AUSTRALIAN EGG CORPORATION LIMITED

Available phosphorus requirement of laying hens

Final Project Report

A report for the Australian Egg Corporation Limited

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January 2016

AECL Publication No 1UQ101A

ISBN 1 920835 49 0

Project Title: Available phosphorus requirement of laying hens

AECL Project Number: 1UQ101

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Published in January 2016

Foreword

Phosphorus (P) has attracted much attention from both scientists and the related industries in the areas of nutrition and environmental protection. Phosphorus supplements for animal feed are derived from rock phosphate that is non-renewable and becoming increasingly scarce and expensive. Phosphorus plays an important role in various biological processes, especially in maintaining optimum egg shell quality. There has been limited research on the actual P requirement of laying hens in recent years. A larger safety margin of P in commercial poultry diets has been the common practice for many years. Excessive dietary P is excreted and causes environmental pollution. To further improve the accuracy of layer feed formulation, it is essential to have a more accurate value for the available P (AP) requirement. Any approach with the potential to reduce the dietary P supplementation of laying hens without affecting their productivity would have a significant impact in reducing the cost of egg production and wastage of P resources.

This is the report of a project with the aim to determine the AP requirement of laying hens and to examine the effect of different dietary AP and calcium (Ca) concentrations on egg production and egg shell quality from the start of lay to 80 weeks of age. The influence of dietary phytase supplementation was also examined.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report is an addition to AECL's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

We appreciate the assistance of our colleagues, Dr Wei Gong Qing, Dr Arun Kumar, Ms Katherine Raymont and Mr Peter Isherwood and our postgraduate students, Miss Xinyu Zhang and Mr Freddy Yang, who helped with bioassays, the collection and analysis of the samples.

We are grateful for support and meaningful comments from Professor Peter Dart during preparation of this report.

The constructive discussions with industrial representatives in particular Drs David Cadogan, Greg Parkinson, Greg Underwood, Louise Edwards, Santiago Ramirez-Cuevas, Kim Huang, Judy O-Keeffe, Alex Chang, Greg Connors and Chris Rowell are greatly appreciated.

This project was supported by the School of Agriculture and Food Sciences, University of Queensland, FeedWorks, Ridley AgriProducts and Hy-Line Australia.

The Australian Egg Corporation Limited provided the funds which supported this project.

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The authors have worked on phosphorus nutrition in laying hens and provided one previous report to AECL related to this area of research, and recently completed one project on *Available Phosphorus Requirement of Broilers* funded by RIRDC.

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Abbreviations

AIADC	Apparent ileal amino acid digestibility coefficient
AIA	Acid insoluble ash
AOAC	Association of Official Analytical Chemists
AP	Available phosphorus
ATP	Adenosine triphosphate
Ca	Calcium
DM	Dry matter
FCR	Feed conversion ratio
GIT	Gastro-intestinal tract
HU	Haugh unit
IDEC	ileal digestible energy coefficient
IP6	Inositol hexakisphosphate
LSD	Least significant difference
MDCP	Monodicalcium phosphate
Na	Sodium
NPP	Nonphytate phosphorus
P	Phosphorus
PTH	parathyroid hormone
PP	Phytate phosphorus
SEM	Standard error of mean
SG	Specific gravity
TP	Total phosphorus
WK	Week

Executive Summary

Phosphorus (P) is an essential nutrient that is involved in numerous body functions of laying hens including bone formation, energy storage, cellular structure, muscular contraction and egg formation. Laying hens meet their requirements for this essential nutrient from the diet. Dietary P content either in excess of or below requirement may adversely affect bird performance. The majority of P in diets is contained in plant feedstuffs where it exists within molecules of phytate which are poorly digested. To overcome this problem the feed enzyme, phytase is routinely added to diets. Moreover, genetic improvement in various laying hen performance parameters makes P requirement a moving target and information on P requirements of modern strains of laying hens is limited.

Understandably, the P requirement of laying hens is an area of ongoing debate as it is a factor that contributes to hen performance and egg quality, especially late in the laying cycle. Part of the uncertainty regarding P requirements is the basal diet fed in experiments (usually corn based) and the variable amounts of phytate P in the diets. For these reasons industry formulates diets to contain 4.0 to 4.5 g/kg of available P (AP). Therefore, within this project, two experiments were undertaken to re-evaluate the AP requirement of brown egg laying hens with or without supplemental phytase from the start of lay to 80 weeks of age and examine the effects of different dietary AP and calcium (Ca) with or without supplemental phytase on egg production, eggshell quality, Ca and P retention, tibia bone and toe ash contents, whereby, to provide safe guidelines to more cost-effectively address P requirements of laying hens.

Experiment 1 was conducted to evaluate the effects of different levels of AP with or without supplemental phytase on egg production and egg shell quality of hens from 20 to 80 weeks of age. A total 720 Hy-Line brown egg laying hens were housed in 6 bird cages in a controlled environmental (22 - 24 ^{o}C) shed with a 16-hour lighting regimen. There were 12 experimental diets and each diet was fed to 10 replicate cages. The experimental diets were based on a sorghum and wheat blend and contained the same levels of Ca (42 g/kg diet), phytate-P (2.6 g/kg diet) with graded levels of AP (1.5, 2.0, 2.5, 3.0, 3.5 and 4.5 g/kg diet) with or without phytase (Phyzyme XP, 1000 FTU/g, 450 g/tonne). Egg production and defective egg shells were recorded daily. Feed intake, bird body weight, egg weight, and egg shell quality (shell colour, specific gravity, shell breaking strength, yolk colour, albumen height, Haugh Unit, shell weight and shell thickness) were measured every four weeks.

The results indicate that egg production and egg shell quality parameters in layers fed diets containing 1.5 g/kg AP were comparable to hens fed diets containing higher AP levels with or without phytase supplementation; and suggest that dietary AP requirement can be substantially reduced. Less than 60% dietary Ca and 30% dietary P were retained in the body. Excreta P contents increased as the dietary AP concentrations increased. Supplemental dietary phytase did not affect P excretion.

Experiment 2 was to investigate the effect of different dietary Ca levels on AP requirement with or without dietary phytase supplementation in laying hens from 16 to 80 weeks of age, utilising the results of Experiment 1. The experimental diets based on sorghum and wheat contained AP levels of 1.5 and 2.5 g/kg diet and each with three levels of Ca (32, 40 and 48 g/kg diet). The diets were prepared with or without phytase supplementation. A total 720 Hy-Line brown egg laying hens were housed in the same controlled environmental shed as in Experiment 1. Each experimental diet was fed to 10 replicate cages with 6 birds per cage.

In this experiment there were no significant effects of dietary AP and Ca concentrations and supplemental phytase on henday egg production, feed intake, egg weight, egg mass, feed

to egg conversion ratio, shell defects, albumen height and Haugh Unit, shell breaking strength and tibia bone ash content.

The eggs from the birds on diets containing 40 and 48 g/kg Ca had significantly higher values in specific gravity, shell weight, shell thickness, shell weight per surface area than those from birds on diet containing 32 g/kg Ca ($P<0.05$) from the start of lay to 50 weeks of age and the same effects of Ca on these measurements were not found after 50 weeks of age. Yolk colour was lower for hens fed on diets contained Ca of 32 g/kg regardless of dietary AP concentrations and phytase supplementation.

The body weight of birds fed on diets containing AP 1.5 g/kg and Ca 32 g/kg without phytase supplementation were significantly smaller ($P<0.05$) than the rest of the treatments. Phytase increased toe ash content and blood P concentration. However, the AP of 1.5 g/kg was adequate to satisfy the need for egg production and feed to egg conversion ratio in the absence of phytase in the diet.

Overall Conclusions

- High levels of egg production throughout both experiments demonstrated that all the dietary AP concentrations fed met the P requirement of hens even at the lowest AP level of 1.5 g/kg diet.

- Dietary Ca levels did not significantly affect egg production and feed to egg conversion ratio. However, hens fed on the diets containing 32 g Ca /kg produced eggs with lighter yolk colour and with lower specific gravity, shell weight, shell thickness and shell breaking strength. Despite these differences, shell defect percentages were not significantly affected.

- The expected significant beneficial effect of phytase was not observed as the lowest concentrations of dietary AP and Ca fed, met the layers' requirements for these minerals.

- The retentions of Ca and P were lower than those reported in the literature partially due to the fact that the diets were not purposely designed to test dietary Ca and P retention.

- Phosphorus excretion was closely related to the dietary P concentrations. Hens fed diets with lower AP levels excreted much less P. Large amounts of Ca were found in excreta.

- The results obtained from the present study are in agreement with overseas reports, which suggest that modern laying hen strains have much lower AP requirements than earlier strains. The results from this project would suggest that the AP requirement is approximately 1.5 g/kg for hens fed wheat and sorghum based diets.

1 General introduction

1.1 Phosphorus

Phosphorus is essential for all forms of life (e.g. bacteria, plants and animals). It is a crucial element for normal muscle growth and egg formation, an important component of the nucleic acids of the genetic code, phospholipids, as well as a co-factor or activator of many enzyme systems. Phosphorus is required to maintain osmotic and acid-base balance and also plays a role in energy metabolism (adenosine triphosphate, ATP), amino acid metabolism and protein production. Therefore P was named “life’s bottleneck” by famous chemist and science writer Isaac Asimov (1974). ‘‘Life can multiply until all the phosphorus has gone, and then there is an inexorable halt which nothing can prevent’’, he wrote. “We may be able to substitute nuclear power for coal, and plastic for wood, and yeast for meat, and friendliness for isolation-but for P there is neither substitute nor replacement”.

Phosphorus occurs in organic and inorganic forms. Most P in feedstuffs of plant origin is provided in organic form of inositol hexakisphosphate (IP6), an inositol ring with six phosphate groups commonly referred to as phytate. Phosphorus and inositol in phytate form are, in general, considered to be poorly used by poultry because poultry lack significant amounts of the digestive enzyme phytase required to remove phosphate from the inositol in the phytate molecule (Cooper and Gowing, 1983; Kornegay, 1996). Others indicated that poultry possess effective endogenous phytase activity in the intestinal mucosa allowing for the utilisation of phytate-P (PP) (Cowieson *et al*., 2011). However, the efficacy of endogenous phytases is thought to be constrained due to high dietary Ca levels rendering phytate insoluble in the small intestine (Tamim *et al*., 2004). Any P that is not bound to the phytate molecule is referred to as nonphytate P (NPP). This NPP can be chemically determined by subtracting analysed PP from analysed total P and assumed to be completely available to poultry and other monogastric animals. Nonphytate P has been considered as AP or used interchangeably with AP (NRC, 1994).

The main ingredients of poultry diets are cereal grains and their by-products. Poultry diets usually require supplementing inorganic P, because not only of low concentrations, but also of low availability of total P. Phosphorus supplements for animal feed are derived from phosphate rock that is non-renewable and becoming increasingly scarce and expensive. Current global P reserves may be depleted in 50-100 years (Cordell *et al.*, 2009). This poses the challenges of obtaining future P supply for the international and national feed industries. Improving PP utilisation and reducing inorganic P supplementation are essential to maintain sustainable poultry production and reducing feed costs.

1.2 Phytate

Phytate is the principal storage form of P (Bryden *et al.*, 2007). The concentration of PP within the same ingredient can vary considerably. Barrier-Guillot *et al.* (1996) found that the concentration of PP ranged from 0.092 to 0.268% dry matter (DM) in wheat and depended on fertilisation of the soil, time of harvesting, stage of maturity and variety.

The availability of PP in different feed ingredients ranges from 0% (Nelson *et al.*, 1976) to almost 50-60% (Simons *et al.*, 1990; Van der Klis and Versteegh, 1996). There are many factors affecting PP utilization: dietary Ca and P concentration, dietary vitamin D3 concentration, age of bird, phytase activity of dietary ingredients, fibre and genotype (Ravindran *et al.*, 1995).

Phytate with a negative charge is capable of binding di- and trivalent cations such as Ca, cobalt, copper, iron, magnesium, manganese, nickel and zinc in very stable complexes (Maenz *et al.*, 1999; Wise, 1983) and reducing the availability of these minerals to the

animal (Pallauf and Rimbach, 1996). Therefore, phytate reduces not only the availability of P, but also of other mineral cations.

Phytate has been known to inhibit activities of some digestive enzymes such as pepsin, α-amylase (Deshpande and Cheryan, 1984) and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). Phytate may inhibit proteolysis by changing the protein configuration of digestive enzymes (Singh and Krikorian, 1982). Phytate may bind with trypsin via Ca forming a tertiary complex, thus inhibiting trypsin activity. Inhibition may also result from the chelation of Ca ions which are essential for the activity of trypsin or possibly from an interaction with the substrate for these enzymes (Liener, 1989). Phytate can also suppressed the α-amylase activity and lead to reduced starch digestion (Thompson and Yoon, 1984; Knuckles and Betschart, 1987) by complexing with the Ca ions required for enzyme activity.

It has also been reported that phytate can reduce fat digestibility by forming insoluble Ca-phytate complexes with fatty acid in the lumen of gut (Leeson, 1993). In its chelated form, the phytate molecule is difficult to hydrolyse by exogenous or endogenous phytases. The pH affects the solubility of phytate. Most phytate mineral complexes are soluble at low pH's (less than 3.5) with maximum insolubility occurring between 4 and 7 (Selle *et al.*, 2000). Champagne (1988) found that Ca phytate complexes precipitate at pH's between 4 and 6, which is the approximate pH of the intestine where the Ca ions should be absorbed. Taylor (1965) has suggested that the primary factor affecting PP utilization is the Ca ion concentration in the small intestine where insoluble Ca-phytate complexes form. A precipitated phytate mineral complex would not be accessible for hydrolysis or absorption in the intestine.

1.3 Calcium

Calcium is an essential element for bone and egg shell formation, blood clotting, muscle contraction and transmission of nerve impulses. Calcium is also involved in the regulation of heartbeat, and can act as an activator or stabiliser of enzymes and some hormone secretions. There is a close relationship between P and Ca in egg production. Calcium is the major structural element in egg shell which is highly mineralised in the form of Ca carbonate making up more than 90% of the shell.

A close relationship has been found between the timing of dietary Ca supply and egg shell quality. Roland and Harmes (1974) indicated that the specific gravity (SG) and shell thickness of eggs laid in the afternoon are greater than those laid in the morning. This finding was confirmed by Washburn and Potts (1975) who used various egg shell quality measurements including shell thickness, shell breaking strength, deformation and SG. Shell quality assessed by any of those methods in all six strains of birds examined was relatively poor for the eggs laid in the morning and progressively greater for eggs laid in the afternoon. All these results suggest that hens laying eggs in the afternoon have more light hours in which to consume dietary Ca during shell formation and therefore, do not need to draw on stored Ca in bones. However, the shell gland is usually active during the dark hours, but Ca absorption from the gut is low at that time (Scanes *et al.*, 1987) because feed is not consumed then and without this dietary Ca supply, the layer relies on other Ca sources, particularly remobilise it from bones.

The medullary bone is a specialised, highly mineralised bone and a readily mobilised source of Ca, thus, it acts as a temporary Ca reserve, releasing Ca when needed at times when supply from feed is insufficient (Etches, 1987). Since Ca is stored almost entirely as hydroxyapatite, a Ca phosphate salt in bone, bone mobilization to fulfil Ca requirement results in elevated levels of plasma P and excretion of P, especially during times of shell formation (Hurwitz and Bar, 1965). These findings explain the differences in shell strength due to time of oviposition as well as suggesting possible management approaches to improve shell quality.

According to the model developed by Dijkstra *et al.* (2006), P can't be efficiently utilised when Ca is insufficient in some hours during the day, therefore supplying more Ca in the diet or ensuring a pattern of Ca absorption that better matches instantaneous Ca requirement can reduce the mobilization from the bone and reduce P excretion. Equally, an increase in P supply may help to resynthesize bone at times when Ca supply is sufficient (during non-shell-forming hours), but this option will increase P excretion in urine.

Increases in dietary P and Ca levels may affect apparent digestion of these nutrients. High levels of dietary Ca and low levels of P had detrimental effects in laying hens (Keshavarz, 1986; 1999). Elevated dietary Ca levels increase pH in the gut and as a result P absorption (Hurwitz and Bar, 1965) and retention (Keshavarz, 1986) are decreased. High plasma P levels decrease Ca absorption from gut (Keshavarz and Austic, 1990). The correct dietary Ca to P ratio is of utmost importance for maximum egg production and an optimum egg shell quality in laying hen.

Limestone, a source of Ca, is an antacid and increases gizzard pH in pullets fed diet with high Ca concentrations (Guinotte *et al.*, 1995). In broilers high dietary Ca significantly ($P<0.05$) increased gastrointestinal pH, which may decrease pepsin activity in the proventriculus/gizzard and reduce apparent ileal crude protein digestibility ($P \leq 0.05$) although growth performance was not affected (Walk *et al.,* 2011).

1.4 Calcium and phosphorus requirements

Calcium and P are closely related so that a deficiency in one can interfere with proper utilization of the other. Phosphorus is the second most significant mineral after Ca, participating in metabolic interaction with vitamin D. In layers, requirement for dietary P is mainly due to the need to store Ca in bones prior to egg shell formation. However, P is also essential for metabolism of carbohydrates and fat, and Ca transport in egg formation.

The AP requirement recommended by NRC (1994) for laying hen diets is 2.5 g/kg diet or 250 mg/hen per day, but the levels commonly fed by industry are much higher. Dietary P content either in excess of or below requirement may adversely affect bird performance. Excess dietary P not only increases the cost of egg production, but also reduces the availability of other divalent cations and also PP by reducing phytate hydrolysis (Ballam *et al*., 1985). Although the P requirement of laying hens has been the subject of numerous investigations, the requirement for this nutrient has not been adequately established. The reported AP requirement for laying hens varied from 1.30 to 3.0 g/kg diet (Miles *et al*., 1983; Vandepopuliere and Lyons, 1992; Summers, 1995; Leeson and Caston, 1996; Gordon and Roland, 1998; Boorman and Gunaratne, 2001; Sohail and Roland, 2002; Keshavarz, 2003; Snow *et al*., 2004; 2005). Leeson and Caston (1996) reported that egg production, egg weight and egg shell deformation of layers fed diets containing AP of 2.8, 3.5 and 4.2 g/kg from 18 to 70 weeks of age were similar ($P>0.05$) (**Table 1-*1***).

Table 1-*1*: Dietary AP concentrations (g/kg) and production performance of layers at different ages (week)

AP (g/kg)	Egg production (%)	Egg weight (g)		Egg shell deformation (μm)	
		34 week	70 week	26 week	70 week
2.8	82.4	56.2	63.1	20.3	23.1
3.5	83.5	56.0	63.3	20.8	24.3
4.2	84.6	56.6	63.3	21.0	24.6

Source: Leeson and Caston (1996)

Summers (1997) suggested that dietary P levels can be reduced by up to 20% for most classes of poultry without any adverse effect on performance. Our previous study (Li and

Bryden, 2006, Li *et al.*, 2007) has shown that a dietary AP at 1.8 g/kg (or 190 mg AP/hen/day) met the P requirement of laying hens for egg production from 23 to 47 weeks of age (**Table 1-*2***). This is much lower than the NRC (1994) recommendation of 250 mg/hen/day.

Table 1-*2*: Dietary AP concentrations, henday egg production, feed conversion ratio (FCR), toe ash, apparent ileal amino acid digestibility coefficient (AIADC) and ileal digestible energy coefficient (IDEC) in hens from 23 to 47 weeks of age

AP (g/kg)	Henday egg production (%)	FCR (g feed/g egg)	Toe ash (%DM)	AIADC	IDEC
4.0	95.8	1.84	14.07	0.75	0.73
2.9	95.8	1.81	14.27	0.74	0.72
1.8	94.5	1.83	14.38	0.76	0.73
P value	>0.05	>0.05	>0.05	>0.05	>0.05

Source: Li et al. *(2007)*

Early researchers suggested approximate 2.0 g NPP kg for optimum egg production (Mikaelian and Sell, 1981; Usayran and Balnave, 1995). Composition of the diet, rearing method, age of the bird and season are known to influence the P requirement. The P requirement is lower on wheat based diets than on other cereal based diets (Salman *et al.*, 1969) which may be a consequence of the endogenous phytase content. The requirement of P for caged layers was known to be higher than on litter (Mathur *et al.*, 1982; Daghir and Farran, 1983). It is believed that birds get extra P from the litter. Scheideler and Sell (1986) reported that P requirement decreased with hen age, and therefore, they suggested different levels of NPP (3.4, 2.5 and 1.5 g /kg diet) at different phases of egg production (24-36, 36-52 and 52-72 weeks of age). Keshavarz (2003) suggested dietary NPP of 2.5, 2.0 and 1.5 g/kg at 20-35, 36-51 and 52-62 weeks of age, respectively, were adequate although there were differences between strains.

There has been limited research on the actual P requirement of laying hens in recent years (Angel, 2010). Information on P requirements of modern strains of laying hens is scarce. Therefore, in practice, the poultry industry often substantially increases the safety margins of P in the diets to insure that birds do not encounter deficiencies during production. For example, research suggests AP requirement is only 1.5 to 2.0 g/kg diet for modern laying hen strains. However, industry has routinely fed upwards of 4.0 g AP/kg diet to ensure that hens receive adequate P. There is a difference between research and industry situations that may prevent direct application of research findings to practice. For example, average daily intake of layers in research trials may be as high as 120-125 g/hen/day compared to industry average of 100 g/hen/day (Applegate and Angel, 2005).

The AP requirement for layers, recommended by NRC (1994) was based on peer-reviewed research published between 1952 and 1983. However, the present commercial layers are very different from the birds prior to 1983 because of genetic selection, improved management and feed related changes (Havenstein *et al.*, 1994; Williams *et al.*, 2000). An updated AP requirement is definitely needed.

Calcium and P balance is critical for optimal egg production and egg shell quality. The relationship between Ca, P, vitamin D_3 and the hormonal system of the layer in Ca metabolism during lay is complicated. Both excess and deficiency of Ca will negatively affect the shell quality. There are very few papers being published recently on the actual requirements for Ca (Angel, 2010). Early studies found that a low dietary Ca intake leads to a reduction in the thickness of the shell. The magnitude of this reduction is not proportional

to the reduced dietary Ca level because of the use of medullary Ca by the hen (Hurwitz and Bar, 1969; Hurwitz, 1978). High dietary Ca level may increase shell thickness (Roland *et al.*, 1977), egg SG and shell breaking strength (Scott *et al.*, 1976). However, this increase may not alter relative differences in shell strength between individuals, strains, or groups (Washburn, 1982).

Egg shell consists of 94-97% Ca in the form of Ca carbonate. Phosphorus is only small part of egg shell, but it plays a crucial role in storing Ca in bones prior to egg shell formation in addition to many other essential functions in the body. The Ca of 32.5 g/kg diet (based on 100 g of feed per day) is required for layers (NRC, 1994). Coutts and Wilson (2007) recommended Ca of 35-40 g/kg in layer diet. Hy-Line International (2014) suggested the Ca level of 40.8 g/kg diet (based on 103 g of feed per day). Calcium requirement of a laying hen is 4 - 6 times that of a non-laying hen. An egg contains almost 2 g Ca. The Ca content of cereal, small grains and soybean meal is low. Most diets are supplemented with Ca to meet the requirement of birds in the form of limestone or an inorganic phosphate source such as Ca-phosphate.

Numerous studies reported the effects of Ca source and particle size on egg shell quality. Feeding oyster shell, limestone or egg shell as the Ca source had no significant effect on egg shell quality if differences in particle size were eliminated (Roland and Harms, 1973). However, a significant improvement in shell quality was observed for larger particle limestone or oyster shell compared with finely ground supplements of each (Watkins *et al.*, 1977). The beneficial effects of larger particle size on shell quality have been attributed to the longer retention of grit particles in the gut (Roland *et al.*, 1972). This promotes a more constant supply of Ca into the circulatory system during night when most of the shell is formed. Manipulating dietary Ca levels to low concentrations during brooding and early lay and high concentrations during late lay can also improve egg shell quality (Ousterhout, 1981).

Total Ca values are currently used for feed formulation since the availabilities of Ca for raw materials have not yet been determined. Available Ca from plant raw materials is expected to be low. This is due to the high phytate content of these raw materials and the low contribution of Ca to the diet. Also, to some extent, phytate will negatively influence the availability of Ca from the dietary source by binding Ca in the forms of mineral-phytate complexes. Therefore the inorganic sources of Ca are more important factor in determining overall dietary availability.

1.5 Calcium and phosphorus absorption, excretion and retention

1.5.1 Calcium and phosphorus absorption and excretion

Early researchers reported that 60% of feed ingested passes through the gizzard within 4 h of consumption (Hurwitz and Bar, 1966; Roland *et al.*, 1972). Most Ca absorption occurs in the duodenum and jejunum in layers and broilers (Hurwitz and Bar, 1970, 1971; van der Klis *et al.*, 1990; van der Klis, 1993) (**Table 1-*3***). In laying hens, absorption also occurs in the lower gastrointestinal tract (GIT) (Pelicia *et al.*, 2009).

Table 1-*3*: Sites of Ca and P absorption or secretion in broilers

Site	Calcium	Phosphorus
Duodenum	Secretion/absorption	Secretion/absorption
Upper jejunum	Absorption	Absorption
Lower jejunum	Absorption	Absorption
Upper ileum	Absorption	No change
Lower ileum	No change	No change

Source: van der Klis (1993)

Calcium source and particle size play a role in Ca level in the gut. The absorption and secretion of Ca by different intestinal segments in laying hens is dependent on the stage of egg shell formation (Waddington *et al.*, 1989). Approximately 40 % dietary Ca was absorbed when the shell gland is inactive and more than 70% when active. Calcium is transported across the intestinal membranes by a saturable, active (transcellular) process and a non-saturable (paracellular) process. The saturable (active) process can be affected by the nutritional and physiological status of the bird (van der Klis, 1993). During Ca restriction, active transport is significantly increased (Hurwitz and Bar, 1969; Hurwitz, 1989). Calicum elimination from the body is primarily through faecal Ca, which would be unabsorbed dietary Ca and endogenous Ca. The Ca eliminated from the kidney is controlled by endocrine factors and to a lesser extent by unabsorbed dietary Ca.

The metabolism of inorganic phosphate is closely related with Ca and its homeostatic control (Anderson 2003). However, the control of P metabolism is different from that of Ca.

The absorption of inorganic phosphate by the gastro-intestinal tract (GIT) is highly efficient and not dependent on the absorption of Ca (Wasserman, 1981). Phosphorus can be absorbed effectively even when it is in excess of requirement because there is limited control of P absorption from the GIT compared to Ca. Unlike broilers in which absorption of P was most efficient from the duodenum to the upper jejunum (Hurwitz and Bar, 1970), with no net absorption occurring in the lower GIT, layers absorb P throughout the whole intestine, but the rate of absorption declines in the lower tract. As is the case with Ca, laying hens show differences in P absorption and excretion based on stage of egg shell formation. As a result of bone mobilisation, plasma P concentration is greatest during the period of egg shell calcification (Mongin and Sauver, 1979). Wasserman and Taylor (1973) suggested that the absorption of P is a saturable and active process.

The concentration of P in the body is tightly regulated by renal excretion in which hormones and metabolic factors are involved in maintaining P homeostasis (Berndt and Knox, 1992). In practical terms, dietary Ca may be available but not absorbed because of the Ca status of the animal, whereas a large portion of dietary P that is available will be absorbed but may be eliminated though the urine (Hegsted, 1973; Leske and Coon, 2002). Birds can tolerate higher P than Ca in their diets. Therefore, excessive amount of P in commercial poultry diets has been the common practice.

Like many other minerals our understanding of the mechanisms of P absorption is still limited. The hormonal form of vitamin D increases P absorption, but much less is known than vitamin D-mediated Ca absorption (Anderson 2003).

Factors which may affect gastrointestinal absorption of Ca and P include dietary concentration and sources, physical and chemical forms of these minerals, vitamin D, passage rate of feed and viscosity of digesta, chelating agents and mineral interactions, GIT pH, and interactions with dietary protein, fat and carbohydrate (Hayes, 1976; van der Klis, 1993). Phosphorus absorption is optimal at pH 5.5-6.0. Excess free fatty acids in the diet can cause a decrease in pH in the GIT and thereby, interfere with Ca and P absorption (Coutts and Wilson, 2007).

The quantity of Ca and P excreted by the urine is dependent on the rates of kidney secretion and reabsorption of the minerals. Factors affecting kidney excretion of Ca and P are parathyroid hormone levels, dietary vitamin D, Ca and P levels and stage of egg shell formation (Wideman, 1987).

The blood inorganic P, but not Ca level was increased proportionately with increase in the dietary NPP level (Reichmann and Connor, 1977; Miles *et al.,* 1983; Keshavarz, 1986; Rama Rao *et al.,* 1999). The increase in serum inorganic P level with increase in dietary NPP content is not reflected either in increased shell quality or increased egg production.

Blood or plasma inorganic P concentrations have only an indirect influence on the amount of P secreted by the kidney (Wideman, 1984). Wideman (1987) suggested that Ca availability for egg shell formation controls urinary Ca and P excretion patterns in laying hens. Urinary P excretion increased and urinary Ca excretion decreased when bone minerals are mobilized for egg shell formation (Coon *et al.*, 2002). Urinary P excretion is also regulated by parathyroid hormone (PTH), which inhibits tubular reabsorption of inorganic P. Both high dietary Ca and low dietary P depress urinary P excretion. A high Ca, low P diet would result in very high urinary Ca excretion (Wideman, 1987). Increased dietary P increases kidney P excretion, whereas low dietary P stimulates P reabsorption by the kidneys (Wideman, 1989). Inadequate Ca in the diet or an incorrect Ca to P ratio may lead to a condition known as secondary nutritional hyperparathyroidism in which PTH secretion increases and the parathyroid glands enlarge. Excessive production of PTH leads to progressive demineralization of bone. Therefore, the optimum Ca to P ratio is vital for laying hens to achieve maximum genetic potential performance.

Li and Bryden (2006) found that P excretion increase as the dietary P increase and approximately 64 to 72% of total P has been excreted (**Table 1-*4***). Summers (1995) reported that more than 70% of total P or 60% of AP could be excreted (**Table 1-*5***).

