The effect of different levels of phytase and available phosphorus on the performance and egg quality in layers

By

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I declare that this thesis, for the degree
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CHAPTER 1 – LITERATURE REVIEW

1.1 INTRODUCTION

Less than one third of the phosphorus (P) contained in feed ingredients of plant origin is biologically available to monogastric animals (NRC, 1994). The remainder of the phosphorus is tied up as phytate (phytic acid or myo-inositol hexaphosphate), and monogastrics as a general rule lack the enzyme(s) necessary to hydrolyse phytate down to phosphorus and inositol (Li *et al.*, 2000). Dietary addition of microbial phytase or the inclusion of high phytase ingredients in poultry diets are now well documented to release a large portion of the naturally occuring phytate phosphorus (P) and thus greatly reduce the amount of inorganic phosphorus that must be added to meet the animal's requirement. The net result is a reduction in phosphorus excretion that can range from 20 - 50%. Microbial phytase was initially used as a tool to reduce phosphorus because modern commercial production of poultry has led to large amounts of manure, that when applied to land in excess results in accumulation of nutrients in and on the soil. This potential for environmental pollution continues to lead to legislation in many countries requiring nutrient management plans for manure (Kornegay, 1999).

It is becoming increasingly clear that the use of adequate amounts of phytase in poultry diets results in improved availability of calcium, zinc, protein/amino acids and energy. This is because seeds or products from seeds, which are major ingredients in poultry diets, contain 60 – 80% of the phosphorus in the form of phytic acid or phytate (Kornegay, 1999). Phytate is known to complex with other nutrients. Thus the unavailable phytate phosphorus and nutrients complexed with it cannot be utilized and are excreted. This chapter will provide a short review of phytate, phytase and the effectiveness of microbial phytase in poultry diets for enhancing the utilization of phosphorus, calcium, zinc, amino acids/protein and energy so as to reduce nutrient excretion. Factors that influence phytase activity will be briefly discussed.

1.2 PHYTATE AND BIO-AVAILABILITY OF NUTRIENTS

Major ingredients in commercial poultry diets are seeds (cereal grains) or products from seeds (oilseed meal and grain by-products). A large portion (60 – 80%) of the phosphorus in these ingredients occurs in the form of phytates, the salts of phytic acid (Table 1.1.).

Table 1.1.Phytate phosphorus (P) content and phytase activity of some common feed ingredients.

Ingredient	**Phytate P (a) (g/kg)**	**Phytate P (a) (% of total P)**	**Phytase activity (units/kg) (b)**
Cereals and by-products			
Corn	2.4	72	15
Wheat	2.7	69	1193
Sorghum	2.4	66	24
Barley	2.7	64	582
Oats	2.9	67	40
Wheat bran	9.2	71	2957
Oilseed meals			
Soybean meal	3.9	60	8
Canola meal	7.0	59	16
Sunflower meal	8.9	77	60
Peanut meal	4.8	80	3
Cottonseed meal	8.4	70	NA

(a) Data adapted from Ravindran (1996) and Ravindran *et al.* (1994, 1995).

(b) Data from Eeckhout and De Paepe (1994). One unit is defined as that amount of phytase that liberates inorganic phosphorus from a 5.1 mM Na-phytate solution at a rate of 1 µmol/min at pH 5.5 and 37 degrees Celcius.

Detailed information on the phytic acid content of various foods and feedstuffs can be found in reviews by Eeckhout and De Paepe (1994), and Ravindran *et al.* (1995). The bio-availability of phytate phosphorus is generally very low for poultry because they have limited capability to utilise phytate phosphorus (Table 1.2.).

Table 1.2. Bio-availability of non-phytate phosphorus for poultry.

Feedstuff	**Nonphytate-P for poultry (% of total)**
Cereal grains	
Corn	28
Oats	33
Barley	36
Triticale	33
Wheat	31
High protein meals-plant origin	
Peanut meal	21
Canola meal	26
Soybean meal, dehulled	35
Soybean meal, 44% protein	40

NRC (1994).

Bio-availability estimates of phosphorus in corn and soybean meal for poultry range from 10 – 40% (Cromwell, 1992 as quoted by Kornegay, 1999). The phytic acid molecule has a high phosphorus content (28.2%) and large chelating potential. Phytic acid can form a wide variety of insoluble salts with di- and trivalent cations such as calcium, zinc, copper, cobalt, manganese, iron and magnesium at neutral pH (Pallauf and Rimbach, 1996). This binding potential renders these minerals unavailable for intestinal absorption.

Zinc may be the trace mineral whose bio-availability is most influenced by phytate (Pallauf and Rimbach, 1996). Phytic acid may have a negative influence on dietary protein and amino acids, with addition of bacterial phytase to a range of feed ingredients it shows a significant improvement in protein and amino acid digestibility (Ravindan *et al.*, 1999).

Phytate-protein or phytate-mineral-protein complexes may reduce the utilization of protein (Knuckles *et al.*, 1985). Starch is also known to be complexed by phytate. The *in vitro* hydrolysis of either wheat or bean starch incubated with human saliva was retarded when sodium phytate was incubated in the mixture, but digestion was restored when calcium was added with the sodium phytate (Thompson *et al.*, 1987 as quoted by Kornegay,1999). The low availability of phytate phosphorus poses two problems for producers:

1) The need to supplement inorganic phosphorus and add higher levels of other nutrients to the diet to ensure that the animals needs are met; and
2) The excretion of large amounts of phosphorus and other nutrients in the manure.

1.3 PHYTASES

Phytases are known to occur widely in micro-organisms, plants and certain animal tissues (Nys *et al.*, 1999). Phytase of microbial origin (3-phytase, E.C.3.1.3.8) hydrolyses the phosphate at the C3 position first, whereas phytase of plant origin (6-phytase, E.C.3.1.3.26) acts first at the C6 position (Pallauf and Rimbach, 1997).

Phytase produced by *Aspergillus* has two pH optima: One at pH 2.5 and the other at pH 5.5. Wheat phytase has only one pH optimum at pH 5.2 (Kornegay, 1999). *Aspergillus* phytase has been shown to be more effective per unit of activity than wheat phytase, probably due to the above-mentioned differences (Eeckhout and De Paepe, 1996). At least three abbreviations are used in the literature for phytase activity: FTU, PU(phytase units/kg) and U(Units/kg).
One unit of phytase activity (FTU) is defined as the quantity of enzyme that liberates 1micromol of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at pH 5.5 and 37 degree celcius.

As the monogastric organism contains no or only negligible amounts of endogenous phytate in the stomach and small intestine, it is therefore dependent on plant or microbial phytase (Pallauf and Rimbach, 1997).

The significance of endogenous phytase and phytase produced by exogenous micro-organisms and resident bacteria is probably negligible.

It has been known for more than 50 years that plant phytase has the ability to hydrolyse phytate, and its effectiveness for improving phosphorus digestibility in poultry has been clearly shown (Qian *et al.*, 1997).

Phytase activity has been reported in a wide range of seeds such as rice, wheat, barley, corn, soybean and oilseeds (Eeckhout and De Paepe, 1994); however phytase activity of seeds varies greatly among species of plants (Table 1.1). With the exception of wheat, rye and triticale, most dormant seeds contain very low phytase activity (Kornegay, 1999). Phytase acivity in corn and soybean meal is so low that it is not of practical importance.

Microbial phytases are found in numerous bacteria, yeast and fungi (Harland and Morris, 1995), but *Aspergillus*, a genus of Ascomycetous fungi, is probably the one most widely used (Irving and Cosgrove, 1974). Research done in the late 1960's and early 1970's by Nelson *et al.* (1971) showed that microbial phytase was effective in improving phytate phosphorus availability for chickens. The cost of adding the enzyme was very high and the lack of environmental pressure to reduce phosphorus excretion delayed interest in commercial application until the late 1980's. The development of commercial phytase that could be economically used in poultry diets was probably a result of advancements in biotechnology that led to genetically modifying fungi, and /or advances in fermentation technology.

In some European countries there are now economic incentives to reduce manure phosphorus loading, and this has stimulated interest in phytase enzyme usage (Leeson and Summers, 1997).
Although some feed ingredients contain native phytase activity, steam pelleting used in the manufacture of many commercial poultry feeds results in significant losses of this intrinsic phytase activity.

Because of variation in phytase activity among and within plant species, damaging effects of pelleting during feed manufacturing, and the lack of availability of feed ingredients of high phytase activity, the presence of residual phytase activity often may not be considered in diet formulation when feeds are pelleted (Kornegay, 1999).
The phytase supplement used until now has been predominantly from the mould *Aspergillus ficuum* var. niger, now called *A. niger*.

Recent developments indicate an improvement in the potency and perhaps the convenience of using the phytase enzyme preparation. Sun, Patterson, Woloshuk and Muir, (1997) reported application of molecular biology to affect the transfer of the gene for the phytase, myo-inositol hexakisphosphate phosphohydrolase, from *A. niger* to the yeast, *Saccharomyces cerevisiae*. By measuring the amount of inorganic P released from sodium phytate *in vitro* a 4-to-11-fold increase in the activity of the transformed yeast over that of the control yeast was estimated. It was suggested that about 20g/kg of the recombinant yeast (dry matter basis) will be required to provide phytase supplementation in poultry feeds.

1.4 EFFECTIVENESS OF MICROBIAL PHYTASE IN POULTRY

1.4.1 Ability of phytase to improve phosphorus bio-availability

Supplemental microbial phytase is well known for its effectiveness in improving phosphorus availability from plant ingredients containing high levels of phytate phosphorus. Scott *et al.* (1999) carried out a study to determine the effect of phosphorus and phytase on the performance of layers fed corn-based diets. The author concluded that the main effects of phosphorus level and phytase supplementation were significant only in the last period of production. Before this time, available phosphorus levels were 0.2 and 0.4% of the diet, and were likely close to being sufficient for maximal production at either level (NRC, 1994).

Boling *et al.* (2000) concluded in his study of the effects of dietary available phosphorus levels and phytase on the performance of young and older laying hens, that the rapid onset of P-deficiency symptoms in the 70 week old hens used in that trial suggests that older hens may be more sensitive to dietary P deficiency than younger birds early in the production cycle.

When available phosphorus levels were reduced to 0.11 and 0.22% after week 55 of production, the lower level of available phosphorus was associated with decreased body weight and egg production, and enzyme supplementation was associated with increased body weight, egg production and improved feed conversion ratio. Clearly the lower level of available phosphorus in the final period was inadequate, and the release of phosphorus by enzyme supplementation was able to compensate for this.

This study demonstrated that phytase enzyme can compensate for reduced available phosphorus levels in layer diets, but it provides an indication that the optimal levels of available phosphorus and enzyme are not the maximum.

Rama Rao *et al.* (1999) concluded in an experiment done to determine the enhancement of phytate phosphorus availability in the diets of commercial layers, that the improved performance of layers fed phytase supplemented diet may be due to increased phosphorus retention.

It thus seems that phytase has the ability to improve phosphorus bio-availability.

1.4.2 Excretion of phosphorus

Keshavarz (2000) conducted an experiment to re-evaluate the non-phytate phosphorus requirement of growing pullets with and without phytase. The information generated from the digestion trials generally indicated that the potential exists for reducing the daily total phosphorus excretion of growing pullets by providing them with diets containing lower non-phytate phosphorus levels than the NRC (1994) recommendation, without comprising performance during the growing period.

Leske and Coon (1999) conducted a bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and layers. Total phosphorus retention for corn were 36.8% and soybean meal 28.6% in this study.

The addition of phytase to the diets of broilers and laying hens significantly increased phytate phosphorus hydrolysis and total phosphorus retention and can be used as a tool to more efficiently provide the birds with their phosphorus needs and reduce excreta phosphorus.

1.4.3 Digested phosphorus vs phosphorus equivalency value of phytase

Phosphorus equivalency value, a term used to describe the replacement or substitution value of phytase, is defined as the amount of inorganic phosphorus that can be removed by a given amount of added or intrinsic phytase (Kornegay, 1999).

If one is to directly compare equivalency value of phytase for phosphorus and digested phosphorus, then equivalency values must be adjusted by the estimated digestibility of the inorganic phosphorus sources that phytase replaces. The retention of phosphorus from several feed grade sources was estimated to be 46.2% for broilers and turkeys (Kornegay, 1999).

Equivalency values (or equations) are usually obtained from non-linear or linear equations generated from body weight gain, bone mineralization and, occasionally, digested phosphorus data obtained by feeding multiple levels of phosphorus without phytase addition and multiple levels of added phytase to a low phosphorus diet. These equations are set equal to one another and solved (Kornegay, 1999). This procedure was described in detail by Denbow *et al.* (1995). In poultry some data suggest that 600 U phytase/kg is equivalent to 1.0g of inorganic phosphorus. The average phosphorus equivalency of 1.0g phosphorus is multiplied by 46.2% (0.462g phosphorus retained per 1g of inorganic phosphorus). The product is 0.462g phosphorus (1.0 * 0.462).

A value of 0.42g phosphorus (0.042%) was obtained for poultry at 600 U phytase/kg (Kornegay, 1999). Equivalency values must be adjusted by the apparent digestibility of the inorganic phosphorus source being replaced.

Dendow *et al.* (1995) conducted a study to determine phosphorus equivalency values of phytase for inorganic phosphorus. The phosphorus equivalency of 250, 500, 750 and 1000 U phytase was 0.56, 0.84, 0.97 and 1.05g, respectively; 821 U of phytase would be equivalent to 1g of phosphorus.

Thirty-one to fifty-eight percent of phytate phosphorus in soybean meal would be released. Based on the similarity of digested phosphorus values calculated from equivalency estimates, and values derived from equations generated in poultry data sets, the estimates of phosphorus excretion should be accurate for a range of situations.

