activity and percentage recovery post-pelleting is shown in Table 3.5. The recovery rate (%) for the respective experimental diets was within an acceptable range. Loop *et al.* (2012) suggests that *in vitro* analysis of phytase activity may not provide sufficient information on activity within the bird. Furthermore, the study done by Loop *et al.* (2012) advised that feed pelleted at 77°C yielded a favourable recovery rate. Similarly, other studies have reported recovery rates that are not in line with expected inclusion rates (Viveros *et al.*, 2000; Aureli *et al.*, 2011; Cowieson *et al.*, 2014). A reason for the variation in expected results and analysed results would be that the recovery rate of phytase varies with the procedure used to analyse the activity (Kerr *et al.*, 2010).

Table 3.5 Analysis of phytase activity and percentage recovery post-pelleting

Treatment diets	Inclusion in feed	Expected phytase units	Analysed activity	Percentage recovery
Starter				
HP200	200g/ton	2000FTU	2099FTU	105%
OP400	400g/ton	1000FTU	1311FTU	131%
QB200	200g/ton	1000FTU	979FTU	98%
HP600	600g/ton	6000FTU	6177FTU	103%
OP1200	1200g/ton	3000FTU	3859FTU	129%
QB600	600g/ton	3000FTU	2778FTU	93%
Grower				
HP200	200g/ton	2000FTU	2211FTU	111%
OP400	400g/ton	1000FTU	1169FTU	117%
QB200	200g/ton	1000FTU	598FTU	60%
HP600	600g/ton	6000FTU	6751FTU	113%
OP1200	1200g/ton	3000FTU	4291FTU	143%
QB600	600g/ton	3000FTU	2403FTU	80%
Finisher				
HP200	200g/ton	2000FTU	2382FTU	119%
OP400	400g/ton	1000FTU	1293FTU	129%
QB200	200g/ton	1000FTU	738FTU	74%
HP600	600g/ton	6000FTU	7004FTU	117%
OP1200	1200g/ton	3000FTU	3472FTU	116%
QB600	600g/ton	3000FTU	2227FTU	74%

FTU- Phytase units

OP400 & OP1200 – OptiPhos ; QB200 & QB600 – Quantum blue; HP200 & HP600 – HiPhos

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3.3.3 Live weight

Table 3.6 shows mean values of live weight, FCR, EPEF, ADG and liveability of broilers raised from day 0 to 34 and fed different phytase treatments.

At day 0 there were no live weight differences ($p > 0.05$) across any treatment groups indicating that birds placed at the beginning of the trial did not differ in weight. By day 7, live weight differences were observed across treatments ($p < 0.05$) with HP200, PC, NC, OP1200, QB200 and QB600 diets showing higher weights than HP600 and OP400. At day 14, PC performed better than HP200, OP1200, QB200 and QB600, whilst lowest weights were observed from treatment diets NC, HP600 and OP400.

There were significant treatment differences on day 21. PC had the highest ($p < 0.05$) weight, whilst NC had the lowest live weight with all other treatments being intermediary. The live weight performance of HP200 (2000FTU) in the current study was similar to that reported by Cowieson *et al.* (2014). However, the performance of QB200 (1000FTU) at day 21 in the current study did not correspond with that reported by Walk *et al.* (2014). The reason for this could be that phytase supplementation did not reduce the wide calcium to phosphorus ratio (2.8:1 grower phase) used in the current study. Furthermore, a study that used citric acid and distiller's grains in diets supplemented with Quantum blue reported better live weight performance than that reported in the current study (Dos Santos *et al.*, 2013).

Treatment differences on day 28 were observed between diets ($p < 0.05$). The live weight trend was similar to that at day 21, although live weight improvement was observed for HP600, with QB600 not performing as well as it did in the previous period.

There were significant differences between treatments on day 34. Live weight was highest for PC and lowest for NC, with all other treatments being intermediary. Intermediary treatments were not equal to either PC or NC, but in some instances being equal to one another. An increase in HiPhos and OptiPhos phytase units did not improve ($p > 0.05$) live weights at day 34. The findings for HiPhos in the current study are similar to reports by Aureli *et al.* (2011). The authors observed that the live weights at day 34 did not differ for broilers fed either 4000FTU or 40000FTU. Furthermore, in the current study an increase in Quantum blue from 1000FTU to 3000FTU led to an increase in live weight at day 34. In addition, at the end of the trial, phytase supplementation to NC diets was not comparable to the PC diets for the current study.

Table 3.6 Mean values (± standard deviation, SD) of live weight, FCR, EPEF, ADG, PER and liveability of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Live weight (g)									
Day 0	46.56 ± 0.75	46.80 ± 0.70	47.03 ± 0.44	46.41 ± 0.73	46.17 ± 0.84	46.25 ± 0.95	46.56 ± 0.82	46.72 ± 0.73	0.3500
Day 7	181.04^{a} ± 9.12	175.47^{a} ± 6.12	154.85^{b} ± 4.82	179.69^{a} ± 6.09	179.08^{a} ± 11.28	149.05^{b} ± 8.65	180.61^{a} ± 4.15	178.44^{a} ± 2.78	<0.0001
Day 14	477.19^{a} ± 11.92	400.07^{c} ± 5.31	400.89^{c} ± 12.73	426.63^{b} ± 24.72	434.19^{b} ± 16.40	377.34^{c} ± 14.61	445.88^{b} ± 17.62	443.37^{b} ± 10.57	<0.0001
Day 21	956.89^{a} ± 24.35	721.01^{d} ± 45.38	813.84^{bc} ± 35.12	816.98^{bc} ± 22.67	826.85^{bc} ± 28.65	780.33^{c} ± 31.49	850.69^{b} ± 18.60	848.77^{b} ± 21.22	<0.0001
Day 28	1494.09^{a} ± 15.85	1155.75^{d} ± 34.49	1236.36^{c} ± 38.20	1246.96^{c} ± 21.97	1289.11^{bc} ± 29.94	1262.85^{bc} ± 35.94	1304.17^{b} ± 50.22	1290.33^{bc} ± 41.55	<0.0001
Day 34	1866.14^{a} ± 26.06	1477.70^{e} ± 52.64	1588.89^{cd} ± 49.15	1577.90^{d} ± 37.90	1663.65^{bc} ± 71.41	1670.8^{bc} ± 42.61	1643.89^{bcd} ± 50.43	1684.34^{b} ± 53.59	<0.0001
Parameter									
FCR[1]	1.66^{b} ± 0.06	1.81^{a} ± 0.05	1.79^{a} ± 0.04	1.80^{a} ± 0.04	1.72^{ab} ± 0.07	1.75^{ab} ± 0.04	1.73^{ab} ± 0.08	1.73^{ab} ± 0.03	<0.0001
PER[2]	2.62 ± 0.19	2.49 ± 0.27	2.73 ± 0.25	2.60 ± 0.19	2.83 ± 0.55	2.72 ± 0.48	2.80 ± 0.49	2.90 ± 0.15	0.3625
ADG[3] (Day0-34)	56.24^{a} ± 0.57	43.30^{d} ± 1.52	47.32^{c} ± 1.51	46.81^{c} ± 0.79	49.12^{b} ± 1.71	48.80^{b} ± 1.51	48.91^{b} ± 1.50	49.63^{b} ± 1.62	<0.0001
Liveability (%)	89.37^{ab} ± 5.08	88.59^{b} ± 4.69	94.22^{ab} ± 3.26	91.09^{ab} ± 3.81	91.09^{ab} ± 1.94	94.82^{a} ± 3.49	93.75^{ab} ± 2.76	90.63^{ab} ± 3.54	<0.0001
EPEF[4]	285.81^{abc} ± 26.39	224.77^{d} ± 27.91	265.45^{bcd} ± 24.30	238.98^{bc} ± 20.98	284.25^{abc} ± 58.96	322.19^{a} ± 33.62	262.77^{bcd} ± 35.76	295.32^{ab} ± 23.25	<0.0001

(^{a,b,c,d}) Means within rows with different superscripts differ significantly ($p<0.05$)

[1]FCR- Feed conversion ratio, [2] PER- Protein efficiency ratio, [3]ADG- Average daily gains , [4] EPEF- European production efficiency factor

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

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3.3.4 Feed conversion ratio, average daily gains, EPEF, PER and liveability

Average daily gains (ADG) were estimated by fitting a linear model (R^2 = 0.946) to the live weight data, the slope of the model represents the rate of change and thus ADG. There were significant treatment differences on day 34 for ADG (Table 3.6). Average daily gain was the best for PC and the worst for NC followed by OP400 and QB200, all other treatments were similar. A study that contained available phosphorus levels of 2.0g/kg and calcium at 6.7g/kg supplemented with OptiPhos observed an increase in ADG with an increase in phytase (Powell *et al.*, 2011). Similarly, in the current study, an increase of 1000FTU to 3000FTU of OptiPhos significantly improved ADG. Other ADG improvements were observed for Quantum blue (from 1000FTU to 3000FTU). This was not the case, however, for HiPhos (from 3000FTU to 6000FTU). Even though incremental levels of phytase led to higher ADG for Quantum blue and OptiPhos, these were not comparable to PC diets.

There were significant treatment differences for feed conversion ratio (FCR). The PC group was statistically equal to OP1200, QB600, HP200 and HP600. Phytase supplementation to QB200 and OP400 diets which were statistically equal to all other treatments except PC. This finding is contrary to a study were supplementation of 500FTU Quantum blue to deficient diets led to improved FCR (Singh *et al.*, 2013). The reason for this might be that even though calcium levels were comparable, phosphorus levels in the current study were lower than those reported by Singh *et al.*, (2013). Furthermore, FCR for both standard and high doses of HiPhos (HP200 & HP600) were the same. Similarly, Aureli *et al.*, (2011) reported that when phytase units were increased from 4000FTU to 40000FTU, the FCR did not differ significantly. It is evident in the current study that FCR improvements for the groups containing phytase at higher doses resulted in increased ADG and higher feed intake. However, FCR improvements for groups supplemented at high phytase doses (HP600, OP1200 and QB600) were not comparable to those of PC.

There were significant differences between treatments on day 34 for liveability (%). The highest liveability was exhibited in the HP600 group, while the lowest liveability was observed in the NC group, with all other treatments being statistically equal and intermediary. Another study found similar results where increased mortality was noted for broilers fed diets that contained 2.0g/kg non-phytate phosphorus (Kornegay *et al.*, 1996). However, the authors observed an improved liveability when diets were supplemented with phytase. In addition, the inclusion of 2.5g/kg non-phytate phosphorus without phytase supplementation was sufficient to reduce mortality (Waldroup *et al.*, 2000). However, inclusion of 1.0g/kg non-phytate phosphorus and 8.0g/kg calcium were adequate for broiler growth and did not adversely affect mortality (Yan *et al.*, 2003). Furthermore, Julian *et al.*, (1986) reported an increase in mortality due to right ventricle failure and ascites.

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The authors indicated that the increased mortality was observed in phosphorus deficient diets. In the current study, available phosphorus levels for PC diets were 3.3g/kg, 3.2g/kg, 3.0g/kg, whilst those for NC diets were 1.7g/kg, 1.5g/kg, and 1.3g/kg for the starter, grower and finisher diets, respectively. Liveability was lower than expected for all treatment diets. The effects of phytase supplementation on the liveability of the flock were inconclusive.

Treatment differences between diets for EPEF were observed ($p < 0.05$). European production efficiency factor (EPEF) is an indicator for the profitability of a flock that takes into account liveability, FCR, live weight and age at depletion. The highest EPEF was observed for the PC group. The lowest EPEF was observed from the NC group with all other treatments being statistically equal and intermediary. Awad *et al.*, (2009) reported EPEF values ranging from 255 to 291. In addition another study investigated the use of selenium in broiler diets and reported EPEF values ranging from 254 to 261 (Perić *et al.*, 2009). The current study reported values that ranged from 224 to 322.