Table 1-*4*: Phosphorus intake (g/h/d), excretion and retention (g/h/d) of ISA brown layers at 47 weeks old

Diet TP (AP) (g/kg)	TP (AP) intake (g/h/d)	Excreta P (g/kg DM)	Excreta P (g/h/d)	P retention (g/h/d)
4.8 (1.8)	0.52 (0.19)	11.7	0.33	0.19
5.9 (2.9)	0.64 (0.32)	16.2	0.46	0.18
7.0 (4.0)	0.77 (0.44)	18.1	0.52	0.25

Source: Li and Bryden (2006)

Table 1-*5*: Phosphorus intake, excretion and retention of laying hens

Age (week)	Diet TP (AP)	Feed Intake	TP (AP) intake	Excreta TP	P retention	Diet AP excreted	Diet AP excreted
	(g/kg)	(g/h/d)	(g/h/d)	(g/kg DM)	(g/b/d)	(g/h/d)	(g/h/d)
25	4.7 (3.0)	103	0.48 (0.31)	17.9	0.40	0.084	0.22
	5.9 (4.0)	104	0.61 (0.42)	20.9	0.49	0.124	0.29
32	4.7 (3.0)	104	0.49 (0.31)	13.9	0.36	0.129	0.18
	5.9 (4.0)	105	0.62 (0.42)	19.1	0.47	0.150	0.27
44	4.7 (3.0)	105	0.49 (0.32)	14.9	0.37	0.124	0.19
	5.9 (4.0)	107	0.63 (0.43)	19.2	0.49	0.141	0.29
60	4.7 (3.0)	100	0.47 (0.30)	17.0	0.37	0.100	0.20
	5.9 (4.0)	102	0.60 (0.41)	20.7	0.48	0.122	0.29

Source: Summers (1995)

Department of Environment (2004) reported that in excreta DM of caged layers Ca averaged 3.9% with a range of 3.6 - 6.0 % and P averaged 2.0 % with a range of 0.5 - 3.4 %. Much higher levels of Ca and P in caged layer manure were reported by Wiedemann *et al.* (2008) (**Table 1-*6***).

Table 1-*6*: Calcium and P concentrations (%DM) in manure of caged laying hen

	Mean (range) (%DM)			
Nutrient	**Cage layer[1]**	**Cage layer[2]**	**Cage layer[3]**	**Cage layer mean**
Ca	10.2 (7.7-14)	12.4 (8.0-15.9	11.6 (7.0-15.1)	11.3 (7.0-15.9)
P	2.0 (1.1-2.4)	2.6 (2.1-3.1)	2.9 (2.1-3.7)	2.5 (1.1-3.7)

[1]Caged layer hen systems with belt removal and No manure drying
[2]Caged layer hen systems with belt removal and manure drying
[3]High rise caged layer hen systems

Source: Wiedemann et al. *(2008)*

1.5.2 Calcium and phosphorus retention

Calcium retention was 50 - 55% when birds were fed a diet balanced for Ca and egg production maintained at 70% or more (Hurwitz and Griminger, 1962). Nahashon *et al.* (1994) reported that retentions from maize and soybean meal diet for laying pullets were 58.9% for Ca and 24.4% for P. At higher daily Ca intakes, percentage retention of Ca decreases (**Table 1-7**) and net retention increases with increasing dietary Ca level at 21 and 24 weeks of age (Rao and Brahmakshatriya, 1976).

Table 1-7: Calcium retention in layers

Diet Ca (g/kg)	Calcium retention (%)		
	18 weeks	**21 weeks**	**24 weeks**
10.5	33.33	32.91	40.9
22.0	48.46	26.99	37.69
33.0	39.9	21.57	37.56

Source: Rao and Brahmakshatriya (1976)

Keshavarz (1986) also found that as Ca concentration increased, net Ca retained increased although percentage retention of Ca decreased (**Table 1-*8***). Same trend was also found in P retention as dietary P intake increases the percentage retention of P decreases. Dietary P has no impact on Ca retention (Coon *et al.*, 2002).

Table 1-*8*: Retention of Ca and P by laying hens at 69 weeks of age

Diet Ca (g/kg)	Ca intake (g/d)	Ca retention (%)	P intake (g/d)	P retention (%)
35	3.48c	49.8a	0.74a	31.3a
45	4.50b	44.9ab	0.75a	25.4b
55	5.42a	39.1b	0.68b	20.2b
Diet NPP(g/kg)				
2.4	4.25b	46.2a	0.54c	30.5a
4.4	4.64a	45.8a	0.75b	25.3ab
6.4	4.51a	41.8a	0.88a	21.2b

Ca x P interaction	*P* < 0.05	NS	*P* < 0.05	*P* < 0.05

a,b,c, Means followed by different letters in each column under Ca and P levels are significantly different (P < 0.05).

Source: Keshavarz (1986)

Calcium retention is affected by age, environmental temperature and metabolisable energy content of the diet (Scott and Balnave, 1991). An addition of fat (10 g/kg diet) increased Ca and P retention of the diet, but more fat addition (30 g/kg diet) produced no further benefits (Nahashon *et al.*, 1994).

Our previous study (Li and Bryden, 2006) showed that total P retention ranged from 28 to 36% of total P intake and it decreased as the dietary P intake increased in layers at 47 weeks of age (**Table 1-*4***). Summers (1995) reported that P retention was less than 30 and 40 % of total P and AP intake (**Table 1-*5***).

1.6 Vitamin D

Vitamin D is essential for absorption and mobilization of Ca during egg shell formation and P utilization. Vitamin D_3 is absorbed from the intestine in association with fats and requires the presence of bile salts for absorption. It is transported via the portal circulation to the liver, where it is accumulated. The first transformation occurs in the liver, where vitamin D_3 is hydroxylated to become 25-hydroxyvitamin D_3 (25-OH D_3). This vitamin D_3 metabolite is then transported to the kidney where it is converted to the most active hormonal compound 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$ D_3). The production of 1,25-$(OH)_2$ D_3 is tightly regulated by parathyroid hormone (PTH) in response to serum Ca. Vitamin D_3 is the major control element in stimulating Ca absorption from the intestine. This process is facilitated by the synthesis of Ca-binding protein. If plasma Ca is low, PTH secretion is induced, which stimulates the hydroxylation of 25-OH D_3 to 1,25-$(OH)_2$ D_3. This compound will increase Ca absorption in the intestine, mobilize Ca from the bones and reduce Ca excretion via the kidney. If plasma Ca is high, first PTH secretion and then 1, 25-$(OH)_2$ D_3 production are suppressed, which results in a reduction of Ca absorption in the gut as well as Ca resorption from the bones and an increase in Ca excretion. Therefore it is of utmost importance for an optimum egg shell quality to optimize Ca supply and secure sufficient vitamin D_3 activity in the laying hen. Any problem that affects the integrity of liver and kidney or the parathyroid gland will have an adverse effect on the action of vitamin D_3 and thereby Ca absorption and metabolism.

Several reports have demonstrated that dietary addition of vitamin D_3 can significantly enhance the retention of P in birds (Sebastian *et al*., 1998). It is possible that Vitamin D_3 promote the absorption of P of birds. However, excess vitamin D_3 and its metabolites have not shown further beneficial effect on egg shell quality when hens are already consuming adequate vitamin D_3. Supplementing Vitamin D to layer diets is common practice in poultry industry.

1.7 Phytase

Maximum utilisation of phytate by bird with supplemental phytase in vivo was approximately 50% on average (Selle *et al.*, 2006). Phytase efficacy is influenced by phytase source and dose; physical factors of feedstuffs, such as source and solubility of phytate; feed particle size; animal physiological factors, such as GIT pH, retention time and Ca; and P status and requirement. Dietary Ca level affects the efficacy of phytase in broilers (Tamim and Angel, 2003), but not to the same extent for all phytases (Augspurger and Baker, 2004). The same source of phytase can produce different responses in different flocks (Angel *et al.*, 2002; Applegate *et al.*, 2003). All these variables make predicting phytase responses very difficult.

There are a number of long term experiments with laying hens which indicate that a diet with 1.0-1.3 g/kg AP (i.e. a typical corn-soybean diet without supplemental sources of P) in

the presence of 100-300 units of microbial phytase per kg diet, can maintain production performance as satisfactorily as diets containing an AP level of 4.0-4.5g/kg of diet that normally is used by industry. Phytate P retention was increased by 15% in the presence of phytase in laying hens from 30 to 42 weeks of age (Keshavarz, 1999, 2000). Total P excretion was reduced by 34 to 47% for hens fed the low NPP regimen with phytase than for the control group without supplemental phytase (Keshavarz, 2000).

1.8 Egg quality and measurements

Kramer (1951) defined quality as "the sum of characteristics of a given food item which influence the acceptability or preference for that food by the consumer". Therefore, it is clear that egg quality will mean different things to different people and the consumer's perception of quality is likely to vary depending on their intended use of the egg and their own preferences.

The colour of egg yolk does not affect the nutritive value of eggs. However, it is an important criteria affecting consumers' expectations of the quality of eggs (Coutts and Wilson, 2007; Schwagele, 2011). The majority of people prefer the egg yolks with darker colour. Most egg marketing authorities require deep-yellow to orange-yellow yolk colours.

The colour of the yolk is mainly from carotenoids. The most important sources of carotenoids in poultry feed are maize, maize gluten, alfalfa (lucerne) and grass meals. These sources contain the pigmenting carotenoids lutein and zeaxanthin, which, together with other oxygen-containing carotenoids, are known by the collective name of xanthophylls. Wheat and sorghum are the most common feed ingredients for poultry in Australian. They contribute limited amount of carotenoids.

The carotenoid content in the ingredients of poultry feed is not constant and the pigmentation properties of the carotenoids can be weakened or lost in a variety of ways. These fluctuations in carotenoid content and availability in feedstuffs concern both the poultry nutritionist and the feed producer. Because of such variations, naturally occurring carotenoids cannot be relied upon to provide the desired yolk colour or to provide a consistent colour. Therefore, nature-identical yellow and red carotenoids, such as apoester and canthaxanthin, are commonly added to feed in order to achieve the desired egg yolk colour. These supplemental carotenoids are readily transferred to the blood and then deposited in the yolk to provide pigmentation in the laying hen.

There are many other factors influencing yolk colour scores. For example, individual birds vary in the genetic capability to absorb and deposit pigment in egg yolk. Colour scores are also affected by the rate of egg production, insufficient pigment, oxidising agents or pigment antagonist in diet, temperature and length of storage of diets (Coon, 2002; Coutts and Wilson, 2007). A colour standard DSM Yolk Colour Fan is used to measure yolk colour by the egg industry worldwide. In general, yolk colour above score 10 is required and 12 is the target. Yolks of more intense colour may be required for specific markets.

Egg shell colour is the result of the porphyrin pigments incorporate with egg shell cuticle, are deposited on the surface of the immobilized egg in the process of egg formation before oviposition. The egg shell colour, like yolk colour, has no relationship to the nutritional value of eggs and is not an indication of internal quality of eggs or shell strength, but consumers prefer brown eggs over white eggs in most markets in Australia and throughout the world (Southeast Asia, New Zealand, United Kingdom, Italy, Portugal and Ireland). Therefore the colour of the egg shell is an important economic parameter.

Shell colour is mainly determined by genetics. However, the same hen may lay eggs with inconsistent shell colour. Hens under strong sun light and high temperatures can produce a fading colour shell. There are also other factors influencing egg shell colour. Stress such as sudden changes to routine, moving to another environment, change to the diet and shocks such as loud noises, bullying within the flock or the presence of predators can affect the colour intensity. These stresses associated with hormonal disturbance will likely lead to

hens retaining their eggs in the shell gland area of the oviduct longer than the normal time of laying and this can result in the deposition of a thin layer of extra-cuticular Ca which makes brown eggs appear paler (Walker and Hughes, 1998).

If the shell colour can be shown to be strength related, it will have economic importance in brown laying hens and other poultries which lay colourful egg shells.

Shell breakage is directly related to shell strength, which is depending on shell thickness (Ca carbonate content) and shell matrix organization.

There are a number of techniques and instruments developed to measure egg shell quality: direct destructive (shell breaking strength), non-destructive method (shell deformation); indirective methods (shell thickness, shell weight, SG) (See Zhang, 1993 for details). Specific gravity is a simple, easy to perform method to determine egg shell thickness, and therefore, egg shell quality.

Specific gravity and egg shell thickness are highly positively correlated and SG measurements are usually all that needs to be taken. Specific gravity of an egg indicates the quantity of shell present relative to other components of the egg. It tends to decrease after approximately forty-five weeks of age. This is partly due to the size of the egg increasing more rapidly than shell weight. Therefore, differences in SG among eggs of similar weights are mainly due to variations in the amount of shell. As SG goes down the number of cracks generally increases. Specific gravity gives the producer an idea of the probability of the eggs being cracked during handling. Determining an egg SG is accomplished by the flotation of the egg in various salt solutions.

The properties of egg shell directly affect the economic value of table-eggs, successful incubation and egg storage. A small crack in the shell enormously decreases the egg keeping quality. Cracked eggs are not only major economic lose to production and marketing, but also the main threat to food safety if they pass through the supply chain system undetected.

Much research on egg shell quality was done decades ago. The genetics of the chicken, diets, house design and management practices have changed dramatically since then. It is likely that more changes will have to be made by the commercial egg industry in future. No matter what changes occur, the egg shell needs to be as strong as possible to maximize the proportion of eggs produced reaching the end user. With current knowledge it is impossible to correct all egg shell quality problems. However, it is possible to make significant reductions in the number of eggs lost due to poor shell quality. Many factors are known to affect egg shell quality such as nutrition, flock health, management practices, environmental conditions and breeding etc. (see Zhang 1993 for details).

One of the most important egg qualities is its freshness, which is usually assessed by the viscosity of egg albumen measured in Haugh units (HU). The HU takes into account egg weight and albumen height, is a measure of egg protein quality and provides a range of values from extremely poor quality eggs to very good quality fresh eggs. The fresher, higher quality eggs have thicker albumen. The higher the HU, the better the quality of the egg is (Monira *et al*., 2003). The albumen heights and HU values decrease as eggs age. The HU is used internationally as the definitive method of defining true egg quality and freshness which is an important industry measure of egg quality next to other measures such as shell thickness and strength.

2 Objectives

Genetic improvement in various laying hen performance parameters makes P requirement a moving target and information on P requirements of modern strains of laying hens is limited. Therefore, the objectives of the project were:

1. To re-evaluate the AP requirement of brown egg laying hens with or without supplemental phytase from lay to 80 weeks of age;
2. To examine the effects of different dietary AP and Ca with or without phytase on egg production, egg shell quality, Ca and P retention, tibia bone and toe ash; and
3. To provide safe guidelines to more cost-effectively address P requirements of brown egg laying hens.

3 General materials and methods

There were 2 experiments conducted in this project: Experiment 1 was entitled “Different AP levels and layer performance with or without phytase supplementation” and Experiment 2 was entitled “Different AP and Ca levels and layer performance with or without phytase supplementation”.

All the layers were housed in an environmentally controlled shed with 16-hour lighting regime and temperature maintained at approximately 22-24^{o}C.

All experimental procedures were approved by the University of Queensland Animal Care and Ethics Committee and complied with the Australia Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.1 Layers

Hy-Line Brown pullets at 16 weeks of age were supplied by Hy-Line Australia for both experiments. All the layers were randomly allocated to cages with 6 layers per cage. The initial body weights of the birds were recorded and the average body weights were similar among treatments (P> 0.05). The coefficients of variation, the standard deviation expressed as percentage of the mean value, were between 3.0 to 4.0 % for Experiment 1 (at 20 weeks of age) and 2.0 to 4.0 % for Experiment 2 (at 16 weeks of age).

3.2 Experimental diets

All the experimental diets for Experiments 1 and 2 were formulated by Ridley ArgiProducts to ensure the experimental diets as close as possible to the current industry practice. Phytase (Feedzyme XP) and xylanase (Feedzyme XBC) were supplied by FeedWorks. There were two phases of diets: for Experiment 1, phase 1 diets were fed to layers from 20 to 50 weeks of age and phase 2 from 51 to 80 weeks of age since the first batch concentrates were delivered approximately 4 weeks after the pullets were delivered. For Experiment 2, phase 1 diets were fed to layers from 16 to 50 weeks of age and phase 2 from 51 to 80 weeks of age. There were four batches of diets prepared in each experiment, two for each phase. The mixed diets were stored in a cool room at a temperature of 12-15^{o}C and relative humidity of approximate 45%. Each batch of diets was fed for 16 weeks. Dietary Ca, AP and sodium (Na) concentrations were not corrected for the use of phytase although the supplier of phytase (FeedWorks) recommending giving a credit of Ca of 1.25 g/kg, AP of 1.3 g/kg and Na of 0.35 g/kg for use of 450 FTU phytase per kg diet, most publications did not mentioned of these mineral corrections. Rovimix® Hy-D® 1.25%

Premix was supplemented to all diets at 0.5 g/kg which provided 6.25 mg of 25-Hydroxycholecalciferol per kg diet.

3.2.1 Experiment 1

The diets were sufficient in all nutrients for layers except for AP, which was included at 1.5, 2.0, 2.5, 3.0, 3.5 and 4.5g/kg diet throughout the experimental period. A fixed Ca concentration of 42 g/kg was used for all diets.

3.2.1.1 Cereal grains

Wheat and sorghum grains from the same harvest were purchased in bulk for the entire experiment and stored in silos at Narrabri, NSW. The grains were treated with hydrogen phosphine gas every 8-10 weeks. The product used was Phostoxin, which comes in pellet form and was placed in bags or on plates in the sealed silos. When the pellets were exposed to moist air a chemical reaction occurs, hydrogen phosphine gas is released to penetrate into the grain and kill weevils and other pests. The grains were left under fumigation for a period of 10 days to allow the pellets to completely react and to change into powder form. The powder residues were removed from the silos and the silos were then opened up for 5-6 hours and allowed to defume before the grains being loaded onto truck for transport from the storage site. Wheat and sorghum gains were hammer milled before being mixed into the diets.

3.2.1.2 Diets

Four batches of six concentrates (1 tonne each), including protein meals, amino acids, pigments, millrun, vitamins and minerals, were supplied by Ridley AgriProducts. Batches 1, 3 and 4 were from Toowoomba mill, Queensland and Batch 2 from Bendigo mill, Victoria. Each of the 6 concentrates was incorporated into two diets either with or without supplementation of phytase to give total 12 experimental diets. The detailed composition of the diets 1 to 6 for phases 1 and 2 are shown in **Table 3-1** and **Table 3-2.** Diets 7 to 12 were essentially the same as diets 1 to 6 except with added phytase.

The experimental diets were prepared by mixing the respective concentrates, cereal grains, oil (Sun-soy oil, Ridley AgriProducts) and xylanase (Feedzyme XBC containing 4000 units/g, at 500 g/tonne) with or without phytase (Feedzyme phytase XP containing 1000 FTU/g, at 450 g/tonne) at Aus Organic Feeds, Greenmount, Queensland. A research team member was present during the diet preparation to ensure the mixing quality and to take representative samples of each grain and diet.

Table 3-*1*: Diet composition for phase 1 from 20 to 50 weeks of age in Experiment 1

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Sorghum	275	272	267	256	154	164
Wheat	303	303	303	300	402	390
Blood meal				6.67	6.67	6.67
Sun-soy oil	30	30.7	32	32	32.7	33.7
Canola meal	40	40	40			
Soybean meal	180	181.3	180.7	194	199	200
Millrun	49.3	48	52	84	77.3	74.7
Limestone	58.7	57.7	56.3	55.8	54.7	52.7
Calgrit (2-4 mm in diameter)	50	50	50	50	50	50
MDCP Biofos	1.5	3.9	6.2	8.6	10.5	15.4

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Salt	2.1	2.1	2.1	2.3	2.3	2.3
Sodium bicarbonate	2.2	2.2	2.2	2.0	2.0	2.0
Choline chloride, 70%	0.2	0.2	0.2	0.2	0.2	0.2
DL-methionine	2.1	2.1	2.1	2.3	2.3	2.3
L-lysine HCL	1.7	1.7	1.7	1.3	1.1	1.1
L-threonine	0.7	0.7	0.7	0.7	0.7	0.7
Rap Poultry BRD premix	2.0	2.0	2.0	2.0	2.0	2.0
Rovimix® Hy-D® premix	0.5	0.5	0.5	0.5	0.5	0.5
Jabiru Gold natural dry pigment	1.2	1.2	1.2	1.2	1.2	1.2
Feedzyme XBC (xylanase)	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000
Calculation (%)						
CP	17.6	17.6	17.5	17.6	17.7	17.7
Fat	4.82	4.88	5.01	5.03	4.97	5.07
ME (kcal /kg)	2781	2781	2780	2780	2781	2780
Ca	4.21	4.21	4.20	4.20	4.20	4.20
P	0.41	0.46	0.51	0.55	0.59	0.69
AP	0.15	0.20	0.25	0.30	0.35	0.45
Phytate-P	0.26	0.26	0.26	0.26	0.26	0.26
Ca: AP	28.1	21.1	16.8	14.0	12.0	9.3
Na	0.18	0.18	0.18	0.18	0.18	0.18
K	0.69	0.69	0.69	0.70	0.70	0.70
Cl	0.22	0.22	0.22	0.22	0.22	0.22
Avail Lys	0.85	0.85	0.85	0.85	0.85	0.85
Avail Met	0.45	0.45	0.45	0.46	0.45	0.45
Avail Met+Cys	0.71	0.71	0.71	0.71	0.71	0.71
Avail Thr	0.60	0.60	0.60	0.60	0.60	0.60
Avail Iso	0.67	0.67	0.67	0.66	0.67	0.67
Avail Try	0.18	0.18	0.18	0.19	0.19	0.19
Avail Arg	0.96	0.96	0.96	0.97	0.99	0.99
Avail Val	0.75	0.76	0.75	0.77	0.77	0.77
Linoleic acid	2.37	2.40	2.47	2.51	2.51	2.56

*Diets 7-12 were supplemented with phytase to diets 1-6

Table 3-*2*: Diet composition for phase *2* from *51* to *80* weeks of age in Experiment 1

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Sorghum	250	250	250	250	250	250
Wheat	387	382	380	375	372	364
Sun-soy oil	20	21	22	23	24	26
Canola meal	40	40	40	40	40	40
Soybean meal	115	116	116	117	117	118
Millrun	65	67	67	69	69	71
Limestone	58.7	58	56.7	56	54.7	52.7
Calgrit (2-4 mm in diameter)	50	50	50	50	50	50
Salt	1.3	1.3	1.3	1.3	1.3	1.3
Sodium bicarbonate	2.7	2.7	2.7	2.7	2.7	2.7
Choline chloride, 70%	0.3	0.3	0.3	0.3	0.3	0.3
DL-methionine	1.7	1.7	1.7	1.7	1.7	1.7
L-lysine HCL	1.9	1.9	1.9	1.9	1.9	1.9
L-Threonine	0.5	0.5	0.5	0.5	0.5	0.5
Rap Poultry BRD premix	2.0	2.0	2.0	2.0	2.0	2.0
Rovimix® Hy-D® premix	0.5	0.5	0.5	0.5	0.5	0.5
Jabiru Gold natural dry pigment	1.2	1.2	1.2	1.2	1.2	1.2
MDCP Biofos	1.0	3.4	5.8	8.2	10.6	15.3
Feedzyme XBC (xylanase)	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000
Calculation (%)						
CP	15.4	15.4	15.4	15.4	15.4	15.4
Fat	3.9	4.0	4.1	4.2	4.3	4.5
ME (kcal/kg)	2751	2751	2750	2750	2749	2749
Ca	4.19	4.20	4.19	4.21	4.20	4.20
AP	0.15	0.20	0.25	0.30	0.35	0.45
Phytate-P	0.26	0.26	0.26	0.26	0.26	0.26
Ca:AP	28.0	21.0	16.8	14.0	12.0	9.4
Na	0.16	0.16	0.16	0.16	0.16	0.16
Cl	0.18	0.18	0.18	0.18	0.18	0.18
Avail Lys	0.71	0.72	0.72	0.72	0.72	0.72
Avail Met	0.38	0.38	0.38	0.38	0.38	0.38
Avail Met+Cys	0.62	0.62	0.62	0.62	0.62	0.62
Avail Thr	0.50	0.50	0.50	0.50	0.50	0.50
Avail Iso	0.56	0.56	0.56	0.56	0.56	0.56
Avail Try	0.16	0.16	0.16	0.16	0.16	0.16

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Avail Arg	0.79	0.79	0.79	0.79	0.79	0.79
Avail Val	0.64	0.64	0.64	0.64	0.64	0.64
Linoleic acid	1.90	1.97	2.00	2.07	2.10	2.21

*Diets 7-12 were supplemented with phytase to diets 1-6

3.2.2 Experiment 2

For Experiment 1, the acquisition of experimental diets was very expensive and time consuming. The cereal grains were shipped from Narrabri, NSW to Queensland, the concentrate preparation and delivery were by Ridley AgriProducts feed mills and all of these ingredients were transferred to Greenmount, Queensland where the experimental diets were mixed. The finished diets were then shipped to the Poultry Science Unit at UQ Gatton Campus and required significant temperature controlled storage capacity. Technically, a minimum of 1000 kg of each concentrate per batch had to be produced but more than 350 kg was not required (wasted) per batch. There were no obvious advantages of such arrangements as used in Experiment 1.

For Experiment 2, therefore, the feedstuffs were purchased locally for each batch (fed for 16 weeks) of diet prepared. The rolled wheat and sorghum, protein meals and millrun were purchased from Riverina Australia. Calgrit (coarse limestone) was bought from Ridley AgriProducts to ensure the product meet the specification of particle size (2-4 mm). The sun-soy oil, sodium bicarbonate, monodicalcium phosphate (MDCP) Biofos and limestone (fine) were also purchased from Ridley AgriProducts. Hy-D® (DSM), L-threonine, DL-methionine, L-lysine, Jabiru Gold Natural Dry pigment 12G, vitamin and mineral premix were obtained from BEC Feed Solution. All the diets were prepared using the feedmill at the University of Queensland, Gatton Campus.

Based on the results of Experiment 1, two concentrations of dietary AP were selected which meet the criteria: one AP concentration is approximately the requirement and the other one is below the requirement. The results from Experiment 1 indicated that hens fed diet containing AP of 2.0 g/kg were the same for all parameters measured when compared with diets with higher AP levels (see Section 4). However, it was unclear whether there was any negative Impact of salt deficiency of diets containing 2.0 g AP/kg at the beginning of lay on later production performance. Therefore, AP concentration of 2.5 g/kg was selected for further examine in Experiment 2.

Without phytase supplementation the birds fed on the diet containing AP of 1.5 g/kg in Experiment 1 was marginally smaller, but was still comparable with the recommendation in Hy-Line management guide and overall egg production from 20 to 80 weeks of age was numerically lower than for the rest of the treatments. Moreover, AP of 1.5 g/kg was the lowest level tested in Experiment 1. Therefore AP of 1.5 g/kg was used in Experiment 2. Each AP level was examined in a factorial arrangement with three levels of Ca (32, 40 and 48 g/kg), with or without phytase (same concentration as in Experiment 1) which produced total 12 experimental diets. The diets 1 to 6 are shown in **Table 3-*3*** and **Table 3-*4*** for phases 1 and 2. Diets 7 to 12 were essentially the same as diets 1 to 6 except with added phytase.

Table 3-*3*: Diet composition for phase 1 from 16 to 50 weeks of age in Experiment 2

Ingredient (g/kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Sorghum	336	282	204	330	275	219
Wheat	300	300	336	300	299	300
Sun-soy oil	11	27	40	13	29	44

Ingredient (g/kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Canola meal	40	40	35	40	40	35
Soybean meal	187	193	202	187	194	204
Millrun	30	41	45	31	43	58
Limestone	32	53	74	30	51	72
Calgrit (2-4 mm in diameter)	50	50	50	50	50	50
Salt	1.5	1.5	2	1.5	1.5	2
Sodium bicarbonate	3.0	3.0	2.0	3.0	3.0	2.0
Choline chloride, 70%	0.2	0.2	0.1	0.2	0.2	0.1
DL-methionine	2.0	2.0	2.1	2.0	2.0	2.1
L-lysine HCL	1.4	1.2	1.1	1.4	1.2	1.0
L-threonine	0.5	0.5	0.5	0.5	0.5	0.5
Rovimix® Hy-D® premix	0.5	0.5	0.5	0.5	0.5	0.5
Jabiru Gold natural dry pigment	1.2	1.2	1.2	1.2	1.2	1.2
MDCP Biofos	1.6	1.5	1.4	6.4	6.3	6.2
Vital poultry premix	2.0	2.0	2.0	2.0	2.0	2.0
Feedzyme XBC (xylanase)	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000
Calculation (%)						
CP	17.3	17.3	17.3	17.3	17.3	17.3
Fat	3.1	4.5	5.7	3.2	4.7	6.1
ME (kcal /kg)	2779	2782	2783	2780	2781	2780
Ca	3.21	4.00	4.79	3.22	4.01	4.79
P	0.41	0.41	0.40	0.51	0.51	0.51
AP	0.15	0.15	0.15	0.25	0.25	0.25
Phytate-P	0.26	0.26	0.26	0.26	0.26	0.26
Ca: AP	21.4	26.8	32.0	12.8	16.0	19.1
Na	0.18	0.18	0.17	0.18	0.18	0.17
Cl	0.18	0.17	0.19	0.18	0.17	0.19
Avail Lys	0.83	0.83	0.83	0.83	0.83	0.83
Avail Met	0.43	0.43	0.44	0.43	0.43	0.44
Avail Met+Cys	0.69	0.69	0.69	0.69	0.69	0.69
Avail Thr	0.58	0.58	0.58	0.58	0.58	0.59
Avail Iso	0.67	0.67	0.67	0.67	0.67	0.68
Avail Try	0.18	0.18	0.19	0.18	0.18	0.19
Avail Arg	0.95	0.97	0.99	0.95	0.97	0.99
Avail Val	0.74	0.74	0.74	0.74	0.74	0.74
Linoleic acid	1.39	2.20	2.85	1.49	2.30	3.07

*Diets 7-12 were supplemented with phytase to diets 1-6

Table 3-*4*: Diet composition for phase 2 from 51 to 80 weeks of age in Experiment 2

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Sorghum	250	250	143	250	250	250
Wheat	449	393	447	444	385	352
Sun-soy oil	1.3	16.7	32.7	2.7	18.7	30
Canola meal	40	40	40	40	40	40
Soybean meal	119	127	133	121	128	136
Millrun	45.3	57.3	66.7	44.7	59.3	51.3
Limestone	32	53.3	74.7	30	51.3	72.7
Calgrit (2-4 mm in diameter)	50	50	50	50	50	50
Salt	1.3	1.3	1.7	1.3	1.7	1.7
Sodium bicarbonate	2.7	2.7	2.0	2.7	2.7	2
Choline chloride, 70%	0.3	0.2	0.1	0.3	0.2	0.2
DL-methionine	1.5	1.6	1.5	1.5	1.6	1.7
L-lysine HCL	1.6	1.5	1.3	1.6	1.5	1.3
L-threonine	0.5	0.4	0.4	0.4	0.4	0.4
Rovimix® Hy-D® premix	0.5	0.5	0.5	0.5	0.5	0.5
Jabiru Gold natural dry pigment	1.2	1.2	1.2	1.2	1.2	1.2
MDCP Biofos	0.9	1.1	0.7	5.7	5.8	6.1
Vital poultry premix	2.0	2.0	2.0	2.0	2.0	2.0
Feedzyme XBC (xylanase)	0.5	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000	1000
Calculation (%)						
CP	15.2	15.1	15.2	15.2	15.1	15.0
Fat	2.10	3.56	4.97	2.22	3.75	4.78
ME (kcal /kg)	2753	2750	2753	2751	2749	2750
Ca	3.19	3.99	4.79	3.19	4.00	4.80
P	0.41	0.41	0.41	0.51	0.51	0.50
AP	0.15	0.15	0.15	0.25	0.25	0.25
Phytate-P	0.26	0.26	0.26	0.26	0.26	0.25
Ca: AP	21.3	26.5	31.9	12.8	16.0	19.2
Na	0.16	0.16	0.15	0.16	0.17	0.16
K	0.60	0.61	0.62	0.60	0.61	0.61
Cl	0.18	0.17	0.18	0.18	0.19	0.19
Avail Lys	0.70	0.70	0.70	0.70	0.70	0.70
Avail Met	0.36	0.36	0.36	0.36	0.36	0.37
Avail Met+Cys	0.60	0.60	0.60	0.60	0.60	0.60
Avail Thr	0.49	0.49	0.49	0.49	0.49	0.49

Ingredient (kg)*	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Avail Iso	0.56	0.56	0.56	0.56	0.56	0.56
Avail Try	0.16	0.16	0.16	0.16	0.16	0.16
Avail Arg	0.79	0.80	0.82	0.79	0.80	0.80
Avail Val	0.63	0.63	0.63	0.63	0.63	0.63
Linoleic acid	0.92	1.71	2.52	0.99	1.82	2.37

*Diets 7-12 were supplemented with phytase to diets 1-6

All experimental diets were in mask form, each was fed to 10 cages each with 6 birds which were located in each of 10 blocks. Birds were allowed to access feed and water *ad libitum*.