1.4.4 Response of layers to phytase supplementation

In a series of trials reported by Van der Klis *et al.* (1997) and Gordon and Roland (1997), phytase supplementation of a low phosphorus diet for layers was very effective as a replacement for inorganic phosphorus. A range of phosphorus equivalencies, 0.5-1.2g phosphorus as monocalcium phosphate, has been reported for 200-300 U phytase/kg. Van der Klis *et al.* (1997) reported that the effect of phytase supplementation (250 and 500 U/kg) on ileal phosphorus absorption was 12% greater when added to a low phosphorus basal diet containing 3.0% calcium compared with a low phosphorus basal diet containing 4.0% calcium.

Leske and Coon (1998) reported that phytate phosphorus retention was 36.7, 29.0 and 14.8% greater with phytase supplementation (300 U phytase/kg), respectively for soybean meal, corn and rice bran. But total phosphorus retention for the same products was only 16.6, 16.1 and 7.1% units greater. Phytase supplementation is very effective at releasing phosphorus in layer diets which results in reduced dietary phosphorus levels and reduced phosphorus excretion. The efficiency appears to be greater for layers than broilers and turkeys. Phytase supplementation of layer diets is also simplified because most diets are fed in a mash form.

1.4.5 Heat stability of phytase

Acceptability of phytase by the feed industry will depend on cost, product stability, consistency of results and ease of application. The fact that mash feed are often used, might raise some controversy over phytase stability during pelleting. Jongbloed and Kemme (1990) reported reduction of phosphorus availability by pelleting of feeds containing phytase activity from wheat. Acitivity loss occurred at temperatures approaching 80 degree celcius. Simons *et al.* (1990) found *A. ficuum* phytase to retain 96% of its activity after pelleting with a conditioning temperature of 50 degree celcius and pellet temperature of 78°C (Table 1.3). Because pelleting temperature may vary from 65-85°C or more depending on conditions, further investigation of the effects of heat on phytase activity is warranted. Enzyme addition after the pellet mill will be one alternative approach to phytase application. Another approach would be to use phytase during processing of high phytase ingredients such as rice bran, cotton seed meal, and soybean meal.

Table 1.3 Effect of pelleting on phytase activity

Temperature before pelleting (°C)	Temperature after pelleting(°C)	Phytase activity (units/kg feed)	Activity remaining (%)
50	78	240	96
50	81	234	94
60	84	208	83
60	87	115	46

Activity before pelleting = 250 units/kg

Simons *et al.*, 1990.

Processors would need to keep processing conditions under close control and monitor the final product for phytic acid and total phosphorus concentrations.

1.4.6 Effects of phytase on calcium bio-availability

Schoner *et al.* (1991, 1993) reported improved calcium retention in broilers fed supplemented phytase. Calcium retention and dry matter digestibility were improved when phytase was added to broiler diets (Kornegay *et al.*, 1996; Yi *et al.*, 1996). Qian *et al.* (1996, 1997) reported that both phosphorus and calcium retention was sensitive to the addition of phytase at varying nonphytate levels or Ca: total phosphorus ratios.

Calcium retention increased linearly as the amount of supplemented phytase increased, and decreased as the Ca: total phosphorus ratios became wider and as the level of phosphorus increased.

1.4.7 Influence of phytase on zinc bio-availability

Using chicks fed a glucose-soy concentrate diet (13mg Zn/kg) with multiple levels of added zinc, phytase or 1, 25-dihydroxycholecalciferol (di-OH-D3). Biehl *et al.* (1995) reported that both phytase and di-OH-D3 supplementation increased growth rate and tibial zinc to a similar extent. Based on Biehl *et al.* (1995) estimates using tibia zinc, the zinc equivalency of 600 and 1200 U phytase/kg was 3.8 and 5.5mg respectively. In contrast, Roberson and Edwards (1994) did not observe a constant improvement in zinc absorption or retention in broilers when 600 to 750 U phytase/kg was added to a corn-soybean diet containing 32mg Zn/kg. Day old male broilers were fed a corn-soy isolate basal diet containing 20mg Zn/kg alone and supplemented with multiple levels of zinc and phytase for 21 days (Yi *et al.*, 1996). Non-linear or linear response equations of the effects of zinc and phytase levels were generated for body weight gain, feed intake, zinc retention, zinc concentration of toes, tibia, and liver, and were used to calculate an average zinc equivalency of 5.4mg Zn/kg for 600 U phytase/kg.

1.4.8 Influence of microbial phytase on amino acids and nitrogen bio-availability

Phytate can bind with protein/amino acids (AA) at low or neutral pH (De Rham and Jost, 1979). Phytate-protein/AA complexes may occur in foodstuffs in the native state and may be formed in the upper gastro-intestinal tract. Complexing of phytate with proteolytic enzymes may also occur in the upper gastro-intestinal tract.

These potential phytate protein complexes thus may reduce the utilisation of proteins and amino acids. If phytate is hydrolyzed, then its inhibitory effects are reduced. Most of the early research with microbial phytase was conducted to measure the effects of phytase on phosphorus utilisation, and when total tract nitrogen digestibility was measured results were inconsistent. Amino acid digestibility was rarely evaluated.

The opportunity to show improvements in protein/AA utilisation is influenced by the dietary level of protein/AA (Kornegay, 1999). If the protein/AA retention is at a maximum, then the potential to show an improvement is greatly reduced.

Furthermore, the use of total tract (fecal) digestibility may not be reliable because of the influence of the microbial population in the large intestine. Ileal digestibility is a more appropriate method of evaluating the influence of phytase on protein/AA utilisation. Ravindran *et al.* (1999) reported that dietary addition of phytic acid as rice pollard reduced ileal digestibility of nitrogen and amino acids (lysine, threonine, isoleucine, leucine, valine, phenylalanine and histidine). These adverse effects of phytic acid were effectively overcome by supplemented phytase.

Apparent nitrogen retention by broilers was improved when phytase was added to 23% crude protein corn-soybean meal diet (Kornegay *et al.*, 1996). Yi *et al.* (1996) using Large White turkey female poults fed a corn-soybean meal diet, reported that apparent and true ileal digestibilities of nitrogen and amino acids were generally improved when 750 U phytase/kg was added to both 22.5 and 28% crude protein diets containing 0.45% non-phytate phosphorus. Improvements however were not observed for birds fed 28.0% crude protein and 0.60% non-phytate phosphorus. Kornegay *et al.* (1998) reported that when dietary protein/AA levels were reduced from 95 to 86% of the NRC (1994) recommendation (1.5 to 2.0% units of crude protein), additions of 300-450 U phytase/kg of diet prevented the decrease in performance (slightly lower), breast meat yield, and ileal crude protein/AA digestibilities observed.

Supplemental phytase (1200 U/kg) was reported by Ravindran *et al.* (1999) to improve ileal digestibilities of protein/AA of three cereals (corn, sorghum and wheat), for oilseed meals (soybean meal, canola meal, cottonseed meal and sunflower meal), and two cereal by-products (wheat middlings and rice polishings). The magnitude of the response varied among feedstuffs and individual AA (Table 1.4). Changes in apparent ileal digestibility (percentage units) of amino acids in several ingredient combinations as influenced by phytase supplementation in broilers. (a,b)

Table 1.4 Changes in apparent ileal digestibility (percentage units) of amino acids in several ingredient combinations as influenced by phytase supplementation in broilers (a, b).The values are given in percentage units.

Amino Acids	Corn	Sorghum	Wheat	Soybean meal	Canola	Cotton Seed meal	Sunflower meal
Arginine	3.0	5.0	7.8	2.0	1.6	2.5	1.7
Histidine	2.4	4.9	7.4	3.5	1.7	2.9	3.9
Isoleucine	2.1	4.5	5.4	2.8	2.4	4.6	3.5
Leucine	0.9	3.3	5.3	2.1	2.2	4.0	3.5
Lysine	2.5	4.7	7.8	3.4	0.7	2.7	2.4
Phenylalanine	1.7	5.2	5.3	3.0	2.1	3.1	3.6
Threonine	4.4	4.5	10.4	5.7	2.9	4.3	4.5
Valine	3.1	4.9	6.8	2.8	2.6	3.9	3.7

a. Phytase added at 1200 U/kg

b. Ravindran *et al.* (1999).

1.4.9 Influence of microbial phytase on energy metabolism

Thompson and Coon (1984) indicated that in the native state phytate could complex with starch. Rajas and Scott (1969) reported that the apparent metabolizable energy (AME) of cottonseed meal and soybean meal for chicks was improved following treatment of the meals with a crude phytase preparation from *Aspergillus ficuum*.

Later Miles and Nelson (1974), using chicks, reported improvements in the AME value of cottonseed meal when treated with a phytase preparation. Small but significant improvements in AME were observed for broilers fed sorghum-soybean meal based diets (Farrel *et al.*, 1993).

Findings from a recent study by Ravindran ***et al.*** (1999), designed to determine the influence of microbial phytase on protein/AA and energy utilization in poultry clearly show that supplemental phytase improves the AME value of wheat and sorghum based poultry diets.

Namkung and Leeson (1999) did an experiment to investigate the effect of phytase on dietary metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks fed diets with lower than normal levels of Ca and P. The diet with supplemental phytase had a higher (+1%) AMEn (nitogen corrected available metabolizable energy) ($P \leq 0.01$) compared with the control diet. They concluded that addition of phytase (1149 FTU/kg) to chick starter diets improves diet AMEn and the digestibilities of total amino acid, non-essential amino acids, Val and Ile and essential amino acids, while replacing some Ca and available phosphorus in the diet.

Ravindran and Bryden (1997) as quoted by Kornegay (1999) reported that phytase increased AME by 3.5% from 3,109 kcal/kg to 3,217 kcal/kg on a Dry Matter (DM) basis. Biehl and Baker (1997) reported that dietary phytase did not affect TMEn (nitogen corrected total metabolizable energy) values (2,388 kcal/kg for control, 2,381 kcal/kg for 600 FTU/kg, and 2,416 kcal/kg for 1,200 FTU/kg).

Cabahug *et al.* (1999) did a study to examine the response of broilers to microbial phytase added to wheat-sorghum-soyabean meal diets. They found that improved body weight gains attributable to the supplemental phytase were associated not only with increased food intake, but also with better food efficiency. Food gain in birds fed on low phytic acid diets were lowered by 1.0% and 2.6% by enzyme additions of 400 and 800 FTU/kg, respectively.

Improvements in food efficiency noted in adequate non-phytate P diets with added phytase might be suggestive of improvements in the utilisation of nutrients other than P.

1.4.10 Influence of dietary calcium and calcium:phosphorus ratio on the effectiveness of phytase

The response to a given level of supplemental phytase will be influenced by dietary calcium level and/or Ca:P ratio, dietary phosphorus level, and dietary phytate level (Lei *et al.*, 1994).

A high molar ratio of Ca:phytate in the diet can lead to the formation of extremely insoluble Ca-phytate complexes under intestinal conditions, making the phytate molecule inaccessible to phytase (Kornegay, 1999). The presence of such strong complexes could explain the apparent inactivity of phytase in calcium rich diets rather than a direct inhibition of the enzyme by Ca-ions (Wise, 1983).

The importance of maintaining a narrow total Ca: total phosphorus ratio (or for that matter available Ca: available P) has been recently demonstrated in broilers (Qian *et al.*, 1997) and turkeys (Qian *et al.*, 1996).

In broilers and turkeys, total Ca:total P ratios of 1.1:1 to 1.4:1 appeared to be equally effective. Feeding diets with wider ratios reduces performance, phosphorus utilisation and bone mineralization (Qian *et al.*, 1996, 1997). In young broilers, using data reported by Qian *et al.* (1997) a 14.5, 8.5 and 8.3% decrease was calculated for body weight gain, phosphorus retention, and toe ash percentage, respectively, when the Ca: total P was increased from 1.1:1 to 2.0:1. In young turkeys, using data reported by Qian *et al.* (1996), a 8.7, 10.8 and 6.6% decrease was calculated for body weight gain, phosphorus retention, and toe ash percentage, respectively, when Ca: total P ratio was increased from 1.1:1 to 2.0:1.

1.5 SUMMARY

When poultry diets are formulated using significant amounts of plant based ingredients that are low in native phytase, microbial phytase supplementation is very effective for improving the availability of phytate phosphorus. Because phytate is known to complex a number of other minerals, amino acids/protein, and even starch, bio-availability of these nutrients is enhanced when phytate is hydrolyzed by phytase.

Thus, the excretion of phosphorus, calcium, zinc and nitrogen can be reduced significantly when diets are properly formulated using phytase.

The dose response curve of phytase for improving phosphorus utilisation is non-linear for poultry (broilers and turkeys) and the response has been described over a wide range of phytase levels. The dose response curves for the effects of phytase on calcium, zinc, amino acid/nitrogen and energy utilisation are not so clearly understood as they are for phosphorus. The dose response of phytase will, however, depend upon: 1) the level of phytase used, 2) the level of total phosphorus in the diet, 3) the level of phytate phosphorus in the diet, 4) the level of calcium and the Ca: P ratio, 5) the intrinsic level of phytase in foodstuffs and, 6) processing and pelleting methods.

CHAPTER 2 – MATERIALS AND METHODS

2.1. INTRODUCTION

The majority of phosphorus in plants is contained in chemical structures called phytic acids, or their salts that are known as phytates (Pallauf and Rimbach, 1997). Phytate phosphorus is relatively unavailable to monogastric animals. Thus, although plants contain substantial amounts of phosphorus, inorganic phosphorus is routinely added to mixed feeds. Phytase, an enzyme that hydrolyzes phytic acid to inositol and phosphoric acid (Liu *et al.*, 1998), making the phosphorus available to animals, is of considerable interest to the poultry industries.

Less than one-third of the phosphorus (P) contained in feed ingredients of plant origin is biologically available to monogastric animals (NRC, 1994). The remainder of the phosphorus is tied up as phytate (phytic acid or myo-inositol hexaphosphate) and monogastrics as a general rule lack the enzyme(s) necessary to hydrolyze phytate down to phosphorus and inositol (Liu *et al.*, 1998).