There were no treatment differences between groups for the protein efficiency ratio (PER) ($p > 0.05$) as shown in Table 3.6. The PER is a measure of the unit of weight gained for every unit of protein intake. As has been described in 2.4.5.5 that phytase has the ability to increase amino acid digestibility. An increase in amino acid digestibility would have an influence on the PER. In the current study, phytase supplementation at both standard and high doses did not have an influence on the PER. Similarly, a study to evaluate the use of phytase in improving the PER on several feed ingredients found that 1200FTU did not increase protein utilization (Boiling-Frankenbach *et al.*, 2001).

3.3.5 Weekly and cumulative gain

Weekly and cumulative gain between treatments were different ($p < 0.05$) for the entire period of the trial (Table 3.7).

After the first week (day 7) of the trial, HP600 and OP400 had the lowest weekly and cumulative gain per bird. Weekly and cumulative weight gain at day 7 was the same for standard dose of Quantum blue (1000FTU) and three times more than that of the standard dose of Quantum blue (3000FTU). Similar to the current study, no differences in cumulative gain at day 7 and 14 were observed when Quantum phytase dose was doubled from 1200FTU to 2400FTU (Cowieson *et al.*, 2006).

The PC group had the highest cumulative gain for both day 14 and 21 with no other treatment being equal. The NC group had the lowest weekly and cumulative gain at day 14, with other treatments being equal or intermediary. In addition, NC group had the lowest weekly and cumulative gain at day 21, with no other treatment being equal. At day

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21, diets that were supplemented with phytase were intermediary but not equal to either PC or NC diet.

At day 28, cumulative live weight gains were highest for PC, lowest for NC and intermediary for all other treatments. Cumulative gains at day 34 had a similar trend to day 28. Furthermore, at day 34 HP600 had the highest weekly gain, while NC had the lowest, with all other treatments being equal to or intermediary to HP600 and NC. Overall, the weight gains that have been reported by Aureli *et al.*, (2011) and Cowieson *et al.*, (2014) are higher than those of the current study. This could be attributed to the low levels of phosphorus and calcium in the negative control diets of the current study.

Table 3.7 Mean values (± Standard deviation, SD) weekly gain and cumulative gain of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Parameter									
Weekly gain/bird (g)									
Day 7	134.47^{a} ± 9.21	128.67^{a} ± 6.22	107.82^{b} ± 4.71	133.28^{a} ± 6.68	132.91^{a} ± 11.87	102.80^{b} ± 8.27	134.04^{a} ± 3.94	131.72^{a} ± 2.58	<0.0001
Day 14	296.15^{a} ± 11.14	224.60^{d} ± 5.10	246.04^{bcd} ± 10.58	246.94^{bc} ± 21.22	255.11^{b} ± 13.51	228.29^{cd} ± 16.42	265.27^{b} ± 15.21	264.94^{b} ± 9.31	<0.0001
Day 21	479.71^{a} ± 21.14	320.94^{c} ± 42.39	412.95^{b} ± 28.31	390.35^{b} ± 24.31	392.65^{b} ± 16.87	402.98^{b} ± 18.84	404.81^{b} ± 11.90	405.34^{b} ± 12.86	<0.0001
Day 28	537.20^{a} ± 18.39	434.74^{bc} ± 33.26	422.52^{c} ± 42.90	429.98^{bc} ± 32.86	462.25^{bc} ± 26.43	482.53^{ab} ± 50.14	453.46^{bc} ± 38.47	441.57^{bc} ± 30.55	<0.0001
Day 34	372.05^{abc} ± 28.07	321.95^{c} ± 34.77	352.53^{abc} ± 30.86	330.94^{bc} ± 35.58	374.54^{abc} ± 63.25	414.82^{a} ± 37.49	339.72^{bc} ± 39.23	394.01^{ab} ± 22.23	<0.0001
Cumulative gain/bird (g)									
Day 7	134.47^{a} ± 9.21	128.67^{a} ± 6.22	107.82^{b} ± 4.71	133.28^{a} ± 6.68	132.91^{a} ± 11.87	102.80^{b} ± 8.27	134.04^{a} ± 3.94	131.72^{a} ± 2.58	<0.0001
Day 14	430.63^{a} ± 11.63	353.27^{c} ± 5.34	353.86^{c} ± 12.90	380.22^{b} ± 24.95	388.03^{b} ± 16.77	331.09^{b} ± 14.51	399.32^{b} ± 17.57	396.66^{b} ± 10.12	<0.0001
Day 21	910.33^{a} ± 24.32	674.21^{d} ± 45.47	766.81^{bc} ± 35.12	770.57^{bc} ± 22.71	780.68^{bc} ± 28.92	734.07^{bc} ± 31.41	804.14^{b} ± 18.86	802.05^{b} ± 20.71	<0.0001
Day 28	1447.53^{a} ± 15.71	1108.95^{d} ± 34.39	1189.33^{c} ± 37.99	1200.55^{c} ± 22.24	1242.94^{bc} ± 30.05	1216.60^{bc} ± 33.56	1257.60^{b} ± 50.07	1243.61^{bc} ± 41.08	<0.0001
Day 34	1819.58^{a} ± 25.91	1430.90^{d} ± 52.52	1541.86^{c} ± 49.07	1531.49^{c} ± 39.98	1617.48^{bc} ± 71.76	1624.44^{bc} ± 42.85	1597.32^{bc} ± 50.46	1637.62^{b} ± 53.15	<0.0001

^{a,b,c}Means within rows with different superscripts differ significantly (p<0.05)

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

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3.3.6 Weekly and cumulative feed intake

Apart from day 7, weekly and cumulative feed intake for the entire period of the trial were different ($p < 0.05$) between dietary treatments as shown in Table 3.8.

There were no differences ($p > 0.05$) in cumulative feed intake at day 7. The weekly feed intakes for day 7 were highest for PC, NC, QB200, HP200, OP1200 and QB600, whilst the lowest feed intake was observed for OP400 and HP600.

At day 14, weekly intake was the highest for PC and the lowest for HP600. Intake of PC was statistically equal to that of QB600, OP1200 and HP200, while HP600 was equal to NC and OP400. Similar to the current study, at day 7 and 14 an increase in feed intake was observed when Quantum levels were increased from 1200FTU to 2400FTU (Cowieson *et al.*, 2006).

Weekly and cumulative feed intakes for day 21 were highest for PC and lowest for the NC group, with all other treatments being intermediary. At day 21, feed intake for broilers fed NC diets supplemented with phytase did not differ. When compared to the current study, the literature shows similarity in feed intake values for QB200 (Quantum blue) (Dos Santos *et al.*, 2013) and HP200 (HiPhos) (Cowieson *et al.*, 2014). However, higher feed intake values are reported by Rutherfurd *et al.*, (2012). The reason for this difference would be that diets reported by Cowieson *et al.*, (2014) were more similar to those reported in the current study. Another study observed no increase in feed intake even after OptiPhos phytase units were increased ten times, from 1000FTU to 10000FTU (Martinez-Amezcua *et al.*, 2006).

Cumulative feed intake at day 28 followed a similar trend to that observed at day 21. While weekly feed intake at day 28 was the highest for the PC diets, with all other treatment being equal or intermediary to NC, which had the lowest feed intake. At day 34, the differences observed in weekly feed intake per bird disappeared. Cumulative intake was highest for PC with no other treatments being equal. The lowest cumulative intake was observed for NC with OP400 and QB200 being statistically equal to NC. At the end of the trial, phytase supplementation did not improve weekly and cumulative feed intake. Furthermore, increasing phytase levels in the diet did not improve feed intake.

Table 3.8 Mean values (± standard deviation, SD) weekly gain and cumulative feed intake of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
<u>Parameter</u>									
Weekly feed intake per bird (g)									
Day 7	181.01^{a} ± 9.12	175.52^{a} ± 6.10	154.93^{b} ± 4.81	179.73^{a} ± 6.10	179.12^{a} ± 11.34	110.10^{b} ± 28.89	180.63^{a} ± 4.24	178.42^{a} ± 2.87	<0.0001
Day 14	473.65^{a} ± 22.24	407.93^{cd} ± 20.31	423.42^{bcd} ± 19.63	435.44^{bc} ± 26.59	437.57^{abc} ± 35.44	396.34^{d} ± 13.37	447.13^{ab} ± 11.80	454.11^{ab} ± 22.51	<0.0001
Day 21	757.03^{a} ± 23.60	578.39^{c} ± 39.54	698.60^{b} ± 35.19	663.55^{b} ± 24.08	663.89^{b} ± 20.37	668.65^{b} ± 25.02	670.68^{b} ± 16.28	687.52^{b} ± 47.28	<0.0001
Day 28	952.90^{a} ± 14.70	798.38^{c} ± 18.22	828.84^{bc} ± 58.79	844.64^{bc} ± 33.44	845.65^{bc} ± 23.56	865.33^{b} ± 34.99	836.2^{bc} ± 41.92	848.05^{bc} ± 39.80	<0.0001
Day 34	754.26^{abc} ± 39.20	693.48^{ab} ± 59.33	693.85^{ab} ± 47.99	686.14^{ab} ± 39.92	718.20^{ab} ± 71.54	784.61^{a} ± 83.49	658.52^{b} ± 79.62	729.77^{ab} ± 61.26	<0.0001
Cumulative feed intake per bird (g)									
Day 7	122.89 ± 15.78	117.53 ± 17.13	110.65 ± 18.88	123.55 ± 16.12	121.42 ± 21.60	110.10 ± 28.89	130.33 ± 13.33	111.08 ± 20.52	0.3520
Day 14	596.55^{a} ± 19.43	525.47^{d} ± 21.00	534.06^{cd} ± 10.65	558.98^{bc} ± 27.33	559.01^{bc} ± 21.49	506.47^{d} ± 22.08	511.54^{ab} ± 14.49	565.19^{abc} ± 9.44	<0.0001
Day 21	1353.58^{a} ± 30.23	1103.87^{d} ± 45.25	1235.67^{bc} ± 39.97	1222.53^{bc} ± 40.77	1222.90^{bc} ± 36.38	1175.12^{c} ± 41.36	1248.15^{b} ± 28.69	1252.71^{b} ± 46.85	<0.0001
Day 28	2270.41^{a} ± 90.94	1902.25^{c} ± 56.81	2061.51^{b} ± 62.65	2067.17^{b} ± 34.86	2068.56^{b} ± 45.30	2040.45^{b} ± 65.08	2109.57^{b} ± 79.97	2100.75^{b} ± 46.92	<0.0001
Day 34	3024.68^{a} ± 113.94	2595.72^{c} ± 89.05	2755.35^{bc} ± 80.05	2753.31^{bc} ± 96.87	2786.76^{b} ± 74.93	2846.02^{b} ± 110.57	2755.40^{b} ± 110.23	2830.52^{b} ± 80.55	<0.0001

^{a,b,c,d} Means within rows with different superscripts differ significantly ($p<0.05$)

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

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3.4 Conclusion

The present study was performed to investigate the effects of three different types of commercial phytases on production performance of commercially raised broilers. The positive control diets were formulated based on the lower end of the NRC recommended values. The negative control diets that contained phytase were formulated on the PC diet less the matrix value of HP200. Phytase supplementation was on two levels: standard and three times the standard level. Calculated nutrient values were used as a reference point in the study and phytase inclusion rates were taken as expected. At the end of the trial, live weight for the NC treatment group was improved with phytase supplementation. However, improvements were not comparable to the PC group. In addition, incremental levels of phytase did not improve live weight. Furthermore, incremental levels of OptiPhos and Quantum blue phytase improved average daily gains. However, standard dose of HiPhos resulted in average daily gains that were similar to those at three times the standard dose (high dose). It is not evident whether phytase supplementation improved feed intake, weekly gain, feed conversion ratio or European production efficiency ratio, as well as the protein efficiency. In conclusion, phytase supplementation to NC diets did not meet the nutrient requirements for improved broiler performance.