3.3 Measurements

3.3.1 Feed intake

Feed intake per cage was recorded every 4 weeks and daily feed consumption was calculated:

Feed intake (g/b/d) = Feed consumed (g)/henday

3.3.2 Body weight

Body weight of layers was weighed in groups monthly and average body weight per bird per cage determined.

3.3.3 Egg production visual shell defects

All eggs laid were collected and inspected manually. Total egg numbers and egg shell defects were recorded daily on an individual cage basis. Shell defects were classified as cracked, broken (part or total egg content lost), deformed and soft shell (partially calcified) or shell less eggs. Egg production was calculated as percentage of henday and henhoused egg production. Egg shell defect was expressed as percentage of total number of eggs.

Henday egg production (%) = Number of eggs laid/hendays x 100

Henhoused egg production (%) = Number of eggs laid/hens housed x 100

Egg shell defect (%) = Number of defect shelled eggs/total eggs laid x 100

Eggs were individually weighed immediately after collecting to avoid weight loss during storage. The average weight (g) per egg in each cage is reported.

Daily egg mass along with feed consumption per hen provides the most important criteria for accurate nutritional management of the flock. Average daily egg mass per hen was calculated using the following formula:

Egg mass (g/hen/d) = egg weight (g) x henday egg production (%)

3.3.4 Feed to egg conversion ratios

Eggs of the whole flock were collected and their weights were recorded every 4 weeks, but this occurred in the middle of period between feed intake measurements. In this way the variation of egg weight changes with hen age would be minimised. The average egg weight per cage multiplied by total number of eggs laid within 4 weeks is referred to as total egg weight and used to calculate feed to egg conversion ratio.

Feed to egg conversion ratio (g feed/g egg) = Total feed intake (g)/total egg weight (g)

3.3.5 Egg and egg shell quality

There are various measurements to determine egg and egg shell quality: egg size, albumen height, HU, yolk colour, shell colour, SG, shell breaking strength, shell weight, shell percentage of egg, shell thickness (mm), shell weight/unit surface area (mg/cm^2).

The first three eggs from right to left in front of each cage (excluding broken and cracked eggs) were labelled and collected monthly, which gave 30 eggs per treatment for measurements of egg and egg shell quality.

3.3.5.1 Albumen height and HU

Albumen (egg white) height was determined using Egg Analyzer^{TM} (EMT-5200). The height correlated with the egg weight, determines the HU rating.

The HU was calculated as follows:

$$HU = 100 \times \log (H - 1.7W^{0.37} + 7.6)$$

Where:

- HU = Haugh unit
- H = height of the albumen in millimeters
- W = egg weight in grams.

3.3.5.2 Egg Yolk colour

Yolk colour is one of the important criteria of consumers' expectations of the quality of eggs even though it does not affect the nutritive value of eggs. The intensity of yolk colour was measured using Egg Analyzer^{TM} (EMT-5200).

3.3.5.3 Egg fractions

The yolk was separated from the albumen and weighed, and the shells were washed in warm water, dried at room temperature for several days and constant weight achieved. Albumen weight was determined by the difference of total egg weight and the weights of yolk and shell. The yolk and albumen were freeze-dried and weighed.

3.3.5.4 Egg shell colour

The egg shell colour was measured monthly using a colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Inc., Japan) and the scale described by Arthur and O'Sullivan (2005). The L* value represents lightness and ranges from 0 to 100, with 0 corresponding to black and 100 to white. The a* value is as a function of redness-greenness and b* represents yellowness-blueness. Positive values of a* represent the amount of redness of the shell colour, whereas negative values of a* indicate the amount of greenness in the shell colour. Similarly, the yellow and blue components in any colour are represented by positive and negative values of b*. The value b* was less important and not included in this report.

3.3.5.5 Specific gravity

The flotation method was used in which eggs were immersed sequentially into a series of saline solutions of ascending SG with SG of 1.075, 1.080, 1.085, 1.090 and 1.095. The SG of any egg is equal to the SG of the solution in which it first floats. Before measurement the SG of the saline solutions was checked with a hydrometer and was adjusted if necessary.

There are many factors known to influence SG measurements such as length of egg storage, temperature of saline solution and time of day when the egg laid. Egg SG declines by an average of 0.001 units for each day the egg is stored in the cooler. The higher the

saline solution temperature the higher will be the egg SG. Eggs laid in the afternoon have higher SG (due to thicker shells) than eggs laid in the morning.

In our experiment we standardised our procedures to minimise possible variations. All eggs were removed about 3:00 pm on the day before egg collection for testing SG and other parameters. Eggs were collected at 12:00 noon and SG was measured immediately after measurements of egg shell colour, within 4 hrs of egg collection. All eggs were immersed into tap water to remove salt residual after measurement of SG and dried with towel and allowed to dry at about 20 ^{o}C overnight for other shell quality measurement.

3.3.5.6 Egg shell breaking strength

Egg shell breaking strength was determined using Egg Shell Force Reader (Orka Food Technology, Israel) which measures the destruction strength of egg shells. Eggs were compressed between two parallel plates by a steadily increasing load until failure results. The egg shell breaking strength was given in terms of the force (expressed as kg) at failure. Egg shell breaking strength between 26 and 46 weeks was not measured in Experiment 2 since the equipment was broken down.

3.3.5.7 Egg shell weight and shell thickness

Egg shells were carefully washed to ensure removal of residual albumen. The shell, with membranes adhering, was left at room temperature for 2-3 days until a constant weight was obtained and then its weight was recorded.

Dried shell weight (%) was calculated as dried shell weight/egg weight x 100.

The thickness of the dried shell was measured using a thickness micrometer gauge. Three small pieces of the shell (membrane included) were taken from the equator of each egg and shell thickness was obtained from the average of the three measurements. Shell weight per unit surface area was calculated according to Curtis *et al.* (1985).

Shell weight/unit surface area (mg/cm^2) = Dried shell weight (g)/3.9782 (W$^{0.7056}$) x 1000

Where W = egg weight (g); 3.9782 (W$^{0.7056}$) = egg surface area (cm^2)

3.3.6 Calcium and P retention

Acid insoluble ash (AIA) served as indigestible marker in diets to determine Ca and P retention at 50 and 80 weeks of age. Celite, as a source of AIA, was added to the experimental diets at 20 g/kg. The diets with AIA were fed to 5 replicate cages per treatment at 49 and 79 weeks of age. After 6 days adaptation, excreta were carefully collected to make sure no feather and other contaminants, for the next 3 days, pooled per cage and mixed thoroughly before subsamples were dried in an oven at 85 ^{o}C for at least 48 hrs.

3.3.7 Sample collection and preparation

Diet samples were collected from 10 places during bagging at feed mill and then mixed thoroughly before sub-samples taken. Both diet and dried excreta samples were ground to pass through 0.5 mm screen for chemical analysis.

Blood samples were taken between 9:00 to 11:00 am into tubes containing lithium heparin (Sarstedt, Australia) at the end of the experiments. Plasma was separated by centrifuging samples at 1500 x g for 10 minutes and stored - 20 ^{o}C before analysis.

At the end of the experiment, 8 birds per treatment were euthanized. Left tibia bones and middle toes were removed. Tibia bones were autoclaved at 121 ^{o}C and 16 psi for 30 minutes and then cleaned to remove all exterior tissues before the measurements of tibia bone DM and ash content.

3.3.8 Chemical analysis

Dry matter was determined by drying the samples at 105 ^{o}C for 48 hrs.

Acid insoluble ash contents in diets and excreta samples were determined using the method described by Li *et al.* (2006). A sample containing about 100 mg of AIA was weighed into a pre-weighed sintered glass crucible (Pyrex, porosity 4, pore size 5–15 µm), dried at 105 ^{o}C for 24 h and re-weighed. The sample was then ashed at 550 ^{o}C for 8 h, boiled with 4 M hydrochloric acid in a crystallising dish for 30 min and then thoroughly washed with purified water. The processes of drying, ashing, boiling and washing were repeated until the ash appeared white. The crucible was then dried and re-weighed.

Total Ca and P contents in diets, ileal digesta and excreta samples were analysed using the AOAC method (1984) by an inductively-coupled plasma emission spectrophotometer (Optima 7300 DV, Perkin Elmer; Wellesley, MA, USA) following digestion with nitric/perchloric acids.

The Ca and P concentrations in plasma samples were analysed by a colorimetric method according to the manufacturer's instruction (Beckman Coulter AU 400, Beckman Coulter Inc, Brea California, USA).

Limestone was isolated from the excreta by soaking the samples in water and separated after repeated washing which may provide for visual evidence since some limestone may be dissolved and losed in the process.

Tibia bones or toes were weighed into pre- cleaned and -ashed crucibles, dried in 105 ^{o}C oven for at least 48 h, weighed until the constant weights achieved. Crucibles with dried tibia bones or toes were ashed at 550 ^{o}C for at least 16 h (Waldroup *et al.*, 2000). Tibia bone DM percentage of bird body weight, tibia bone ash percentage of DM and percentage of body weight were calculated. Toe ash contents were expressed as percentages of dry toe.

3.3.9 Calculations

Tibia bone DM (% bird body weight) = Tibia bone DM / body weight x 100

Tibia bone ash (% DM) = Tibia bone ash / tibia bone DM x 100

Tibia bone ash (% body weight) = Tibia bone ash / body weight x 100

Toe ash (%DM) = toe ash / toe DM x 100

Calcium and P retention and dietary retainable Ca and P were calculated, for example:

$$\text{P retention (\%)} = \frac{(P / AIA)d - (P / AIA)e}{(P / AIA)d} \times 100$$

Where, (P/ AIA) d = ratio of P to AIA in the diet, and (P/ AIA)e = ratio of P to AIA in excreta.

Dietary retainable Ca and P contents were calculated by multiplying dietary Ca or P content by Ca or P retention, respectively.

Dietary retainable P (g/kg) = diet P (g/kg) x P retention (%)

3.4 Statistical analysis

General linear model with the Tukey option and Minitab program (version 16.0) were used to analyse all the data according to the principle of Steele *et al*. (1991).

A factorial analysis was conducted to test main effects of dietary AP levels and phytase in Experiment 1; dietary AP, Ca levels and phytase in Experiment 2. Body weight, feed intake, feed to egg conversion ratio, egg production, egg weight, egg mass, egg shell detect percentage, egg shell colour, SG, albumen height, yolk colour, HU, egg shell breaking

strength, shell thickness, shell weight, shell weight percentage of egg and shell weight per unit surface area were response variables tested. To provide basic data in more details for future bench mark, No further analysis were conducted with pooled factors even though some of the factors had no effects on all the parameters measured and the interactions between the main factors in each experiment were non-significant. The means under each of main factors were presented and the standard error of mean (SEM) and the least significant difference (LSD) are given in the respective tables. The significant threshold is $P < 0.05$.

4 Results

4.1 Effect of dietary AP levels and supplemental phytase on layer performance (Experiment 1)

4.1.1 Feed intake

Feed intake was not influenced by dietary AP concentrations or phytase supplementation throughout the experimental period (**Table 4-1**) except at the beginning of the trial when the feed intake was significantly (P<0.05) reduced in birds fed on diets 2 and 8 (containing 2.0 g/kg AP with or without phytase), 4 and 10 (containing 3.0 g/kg AP with or without phytase) compared to the rest of treatment diets. A series of investigations of possible cause were conducted and the results of chemical analysis of the diets showed that Na concentrations were much lower in these diets. Further analysis revealed that the Na contents were much lower in 2 out of 6 concentrates from which the affected diets were made from. Salt was immediately (26 weeks of age) added to the 4 affected diets initially at the level of 5.0 g/kg as suggested by the nutritionist. However, it was observed that the excreta from the affected birds was wetter than the rest of birds, therefore the salt supplementation was reduced from 5.0 g/kg to 4.0 g/kg diet. In the meantime, the second batch of diets was prepared as soon as the new concentrates were ready. The feed intake was back to normal shortly when correct salt content was presented in the diets.

Interestingly, phytase significantly (P< 0.05) improved feed intake of birds fed diets with salt deficiency. The birds fed on diet contained AP of 3.0 g/kg with phytase supplementation had significantly (P<0.05) higher feed intake than those on the salt deficiency diet without phytase supplementation.

Feed intake was marginal lower for weeks 49 to 52 and 76 to 80 compared to other months. The birds were fed diets with 2.0 g/kg indigestible marker in weeks 49 and 79 for determination of Ca and P retention. To make sure that birds finish all feed offered including fine particles marginal less feed was offered during these periods. In this way the marker method to estimate Ca and P retention is more reliable.

Table 4-1: Effect of dietary AP concentrations and supplemental phytase on feed intake (g/b/d) of birds from 20 to 80 weeks of age in Experiment 1

		Age (week)														
AP (g/kg)	**Phytase**	**20-24**	**25-28**	**29-32**	**33-36**	**37-40**	**41-44**	**45-48**	**49-52***	**53-56**	**57-60**	**61-64**	**65-68**	**69-72**	**73-76**	**77-80***
1.5	**-**	94.7	107.9	111.6	112.9	108.6	108.0	109.3	100.5	106.6	108.6	106.0	104.2	111.1	110.5	105.3
	+	96.8	107.8	112.8	111.4	106.6	105.8	105.2	99.5	107.0	106.6	103.4	106.4	107.7	109.0	107.1
2.0	**-**	78.7	97.7	112.2	112.8	106.2	106.3	108.3	100.9	106.4	106.2	105.6	107.4	105.9	106.6	104.9
	+	80.9	99.8	110.2	112.9	103.6	105.3	107.1	97.7	102.0	103.6	103.5	101.9	106.9	103.8	99.9
2.5	**-**	95.2	103.0	110.4	111.9	105.5	106.5	106.7	99.0	105.2	105.5	103.5	104.9	107.9	107.8	104.8
	+	94.2	102.0	109.0	111.2	108.7	108.8	107.6	98.8	106.8	108.7	106.0	104.3	107.9	106.8	105.1
3.0	**-**	79.2	97.0	112.4	112.9	104.1	106.9	109.8	99.5	103.9	104.1	100.9	102.2	106.8	104.0	103.3
	+	83.6	103.2	115.7	114.3	109.9	107.7	110.9	100.9	104.5	109.9	105.9	102.9	110.5	109.8	104.8
3.5	**-**	99.2	109.1	112.1	110.7	105.8	106.6	106.1	98.5	104.0	105.8	102.2	101.1	109.4	106.7	103.3
	+	100.8	106.7	107.9	109.8	103.9	105.4	105.2	101.2	105.8	103.9	101.7	104.1	108.0	105.0	102.8
4.5	**-**	95.9	103.6	104.5	109.0	102.7	105.8	105.9	97.6	105.9	102.7	104.3	105.4	106.9	104.4	102.5
	+	98.0	103.2	107.4	111.4	107.3	107.1	107.8	100.3	107.1	107.3	103.0	105.4	109.9	108.0	102.9
Main effect																
AP	**Pooled SEM**	1.11	1.42	1.17	1.46	1.11	1.06	1.34	1.02	1.13	1.35	1.21	1.35	1.36	1.37	1.21
	$LSD_{0.05}$	3.10	3.99	3.29	4.09	3.12	2.97	3.75	2.86	3.16	3.80	3.40	3.79	3.82	3.85	3.39
	P value	0.000	0.000	0.000	0.498	0.199	0.789	0.247	0.916	0.530	0.488	0.540	0.537	0.721	0.255	0.204
Phytase	**Pooled SEM**	0.64	0.82	0.68	0.84	0.64	0.61	0.77	0.59	0.65	0.78	0.70	0.78	0.79	0.79	0.70
	$LSD_{0.05}$	1.79	2.30	1.90	2.36	1.80	1.71	2.16	1.65	1.83	2.19	1.97	2.19	2.21	2.22	1.96
	P value	0.038	0.471	0.978	0.906	0.681	0.999	0.706	0.658	0.668	0.288	0.879	0.604	0.670	0.723	0.803
AP x Phytase	**Pooled SEM**	1.57	2.01	1.66	2.06	1.57	1.50	1.89	1.45	1.60	1.92	1.72	1.91	1.93	1.94	1.71
	$LSD_{0.05}$	4.39	5.64	4.65	5.78	4.41	4.20	5.30	4.05	4.47	5.37	4.81	5.36	5.40	5.44	4.79
	P value	0.677	0.347	0.160	0.927	0.253	0.665	0.662	0.275	0.348	0.096	0.197	0.507	0.464	0.171	0.396

4.1.2 Body weight

The average bird body weights per diet for the entire experimental period are presented in **Figure 4-1**. As expected, the body weight increased as birds aged. Birds fed on lower AP (1.5 g/kg) diets tended to have lower body weight and the differences became more pronounced after 50 weeks of age. Lower salt concentrations in diets 2, 8, 4 and 10 at the beginning of the experiment affected the body weights of birds on these diets (**Figure 4-1**). Body weights of affected birds were recovered after dietary salt deficiency was identified and rectified. Phytase supplementation had no effect on body weight (P>0.05).

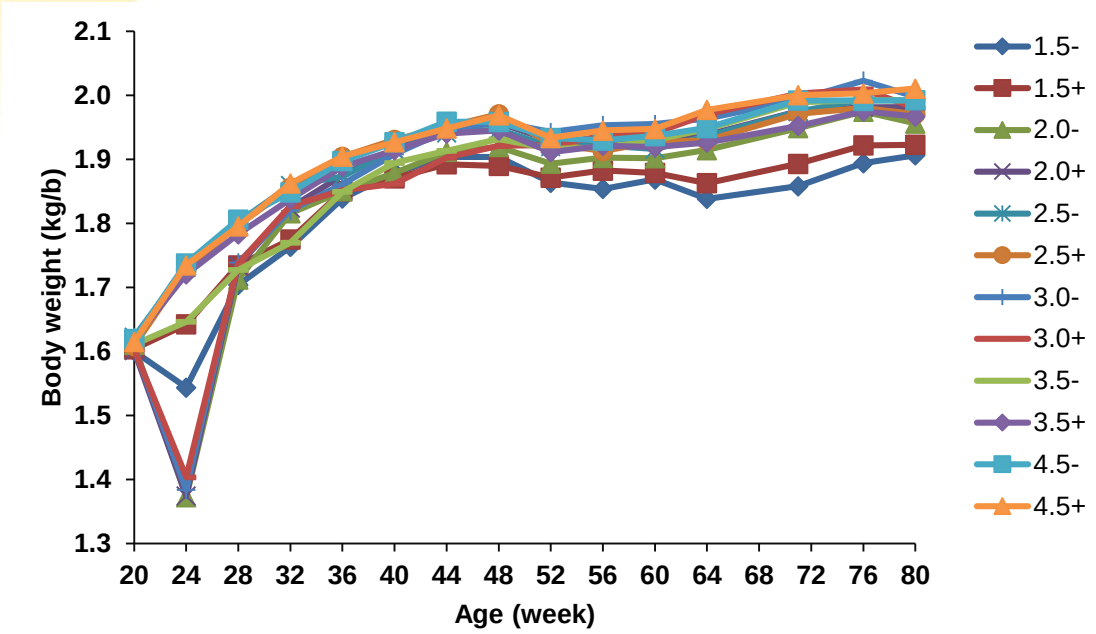

Figure 4-*1*: Effect of dietary AP concentrations and supplemental phytase on body weight of birds from 20 to 80 weeks of age in Experiment 1

4.1.3 Egg production

There were no significant differences in henday egg production percentages between layers fed on diets with different dietary AP concentrations although those on diets with low AP had marginally lower egg production at the beginning of lay (**Figure 4-*2***). Inadequate salt in diets containing AP concentrations of 2.0 and 3.0 g/kg at the beginning of the trial resulted in significantly lower egg production (P<0.05) compared to the rest of the treatments until the salt deficiency was corrected.

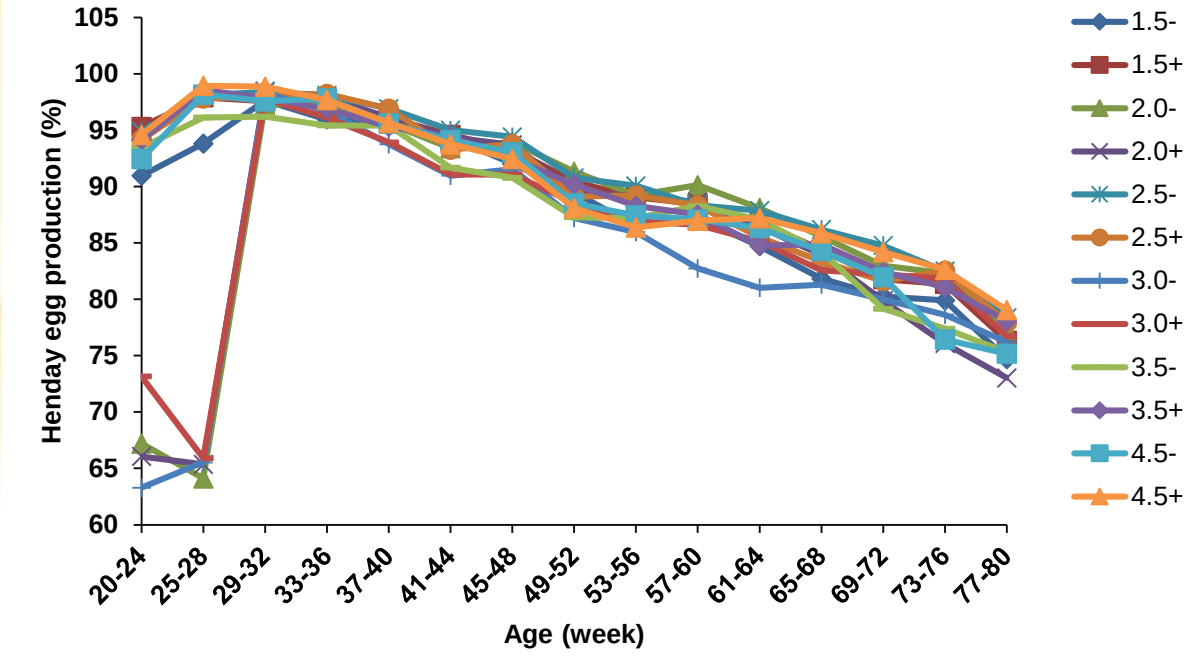

Figure 4-*2*: Effect of dietary AP concentrations and supplemental phytase on henday egg production of birds from 20 to 80 weeks of age in Experiment 1

Henhoused egg production was the same as henday egg production up to 52 weeks of age since no mortality occurred from 20 to 52 weeks of age (**Figure 4-3**).

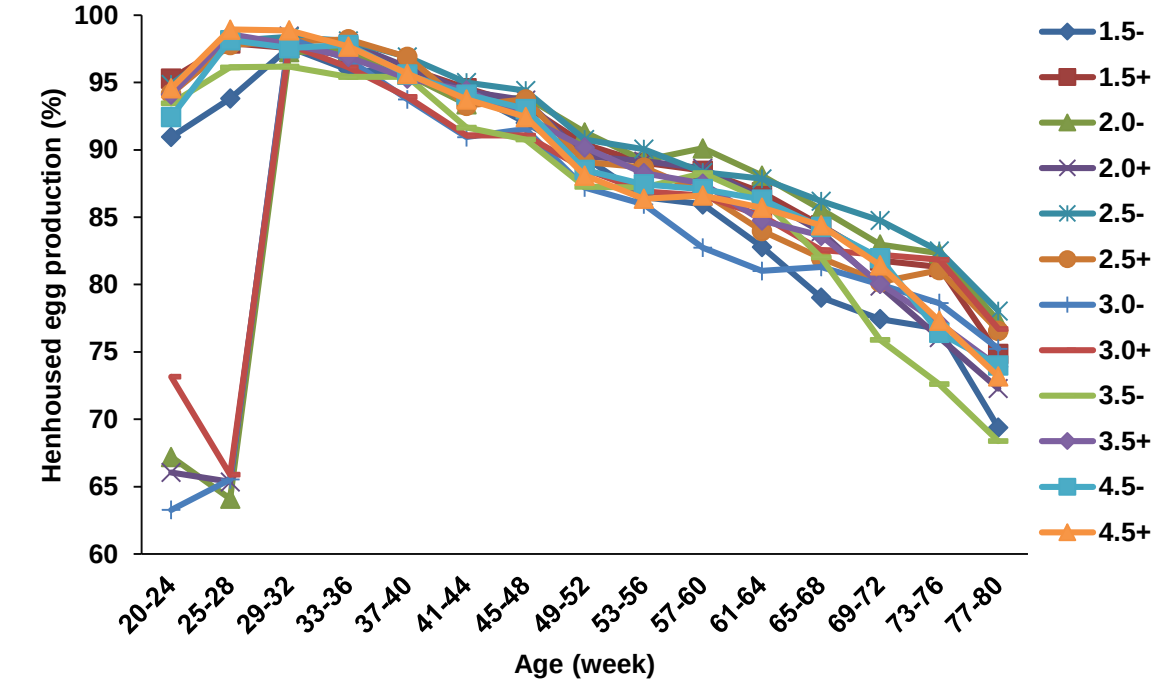

Figure 4-*3*: Effect of dietary AP concentrations and supplemental phytase on henhoused egg production of birds from 20 to 80 weeks of age in Experiment 1

Henday egg production tended to be higher with phytase addition in diet containing lower AP (1.5 g.kg), but this effect failed to attain statistical significance (**Figure 4-*4***). Similar effect was not observed in henhoused egg production.

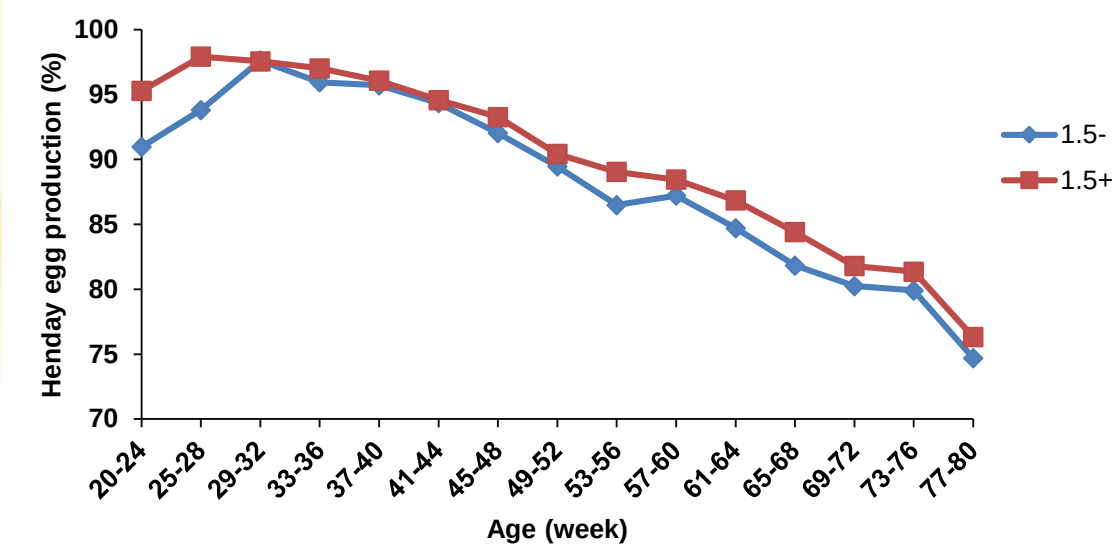

Figure 4-*4*: Phytase marginally improved henday egg production of birds fed diet containing AP of 1.5 g/kg from 20 to 80 weeks of age in Experiment 1

Egg weight is a basic parameter for evaluating egg quality. Average egg weight increased as the hens became older (**Figure 4-*5***) and was not significantly affected by AP levels and phytase supplementation in diets. Hens on salt deficient diets containing AP concentrations of 2.0 and 3.0 g/kg produced significantly smaller eggs (P<0.05) than the other treatments at the beginning of the trial. Salt was added to the affected diets at 26 weeks of age and the egg weights were comparable with those of the birds at 28 weeks of age.

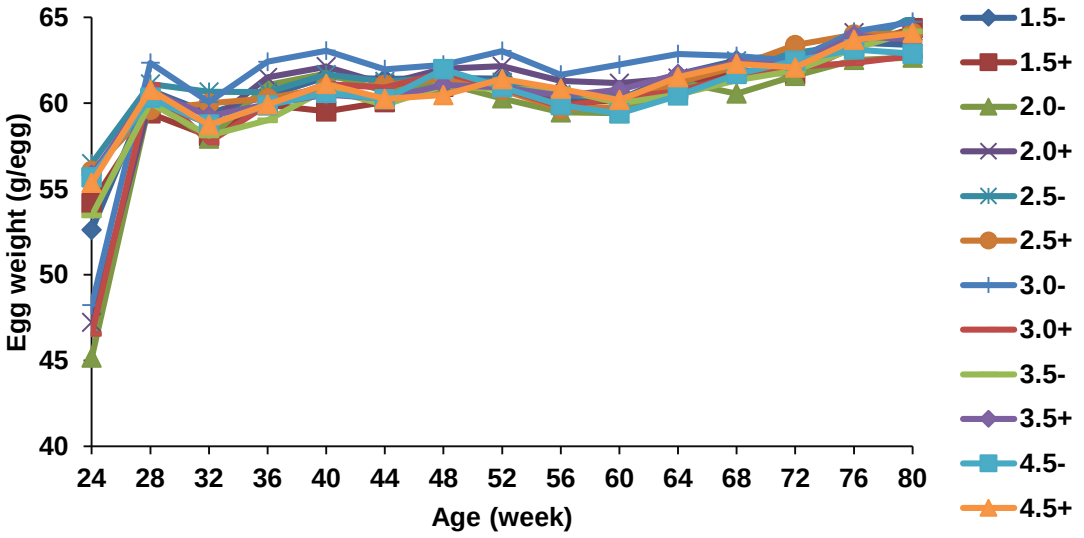

Figure 4-*5*: Effect of dietary AP concentrations and supplemental phytase on egg weight of birds from 24 to 80 weeks of age in Experiment 1

There were large variations in egg weight within the treatment (**Table 4-*2***), even within each replicate cage.