Therefore, inorganic sources of phosphorus (monocalcium phosphate, dicalcium phosphate, etc.) which have a high biological availability to the animal are supplemented in the feed to provide adequate intake levels necessary for bone and tissue growth and development as well as other metabolic needs (ATP/ADP, enzymes, egg and shell production, etc). The unavailable plant phosphorus (phytate) is passed undigested from the animal in the manure.

The usual method of manure disposal on most livestock operations is land application. There has been increasing concern in recent years relative to nutrient accumulation in soils upon which poultry (and other livestock) manure is applied as microorganisms in the soil can break down phytate. Phosphorus is one of the two nutrients that have received the most attention in this regard (nitrogen is the other). In the soil, phosphorus forms insoluble complexes with elements such as iron(Fe), aluminium(Al) an calcium(Ca) and tends to be immobile in the soil; thus it is not readily leached out of the soil into ground water like nitrogen.

Phosphorus levels in the soil tend to build up quite high with continued application of animal waste. Run-off due to improper manure application or weather conditions that cause erosion of soils into lakes or waterways can result in phosphorus pollution and eutrophication of those bodies of water.

Large concentrations of laying hens on individual farms results in the generation of very large amounts of manure in numerous localized areas. Eggshell quality in birds is highly influenced by the available phosphorus as well as the calcium in the feed. Since land spreading has been the traditional disposal method of animal waste, reducing the amount of phosphorus in the bird manure could have significant implications for being able to continue this method of disposal. In the past few years, several enzymes which can be added to the feed have become commercially available from several companies, and these may release some or all of the unavailable phosphorus in the feed grains of poultry diets and make it available. This could decrease the need for inorganic phosphorus supplements in the feed, and thereby decrease the feed cost. The use of these enzymes as a feed supplement and the effect on animal health, productivity and manure phosphorus content is currently being investigated.

2.2. MATERIALS AND METHODS

960 Point-of-lay Hy-line layers (20 weeks of age) were grouped in a 16 x 5 x 12 factorial. The layers were housed in a convection layer house for a period of 28 weeks (starting at an age of 20 weeks). The experimental period started at an age of 20 weeks, and was terminated at an age of 48 weeks. A commercial layer diet with different levels of available phosphorus and phytase (Natuphos BASF LTD) were fed. The 16 treatments (Table 2.1.) were randomly allocated in the convection layer house using random digits (Sameuls, 1991). Four basal feeds were mixed initially, and the 16 treatments were mixed out of these basal feeds. The mixing proportions are given in Table 2.2. The four basal feeds were formulated using least cost feed formulation software. The formulations are set out in Tables 2.3.1, 2.3.2, 2.3.3 and 2.3.4. A composition of the vitamin and mineral premix for layers that were used in the four basal diets is given in Table 2.3.5.

Table 2.1. Treatments

Treatment	**P-level (g/kg)**	**Phytase (FTU's)**
1	1.5	0
2	2.5	0
3	3.5	0
4	4.5	0
5	1.5	150
6	2.5	150
7	3.5	150
8	4.5	150
9	1.5	300
10	2.5	300
11	3.5	300
12	4.5	300
13	1.5	450
14	2.5	450
15	3.5	450
16	4.5	450

The treatments were mixed according to the following proportions:

Table 2.2. Mixing proportions (%).

Treatment	Basal feed 1	Basal feed 2	Basal feed 3	Basal feed 4
1	100	-	-	-
2	67	33	-	-
3	33	67	-	-
4	-	100	-	-
5	67	-	33	-
6	45	22	22	11
7	22	45	11	22
8	-	67	-	33
9	33	-	67	-
10	22	11	45	22
11	11	22	22	45
12	-	33	-	67
13	-	-	100	-
14	-	-	67	33
15	-	-	33	67
16	-	-	-	100

Table 2.3.1. Basal feed 1.

Ingredient	Percent (%)	Mix (kg)
Yellow maize	59.32	593.18
Bran	8.95	89.55
Soya o/c 47%	11.31	113.09
Sunflower o/c 38%	10.00	100.00
Lysine HCL	0.09	0.89
DL Methionine	0.10	1.02
L Threonine	0.01	0.11
Monocalcium Phosphate	0.29	2.90
Limestone	9.29	92.93
Salt	0.38	3.82
Vits & Meds	0.25	2.50

Table 2.3.2. Basal feed 2.

Ingredient	Percent (%)	Mix (kg)
Yellow maize	60.15	601.53
Bran	7.10	71.02
Soya o/c 47%	11.56	115.6
Sunflower o/c 38%	10.00	100.00
Lysine HCL	0.09	0.89
DL Methionine	0.10	1.04
L Threonine	0.01	0.13
Monocalcium Phosphate	2.11	21.11
Limestone	8.24	82.35
Salt	0.38	3.84
Vits & Meds	0.25	2.50

Table 2.3.3. Basal feed 3.

Ingredient	Percent (%)	Mix (kg)
Yellow maize	59.33	593.28
Bran	8.93	89.33
Soya o/c 47%	11.31	113.12
Sunflower o/c 38%	10.00	100.00
Lysine HCL	0.09	0.89
DL Methionine	0.10	1.02
L Threonine	0.01	0.11
Monocalcium Phosphate	0.29	2.90
Limestone	9.29	92.93
Salt	0.38	3.82
Vits & Meds	0.25	2.50
Natuphos	0.01	0.09

Table 2.3.4. Basal feed 4.

Ingredient	Percent (%)	Mix (kg)
Yellow maize	60.16	601.63
Bran	7.08	70.80
Soya o/c 47%	11.56	115.63
Sunflower o/c 38%	10.00	100.00
Lysine HCL	0.09	0.89
DL Methionine	0.10	1.04
L Threonine	0.01	0.13
Monocalcium Phosphate	2.11	21.11
Limestone	8.24	82.35
Salt	0.38	3.84
Vits & Meds	0.25	2.50
Natuphos	0.01	0.09

A vitamin and mineral premix as well as the phytase (Natuphos) were obtained from BASF Neuvet (PTY) LTD and were used in the basal feeds.

Table 2.3.5. Vitamin and Mineral premix for layers that were used in the basal diets.

Vitamin A	10 000 000 iu
Vitamin D3	3 500 000 iu
Vitamin E	15 000 iu
Vitamin K3	1.5g
Vitamin B1	2g
Vitamin B2	4g
Niacin	28g
Cal Pan	7g
Vitamin B12	20mg
Vitamin B6	2.5g
Choline	300g
Folic Acid	0.5g
Biotin	25mg
Manganese	70g
Zinc	30g
Copper	6g
Iodine	1g
Cobalt	0.5g
Ferrous	30g
Selenium	0.15g

2.2.1 Vaccination

A specifically designed vaccination program (as set out in Table 2.4.) obtained from OTK Hy-line was followed.

The IB and NCD vaccinations were done through the drinking water, with milk powder used to bind the chlorine in the water before the vaccinations were admitted.

Table 2.4. Vaccination program.

Age	Disease	Vaccine	Administration method
Day 1	Mareks NCD IBD	Mareks rispins VG/GA BUR-706	Inject in neck Coarse Spray
Day 7-9	NCD & IBD	Gumbopest	Inject in neck
Day 14	IBD	Mid-intermediate	Drinking water
Day 18	NCD	Clone 30	Aerosol
Day 20	IBD	Mid-Intermediate	Drinking water
Day 28	IB	H-120	Aerosol
Day 35	NCD	VG/GA	Drinking Water
Week 6	ILT	LT VAXI	Eye Drop
Week 8	NCD	Clone 30	Aerosol
Week 9	Pox	Pox Vaccine	Wing Stab
Week 10	NCD	VG/GA	Drinking Water
Week 11	Coryza & EDS NCD	Oil adjuvant type Inactivated oil	Inject Inject
Week 12	IB	H-120	Aerosol
Week 14	AE	AE Vaccine	Drinking Water
Week 15	NCD NCD/IB Coryza & EDS	VG/GA Inactivated oil combination Oil adjuvant type	Drinking Water Inject Inject in breast
Week 16	ILT	Live ILT vaccine	
Week 20	NCD	La Sota	Drinking water (thereafter every 6 weeks)
Week 23	IB	H-120	Drinking water (every 6 weeks)

2.2.2 Lighting program

A layout of the total lighting program is given in Table 2.5, but was only followed from 20 weeks of age when the birds were received from their rearing houses.

The following lighting program was followed up to 17 weeks:

Day 1-2: 24 hours at 10lux intensity

Day 3-21: 16 hours per day at 5lux intensity

Three weeks – 17 weeks: 10 hours per day at 5lux intensity.

Table 2.5. Lighting program

Age	Total daylight	Lights on	Lights off
17 weeks	10 and ½ hours	06:30am	17:00pm
18 weeks	11 hours	06:00am	17:00pm
19 weeks	12 hours	06:00am	18:00pm
20 weeks	12 and ½ hours	05:30am	18:00pm
21 weeks	12 and ½ hours	05:30am	18:00pm
22 weeks	13 hours	05:30am	18:30pm
23 weeks	13 hours	05:30am	18:30pm
24 weeks	13 and ½ hours	05:00am	18:30pm
25 weeks	13 and ½ hours	05:00am	18:30pm
26 weeks	14 hours	04:30am	18:30pm
27 weeks	14 and ½ hours	04:30am	19:00pm
28 weeks	15 hours	04:00am	19:00pm
29 weeks	15 hours	04:00am	19:00pm
30 weeks	15 and ½ hours	03:30am	19:00pm
31 weeks	16 hours	03:30am	19:30pm
32 weeks	16 hours	03:30am	19:30pm
33 weeks	16 and ½ hours	03:30am	20:00pm
Keep at	16 and ½ hours	03:30am	20:00pm

2.2.3 Parameters measured

Week 20-48

Mortalities were recorded over the twenty eight-week period. The dead birds were removed and incinerated. Post-mortems were not conducted due to financial constraints. Average body weights (kg) of groups were measured weekly. The same four birds out of each group were weighed each week, and an average weight was determined. Egg production was determined weekly for each group and expressed in percentage units (%). The breaking strength (Newton) of a randomly selected representative sample of the egg population was determined every 4 weeks (starting at week 20). One egg was taken from each group in each replicate for breaking strength determination (total amount of eggs broken every four weeks amounted to 80 eggs per week). Eggshell thickness (mm) was determined with a vernion gauge on the eggs that were broken every four weeks (starting at week 20). The eggs were left to air-dry before eggshell thickness was determined. Eggshell thickness was measured at the equator. Egg weights (g) were determined weekly by taking the average weight of the total weekly eggs layed within each group.

2.3 PROCEDURES FOR CHEMICAL ANALYSES

The purpose of chemical analysis is to determine the potential nutritional value of the feeds (16 treatments). The nutritional values determined by chemical analysis must be the same or very near to the values that were used for formulation of the diets. Knowledge of the chemical values, protein, amino acids, crude fiber, ether extractable lipid, calcium, phosphorus and DM (dry matter) are crucial for optimal formulation. The values obtained in these analyses are given in Table 2.6. The comparative theoretical values are given in Table 2.7. Feeds analyzed by other laboratories can be questioned. These include those for phytate P, TMEn and available Lys (lysine).

Table 2.6. Analyzed nutrient composition of experimental diets.

Treatment	Crude fibre%	Phytate P (g/kg)	Non phytate P (g/kg)	Total P (g/kg)	Ca %	Crude protein %	Available Lys (g/kg)	Fat %	DM %	Ash %	AMEn (MJ/kg)	TMEn (MJ/kg)
Treatment 1	5.49	2.00	2.58	4.58	3.14	15.47	5.75	1.66	99.60	10.63	10.02	10.43
Treatment 2	5.37	2.06	3.76	5.82	3.69	15.23	5.86	1.66	99.60	11.85	10.38	10.79
Treatment 3	5.09	2.13	5.01	7.14	3.41	15.32	5.97	1.57	99.64	11.61	10.47	11.15
Treatment 4	4.85	2.20	5.55	7.75	3.48	15.27	6.08	1.54	99.64	11.99	11.09	11.50
Treatment 5	5.09	1.34	3.16	4.50	3.23	15.26	6.08	1.67	99.65	11.73	10.48	10.89
Treatment 6	5.07	1.44	4.45	5.89	3.71	15.39	6.10	1.72	99.63	12.04	10.66	11.07
Treatment 7	4.38	1.54	4.97	6.51	3.40	15.23	6.11	1.68	99.69	11.67	10.85	11.26
Treatment 8	4.64	1.64	6.09	7.73	3.28	15.24	6.07	1.67	99.60	11.80	11.03	11.44
Treatment 9	5.33	0.66	4.24	4.90	3.51	15.37	6.42	1.65	99.73	11.67	10.96	11.37
Treatment 10	4.97	0.79	5.37	6.16	3.29	15.78	6.34	1.84	99.69	11.70	10.96	11.37
Treatment 11	4.64	0.93	6.20	7.13	3.15	15.37	6.26	1.67	99.71	11.36	10.96	11.37
Treatment 12	5.11	1.06	7.20	8.26	3.43	15.13	6.18	1.57	99.65	11.79	10.97	11.38
Treatment 13	5.03	0.00	5.15	5.15	3.49	15.58	6.75	1.72	99.65	11.58	11.42	11.83
Treatment 14	4.64	0.17	5.81	5.98	3.46	15.67	6.58	1.57	99.46	12.06	11.25	11.66
Treatment 15	4.79	0.34	7.01	7.35	3.40	15.55	6.40	1.62	99.60	11.97	11.08	11.49
Treatment 16	4.67	0.50	7.62	8.12	3.46	15.53	6.23	1.46	99.69	11.90	10.91	11.32

Table 2.7 Theoretical nutrient composition of experimental diets.