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Chapter 4

An investigation of the effects of phytase supplementation on broiler carcass characteristics, internal organ weights and intestinal morphology

Abstract

A 34 day experiment was conducted to determine the effect of three different types (HiPhos, OptiPhos and Quantum blue) of phytase on carcass characteristics, internal organs and intestinal morphology of broilers. Experimental diets were supplemented with phytase at standard inclusion levels and three times the standard level. Positive control (PC) diets were formulated based on the lower end of recommended NRC values. Whilst negative control (NC) diets were formulated on the PC diets less the matrix value for HiPhos 2000FTU (HP200). A positive control diet and negative control diet were compounded for three phases: starter, grower and finisher. Therefore, eight diets were mixed: 1. Positive control (PC); 2. Negative control (NC); 3. NC + 2000FTU HiPhos (HP200); 4. NC + 1000FTU OptiPhos (OP400); 5. NC + 1000FTU Quantum blue (QB200); 6. NC + 6000FTU HiPhos (HP600); 7. NC + 3000FTU OptiPhos (OP1200) 8. NC + 3000FTU Quantum blue (QB600). At the beginning of the trial 5120 old broilers were allocated to a completely randomised design with eight treatment diets and eight replications. On day 34, sixty-four broilers were slaughtered to determine cold carcass weight, portion weight, breast colour and pH, as well as temperature. In addition, internal organ weights were measured, gizzards were scored and intestinal histology was performed. Breast colour, pH, temperature as well as dressing percentage, thigh and wing portions did not differ between treatments ($p > 0.05$). Cold carcass weight, breast and drumstick portions differed ($p < 0.05$) between treatments. Internal organ parameters did not differ ($p > 0.05$). However, gizzard weight expressed as a percentage of live weight, differed ($p < 0.05$) between treatments. Differences ($p < 0.05$) between treatments were also observed for intestinal morphometric observations.

Keywords: Poultry, muscle pH, breast colour, portion weights, gizzard

4.1 Introduction

Advancements in poultry nutrition that led to efficient production of broiler meat had implications on carcass characteristics and intestinal morphology (Petracci & Cavani, 2011). Feed additives are an advancement that has positively influenced broiler meat yields (Selle

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& Ravindran, 2007). Phytase, a commonly used non-nutritive feed additive, makes phosphorus from the phytate complex of feed ingredients available in the gastro-intestinal tract (Greiner & Konietzny, 2010).

Currently, broiler producers use standard doses of phytase that are recommended by the manufacturer. Several studies have suggested that levels above standard doses (high levels) of phytase have the potential to increase broiler meat yields due to 'extra-phosphoric effects' (Cabahug *et al.*, 1999; Cowieson *et al.*, 2006; Shirley & Edwards, 2003). 'Extra-phosphoric effects' are benefits from the phytate complex beyond the release of phosphorus. These effects include the release of amino acids, energy and bound cations such as: calcium, magnesium and iron (Cowieson *et al.*, 2004; Ravindran *et al.*, 2000). Such benefits have been suggested to lead to improved carcass characteristics (Angel *et al.*, 2006; Çimrin & Demirel, 2008). For instance, improved commercial portion yields such as the breast (Musavi *et al.*, 2009), wing and back (Çimrin & Demirel, 2008) and leg quarter (Teixeira *et al.*, 2013). A possible explanation for the effect of above standard doses of phytase includes: increased phosphate availability, higher nitrogen retention and increased phytate complex solubility in the gastro-intestinal tract (Shirley & Edwards, 2003).

Gastro-intestinal health and integrity are important for efficient digestion and absorption of nutrients. Broilers at an early stage have to develop a functioning gastro intestinal tract (GIT) to cope with nutritional demands resulting from genetic selection for fast growth (Iji et *al.*, 2001; Nitsan *et al.*, 1991b; Uni *et al.*, 1996). That means the mucosa of the small intestine has to develop concurrently with the growing bird (Uni *et al.*, 1998). However, reduced enzyme activity during the first eight days can impede digestion (Nitsan *et al.*, 1991a). More especially when marginal diets with anti-nutritional factors are fed. For instance, the myo-inositol hexaphosphate (IP6) compound exhibits anti-nutritional effects and occurs in grains bound to phosphorus as phytate. Reports suggest that inclusion of IP6 to broiler diets had a negative influence on endogenous amino acids, phosphorus and calcium digestion (Cowieson *et al.*, 2013; Żyła *et al.*, 2012).

Phytase is a phosphatase that makes available phosphorus from the myo-inositol ring (Cowieson *et al.*, 2013). Therefore, phytase can aid the process of digestion and increase utilization of nutrients (Bedford & Partridge, 2001). Furthermore, other studies have observed that supplementation of phytase to maize-soybeans diets increased phosphorus utilization (Augspurger & Baker, 2004; Aureli *et al.*, 2011), amino acid absorption (Cowieson *et al.*, 2004) and digestibility of feed ingredients (Rutherfurd *et al.*, 2002). In addition, phytase supplementation to diets with adequate levels of phosphorus led to an increase in the villi length of the duodenum (Wu *et al.*, 2004). Thomson (2007) observed that nutrient

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digestibility and enzyme inclusion in diets had an effect on the intestinal morphology. In addition, *E. coli* and *A. oryzae* have specific pH ranges that are optimal for phosphorus release (Igbasan *et al.*, 2000). Furthermore, diets formulated with phytase tend to have higher inclusion rates of limestone which could increase the pH of the ileum and crop contents (Shafey *et al.*, 1991).

Therefore, poor GIT health resulting from unfavourable intestinal environment and impaired gizzard development can have an impact on the welfare of the birds. This may induce stress factors that affect the immune status of the bird and hence impair development of internal organs such as the bursa, liver, spleen and heart. In addition, reports have indicated that dietary calcium levels had an effect on the bursa (Nourmohammadi *et al.*, 2011) and other internal organs such as liver, heart, spleen and gizzard (Talpur *et al.*, 2012). Similarly, liver and spleen weight were reduced due to a decrease in non-phytate phosphorus (Viveros *et al.*, 2002).

Although research has been conducted on the effects of phytase addition on the carcass characteristics and gut morphology of broilers, researchers are continuously seeking means of improving the production efficiency of broilers without negatively influencing the meat quality. Such as increasing the amount of phytase included in the diet substantially. The objectives of the study were to:

i. Assess the effects of three different types of phytase at various inclusion levels on carcass characteristics (muscle pH, colour and portion weights) of broilers raised under commercial conditions.

ii. Determine the effects of three different types of phytase at various inclusion levels on organ weights, intestinal pH and morphology.

4.2 Materials and methods

Experimental procedures for the birds' housing system, diets and phytase inclusion rates are described in 3.2 and 3.2.1. Briefly, eight treatment diets consisting three different types of phytases were fed *ad libitum* to broilers for 34 days. The description of diets that were used for the entire duration of the trial is as shown in Table 3.1.

4.2.1 Data collection and experimental procedure

On day 34, Sixty- four Cobb 500 broilers were selected, one per pen, from the middle weight group. Live broiler weights were recorded using a Mettler scale (Model ICS 429, Switzerland). Slaughter procedures were performed at the Mariendahl experimental farm (Stellenbosch University, Western Cape). Slaughtering of broilers was done according to acceptable commercial standards through immobilization by electrical stunning then followed by exsanguination. Broilers were then scalded, de-feathered and eviscerated (removal of internal organs, feet and neck).

4.2.2 Data collection and experimental procedure for carcass characteristics

The initial pH (pH_i) measurements for the thigh and breast muscle were obtained using a portable Crison 506 pH-meter fifteen minutes after slaughter at the Mariendahl experimental farm (Stellenbosch University, Western Cape). The ultimate pH (pH_u) was measured 24h post-mortem using the same procedure at the Animal Sciences department (Stellenbosch University, Western Cape). The pH meter was calibrated (standard buffers pH 4.0 and 7.0 at 25°C) and stabilized before being placed at an angle in the muscle. Distilled water was used to clean the probe of the pH meter between each reading, which was placed and stored in 3M KCl electrolytic solution. After obtaining the $pH_{i,}$ carcasses were hung in a cold room at 4°C for 3h before transportation to a 4°C cold room for 24h at the Animal Sciences Department (Stellenbosch University, Western Cape).

Cold carcass weights were obtained 24h post-mortem and pH_u was measured. The cold dressing percentage was expressed as a percentage of cold carcass weight relative to live weight. Thereafter, the weight of each portion was measured using a Ragwag scale with an accuracy of 0.01 grams (Model PS 4500/C/1, Poland). A portion cutter was used to separate leg quarters from the rest of the carcass. The leg quarter was subsequently divided into thigh and drumstick by cutting at the joint of the tibia and femur. The wing portion was obtained by cutting through the scapula and coracoids joint. The percentage of each commercial portion relative to the carcass weight was determined.

The breast portion was obtained by cutting from the clavicle *furcula* bone alongside the carina (keel) bone. The right side of the breast was weighed and was expressed as a

percentage relative to the carcass weight. The left side of the breast was partitioned into flesh, skin and subcutaneous fat, as well as bone. The portions were then weighed and expressed as a percentage relative to the left breast weight. After the skin of the left breast was removed, the muscle was allowed to bloom for 60min, thereafter colour measurements were determined. Colour measurements L*, a* and b* were taken in triplicate with a Minolta® Chroma meter (Model CR200, Japan). L* represents brightness, a* represents the red-green range and b* represents the blue-yellow range. Triplicate readings were done over the total area of the muscle and the average calculated. Positive b* values are a measure of yellowness and negative b* values indicates blueness. The Hue and Chroma of the breast portion was then calculated as shown in Equation 4.1 and Equation 4.2

Equation 4.1

$$\text{Hue angle } (\mathbf{h_{ab}}) = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Equation 4.2

$$\text{Chroma } (\mathbf{C^*}) = \sqrt{(a^*)^2 + (b^*)^2}$$

4.2.3 Data collection and experimental procedure for gizzard weight

After slaughtering the broilers, the internal organs were removed and gizzards were sectioned off at the proventriculus and the pyloric sphincter at the Mariendahl experimental farm (Stellenbosch University, Western Cape). A longitudinal section was done on the gizzards and contents were rinsed out under running water. Gizzards were then allowed to dry at room temperature and the cuticle-layer was not removed. Thereafter, weights of cleaned gizzards were noted using a Ragwag scale with an accuracy of 0.01 grams (Model PS 4500/C/1, Poland).

4.2.4 Data collection and experimental procedure for organs and intestinal pH

Organ weights and intestinal pH were taken at the Mariendahl experimental farm (Stellenbosch University, Western Cape). The liver, heart, spleen and bursa were sectioned off with a scalpel taking care not to cut the organs. The weights of the organs were then measured using a Ragwag scale with an accuracy of 0.01 grams (Model PS 4500/C/1, Poland). Organs weights were expressed as a percentage of live weight. The determination of *in-situ* intestinal pH was performed as recommended by Morgan *et al.*, (2014). The intestinal pH was taken using a calibrated portable Crison 506 pH meter (standard buffers pH 4.0 and 7.0 at 25°C). Each reading was taken after the pH meter was allowed to

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stabilise, at the place that was sectioned off for histological analysis. After each reading, the probe of the pH meter was rinsed with distilled water and rested in a KCL 3M electrolytic solution. The ileum, duodenum and jejunum pH measurements were taken by describing the ileum region as a point 40mm proximal to the ileocecal junction to the Meckel's diverticulum. The jejunum portion extended from the bile duct entrance to Meckel's diverticulum.