Table 4-*2*: Variation in egg weight (g/egg) of birds fed different dietary AP concentrations with or without phytase supplementation from 24 to 80 weeks of age in Experiment 1

Age (week)	Phytase	AP (g/kg)											
		1.5		2.0*		2.5		3.0*		3.5		4.5	
		-	+	-	+	-	+	-	+	-	+	-	+
24	Range	45.1-59.7	47.2-82.6	40.0-54.7	34.8-54.6	48.9-85.1	47.8-63.7	39.4-56.9	40.5-52.2	45.2-60.7	47.8-78.1	45.8-63.8	47.8-62.2
	Mean	52.6	54.2	45.2	47.2	56.5	56.0	48.2	46.5	53.4	55.8	55.7	55.4
28	Range	52.2-68.4	49.5-68.8	50.4-68.4	51.7-65.0	53.3-69.6	53.4-71.2	49.7-68.0	51.7-64.6	50.9-63.2	52.2-71.4	53.5-62.9	52.2-67.5
	Mean	59.4	58.1	57.9	58.8	60.7	60.0	60.1	57.7	58.1	59.2	58.8	58.7
32	Range	53.5-69.3	53.5-68.2	51.3-71.2	50.6-69.5	55.6-68.1	53.4-67.7	54.8-71.6	51.6-66.7	52.4-68.8	54.5-67.0	49.6-67.8	51.7-65.2
	Mean	60.4	59.9	61.1	61.5	60.7	60.3	62.4	59.9	59.0	59.9	59.9	59.9
36	Range	51.6-70.0	51.0-70.0	51.1-70.3	53.3-70.3	54.6-69.4	54.5-68.8	54.8-70.5	51.7-68.8	50.6-69.3	52.8-67.2	53.3-66.0	50.0-73.3
	Mean	61.5	59.5	61.7	62.1	61.7	60.9	63.1	61.1	60.8	60.5	60.6	61.1
40	Range	53.4-70.4	48.4-70.5	51.9-73.3	50.6-70.4	52.0-71.8	51.4-68.3	53.7-68.7	51.0-69.0	47.1-68.3	53.0-73.4	46.3-66.1	51.5-69.9
	Mean	61.4	60.1	60.7	61.1	61.3	61.1	62.0	60.9	59.9	60.3	60.2	60.3
44	Range	51.1-73.0	48.2-73.8	52.2-70.7	50.2-72.3	51.7-72.1	53.7-71.7	52.2-72.9	52.5-74.3	51.0-71.1	52.5-71.6	49.7-68.6	49.0-69.2
	Mean	61.5	61.0	61.2	62.0	61.5	61.4	62.2	61.8	61.0	61.1	62.0	60.5
48	Range	53.4-70.9	48.2-75.4	51.5-68.9	50.1-76.5	50.8-74.3	53.5-74.5	54.5-86.6	52.9-70.9	53.5-79.1	52.4-70.8	49.6-69.5	50.8-77.5
	Mean	61.4	61.0	60.3	62.2	61.2	60.8	63.0	60.9	60.8	60.9	60.9	61.4
52	Range	48.6-70.7	49.2-74.4	48.2-67.6	51.2-76.0	49.4-68.8	36.4-69.4	51.2-71.9	50.9-69.1	49.4-79.3	51.0-73.6	50.7-67.3	51.1-70.4
	Mean	60.2	59.9	59.5	61.3	60.5	59.7	61.7	59.8	60.8	60.5	59.9	60.8
56	Range	51.5-70.8	47.2-75.4	48.2-77.8	49.5-71.8	51.2-74.9	50.9-70.1	52.6-72.0	52.1-71.3	50.1-72.0	49.9-75.5	46.9-75.6	48.4-71.2
	Mean	60.8	59.4	60.2	60.1	61.1	59.6	62.3	61.0	60.0	60.7	60.3	60.8
60	Range	51.3-69.6	51.0-72.8	47.6-71.8	48.6-73.0	51.0-72.5	52.0-75.3	51.8-71.9	50.5-69.8	50.5-74.4	50.7-77.8	48.2-68.9	48.4-73.5
	Mean	60.2	60.2	59.4	61.2	60.3	59.8	62.3	60.3	60.0	60.8	59.4	60.2
64	Range	52.3-73.0	48.1-80.0	49.7-71.5	50.9-75.1	51.9-71.8	49.4-71.1	49.9-73.3	52.2-71.4	48.8-72.5	54.0-77.0	49.3-71.9	48.9-72.4
	Mean	61.7	60.8	61.3	61.5	61.1	61.2	62.9	60.7	60.5	61.7	60.4	61.5
68	Range	51.0-73.9	50.5-76.5	49.7-70.1	52.3-73.7	50.1-80.5	52.3-76.1	52.9-71.4	50.6-73.0	52.1-76.3	51.0-74.1	51.0-82.1	49.7-74.1
	Mean	62.5	61.9	60.6	62.3	62.5	62.1	62.8	61.6	61.3	62.4	61.7	62.3
72	Range	50.0-79.5	51.1-78.1	49.0-72.6	49.9-78.1	49.3-82.4	55.6-75.1	51.4-76.1	47.6-71.6	49.7-77.0	51.6-73.6	53.4-71.1	50.0-74.5
	Mean	62.7	61.7	61.6	62.6	62.9	63.4	62.5	62.1	61.9	62.2	62.5	62.1
76	Range	53.3-72.6	47.6-81.6	50.6-75.3	51.5-75.8	54.0-73.3	54.6-77.8	55.3-78.2	53.9-73.0	54.6-78.1	55.0-76.7	49.9-75.4	50.8-75.3

Age (week)	Phytase	AP (g/kg)											
		1.5		2.0*		2.5		3.0*		3.5		4.5	
		-	+	-	+	-	+	-	+	-	+	-	+
	Mean	63.5	63.3	62.5	64.1	63.4	64.0	64.2	62.3	63.2	63.9	63.1	63.7
80	Range	55.9-74.0	49.0-76.4	50.1-73.1	52.0-83.3	51.7-75.4	52.2-75.2	55.4-75.9	54.1-74.9	52.6-82.0	52.6-74.3	49.3-91.5	55.1-74.3
	Mean	63.4	64.4	62.7	63.6	65.0	64.1	64.7	62.8	64.2	63.7	62.9	64.1

Dietary AP concentrations and phytase supplementation had no significant effect on egg mass output (P>0.05). Egg mass followed trends to egg production, reached the highest values during the peak egg production and decreased as egg production declined (**Figure 4-*6***). As expected, egg mass of hens fed diets containing AP concentrations of 2.0 and 3.0 g/kg with salt deficiency was lower than the rest of treatments at the beginning of the experiment. Based on the egg mass results for the entire experiment, an AP of 1.5 g/kg was adequate to satisfy the need for egg mass production in the absence of phytase in the diet.

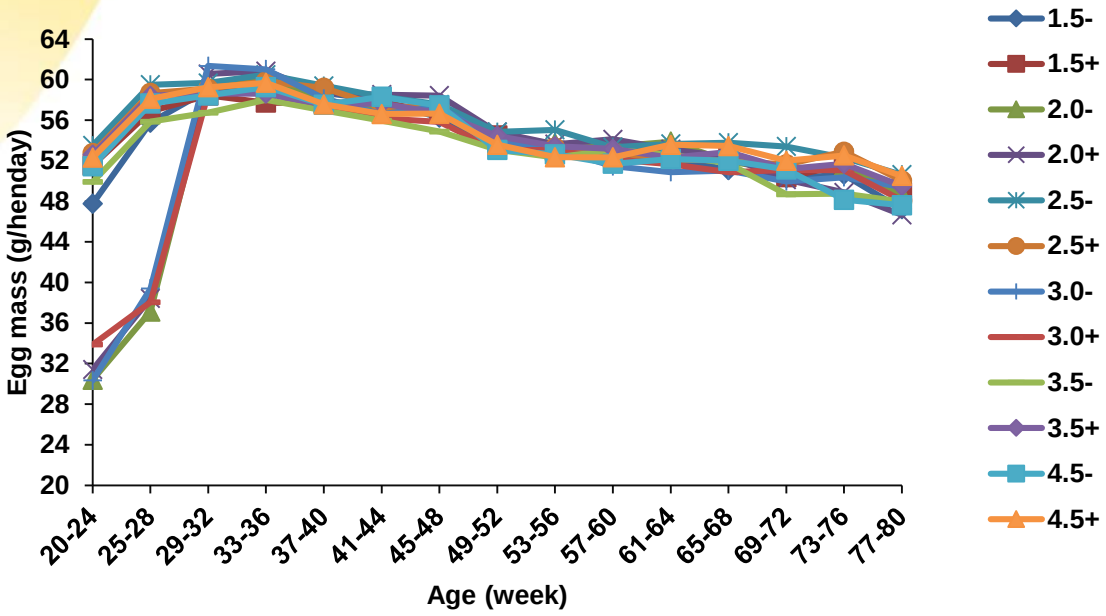

Figure 4-*6*: Effect of dietary AP concentrations and supplemental phytase on egg mass of birds from 20 to 80 weeks of age in Experiment 1

4.1.4 Feed to egg conversion ratio (g feed/g egg)

There was no significant effect of AP contents and phytase supplementation on feed to egg conversion ratios except for the period of salt deficiency at the beginning of the experiment (**Table 4-*3***).

The feed to egg conversion data indicated that in general a dietary AP of 1.5 g/kg was adequate to fulfil the need for a satisfactory feed conversion.

Overall egg production, egg shell defects, egg mass, feed intake and feed to egg conversion ratio from 20 to 80 weeks of age are summarised in **Table 4-*4***. The number of eggs laid per hen housed and henhoused egg production from 20 to 80 weeks of age was significantly higher for hens fed AP of 1.5 g/kg diets with phytase supplementation than those without (P<0.05). This was partially related to the higher mortality rate of hens fed low AP (1.5 g/kg) diets without phytase supplementation.

It should be mentioned that there were no significant differences in overall henday and henhoused egg production (%), total egg numbers, shell defect (%) and egg mass of hens fed diets contained 2.0 and 3.0 g/kg AP from other treatments from 29 to 80 weeks.

The negative effects of salt deficiency in diets containing AP 2.0 and 3.0 g/kg at the beginning of the trial were clearly evident in the results of all production performance except for egg shell defects.

Table 4-*3*: Effect of dietary AP concentrations and supplemental phytase on feed conversion (g feed/g egg) of hens from 20 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		20-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52*	53-56	57-60	61-64	65-68	69-72	73-76	77-80*
1.5	-	1.98	1.96	1.89	1.92	1.89	1.86	1.94	1.87	2.01	2.06	2.03	2.07	2.22	2.18	2.05
	+	1.88	1.91	1.93	1.93	1.84	1.84	1.86	1.83	2.02	2.01	1.96	2.03	2.15	2.15	2.03
2.0	-	2.60	2.66	1.89	1.88	1.86	1.88	1.89	1.86	1.98	1.99	1.96	2.07	2.09	2.08	2.01
	+	2.62	2.61	1.82	1.86	1.87	1.80	1.83	1.78	1.90	1.92	1.95	1.95	2.16	2.17	1.98
2.5	-	1.78	1.73	1.85	1.85	1.84	1.83	1.86	1.81	1.92	1.98	1.93	1.95	2.03	2.07	1.92
	+	1.79	1.74	1.84	1.86	1.83	1.90	1.88	1.86	2.01	2.06	2.04	2.01	2.10	2.03	1.97
3.0	-	2.62	2.47	1.83	1.85	1.86	1.90	1.92	1.85	1.96	2.03	2.00	2.01	2.16	2.07	1.96
	+	2.48	2.72	1.98	1.95	1.94	1.92	1.99	1.91	1.98	2.12	2.06	2.02	2.17	2.15	2.04
3.5	-	1.99	1.96	1.98	1.91	1.86	1.91	1.93	1.86	2.02	2.01	1.94	2.00	2.27	2.21	2.02
	+	1.92	1.83	1.84	1.87	1.84	1.83	1.85	1.86	1.98	1.96	1.96	1.97	2.13	2.03	1.96
4.5	-	1.86	1.80	1.79	1.84	1.90	1.82	1.85	1.84	2.02	1.99	2.00	2.04	2.10	2.17	1.99
	+	1.87	1.78	1.81	1.87	1.89	1.89	1.90	1.87	2.05	2.05	1.92	1.98	2.12	2.06	1.92
Main effect																
AP	Pooled SEM	0.038	0.036	0.023	0.026	0.022	0.022	0.025	0.021	0.027	0.031	0.030	0.043	0.044	0.049	0.038
	$LSD_{0.05}$	0.106	0.100	0.064	0.073	0.060	0.060	0.070	0.059	0.075	0.088	0.083	0.119	0.124	0.136	0.105
	P value	0.000	0.000	0.004	0.351	0.152	0.219	0.097	0.442	0.164	0.108	0.438	0.361	0.246	0.674	0.525
Phytase	Pooled SEM	0.022	0.021	0.013	0.015	0.012	0.012	0.014	0.012	0.015	0.018	0.017	0.025	0.026	0.028	0.022
	$LSD_{0.05}$	0.061	0.058	0.037	0.042	0.035	0.035	0.040	0.034	0.043	0.051	0.048	0.069	0.072	0.079	0.061
	P value	0.143	0.891	0.894	0.499	0.967	0.991	0.614	0.817	0.621	0.681	0.877	0.882	0.852	0.442	0.741
AP x Phytase	Pooled SEM	0.053	0.050	0.032	0.037	0.030	0.031	0.035	0.030	0.038	0.045	0.042	0.060	0.063	0.069	0.053
	$LSD_{0.05}$	0.150	0.141	0.091	0.104	0.085	0.086	0.099	0.084	0.106	0.125	0.118	0.169	0.175	0.193	0.149
	P value	0.565	0.011	0.001	0.589	0.440	0.039	0.083	0.134	0.390	0.273	0.212	0.668	0.493	0.328	0.646

Table 4-*4*: Effect of dietary AP concentrations and supplemental phytase on over all egg production, feed intake and feed to egg conversion ratio of hens from 20 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Eggs/ hen housed	Henday egg production (%)	Henhouse egg production (%)	Defect shelled egg (%)	Egg mass (g/henday)	Feed intake (g/b/d)	FCR (g feed/g egg)
1.5	**-**	371.0	88.2	86.9	2.5	53.6	107.2	1.99
	+	382.9	89.8	89.7	2.3	54.2	106.4	1.96
2.0	**-**	368.2	86.2	86.2	2.3	51.8	104.5	2.01
	+	362.9	85.1	85.0	1.9	52.0	103.3	1.98
2.5	**-**	387.5	90.8	90.7	2.6	55.6	105.5	1.89
	+	380.7	89.8	89.2	1.5	54.7	105.9	1.93
3.0	**-**	355.9	83.4	83.3	1.9	51.6	103.5	2.00
	+	363.7	85.2	85.2	1.9	51.3	106.7	2.07
3.5	**-**	370.8	88.3	86.8	2.4	53.1	105.5	1.98
	+	379.0	89.7	88.8	1.8	54.6	104.8	1.91
4.5	**-**	378.9	88.8	88.7	3.1	53.8	104.2	1.93
	+	378.9	90.1	88.7	3.5	54.7	105.8	1.93
Main effect								
AP	**Pooled SEM**	3.03	0.63	0.71	0.42	0.45	0.86	0.019
	$LSD_{0.05}$	8.50	1.76	1.99	1.18	1.27	2.41	0.053
	P value	0.00	0.00	0.00	0.20	0.00	0.293	0.000
Phytase	**Pooled SEM**	1.75	0.36	0.41	0.24	0.26	0.497	0.011
	$LSD_{0.05}$	4.91	1.02	1.15	0.68	0.73	1.39	0.031
	P value	0.29	0.22	0.29	0.41	0.42	0.575	0.882
AP x Phytase	**Pooled SEM**	4.29	0.89	1.00	0.59	0.64	1.22	0.027
	$LSD_{0.05}$	12.02	2.50	2.82	1.66	1.80	3.41	0.075
	P value	0.15	0.35	0.15	0.81	0.50	0.428	0.098

4.1.5 Egg and egg shell quality

4.1.5.1 Fraction of egg yolk and egg albumen

Dietary AP concentrations and supplemental phytase had no effects on proportions of egg yolk and white. In general, a fresh egg weighing 61.4 g consisted of approximately 25% yolk and 65 % white (**Table 4-*5***) from hens at 50 weeks of age. Dry matter contents of whole egg, yolk and white were 31, 51 and 12 %. The egg contained total of 18.7 g of DM, in which there were 8.0 g of dry egg yolk and 5.0 g of dry white and 5.7 g of shell.

Egg weight increased with hen age, however, the proportion of yolk and white fractions were marginally changed (**Table 4-*6***) at 80 weeks of age compared to smaller eggs at 50 weeks of age.

Table 4-*5*: Effect of dietary AP concentrations and supplemental phytase on egg fractions of hens at 50 weeks of age in Experiment 1

AP (g/kg)	Phytase	Egg weight (g/egg)	Fresh yolk (% of egg)	Yolk DM (%)	Fresh white (% of egg)	White DM (%)
1.5	**-**	61.9	25.4	51.1	65.2	12.3
	+	60.3	25.8	51.3	64.9	12.5
2.0	**-**	60.7	25.2	51.2	65.4	12.6
	+	63.5	25.1	52.0	65.8	12.5
2.5	**-**	61.0	25.1	51.1	65.7	12.4
	+	60.8	25.2	51.2	65.7	12.6
3.0	**-**	62.0	25.2	51.0	65.4	12.2
	+	60.5	25.9	51.3	64.6	12.2
3.5	**-**	61.0	26.2	51.1	64.6	12.2
	+	61.8	25.7	51.4	64.9	12.2
4.5	**-**	61.5	26.2	51.5	64.5	12.5
	+	61.2	25.4	51.1	65.4	12.3
Main effect						
AP	**Pooled SEM**	0.60	0.24	0.18	0.26	0.12
	$LSD_{0.05}$	1.66	0.67	0.50	0.73	0.32
	P value	0.83	0.11	0.45	0.08	0.16
Phytase	**Pooled SEM**	0.34	0.14	0.10	0.15	0.07
	$LSD_{0.05}$	0.96	0.39	0.29	0.42	0.19
	P value	0.98	0.80	0.16	0.73	0.95
APxphytase	**Pooled SEM**	0.84	0.34	0.25	0.37	0.16
	$LSD_{0.05}$	2.34	0.95	0.70	1.03	0.46
	P value	0.10	0.22	0.40	0.26	0.76

Table 4-*6*: Effect of dietary AP concentrations and supplemental phytase on egg fractions of hens at 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Egg weight (g/egg)	Fresh yolk (% of egg)	Yolk DM (%)	Fresh white (% of egg)	White DM (%)
1.5	**-**	64.1	26.9	51.6	64.2	11.6
	+	63.4	26.7	52.0	64.1	11.2
2	**-**	63.5	27.9	51.5	63.3	11.0
	+	66.6	26.1	51.3	64.5	11.6
2.5	**-**	65.1	25.6	51.4	65.5	11.5
	+	64.5	27.1	51.2	64.0	11.0
3	**-**	65.9	25.7	51.8	65.4	11.2
	+	64.3	26.3	51.5	64.7	11.6
3.5	**-**	63.0	26.6	51.3	64.6	11.1
	+	64.0	26.6	51.5	64.6	11.2
4.5	**-**	62.1	26.4	51.3	64.5	11.3
	+	62.7	26.2	51.6	64.8	11.4
AP	**Pooled SEM**	1.15	0.50	0.21	0.53	0.15
	$LSD_{0.05}$	3.19	1.39	0.59	1.48	0.42
	P value	0.489	0.757	0.526	0.681	0.812
Phytase	**Pooled SEM**	0.66	0.29	0.12	0.31	0.09
	$LSD_{0.05}$	1.84	0.80	0.34	0.86	0.25
	P value	0.75	0.950	0.871	0.748	0.678
APxphytase	**Pooled SEM**	1.62	0.71	0.30	0.75	0.22
	$LSD_{0.05}$	4.51	1.97	0.84	2.09	0.60
	P value	0.750	0.313	0.805	0.550	0.094

4.1.5.2 Albumen height and HU

Dietary AP concentrations and phytase supplementation had no significant effect on albumen heights (**Table 4-*7***) and HU (**Table 4-8**). Albumen viscosity decreased significantly with bird age HU of 90% at 24 weeks of age declining to 70% at 80 weeks of age, but no effects were observed by dietary AP concentrations and phytase supplementation. The albumen height measurement showed a similar pattern as the HU results.

The impacts of salt deficiency in diets containing 2.0 and 3.0g AP/kg at the beginning of the trial were still visible in the results of albumen height and HU.

Table 4-7: Effect of dietary AP concentrations and supplemental phytase on albumen height (mm) of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	8.0	7.5	7.9	7.7	7.2	7.4	6.6	7.3	7.1	6.9	6.7	6.6	6.1	6.2	6.2
	+	7.7	7.4	7.8	7.3	7.1	6.8	6.7	6.7	6.7	6.4	6.5	6.4	5.9	6.0	6.2
2.0	-	8.0	7.8	8.3	7.9	7.2	7.1	6.7	7.1	7.0	6.9	6.4	6.5	6.3	6.7	6.3
	+	8.1	7.8	8.1	7.7	7.4	7.5	6.8	7.0	6.7	6.8	6.7	6.6	6.4	6.7	6.1
2.5	-	7.9	7.4	7.9	7.7	7.2	7.0	6.7	7.1	6.7	6.6	6.4	6.3	6.1	5.7	6.2
	+	7.9	7.4	8.0	7.5	7.2	7.0	6.6	7.1	6.8	6.8	6.7	6.7	6.0	6.0	5.8
3.0	-	8.5	7.8	8.2	7.7	7.4	7.2	6.7	7.0	6.8	6.6	7.2	6.5	6.4	6.6	5.9
	+	7.8	7.8	8.1	7.6	7.4	7.0	6.7	7.2	7.1	6.9	6.5	6.5	6.5	6.4	6.3
3.5	-	7.6	7.3	8.0	7.6	7.2	6.6	6.5	6.8	6.7	6.4	6.7	6.2	6.0	6.1	6.0
	+	7.9	7.2	7.9	7.6	7.0	7.3	6.5	6.8	6.7	6.9	6.8	6.7	6.1	6.4	6.2
4.5	-	8.0	7.7	8.1	7.6	7.1	6.9	6.7	7.1	6.9	6.9	6.6	6.6	5.9	6.6	6.1
	+	7.8	7.2	8.3	7.6	7.1	7.3	6.7	7.0	6.7	6.4	6.2	6.4	6.0	6.1	6.0
Main effect																
AP	Pooled SEM	0.12	0.10	0.09	0.09	0.10	0.10	0.11	0.12	0.11	0.13	0.14	0.14	0.15	0.16	0.17
	$LSD_{0.05}$	0.33	0.27	0.25	0.26	0.27	0.28	0.31	0.33	0.32	0.35	0.39	0.38	0.42	0.46	0.47
	P value	0.133	0.000	0.005	0.513	0.307	0.394	0.668	0.566	0.676	0.833	0.182	0.999	0.086	0.004	0.925
Phytase	Pooled SEM	0.07	0.06	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.09	0.09	0.10
	$LSD_{0.05}$	0.19	0.15	0.14	0.15	0.16	0.16	0.18	0.19	0.18	0.20	0.22	0.22	0.24	0.26	0.27
	P value	0.122	0.265	0.507	0.115	0.819	0.111	0.716	0.280	0.568	0.919	0.426	0.367	0.800	0.616	0.853
AP x Phytase	Pooled SEM	0.17	0.14	0.13	0.13	0.14	0.14	0.16	0.17	0.16	0.18	0.20	0.20	0.21	0.23	0.24
	$LSD_{0.05}$	0.46	0.38	0.35	0.37	0.39	0.40	0.44	0.46	0.45	0.50	0.55	0.54	0.59	0.65	0.66
	P value	0.066	0.531	0.702	0.483	0.773	0.000	0.952	0.188	0.268	0.015	0.049	0.418	0.927	0.484	0.692

Table 4-8: Effect of dietary AP concentrations and supplemental phytase on Haugh Unit of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	**-**	91.2	86.7	88.9	87.5	84.7	85.5	79.8	85.2	83.4	82.4	80.3	79.8	76.3	75.8	76.4
	+	89.3	86.8	88.5	85.8	84.3	82.0	81.8	80.3	82.2	77.7	78.6	78.3	74.2	75.1	76.3
2.0	**-**	93.4	89.1	90.6	88.3	84.3	83.4	81.7	83.8	82.9	83.1	78.2	79.5	78.4	80.7	77.2
	+	93.3	89.0	89.9	87.3	85.7	86.1	81.4	82.8	81.4	81.6	80.0	79.4	77.9	79.8	75.5
2.5	**-**	90.5	85.8	88.4	87.5	84.6	83.3	81.3	83.4	81.2	80.2	78.6	77.4	75.1	71.2	74.3
	+	90.3	86.5	89.2	86.1	84.4	83.5	80.4	84.2	82.7	82.1	81.3	80.7	75.1	74.8	73.2
3.0	**-**	95.1	88.5	90.3	86.6	85.1	83.9	80.9	82.5	81.1	80.6	83.6	78.9	77.8	79.0	73.0
	+	91.8	88.8	89.8	87.2	85.4	83.1	81.3	84.7	83.5	82.6	78.8	79.4	78.2	77.8	78.3
3.5	**-**	89.0	86.4	89.6	86.8	85.0	80.7	80.1	82.1	81.0	78.7	80.8	77.2	74.9	75.2	74.5
	+	90.3	85.4	89.1	87.3	83.0	85.3	79.9	81.7	81.5	82.9	80.6	79.7	76.4	77.5	75.4
4.5	**-**	90.8	88.0	90.5	87.1	83.8	82.5	81.2	84.0	82.3	82.7	80.1	80.0	74.5	79.7	76.2
	+	89.5	86.4	91.4	87.1	84.5	85.3	81.4	82.7	80.7	79.8	76.3	78.4	74.9	74.3	73.8
Main effect																
AP	**Pooled SEM**	0.65	0.54	0.51	0.56	0.61	0.67	0.75	0.83	0.78	0.89	1.08	1.10	1.26	1.39	1.47
	$LSD_{0.05}$	1.80	1.50	1.41	1.55	1.70	1.87	2.10	2.30	2.18	2.47	3.00	3.06	3.51	3.87	4.10
	P value	0.000	0.000	0.010	0.752	0.684	0.580	0.749	0.604	0.747	0.596	0.411	0.994	0.200	0.008	0.806
Phytase	**Pooled SEM**	0.37	0.31	0.29	0.32	0.35	0.39	0.44	0.48	0.45	0.51	0.62	0.63	0.73	0.80	0.85
	$LSD_{0.05}$	1.04	0.86	0.81	0.89	0.98	1.08	1.21	1.33	1.26	1.43	1.73	1.76	2.03	2.23	2.37
	P value	0.075	0.540	0.885	0.282	0.924	0.077	0.729	0.251	0.979	0.812	0.247	0.576	0.965	0.752	0.906
AP x Phytase	**Pooled SEM**	0.91	0.76	0.72	0.79	0.86	0.95	1.07	1.17	1.11	1.26	1.52	1.55	1.79	1.97	2.09

4.1.5.3 Yolk colour

Yolk colour was lower from 52 to 68 weeks of age, especially for the hens fed diets containing AP of 1.5 g/kg. Birds were offered batch 3 diets from 50 weeks to 66 weeks of age. It was most likely that the pigment supplementation was inadequate in this batch of concentrates/diets. The diets containing AP of 1.5 g/kg with or without phytase supplementation were derived from the same concentrate and hens on these diets produced eggs with much lighter yolk colour. The batch 4 diets were offered from 66 weeks of age and yolk colour was restored although hens fed on diet containing AP of 1.5 g/kg without phytase supplementation were recovered significantly slower than other treatments (**Figure 4-*7***).

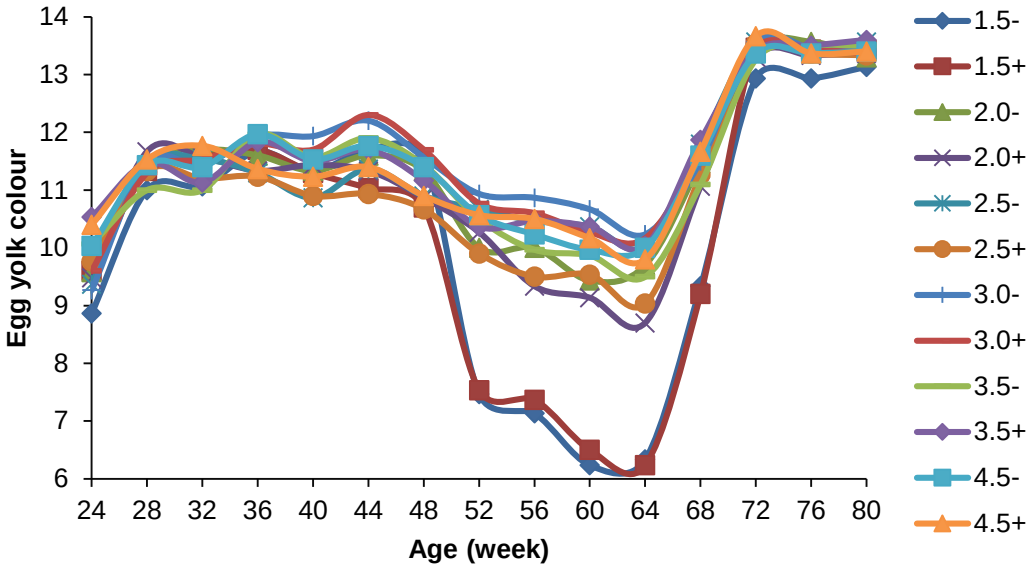

Figure 4-*7*. Effect of dietary AP concentrations and supplemental phytase on yolk colour of birds from 24 to 80 weeks of age in Experiment 1

4.1.5.4 Egg shell defects

Layers on diets with AP concentration of 4.5 g/kg with phytase supplementation produced significantly ($P<0.05$) more defect shells or more fragile eggs than the birds fed on other diets from 33 to 60 weeks of age (**Figure 4-*8***).