Treatment	Crude fibre %	Phytate P (g/kg)	Non phytate P (g/kg)	Total P (g/kg)	Ca %	Crude protein %	Available Lys (g/kg)	Fat %	TMEn (MJ/kg)
Treatment1	4.59	3.15	1.5	4.65	3.5	15.36	6.54	3.20	11.30
Treatment2	4.54	3.38	2.5	5.88	3.5	15.33	6.54	3.20	11.30
Treatment3	4.49	3.62	3.5	7.12	3.5	15.30	6.54	3.20	11.30
Treatment4	4.44	3.85	4.5	8.35	3.5	15.27	6.54	3.20	11.30
Treatment5	4.59	3.15	1.5	4.65	3.5	15.36	6.54	3.20	11.30
Treatment6	4.54	3.38	2.5	5.88	3.5	15.33	6.54	3.20	11.30
Treatment7	4.49	3.62	3.5	7.12	3.5	15.30	6.54	3.20	11.30
Treatment8	4.44	3.85	4.5	8.35	3.5	15.27	6.54	3.20	11.30
Treatment9	4.59	3.15	1.5	4.65	3.5	15.36	6.54	3.20	11.30
Treatment10	4.54	3.38	2.5	5.88	3.5	15.33	6.54	3.20	11.30
Treatment11	4.49	3.62	3.5	7.12	3.5	15.30	6.54	3.20	11.30
Treatment12	4.44	3.85	4.5	8.35	3.5	15.27	6.54	3.20	11.30
Treatment13	4.59	3.15	1.5	4.65	3.5	15.36	6.54	3.20	11.30
Treatment14	4.54	3.38	2.5	5.88	3.5	15.33	6.54	3.20	11.30
Treatment15	4.49	3.62	3.5	7.12	3.5	15.30	6.54	3.20	11.30
Treatment16	4.44	3.85	4.5	8.35	3.5	15.27	6.54	3.20	11.30

The theoretical nutrient composition was obtained by using the mixing proportions as indicated in Table 2.2, and multiplying these values with the least-cost formulation values (Table 2.3.1; 2.2.2; 2.2.3 and 2.3.4). The analysed nutrient composition of the diets was obtained from independent companies and analyses done personally within the laboratories of the University of Pretoria. As large differences between the raw materials' nutrient values are possible due to the use of average figures and formulations (as opposed to exact values), differences between the theoretical and analysed nutrient composition values will be evident. Also, due to human error in the laboratories, various analysed values may not be accurately measured, with differences occurring between theoretical and analysed nutrient compositions.

The dry matter determinations obtained personally within the University of Pretoria (Table 2.6) differed by approximately 10% to values obtained through similar experiments within the University of Natal. These differences cannot be thoroughly explained, as it is believed that all University of Pretoria's experimental procedures and formulations were accurately followed as according to the A.O.A.C. (1980) method. Concurrently, there is an approximate 51% difference between theoretical and analysed fat values. As above, these differences cannot be accurately explained, but may also be due to the use of average values instead of specific values. The phytate phosphorus analysis was conducted by ARC (Agricultural Research Centre). There is a large difference between theoretical and analysed nutrient composition values. These differences may once again be due to human error, as certain results ended up as 'zero' – practically impossible within this experiment. Therefore, due to the variances seen these values may be questioned.

Further analytical studies are required in order to fully understand the nature of all the above-mentioned differences.

The following chemical analyses were done on the 16 treatments:

2.3.1 Dry matter determinations

The dry matter determination was conducted using the A.O.A.C. (1980) method. Firstly, a specific amount (3 to 5g) of the treatments (1-16) were weighed into a silica crucible. The sample was then placed in an oven at a temperature of a 100°C and dried until a constant mass was reached. After the sample was cooled in a dessicator, the weight was determined.

The percentage moisture and dry matter were then determined as follows:

$$\frac{\text{Loss in weight during drying}}{\text{Initial weight}} \times \frac{100}{1} = \% \text{ moisture}$$

Weight of sample after drying / Initial weight x 100/1 = % dry matter

2.3.2. Crude protein determinations

This analysis was done with the help of a Büchi apparatus, as described by the A.O.A.C. (1980). The method is as follows:

Reagents:

1. "Kjeltabs" with 5g K_2SO_4.
2. Concentrated sulfuric acid 98%.
3. NaOH-solution – 10kg NaOH diluted in 15l distilled water, and then filled up to 20L with distilled water.
4. Boric acid-solution – 800g boric acid in boiling distilled water, and fill to 20L after cooling down.
5. 0.1 N HCl-solution.
6. Buffer solution – pH 4, in which the electrodes were placed.
7. Anhidric coppersulfate.

Sample digestion method:

- 30g light dry sample was milled through a 1mm sieve.
- 350mg sample was weighed into a Büchi digestion tube. Two "Kjeltabs", 0.5g coppersulfate and two glass beads were also put into the tube.
- 20ml H_2SO_4 was added, and then the tube placed on the Büchi 430 digestion unit. The sample was heated until all the carbon was digested, and the sample was clear.
- With the help of the Büchi 322 and 342 operation unit the nitrogen in the digested solution was distilled to the boric acid solution.
- The boric acid solution was mixed and titrated with the E649 Metrohm and E526 Metrohm, respectively.
- A blank was also prepared following the same steps, but without the sample (treatment 1-16).

The nitrogen content (%) of the sample was determined as follows:

$$\%N = \frac{T \times 0.1 \times 14 \times 100}{1000 \times \text{sample weight (g)}}$$

Total crude protein = %N x 6.25
Where N = nitrogen in sample.

2.3.3 Available amino acids

The laboratory of the University of Natal – Department of Animal Science and Poultry Science, determined the available amino acids. The Beckman Amino Acid Analyser System 6300 was used Applications data A6300-An-002 (Beckman Instruments, Inc., Spinco Division, Palo Alto, California. October 1983). The method of Moore and Stein (1948) was used.

2.3.4. Crude fiber

The determination of crude fiber were done as described by the A.O.A.C. (1980), using the Fibertec system. The sample must be air dried, and then milled through a 1mm sieve. If the sample contain more than 10 % fat, it must first be treated with petroleum ether to remove the fat before analysis.

The 'sinterglass' crucible where the weighed sample was placed into must be free of any residual ash, and therefore flushed with a 5% HCl-solution beforehand.

Reagents:

1. 0.128-M sulfuric acid solution - 6.96ml of a H_2SO_4 per liter distilled water.
2. 0.313-M sodiumhydroxide – 12.5g NaOH per liter distilled water.
3. n-Octanol.
4. Acetone.

Method:

- 1g of the sample is weighed into the crucible. The crucible is then placed on a warm extraction unit, in such a way that it fits tightly.
- The water-cooling is opened to ensure that the reagent does not boil over.
- 150ml cooked 0.128M H_2SO_4 is then added into the condensation tube above the crucible, with a funnel. Three drops of octanol is also added into the tube.
- The lid of the heating unit is then placed into position and the heating element turned to maximum. The sample is then brought to boiling point and boiled for 30 minutes.
- The heating element is then turned off, and the sample is filtrated by using a vacuum pump.
- The sample is then flushed three times with 30ml warm distilled water per flush.
- 150ml boiling 0.313M NaOH is placed into the tube.
- Three drops of octanol are also placed into the tube. The sample is then brought to boiling point and kept there for 30 minutes. The heating element is then turned off, and the sample filtrated.
- The sample is flushed three times with 30ml distilled water per flush.
- All the material of the sample must be flushed into the crucible (nothing must be left in the tube).
- The material in the crucible is then washed three times with acetone, and filtrated dry with the vacuum pump. The crucible is placed into the drying oven.
- The crucible is dried overnight at 105°C.
- The sample in the crucible is cooled down in a dessicator for 30 minutes before weighing.
- The sample is then ashed at a temperature of 500°C for a minimum of four hours.
- The crucible is left to cool down to a temperature below 250°C, before it is removed from the oven. The sample is then cooled in a dessicator for 30 minutes before being weighed.

$$\% \text{ Crude fiber} = \frac{Wrd - Wra}{ws}$$

Wrd = weight of residue after drying in crucible.

Wra = weight of residue after ashing in crucible.

Ws = weight of initial sample.

2.3.5. Ether extractable lipid

The "Tecator Soxtec" system 1040 extraction apparatus was used. The "Tecator" method was used the same as the A.O.A.C.-method, where petroleum ether (40-60°C boiling point) was used at 105°C for 30 minutes for drying.

Reagents:

Petroleum ether (40 – 60°C).

Method:

- 2g-milled sample (through 1mm sieve) was accurately weighed into an extraction thimble.
- The extraction tubes and four glass beads are dried in an oven at 105°C for 2 hours, before it is cooled down in a dessicator and weighed.
- 50ml petroleum ether is then placed into the tube and positioned on the apparatus.
- The tube is then heated for 30-40 minutes, while the fat is extruded.
- The tube is dried in the oven, and then weighed accurately.

$$\% \text{ Ether extract} = \frac{Wte - Wt \times 100}{Ws}$$

Wte = weight of tube and ether extract.

Wt = weight of tube.

Ws = weight of sample.

2.3.6 Calcium analysis and 2.3.7 Phosphorus analysis

Apparatus needed:

1. Calcium (Ca) is determined with an atom absorption spectrophotometer in the presence of LanthanumOxide (La_2O_3) that acts as a suppressant for any phosphorus that may be present in the sample.
2. Phosphorus is determined colometrically with molibdovanate used as a color reagent, using the Auto Analyzer apparatus.
3. Parameters: Ca – 5 to 10 ppm (parts per million).
   P - 1 to 100 ppm.

Determination of Ca.

Reagents:

- La solution: 50g La/liter of 50 000 ppm. Use La_2O_3 (99.99 % AR) and weigh 58.65g of that into a 600ml glass cup, and slowly add 250ml HCl (34 %) while stirring. Fill the solution up to 1000ml in a volumetric flask with distilled water.
- Ca-solution: 500mg Ca/liter. Weigh 1.249g dry $CaCO_3$ and dilute in 50ml 4M HCl before it is made up to 1000ml with distilled water in a volumetric flask.
- Standard solution of Ca was made up to give a series that falls into the parameters of the apparatus. La-solution must be added to the Ca standard to get a final solution of 1 % La.

Sample preparation.

Dilute directly after sample was filtrated so that the concentration falls into the parameters as mentioned above.

Solid samples: weigh 0.5 to 2g of the sample into a silica crucible, and place into cold ashing oven. Ash at 550°C for three hours. Cool down and add 10ml of a 4M HCl solution to the ashed sampled. Heat slowly in waterbath at 70°C for 20 minutes. Cool down and filtrate into a 100ml volumetric flask – flush 3 times with distilled water and make up to volume (100ml).

Dilutions must be made up with 0.1M HCl to fall into the parameters of the apparatus. Diluted sample can be read on the atomic absorption spectrophotometer.

Determination of P.

Reagents:

- KH_2PO_4
- NH_4 Molibdate
- NH_4 Monovanadate
- 70% $HClO_4$

1. P-solution: 1000mg P/liter. Weigh 4.394 dry KH_2PO_4 and dilute into 100ml water. Add 1ml HCl (34%) and make up to 1000ml with distilled water in a volumetric flask.
2. NH_4 Molibdate: Weigh 40g NH_4 molibdate into a 50ml glass cup, add 400ml distilled water and heat (60°C) to dilute. Cool down.
3. NH_4 monovanadate: Weigh 2g NH_4 monovanadate, place into a 1liter-glass cup, add 250ml distilled water and heat to 60°C (to dilute). Cool down and add 450ml 70% $HClO_4$ while stirring.
4. Add solution (2) slowly to (3) while stirring. Cool down and make up to 2000ml with distilled water in a volumetric flask.

Sample preparation:

1. Solid samples: Samples ashed and made up the same as for Ca. Dilutions made up with 0.2M HCl, the same as for Ca.
2. Use an Auto Analyzer to read diluted samples, after standardization with standards.

2.3.8 Phytate phosphorus determination

ARC did the phytate phosphorus determinations – Irene Analytical Services (Pretoria). A method for phytic acid determination in wheat and wheat fractions were used (Wheeler and Ferrel, 1971).

2.3.9 Non-phytate phosphorus (NPP)

Non-phytate phosphorus was determined by subtracting the phytate bound phosphorus from the total phosphorus, using the formula:

NPP = TP – PP

Where:

NPP = Non-phytate phosphorus

TP = Total phosphorus

PP = Phytate phosphorus

2.3.10 Available metabolizable energy (AMEn)

The University of Natal – Department of Animal Sciences and Poultry Science, did the determination of the nitrogen corrected available metabolizable energy. The AMEn were determined using the method of Sibbald (1976).

2.3.11 Ash determination

The ash determinations were done following the A.O.A.C. (1980) method. After determining dry matter (as mentioned before hand), the samples are placed into an incineration oven for 4 hours at 450°C. The oven is then left to cool down. The crucibles are then placed into a dessicator to cool down for 30 minutes. After cooling down the crucibles are weighed and % ash is determined as follows:

$$\% \text{ Ash} = \frac{\text{ash weight} \times 100}{\text{Sample weight}}$$

2.3.12 Breaking strength

Method:

The Instron Model 1011 was used to determine breaking strength.

Calibration was firstly done as follows:

Attach the compression probe before calibrating, make sure that the washer is used.

Switch the machine on 15 minutes beforehand.

Press function: Units [Enter]

SI [Enter]

Press transducer: 50kg/500N [Enter]

Speed: Set to 100mm/min [Enter]

Balance knobs to zero and lock.

Hang 5kg weight to attachment.

Use screwdriver to adjust setting: set to 50N.

Removes 5kg weight and set the gauge length.

Set compression.

Break action: set to return.

Unlock balance knobs again and set to zero, and lock.

Data selection: set to break.

The egg is placed sharp end up before breaking.

Breaking strength is red on the Instron screen in Newton.

Eggs are collected randomly from every treatment, and broken within 24 hours of lay.

2.3.13 Shell thickness

Method:

After the eggs were broken (as described above), they were further broken open along the equator. The eggs were left to air-dry. The eggshell thickness was measured on the equator.

2.4 Statistical analyses

An analysis of variance with the ANOVA model (Statistical Analysis Systems, 1994) was used to determine the significance between different treatments for the balanced data. Means and standard deviations (SD) were calculated.