4.2.5 Data collection and experimental procedure for intestinal histology

Experimental procedures followed for intestinal histology were as indicated by Van Emmenes (2014). After taking the intestinal pH, gut samples were taken from the duodenum at the beginning of the pancreas and on the side proximal to the gizzard. The gut sample from the jejunum was taken from the centre and that of the ileum was obtained 5mm from Meckel's diverticulum to the ileocecal junction. Obtained samples were then rinsed with 9% saline solution. Thereafter sectioned gut samples were placed in buffered 10% w/v formalin solution until further analysis. Fixing and staining of gut sections was performed at the Physiology Department (Stellenbosch University, Western Cape). The gut sections were cut to 4µm (four cross-sections per sample) using a rotary microtome (Reichert Jung, Austria). An automated tissue processor (Tissue Tek II, model 4640B, Lab division, Miles Laboratories, Illinois) embedded the 4µm gut sections with paraffin wax and fixed them on slides. Thereafter embedded sections were stained with haematoxylin and eosin. An Olympus microscope model IX70 (Olympus Cooperation, America) was used for examination of slides using X4 magnification. The crypt depth, villi length and villi parameter were then measured in micro meters. The villi length was measured from the villi tip to the villi-crypt junction. The crypt depth was defined as the depth of the invagination between two villus. The perimeter was measured as the boundary for each villi from the tip of the villi around to the villi crypt junction and back to the villi tip.

4.2.6 Statistical analysis

One-way analysis of variance (ANOVA) was performed using the general linear model procedure of SAS enterprise guide 5.1. Treatment means were compared by least significant difference ($p < 0.05$). Parameters were tested for normality and homoscedasticity. A *post hoc* test of least significant difference was performed on treatment means using Bonferoni's test. For large variation in the standard deviation of intestinal morphology Welch's ANOVA test was used.

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4.3 Results and discussion

4.3.1 Colour and pH

Table 4.1 shows results of temperature, pH measurements, colour values L*, a* and b* as well as the Hue and Chroma.

The colour values of the left breast (L*, a*, b*, Hue and Chroma) did not differ ($p > 0.05$). Similar to results from Han *et al.* (2009), colour values were not influenced by phytase supplementation. Furthermore, colour calculations determined that the Hue and Chroma were not affected ($p > 0.05$) by phytase addition to the diets. The L* values across all dietary treatments were greater than 53 and these are considered lighter than normal (Qiao, *et al*., 2001). Temperature and pH measurements of left breast and thigh did not differ ($p > 0.05$). The ultimate pH across treatments was less than 5.8 and this is consistent with results noted Fletcher (2002) who indicated that a decline in pH is associated with a rise in L* values. High L* values and low ultimate pH values indicate pale, soft and exudative meat (PSE) (Woelfel, *et al*., 2002; Kralik *et al*., 2014). In the current study, left breast portions in all treatment groups exhibited pale, soft and exudative meat (PSE). However, indications are that the PSE meat in this study was not caused by phytase supplementation, but rather by other factors such as ante-mortem handling and/or post-mortem storage processing (Qiao *et al*., 2001; Han *et al*., 2009; Ristic & Damme, 2010). In the current study, carcasses may have been chilled too slow resulting in PSE meat. Generally, a* values that are equal to 1.4 and b* values that are equal to 10.3 are considered normal (Totosaus *et al*., 2007). In the current study, a* values across all treatments were above 1.4, which is an indication of redness in meat. The b* values in all treatments were positive and above 10.3, indicating yellowness. Temperature fluctuations during post-mortem processing in the current study may have resulted in left breast portions that are more red and yellow in colour (Savell *et al*., 2005; Rathgeber *et al*., 1999).

Table 4.1 Mean values (± Standard deviation, SD) of colour measurements (CIE-Lab), pH of breast and thigh, and temperature measurements of the breast and thigh of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Parameter									
Colour measurements									
Ave L*	58.67 ± 2.87	60.52 ± 3.97	57.40 ± 3.14	61.04 ± 2.88	58.10 ± 3.70	57.42 ± 4.37	58.26 ± 5.10	58.59 ± 3.75	0.6147
Ave a*	3.25 ± 0.70	2.80 ± 1.35	3.57 ± 1.49	3.17 ± 1.25	3.01 ± 0.73	3.61 ± 1.75	3.38 ± 0.60	2.46 ± 0.75	0.7912
Ave b*	13.28 ± 2.05	11.69 ± 2.25	11.94 ± 2.18	11.69 ± 2.09	12.94 ± 2.48	12.81 ± 2.16	12.57 ± 2.81	11.45 ± 2.34	0.8584
Hue	76.32 ± 1.06	76.48 ± 6.14	73.48 ± 6.79	74.10 ± 8.52	76.37 ± 4.77	74.89 ± 5.65	74.46 ± 3.64	77.06 ± 6.43	0.9524
Chroma	13.68 ± 2.15	12.08 ± 2.30	12.52 ± 2.22	12.22 ± 1.74	13.32 ± 2.34	13.36 ± 2.47	13.04 ± 2.75	11.76 ± 2.14	0.8401
pH & temperature measurements									
pH_i breast	6.14 ± 0.18	6.19 ± 0.23	6.19 ± 0.23	6.35 ± 0.12	6.25 ± 0.33	6.12 ± 0.18	6.13 ± 0.22	6.14 ± 0.33	0.5294
pH_u breast	5.69 ± 0.14	5.64 ± 0.16	5.75 ± 0.11	5.67 ± 0.08	5.72 ± 0.08	5.76 ± 0.07	5.69 ± 0.20	5.68 ± 0.08	0.3922
$Temp_i$ breast	32.40 ± 4.02	31.25 ± 4.40	31.06 ± 2.23	31.04 ± 3.10	31.15 ± 2.45	31.10 ± 1.07	30.94 ± 2.53	30.80 ± 2.26	0.2498
$Temp_u$ breast	6.75 ± 0.80	7.34 ± 1.16	7.21 ± 1.92	7.27 ± 1.50	7.00 ± 0.61	7.57 ± 0.83	7.08 ± 2.13	6.58 ± 1.06	0.7661
pH_i thigh	6.12 ± 0.20	6.18 ± 0.15	6.27 ± 0.22	6.06 ± 0.09	6.19 ± 0.16	6.24 ± 0.12	6.22 ± 0.15	6.36 ± 0.31	0.1023
pH_u thigh	5.72 ± 0.09	5.79 ± 0.15	5.76 ± 0.10	5.78 ± 0.12	5.82 ± 0.09	5.83 ± 0.09	5.73 ± 0.16	5.76 ± 0.10	0.5927
$Temp_i$ thigh	32.10 ± 4.34	30.76 ± 4.98	30.90 ± 2.53	30.53 ± 3.38	31.36 ± 3.23	31.07 ± 1.93	30.70 ± 3.39	30.22 ± 1.30	0.2182
$Temp_u$ thigh	6.53 ± 1.15	6.89 ± 1.30	6.24 ± 1.50	6.57 ± 1.69	6.60 ± 0.64	7.20 ± 1.04	6.90 ± 1.91	6.02 ± 0.83	0.1290

^{a,b} Means within rows with different superscripts differ significantly ($p < 0.05$)

Ave- Average. pH_i - Initial pH. pH_u- Ultimate pH.

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

4.3.2 Dressing percentage and portion percentages

As shown in Table 4., dressing percentage did not differ between phytase dietary treatments ($p > 0.05$). This is consistent with results found by Kozłowski *et al.* (2009). However, Çimrin & Demirel (2008) and Kozłowski *et al.* (2010) in a later study noted improvements in dressing percentages due to phytase supplementation. In the current study, percentage breast of carcass and the percentage drumstick of carcass did not differ significantly. In addition, the percentage thigh, percentage wings and the percentage back of carcass did not differ significantly between treatments. Similar to the current study, when portion weights were expressed as a percentage of body weight no differences between treatments were observed by Kozłowski *et al.* (2010).

4.3.3 Carcass weight

As shown in Table 4., at the end of the trial, live weights and cold carcass differed significantly between treatment groups. The positive control group (PC) had the highest cold carcass weight with all other treatments being equal. In a study by Angel *et al.* (2006), phytase derived from *A. oryzae* led to an improved carcass weight. Another study which used three types of commercial microbial phytase derived from *A. niger, Penicillium canescens* and *Pichia* noted that carcass yields were improved with phytase supplementation (Kozłowski *et al.*, 2009). In addition, a follow up experiment by Kozłowski *et al.* (2010) which involved diets with different amounts of *E. coli* phytase (250, 500 and 750FTU) at low levels of available phosphorus (2.0g/kg) included in the grower diet showed an increase in carcass yields. In the current study, a similar trend for live weight was observed, however the NC diets did not perform equally to other diets.

In another study, broilers that were fed diets with insufficient metabolisable energy (12.3MJ/kg) and low levels of crude protein (140g/kg, 160g/kg) during the finisher phase had carcass weights at 42 days that were not affected by inclusion of 500 FTU (Nagata *et al.*, 2011). This finding is similar to a study by Gomide *et al.* (2012), where the carcass percentage at 35 days was not improved with the addition of phytase to diets that contained crude protein of up to 160g/kg. Crude protein levels in diets of the current study were above 180g/kg. However, reduced levels of apparent metabolisable energy for the NC diets may have contributed to reduced carcass weights.

4.3.4 Portion weight

Table 4. shows results of breast, drumstick, thigh and wing portions. The absolute weights of the breast portion, breast lean meat, drumstick, skin and fat as well as back yield were different

($p < 0.05$) between treatments. However, differences disappeared when absolute weights were expressed as a percentage of cold carcass weight or breast portion weight.

Breast weights differed significantly between treatments. In a study by Musavi *et al.* (2009), breast weights were improved with phytase supplementation. The authors suggests that these improvements were due to a rise in amino acids made available from the phytate complex because of the extra phosphoric effects that supply amino acids for breast growth. Similarly, Çimrin & Demirel (2008) found that breast portions of broilers that were not supplemented with phytase were not as heavy as those that were supplemented with phytase. However, Kozłowski *et al.* (2010) observed that breast portions were neither influenced by varying amounts of available phosphorus nor phytase addition. In addition, Gomide *et al.* (2012) observed that breast portions were not affected by addition of phytase to diets with metabolisable energy of 12.9MJ/kg and crude protein levels ranging from 160g/kg to 190g/kg.

Findings from studies that are consistent with the current study indicate that despite overall bird live weight improvement due to phytase supplementation, thigh portions (Kozłowski *et al.*, 2010) and wing yields (Angel *et al.*, 2006) did not improve. However, other studies found that thigh percentages and wing portions of male broilers (Çimrin & Demirel, 2008; Musavi *et al.*, 2009), back and leg quarter (Angel *et al.*, 2006; Çimrin & Demirel, 2008; Teixeira *et al.*, 2013) improved with phytase supplementation to diets. The diets of Çimrin & Demirel (2008) and Musavi *et al.* (2009) contained adequate levels of total calcium (10g/kg) but reduced levels of available phosphorus (2.0g/kg). In addition, Musavi *et al.* (2009) reported diets that contained 9.4g/kg total calcium and 2.1g/kg available phosphorus. In comparison, Kozłowski *et al.* (2010) reported diets with reduced levels of both calcium (7.52g/kg) and available phosphorus (1.95g/kg) during the grower phase. Similarly, the current study had deficient diets that contained both reduced levels of calcium and phosphorus and this could be the reason for the lack of improvements on portion weight.

Drumstick weights differed significantly between treatments. The drumstick % portions of broilers fed diets that contained phytase at standard levels (HP200, OP400 and QB200) were numerically lower than those fed diets at the higher dosage (HP600, OP1200 and QB600), but were not significantly different.