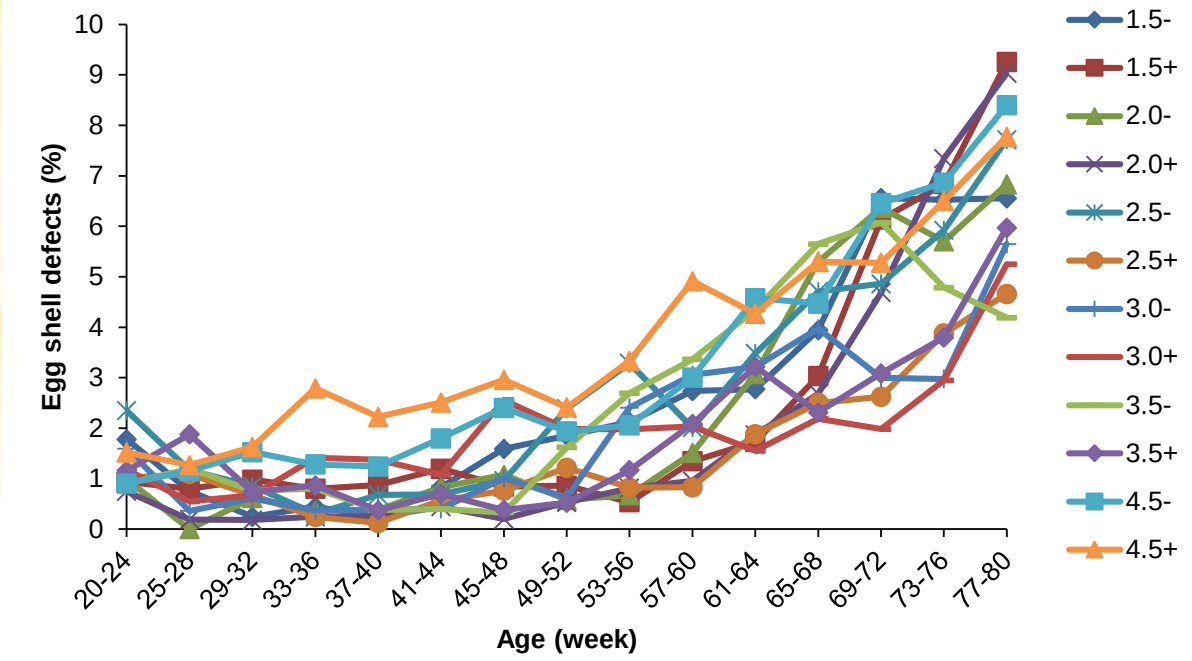

Figure 4-*8*: Effect of dietary AP concentrations and supplemental phytase on egg shell defects of hens from 20 to 80 weeks of age in Experiment 1

4.1.5.5 Egg shell colour

Egg shell colours became lighter as the hens aged, as evidenced by the lightness (L*) values increasing in time and a decrease in the amount of redness (a*) which was associated with a decrease in pigmentation in per unit egg shell. There were no differences in these parameters between dietary AP concentrations or those with or without phytase. There were large variations within each treatment. The detailed L* and a* values are presented in Appendix **Table 10-1** and **Table 10-2**.

4.1.5.6 Specific gravity

The effects of dietary AP concentrations with or without supplemental phytase on SG for the entire experiment are shown in **Table 4-*9***. In general, SG decreased with hen age and was not affected by either dietary AP concentrations or phytase supplementation.

Table 4-9: Effect of dietary AP concentrations and supplemental phytase on specific gravity of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	1.091	1.086	1.088	1.088	1.089	1.088	1.089	1.088	1.087	1.085	1.084	1.085	1.085	1.083	1.084
	+	1.092	1.085	1.087	1.089	1.086	1.088	1.088	1.087	1.089	1.086	1.087	1.086	1.085	1.083	1.083
2.0	-	1.092	1.088	1.088	1.087	1.088	1.088	1.089	1.090	1.088	1.087	1.088	1.087	1.085	1.082	1.083
	+	1.092	1.087	1.088	1.087	1.088	1.087	1.088	1.089	1.089	1.088	1.088	1.088	1.085	1.084	1.084
2.5	-	1.092	1.084	1.087	1.087	1.087	1.087	1.088	1.088	1.088	1.087	1.086	1.084	1.085	1.083	1.083
	+	1.093	1.085	1.087	1.086	1.086	1.088	1.088	1.089	1.087	1.086	1.086	1.085	1.084	1.081	1.084
3.0	-	1.089	1.086	1.088	1.088	1.088	1.090	1.089	1.089	1.089	1.086	1.087	1.086	1.085	1.082	1.083
	+	1.093	1.087	1.089	1.088	1.087	1.089	1.090	1.090	1.089	1.087	1.087	1.086	1.084	1.085	1.084
3.5	-	1.092	1.086	1.087	1.087	1.088	1.088	1.088	1.089	1.088	1.086	1.086	1.085	1.085	1.081	1.082
	+	1.092	1.085	1.086	1.087	1.088	1.088	1.090	1.088	1.088	1.086	1.085	1.084	1.084	1.082	1.081
4.5	-	1.092	1.086	1.088	1.088	1.086	1.088	1.088	1.089	1.089	1.087	1.087	1.083	1.082	1.083	1.085
	+	1.092	1.085	1.087	1.086	1.088	1.088	1.088	1.088	1.087	1.085	1.085	1.084	1.083	1.079	1.082
Main effect																
AP	Pooled SEM	0.0006	0.0004	0.0005	0.0005	0.0005	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0007	0.0008	0.0008	0.0007
	$LSD_{0.05}$	0.0018	0.0011	0.0013	0.0013	0.0015	0.0016	0.0016	0.0016	0.0016	0.0017	0.0017	0.0020	0.0022	0.0023	0.0020
	P value	0.727	0.000	0.102	0.075	0.274	0.279	0.372	0.035	0.640	0.247	0.036	0.016	0.336	0.196	0.219
Phytase	Pooled SEM	0.0004	0.0002	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0004	0.0004	0.0004	0.0005	0.0005	0.0004
	$LSD_{0.05}$	0.0010	0.0006	0.0007	0.0007	0.0009	0.0009	0.0009	0.0009	0.0009	0.0010	0.0010	0.0011	0.0013	0.0014	0.0012
	P value	0.047	0.204	0.332	0.707	0.144	0.878	0.908	0.544	0.954	0.787	0.697	0.289	0.447	0.573	0.963
AP x Phytase	Pooled SEM	0.0009	0.0006	0.0006	0.0006	0.0008	0.0008	0.0008	0.0008	0.0008	0.0009	0.0009	0.0010	0.0011	0.0012	0.0010
	$LSD_{0.05}$	0.0025	0.0016	0.0018	0.0018	0.0022	0.0023	0.0023	0.0022	0.0023	0.0025	0.0024	0.0028	0.0032	0.0033	0.0029
	P value	0.314	0.027	0.215	0.443	0.145	0.891	0.474	0.458	0.510	0.457	0.237	0.791	0.987	0.053	0.231

4.1.5.7 Egg shell breaking strength, egg shell weight, shell thickness and shell percentage of egg

In general, there were no effects of treatments either AP concentrations or supplemental phytase in diets on egg shell breaking strength (**Table 4-*10***). Egg shell thickness (**Table 4-11**) and shell weight (**Table 4-12**) were affected by dietary AP concentrations from 24 to 36 weeks of age. The statistical differences in egg shell thickness and shell weight between AP concentrations were not detected after 36 weeks of age. The hens fed on salt deficient diets had significantly lower feed intake and produced eggs with thinner and lighter shells at 24 weeks age. Salt was supplemented at 26 weeks of age. The affected birds layed eggs with thicker and heavier shells than the other treatments at 28 and 32 weeks of age.

Egg shell breaking strength and thickness decreased with bird age, whereas egg shell weight increased with bird age as egg size increased when hens got older. Phytase supplementation had no effect on overall egg shell thickness and shell weight throughout the experimental period. Although both egg weight and shell weight increased with hen age, the ratio of shell weight to egg weight, shell percentage of egg, decreased with age (**Table 4-13**). Shell weight per surface area (mg/cm^2) was not affected by any dietary treatment (Appendix **Table 10-3**).

4.1.6 Mortality

Mortality was low throughout this trial, with a total of 25 out of 720 birds dying (3.5 %) by 80 weeks of age and the majority of deaths occurred after 60 weeks of age. No hens died before 50 weeks of age. Mortality of hens on low AP (1.5 g/kg) diet without phytase supplementation was 6.7 % which was comparable with the standard of Hy-Line Brown commercial layers, but higher than those on diets with the same level of AP supplemented with phytase (1.7 %).

Table 4-*10*: Effect of dietary AP concentrations and supplemental phytase on egg shell breaking strength (kg) of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	**-**	3.9	4.1	3.8	3.5	4.0	3.7	3.6	3.2	3.4	2.9	3.2	2.9	3.1	3.0	3.0
	+	4.2	4.2	3.7	4.0	3.8	3.7	3.5	3.4	3.6	3.2	3.1	2.9	2.7	3.2	2.9
2.0	**-**	4.1	4.3	3.7	3.5	3.7	3.6	3.5	3.5	3.4	3.2	3.1	3.3	3.0	3.1	2.7
	+	4.2	4.1	3.8	4.0	3.7	3.5	3.4	3.5	3.4	3.3	3.4	3.4	2.8	3.1	2.9
2.5	**-**	4.0	4.0	3.6	3.8	3.7	3.6	3.4	3.4	3.4	3.0	3.4	3.0	3.2	3.0	3.0
	+	4.1	4.0	3.6	3.8	3.6	3.6	3.4	3.5	3.1	3.2	3.2	3.1	3.0	2.8	3.1
3.0	**-**	3.6	4.0	3.8	3.9	3.9	3.9	3.5	3.6	3.5	3.2	3.3	3.3	3.1	3.0	3.1
	+	4.1	4.2	3.9	4.0	3.6	3.9	3.6	3.8	3.5	3.1	3.4	3.3	2.9	2.7	3.2
3.5	**-**	4.1	4.2	3.6	3.8	3.7	3.7	3.4	3.5	3.5	3.3	3.2	3.3	2.9	3.1	2.7
	+	4.1	4.0	3.6	3.9	4.0	3.9	3.4	3.6	3.4	3.2	3.2	3.1	3.0	3.0	2.7
4.5	**-**	4.2	4.2	3.8	4.1	3.8	3.7	3.3	3.5	3.5	3.4	3.4	2.9	2.9	3.0	3.1
	+	4.0	4.0	3.5	3.7	3.7	3.6	3.4	3.3	3.2	3.2	3.1	3.2	2.9	2.9	2.9
Main effect																
AP	**Pooled SEM**	0.09	0.09	0.09	0.10	0.09	0.09	0.10	0.10	0.09	0.10	0.10	0.11	0.10	0.09	0.10
	$LSD_{0.05}$	0.26	0.25	0.24	0.28	0.26	0.25	0.27	0.28	0.26	0.29	0.26	0.31	0.28	0.26	0.27
	P value	0.318	0.663	0.095	0.670	0.373	0.131	0.551	0.030	0.501	0.516	0.736	0.055	0.707	0.286	0.038
Phytase	**Pooled SEM**	0.05	0.05	0.05	0.06	0.05	0.05	0.06	0.06	0.05	0.06	0.05	0.06	0.06	0.05	0.06
	$LSD_{0.05}$	0.15	0.14	0.14	0.16	0.15	0.15	0.15	0.16	0.15	0.17	0.15	0.18	0.16	0.15	0.16
	P value	0.116	0.670	0.658	0.124	0.382	0.929	0.990	0.597	0.426	0.810	0.640	0.432	0.051	0.232	0.789
AP x Phytase	**Pooled SEM**	0.13	0.13	0.12	0.14	0.13	0.13	0.14	0.14	0.13	0.15	0.13	0.16	0.14	0.13	0.14
	$LSD_{0.05}$	0.37	0.35	0.34	0.40	0.37	0.36	0.38	0.39	0.36	0.41	0.37	0.43	0.40	0.37	0.39
	P value	0.062	0.401	0.554	0.012	0.275	0.763	0.943	0.736	0.217	0.457	0.239	0.660	0.583	0.500	0.540

Table 4-11: Effect of dietary AP concentrations and supplemental phytase on shell thickness (mm) of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	0.40	0.40	0.40	0.40	0.41	0.39	0.39	0.39	0.38	0.34	0.36	0.34	0.38	0.37	0.38
	+	0.40	0.39	0.39	0.41	0.40	0.39	0.38	0.38	0.38	0.35	0.36	0.35	0.36	0.37	0.38
2.0	-	0.39	0.40	0.40	0.40	0.40	0.39	0.39	0.39	0.39	0.35	0.36	0.34	0.36	0.37	0.37
	+	0.40	0.41	0.40	0.41	0.40	0.38	0.39	0.39	0.39	0.36	0.37	0.36	0.37	0.37	0.38
2.5	-	0.40	0.39	0.39	0.40	0.40	0.39	0.38	0.39	0.38	0.36	0.36	0.34	0.37	0.37	0.38
	+	0.40	0.39	0.38	0.39	0.39	0.38	0.38	0.39	0.37	0.35	0.35	0.32	0.36	0.36	0.38
3.0	-	0.39	0.40	0.40	0.41	0.40	0.40	0.39	0.40	0.39	0.35	0.35	0.35	0.37	0.36	0.37
	+	0.39	0.40	0.40	0.40	0.39	0.39	0.39	0.40	0.38	0.35	0.36	0.34	0.37	0.37	0.38
3.5	-	0.41	0.39	0.39	0.39	0.40	0.39	0.38	0.39	0.37	0.36	0.35	0.35	0.36	0.36	0.37
	+	0.40	0.39	0.38	0.39	0.40	0.38	0.39	0.39	0.38	0.35	0.34	0.33	0.37	0.36	0.36
4.5	-	0.41	0.40	0.40	0.40	0.39	0.39	0.38	0.39	0.38	0.35	0.35	0.33	0.36	0.37	0.38
	+	0.40	0.39	0.39	0.39	0.40	0.39	0.38	0.39	0.37	0.33	0.34	0.34	0.36	0.35	0.36
Main effect																
AP	**Pooled SEM**	0.004	0.003	0.003	0.003	0.003	0.004	0.004	0.003	0.004	0.004	0.005	0.005	0.006	0.004	0.005
	$LSD_{0.05}$	0.010	0.008	0.008	0.009	0.009	0.010	0.010	0.009	0.011	0.011	0.013	0.014	0.016	0.012	0.013
	P value	0.018	0.004	0.000	0.011	0.102	0.416	0.489	0.115	0.043	0.182	0.022	0.053	0.861	0.711	0.127
Phytase	**Pooled SEM**	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.003
	$LSD_{0.05}$	0.006	0.005	0.004	0.005	0.005	0.006	0.006	0.005	0.007	0.006	0.008	0.008	0.009	0.007	0.007
	P value	0.806	0.403	0.037	0.411	0.164	0.084	0.985	0.414	0.332	0.204	0.784	0.935	0.658	0.323	0.905
AP x Phytase	**Pooled SEM**	0.005	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.006	0.006	0.007	0.007	0.008	0.006	0.006
	$LSD_{0.05}$	0.014	0.011	0.011	0.012	0.012	0.014	0.014	0.013	0.016	0.016	0.019	0.020	0.022	0.018	0.018
	P value	0.652	0.283	0.594	0.193	0.051	0.930	0.757	0.894	0.411	0.057	0.398	0.022	0.744	0.100	0.238

Table 4-12: Effect of dietary AP concentrations and supplemental phytase on shell weight (g) of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	5.1	5.8	5.8	6.0	5.9	5.8	5.8	5.7	5.8	5.4	5.7	5.7	5.9	5.9	5.9
	+	5.3	5.6	5.7	5.8	5.7	5.8	5.6	5.7	5.7	5.6	5.8	5.8	5.6	5.6	5.9
2.0	-	4.5	5.7	5.9	5.9	5.8	5.8	5.7	5.8	5.8	5.5	5.9	5.7	5.7	5.7	5.7
	+	4.7	5.8	5.9	6.0	5.8	5.8	5.8	5.9	5.8	5.8	6.0	5.9	5.8	5.9	5.9
2.5	-	5.2	5.7	5.7	5.9	5.8	5.8	5.6	5.8	5.8	5.7	5.8	5.6	5.8	5.7	5.9
	+	5.4	5.7	5.7	5.7	5.6	5.7	5.6	5.8	5.5	5.5	5.7	5.7	5.7	5.7	6.0
3.0	-	4.6	5.8	6.0	6.1	6.0	6.0	5.8	6.0	5.9	5.7	5.8	5.8	5.9	5.8	5.9
	+	4.7	5.7	5.9	5.9	5.8	5.9	5.8	6.0	5.8	5.7	6.0	5.7	5.7	5.7	5.8
3.5	-	5.2	5.6	5.7	5.8	5.8	5.8	5.6	5.9	5.6	5.7	5.6	5.8	5.7	5.7	5.7
	+	5.2	5.5	5.6	5.8	5.8	5.8	5.8	5.9	5.8	5.5	5.7	5.6	5.7	5.7	5.6
4.5	-	5.5	5.6	5.7	5.8	5.6	5.7	5.7	5.8	5.8	5.6	5.7	5.4	5.6	5.8	5.7
	+	5.3	5.6	5.7	5.7	5.7	5.7	5.6	5.7	5.7	5.5	5.7	5.6	5.6	5.5	5.6
Main effect																
AP	Pooled SEM	0.06	0.06	0.06	0.06	0.06	0.07	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.08
	$LSD_{0.05}$	0.17	0.18	0.15	0.17	0.16	0.19	0.18	0.18	0.19	0.19	0.20	0.23	0.24	0.21	0.22
	P value	0.000	0.128	0.000	0.014	0.180	0.321	0.241	0.011	0.391	0.498	0.055	0.135	0.478	0.756	0.020
Phytase	Pooled SEM	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.04	0.05
	$LSD_{0.05}$	0.10	0.10	0.09	0.10	0.09	0.11	0.10	0.10	0.11	0.11	0.11	0.14	0.14	0.12	0.13
	P value	0.182	0.189	0.168	0.076	0.162	0.545	0.914	0.682	0.375	0.607	0.272	0.306	0.235	0.172	0.913
AP x Phytase	Pooled SEM	0.08	0.09	0.08	0.08	0.08	0.10	0.09	0.09	0.09	0.10	0.10	0.12	0.12	0.11	0.11
	$LSD_{0.05}$	0.23	0.25	0.22	0.23	0.23	0.27	0.25	0.25	0.26	0.27	0.28	0.33	0.33	0.30	0.31
	P value	0.232	0.328	0.948	0.372	0.485	0.958	0.109	0.967	0.579	0.094	0.793	0.630	0.682	0.191	0.663

Table 4-13: Effect of dietary AP concentrations and supplemental phytase on egg shell percentage of egg (%) of hens from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	**-**	9.7	9.8	9.7	9.7	9.6	9.5	9.4	9.4	9.4	9.1	9.0	9.0	9.3	9.2	9.3
	+	9.9	9.6	9.5	9.7	9.4	9.5	9.3	9.5	9.6	9.3	9.4	9.3	9.1	9.0	9.3
2.0	**-**	10.0	9.8	9.7	9.6	9.5	9.4	9.4	9.6	9.5	9.5	9.5	9.4	9.3	9.0	9.0
	+	10.1	9.9	9.7	9.7	9.5	9.3	9.2	9.6	9.6	9.5	9.5	9.5	9.1	9.4	9.4
2.5	**-**	9.6	9.4	9.4	9.6	9.4	9.4	9.2	9.5	9.4	9.4	9.4	9.0	9.3	9.0	9.1
	+	9.7	9.5	9.4	9.4	9.1	9.5	9.2	9.6	9.3	9.3	9.2	9.1	9.1	9.0	9.3
3.0	**-**	9.5	9.6	9.5	9.7	9.5	9.7	9.4	9.6	9.6	9.3	9.2	9.2	9.3	9.0	9.1
	+	10.0	9.9	9.8	9.7	9.4	9.6	9.5	9.8	9.5	9.4	9.5	9.3	9.1	9.3	9.4
3.5	**-**	9.9	9.7	9.6	9.5	9.6	9.4	9.3	9.6	9.3	9.4	9.2	9.3	9.2	9.0	9.1
	+	9.6	9.2	9.4	9.6	9.5	9.5	9.5	9.5	9.5	9.2	9.0	9.0	9.2	8.9	8.7
4.5	**-**	9.8	9.6	9.6	9.6	9.3	9.4	9.2	9.6	9.5	9.4	9.3	8.8	9.0	9.2	9.3
	+	9.7	9.5	9.5	9.4	9.5	9.5	9.2	9.4	9.3	9.2	9.1	9.0	9.0	8.6	8.9
Main effect																
AP	**Pooled SEM**	0.10	0.09	0.07	0.08	0.08	0.09	0.09	0.09	0.10	0.10	0.10	0.12	0.12	0.12	0.11
	$LSD_{0.05}$	0.27	0.26	0.20	0.23	0.22	0.25	0.25	0.24	0.27	0.29	0.27	0.34	0.34	0.34	0.31
	P value	0.122	0.004	0.020	0.163	0.110	0.271	0.328	0.152	0.485	0.481	0.075	0.037	0.694	0.342	0.109
Phytase	**Pooled SEM**	0.06	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.12	0.07	0.07	0.06
	$LSD_{0.05}$	0.16	0.15	0.11	0.13	0.13	0.15	0.14	0.14	0.15	0.17	0.16	0.34	0.19	0.20	0.18
	P value	0.506	0.513	0.362	0.612	0.218	0.916	0.848	0.891	0.488	0.916	0.932	0.495	0.174	0.704	0.864
AP x Phytase	**Pooled SEM**	0.14	0.13	0.10	0.12	0.11	0.13	0.13	0.12	0.14	0.15	0.14	0.17	0.17	0.17	0.16
	$LSD_{0.05}$	0.38	0.36	0.28	0.32	0.31	0.36	0.35	0.34	0.38	0.41	0.38	0.46	0.48	0.48	0.44
	P value	0.030	0.065	0.332	0.456	0.240	0.902	0.515	0.613	0.345	0.669	0.130	0.565	0.974	0.048	0.075

4.1.7 Blood Ca and P concentrations

Plasma Ca and P concentrations are shown in **Table 4-*14***. There was no consistent effect of dietary AP concentrations or supplemental phytase on Ca and P levels in blood. There were considerable variations in Ca and P concentrations although the time for blood sampling was standardised.

Table 4-*14*: Effect of dietary AP concentrations and supplemental phytase on plasma Ca and P contents of hens at 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Mean (range)	
		Ca (mmol/L)	P (mmol/L)
1.5	-	5.9 (3.4-7.3)	1.5 (0.6-2.4)
	+	5.7 (1.8-7.3)	1.7 (0.9-2.2)
2.0	-	7.5 (4.0-9.4)	2.0 (1.0-3.1)
	+	6.9 (6.4-7.4)	1.8 (1.7-2.0)
2.5	-	5.8 (4.5-8.0)	1.7 (1.0-2.3)
	+	7.5 (6.1-8.2)	2.3 (1.6-3.0)
3.0	-	5.5 (3.8-6.4)	1.5 (1.1-1.7)
	+	5.2 (3.3-7.1)	1.5 (0.9-2.1)
3.5	-	7.9 (6.5-9.7)	2.1 (1.8-2.3)
	+	5.9 (3.6-7.3)	1.8 (0.7-2.3)
4.5	-	6.1 (4.3-8.7)	2.1 (1.1-2.9)
	+	6.5 (3.7-8.2)	2.1 (1.4-2.7)
Main effect			
AP	Pooled SEM	0.61	0.22
	$LSD_{0.05}$	1.72	0.61
	P value	0.287	0.325
Phytase	Pooled SEM	0.35	0.12
	$LSD_{0.05}$	0.99	0.35
	P value	0.754	0.770
AP x phytase	Pooled SEM	0.87	0.31
	$LSD_{0.05}$	2.43	0.86
	P value	0.460	0.746

4.1.8 Calcium and P retention and excretion

Dietary Ca was approximately 50 to 60% retained in the body of the layers at 50 weeks old (**Table 4-*15***) and much less at 80 weeks old (**Table 4-*16***). High levels of Ca and P appeared in excreta. The percentages of Ca in excreta varied although Ca concentration was the same across all diets. Particle size variation of limestone may have contributed to some of the variations. Lower Ca retention at 80 weeks than 50 weeks of age was associated with less demand of Ca for egg production at 80 weeks old.

Dietary P retention was less than 30% at both 50 and 80 weeks of age. There was poor correlation (R = 0.017 and 0.001 for 50 and 80 weeks of age) between dietary AP levels and P retention percentages; however the amount of retainable P increased as dietary AP level increased (**Table 4-*15*** and **Table 4-*16***). Phytase supplementation numerically increased percentage of P retention at 50 weeks of age but no consistent effect observed at 80 weeks of age. There were large variations within some of the replicates.

Dietary total Ca to AP ratios ranged from 9.3 to 28 and determined retainable Ca to P ratios from 14 to 22 and from 7 to 11 at 50 and 80 weeks of age.

Table 4-*15*: Calcium and P retention, determined dietary retainable Ca and P of hens at 50 weeks of age in Experiment 1

AP (g/kg)	Phytase	Ca retention (%)	P retention (%)	Dietary retainable Ca (g/kg)	Dietary retainable P (g/kg)
1.5	**-**	52.5	22.8	31.4	1.41
	+	60.2	26.4	32.5	1.52
2.0	**-**	60.5	24.5	37.1	1.74
	+	60.2	26.5	29.4	1.86
2.5	**-**	61.1	24.7	31.6	1.81
	+	54.1	26.4	32.1	2.08
3.0	**-**	48.6	23.7	29.3	1.94
	+	56.3	27.7	30.5	2.20
3.5	**-**	52.1	26.8	36.5	2.43
	+	58.0	27.4	32.8	2.39
4.5	**-**	57.8	26.1	34.6	2.71
	+	59.5	26.8	37.8	3.01

Table 4-*16*: Calcium and P retention, determined dietary retainable Ca and P of hens at 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Ca retention (%)	P retention (%)	Dietary retainable Ca (g/kg)	Dietary retainable P (g/kg)
1.5	**-**	33.3	28.9	12.2	1.54
	+	41.9	25.4	14.2	1.30
2.0	**-**	37.7	27.7	16.7	1.60
	+	34.2	27.2	15.1	1.49
2.5	**-**	33.8	27.0	13.8	1.70
	+	29.6	26.1	12.5	1.72
3.0	**-**	27.2	22.2	10.4	1.43
	+	43.6	26.2	20.1	1.88
3.5	**-**	33.0	22.1	13.9	1.65
	+	38.9	30.5	16.5	2.35

AP (g/kg)	Phytase	Ca retention (%)	P retention (%)	Dietary retainable Ca (g/kg)	Dietary retainable P (g/kg)
4.5	**-**	45.2	34.7	20.8	3.19
	+	40.4	24.7	16.9	2.20

Calcium contents in excreta were 5.6-8.3 and 6.0-8.8 % DM for 50 and 80 weeks of age, which is equivalent to 15-18 and 16-24 % limestone in excreta DM for 50 and 80 weeks of age (assuming limestone contains 37 % Ca). The following picture (**Figure 4-*9***) clearly demonstrates the large amount of limestone excreted. Limestone was also recovered from excreta of birds fed commercial layer diet (in a different trial).

Figure 4-*9*: Limestone recovered from excreta of layers

Dietary AP and supplemental phytase had no consistent effect on the excreta Ca content.

Excreta P contents increased as the dietary AP concentrations increased for hens at 50 (**Figure 4-*10***) and 80 (**Figure 4-*11***) weeks of age. Supplemental phytase in the diet did not affect P excretion.

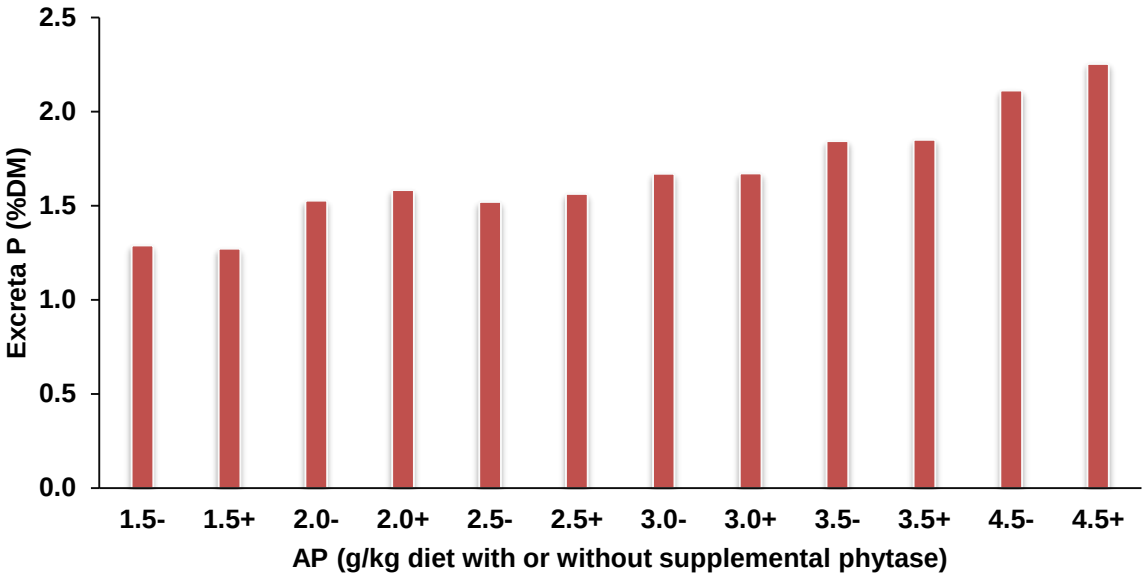

Figure 4-*10*: Effect of dietary AP concentrations and supplemental phytase on excreta P content of hens at 50 weeks of age in Experiment 1

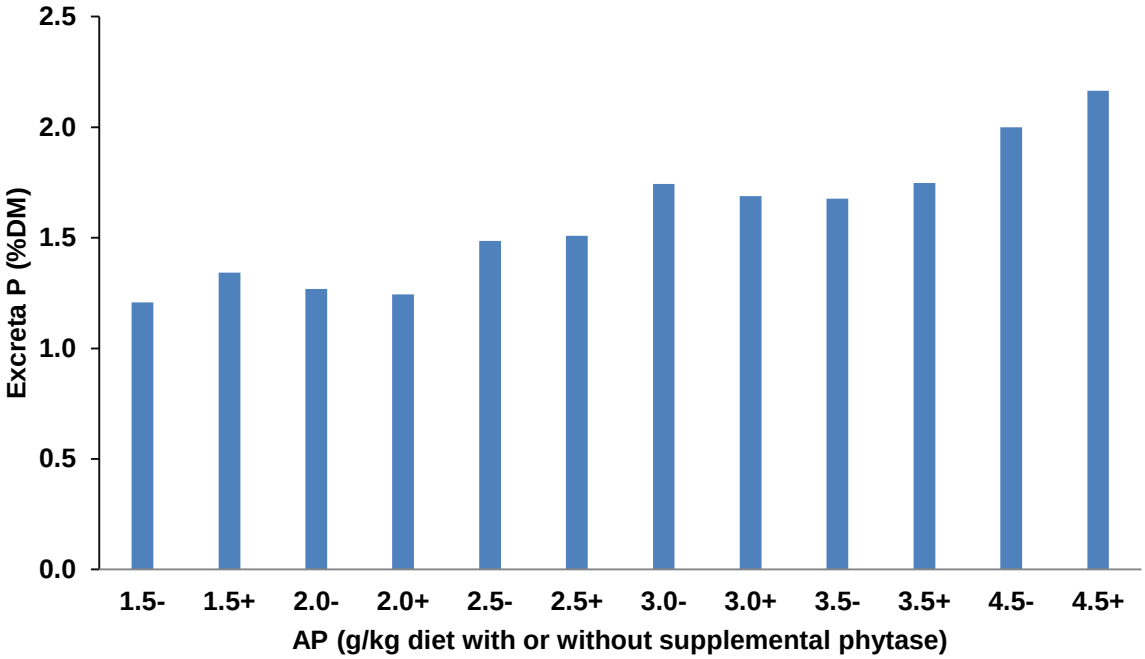

Figure 4-*11*: Effect of dietary AP concentrations and supplemental phytase on excreta P content of hens at 80 weeks of age in Experiment 1

4.2 Effect of dietary AP and Ca concentrations and supplemental phytase on layer performance (Experiment 2)

4.2.1 Body weight

Body weight of birds increased dramatically from 16 to 20 weeks of age and then kept steadily increasing up to 50 weeks of age and only marginally changed after that. Dietary AP and Ca levels had significant effect on body weight. Birds fed on diets containing AP 1.5 and Ca 32 g/kg without phytase supplementation had smaller body weights (P <0.05) than the rest of treatments (**Figure 4-*12***).