Significance of difference (5%) between means was determined by multiple comparisons using Tukey t-test (Sameuls, 1989).

CHAPTER 3 – RESULTS AND DISCUSSION

3.1 RESULTS - Introduction

An analysis of variance with the ANOVA model (Statistical Analysis Systems, 1994) was used to determine the significance between different treatments for the balanced data. Means and standard deviations (SD) were calculated. Significance of difference (5%) between means was determined multiple comparisons using Tukey t-test (Sameuls, 1989).

The significant differences in 4-week periods were minimal, therefore the averages over the 28 week period were used. More significant differences were found between the averages over the 28-week period.

Means and standard deviations can be found in Chapter 7 – Appendix D.

3.1.1 Body weights (kg)

The mean weight changes (kg) over the 28-week experimental period are represented in Table 3.1.1. and Table 3.1.2. There were significant differences in change in weight with regard to P-level. Significant differences were found between 1.5g/kg available phosphorus and 3.5g/kg available phosphorus. A significant difference was also found between 2.5g/kg available phosphorus and 3.5g/kg available phosphorus. Boling *et al.* (2000) found that hens fed 0.15% aP with no phytase had significantly lower body weights ($P<0.05$) than hens fed higher aP levels. Gordon and Roland (1998) obtained an increase in body weights of 4.4% with phytase inclusion, and increasing P from 0.1 to 0.3% resulted in a 3.1% increase. The present study only showed an increase in body weight with an increase in P-level.

There were no significant differences in change in weight with regard to phytase level.

Table 3.1.1. P-level with regard to change in weight (mean over 28 weeks).

P-level (g/kg)	Mean weight change (kg)	SD (±)
1.5	0.047950[a]	0.0137246
2.5	0.047150[a]	0.0160862
3.5	0.060300[b]	0.0145678
4.5	0.054050[ab]	0.0133040

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.2. Phytase level with regard to change in weight (mean over 28 weeks).

Phytase level (FTU's)	Mean weight change (kg)	SD (±)
0	0.0484500[a]	0.0173159
150	0.0514000[a]	0.0182711
300	0.0543500[a]	0.0097564
450	0.0552500[a]	0.0139958

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.2 Feed intake (g)

Table 3.1.3. and Table 3.1.4. show that feed intake with regard to P-level and phytase level, respectively, had no significant differences. Gordon and Roland (1998) found that phytase supplementation significantly increased feed consumption as early as week 1 (from 58 weeks of age) in their experimental period. Gordon and Roland (1998) also found that increasing dietary P resulted in increased feed consumption when diets were not supplemented with phytase. The same results were not obtained in the present study with no significant differences found in feed intake with regard to phytase and phosphorus levels.

Table 3.1.3. P-level with regard to feed intake per day (mean over 28 weeks).

P-level (g/kg)	Mean feed intake (g/day)	SD (±)
1.5	124.0038[a]	2.2789866
2.5	125.2650[a]	2.3163819
3.5	124.5697[a]	2.7232542
4.5	124.5974[a]	2.5911566

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.4. Phytase level with regard to feed intake per day (mean over 28 weeks).

Phytase level (FTU's)	Mean feed intake (g/day)	SD (±)
0	123.87890[a]	2.7696959
150	125.02215[a]	2.7050406
300	125.16415[a]	2.3014312
450	124.37080[a]	2.0297640

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.3 Egg production (%)

Table 3.1.5. and Table 3.1.6. show that egg production (%) with regard to P-level and phytase level, respectively, had no significant differences. Boling *et al* (2000) indicated that although 0.10% aP was inadequate for maintaining egg production, 0.10% aP + 300U/kg phytase or 0.15% aP with no supplemental phytase supported optimum egg production throughout the experimental period. It seems that the lowest aP level (1.5g/kg) was not low enough to depress production in the present study, as no significant drop in production was observed.

Gordon and Roland (1998) found that when diets contained 0.1% NPP (non-phytate phosphorus), egg production of unsupplemented (without phytase) hens significantly decreased.

Table 3.1.5. P-level with regard to % production (mean over 28 weeks).

P-level (g/kg)	Mean % production	SD (±)
1.5	92.6353[a]	2.5582608
2.5	93.0572[a]	1.1499468
3.5	92.0934[a]	1.3849674
4.5	91.4967[a]	2.2810241

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.6. Phytase level with regard to % production (mean over 28 weeks).

Phytase level (FTU's)	Mean % production	SD (±)
0	91.66435[a]	2.8740807
150	92.40590[a]	1.1236712
300	92.36085[a]	2.1895415
450	92.85155[a]	1.1644507

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.4 Egg weight (g)

The average egg weights with regard to P-level and phytase level are presented in Table 3.1.7. and Table 3.1.8., respectively. There were no significant differences between egg weight with regard to P-level. A significant difference was found between a phytase level of 0 FTU's and 300 FTU's. Gordon and Roland (1998) found that supplemental phytase significantly increased egg weights during weeks 4 and 6 (starting at 58 weeks of age) when diets contained 0.1% NPP (non phytate phosphorus). In accordance with the present study Gordon and Roland (1998) found that there was no evidence of P effect on egg weight when phytase was included in the diet.

Table 3.1.7. P-level with regard to egg weight (mean over 28 weeks).

P-level (g/kg)	Mean egg weight (g)	SD (±)
1.5	54.89920[a]	1.0751901
2.5	55.04310[a]	0.8886536
3.5	55.29980[a]	0.7011716
4.5	55.38505[a]	1.1915695

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.8. Phytase level with regard to egg weight (mean over 28 weeks).

Phytase level (FTU's)	Mean egg weight (g)	SD (±)
0	54.77605[a]	0.9338906
150	54.97775[ab]	0.9078675
300	55.68570[b]	1.2116397
450	55.18765[ab]	0.6089250

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.5 Egg output (g/day)

Table 3.1.9. and Table 3.1.10. show that egg output (g/day) with regard to P-level and phytase level, respectively, have no significant differences.

Table 3.1.9. P-level with regard to egg output (mean over 28 weeks).

P-level (g/kg)	Mean egg output (g/day)	SD (±)
1.5	50.96580[a]	1.9806565
2.5	51.22630[a]	1.2657250
3.5	50.93030[a]	1.1437370
4.5	50.67140[a]	1.5354400

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.10. Phytase level with regard to egg output (mean over 28 weeks).

Phytase level (FTU's)	Mean egg output (g/day)	SD (±)
0	50.21850[a]	2.0125587
150	50.80080[a]	0.9417794
300	51.43140[a]	1.6420632
450	51.24320[a]	0.8977097

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.6 Breaking strength (Newton)

Table 3.1.11. and Table 3.1.12. show that breaking strength (N) with regard to P-level and phytase level, respectively, have no significant differences.

Table 3.1.11. P-level with regard to breaking strength (mean over 28 weeks).

P-level (g/kg)	Mean breaking strength (N)	SD (±)
1.5	35.24780[a]	4.7498065
2.5	33.88625[a]	5.2010645
3.5	33.12930[a]	3.1929200
4.5	33.92005[a]	4.4981060

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.12. Phytase level with regard to breaking strength (mean over 28 weeks).

Phytase level (FTU's)	Mean breaking strenght (N)	SD (±)
0	33.04790[a]	4.0057069
150	34.62945[a]	4.4360145
300	32.77265[a]	4.4673996
450	35.73340[a]	4.5492225

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.1.7 Shell thickness (mm)

The average shell thickness values with regard to P-level and phytase levels are presented in Table 3.1.13. and Table 3.1.14., respectively. A significant difference was found between a P-level of 1.5 g/kg and 4.5 g/kg. A significant difference was also found in a phytase level of 0 FTU's and 300 FTU's.

Table 3.1.13. P-level with regard to shell thickness (mean over 28 weeks).

P-level (g/kg)	Mean shell thickness (mm)	SD (±)
1.5	0.367250[a]	0.0197214
2.5	0.363300[ab]	0.0080792
3.5	0.358300[ab]	0.0117433
4.5	0.356300[b]	0.0066499

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

Table 3.1.14. Phytase level with regard to shell thickness (mean over 28 weeks).

Phytase level (FTU's)	Mean shell thickness (mm)	SD (±)
0	0.3656[a]	0.0096914
150	0.3604[ab]	0.0089290
300	0.3554[b]	0.0105451
450	0.3637[ab]	0.0191121

*Means with different superscripts are significantly different (P<0.05) according to Tukey's Multiple Range Test.

3.2 PRODUCTION RESULTS (WEEK 20 – 48)

3.2.1 Mortalities

Mortalities were recorded over the 28-week experimental period as depicted in Table 3.2.1. Mortalities were not linked to treatments.

Table 3.2.1. Mortalities over 28 week experimental period.

Treatment	Total number of mortalities
1	2
2	1
3	1
4	1
5	1
6	1
7	1
8	0
9	0
10	1
11	1
12	1
13	1
14	0
15	2
16	0

*Post mortems were not done due to financial restrictions.

3.2.2 Temperatures (°C)

Temperatures were taken daily over the whole 28-week experimental period. Thermometers were placed randomly in 4 different spots in the convection layer house. A list with the temperatures can be found in Chapter 7 - Appendix A.

3.3 General conclusions

Results show that phosphorus and phytase has an influence on weight change in accordance with work done by Boling *et al.* (2000).

No significant differences were found in feed intake, but this could be attributed to the trial being terminated too early (48 weeks). The accuracy of intake determination was also hampered by the large amount of feed that was wasted. Gordon and Roland (1998) found increased feed intake with phytase supplementation at 58 weeks of age.
The egg production was not significant between treatments. Egg production seems to be influenced only if the aP levels are lowered to 0.1% (Gordon and Roland, 1998). The lowest level of aP in the present study was 1.5g/kg.

A significant difference in egg weight was found between a phytase level of 0 FTU's and 300 FTU's. Gordon and Roland (1998) found that phytase increased egg weights similarly as in the present study.

Significant differences with respect to shell thickness were found in a phosphorus level of 1.5g/kg and 4.5g/kg, as well as a phytase level of 0 FTU's and 300 FTU'.

CHAPTER 4 – ECONOMICS OF EXPERIMENT

4.1 ECONOMICS

Although an economic conclusion was not the objective of this experiment, it was considered a worthwhile exercise to quantify the value of using phytase as a supplement. It must be noted that this was not a commercial production enterprise as many of the overheads one accounted for in commercial enterprise weren't included in this calculations.

The financial layout of the experiment consisted of the following:

4.1.1 Income

Income was obtained from selling the eggs (referring to Table 4.1). The price per egg escalated from 13 cents to 33 cents over the 28-week period. This escalation can be explained by the increase in egg size. The amount of egg trays lessened as the percentage production decreased. After the experiment was finished, the layers were sold at an average price of R13.50 per bird. The feed was sold to a private buyer. Therefore, the total income of the experiment was obtained from egg sales, leftover feed sales and the sales of the layers.

4.1.2 Direct costs

The direct costs consisted primarily of the cost of the feed and the layers (Referring to Table 4.2). Four tons of feed was mixed every month (16 treatments were mixed out of these four tons, as previously mentioned). As the experiment took place over seven months, 28 tons of feed was mixed over the entire duration of the research. The premix for the feed was mixed in heavy-duty bags.

Milk powder was bought to mix with water during vaccinations.

Electricity was provided by the experimental farm and therefore proved insignificant as a direct cost pertaining to the experiment.

Veterinary services were not required.

Karbadust was bought for the purpose of controlling Northern Fowl Mite.

A vernion gauge was bought to measure shell thickness.

Stationery was used to mark the cages and the feed bags. Ziploc bags were used to collect feed and manure (faeces) samples. Keys were cut for the different locks leading to the convection layer house, and thermometers bought and placed in determined locations for temperature measurement in the layer house.

The costs originating from analysis of feeds are self-explanatory.

4.1.3 Bank reconciliation statement

A bigger profit could have been generated had the experiment been extended over a longer time period (Referring to Table 4.3)

4.1.4 Proposed budget

The price of the layers and the feed was known beforehand, so an accurate estimation could be made (Referring to Table 4.4). Vaccinations and milk powder were predetermined costs, as were the thermometers and vernion gauge – used for specific measurements during the trial. A fuel budget was made for feed collection.

4.1.5 Conclusion

Due to the large initial financial outlay the experiment only started to show a profit in the sixth month of production. This initial financial outlay was comprised mainly of the layers and the feed. There was a tendency for the feeds to be less expensive when phytase was included in the feed. A higher profit would have been realized if the experiment was done over the whole production cycle of the layers.