Table 4.2 Mean values (± Standard deviation, SD) of cold carcass weight (g), portion weights (g) and portion yields expressed as a percentage of carcass weight (%),breast lean meat, bone and skin (%) of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Live weight (g)	1866.14[a] ± 26.06	1477.70[e] ± 52.64	1588.89[cd] ± 49.15	1577.90[d] ± 37.90	1663.65[bc] ± 71.41	1648.12[bc] ± 75.27	1643.89[bcd] ± 50.40	1684.34[b] ± 53.61	<0.0001
Carcass weight (g)	1300.00[a] ± 94.87	1039.15[b] ± 120.40	1021.43[b] ± 75.59	1035.71 [b] ± 110.73	1100.00[b] ± 70.71	1114.29[b] ± 94.49	1062.50[b] ± 74.40	1100.00[b] ± 61.24	<0.0001
Dressing percentage	66.9 ± 2.7	63.0 ± 3.8	64.7 ± 1.2	63.3 ± 4.9	66.3 ± 2.4	64.5 ± 3.4	63.7 ± 2.3	64.4 ± 3.6	0.278
Left breast (g)	249.33[a] ± 26.73	200.01[ab] ± 39.30	192.32[b] ± 24.71	211.52[ab] ± 31.43	215.72[ab] ± 20.54	217.63[ab] ± 21.51	204.34[ab] ± 26.90	209.23[ab] ± 40.81	0.052
Right breast (g)	244.24[a] ± 23.20	199.99[ab] ± 37.94	194.59[b] ± 29.21	206.61[ab] ± 25.71	205.61[ab] ± 22.87	212.74[ab] ± 25.04	203.51[ab] ± 23.10	201.93[ab] ± 14.31	0.048
Drumsticks (g)	175.63[a] ± 24.12	142.31[b] ± 11.52	141.84[b] ± 11.07	133.92[b] ± 15.22	146.38[b] ± 9.01	160.14[ab] ± 10.29	153.23[ab] ± 13.60	152.63[ab] ± 12.70	<0.0001
Thigh (g)	314.73 ± 44.43	268.14 ± 335.38	263.16 ± 16.91	264.98 ± 39.81	287.20 ± 31.72	304.01 ± 37.18	273.22 ± 34.38	280.95 ± 27.01	0.067
Wing (g)	162.31 ± 23.49	141.90 ± 13.05	142.18 ± 10.43	138.13 ± 11.21	141.56 ± 11.24	143.27 ± 9.57	147.82 ± 12.92	148.75 ± 27.88	0.137
Back (g)	97.73[a] ± 11.56	76.31[ab] ± 7.52	80.55[b] ± 9.51	77.55[ab] ± 7.14	84.38[ab] ± 14.02	77.93[ab] ± 8.13	84.38[ab] ± 11.35	75.66[ab] ± 6.16	0.037
Breast lean meat (g)	188.11 ± 24.24	153.60 ± 26.06	145.14 ± 20.89	165.71 ± 28.88	163.76 ± 21.36	163.48 ± 16.98	161.14 ± 25.52	163.58 ± 24.57	0.180
Breast bone (g)	34.26 ± 6.44	28.41 ± 8.92	35.27 ± 9.12	29.01 ± 4.00	30.22 ± 7.25	35.72 ± 5.01	31.17 ± 4.45	29.84 ± 14.59	0.859
Breast, skin & fat (g)	18.26[a] ± 2.80	14.15[ab] ± 2.94	12.41[b] ± 2.83	13.96[b] ± 1.69	15.68[ab] ± 2.99	14.73[ab] ± 3.21	14.25[ab] ± 3.31	12.24[b] ± 2.74	0.033
Breast (%)	39.14 ± 2.9	38.54 ± 3.6	38.15 ± 2.4	40.39 ± 3.2	38.27 ± 2.9	38.58 ± 2.7	38.40± 2.9	38.18 ± 3.9	0.898
Thigh (%)	24.67 ± 2.2	26.13 ± 1.4	26.11 ± 1.3	25.53 ± 2.2	26.10 ± 2.3	27.24 ± 1.6	25.67 ± 2.1	26.15 ± 0.8	0.464
Drumstick (%)	13.85 ± 0.9	13.86 ± 1.1	14.08 ± 1.1	12.97 ± 1.3	13.34 ± 0.9	14.41 ± 0.9	14.45 ± 1.2	14.20 ± 1.1	0.145
Wing (%)	12.79 ± 1.3	13.87 ± 1.6	14.14 ± 1.4	13.39 ± 0.9	12.92 ± 1.3	12.89 ± 0.8	13.95 ± 1.3	13.88 ± 2.9	0.483
Back (%)	7.10 ± 1.1	7.56 ± 0.7	7.99 ± 0.9	7.57 ± 1.2	7.68 ± 1.3	7.01 ± 0.6	7.95 ± 0.9	7.04 ± 0.6	0.466
Breast lean meat (%)	75.7 ± 1.7	76.25 ± 1.8	75.5 ± 4.2	77.9 ± 2.3	76.8 ± 4.0	75.4 ± 3.5	77.4 ± 2.4	78.6 ± 3.8	0.578
Breast bone (%)	13.9 ± 2.7	14.3 ± 2.6	16.7 ± 3.9	13.8 ± 2.0	14.5 ± 4.6	15.2 ± 2.7	15.1 ± 2.4	13.8 ± 4.3	0.781
Breast, skin & fat (%)	7.48 ± 1.7	7.25 ± 1.3	6.52 ± 1.6	6.73 ± 1.4	7.35 ± 1.0	6.85 ± 1.7	6.83 ± 1.0	5.85 ± 0.7	0.633

[a,b,c,d,e] Means within rows with different superscripts differ significantly ($p < 0.05$)

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

The back portion weights and breast skin and fat differed significantly between treatment groups. Çimrin & Demirel (2008) reported an increase in back weight for broilers that were fed diets supplemented with phytase. The weight of back portions followed a similar trend to that of the breast portions. Similar to breast portions, when the back portion weights are expressed as a percentage of carcass weight the differences disappear.

4.3.5 Intestinal pH

There were no differences ($p > 0.05$) in the pH of the duodenum, ileum and jejunum as shown in Table 4.. Neither type nor level of phytase had an effect on the intestinal pH. The pH range for the duodenum is 5.5 - 6.2, jejunum 5.8 - 6.9 and ileum 6.3 - 8.0 (Blok, 2002). In the current study, the pH range of the duodenum (5.57 - 5.89) and jejunum (5.89 - 6.11) was within the pH range as reported by Blok (2002). In addition, the pH of the ileum for the PC, HP200, OP400 and QB200 were similar to the range reported (Blok, 2002). However, the NC, HP600, OP1200 and QB600 groups had pH values that were lower than that reported (Blok, 2002). Furthermore, as expected, the GIT pH increased from the duodenum to the ileum for each treatment. Contrary to the current study, an increase in the levels of phytase from 500FTU to 5000FTU led to an increase in duodenal and distal ileum pH (Walk *et al.*, 2012). The possible reason for the difference in results noted by Walk *et al.*, (2012) might be due to the higher levels of dietary calcium (6.0g/kg) and a higher level of phytase as compared to the 4.7g/kg total calcium and 3000FTU reported in the current study. The ranges of pH values found in the current study are suitable for *A. oryzae* and *E. coli* phytase as has been described in section 2.1.4.2. In addition, activity from supplemented phytase has been detected in the intestinal brush border (Maenz & Classen, 1998). However, phytase activity was not observed in digesta from the jejunum and ileum (Onyango *et al.*, 2005; Yu *et al.*, 2004). Therefore, the possibility of phytase activity in the small intestine would be negligible. In the current study, acidity in the lumen would be the result of the formation of phytate complexes leading to an increase in excreted phosphorus and calcium. However, the benefit of lower lumen pH is a healthy GIT environment due to a reduced microbial population.

4.3.6 Weight of internal organs

The results for absolute weights of organs and the organ weight expressed as a percentage of live weight are shown in Table 4.. Phytase supplementation did not have an effect ($p > 0.05$) on the relative weights of the liver, spleen, bursa, gizzard and heart. However, when the gizzard and heart were expressed as a percentage of live weight, differences ($p < 0.05$) were noted. Similarly, gizzard weights were neither influenced by phosphorus levels (Atapattu & Gamage,

2007) nor by phytase derived from *Aspergillus sp.* (Hang *et al.*, 2008). Akyurek *et al.*, (2011) observed a reduction in gizzard weight expressed as a percentage of body weight when deficient diets (2.0g/kg phosphorus) were supplemented with phytase. Even though phytase had no influence on gizzard weight in the current study, other authors have noted phytase activity in the gizzard (Onyango *et al.*, 2005; Yu *et al.*, 2004). In addition, a reduction in gizzard inositol levels was observed due to phytase supplementation (Walk *et al.*, 2013). A reduction in gizzard inositol levels would then affect passage time and particle size of contents, which would then affect gizzard development (Watson *et al.*, 2006).

Viveros *et al.* (2002) reported that the absolute weights of the spleen and liver were not influenced by phytase supplementation. In addition, the spleen and liver expressed as a percentage of body weight were not affected by phytase derived from *Aspergillus sp.* (Hang *et al.*, 2008). However, phytase inclusion to diets with low levels of non-phytate phosphorus resulted in reduced liver weight when expressed as a percentage of body weight (Atapattu & Gamage, 2007; Viveros *et al.*, 2002). Akyurek *et al.*, (2009) also reported that the inclusion of phytase to diets with adequate non-phytate phosphorus levels 5.0g/kg led to an increase in liver weight.

Several studies have reported different inclusion rates of phytase, calcium and phosphorus levels in diets. For instance, phytase was supplemented with either adequate diets containing 4.1g/kg phosphorus and 8.1g/kg calcium (Wang *et al.*, 2013) or diets deficient in available phosphorus 2.0g/kg and calcium 10.0g/kg (Akyurek *et al.*, 2011). Similar to the current study, the bursa were not influenced by phytase addition to either deficient diets (Akyurek *et al.*, 2011) or adequate diets (Nourmohammadi *et al.*, 2011). However, Akyurek *et al.* (2009) reported an increase in the spleen and bursa weight with an increase in phytase supplementation. In addition, when phytase levels in adequate diets were increased, a corresponding increase in the bursa weight was noted (Wang *et al.*, 2013). Furthermore, in the current study there were no treatment differences for the spleen to bursa ratio ($p > 0.05$). This ratio was the same for diets supplemented with phytase and those not supplemented with phytase.