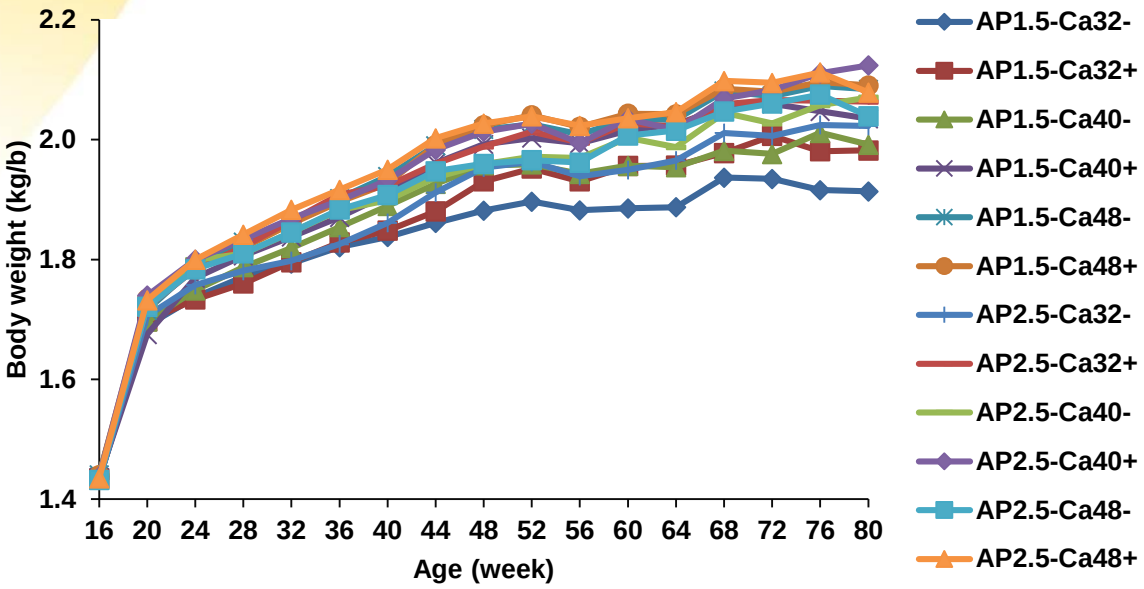

Figure 4-*12*: Effect of dietary AP and Ca concentrations and supplemental phytase on body weight of hens from 16 to 80 weeks of age in Experiment 2

4.2.2 Feed intake

In general, there were no significant effects of dietary AP and Ca concentrations and supplemental phytase on feed intake throughout the experimental period (**Table 4-*17***). Some decline and variation in feed intake observed near the end of the trial between the birds fed on diets with different AP levels could be due to the decline of egg production.

Table 4-*17*: Effect of dietary AP and Ca concentrations and supplemental phytase on feed intake (g/b/d) of hens from 17 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)															
			17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52	53-56	57-60	61-64	65-68	69-72	73-76	77-80
1.5	32	-	78.1	98.5	103.0	103.5	107.2	106.7	108.1	109.0	108.3	109.6	110.0	110.3	112.6	111.0	104.1	106.3
		+	81.4	96.5	101.8	104.3	105.5	106.5	107.8	107.5	108.8	106.4	109.2	109.4	108.5	106.1	101.6	102.6
1.5	40	-	78.9	98.0	103.4	106.0	107.1	108.1	109.8	108.7	108.2	107.7	108.5	110.7	109.2	108.5	104.9	100.9
		+	80.6	99.3	101.1	104.6	105.8	106.1	109.5	109.2	108.0	110.1	109.2	110.8	110.6	109.9	106.0	102.5
1.5	48	-	80.2	101.0	104.8	105.9	106.5	108.2	109.4	110.2	107.3	107.2	108.7	110.3	111.1	107.9	103.5	103.2
		+	81.4	99.4	102.5	104.2	105.9	105.7	107.7	107.9	107.1	104.4	108.6	108.2	111.6	108.7	101.5	108.0
2.5	32	-	79.3	97.1	102.0	102.8	105.4	105.6	106.6	108.6	107.8	107.7	107.1	108.1	110.9	109.7	107.3	109.9
		+	82.6	97.2	104.5	106.0	106.1	109.5	110.4	111.3	110.9	111.3	110.3	111.4	114.7	109.0	105.3	105.9
2.5	40	-	79.8	97.5	100.9	103.1	106.3	106.7	108.0	109.0	107.8	112.0	109.6	110.0	110.5	109.6	107.5	109.7
		+	83.0	99.7	103.6	105.4	107.4	108.7	110.0	110.7	108.5	109.1	110.3	111.8	113.5	111.3	109.0	108.8
2.5	48	-	81.5	99.8	103.9	103.8	108.1	108.7	108.4	107.1	106.2	111.9	110.7	111.3	111.0	109.6	109.8	107.2
		+	81.9	101.5	103.5	106.0	107.1	108.4	109.3	108.9	107.4	108.3	110.8	112.2	110.4	110.5	107.7	108.5
Main effect																		
AP	Pooled SEM		0.47	0.61	0.56	0.60	0.58	0.54	0.44	0.45	0.61	0.72	0.62	0.62	0.70	0.61	0.96	0.95
	$LSD_{0.05}$		1.33	1.72	1.57	1.67	1.63	1.51	1.22	1.25	1.70	2.02	1.73	1.75	1.95	1.71	2.68	2.66
	P value		0.068	0.978	0.669	0.798	0.617	0.169	0.932	0.437	0.883	0.017	0.223	0.341	0.208	0.151	0.003	0.001
Ca	Pooled SEM		0.58	0.75	0.68	0.73	0.71	0.66	0.53	0.55	0.74	0.88	0.76	0.77	0.85	0.75	1.17	1.16
	$LSD_{0.05}$		1.63	2.10	1.92	2.05	2.00	1.85	1.50	1.53	2.08	2.47	2.12	2.14	2.39	2.09	3.28	3.26
	P value		0.525	0.016	0.331	0.715	0.688	0.778	0.352	0.539	0.179	0.375	0.727	0.648	0.789	0.689	0.388	0.729
Phytase	Pooled SEM		0.47	0.61	0.56	0.60	0.58	0.54	0.44	0.45	0.61	0.72	0.62	0.62	0.70	0.61	0.96	0.95
	$LSD_{0.05}$		1.33	1.72	1.57	1.67	1.63	1.51	1.22	1.25	1.70	2.02	1.73	1.75	1.95	1.71	2.68	2.66
	P value		0.001	0.733	0.843	0.289	0.547	0.840	0.248	0.455	0.319	0.297	0.210	0.545	0.489	0.864	0.454	0.925

4.2.3 Egg production

Hens started to lay at approximate 18 weeks of age, average henday egg production was about 40 % for the first 3 weeks of lay and then sharply increased to above 90% at 21 weeks of age. Average henday egg production was over 95% from 22 to 40 weeks of age (data not presented) and overall average of 85% from 17 to 80 weeks of age (**Figure 4-*13***). Henhoused egg production followed the same trends as henday egg production except for hens on diet containing AP of 2.5 and Ca of 4.8 g/kg without supplemental phytase (**Figure 4-*14***) in which average henhoused egg production from 17 to 80 weeks of age was 83.3%. In general, dietary AP and Ca concentrations and supplemental phytase had no significant impact on overall egg production.

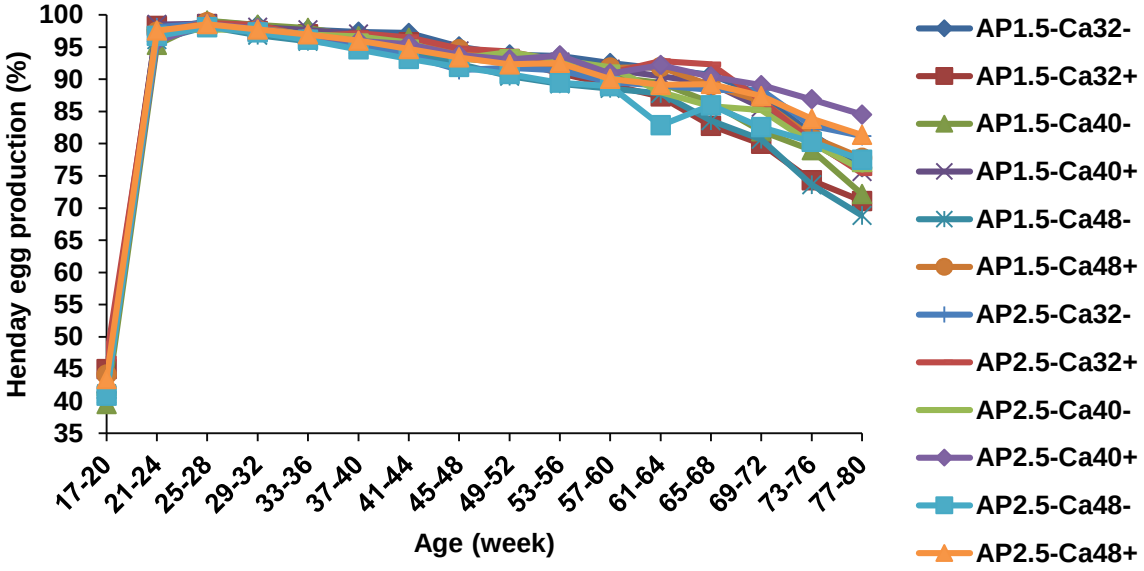

Figure 4-*13*: Effect of dietary AP and Ca concentrations and supplemental phytase on henday egg production of hens from 17 to 80 weeks of age in Experiment 2

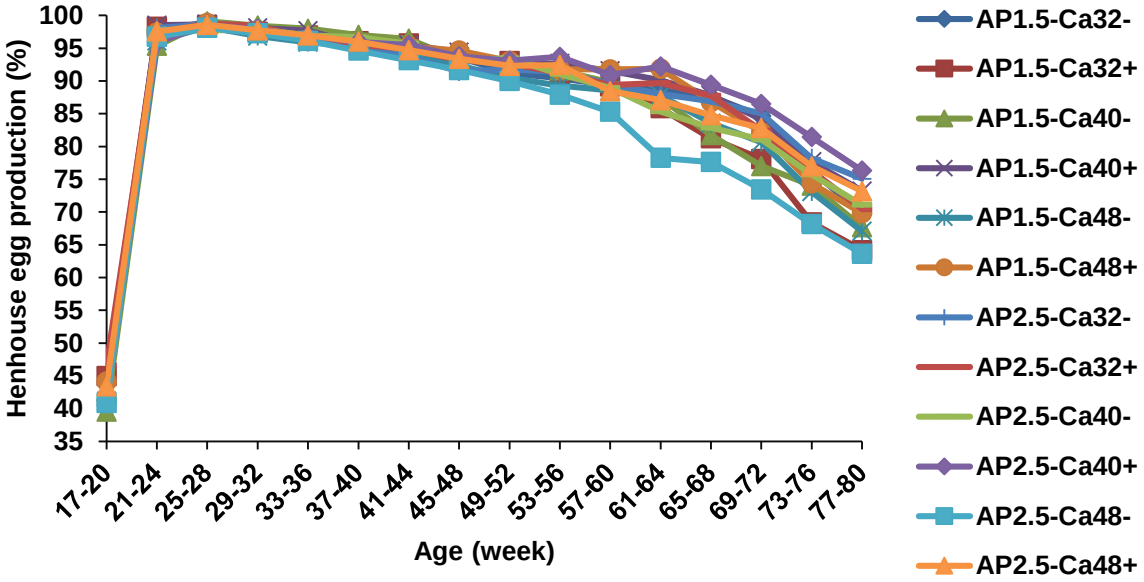

Figure 4-*14*: Effect of dietary AP and Ca concentrations and supplemental phytase on henhoused egg production of hens from 17 to 80 weeks of age in Experiment 2

4.2.4 Egg shell defects

Egg shell defect percentage was low from first lay to 50 weeks of age and increased with hen age (**Figure 4-*15***). In general, there were no effects of dietary AP and Ca concentration and phytase supplementation on overall egg shell defects.

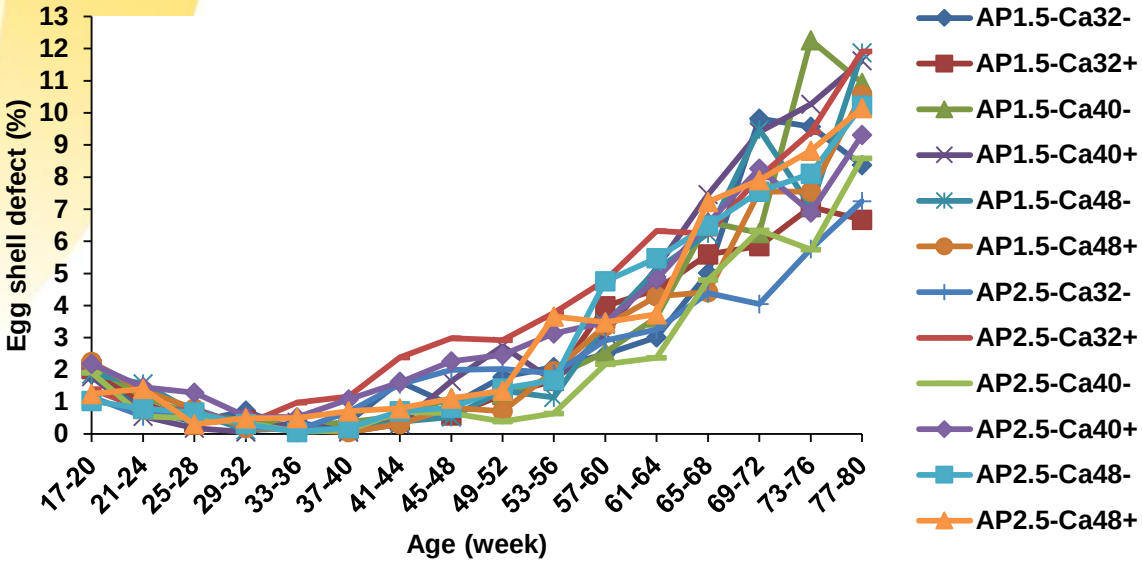

Figure 4-*15*: Effect of dietary AP and Ca concentrations and supplemental phytase on egg shell defects of hens from 17 to 80 weeks of age in Experiment 2

4.2.5 Egg weight and egg mass

Egg weight increased up to 50 weeks of age and then flat out. Diets changing from phase 1 to phase 2 may have affected the egg weight from 52 to 62 weeks of age (**Figure 4-*16***). There were large variations in egg weights within each treatment group (**Table 4-*18***). Egg mass per henday was increased as egg production reached the peak and slowly drifted downward (**Figure 4-*17***) as egg production decreased. There were no significant treatment effects on both egg weight and egg mass.

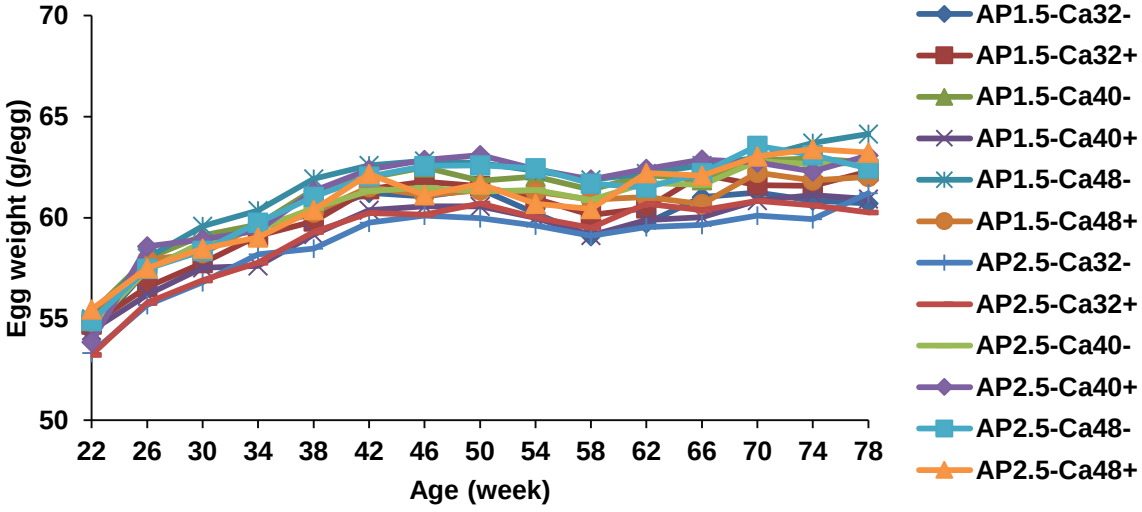

Figure 4-*16*: Effect of dietary AP and Ca concentrations and supplemental phytase on egg weight of hens from 22 to 80 weeks of age in Experiment 2

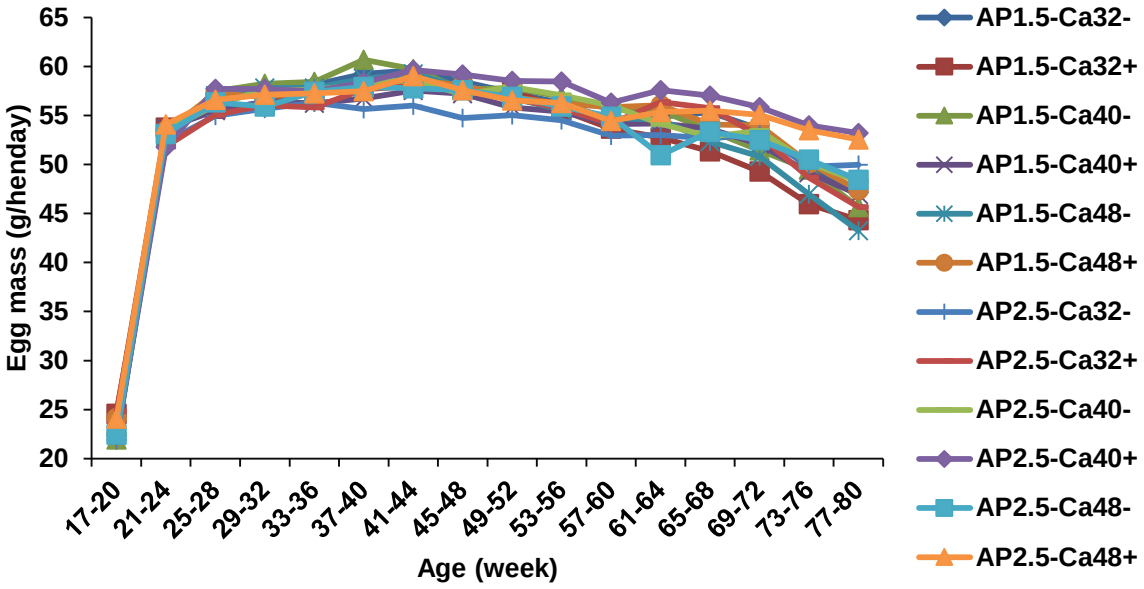

Figure 4-*17*: Effect of dietary AP and Ca concentrations and supplemental phytase on egg mass of hens from 17 to 80 weeks of age in Experiment 2

Table 4-*18* Variations in egg weight of hens fed different dietary AP and Ca concentrations and phytase supplementation from 22 to 80 weeks of age in Experiment 2

Age (week)	AP (g/kg)	1.5						2.5					
	Ca (g/kg)	32		40		48		32		40		48	
	Phytase	-	+	-	+	-	+	-	+	-	+	-	+
22	Range	45.1-66.7	47.2-67.9	47.4-67.6	47.4-71.0	39.0-66.0	48.3-62.1	39.6-61.6	47.6-63.0	49.5-63.8	44.7-61.9	49.5-63.3	48.1-76.5
	Mean	54.4	54.7	55.3	54.4	55.0	54.9	53.3	53.2	54.6	53.9	54.9	55.5
26	Range	50.0-70.5	47.0-63.3	48.7-73.1	46.8-67.8	49.1-67.7	50.8-65.5	49.2-64.2	49.5-64.5	50.0-67.9	51.2-89.0	50.1-67.4	46.9-66.0
	Mean	57.4	56.6	58.0	56.2	58.0	57.9	55.7	55.8	57.4	58.6	57.5	57.5
30	Range	47.7-86.4	49.8-69.6	50.1-70.0	50.7-80.6	52.1-67.2	50.3-66.8	44.4-66.6	47.2-65.5	50.2-67.7	49.6-68.0	51.3-66.9	48.8-66.4
	Mean	58.8	57.8	59.1	57.5	59.6	58.3	56.8	56.9	58.8	58.9	58.4	58.5
34	Range	49.0-70.2	51.4-66.7	54.1-68.9	51.8-64.9	52.6-70.4	50.3-66.7	50.2-65.6	50.8-66.0	51.0-72.0	51.3-68.5	51.5-67.5	52.8-68.6
	Mean	59.5	59.1	59.7	57.6	60.4	59.6	58.2	57.8	59.4	59.4	59.8	59.0
38	Range	49.0-71.9	54.0-68.8	52.3-73.5	50.0-70.3	50.9-72.1	52.3-71.6	48.0-66.7	52.4-67.6	53.0-72.3	55.5-71.9	54.5-74.5	51.3-68.1
	Mean	60.2	59.8	61.4	59.2	61.9	60.3	58.5	59.3	60.5	61.3	61.0	60.4
42	Range	51.4-71.7	52.4-70.4	50.9-73.4	52.9-68.7	53.8-74.6	53.2-71.9	52.1-67.8	52.3-68.5	55.5-67.8	56.8-69.7	51.9-72.5	53.2-72.6
	Mean	61.2	61.4	62.0	60.4	62.6	61.5	59.8	60.2	61.3	62.4	62.0	62.2
46	Range	51.4-74.5	52.7-73.3	50.1-76.1	51.6-69.2	50.6-74.0	52.7-72.2	49.8-71.0	51.4-69.3	55.1-69.8	49.9-77.0	52.5-75.0	51.8-70.4
	Mean	61.1	61.8	62.5	60.6	62.8	61.1	60.1	60.2	61.5	62.9	62.6	61.1
50	Range	51.4-73.3	52.3-69.3	51.5-73.6	52.0-70.1	54.5-73.8	52.9-72.2	48.0-67.9	51.6-72.6	53.9-71.1	53.8-74.0	53.3-69.6	52.4-71.6
	Mean	61.4	61.6	61.8	60.6	62.7	61.3	60.0	60.7	61.3	63.1	62.6	61.7
54	Range	52.5-69.9	53.7-66.5	52.5-74.9	52.2-66.6	54.3-70.5	54.2-68.3	53.4-67.9	53.1-67.3	53.8-66.2	49.7-71.3	56.7-69.4	54.6-66.5
	Mean	60.2	60.9	62.0	60.0	62.3	61.3	59.6	60.0	61.4	62.4	62.5	60.7
58	Range	49.4-69.8	52.3-68.4	52.2-73.7	51.0-68.6	52.5-73.9	50.9-69.4	49.9-72.2	50.9-70.5	51.6-70.0	49.6-69.9	54.3-71.7	51.4-66.8
	Mean	59.1	60.1	61.4	59.1	61.9	60.9	59.1	59.5	60.9	61.9	61.7	60.4
62	Range	50.6-71.0	52.4-71.6	52.7-76.6	51.5-68.5	54.2-76.3	51.9-70.8	51.9-67.4	49.5-70.0	55.7-69.9	53.4-71.9	54.2-76.2	50.5-72.5
	Mean	59.7	60.5	62.1	59.9	62.2	61.0	59.5	60.7	61.7	62.4	61.5	62.2

Age (week)	AP (g/kg)	1.5						2.5					
	Ca (g/kg)	32		40		48		32		40		48	
	Phytase	-	+	-	+	-	+	-	+	-	+	-	+
66	Range	49.9-72.2	52.3-71.4	48.9-72.8	44.2-69.2	55.0-74.5	51.2-72.0	49.0-70.4	53.3-73.0	53.5-70.9	56.0-74.1	53.1-73.8	51.4-68.8
	Mean	61.0	62.1	61.9	60.0	62.6	60.7	59.6	60.4	61.6	62.9	62.2	62.1
70	Range	50.9-75.7	53.76.5	51.4-73.3	52.5-75.2	50.5-76.5	48.9-74.9	49.0-69.1	51.4-71.9	54.1-70.9	52.0-71.3	54.5-76.9	53.6-73.9
	Mean	61.3	61.6	62.8	60.8	63.0	62.2	60.1	60.8	62.8	62.7	63.6	63.0
74	Range	47.6-75.1	53.0-81.2	53.2-73.6	52.4-75.0	55.0-76.0	52.6-72.5	50.1-72.1	52.6-72.2	52.3-71.6	51.5-81.9	56.9-79.2	52.1-76.8
	Mean	60.9	61.6	62.9	61.1	63.7	61.8	59.9	60.6	62.7	62.3	63.1	63.4
78	Range	51.8-71.2	53.5-75.3	52.0-72.8	53.5-70.6	55.4-77.5	50.4-76.2	50.0-70.3	52.5-71.7	56.0-73.4	54.1-72.4	54.4-72.7	51.8-76.5
	Mean	60.7	62.3	62.7	60.9	64.1	62.0	61.2	60.3	62.7	63.1	62.4	63.2

4.2.6 Feed to egg conversion ratio

As expected from the results of feed intake and egg production, there were no significant effects of dietary AP and Ca concentrations with or without phytase supplementation on feed to egg conversion ratios from 17 to 80 weeks of age (**Table 4-*19***).

In summary, dietary AP and Ca concentrations had no significant effect on total egg numbers per hen housed from 17 to 80 weeks of age. Phytase marginally increased total egg numbers per hen housed from 17 to 80 weeks of age (392 vs. 387) and the effect only occurred in hens fed diets containing Ca of 40 and 48 g/kg. Hens fed on diets containing Ca 48 g/kg without phytase supplementation tended to have less number of eggs than others regardless of AP concentrations (**Table 4-*20***).

4.2.7 Mortality

Cumulative mortality was 1.4 % at 60 weeks of age, 4.4% at 70 weeks and more than half the birds died between 71 and 80 weeks of age.