Year	**05/29/2000 - 12/04/2000**	**Table 4.1**
Enterprise Budget	**Layers**	
Layers	**960.00**	

INCOME	**PRICE/UNIT**	**RAND/EGG**	**UNITS**		**TOTAL**	**30/6/2000**	**31/7/2000**	**31/8/2000**	**30/9/2000**	**31/10/2000**	**30/11/2000**
Sales											
Eggs: June	R 4.00	R 0.13	837.00	trays	R 3,348.00	R 3,348.00					
July	R 5.00	R 0.17	889.00	trays	R 4,445.00		R 4,445.00				
August	R 6.00	R 0.20	868.00	trays	R 5,208.00			R 5,208.00			
September	R 8.00	R 0.27	876.00	trays	R 7,008.00				R 7,008.00		
October	R 9.00	R 0.30	866.00	trays	R 7,794.00					R 7,794.00	
November	R 10.00	R 0.33	820.00	trays	R 8,200.00						R 8,200.00
Layers	R 13.50		946.00	layers	R 12,771.00						R 12,771.00
Feed (left over)	R 0.33		600.00	kg	R 200.00						R 200.00
TOTAL					**R 48,974.00**	**R 3,348.00**	**R 4,445.00**	**R 5,208.00**	**R 7,008.00**	**R 7,794.00**	**R 21,171.00**

Table4.2 DIRECT COSTS	UNITS	UNITS/ LAYER		RAND/UNIT	UNITS (layers)		RAND/ LAYER		TOTAL		30/6/2000		31/7/2000		31/8/2000		30/9/2000		31/10/2000		30/11/2000	
Layer 105 Diet I	6.00	0.025	R	783.17	240.00	R	3.26	R	4,699.02	R	783.17	R	783.17	R	783.17	R	783.17	R	783.17	R	783.17	
Layer 105 Diet II	6.00	0.025	R	827.13	240.00	R	3.45	R	4,962.78	R	827.13	R	827.13	R	827.13	R	827.13	R	827.13	R	827.13	
Layer 105 Diet III	6.00	0.025	R	794.91	240.00	R	3.31	R	4,769.46	R	794.91	R	794.91	R	794.91	R	794.91	R	794.91	R	794.91	
Layer 105 Diet IV	6.00	0.025	R	834.96	240.00	R	3.48	R	5,009.76	R	834.96	R	834.96	R	834.96	R	834.96	R	834.96	R	834.96	
Salary: Permanent								R	2,466.20	R	493.24	R	493.24	R	493.24	R	493.24	R	493.24	R	493.24	
Dose																						
Vacc : NCD La Sota-(New Castle Disease)	5.00	0.005	R	7.50	960.00	R	0.01	R	37.50	R	7.50	R	7.50	R	7.50	R	7.50	R	7.50	R	-	
IB (Infectious Brongitis)	5.00	0.005	R	9.01	960.00	R	0.01	R	45.03	R	9.01	R	9.01	R	9.01	R	9.01	R	9.01	R	-	
Vet																						
Electricity																						
Transport (fuel)	312.00		R	3.25				R	1,014.00	R	169.00	R	169.00	R	169.00	R	169.00	R	169.00	R	169.00	
Divers																						
Purchase of layers (vat incl.)					960.00	R	17.10	R	16,416.00	R	16,416.00	R	-	R	-	R	-	R	-	R	-	
Milk powder	6.00	0.006	R	6.00	960.00	R	0.01	R	36.00	R	6.00	R	6.00	R	6.00	R	6.00	R	6.00	R	6.00	
Stationary	4.00		R	10.00				R	40.00	R	40.00	R	-	R	-	R	-	R	-	R	-	
Ziploc Bags	2.00		R	8.99				R	17.98	R	17.98	R	-	R	-	R	-	R	-	R	-	
Karbadust 5% 500g	1.00	0.001	R	23.72	960.00	R	0.02	R	23.72	R	23.72	R	-	R	-	R	-	R	-	R	-	
Keys	3.00		R	10.33				R	31.00	R	31.00	R	-	R	-	R	-	R	-	R	-	
Thermometers	4.00		R	84.00				R	-	R	336.00	R	-	R	-	R	-	R	-	R	-	
Heavy duty bags	1.00		R	9.49				R	9.49	R	9.49	R	-	R	-	R	-	R	-	R	-	
Vernion gauge	1.00		R	247.00				R	247.00	R	247.00	R	-	R	-	R	-	R	-	R	-	
Costs of analysis: ARC Irene								R	3,374.40	R	-	R	-	R	-	R	-	R	-	R	3,374.40	
University of Natal								R	3,220.00	R	-	R	-	R	-	R	-	R	-	R	3,220.00	
Other								R	17.15	R	17.15	R	-	R	-	R	-	R	-	R	-	
TOTAL						**R**	**30.62**	**R**	**46,436.49**	**R**	**21,063.26**	**R**	**3,924.92**	**R**	**3,924.92**	**R**	**3,924.92**	**R**	**3,924.92**	**R**	**10,502.81**	
MARGIN ABOVE DIRECT COST								**R**	**2,537.51**	**R**	**-17,715.26**	**R**	**520.08**	**R**	**1,283.08**	**R**	**3,083.08**	**R**	**3,869.08**	**R**	**10,668.19**	

Table 4.3
Bankreeconciliation statement

	June	July	Aug	Sept	Oct	Nov
Begin balance	R -	R -17,715.26	R -17,195.18	R -15,912.10	R -12,829.02	R -8,959.94
Profit / Loss	R -17,715.26	R 520.08	R 1,283.08	R 3,083.08	R 3,869.08	R 10,668.19
End saldo	R -17,715.26	R -17,195.18	R -15,912.10	R -12,829.02	R -8,959.94	R 1,708.25

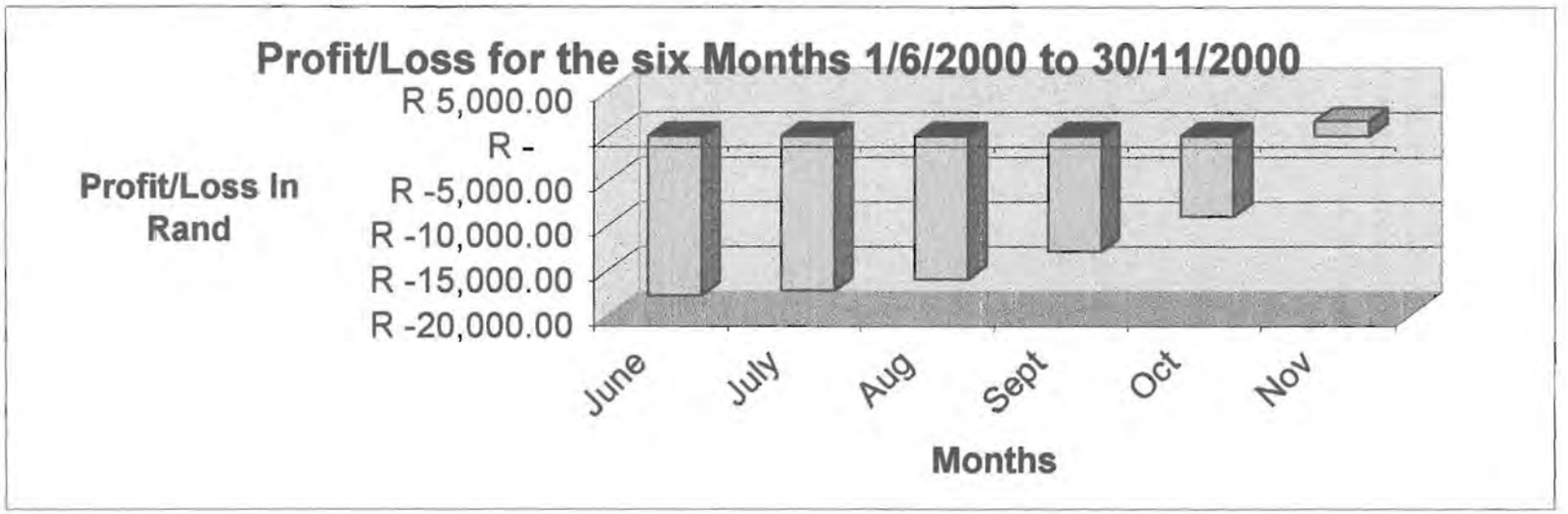

Table 4.4
PROPOSED BUDGET

Income	**R**	**53,518.50**										
Egg Sales:				Price/Unit		Units						
June	R	6,885.00	R	8.50		810						
July	R	7,114.50	R	8.50		837						
August	R	7,114.50	R	8.50		837						
September	R	6,885.00	R	8.50		810						
October	R	7,114.50	R	8.50		837						
November	R	6,885.00	R	8.50		810						
Layer Sales	R	11,520.00	R	12.00		960						
Expenditures	**R**	**37,450.06**										
				Jun		*Jul*		*Aug*		*Sept*		*Oct*		*Nov*
Layers (960 poults@ R17.10)	R	16,416.00	R	16,416.00										
Feed	R	19,416.00	R	3,236.00	R	3,236.00	R	3,236.00	R	3,236.00	R	3,236.00	R	3,236.00
Vaccination:														
IB (Infectious Brongitis)	R	45.00	R	7.50	R	7.50	R	7.50	R	7.50	R	7.50	R	7.50
ND (Newcastle Disease)	R	54.06	R	9.01	R	9.01	R	9.01	R	9.01	R	9.01	R	9.01
Fuel	R	900.00	R	150.00	R	150.00	R	150.00	R	150.00	R	150.00	R	150.00
Thermometers	R	336.00	R	336.00										
Vernion gauge	R	247.00	R	247.00										
Milk powder	R	36.00	R	6.00	R	6.00	R	6.00	R	6.00	R	6.00	R	6.00
Net income	[illegible]													

CHAPTER 5 – CRITICAL EVALUATION, FUTURE RESEARCH PROPOSALS AND GENERAL CONCLUSION

5.1 CRITICAL EVALUATION

Egg quality indices were monitored subjectively and only a few of these indices were measured. Qualities such as blood and meat spots, the Hough unit measure of internal egg quality and specific gravity were not determined in this trial.

Hens were caged two in a cage. It might be better to cage the hens individually for more accurate measurements. Due to technical reasons and a lack of labour this practice was not followed.

The trial was stopped to soon, because more significant differences were expected later in the production cycle. Due to financial reasons and technical difficulties the trial had to be stopped. Boling *et al.* (2000) found that the P-deficiency symptoms becomes more pronounced at a later stage in the production cycle (70 weeks and older).

Representative samples (hens) were weighed each week. In future each hen could be weighed individually if enough labour is present.

The feeding system in the layer house did not allow for accurate feed intake measurement. Too much of the feed is wasted over the edge of the feed troughs. A new feeding system should be considered.

5.2 FUTURE RESEARCH PROPOSALS

The influence of phytase and available phosphorus on older laying hens (65 weeks +): Boling *et al.* (2000) found that at lower available phosphorus levels the egg production differences became more pronounced by 73 weeks of age. The determining of specific available phosphorus levels for older laying hens, with the inclusion of phytase.

Boling *et al.* (2000) suggested that the rapid onset of P-deficiency symptoms in the 70 week old hens used in his experiment was attributed to the fact that the older hens may be more sensitive to dietary P deficiency than are younger birds early in the production cycle.

The effect of phytase, different available phosphorus levels and choice feeding on layers should be investigated. Specific available phosphorus levels could then be determined for layers at a specific age.

Alternative sources of phosphorus for laying hens could be investigated.

The efficacy of different phytases and different phytase levels needs to be investigated.

The effect of phytase on bio-availability of phosphorus and calcium in layers. Ahmad *et al.* (2000) found an increase in body weight in broilers, and a decrease of Ca and P in the excreta.

The effect of 1, 25–Dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by layers: It seems that 1, 25 – Dihydroxycholecalciferol and phytase has a positive effect on the utilization of phytate phosphorus, but further investigation is needed.

CHAPTER 6 - REFERENCES

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CHAPTER 7 – APPENDIX

APPENDIX A. TEMPERATURES (°C)

APPENDIX B. SELECTED RAW DATA.

Results of laboratory analyses (University of Natal)
Results of laboratory analyses (ARC – Irene Analytical Services)

APPENDIX C. CERTIFICATION OF PREMIX
APPENDIX D. MEANS AND STANDARD DEVIATIONS

APPENDIX A: TEMPERATURES (°C)

Date	Min	Max	Min	Max	Min	Max	Min	Max
29-May	7	18	5	20	6	21	6	21
30-May	7	18	6	18	6	20	5	19
31-May	7	20	6	19	6	21	6	19
01-Jun	7	20	5	21	5	21	5	22
02-Jun	7	18	6	19	6	21	5	21
03-Jun	7	18	5	18	8	21	6	19
04-Jun	11	20	11	19	13	21	11	21
05-Jun	10	18	10	18	9	19	10	18
06-Jun	10	18	10	19	10	20	9	19
07-Jun	11	20	9	21	9	19	7	19
08-Jun	11	20	11	20	10	21	9	21
09-Jun	10	20	9	21	10	20	9	22
10-Jun	11.5	20	11	21	13	19	10	23
11-Jun	12	20	12	19	12	19	10	17
12-Jun	11	21	10	21	11	23	9	22
13-Jun	11	21	10	22	11	24	10	23
14-Jun	11	21	10	22	11	24	10	23
15-Jun	11	21	10	22	10	23	7	22
16-Jun	11	21	10	22	10	23	7	22
17-Jun	14	18	14	18	10	24	13	18
18-Jun	11	20	11	21	13	21	10	22
19-Jun	12	21	13	22	13	21	11	23
20-Jun	11	18	10	18	12	17	10	19
21-Jun	11	14	11	14	13	16	10	15
22-Jun	9	18	8	19	9	17	6	19
23-Jun	8	18	10	18	9	20	7	19
24-Jun	11	18	10	18	9	17	7	19
25-Jun	11	17	10	17	10	18	7	18
26-Jun	10	18	9	18	9	19	6	19

27-Jun	7	20	7	21	9	21	6	21
28-Jun	7	20	6	21	9	21	6	22
29-Jun	7	20	7	21	8	21	6	21
30-Jun	7	20	7	21	9	21	6	22

Date	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Jul	8	18	8	18	9	20	6	19
02-Jul	8	18	7	19	9	21	6	19
03-Jul	9	19	9	18	9	20	7	21
04-Jul	8	18	8	18	8	19	6	20
05-Jul	8	18	8	18	8	19	6	20
06-Jul	7	18	7	18	9	21	5	21
07-Jul	7	18	7	18	9	20	5	20
08-Jul	8	18	7	18	10	20	6	19
09-Jul	7	18	7	18	9	20	6	18
10-Jul	7	18	7	18	9	20	6	18
11-Jul	8	20	8	21	9	21	7	22
12-Jul	11	19	11	20	13	20	10	20
13-Jul	11	19	11	20	13	20	10	20
14-Jul	11	19	11	20	10	21	10	20
15-Jul	10	19	9	21	9	21	9	20
16-Jul	4	20	8	20	9	20	6	21
17-Jul	4	14	3	15	5	17	3	15
18-Jul	5	18	6	18	5	21	3	20
19-Jul	8	20	9	20	9	20	9	19
20-Jul	4	17	5	18	5	18	3	19
21-Jul	4	18	4	19	6	19	4	20
22-Jul	7	19	7	18	9	20	6	18
23-Jul	7	19	7	19	9	20	6	19
24-Jul	8	20	10	21	9	20	7	21
25-Jul	8	20	10	21	9	20	7	21