Table 4.3 Mean values (± Standard deviation, SD) of intestinal pH measurements, internal organ weight (g), and liver, heart and spleen expressed as percentage (%) of live weight of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Parameter									
pH duodenum	5.69 ± 0.44	5.79 ± 0.33	5.76 ± 0.27	5.62 ± 0.16	5.61 ± 0.35	5.85 ± 0.23	5.61 ± 0.48	5.57 ± 0.42	0.693
pH jejunum	6.00 ± 0.21	5.91 ± 0.27	6.11 ± 0.25	5.89 ± 0.32	6.08 ± 0.34	6.14 ± 0.19	5.91 ± 0.25	5.95 ± 0.25	0.386
pH ileum	6.39 ± 0.29	6.25 ± 0.33	6.41 ± 0.24	6.32 ± 0.47	6.35 ± 0.43	6.21 ± 0.27	6.18 ± 0.34	6.20 ± 0.37	0.794
Liver weight (g)	44.51 ± 7.03	37.76 ± 4.50	35.81 ± 4.13	40.46 ± 5.76	38.93 ± 5.89	37.10 ± 4.78	38.85 ± 5.22	37.99 ± 3.90	0.069
Gizzard weight (g)	33.91 ± 6.07	28.76 ± 3.59	30.41 ± 3.81	29.52 ± 5.12	30.87 ± 4.40	28.88 ± 4.80	34.53 ± 5.55	28.01 ± 3.52	0.063
Heart weight (g)	9.61 ± 1.95	9.38 ± 1.07	9.56 ± 0.91	8.35 ± 0.79	10.05 ± 1.79	8.97 ± 1.31	8.34 ± 1.34	9.11 ± 1.33	0.179
Spleen weight (g)	2.54 ± 0.58	1.96 ± 0.58	1.93 ± 0.36	2.03 ± 0.14	2.31 ± 0.56	2.02 ± 0.52	1.98 ± 0.42	2.01 ± 0.73	0.234
Bursa weight (g)	2.95 ± 0.49	2.51 ± 0.57	2.75 ± 0.85	2.93 ± 0.66	3.24 ± 0.72	3.05 ± 0.59	2.60 ± 0.61	3.37 ± 1.18	0.314
Spleen : Bursa	0.88 0.25	0.82 0.35	0.78 0.35	0.72 0.17	0.76 0.31	0.67 0.18	0.79 0.23	0.63 0.25	0.654
Liver weight (% BW)	2.37 ± 0.42	2.42 ± 0.24	2.28 ± 0.32	2.46 ± 0.24	2.33 ± 0.39	2.14 ± 0.25	2.33 ± 0.26	2.21 ± 0.24	0.452
Gizzard weight (% BW)	1.79^{ab} ± 0.26	1.84^{ab} ± 0.14	1.94^{ab} ± 0.32	1.80^{ab} ± 0.27	1.84^{ab} ± 0.23	1.67^{ab} ± 0.27	2.07^{a} ± 0.34	1.62^{b} ± 0.17	0.026
Heart weight (% BW)	0.51 ± 0.11	0.61 ± 0.09	0.61 ± 0.05	0.51 ± 0.03	0.60 ± 0.12	0.52 ± 0.06	0.50 ± 0.07	0.53 ± 0.07	0.090
Spleen weight (% BW)	0.14 ± 0.03	0.13 ± 0.04	0.12 ± 0.02	0.12 ± 0.01	0.14 ± 0.04	0.12 ± 0.03	0.12 ± 0.02	0.12 ± 0.04	0.760

^{a,b} Means within rows with different superscripts differ significantly ($p<0.05$)

BW- Body weight,

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

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4.3.7 Intestinal morphology

The duodenum and jejunum crypt depth, villi length, villi perimeter, as well as the ratio of the villi length to the crypt depth of broilers raised up to day 34 are shown in Table 4.. The crypt depths of the duodenum differed ($p < 0.05$) between treatments. The QB200 treatment group was significantly different from OP1200 group; all other treatments were intermediary being equal to either QB200 or OP1200. The length and villi perimeter of the duodenum differed significantly between diets. HP600 was different ($p < 0.05$) from NC with other treatments being intermediary.

The crypt depths of the jejunum differed ($p < 0.05$) between treatment groups; the groups OP400 and HP600 differed from HP200, NC and PC. All other treatments were intermediary and equal to PC, NC, HP200, HP600 and OP400. The villi length also differed ($p < 0.05$) between treatment groups; the villi length of the PC group was significantly different from OP400, with all other treatment groups being intermediary and equal to PC and OP400. There were no differences ($p > 0.05$) in jejunum villi perimeter between treatment groups.

Phytase supplementation increases the nutritive value of feed ingredients. In addition, dietary phytase improves digestive and absorptive efficiency which is directly related to an increase in villi size (Blok, 2002). Nourmohammadi & Afzali (2013) reported the use of maize-soybean meal diets that contained citric acid and microbial phytase supplemented at 500FTU, as well as 1000FTU and concluded that 1000FTU improved the crypt depth of the duodenum, however the villi length to crypt depth ratio was reduced. Furthermore, Nourmohammadi & Afzali, (2013) reported that duodenum villi length, jejunum villi length, jejunum crypt depth and the jejunum villi length to crypt depth ratio were not influenced by phytase supplementation. In the current study, it is not apparent that phytase supplementation had an influence on the intestinal morphology. As has been recommended for anatomic pathological tissue collection, histological processing of intestinal sections should be 1-2d after formaldehyde fixation. However, due to logistic constraints, processing of histological sections in the current study was not done after 1-2d.

Table 4.4 Mean values (± Standard deviation, SD) of duodenum and jejunum crypt depth, villi length , villi perimeter and the ratio of the villi length to the crypt depth of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Duodenum									
Crypt depth	$263.72^{ab} \pm 58.45$	$252.81^{ab} \pm 56.30$	$237.59^{bc} \pm 26.39$	$291.15^{a} \pm 72.92$	$254.89^{ab} \pm 55.11$	$278.21^{ab} \pm 65.93$	$209.15^{c} \pm 42.80$	$239.56^{bc} \pm 62.49$	<0.000
Villi length	$1195.79^{bc} \pm 405.16$	$1025.96^{c} \pm 371.41$	$1202.41^{abc} \pm 465.29$	$1233.19^{abc} \pm 326.40$	$1376.94^{ab} \pm 249.42$	$1505.13^{a} \pm 588.95$	$1166.81^{bc} \pm 241.33$	$1276.31^{abc} \pm 238.97$	<0.000
Villi Perimeter	$2713.45^{bc} \pm 845.02$	$2386.45^{c} \pm 744.04$	$2740.32^{bc} \pm 956.76$	$2836.83^{bc} \pm 648.45$	$3153.24^{ab} \pm 571.28$	$3475.61^{a} \pm 1258.18$	$2670.41^{bc} \pm 485.64$	$2960.12^{abc} \pm 481.07$	<0.000
Villi length to crypt depth ratio	$4.82^{bc} \pm 1.89$	$4.33^{c} \pm 2.12$	$5.12^{abc} \pm 1.89$	$4.38^{c} \pm 1.23$	$5.63^{a} \pm 1.36$	$5.45^{ab} \pm 1.74$	$5.74^{a} \pm 1.36$	$5.62^{ab} \pm 1.57$	0.000
Jejunum									
Crypt depth	$238.24^{b} \pm 65.78$	$240.61^{b} \pm 59.32$	$295.28^{a} \pm 53.87$	$271.11^{ab} \pm 55.78$	$240.28^{b} \pm 50.30$	$284.41^{a} \pm 70.94$	$270.44^{ab} \pm 59.19$	$272.50^{ab} \pm 47.79$	<0.000
Villi length	$1030.97^{a} \pm 228.29$	$922.41^{ab} \pm 175.59$	$829.37^{b} \pm 189.71$	$911.16^{ab} \pm 128.95$	$919.15^{ab} \pm 257.73$	$907.34^{ab} \pm 20.01$	$1009.94^{ab} \pm 210.83$	$974.41^{ab} \pm 283.44$	0.01
Villi perimeter	2421.81 ± 461.73	2312.09 ± 560.12	2030.69 ± 347.79	2110.57 ± 250.72	2173.48 ± 711.72	2144.74 ± 466.75	2298.94 ± 468.99	2279.89 ± 638.46	0.08
Villi length to crypt depth ratio	$4.91^{a} \pm 2.88$	$4.36^{ab} \pm 1.84$	$2.82^{d} \pm 0.71$	$3.43^{cd} \pm 0.88$	$4.02^{bc} \pm 1.37$	$3.35^{cd} \pm 1.06$	$3.84^{bc} \pm 0.88$	$3.66^{bc} \pm 0.93$	<0.000

^{a,b,c,} Means within rows with different superscripts differ significantly (p<0.05)

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

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4.4 Conclusion

The current study assessed the effects of three different types of phytase on carcass characteristics of broilers reared under commercial conditions. In addition, the effects of three different types of phytase on organ weights, intestinal pH and morphology were investigated.

Phytase supplementation to NC diets at high levels had an effect on drumstick yields. However, addition of phytase to NC diets at standard levels (OP400, HP200, and QB200) did not improve drumstick portion weights. The thigh and wing portions were not influenced by phytase supplementation. Cold carcass weight was not improved with phytase supplementation at either standard or high levels to NC diets. Overall, 'extra phosphoric effects' were not observed in the current study as not all portion weights were increased.

The type or dosage of phytase did not have a negative influence on breast colour, thigh muscle pH and breast muscle pH. Therefore, it may be concluded that the supplementation of phytase to NC diets fed to broilers would not result in unfavourable carcass quality characteristics.

The internal organ weights were the same for all three types of phytase; at both standard and high phytase doses. Furthermore, the effects of phytase supplementation on intestinal morphometric results were inconclusive.

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Chapter 5

Determination of bone mineralisation and computed tomography scan of tibia of 34 day old broilers fed diets supplemented with phytase

Abstract

A 34 day experiment was conducted to determine the effect of three different types (HiPhos, OptiPhos and Quantum blue) of phytase on broiler tibia mineralisation. Experimental diets were supplemented with phytase at standard levels and three times the standard level. Positive control (PC) diets were formulated based on the lower end of recommended NRC values. Negative control (NC) diets were formulated on the PC diets less the matrix value for HiPhos 2000FTU (HP200). A positive control diet and negative control diet were compounded for three phases; starter, grower and finisher. Therefore, eight diets were mixed: 1. Positive control (PC); 2. Negative control (NC); 3. NC + 2000FTU HiPhos (HP200); 4. NC + 1000FTU OptiPhos (OP400); 5. NC + 1000FTU Quantum blue (QB200); 6. NC + 6000FTU HiPhos (HP600); 7. NC + 3000FTU OptiPhos (OP1200) 8 .NC + 3000FTU Quantum blue (QB600). At the beginning of the trial five thousand one hundred and twenty day old broilers were allocated to a completely randomised design with eight treatment diets and eight replications. Sixty-four broilers were selected at day 34 and the tibiae were obtained for bone mineral, bone strength and computed tomography scan analysis. No treatment differences ($p > 0.05$) were observed for tibia length, tibia diameter, robusticity index, bone breaking strength, percent ash, percent phosphorus and percent calcium. Dry tibia weight, calcium to phosphorus ratio and the length of the tibia in relation to live weight differed ($p < 0.05$) between treatments. The computed tomography scans were a rapid tool that showed tibia structural abnormalities at day 34. Overall, phytase supplementation to negative control diets did not meet the calcium and phosphorus requirements for proper bone formation of broilers at day 34.

Keywords: bone ash, bone strength, CT scan, computer tomography scans

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5.1 Introduction

Genetic selection for fast growing broilers has a direct implication for bone development. A rapid increase in live weight may not occur at the same rate as bone development and could result in a loss of structural bone integrity. In addition, reduced levels of dietary phosphorus and calcium may lead to bone deformities and fractures in heavier birds. Phosphorus deficiency predisposes chicks to skeletal disorders such as tibial dyschondroplasia (Liem *et al.*, 2008). In addition, poor bone mineralisation may lead to osteodystrophy. Furthermore, lameness and deformities typically result in an increase in broken bones during transportation and processing (Driver *et al.*, 2006).

In order to measure bioavailable phosphorus, qualitative response criteria such as bone ash and strength are used. Bone ash has been used to determine the amount of phosphorus deposited in the bone. In addition, bone breaking force has been used as an indicator for strength. Bone breaking strength is the force that is applied per square area at the time of break (Turner & Burr, 1993).

The bone structure is continuously modelling and remodelling based on physical activity and load stress. When the cortical bone is lost due to an increase in load stress, fractures and broken bones are more likely to occur (Lanyon, 1993). A computed tomography scan shows a three dimension image of the bone structure. Apart from it being a very fast method which is non-destructive, bone density can be determined. The disadvantage of computed tomography is that it is expensive.

Most of the phosphorus found in broiler feed ingredients is bound in the form of phytate (O'Dell *et al.*, 1972). The supplementation of phytase to diets has led to improved broiler phosphorus utilization and reduced phosphorus excretion in manure (Jongbloed & Lenis, 1992). There are different types of phytase that are available commercially, derived mainly from *Escherichia coli* and *Aspergillus oryzae.*

However, information on the effect of phytase supplementation, especially at higher than recommended levels is not that abundant. Therefore, the objectives of the current study were to:

i. Investigate the effects of three different types of phytase at various inclusion levels on tibia ash, calcium and phosphorus content.
ii. Determine the effects of three different types of phytase at various inclusion levels on tibia bone breaking strength.