Table 4-*19*: Effect of dietary AP and Ca concentrations and supplemental phytase on feed to egg conversion ratio of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)															
			17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52*	53-56	57-60	61-64	65-68	69-72	73-76	77-80*
1.5	32	-	3.60	1.86	1.81	1.80	1.85	1.80	1.82	1.87	1.89	1.95	2.02	2.01	2.04	2.08	2.07	2.30
		+	3.35	1.79	1.83	1.85	1.84	1.84	1.84	1.87	1.89	1.92	2.04	2.08	2.14	2.17	2.14	2.24
1.5	40	-	3.88	1.86	1.80	1.82	1.83	1.79	1.84	1.89	1.88	1.91	1.96	2.00	2.06	2.17	2.11	2.30
		+	3.51	1.85	1.82	1.86	1.88	1.88	1.91	1.91	1.94	1.99	2.02	2.06	2.07	2.11	2.17	2.21
1.5	48	-	3.63	1.91	1.84	1.84	1.84	1.85	1.85	1.91	1.90	1.94	1.99	2.01	2.13	2.15	2.23	2.42
		+	3.50	1.86	1.79	1.83	1.85	1.82	1.84	1.86	1.89	1.86	1.95	1.93	2.07	2.03	2.05	2.30
2.5	32	-	3.63	1.86	1.86	1.85	1.87	1.90	1.91	1.99	1.96	1.98	2.03	2.05	2.11	2.07	2.17	2.21
		+	3.39	1.88	1.90	1.89	1.90	1.90	1.91	1.94	1.96	2.00	2.04	1.98	2.06	2.07	2.16	2.36
2.5	40	-	3.64	1.82	1.80	1.79	1.85	1.84	1.84	1.91	1.86	1.96	1.96	2.04	2.10	2.06	2.15	2.29
		+	3.79	1.93	1.80	1.83	1.87	1.87	1.85	1.87	1.86	1.87	1.96	1.94	1.99	2.00	2.06	2.05
2.5	48	-	3.73	1.88	1.85	1.83	1.89	1.89	1.88	1.85	1.87	2.00	2.03	2.15	2.10	2.08	2.22	2.23
		+	3.48	1.88	1.83	1.86	1.87	1.89	1.86	1.89	1.90	1.93	2.04	2.03	1.99	2.02	2.03	2.09
Main Effect																		
AP	Pooled SEM		0.085	0.011	0.009	0.011	0.011	0.015	0.012	0.014	0.015	0.018	0.016	0.016	0.019	0.029	0.029	0.035
	$LSD_{0.05}$		0.237	0.031	0.026	0.031	0.030	0.041	0.033	0.040	0.043	0.049	0.044	0.045	0.054	0.080	0.082	0.098
	P value		0.797	0.229	0.094	0.355	0.090	0.016	0.155	0.236	0.949	0.237	0.433	0.497	0.385	0.074	0.912	0.072
Ca	Pooled SEM		0.104	0.014	0.011	0.013	0.013	0.018	0.015	0.017	0.019	0.021	0.019	0.020	0.024	0.035	0.036	0.043
	$LSD_{0.05}$		0.290	0.038	0.032	0.038	0.037	0.050	0.041	0.049	0.053	0.060	0.054	0.056	0.066	0.098	0.101	0.121
	P value		0.353	0.211	0.030	0.372	0.950	0.643	0.910	0.368	0.252	0.518	0.205	0.725	0.613	0.822	0.972	0.565
Phytase	Pooled SEM		0.085	0.011	0.009	0.011	0.011	0.015	0.012	0.014	0.015	0.018	0.016	0.016	0.019	0.029	0.029	0.035
	$LSD_{0.05}$		0.237	0.031	0.026	0.031	0.030	0.041	0.033	0.040	0.043	0.049	0.044	0.045	0.054	0.080	0.082	0.098
	P value		0.137	0.997	0.859	0.083	0.358	0.285	0.639	0.527	0.643	0.239	0.345	0.079	0.173	0.399	0.153	0.108

Table 4-*20*: Summary of egg production, feed intake and feed to egg conversion ratio (FCR) of hens from 17 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Eggs/hen housed	Henday egg production (%)	Henhouse egg production (%)	Egg shell defects (%)	Egg mass (g/h/d)	Feed intake (g/b/d)	FCR (g feed/g egg)
1.5	**32**	**-**	390.4	89.4	87.1	2.8	53.6	105.2	1.97
		+	384.3	87.1	85.8	2.4	52.3	103.8	1.98
1.5	**40**	**-**	386.1	87.9	86.2	2.7	53.7	104.9	1.96
		+	395.1	88.8	88.2	3.2	52.6	105.2	2.00
1.5	**48**	**-**	383.9	85.9	85.7	2.9	52.7	105.3	2.00
		+	392.5	89.0	87.6	2.6	53.6	104.5	1.95
2.5	**32**	**-**	391.8	88.5	87.5	2.3	52.0	104.7	2.02
		+	392.3	89.5	87.6	3.8	52.9	106.5	2.01
2.5	**40**	**-**	388.3	88.3	86.7	2.0	53.4	105.4	1.97
		+	397.4	89.8	88.7	3.3	54.9	107.0	1.95
2.5	**48**	**-**	381.0	86.9	83.3	2.8	52.8	105.9	2.01
		+	391.0	89.0	87.3	3.1	54.2	106.4	1.96
Main effect									
AP	**Pooled SEM**		2.32	0.38	0.55	0.31	0.28	0.43	0.01
	$LSD_{0.05}$		6.51	1.08	1.55	0.87	0.78	1.20	0.03
	P value		0.633	0.239	0.938	0.789	0.487	0.058	0.425
Ca	**Pooled SEM**		2.85	0.47	0.68	0.38	0.34	0.52	0.01
	$LSD_{0.05}$		7.98	1.32	1.90	1.07	0.96	1.47	0.03
	P value		0.521	0.257	0.297	0.999	0.139	0.744	0.345
Phytase	**Pooled SEM**		2.32	0.38	0.55	0.31	0.28	0.43	0.01
	$LSD_{0.05}$		6.51	1.08	1.55	0.87	0.78	1.20	0.03
	P value		0.118	0.057	0.067	0.264	0.338	0.577	0.520

4.2.8 Egg and egg shell quality

Egg shell colour became lighter with hen age which was confirmed in Experiment 2, in which the L* value increased and the a* value decreased as hens aged (Appendix **Table 10-4** and **Table 10-5**).

Albumen height and HU decreased with hen age, but were not affected by dietary AP and Ca concentrations or supplemental phytase (P>0.05) (Appendix **Table 10-6** and **Table 10-7**).

Yolk colour tended to be lighter for hens fed on diets with lower Ca, as can be seen in Figure 4-18 (Appendix **Table 10-8**). Yolk colour was lower for hens fed on diets containing Ca of 32 g/kg regardless of dietary AP concentrations. Storage of diets for 4 months may have had some negative effect on yolk colour as indicated by lower colour readings at 34 and 50 weeks of age which were at the end of each batch of the diets (**Figure 4-*18***).

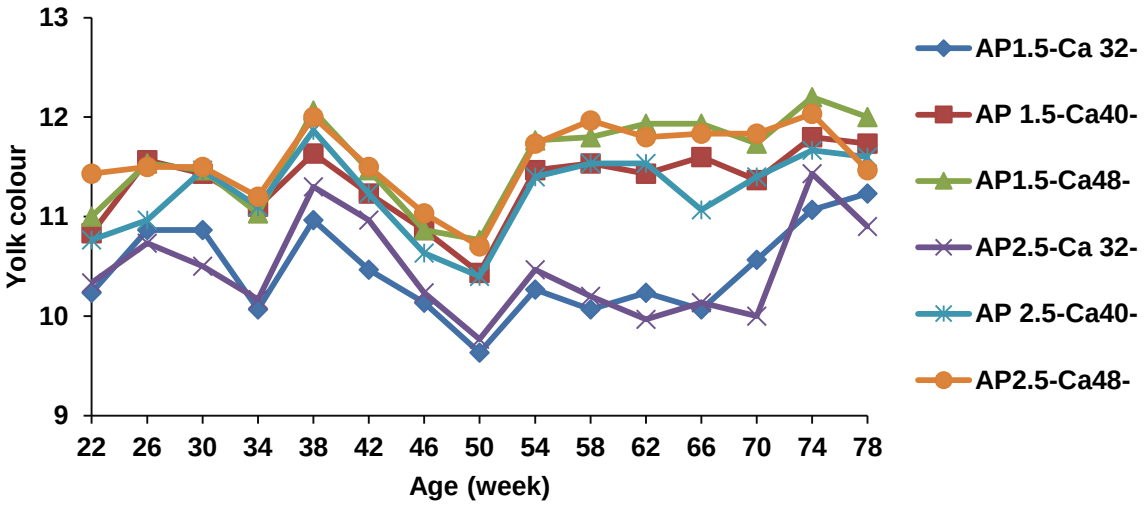

Figure 4-*18*: Yolk colour of hens fed different dietary AP and Ca concentrations without phytase supplementation from 22 to 78 weeks of age in Experiment 2

Specific gravity decreased with hen age and it was not affected by dietary AP concentrations and supplemental phytase (P > 0.05). The Ca concentration affected SG from first lay to 50 weeks of age during which the SG of eggs laid by birds on diets containing 40 and 48 g/kg Ca was significantly higher than those from birds on diet containing 32 g/kg Ca (P<0.05) (**Table 4-*21***). Such effects of Ca on SG were not detected after 50 weeks of age.

There were no effects of AP and supplemental phytase on egg shell weight (Appendix **Table 10-9**), shell percentage of egg (Appendix **Table 10-10**), shell thickness (**Table 10-11**) and shell weight per surface area (Appendix **Table 10-12**). All these measurements were significantly higher for birds fed on diets containing Ca of 40 and 48 g/kg than those on 32 g/kg (P<0.05) from first lay to 50 weeks of age, which was consistent with SG results.

Table 4-*21*: Effect of AP and Ca concentrations with or without supplemental phytase on egg specific gravity of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	1.089	1.090	1.087	1.088	1.085	1.084	1.085	1.083	1.085	1.088	1.088	1.085	1.083	1.085	1.082
		+	1.089	1.090	1.090	1.084	1.084	1.084	1.084	1.085	1.085	1.084	1.084	1.084	1.083	1.082	1.081
1.5	40	-	1.092	1.092	1.090	1.087	1.085	1.085	1.087	1.086	1.085	1.086	1.085	1.086	1.083	1.085	1.083
		+	1.093	1.092	1.090	1.089	1.087	1.086	1.086	1.086	1.085	1.085	1.086	1.084	1.082	1.084	1.081
1.5	48	-	1.092	1.091	1.091	1.088	1.086	1.086	1.086	1.085	1.085	1.085	1.088	1.085	1.082	1.083	1.082
		+	1.093	1.092	1.091	1.088	1.087	1.087	1.087	1.086	1.087	1.087	1.086	1.087	1.084	1.085	1.082
2.5	32	-	1.091	1.090	1.087	1.086	1.086	1.087	1.087	1.084	1.087	1.085	1.087	1.085	1.084	1.085	1.082
		+	1.091	1.090	1.089	1.085	1.084	1.085	1.085	1.083	1.085	1.086	1.084	1.083	1.083	1.083	1.081
2.5	40	-	1.092	1.088	1.089	1.088	1.086	1.087	1.087	1.086	1.087	1.086	1.087	1.087	1.082	1.085	1.080
		+	1.093	1.091	1.090	1.089	1.087	1.086	1.086	1.086	1.086	1.087	1.087	1.085	1.083	1.085	1.083
2.5	48	-	1.094	1.092	1.090	1.089	1.087	1.087	1.086	1.085	1.086	1.086	1.082	1.084	1.081	1.085	1.080
		+	1.093	1.092	1.090	1.089	1.087	1.086	1.086	1.087	1.085	1.087	1.086	1.083	1.081	1.085	1.082
AP	Pooled SEM		0.0003	0.0005	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0005	0.0003	0.0004	0.0004	0.0004
	$LSD_{0.05}$		0.0009	0.0013	0.0009	0.0009	0.0009	0.0010	0.0009	0.0009	0.0009	0.0008	0.0013	0.0009	0.0011	0.0011	0.0011
	P value		0.047	0.330	0.138	0.952	0.131	0.115	0.447	0.771	0.153	0.509	0.370	0.165	0.137	0.155	0.604
Ca	Pooled SEM		0.0004	0.0006	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0004	0.0006	0.0004	0.0005	0.0005	0.0005
	$LSD_{0.05}$		0.0010	0.0016	0.0011	0.0011	0.0012	0.0012	0.0011	0.0011	0.0011	0.0010	0.0016	0.0012	0.0013	0.0013	0.0013
	P value		0.000	0.062	0.000	0.000	0.003	0.040	0.017	0.000	0.626	0.479	0.578	0.195	0.264	0.571	0.876
Phytase	Pooled SEM		0.0003	0.0005	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	0.0005	0.0003	0.0004	0.0004	0.0004
	$LSD_{0.05}$		0.0009	0.0013	0.0009	0.0009	0.0009	0.0010	0.0009	0.0009	0.0009	0.0008	0.0013	0.0009	0.0011	0.0011	0.0011
	P value		0.564	0.417	0.064	0.433	0.561	0.310	0.310	0.382	0.494	0.692	0.388	0.021	0.764	0.361	0.835

Egg shell breaking strength of young birds fed diets containing Ca of 32 g/kg was significantly lower (P<0.01) than those fed on diets containing Ca of 40 and 48 g/kg (3.8 kg vs. 4.1 and 4.2 kg at 22 weeks old; 3.5 kg vs. 3.7 and 3.7 kg at 50 weeks old). This effect became less significant when birds got older (Appendix **Table 10-13**).

4.2.9 Tibia bone and toe ash contents

Dietary AP and Ca concentrations had no effect on tibia bone or toe DM and ash contents. Phytase supplementation in diet marginally increased tibia bone DM content expressed as the percentage of body weight (0.36 vs. 0.34 % of body weight) although the differences were just not statistically significant (P=0.066) (**Table 4-22**).

Tibia bone ash content (as % DM) was numerically higher for birds fed diets with supplemental phytase than those without (54.6 vs. 52.9 %DM, P > 0.05). However, when tibia bone ash content was expressed as percentage of body weight, phytase supplementation significantly increased tibia bone ash content (0.20 vs. 0.18% of body weight, P<0.05).

Toe ash percentage of DM was significantly higher for birds fed on diet with phytase supplementation than those without phytase supplementation (15.03 vs. 14.19 % DM) (P<0.05) (**Table 4-22**). There was no close correlation between tibia bone and toe ash contents.

Table 4-22: Effect of AP and Ca concentrations and supplemental phytase on tibia bone and toe ash contents of hens at 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Toe ash (%DM)	Tibia bone ash (%DM)	Tibia bone DM (% of body weight)	Tibia bone ash (% of body weight)
1.5	**32**	**-**	14.1	49.9	0.33	0.16
		+	15.8	55.5	0.38	0.21
1.5	**40**	**-**	14.4	53.3	0.35	0.19
		+	15.0	52.9	0.38	0.20
1.5	**48**	**-**	14.0	52.8	0.35	0.18
		+	14.4	55.4	0.36	0.20
2.5	**32**	**-**	14.9	52.4	0.33	0.17
		+	15.7	55.1	0.35	0.19
2.5	**40**	**-**	13.7	54.7	0.36	0.20
		+	14.8	55.1	0.35	0.19
2.5	**48**	**-**	14.1	54.4	0.35	0.19
		+	14.5	53.6	0.36	0.19
Main effect						
AP	**Pooled SEM**		0.24	0.73	0.006	0.005
	$LSD_{0.05}$		0.67	2.06	0.018	0.014
	P value		0.992	0.366	0.509	0.987
Ca	**Pooled SEM**		0.29	0.90	0.011	0.006
	$LSD_{0.05}$		0.82	2.52	0.030	0.017
	P value		0.084	0.762	0.322	0.435

Phytase	**Pooled SEM**		0.24	0.73	0.006	0.005
	$LSD_{0.05}$		0.67	2.06	0.018	0.014
	P value		0.016	0.109	0.066	0.028

4.2.10 Blood Ca and P concentrations

Diet AP and Ca had no effect on blood Ca and P concentrations (**Table 4-*23***) of layers at 80 weeks of age. Phytase significantly increase blood P concentration (1.93 vs. 1.63, P = 0.045).

Table 4-*23*: Effect of dietary AP and Ca concentrations and supplemental phytase on blood Ca and P contents of hens at 80 weeks of age in Experiment 2

			Mean Range	
AP (g/kg)	**Ca (g/kg)**	**Phytase**	**Ca (mmol/L)**	**P (mmol/L)**
1.5	**32**	**-**	6.9 (4.5-8.1)	1.7 (1.2-2.0)
		+	6.4 (3.1-8.4)	1.6 (1.4-2.1)
1.5	**40**	**-**	7.2 (5.7-9.1)	1.6 (1.1-1.9)
		+	8.8 (6.3-10.1)	2.3 (1.6-2.8)
1.5	**48**	**-**	6.2 (4.9-6.9)	1.6 (1.0-2.0)
		+	7.3 (6.3-8.4)	1.8 (1.6-2.0)
2.5	**32**	**-**	6.5 (4.9-8.3)	1.5 (1.2-1.9)
		+	6.8 (5.6-8.4)	1.9 (1.2-2.4)
2.5	**40**	**-**	6.9 (4.6-9.3)	1.9 (1.2-2.7)
		+	8.3 (5.4-9.9)	2.2 (1.6-2.6)
2.5	**48**	**-**	7.2 (6.8-7.8)	1.6 (1.4-1.7)
		+	6.5 (3.5-8.4)	1.8 (1.0-3.0)
Main effect				
AP	**Pooled SEM**		0.33	0.10
	$LSD_{0.05}$		0.91	0.28
	P value		0.831	0.782
Ca	**Pooled SEM**		0.40	0.12
	$LSD_{0.05}$		1.12	0.35
	P value		0.119	0.189
Phytase	**Pooled SEM**		0.33	0.10
	$LSD_{0.05}$		0.91	0.28
	P value		0.281	0.045

5 Discussion

Two experiments presented in this report were designed to closely reflect current poultry industry practice in Australia, as all experimental diets were formulated by the industry. The main aims of the experiments were to examine the influence of different levels of AP with or without supplemental phytase (Experiment 1) or different levels of AP and Ca with or without supplemental phytase (Experiment 2) on egg production, quality and nutrient retention.

The requirement of P has been debated for many decades with conflicting and inconclusive outcomes from numerous researches. The recommendation of NRC (1994) for laying hen diets is 2.5 g/kg AP or 250 mg per hen per day (based on 100 g feed/hen/day), but the levels commonly fed by industry are much higher. Our previous research (Li and Bryden, 1996) has shown that the dietary AP of 1.8 g/kg (or 190 mg AP/hen/day) met P requirement of laying hens from 23 to 47 weeks of age. In current experiments, the lowest concentration of AP tested was 1.5 g/kg diet. Within each treatment, the birds received the same Ca and AP concentrations in their diets from first lay to 80 weeks of age. Egg production performance was satisfactory across all treatment diets, including the low AP (1.5 g/kg) diets in both experiments although the body weights of hens fed the diets containing AP 1.5 g/kg in Experiment 1 and those containing AP 1.5 g/kg and 32 g/kg Ca in Experiment 2 tended to be smaller than those on the other treatments. However, it was still within the industry performance standard (Hy-Line International, 2014). This marginally lower body weight did not have detrimental effect on either egg production or egg shell quality. Average henday egg production was maintained exceptionally high across all the treatments and peaked over 94% at 21 weeks of age to over 90% at 47 weeks of age in Experiment 1, and over 94% at 22 weeks of age to over 90% at 50 weeks of age in Experiment 2. The results support the finding by Boling *et al.* (1998) who suggested that an AP level of 1.5 g/kg diet in the absence of phytase was sufficient to support optimum performance when compared with hens fed 4.5 g/kg AP from 20 to 70 weeks of age. Snow *et al.* (2004) found that the hens fed 1.5 g/kg AP maintained egg production similar to, though numerically lower, than hens fed 4.5 g/kg AP. However, in separate experiment they found that a dietary supply of AP 1.6 g/kg or lower had negative impact on egg production and egg mass from 23 to 63 weeks of age.

Phytase supplementation generally had no effects on all the parameters measured monthly in this project. However when the data were summarised from 20 to 80 weeks of age in Experiment 1, the number of eggs per hen housed and henhoused egg production were significantly higher for hens fed low AP (1.5 g/kg) diets with phytase supplementation than those fed the same level of AP without supplemental phytase ($P<0.05$), which can be explained by higher mortality of hens on low AP (1.5 g/kg) diets without phytase supplementation (7% vs. 2%). Phytase also numerically improved the body weight of hens on low AP (1.5 g/kg) diets. Phytase supplementation of diets containing 2.0 to 4.5 g/kg AP gave no further improvements in performance. Similar results were reported by Gordon and Roland (1997), who found that the production performance was not different among hens fed 2.0 to 5.0 g/kg AP, and phytase did not have an effect on performance of these hens.

In Experiment 2, phytase marginally increased total egg numbers per hen housed of hens fed diets containing Ca of 40 and 48 g/kg from 17 to 80 weeks of age regardless of dietary AP levels. Hens fed on diets containing Ca 48 g/kg without phytase supplementation tended to produce fewer eggs than in other treatments regardless of AP concentrations.

Several publications indicate that layers fed on diets containing NPP levels from 1.0 to 1.3 g/kg with supplemental phytase maintain their productivity at the same level as a control with a normal NPP level (Van Der Klis *et al.*, 1996; Gordon and Roland, 1997; Punna and Roland, 1999; Boling *et al.*, 2000b; Francesch *et al.*, 2005). Van Der Klis *et al.* (1996) found

that 1.2 g/kg AP was not adequate to satisfactorily maintain egg production performance. However, the addition of 0.6 g/kg P from monocalcium phosphate or adding 200 unit phytase / kg diet overcame all the signs of the P deficiency.

Gordon and Roland (1997) reported that an AP level of 1.0 g/kg was not adequate to support egg production performance. However, the addition of 300 units of phytase/kg diet was effective in alleviating all the deficiency signs attributed to feeding 1.0 g/kg AP (high mortality, low bone density and bone breaking strength, inferior egg production and egg weight and shell quality). Punna and Roland (1997) reported that supplemental phytase at 300 units/kg diet was effective in overcoming all the adverse effects of 1.0 g/kg AP on hens performance during the growing and laying periods. If 250 FTU phytase (from *A. niger*) per kg diet (corn-soybean meal) is equivalent to 0.8 g monocalcium phosphate P (Van der Klis *et al.*, 1997), the NPP concentration of 1.0 g/kg diets with supplemental phytase was higher than the lowest level of 1.5 g/kg AP currently tested in this project.

Unlike some of the other shorter-term research, the two experiments reported here were conducted from the start of lay to 80 weeks of age. With the high level of production performance achieved it can be concluded that all the dietary P concentrations met the hens P requirement even at the lowest level of 1.5 g/kg AP. Other studies reported by Mikaelian and Sell (1981), Miles *et al.* (1983), Simmons *et al.* (1992), Usayran and Balnave (1995) and (Parsons, 1999) have reached similar conclusions. Our current results (AP 158 mg/hen/day) indicate that the AP requirement of laying hens for egg production is lower than the AP 1.8 g/kg (or 190 mg/b/d), which was the lowest level tested in our previous project (Li and Bryden, 2006) and the NRC (1994) recommendation of 250 mg per bird per day.

The present study indicates that a diet with AP of 1.5 g/kg, which was sufficient to support all production parameters, was also adequate for maintaining normal egg and egg shell quality.

Hens fed on the diets containing Ca of 32 g/kg laid eggs with significantly lower SG, shell weight, shell thickness and shell breaking strength compared with those on diets with Ca of 40 and 48 g/kg. However, the effect mainly occurred in the first phase from 16 to 50 weeks of age. More importantly all the egg production, shell defects and feed to egg conversion ratios were similar between all Ca levels for the entire experimental period. Therefore, dietary Ca of 32 g/kg was adequate to maintain normal egg production.

Dietary Ca to P ratio is important to maintain optimum P utilisation. A wide Ca to P ratio reduces absorption of P especially when P levels approach its requirement. It was suggested that a ratio of 13:1 of total Ca to NPP should be maintained for brown egg laying hens (NRC, 1994). This Ca to P ratio was based on comparing total Ca, not available Ca, to NPP, not biologically determined AP. Hy-Line International (2014) recommended that total Ca to AP ratios are from 9 to 11:1 throughout the production cycle. However, in our current study, the wide range of total Ca to AP ratios from 9.3 to 28:1 appeared to have no significant negative impact on egg production and egg shell quality.

The determined retainable Ca to P ratios were 14 to 22 for 50 weeks of age and 7 to 11 for 80 weeks of age. These retainable Ca to P ratios were not associated with the Ca to P ratios in the diets. The retention values of Ca and P presented in this report may have been underestimated because they were determined in the birds fed the experimental diets which were not designed for determination of Ca or P retention. The P levels in these diets varied from close to, or much higher than, hen actual P requirement. Most researches on availability or retention of P in feedstuffs have been conducted using low P levels or even marginally P deficient diets. Moreover, dietary Ca level affects both Ca and P retention (Tamim *et al.*, 2004). In the present study Ca concentration was the same for all AP levels in the diets and possibly too high for the determination of Ca and P retention.

It is not surprising that there were large variations in the values of Ca and P retention between replicates within the treatments at 80 weeks of age as the egg production of the older layers varied considerably. Some layers may be in the process of moulting and some may have moved to second peak of lay after moulting. Therefore the demand for Ca and P from old hens for egg production was very different. The Ca content of the egg shell is approximately 1.7-2.5 g. Calcium requirement of a laying hen is 4 - 6 times of that of a non-laying hen (Coutts and Wilson, 2007). The P concentrations of egg contents and egg shell are 120 and 20 mg (Coutts and Wilson, 2007). Literature clearly demonstrates that P requirements for laying and non-laying hens are different. Moreover intestinal Ca absorption varies depending on demand during the stages of egg formation. For example, absorption of the Ca in the diet is about 40% when the shell gland is inactive, but reaches to 72% when the shell gland is active (Coutts and Wilson, 2007). Therefore to determine Ca and P retention, factors such as dietary Ca and P levels, rate and time of lay should be taken into account.

The speed of Ca absorption is higher than that of any other ion, except for Na (Pelicia *et al.*, 2009). It is generally agreed that majority of dietary Ca is absorbed when the shell gland is active. This time closely coincides with late afternoon or the dark hours for the layer. To ensure Ca available to be taken from diet into the gut and minimised its mobilisation from bone, it is commonly recommended to provide higher Ca levels with large particle sizes for the Ca sources in the gut during this time. However, from the present study, up to 8.8% Ca was found in the dry excreta of layers in Experiment 1 which is equivalent to 24% limestone in excreta DM assuming limestone contains 37% Ca. With the same assumption, up to 25% of limestone was found in the dry excreta in Experiment 2. The recommendation for particle size of Ca source is 50% coarse (2 - 4 mm) for pre-lay and peak lay, and 60% when egg production is 90-84% (Hy-Line International, 2014). Calgrit (limestone with particle size of 2 - 4 mm) of 46-48% was included in the diets in Experiment 1 and 40-60% in Experiment 2. Majority of dietary Ca is actively absorbed in the duodenum and the jejunum although it can be absorbed in all intestinal segments (Pelicia *et al.*, 2009). The observations from our two experiments indicated considerable amount of limestone in the diets has passed through the GIT and excreted into faeces, which may result from either excessive Ca in the diets or poor availability of Ca from limestone. Excess Ca and P in layer diet excreted into layer manure appear to be common in Australia. Wiedemann *et al.* (2008) surveyed a few layer farms and found up to 15.9% for Ca and 3.7% for P in manure DM of caged layers.

The detrimental effect of excessive dietary Ca has been well documented. High dietary Ca increases the pH in the gut. Phosphorus absorption is optimal at pH 5.5 - 6.0. The absorption and retention of P markedly decrease when the pH is higher than 6.5 (Hurwitz and Bar, 1965; Keshavarz, 1986). Excessive Ca in diet can bind with phytate to form insoluble complexes, which make Ca and P unavailable and also reduce hydrolysis of phytate due to the less accessible by phytase. Extra dietary Ca can lead to a reduced availability of other minerals, such as magnesium, manganese and zinc. It may also directly suppress phytase by competing for the active sites of the enzymes. This could explain partially why the improvement of Ca utilization by dietary phytase (Gordon and Roland, 1998) was not observed in the present study.

The increases in shell defects and decreases in shell thickness, shell percentage and shell breaking strength with hen age observed in the current study were due to the definite capacity of hens to deposit Ca in the shell (Roland *et al.*, 1975; Rajkumar *et al*., 2009). As hens age the increase in egg weight is not followed by proportional increase in shell deposition and a similar amount of Ca is distributed spread over a larger area of shell which results in a concomitant decrease in shell thickness and percentage of Ca in the shell. Therefore, eggs that had the greater increase in size throughout the entire laying cycle also had the greater decline in shell quality (Roland, 1979). Egg SG is an indirect measurement of shell thickness and it declines with hen age. This is partly due to the fact that the

increase in egg size is more rapidly than that of shell weight. Variations in SG among eggs of similar weights are mainly due to differences in the proportion of shell. In contrast, there is evidence which does not support the concept that shell quality suffers as a result of the increase in egg size with age or that a reduction in egg weight results in improved shell strength (Doyon *et al.*, 1985).

Egg shell colour is another parameter directly affected by the age of layers. As the hen ages, the pigments of the brown egg shell are deposited on a larger surface area due to increase in egg size. These may explain the results that egg shell colour became paler with hen age in the present project. No evidence suggests a variation in the amount of pigment produced according to egg size (Solomon, 1997).

Egg yolk colour is an important criteria of consumers' expectations around the quality of eggs (Coutts and Wilson, 2007; Schwagele, 2011). Many factors affect it, such as individual bird differences in capability to absorb and deposit pigment in egg yolk, the rate of egg production, quantity of carotenoids and oxidising agents or pigment antagonist in the feed, and the conditions and length of feed storage (Coon, 2002; Coutts and Wilson, 2007). Therefore, a wide variation in colour may occur in the yolks from any flock. If a flock average of yolk colour score 10 on the DSM Yolk Colour Fan, approximate 66% eggs laid by the flock will score between 9 and 11, 5% will score less than 8 and another 5% greater than 12. However, extremely low yolk colour scores found in some of the experimental diets in Experiment 1 were most likely due to the inadequate pigment supply in these diets. In Experiment 2, hens fed on diet contained lower Ca (32 g/kg) produced eggs with lighter yolk colour than those fed on diets with higher Ca levels. Diets containing lower Ca (32 g/kg) had much lower contents of oil than the diets with higher Ca (40 and 48 g/kg) levels. However, it is not clear how this difference in dietary oil content contributes to the difference in yolk colour or how oil, pigment and Ca interacts to affect yolk colour. There was no such effect observed on egg shell colour.

The results of blood Ca and P concentrations in the current study varied considerably within each treatment. This may be due to differences between hens in their stages of egg formation or after lay when the blood samples were taken. During egg shell formation, plasma Ca turnover is extremely fast (1 min half-life) (Pelicia *et al.*, 2009). Therefore, better timing is needed to make sure the blood samples are taken when the hens are all in the same stage of egg formation.

The unexpected incident of salt deficiency at the beginning of the Experiment 1 led to the significant decline in feed intake, egg production and body weight of hens in the four affected diets. All the measurement parameters were subsequently recovered after the problem was identified and salt added to the affected diets. However, any long-term impact of salt deficiency at early lay is not clear, although most production parameters were not significantly different among birds fed on diets with different AP concentrations or phytase supplementation.

Tibia bone ash content has been used as an important criterion for assessing bone mineralisation and estimating P requirement in poultry for many decades. A positive response of tibia bone ash content to dietary P levels has been found in young broilers (21 days old), but not found in old broilers (49 days old) (Li *et al.*, 2013). There was no close correlation between tibia bone ash content and bone strength which was not related to egg production (Rowland *et al.*, 1972). The current trial results did not show a correlation between tibia bone and toe ash contents and layer egg production. Also, there was no obvious relationship between layer tibia bone or toe ash contents and dietary Ca or AP levels at 80 weeks of age. Based on this finding, it is questionable whether the tibia bone or toe ash contents of layers are suitable criteria for assessing Ca and AP requirements of layers.

6 Conclusions

These experiments were undertaken on mash rations containing 5% particulate Ca in size from 2-4 mm that provided 40-60% of the dietary calcium. All the experimental diets were supplemented with 6.25 mg of 25-Hydroxycholecalciferol per kg.

The evidence of high level of egg production in both experiments clearly indicated that all the dietary AP concentrations met the P requirement of hens, even at the lowest AP level of 1.5 g/kg diet.

Experiments illustrated that smaller body weight was associated with the lowest level of dietary AP (1.5 g/kg) with Ca at 42 g/kg. Higher mortality (6.7%) and reduced hen housed egg production at the same AP level without phytase were observed compared to those with supplemental phytase. Therefore, this particular dietary combination may act as negative control illustrating a potential threshold for P deficiency.

Dietary Ca did not significantly affect egg production and feed to egg conversion ratio. However, hens fed on the diets containing Ca of 32 g/kg produced the eggs with lighter yolk colour and lower values of SG, shell weight, shell thickness and shell breaking strength irrespective of AP levels and phytase inclusion. Interestingly, shell defect percentages were not significantly affected.

The significant beneficial effect of phytase was not observed possibly due to the fact that dietary AP and Ca met layers' requirements for these minerals.

Egg weight increased, while egg production and egg shell quality (egg shell breaking strength, shell colour, shell thickness, albumen height and HU) decreased with hen age.