26-Jul	7	19	9	20	8	19	6	20
27-Jul	7	19	9	20	8	19	6	20
28-Jul	8	23	8	18	9	21	6	19
29-Jul	8	20	8	22	10	23	6	20
30-Jul	11	21	9	21	10	21	7	22
31-Jul	7	21	8	20	10	20	9	20

Date	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Aug	11	21	9	22	10	23	7	22
02-Aug	11	20	11	21	13	21	10	22
03-Aug	11	20	9	21	10	21	7	21
04-Aug	10	21	10	20	10	22	8	20
05-Aug	11	22	10	22	10	24	10	23
06-Aug	11	21	11	21	13	20	11	19
07-Aug	11	21	11	23	13	23	10	22
08-Aug	10	20	9	22	10	24	7	23
09-Aug	11	22	9	22	10	24	7	23
10-Aug	11	21	9	22	10	24	7	23
11-Aug	11	21	9	22	10	24	7	23
12-Aug	11	24	10	22	14	24	9	23
13-Aug	11	24	10	22	14	24	9	23
14-Aug	11	22	11	22	13	24	14	19
15-Aug	12	21	9	22	9	24	7	23
16-Aug	12	21	9	22	9	24	7	23
17-Aug	11	21	10	22	10	21	9	23
18-Aug	12	13	10	24	11	21	10	25
19-Aug	12	23	10	24	11	24	10	25
20-Aug	12	21	12	22	13	24	11	23
21-Aug	12	21	11	22	13	24	10	23
22-Aug	11	24	11	25	13	24	10	26
23-Aug	11	24	11	25	13	24	10	26

24-Aug	13	24	14	25	13	25	11	25
25-Aug	13	24	12	25	13	24	11	25
26-Aug	13	24	12	25	13	24	11	25
27-Aug	14	25	14	25	13	26	11	26
28-Aug	14	25	14	25	13	26	11	26
29-Aug	12	25	11	26	13	26	11	26
30-Aug	12	25	11	26	13	26	11	26
31-Aug	12	27	11	26	13	26	11	26

Date	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Sep	12	27	12	27	14	27	12	27
02-Sep	12	27	12	27	14	27	12	27
03-Sep	12	27	12	27	14	27	12	27
04-Sep	12	27	12	27	14	27	12	27
05-Sep	15	27	14	25	14	24	14	26
06-Sep	15	27	14	25	14	24	14	26
07-Sep	12	27	11	26	14	28	11	26
08-Sep	12	27	11	26	14	28	11	26
09-Sep	12	25	13	25	14	27	11	26
10-Sep	12	25	13	25	14	27	11	26
11-Sep	15	27	14	26	14	27	14	27
12-Sep	15	27	14	26	14	27	14	27
13-Sep	12	28	10	29	10	29	10	29
14-Sep	12	28	10	29	10	29	10	29
15-Sep	14	29	16	29	18	30	16	29
16-Sep	11	24	15	25	17	24	14	25
17-Sep	11	22	11	22	13	21	10	22
18-Sep	11	22	11	22	13	21	10	22
19-Sep	11	25	11	25	13	24	11	26
20-Sep	11	21	10	22	13	21	11	23
21-Sep	11	21	10	22	13	21	11	23

22-Sep	12	25	13	26	14	24	14	22
23-Sep	12	25	14	26	14	24	14	26
24-Sep	15	28	14	28	14	28	14	29
25-Sep	15	28	15	28	14	29	15	28
26-Sep	15	28	14	28	15	28	15	28
27-Sep	14	27	14	28	14	28	15	29
28-Sep	15	28	15	28	15	25	15	28
29-Sep	15	28	15	28	15	28	15	28
30-Sep	16	30	17	30	17	31	17	30

Date	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Oct	18	29	17	28	17	30	17	28
02-Oct	15	28	16	29	18	28	16	29
03-Oct	18	27	18	30	19	29	17	30
04-Oct	17	28	17	30	18	28	17	30
05-Oct	16	28	18	27	17	30	18	29
06-Oct	17	28	18	30	19	28	18	30
07-Oct	14	28	15	29	15	26	14	30
08-Oct	13	24	14	25	13	23	13	24
09-Oct	16	29	17	29	15	28	15	30
10-Oct	16	29	17	29	17	31	16	31
11-Oct	17	27	15	26	16	27	15	27
12-Oct	15	25	14	25	14	24	14	26
13-Oct	16	31	15	30	17	32	15	30
14-Oct	16	28	14	29	16	30	15	29
15-Oct	16	31	15	31	17	32	15	32
16-Oct	15	33	15	33	15	33	15	33
17-Oct	16	33	16	33	16	25	16	33
18-Oct	16	29	15	28	21	26	15	30
19-Oct	16	29	16	29	18	24	16	29
20-Oct	16	25	16	24	16	24	15	26

21-Oct	16	26	16	26	16	25	16	24
22-Oct	14	25	14	26	16	26	16	26
23-Oct	14	25	14	26	16	28	15	26
24-Oct	16	28	15	28	17	26	15	29
25-Oct	14	24	14	24	15	27	14	26
26-Oct	15	25	14	26	17	26	15	26
27-Oct	16	26	16	26	17	26	15	26
28-Oct	15	25	16	26	16	27	15	26
29-Oct	16	26	16	26	15	26	14	26
30-Oct	15	25	14	26	15	26	15	26
31-Oct	15	25	15	25	16	27	15	25

Date	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Nov	14	25	14	25	14	25	14	24
02-Nov	15	28	14	28	14	28	14	27
03-Nov	15	28	14	28	14	28	15	27
04-Nov	15	27	14	26	15	27	14	27
05-Nov	18	28	18	30	18	31	17	30
06-Nov	17	28	17	28	17	28	14	30
07-Nov	17	28	17	28	17	28	14	30
08-Nov	17	29	17	27	15	28	14	26
09-Nov	18	29	18	30	17	29	14	30
10-Nov	18	31	17	30	19	31	18	30
11-Nov	17	30	17	30	19	30	17	30
12-Nov	19	29	18	30	18	30	18	28
13-Nov	17	28	18	30	17	29	17	29
14-Nov	19	29	18	29	18	29	18	29
15-Nov	19	29	17	29	19	29	19	30
16-Nov	17	28	17	28	18	28	18	29
17-Nov	19	25	17	26	17	26	17	25
18-Nov	19	24	15	22	15	23	14	24

19-Nov	15	21	14	22	15	24	15	22
20-Nov	15	23	15	23	15	23	15	23
21-Nov	15	23	14	22	15	24	14	23
22-Nov	15	24	14	22	15	24	15	22
23-Nov	15	30	15	30	15	30	15	28
24-Nov	15	30	15	30	15	30	15	28
25-Nov	15	30	15	31	15	30	15	30
26-Nov	15	29	15	29	15	29	15	29
27-Nov	15	28	15	25	15	28	15	28
28-Nov	15	25	15	25	15	25	15	26
29-Nov	15	27	15	27	15	27	15	27
30-Nov	15	27	15	27	15	27	15	27

Date	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
01-Dec	15	28	15	28	15	28	15	28
02-Dec	15	29	15	28	15	28	15	28
03-Dec	21	32	21	28	21	32	21	32
04-Dec	21	32	21	30	21	32	21	32
05-Dec	21	34	21	34	21	34	21	34
06-Dec	21	32	21	34	21	34	21	34
07-Dec	19	30	19	33	19	20	20	34
08-Dec	19	24	19	24	19	24	19	24
09-Dec	20	26	20	26	20	27	20	26
10-Dec	20	24	20	24	20	25	20	24
11-Dec	20	24	19	24	20	25	19	24

APPENDIX B: SELECTED RAW DATA.

Results of laboratory analyses (University of Natal)
Results of laboratory analyses (ARC – Irene Analytical Services)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
UNIVERSITY OF NATAL

Department of Animal Science and Poultry Science

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P.O. Box 14461
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14-Nov-00

RESULTS OF LABORATORY ANALYSES

Results on ~~a dry~~/as is basis

OUR CODE	DATE RECEIVED	DESCRIPTION	PROTEIN (%)	MOIST (%)	MILLED MOIST (%)	AMEn (MJ/kg)	TMEn (MJ/kg)	AV. AMINO ACIDS
R1	29-09-00	1.	15.37	10.20	9.50	10.023	10.433	ATTACHED
R2		2.	15.91	10.25	8.70	11.090	11.501	ATTACHED
R3		3.	15.42	10.10	8.70	11.417	11.828	ATTACHED
R4		4.	15.55	9.60	8.50	10.908	11.318	ATTACHED

Chief Technician

Important Notes:
The report covers the sample submitted and does not guarantee the composition of the bulk of the material from which the sample was drawn
While every care is taken to ensure the accuracy of any work undertaken by the feed evalution unit, the University of Natal accepts no responsibility for losses, claims or litigation arising from the sale or use of the material examined.

Private bag X01 : Scottsville 3209 : South Africa : Tel (033) 2605480 : Fax (033) 2605067

ANIMAL AND POULTRY SCIENCE
UNIVERSITY OF NATAL
SCOTTSVILLE
3209

DATE 9th November 2000

CUSTOMER **University of Pretoria**
Jaco Hattingh

DESCRIPTION Sample No 1

LAB CODE R1

	% SAMPLE	AVAIL	% AVAIL
Protein	15.086		
Aspartic	1.383	1.173	84.81
Threonine	0.538	0.448	83.22
Serine	0.574	0.511	89.14
Glutamic	2.906	2.630	90.50
Proline	0.948	0.823	86.85
Glycine	0.691		
Alanine	0.821	0.687	83.69
Valine	0.819	0.657	80.22
Methionine	0.289	0.278	96.18
Isoleucine	0.687	0.585	85.14
Leucine	1.331	1.183	88.87
Tyrosine	0.375	0.329	87.63
Phenylalanine	0.756	0.656	86.77
Histidine	0.442	0.362	81.92
Lysine	0.824	0.575	69.85
Arginine	0.992	0.871	87.85
Ammonia	1.240		

Protein calculated on amino acid recovery.
Results on an as is base
Total Protein (Leco) 15.37

TECHNICIAN Marianne Hundley

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DATE 9th November 2000

CUSTOMER **University of Pretoria**
Jaco Hattingh

DESCRIPTION Sample No 3

LAB CODE R3

	% SAMPLE	AVAIL	% AVAIL
Protein	14.778		
Aspartic	1.366	1.200	87.82
Threonine	0.550	0.475	86.43
Serine	0.599	0.545	90.99
Glutamic	2.928	2.694	92.00
Proline	0.964	0.860	89.27
Glycine	0.693		
Alanine	0.807	0.714	88.47
Valine	0.821	0.697	84.97
Methionine	0.269	0.260	96.80
Isoleucine	0.677	0.596	88.15
Leucine	1.316	1.197	90.91
Tyrosine	0.374	0.341	91.06
Phenylalanine	0.742	0.660	89.02
Histidine	0.438	0.376	85.89
Lysine	0.764	0.608	79.57
Arginine	0.958	0.868	90.65
Ammonia	0.998		

Protein calculated on amino acid recovery.
Results on an as is base
Total Protein (Leco) 15.42

TECHNICIAN Marianne Hundley

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DATE 9th November 2000

CUSTOMER **University of Pretoria**
Jaco Hattingh

DESCRIPTION Sample No 2

LAB CODE R2

	% SAMPLE	AVAIL	% AVAIL
Protein	15.507		
Aspartic	1.386	1.250	90.19
Threonine	0.573	0.511	89.30
Serine	0.654	0.599	91.55
Glutamic	3.017	2.834	93.92
Proline	0.980	0.888	90.59
Glycine	0.708		
Alanine	0.824	0.755	91.66
Valine	0.820	0.719	87.70
Methionine	0.324	0.319	98.47
Isoleucine	0.683	0.622	91.12
Leucine	1.318	1.228	93.23
Tyrosine	0.388	0.367	94.78
Phenylalanine	0.749	0.687	91.68
Histidine	0.438	0.386	88.01
Lysine	0.798	0.675	84.55
Arginine	1.017	0.945	92.88
Ammonia	1.336		

Protein calculated on amino acid recovery.
Results on an as is base
Total Protein (Leco) 15.91

TECHNICIAN Marianne Hundley

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3209

DATE 9th November 2000

CUSTOMER **University of Pretoria**
Jaco Hattingh

DESCRIPTION Sample No 4

LAB CODE R4

	% SAMPLE	AVAIL	% AVAIL
Protein	14.968		
Aspartic	1.370	1.209	88.20
Threonine	0.521	0.448	85.94
Serine	0.535	0.466	87.00
Glutamic	2.921	2.716	92.96
Proline	0.954	0.850	89.16
Glycine	0.697		
Alanine	0.820	0.738	89.93
Valine	0.832	0.720	86.54
Methionine	0.305	0.298	97.57
Isoleucine	0.691	0.618	89.37
Leucine	1.327	1.220	91.96
Tyrosine	0.362	0.338	93.39
Phenylalanine	0.751	0.680	90.52
Histidine	0.438	0.380	86.58
Lysine	0.762	0.623	81.74
Arginine	0.982	0.896	91.21
Ammonia	1.189		

Protein calculated on amino acid recovery.
Results on an as is base
Total Protein (Leco) 15.55

TECHNICIAN Marianne Hundley

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

ARC-Irene Analytical Services
LNR-Irene Analitiese Dienste

SANAS ACCREDITED LABORATORY T0063

Private Bag/Privaatsak X2, Irene, 0062 Tel: (012) 672 9294 Fax: (012) 665 1605

in association with
in samewerking met

Taurus Milk Laboratory/Taurus Melk Laboratorium

Private Bag/Privaatsak X5, Irene, 0062 Tel: (012) 672 9390 Fax: (012) 665 1579

Navrae: J Collier 2001-01-30
Tel: (012) 672 9040

Die Bestuurder
Dept Vee & Wildkunde
Univ ersitiet van Pretoria
PRETORIA
0001

Tel nr.: 012 420 2999
Faks nr.: 012 420 3290

Aandag: S M Hattingh / Jaco Hattingh

TOETSVERSLAG

Datum ingehandig: 2001-01-16
Datum afgehandel: 2001-01-25
Toetsverslag no.: 01/023

RESULTATE VAN HOENDERMIS

Neem asb kennis dat:

1. Toetsuitslae is slegs van toepassing op die monsters getoets.
2. Hierdie verslag mag nie versprei word sonder die toestemming van die kwaliteitsbestuurder nie.
3. Die monster ontvang, is deeglik gemeng voor toetsing.
4. Monsters sal vir een maand gestoor word vir enige navrae.
5. Chromatogramme is beskikbaar op aanvraag.
6. Enige opinies, afleidings en/of opmerkings uitgespreek in hierdie verslag, word nie deur SANAS onderskryf nie.