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iii. Use computer tomography scans as a tool for rapid measure in the detection of structural deformities in the tibia bone.

5.2 Materials and methods

Experimental procedure for animal housing system, diets and phytase inclusion rates are described in 3.2 and 3.2.1. Briefly, eight treatment diets consisting three different types of phytase were fed *ad libitum* to broilers for 34 days. The description of diets that were used for the entire duration of the trial are as shown in Table 3.1.

5.2.1 Data collection and experimental procedures

On day 34, Sixty- four Cobb 500 broilers were selected, one per pen, from the middle weight group. Slaughtering of broilers was done according to acceptable commercial standards through immobilization by electrical stunning then followed by exsanguinations. Thereafter, drumsticks were collected after cutting off the feet and by cutting at the joint of the tibia and femur. The skin and flesh of the drumstick was removed and cleaned tibiae were stored at -20°C until further analysis was performed. The tibia length and weight measurements were taken after thawing and removing of adhering muscle. All measurements were done after tibia brought to room temperature. The diaphysis diameter was obtained with an electronic digital calliper. The robusticity index was determined as the ratio of the bone length to the cube root of dry bone weight as described by Riesenfeld, (1972).

5.2.2 Bone ash and mineral determination of left tibiae

Ash and mineral determination was done as previously described in 3.2.2.1.2 and 3.2.2.1.3. The dry tibia and ash content determination on cleaned bones was according to the Association of Official Analytical Chemists International (AOAC, 2002) Official method 934.01 and Official method 942.05, respectively. Ash samples were then used for mineral analysis according to the Agricultural Laboratory Association of Southern Africa (ALASA), method 6.1.1.

Briefly, clean empty porcelain crucible were placed in an oven for 2h at 100°C to dry. The crucible for each sample was then placed in a desiccator and allowed to cool. The weight of the empty crucible was taken and the scale was tarred to measure the weight of the cleaned tibia. Secondly, bones were dried to obtain constant weight and the dry matter (dry bone) percentage was calculated. Thirdly, the tibiae were ashed in a muffle furnace for 24h at 600°C. The weight of the ash was obtained to determine the percentage ash after placing crucibles in a desiccator to cool off. The dry tibiae and ash percentage were then calculated as shown in Equation 3.1 and Equation 3.2.

5.2.3 Bone breaking strength test of right tibiae

The bone breaking strength was determined with a three-point destructive bending test using an Instron testing machine as described by Fleming *et al.*, (1998). The mid-point of each tibia was marked and the diameter at the mid-point was taken. The tibia was placed in between two 10mm diameter retaining bars, set 40mm apart. Thereafter, the 10mm diameter crosshead probe approached the tibia at 30mm/min until the tibia was broken. A connected and calibrated plotter showed a curve having a peak at bone breaking force in Newton.

5.2.4 Computed tomography Scan

The computed tomography scan uses collimated x-rays. Eight tibiae were exposed to x-rays one at a time and the absorbed radiation was measured on the opposite side. The procedure was then repeated from different angles around each tibia to obtain a three dimension reconstruction. The equipment used was a General Electric Phoenix V|Tome|X L240/NF180. X-ray settings were at 160 kV and 100 microA and 2000 images were acquired in a full rotation at image acquisition time of 500 µs per image, with no averaging and no skipping of images. Detector shift was activated to minimize ring artefacts. Background calibration was performed and the scan time was approximately 40mins per tibia scan. Reconstruction was done with system-supplied Datos reconstruction software. Analysis was performed with Volume Graphics VGStudio Max 2.1 software package.

5.2.5 Statistical analysis

One-way analysis of variance (ANOVA) was performed using the general linear model procedure of SAS enterprise guide 5.1. Treatment means were compared by least significant difference ($p < 0.05$). A *post hoc* test of least significant difference to was performed on treatment means using Bonferoni's test.

5.3 Results and discussion

5.3.1 Bones ash and minerals

The results for tibia ash, phosphorus and calcium are shown in Table 5.1. No treatment differences ($p > 0.05$) were observed for tibia ash %, phosphorus % and calcium %. Similarly, Kozłowski *et al.*, (2010) reported that calcium levels in the bone were not improved with phytase supplementation. However, bone ash and phosphorus amounts were improved for diets that contained 250FTU and 750FTU of OptiPhos (Kozłowski *et al.*, 2010).

In another study by Pillai *et al.*, (2006) containing low levels of available phosphorus (1.3g/kg) and calcium (8.8g/kg) with various levels of OptiPhos (250, 500, 1000, 2000, or 4000FTU), reducing the amount of inorganic phosphorus and calcium in the diet resulted in

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lower tibia ash levels. However, Pillai *et al*., (2006) noted that incremental increase in phytase levels resulted in a linear rise in tibia weight and ash. Powell *et al*., (2011) also reported that bone weight, ash weight and tibia ash percentage improved with phytase supplementation and with incremental calcium levels.

Contrary, to the current study, tibia ash was improved with supplementation of 750FTU of HiPhos (Martins *et al*., 2013). The difference in the results when compared to the current study, might be that diets reported by Martins *et al*., (2013) contained higher levels of phosphorus (4.0g/kg). Also, contradictory to the results of this study, a higher amount of tibia ash at day 18 was observed for broilers that were fed Quantum phytase at concentrations higher than the required levels (Karimi *et al*., 2013).

Furthermore, tibia ash, calcium and phosphorus levels were positively influenced by an increase in Quantum blue phytase and dietary calcium that was more than 4.5g/kg (Walk *et al*., 2012). In addition, birds fed diets that contained limestone at 9.0g/kg had tibias that weighed more than those birds fed diets that contained high soluble calcium at 9.0g/kg (Walk *et al*., 2012). Contrary to the current study, Singh *et al*., (2013) reported that the addition of 500FTU of Quantum blue to deficient diets led to a rise in tibia calcium at day 42.

In the current study, there were no differences ($p > 0.05$) between treatment diets for both left and right tibiae length and diameter. However a 42 day experiment that contained 500FTU of Quantum blue phytase observed a positive influence on tibia length (Singh *et al*., 2013). Furthermore, determined ratios such as the robusticity index and mass of bone to live weight did not differ between diets ($p > 0.05$). In the current study, the dry tibia weight and the tibia calcium to phosphorus ratio differed ($p < 0.05$) between diets. Similar to the study by Powell *et al*., (2011), the PC treatment group in the current study had the highest tibia calcium to phosphorus ratio.

5.3.2 Bone strength

The bone breaking force in Newton per square meter did not differ ($p > 0.05$) between treatments diets. A study that contained diets with 2.0g/kg non-phytate phosphorus and three calcium levels (6.7g/kg, 3.3g/kg and 0.66g/kg) resulted in an improvement in bone strength (Powell *et al*., 2011). The authors also reported that the inclusion of 500 FTU OptiPhos to diets had a positive effect on bone breaking strength.

Table 5.1 Mean values (± Standard deviation, SD) of day 34 tibiae bone ash, phosphorus, calcium, length, diameter, Robusticity index, breaking force in Newton and Pascal's of broilers raised from day 0 to 34 and fed different phytase treatments

Phytase	-	-	OP400	QB200	HP200	HP600	OP1200	QB600	P-value
Diet	Positive Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	Negative Control	
Left tibia									
Dry tibia weight (g)	$4.14^{a} \pm 0.56$	$3.09^{b} \pm 0.53$	$3.36^{b} \pm 0.38$	$3.17^{b} \pm 0.42$	$3.25^{b} \pm 0.42$	$3.46^{ab} \pm 0.34$	$3.19^{b} \pm 0.44$	$3.48^{ab} \pm 0.31$	0.0001
Ash %	41.86 ± 2.89	38.78 ± 5.25	37.59 ± 2.67	38.05 ± 2.57	40.22 ± 2.61	39.82 ± 2.67	41.50 ± 5.28	38.80 ± 2.06	0.148
Phosphorus %	23.34 ± 0.75	23.32 ± 0.78	23.11 ± 1.63	23.66 ± 0.77	22.89 ± 0.86	23.46 ± 1.48	22.85 ± 0.87	23.36 ± 0.95	0.797
Calcium %	48.10 ± 2.66	46.00 ± 3.08	45.25 ± 3.26	46.15 ± 2.01	44.80 ± 2.05	46.85 ± 4.54	44.74 ± 1.37	46.75 ± 3.42	0.337
Calcium: Phosphorus	$2.05^{a} \pm 0.01$	$1.94^{b} \pm 0.01$	$1.95^{b} \pm 0.03$	$1.93^{b} \pm 0.02$	$1.95^{b} \pm 0.04$	$1.99^{ab} \pm 0.05$	$1.92^{b} \pm 0.04$	$1.99^{ab} \pm 0.04$	<0.0001
Bone length (cm)	8.87 ± 0.19	8.41 ± 0.43	8.60 ± 0.13	8.58 ± 0.44	8.49 ± 0.63	8.36 ± 0.58	8.65 ± 0.33	8.50 ± 0.24	0.284
Bone diameter (mm)	6.46 ± 0.43	6.64 ± 0.54	6.42 ± 0.52	6.13 ± 0.39	6.47 ± 0.72	6.54 ± 0.69	6.31 ± 0.34	6.38 ± 0.28	0.649
Robusticity index	5.55 ± 0.26	5.69 ± 0.33	5.75 ± 0.24	5.81 ± 0.14	5.74 ± 0.23	5.54 ± 0.43	5.89 ± 0.14	5.62 ± 0.18	0.098
Right tibia									
Bone length (mm)	88.94 ± 1.83	84.52 ± 2.92	86.20 ± 1.20	84.70 ± 4.09	84.33 ± 5.92	85.88 ± 3.16	87.89 ± 2.49	86.38 ± 2.13	0.185
Bone diameter (mm)	6.75 ± 0.43	6.42 ± 0.54	6.66 ± 0.27	6.55 ± 0.88	6.28 ± 0.53	6.68 ± 0.69	6.24 ± 0.35	6.41 ± 0.32	0.580
Mega Pascal(N/mm^2)	71.72 ± 15.21	57.91 ± 17.02	59.46 ± 19.82	58.33 ± 21.51	63.80 ± 8.94	65.53 ± 18.62	62.73 ± 15.73	63.88 ± 10.43	0.857

^{a,b} Means within rows with different superscripts differ significantly ($p<0.05$)

OP400 & OP1200 – OptiPhos, QB200 & QB600 – Quantum blue, HP200 & HP600 – HiPhos

5.3.3 Computed tomography scan

Figure 5.1 shows scans of the whole tibia bone for different dietary treatments. A vertical slice view of the metaphysis is shown in Figure 5.2 and a horizontal slice view of the region between the tip of the tibia and the diaphysis is shown in Figure 5.3. Diaphysis horizontal sections are shown in Figure 5.4 and

Figure 5.5.

As can be seen in Figure 5.1, prominent bending that might be an indication of rickets is shown in a tibia collected from the HP600 group (double arrowed line). An enlarged metaphysis and poor bone modelling, an indication of calcium rickets, are observed in tibia from treatment groups QB200, QB600, OP400, OP1200 and PC (single arrowed line in Figure 5.1). In the case of phosphorus rickets, poor bone modelling is a rare occurrence. Poor mineralisation due to an imbalanced dietary calcium to phosphorus ratio or the deficiency of either calcium or phosphorus encourages the occurrence of rickets.

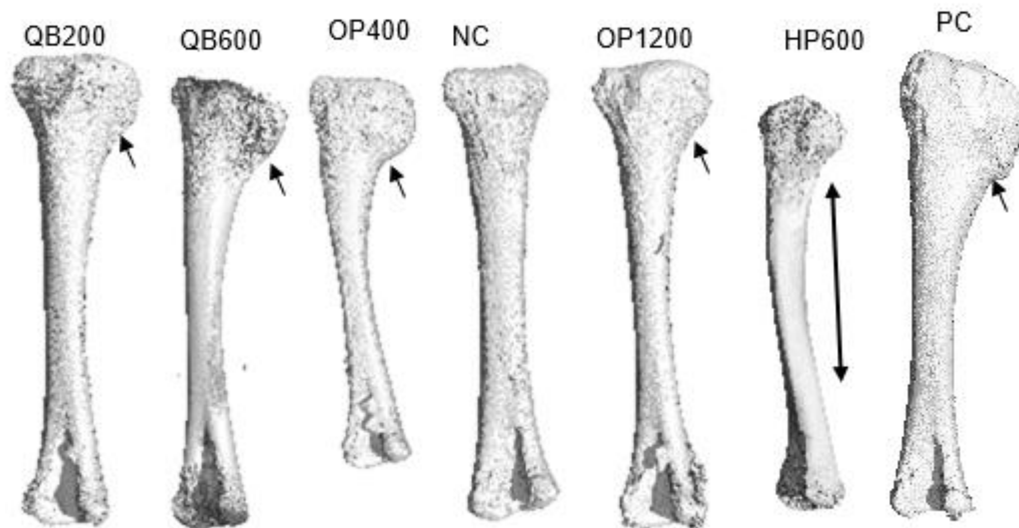

Figure 5.1 Whole tibia scan of broilers fed diets with different types phytase for 34 days

In the current study the growth plate will be termed as the physis. The metaphysis will be the region proximal to the physis while the diaphysis will be the midpoint of the tibia bone. The physis has four regions, namely the cartilage zone, zone of proliferation, pre-hypertrophic zone and the zone of hypertrophy.

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A vertical slice view of the metaphysis is shown in Figure 5.2. Abnormal cartilage formation in the proximal end of the tibia is present in the region marked A due to poor bone mineralisation. The physis zones, marked B and C, are irregular, enlarged and poorly formed. The physis region is not clearly distinct due to a lack of calcification. The bone marrow marked D fails to extend to the right side of the bone due to poor blood vessel formation. Riddell (1975) describes morphometric abnormalities that are similar to the current study which are indicative of tibial dyschondroplasia (TD). Figure 5.3 shows a horizontal slice view of the proximal end of the tibia from treatment group PC. Similar to observations noted in Figure 5.2 morphology abnormalities are prominent in regions A, B and C as shown in Figure 5.3

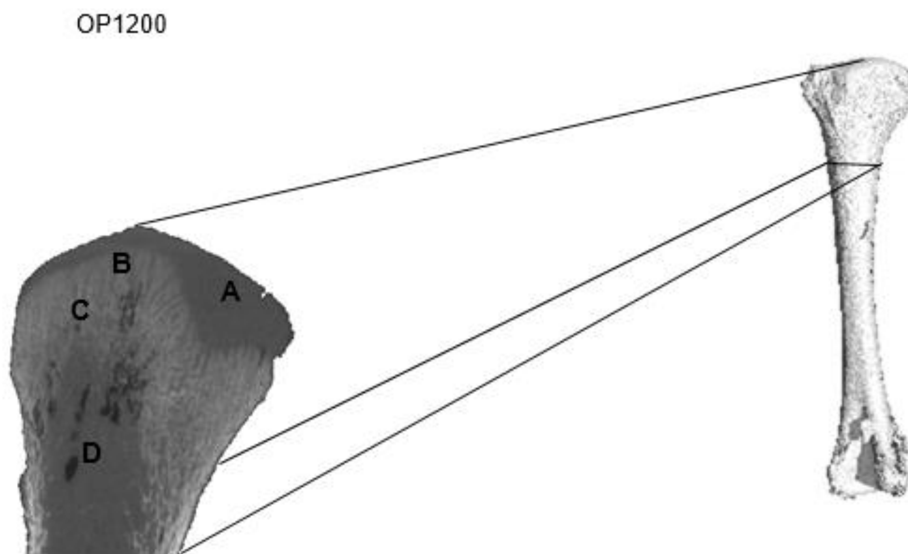

Figure 5.2 Vertical slice view of tibia of of broilers fed diets with different types phytase for 34 days. Abnormal development of areas marked A,B,C and D.

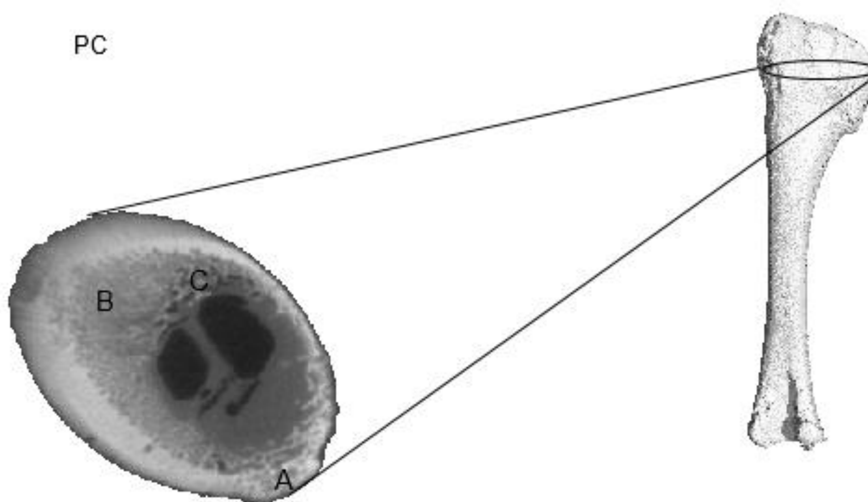

Figure 5.3 Horizontal slice view of tibia of broilers fed diets with different types phytase for 34 days. Regions marked A, B and C are irregularly developed.

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For the tibia that are shown in Figure 5.4 , cortical bone thickness is irregular as shown in the location labelled A. Some areas are thinner than others due to uneven mineral deposition. An assumption may be made that increased mineral deposition is prominent in the regions that bear the weight of the bird. Williams *et al.*, (2004) reported that there was a marked thickening on the concave side of the bone due to reduced mineral resorption from the osteoclasts. Fibrous portions are observed in the bone marrow area B at midpoint sections of the tibiae in

Figure 5.5. Furthermore, Williams *et al.*, (2004) observed that the growth rate for broilers selected for increased performance contributed to reduced mineralisation and higher cortical porosity.

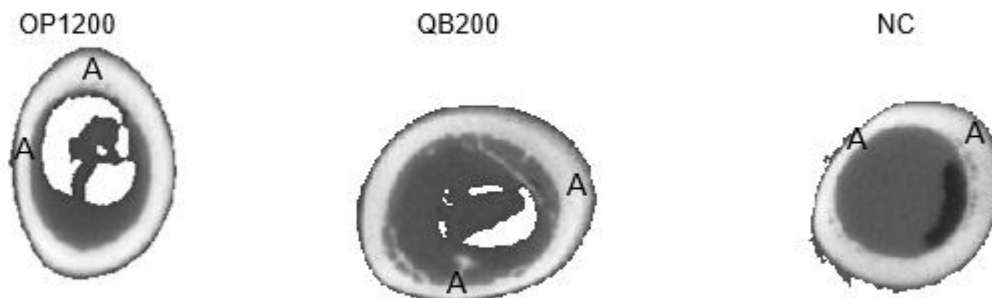

Figure 5.4 Diaphysis horizontal cross-section view of tibia of broilers fed different dietary treatments for 34 days. Area marked A is the cortical region (unevenly distributed)

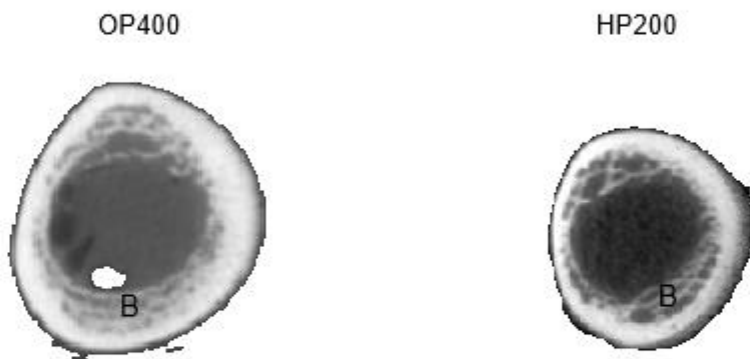

Figure 5.5 Diaphysis horizontal cross-sections of tibia of broilers fed different dietary treatments for 34 days. The region marked B shows fibrous extensions in the cortical area.

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5.4 Conclusion

The current study determined the effects of three different types of phytase on bone mineralisation. A computed tomography scan was performed to evaluate bone development at day 34 in selected tibia.

Phytase supplementation did not improve the tibia ash percentage, phosphorus and calcium levels and the bone breaking strength. The tibia calcium to phosphorus ratio was numerically higher in the HP600 and QB600 dietary treatments. The numerical increase in the ratio did not significantly differ from the NC group. The computed tomography scan (CT scan) was used as an observational tool that evaluated the influence of phytase supplementation on leg disorders.

The results in the current study showed that phytase was not able to release adequate phosphorus for proper bone development. Therefore, a conclusion might be drawn that the calcium to phosphorus ratio in the dietary treatments were insufficient to meet the nutritional requirements of the broilers at day 34.

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Chapter 6

General conclusion

The present study was performed to investigate the effects of three different types of commercial phytases that are available in South Africa on broiler performance. Outcomes of the study were determined on production performance, carcass characteristics, organ weights, bone mineralisation, intestinal pH and intestinal morphology of commercially raised broilers. In addition, the potential innovation of inclusion level of phytase that are three times higher than the recommended doses were evaluated.

Phytase supplementation improved live weight performance at day 34. A higher dose of Quantum blue resulted in improved live weight. However, neither standard dose nor high dose of both HiPhos and OptiPhos resulted in differences in live weight. The feed conversion ratio was not improved at both standard and high dosage levels. Average daily gains were improved with dietary phytase. Higher doses of both OptiPhos and Quantum blue led to an increase in ADG as compared to standard doses of these two phytases. However, in the case of HiPhos, both standard and high doses performed equally. Phytase supplementation did not have an influence on the PER, but increased the EPEF, with both standard and high doses being comparable. The effects of phytase on mortality are inconclusive as liveability outcomes were comparable between treatment groups. At the end of the study carcass weights were not improved by dietary phytase. In addition, the influence of phytase on commercial portions was inconclusive. Furthermore, both standard and high doses of phytase did not negatively influence muscle colour measurements, pH and temperature readings. Therefore, an assumption may be made that these muscle post mortem characteristics were in accordance with meat of acceptable standard. The pH of the intestinal segments and the weight of internal organs were neither influenced by type nor dosage of phytase. In addition, the morphometric differences in intestinal segments could not be entirely attributed to dietary phytase. The bone parameters that were measured were not influenced by phytase supplementation. Even though treatment differences were observed for the bone calcium to phosphorus ratio, these improvements were observed for the PC group. The dietary calcium to phosphorus ratio might have had an influence on the outcome of the results of this study.

The current study has demonstrated that dietary phytase supplemented at both standard and high doses could not achieve optimal broiler performance. Generally, the three different types of phytases were comparable in performance for most performance response criteria.

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The limitation of the study was that the calculated nutrient composition of diets was based on existing nutrient content values of ingredients. Therefore, wet chemistry and NIR values could not completely verify the calculated nutrient values of the diets. In this case, a recommendation would be that the nutrient content of ingredients be determined prior to feed formulation. Furthermore, a suggestion that an investigation on higher inclusion rates of phytase on actual phosphorus and calcium NRC recommended values is performed.