Die uwe

BESTUURDER: LNR-IRENE

TOETSVERSLAG

Datum ingehandig: 2001-01-16
Datum afgehandel: 2001-01-25
Toetsverslag no.: 01/023

RESULTATE VAN HOENDERMIS

Die resultate is uitgedruk op 'n nat basis, met ander woorde soos monster ontvang is

Ontleding	Droë materiaal	Vog	Fosfor
Akkreditasie nommer	ASM 047	ASM 047	ASM 045
Eenhede	%	%	%
Monsternommer			
1	21.65	78.35	0.13
2	21.18	78.82	0.30
3	22.70	77.30	0.29
4	19.77	80.23	0.26
5	21.80	78.20	0.29
6	26.04	73.96	0.24
7	20.16	79.84	0.39
8	21.18	78.82	0.34
9	22.71	77.29	0.16
10	25.07	74.93	0.33
11	22.15	77.85	0.32
12	21.08	78.92	0.27
13	23.52	76.48	0.25
14	21.58	78.42	0.33
15	21.25	78.75	0.34
16	18.28	81.72	0.40

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TOETSVERSLAG

Datum ingehandig: 2001-01-16
Datum afgehandel: 2001-01-25
Toetsverslag no.: 01/023

RESULTATE VAN HOENDERMIS

Resultate uitgedruk op 'n droë basis (uitgesonderd Droë Materiaal en Vog wat uitgedruk is op 'n nat basis, dus soos monster ontvang is)

Ontleding	Droë materiaal	Vog	Fosfor
Akkreditasie nommer	ASM 047	ASM 047	ASM 045
Eenhede	%	%	%
Monsternommer			
1	21.65	78.35	0.58
2	21.18	78.82	1.40
3	22.70	77.30	1.27
4	19.77	80.23	1.30
5	21.80	78.20	1.32
6	26.04	73.96	0.92
7	20.16	79.84	1.94
8	21.18	78.82	1.63
9	22.71	77.29	0.70
10	25.07	74.93	1.31
11	22.15	77.85	1.43
12	21.08	78.92	1.26
13	23.52	76.48	1.08
14	21.58	78.42	1.53
15	21.25	78.75	1.62
16	18.28	81.72	2.17

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ARC • LNR

ARC-Irene Analytical Services
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SANAS ACCREDITED LABORATORY T0063

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in association with
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Private Bag/Privaatsak X5, Irene, 0062 Tel: (012) 672 9390 Fax: (012) 665 1579

Navrae: J Collier
Tel: (012) 672 9040

2001-02-05

Die Bestuurder
Departement Vee en Wildkunde
Universiteit van Pretoria
0002

Tel nr.: 012 420 2999
Faks nr.: 012 420 3290

Aandag: S M Steenkamp / Jaco Hattingh

TOETSVERSLAG

Datum ingehandig: 2001-01-18
Datum afgehandel: 2001-02-02
Toetsverslag no.: 01/022

RESULTATE VAN VOERE

Neem asb kennis dat:

1. Toetsuitslae is slegs van toepassing op die monsters getoets.
2. Hierdie verslag mag nie versprei word sonder die toestemming van die kwaliteitsbestuurder nie.
3. Die monster ontvang, is deeglik gemeng voor toetsing.
4. Monsters sal vir een maand gestoor word vir enige navrae.
5. Chromatogramme is beskikbaar op aanvraag.
6. Enige opinies, afleidings en/of opmerkings uitgespreek in hierdie verslag, word nie deur SANAS onderskryf nie.

Die uwe

BESTUURDER: LNR-IRENE

TOETSVERSLAG

Datum ingehandig: 2001-01-18
Datum afgehandel: 2001-02-02
Toetsverslag no.: 01/022

RESULTATE VAN VOERE

Die resultate is uitgedruk op 'n nat basis, met ander woorde soos monster ontvang is

\# Nie 'n SANAS geakkrediteerde metode

Ontleding:	Phytaat fosfaat #
Eenheid:	%
Monsternommer	
Proef 1 Ransoen 1	0.20
Proef 4 Ransoen 2	0.22
Proef 13 Ransoen 3	0.00
Proef Ransoen 4	0.05

APPENDIX C. CERTIFICATION OF PREMIX.

UNIVERSITEIT VAN PRETORIA
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YUNIBESITHI YA PRETORIA

BASF Neuvet (PTY) LTD (EDMS) BPK
Reg. No. 92/01478/07
Animal Feed Additives/Dierevoerbyvoegsels

P.O. Box/Posbus 1783
Kempton Park 1620
South Africa/Suid-Afrika
Tel: (W) (011) 974 4171
Fax/Faks: (011) 392 5718

LYSINE

1 X 15KG

BATCH NO: 030307
EXPIRY DATE: NOVEMBER 2000

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BASF Neuvet (PTY) LTD
(EDMS) BPK
Reg. No. 92/01478/07
Animal Feed Additives/Dieravoerbyvoegsels

P.O. Box/Posbus 1783
Kempton Park 1620
South Africa/Suid-Afrika
Tel: (W) (011) 974 4171
Fax/Faks: (011) 392 5718

METHIONINE

1 X 17KG

BATCH NO: 171797
EXPIRY DATE: NOVEMBER 2000

BASF Neuvet (PTY) LTD
(EDMS) BPK
Reg. No. 92/01478/07
Animal Feed Additives/Dierevoerbyvoegsels

P.O. Box/Posbus 1783
Kempton Park 1620
South Africa/Suid-Afrika
Tel: (W) (011) 974 4171
Fax/Faks: (011) 392 5718

NATUPHOS

1.2KG

BATCH NO: 210518
EXPIRY DATE: NOVEMBER 2000

UNIVERSITEIT VAN PRETORIA
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BASF Neuvet (PTY) LTD
(EDMS) BPK
Reg. No. 92/01478/07
Animal Feed Additives/Diereavoerbyvoegsels

P.O. Box/Posbus 175
Kempton Park 1620
South Africa/Suid-Afrika
Tel: (W) (011) 974 4171
Fax/Faks: (011) 392 5718

THREONINE

1 X 1KG

BATCH NO: 8298
EXPIRY DATE: NOVEMBER 2000

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

BASF Animal Nutrition S.A. (Pty) Ltd. (Edms) Bpk.
Reg. No. 1987/000152/07
Animal Feed Additives/Dierevoerbyvoegsels

7 Foundry Rd/Weg, Isando
P.O. Box /Posbus 1783
Kempton Park 1620
South Africa/Suid-Afrika
Tel: +27 11 974-4171
Fax/Faks: +27 11 392-5718
e-mail: neuvet@pop.onwe.co.za

NEUVET STANDARD LAYER PREMIX (1 TON)
NEUVET STANDAARD LÊ VOORMENGSEL (1 TON)
(Class: Vitamin and Mineral premix for Layers)
(Klas: Vitamien en Minerale voormengsel vir Le-Henne)
Reg. No. V10355 Act/Wet 36 of 1947

EACH UNIT CONTAINS / ELKE EENHEID BEVAT

Vitamin A / Vitamien A	10 000 000	iu/ie
Vitamin D3 / Vitamien D3	3 500 000	iu/ie
Vitamin E / Vitamien E	15 000	iu/ie
Vitamin K3 / Vitamien K3	1.5	g
Vitamin B1 / Vitamien B1	2	g
Vitamin B2 / Vitamien B2	4	g
Niacin / Niasien	28	g
Cal Pan / Ca Pantotenaat	7	g
Vitamin B12 / Vitamien B12	20	mg
Vitamin B6 / Vitamien B6	2,5	g
Choline / Cholien	300	g
Folic Acid / Foliensuur	0,5	g
Biotin / Biotien	25	mg
Manganese / Mangaan	70	g
Zinc / Sink	30	g
Copper / Koper	6	g
Iodine / Jodium	1	g
Cobalt / Kobalt	0.5	g
Ferrous / Yster	30	g
Selenium / Selenium	0.15	g

2.5KG MASS / MASSA 2.5KG

USAGE RATE

Add 1 unit to 1 Ton final feed
Store in a cool dry place

GEBRUIKSAANWYSINGS

Voeg 1 eenheid by 1 Ton finale voer
Berg op 'n koel droë plek

BATCH NO 33415
EXPIRY DATE MARCH 2001

Appendix D. Means and standard deviations

Mean weight change over 28-week period (kg ±SD)

P-level (g/kg)	Phytase(FTU's) 0	150	300	450
1.5	0.0436 (±0.017067)	0.0438 (±0.0131795)	0.0486 (±0.0107842)	0.0558 (±0.0138094)
2.5	0.0374 (±0.0222666)	0.0436 (±0.018743)	0.0546 (±0.0082644)	0.053 (±0.0081854)
3.5	0.0614 (±0.0090167)	0.064 (±0.024052)	0.0582 (±0.0082583)	0.0576 (±0.0157099)
4.5	0.0514 (±0.0123814)	0.0542 (±0.0112116)	0.056 (±0.0116619)	0.0546 (±0.202929)

Mean feed intake over 28-week period (g ±SD)

P-level (g/kg)	Phytase(FTU's) 0	150	300	450
1.5	123.8152 (±0.7024153)	123.1376 (±2.8002099)	123.6958 (±2.8019311)	125.3666 (±2.2500276)
2.5	125.7538 (±2.106998)	126.6126 (±2.7978952)	124.723 (±1.927391)	123.9706 (±2.1151815)
3.5	122.3904 (±3.4650805)	124.7616 (±1.0997315)	126.5386 (±2.266318)	124.5884 (±2.4587161)
4.5	123.5562 (±3.4928239)	125.5768 (±3.1316738)	125.6992 (±1.6731286)	123.5576 (±1.2893003)

Mean % production over 28-week period (±SD)

P-level (g/kg)	Phytase(FTU's) 0	150	300	450
1.5	91.8806 (±4.9040558)	92.9582 (±0.9699068)	92.533 (±1.7052564)	93.1694 (±1.4060506)
2.5	92.334 (±1.6402954)	93.2734 (±0.8983659)	93.6104 (±0.9772885)	93.011 (±0.8550810)
3.5	91.0726 (±1.3714411)	91.2748 (±0.7111418)	92.9738 (±1.2165577)	93.0524 (±0.9929244)
4.5	91.3702 (±3.0741782)	92.1172 (±0.8477112)	90.3262 (±3.1298215)	92.1734 (±1.4011955)

Mean egg weight over 28-week period (g±SD)

	Phytase(FTU's)			
P-level (g/kg)	0	150	300	450
1.5	54.3306 (±1.0326317)	54.4626 (±0.750919)	55.4696 (±1.2787569)	55.334 (±0.9696997)
2.5	55.0646 (±1.172253)	54.4328 (±0.7432299)	55.7702 (±0.5911858)	54.9048 (±0.5693147)
3.5	54.7796 (±0.6062098)	55.4676 (±0.8213786)	55.6506 (±0.7711007)	55.3014 (±0.4138397)
4.5	54.9294 (±0.9763223)	55.548 (±0.8454398)	55.8524 (±2.0713386)	55.2104 (±0.426558)

Mean egg output over 28-week period (g/day±SD)

	Phytase(FTU's)			
P-level(g/kg)	0	150	300	450
1.5	51.0802 (±2.1248189)	51.3402 (±0.937074)	51.4088 (±1.6016904)	51.6362 (±1.5507758)
2.5	51.5862 (±1.6669807)	51.4108 (±0.9896887)	52.2902 (±1.0650252)	51.1218 (±0.5430149)
3.5	50.6822 (±0.7005308)	50.7134 (±0.8230813)	51.820 (±1.2621834)	51.513 (±0.3462116)
4.5	50.91180 (±1.6636718)	51.2156 (±1.1259531)	49.595 (±1.1484783)	50.9698 (±0.6951498)

Mean breaking strenght over 28-week period (N±SD)

	Phytase(FTU's)			
P-level(g/kg)	0	150	300	450
1.5	37.2384 (±2.5847952)	34.9396 (±6.3015578)	33.3336 (±6.2482655)	35.4796 (±3.465878)
2.5	28.619 (±1.7571646)	34.6294 (±2.3413416)	32.9006 (±5.9657984)	39.396 (±3.130659)
3.5	33.5636 (±3.7825978)	32.7336 (±3.8446324)	32.6092 (±1.6758837)	33.6108 (±3.9329059)
4.5	32.7706 (±2.2896108)	36.2152 (±5.0737118)	32.2472 (±4.0701854)	34.4472 (±6.0393453)

Mean shell thickness over 28-week period (mm±SD)

P-level(g/kg)	Phytase(FTU's) 0	150	300	450
1.5	0.3736 (±0.0090443)	0.3586 (±0.0107378)	0.3596 (±0.0129151)	0.3772 (±0.0338038)
2.5	0.362 (±0.0072801)	0.3668 (±0.0074632)	0.361 (±0.0116619)	0.3634 (±0.0064265)
3.5	0.3706 (±0.0077974)	0.358 (±0.0075166)	0.348 (±0.0058310)	0.3566 (±0.0133529)
4.5	0.3564 (±0.0041593)	0.3582 (±0.0090940)	0.353 (±0.0073824)	0.3576 (±0.0059414)