The differences in Ca to P ratios between diets may have impact on shell quality. Higher levels of defective shells were recorded in the diets using the highest AP (4.5 g/kg) with Ca at 42 g/kg with phytase included, and these impacts were reversed by removing phytase. Therefore, this dietary treatment may act as a negative control illustrating a threshold for excess P.

The retention of Ca and P was lower than those reported in the literature partially due to the fact that the diets were not purposely designed to test dietary Ca and P retention.

Phosphorus excretion was closely related to the dietary P concentrations and blood P was significantly increased by phytase inclusion irrespective of the dietary P level. Birds fed diets with lower AP levels excreted much less P. Large amount of Ca was found in excreta.

Inadequate salt in diets containing AP concentrations of 2.0 and 3.0 g/kg resulted in significantly lower egg production and egg weight than the other treatments. Salt deficiency also affected shell weight and thickness. The impact of short-term salt deficiency (6 weeks) at the beginning of the laying cycle on long-term egg production and quality parameters is unclear.

7 Implications

The AP content of layer diets used by the industry is considerably higher than the reported values and also the NRC (1994) recommendation. This suggests that current dietary usage of P is in excess of the layers' requirement.

Large quantity of Ca excreted by birds even fed the diet with the lowest Ca level; a level lower than the values used by the industry. This demonstrated that Ca in commercial layer diet is also in excess of the layers' requirement and worthy of further research.

Total Ca value was used in this project because there is no available Ca information for feedstuffs fed to poultry. Further investigation is warranted with regard to the particle size of Ca sources and Ca availability of different raw materials (e.g. limestone sources) for different phases of egg production. This information would assist the industry to optimise nutrient utilisation and the cost of production.

Available Ca requirement and available Ca to AP ratio should be determined.

The increment of dietary Ca concentrations in the current project was 8 g/kg, which should be reduced in future investigation.

There was considerable variation in some of the parameters measured within the replicate due to the limited number of layers used. Therefore, a large-scale experiment in commercial condition is recommended.

Age affected the production parameters measured and it may also affect the utilisation of Ca and P in the diets. Therefore it is not recommended the same level of Ca and AP be used in layer diets throughout the egg production cycle. This refinement in Ca and P nutrition of laying hens should be the subject of further research.

The productive life of commercial layers is likely to be extended beyond 80 weeks of age. There is a strong justification to undertake more fundamental research on Ca and P metabolism and maintain optimal shell quality in older layers.

Vitamin D plays an important role in absorption and mobilization of Ca during egg shell formation and P utilization. It may interact with phytase to influence Ca and P uptake. All future research experiments must be supported by an analysis of 25-Hydroxycholecalciferol in blood to advance the scientific understanding and enable experiments to be well standardised.

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9 Plain English Summary

Project Title:	Dietary available phosphorus requirement of laying hens
AECL Project No	1UQ101
Researchers Involved	X. Li, W.L. Bryden and D. Zhang
Organisations Involved	School of Agriculture and Food Sciences, University of Queensland, Gatton Campus, Gatton QLD 4343
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Objectives	This project aimed to re-evaluate the AP requirement of brown egg laying hens with or without supplemental phytase from lay to 80 weeks of age and examine the effects of different AP and Ca with or without phytase on egg production, egg shell quality, Ca and P retention, tibia bone and toe ash.
Background	The established adequate Ca and P levels for layers have been challenged due to the continuous advances in genetic improvement, nutrition, environment, and management.
Research	Wheat and sorghum diets contained Ca of 42 g/kg diet and the AP of 1.5, 2.0, 2.5, 3.0, 3.5 and 4.5g/kg diet in Experiment 1; and the AP levels of 1.5 and 2.5 g/kg diet and each with three levels of Ca (32, 42 and 48 g/kg diet) in Experiment 2. The diets with or without phytase fed Hy-Line brown egg laying hens from the first lay to 80 weeks of age. Egg production, feed intake, body weight, egg and egg shell quality parameters were measured. Retention of Ca and P, tibia and toe ash contents and Ca and P in blood were tested at the end of experiments.
Outcomes	High level of egg production was maintained in both experiments and it appears that all the dietary AP concentrations met the P requirement of hens even at the lowest level of 1.5 g/kg diet. Large quantity of Ca excreted even with lowest dietary Ca. Dietary supplemental phytase had no significant beneficial effect on egg production, feed to egg conversion ratio and shell quality measurements due to the fact that dietary AP and Ca met layers' requirements for these minerals.
Implications	The AP and Ca contents used by the poultry industry are in excess of the layers' requirement. Further study is required on Ca availability of different sources at the different stage of laying cycle. There was considerable variation in some of the parameters measured within the replicate due to the limited number of layers used. Therefore, a large scale experiment in commercial condition is recommended.
Key Words	Calicum, Phosphorus, requirement, retention, laying hens

Publications	Zhang, X., Wei, G., Zhang, D., Li, X. and Bryden, W. L. 2013. Dietary non-phytate phosphorus levels and layer performance in the last phase of lay. Aust. Poult Sci. Symp. 24:51. Yang, T.Y., Setiyawan, E., Li, X, Zhang, D. and Bryden, W.L. 2013. Dietary calcium levels and non-phytate phosphorus requirement of broilers from days 22-49. Recent Advances in Animal Nutrition Australia 2013, P5

10 Appendices

Table 10-1: Effect of dietary AP and supplemental phytase on egg shell colour L* from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	56.7	52.4	52.5	54.1	61.9	60.3	61.8	61.7	62.7	61.9	63.1	63.0	62.7	63.3	63.1
	+	57.2	52.3	52.5	53.2	61.6	60.5	62.4	63.1	62.5	61.2	60.1	61.9	62.6	63.7	62.8
2.0	-	55.0	52.7	52.3	54.0	61.9	60.8	62.4	62.4	62.6	61.1	61.6	62.3	62.6	62.6	64.9
	+	56.1	52.1	51.5	52.9	61.5	61.4	63.5	62.4	62.4	61.2	61.6	61.2	63.0	61.6	62.9
2.5	-	57.6	54.0	52.8	54.1	62.0	60.8	62.5	63.8	62.6	61.7	62.3	62.2	63.0	62.7	62.7
	+	56.4	52.7	52.2	53.0	62.1	59.9	62.3	62.1	63.0	61.4	61.2	62.7	61.9	63.2	62.2
3.0	-	54.8	50.9	50.6	51.4	60.4	59.3	61.6	60.1	60.8	60.0	61.4	62.1	60.9	61.2	61.7
	+	55.2	52.3	50.7	53.3	61.5	60.0	62.1	61.2	61.7	60.6	61.6	61.2	62.3	61.6	62.7
3.5	-	57.2	52.5	52.7	53.8	61.0	60.0	63.0	61.3	62.7	60.5	61.7	61.2	61.2	61.3	62.3
	+	58.2	54.0	52.7	54.1	62.1	61.0	62.7	63.1	62.9	61.5	62.4	62.0	63.6	63.1	64.2
4.5	-	57.0	52.9	53.1	53.9	63.1	61.4	63.4	62.4	62.1	62.0	61.7	63.5	62.8	62.7	62.7
	+	57.3	53.6	52.8	54.1	62.8	61.3	62.2	62.4	63.0	62.2	61.3	61.9	62.7	63.4	63.5
Main effect																
AP	Pooled SEM	0.39	0.42	0.39	0.44	0.41	0.42	0.46	0.43	0.45	0.44	0.41	0.52	0.55	0.53	0.51
	$LSD_{0.05}$	1.10	1.18	1.08	1.23	1.14	1.16	1.29	1.18	1.25	1.22	1.15	1.44	1.52	1.47	1.42
	P value	0.000	0.020	0.001	0.092	0.031	0.060	0.465	0.006	0.140	0.087	0.938	0.530	0.656	0.066	0.257
Phytase	Pooled SEM	0.23	0.24	0.22	0.26	0.24	0.24	0.27	0.25	0.26	0.25	0.24	0.30	0.31	0.30	0.30
	$LSD_{0.05}$	0.63	0.68	0.62	0.71	0.66	0.67	0.74	0.68	0.72	0.70	0.67	0.83	0.88	0.85	0.82
	P value	0.255	0.449	0.394	0.722	0.501	0.469	0.833	0.241	0.378	0.691	0.084	0.187	0.289	0.310	0.735
AP x Phytase	Pooled SEM	0.56	0.60	0.55	0.62	0.58	0.59	0.66	0.60	0.64	0.62	0.59	0.73	0.77	0.75	0.72
	$LSD_{0.05}$	1.55	1.66	1.53	1.74	1.61	1.64	1.82	1.67	1.77	1.72	1.63	2.04	2.15	2.07	2.01
	P value	0.335	0.092	0.953	0.131	0.554	0.695	0.582	0.053	0.890	0.828	0.019	0.467	0.270	0.596	0.119

Table 10-2: Effect of dietary AP and supplemental phytase on egg shell colour a* from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	21.8	19.4	18.0	17.8	19.5	20.4	18.7	18.4	17.6	17.9	16.0	17.0	16.6	16.6	18.0
	+	21.7	19.4	18.2	18.1	19.7	19.9	18.4	17.8	17.8	18.5	17.8	17.4	16.7	16.3	18.2
2.0	-	22.6	19.3	17.9	17.8	19.7	19.6	18.1	17.9	17.7	19.0	17.1	17.4	16.6	17.1	17.3
	+	22.0	19.6	18.4	18.2	19.8	19.4	17.9	18.2	17.8	18.6	17.1	18.0	16.2	17.4	18.7
2.5	-	21.4	18.6	18.0	17.6	19.3	19.6	18.4	17.4	17.7	18.5	17.1	17.7	16.6	17.1	19.4
	+	22.0	19.4	18.2	18.5	19.5	20.6	18.4	18.2	17.4	17.5	17.6	16.9	17.2	16.9	18.8
3.0	-	22.6	20.0	19.1	18.9	20.5	20.5	18.6	19.6	18.9	19.5	17.5	17.6	17.7	18.4	19.7
	+	22.5	19.6	18.8	18.3	19.8	20.5	18.5	18.9	18.4	18.2	17.2	18.2	16.9	18.1	17.3
3.5	-	21.7	19.3	17.9	18.2	20.2	20.2	18.0	19.0	17.6	18.9	17.2	18.0	17.6	18.0	18.1
	+	20.9	18.6	18.0	18.0	19.5	19.5	18.0	17.6	17.1	18.0	16.9	17.7	15.7	16.9	16.4
4.5	-	21.9	19.3	17.9	17.5	18.7	19.3	17.8	18.0	18.0	18.2	16.8	16.6	16.7	17.3	18.4
	+	21.6	19.0	18.0	17.7	19.1	19.1	18.4	18.2	17.2	17.7	17.3	17.5	16.7	16.7	19.5
Main effect																
AP	Pooled SEM	0.26	0.21	0.19	0.22	0.27	0.30	0.33	0.29	0.31	0.48	0.29	0.36	0.38	0.38	0.68
	$LSD_{0.05}$	0.72	0.58	0.53	0.63	0.75	0.83	0.91	0.82	0.87	1.34	0.81	1.01	1.05	1.05	1.89
	P value	0.008	0.040	0.002	0.084	0.034	0.027	0.593	0.014	0.071	0.678	0.841	0.468	0.697	0.024	0.402
Phytase	Pooled SEM	0.15	0.12	0.11	0.13	0.16	0.17	0.19	0.17	0.18	0.28	0.17	0.21	0.22	0.22	0.39
	$LSD_{0.05}$	0.42	0.33	0.31	0.36	0.44	0.48	0.52	0.47	0.50	0.77	0.47	0.58	0.61	0.60	1.09
	P value	0.252	0.650	0.410	0.421	0.859	0.655	0.983	0.296	0.298	0.133	0.125	0.434	0.176	0.249	0.559
AP x Phytase	Pooled SEM	0.37	0.29	0.27	0.32	0.38	0.42	0.46	0.42	0.44	0.68	0.41	0.51	0.54	0.53	0.96
	$LSD_{0.05}$	1.02	0.82	0.76	0.88	1.07	1.17	1.28	1.16	1.23	1.89	1.15	1.43	1.49	1.48	2.68
	P value	0.483	0.106	0.733	0.229	0.513	0.412	0.959	0.111	0.906	0.792	0.111	0.576	0.269	0.880	0.291

Table 10-3: Effect of dietary AP and supplemental phytase on shell weight per surface area (mg/cm2) from 24 to 80 weeks of age in Experiment 1

AP (g/kg)	Phytase	Age (week)														
		24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
1.5	-	78.3	81.8	81.3	81.8	81.3	79.9	79.6	79.2	79.1	76.2	76.9	76.4	79.3	78.9	79.2
	+	79.9	79.7	79.8	81.5	79.0	79.7	77.8	79.4	80.3	78.0	79.2	78.7	76.9	76.3	79.0
2.0	-	77.3	81.7	82.0	81.5	79.7	79.1	79.1	81.0	79.8	78.6	80.3	79.1	78.3	76.9	76.7
	+	78.7	82.8	81.7	82.5	80.0	78.9	78.3	81.3	81.0	80.4	80.9	80.7	77.7	79.8	79.7
2.5	-	78.4	79.0	79.2	81.0	79.3	79.3	77.4	80.1	79.4	78.6	79.3	76.3	79.0	77.0	78.1
	+	79.3	79.8	78.8	79.1	77.1	79.4	77.1	80.3	78.0	77.7	77.7	77.0	77.0	76.5	79.8
3.0	-	74.7	80.8	81.0	82.3	80.7	82.3	79.3	81.8	81.3	78.0	78.4	78.4	79.1	77.0	78.1
	+	78.2	81.9	82.0	81.5	79.2	80.8	80.0	82.8	80.3	79.0	80.6	78.6	77.3	78.6	79.3
3.5	-	80.0	80.6	80.0	80.0	80.3	79.5	78.0	81.3	78.0	78.7	77.8	79.1	77.7	76.3	77.0
	+	77.9	77.0	78.9	80.7	79.9	80.4	80.1	80.1	80.1	77.3	76.5	76.4	77.8	76.5	74.1
4.5	-	80.7	80.1	80.4	80.9	77.9	79.3	78.0	80.3	80.0	78.7	78.7	74.6	76.1	78.2	78.2
	+	78.9	79.4	79.3	78.9	79.4	79.6	77.4	78.9	78.6	76.9	77.6	76.3	75.9	73.3	76.0
Main effect																
AP	Pooled SEM	0.77	0.75	0.58	0.65	0.65	0.74	0.71	0.69	0.78	0.83	0.80	1.00	1.00	0.96	0.9
	$LSD_{0.05}$	2.13	2.08	1.60	1.81	1.81	2.06	1.98	1.92	2.16	2.30	2.24	2.78	2.79	2.67	2.6
	P value	0.036	0.011	0.001	0.053	0.156	0.216	0.196	0.031	0.378	0.466	0.047	0.040	0.616	0.394	0.054
Phytase	Pooled SEM	0.44	0.43	0.33	0.38	0.38	0.43	0.41	0.40	0.45	0.48	0.46	0.58	0.58	0.55	0.5
	$LSD_{0.05}$	1.23	1.20	0.92	1.04	1.04	1.19	1.14	1.11	1.25	1.33	1.29	1.61	1.61	1.54	1.5
	P value	0.344	0.352	0.237	0.303	0.160	0.872	0.839	0.784	0.843	0.918	0.741	0.411	0.167	0.473	0.866
AP x Phytase	Pooled SEM	1.08	1.06	0.81	0.92	0.92	1.05	1.00	0.97	1.10	1.17	1.14	1.41	1.42	1.36	1.3
	$LSD_{0.05}$	3.01	2.95	2.26	2.56	2.56	2.91	2.79	2.71	3.06	3.26	3.16	3.93	3.94	3.78	3.6
	P value	0.069	0.123	0.714	0.436	0.251	0.919	0.458	0.810	0.436	0.422	0.298	0.561	0.924	0.063	0.170

Table 10-4: Effect of dietary AP and Ca and supplemental phytase on shell colour L* of birds from 22 to 78 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	55.6	58.3	59.0	59.7	58.0	59.5	60.1	60.5	61.9	61.0	60.8	62.0	55.8	61.5	61.5
		+	56.8	58.9	59.5	59.8	58.6	59.2	60.2	60.0	61.8	61.2	60.7	62.2	54.6	62.2	61.7
1.5	40	-	56.3	58.0	59.2	58.8	58.5	58.5	59.9	60.5	62.4	61.7	61.1	62.6	55.1	60.5	60.7
		+	56.6	57.8	58.6	58.2	57.6	58.2	59.5	59.2	60.6	60.2	60.0	61.7	53.9	60.6	60.9
1.5	48	-	57.4	58.9	59.6	59.7	57.8	59.8	60.5	60.6	61.8	61.6	61.5	62.5	55.7	61.9	61.3
		+	57.9	57.8	58.8	58.5	57.3	58.8	60.0	60.7	62.2	59.9	59.7	61.9	54.6	62.0	61.0
2.5	32	-	56.8	57.4	60.1	60.6	58.3	58.8	60.6	59.6	60.6	60.8	59.6	61.4	54.3	60.3	61.0
		+	57.3	57.9	59.2	59.0	59.0	59.4	61.1	59.9	61.1	60.7	61.0	62.5	55.3	60.8	62.3
2.5	40	-	57.1	58.6	59.0	60.3	58.5	59.0	60.1	60.0	61.7	61.9	61.1	62.6	55.2	61.5	62.1
		+	55.4	56.8	57.7	58.0	56.6	58.4	59.4	58.8	61.3	59.8	59.6	60.9	53.6	60.6	60.8
2.5	48	-	57.0	59.1	59.3	58.5	59.8	59.2	60.7	61.3	61.9	61.5	61.3	63.1	55.2	62.7	62.5
		+	56.8	58.2	59.1	57.8	57.1	59.0	59.0	59.5	61.1	61.0	61.3	62.0	54.7	60.9	61.2
Main effect																	
AP	Pooled SEM		0.23	0.22	0.20	0.21	0.24	0.21	0.20	0.21	0.24	0.22	0.21	0.25	0.27	0.27	0.28
	$LSD_{0.05}$		0.64	0.61	0.56	0.58	0.66	0.59	0.55	0.57	0.67	0.60	0.60	0.70	0.74	0.74	0.78
	P value		0.897	0.430	0.768	0.818	0.439	0.904	0.607	0.208	0.121	0.941	0.963	0.821	0.505	0.412	0.265
Ca	Pooled SEM		0.28	0.27	0.25	0.26	0.29	0.26	0.24	0.25	0.29	0.27	0.26	0.31	0.33	0.33	0.34
	$LSD_{0.05}$		0.78	0.75	0.69	0.71	0.81	0.72	0.68	0.70	0.81	0.74	0.73	0.86	0.90	0.91	0.95
	P value		0.056	0.176	0.051	0.004	0.229	0.093	0.084	0.041	0.656	0.953	0.394	0.567	0.385	0.069	0.570
Phytase	Pooled SEM		0.23	0.22	0.20	0.21	0.24	0.21	0.20	0.21	0.24	0.22	0.21	0.25	0.27	0.27	0.28
	$LSD_{0.05}$		0.64	0.61	0.56	0.58	0.66	0.59	0.55	0.57	0.67	0.60	0.60	0.70	0.74	0.74	0.78
	P value		0.754	0.117	0.054	0.001	0.021	0.311	0.110	0.014	0.269	0.002	0.074	0.160	0.046	0.547	0.611

Table 10-5: Effect of dietary AP and Ca and supplemental phytase on shell colour a* of birds from 22 to 78 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	22.8	21.0	20.2	19.4	19.9	19.4	19.5	19.3	18.2	14.7	18.1	17.2	14.9	17.6	17.4
		+	21.8	20.8	20.5	19.6	20.0	19.9	19.7	20.0	18.7	15.0	18.6	17.8	15.8	17.3	17.5
1.5	40	-	21.8	21.1	20.1	20.1	19.6	20.2	19.8	19.5	17.9	14.4	18.1	16.9	15.4	18.1	17.9
		+	22.0	21.6	21.3	20.2	20.8	20.5	20.3	20.5	19.3	15.5	19.1	18.0	16.2	18.4	18.1
1.5	48	-	21.7	20.8	20.3	19.4	20.4	19.5	19.3	19.6	18.2	14.7	17.7	17.4	15.1	17.4	17.8
		+	20.8	21.5	20.8	20.2	20.5	20.1	19.9	19.5	18.2	16.0	19.2	17.8	15.8	17.5	17.7
2.5	32	-	21.7	22.1	20.2	19.2	19.8	20.1	19.2	20.2	19.3	15.4	19.1	18.3	15.8	18.3	17.9
		+	21.4	21.5	20.1	20.2	19.8	19.8	18.8	19.8	18.9	15.1	18.2	17.4	15.1	18.1	17.0
2.5	40	-	21.7	21.2	20.5	19.3	20.0	19.8	19.6	19.5	18.2	14.5	18.1	17.2	15.4	17.5	17.2
		+	22.6	22.1	21.3	20.6	20.9	20.4	19.9	20.7	18.8	15.9	19.0	18.6	16.0	17.9	17.8
2.5	48	-	21.0	20.5	20.5	20.1	19.7	19.8	19.4	19.2	18.2	14.8	18.3	17.1	15.6	16.9	16.9
		+	22.1	21.4	20.3	20.5	20.7	20.1	20.4	20.2	18.7	14.9	18.2	17.6	15.5	18.1	17.6
Main effect																	
AP	Pooled SEM		0.14	0.15	0.13	0.13	0.15	0.14	0.14	0.14	0.18	0.16	0.15	0.17	0.15	0.19	0.19
	$LSD_{0.05}$		0.39	0.42	0.37	0.38	0.41	0.40	0.39	0.40	0.49	0.44	0.41	0.47	0.41	0.53	0.53
	P value		0.781	0.131	0.741	0.326	0.784	0.680	0.386	0.313	0.319	0.838	0.918	0.467	0.790	0.882	0.284
Ca	Pooled SEM		0.17	0.19	0.16	0.17	0.18	0.18	0.14	0.18	0.21	0.19	0.18	0.21	0.18	0.23	0.23
	$LSD_{0.05}$		0.47	0.52	0.45	0.46	0.50	0.49	0.39	0.49	0.60	0.54	0.50	0.58	0.50	0.65	0.65
	P value		0.021	0.181	0.062	0.070	0.134	0.203	0.044	0.207	0.364	0.973	0.751	0.716	0.397	0.321	0.636
Phytase	Pooled SEM		0.14	0.15	0.13	0.13	0.15	0.14	0.14	0.14	0.18	0.16	0.15	0.17	0.15	0.19	0.19
	$LSD_{0.05}$		0.39	0.42	0.37	0.38	0.41	0.40	0.39	0.40	0.49	0.44	0.41	0.47	0.41	0.53	0.53
	P value		0.954	0.071	0.030	0.001	0.006	0.106	0.043	0.006	0.071	0.004	0.021	0.037	0.058	0.369	0.758

Table 10-6: Effect of dietary AP and Ca concentrations and supplemental phytase on albumen height (mm) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	8.3	7.8	8.0	8.0	7.9	7.6	7.6	7.2	7.0	6.9	6.9	6.5	6.4	6.4	5.9
		+	8.3	7.9	7.6	8.0	7.7	7.7	7.6	7.5	7.2	6.7	6.7	6.3	6.4	6.2	5.9
1.5	40	-	8.1	7.5	7.7	7.7	7.6	7.7	7.3	7.2	7.0	6.4	6.5	6.0	6.4	6.2	6.2
		+	8.3	7.7	7.8	7.5	7.6	7.7	7.3	7.3	7.3	6.3	6.6	6.2	6.3	6.1	6.1
1.5	48	-	8.4	8.0	7.8	8.0	7.8	7.5	7.5	7.4	7.0	7.0	6.7	6.3	6.5	5.9	5.7
		+	8.0	7.9	7.7	7.8	7.6	7.5	7.6	7.6	7.3	6.8	6.7	6.6	6.0	6.2	6.0
2.5	32	-	7.9	7.5	7.8	8.0	7.7	7.5	7.2	7.2	7.0	6.9	6.7	6.4	6.7	6.5	6.0
		+	8.1	7.9	7.8	7.7	7.6	7.3	7.5	7.3	7.0	6.6	6.1	6.5	6.1	6.4	5.8
2.5	40	-	7.9	8.0	7.6	7.8	7.7	7.8	7.5	7.4	7.0	6.8	6.7	6.5	6.3	5.9	5.9
		+	8.1	7.9	7.7	7.9	7.7	7.7	7.5	7.4	7.3	6.8	6.5	6.5	6.6	6.1	6.0
2.5	48	-	8.1	7.8	8.0	7.9	7.6	7.5	7.5	7.3	7.1	6.5	6.4	6.1	6.0	5.8	5.9
		+	7.9	7.8	7.7	7.7	7.7	7.5	7.6	7.2	7.1	6.5	6.8	6.1	6.4	6.0	6.0
Main effect																	
AP	Pooled SEM		0.06	0.06	0.06	0.07	0.06	0.06	0.064	0.06	0.07	0.08	0.08	0.09	0.09	0.09	0.09
	$LSD_{0.05}$		0.17	0.18	0.18	0.18	0.18	0.17	0.178	0.17	0.18	0.22	0.22	0.24	0.26	0.26	0.26
	P value		0.014	0.736	0.981	0.895	0.529	0.577	0.713	0.584	0.434	0.976	0.157	0.715	0.752	0.779	0.728
Ca	Pooled SEM		0.07	0.08	0.08	0.08	0.08	0.07	0.064	0.08	0.08	0.10	0.10	0.11	0.11	0.11	0.11
	$LSD_{0.05}$		0.20	0.21	0.22	0.22	0.22	0.21	0.178	0.21	0.22	0.27	0.27	0.29	0.32	0.32	0.32
	P value		0.873	0.473	0.536	0.197	0.645	0.165	0.341	0.832	0.581	0.381	0.870	0.572	0.376	0.032	0.545
Phytase	Pooled SEM		0.06	0.06	0.06	0.07	0.06	0.06	0.064	0.06	0.07	0.08	0.08	0.09	0.09	0.09	0.09
	$LSD_{0.05}$		0.17	0.18	0.18	0.18	0.18	0.17	0.178	0.17	0.18	0.22	0.22	0.24	0.26	0.26	0.26
	P value		0.963	0.290	0.250	0.087	0.363	0.979	0.352	0.388	0.040	0.295	0.354	0.454	0.402	0.703	0.886

Table 10-7: Effect of dietary AP and Ca concentrations and supplemental phytase on Haugh Unit of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	92.4	88.6	89.2	89.2	88.7	86.3	86.1	84.4	83.2	82.6	82.3	78.1	78.3	78.8	74.6
		+	92.3	89.6	87.5	89.1	87.7	87.2	86.1	85.5	83.6	80.6	80.2	76.8	78.2	76.9	73.6
1.5	40	-	91.1	87.1	87.5	87.8	86.6	86.3	84.0	84.0	82.5	78.4	78.9	73.8	77.5	75.5	76.4
		+	92.3	88.7	88.9	86.6	87.2	87.0	84.9	84.6	85.2	78.5	80.4	77.1	76.6	75.7	75.4
1.5	48	-	92.6	89.7	88.5	89.2	88.1	85.4	85.8	84.7	82.6	82.4	80.7	77.2	77.6	73.4	71.8
		+	90.9	89.7	87.6	88.3	86.7	85.8	86.7	86.2	85.1	81.2	80.7	80.8	74.1	72.0	74.8
2.5	32	-	91.0	87.3	88.8	90.0	88.1	86.5	84.2	84.3	82.6	82.6	81.2	79.0	80.7	79.2	75.4
		+	91.5	89.8	88.7	87.9	87.3	85.3	86.1	85.0	83.1	79.8	74.4	79.3	75.8	79.0	72.6
2.5	40	-	90.5	90.0	87.2	88.5	87.5	87.6	85.6	85.3	82.4	81.2	81.0	79.9	77.6	73.9	73.6
		+	91.6	89.9	87.5	88.6	87.2	86.3	85.2	84.7	84.0	80.1	78.7	78.3	79.2	75.5	74.9
2.5	48	-	91.4	89.0	90.2	88.8	86.9	85.8	85.5	84.4	83.0	78.7	79.0	76.1	74.4	73.8	74.3
		+	90.3	88.7	88.2	88.1	87.4	85.8	86.3	84.2	83.8	79.9	80.7	75.2	77.0	75.2	75.0
Main effect																	
AP	Pooled SEM		0.32	0.35	0.37	0.38	0.37	0.37	0.40	0.39	0.43	0.59	0.67	0.71	0.80	0.99	0.84
	$LSD_{0.05}$		0.88	0.97	1.04	1.06	1.04	1.02	1.10	1.09	1.19	1.64	1.87	1.98	2.22	2.76	2.33
	P value		0.043	0.667	0.672	0.585	0.849	0.823	0.830	0.685	0.357	0.767	0.144	0.526	0.711	0.621	0.909
Ca	Pooled SEM		0.39	0.43	0.46	0.47	0.46	0.45	0.48	0.48	0.52	0.72	0.82	0.87	0.98	1.21	1.03
	$LSD_{0.05}$		1.07	1.19	1.27	1.30	1.27	1.25	1.35	1.33	1.46	2.01	2.29	2.42	2.71	3.38	2.86
	P value		0.631	0.720	0.341	0.203	0.413	0.246	0.239	0.937	0.767	0.203	0.812	0.631	0.165	0.015	0.703
Phytase	Pooled SEM		0.32	0.97	0.37	0.38	0.37	0.37	0.40	0.39	0.43	0.59	0.67	0.71	0.80	0.99	0.84
	$LSD_{0.05}$		0.88		1.04	1.06	1.04	1.02	1.10	1.09	1.19	1.64	1.87	1.98	2.22	2.76	2.33
	P value		0.960	0.102	0.347	0.132	0.460	0.873	0.229	0.343	0.019	0.239	0.165	0.564	0.441	0.978	0.978

Table 10-8: Effect of dietary AP and Ca concentrations and supplemental phytase on yolk colour of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	10.2	10.9	10.9	10.1	11.0	10.5	10.1	9.6	10.3	10.1	10.2	10.1	10.6	11.1	11.2
		+	10.1	10.2	9.9	9.3	11.0	10.4	9.4	10.1	10.7	11.2	11.5	11.3	11.5	11.5	11.4
1.5	40	-	10.8	11.6	11.4	11.1	11.6	11.2	10.9	10.4	11.5	11.5	11.4	11.6	11.4	11.8	11.7
		+	10.7	10.8	10.6	9.2	11.5	11.8	10.9	10.6	11.4	11.7	11.3	11.1	11.1	12.1	11.6
1.5	48	-	11.0	11.5	11.5	11.0	12.1	11.5	10.9	10.8	11.8	11.8	11.9	11.9	11.7	12.2	12.0
		+	11.0	11.0	10.8	9.9	11.9	12.2	11.9	10.7	11.4	11.7	11.6	11.5	11.5	11.4	11.7
2.5	32	-	10.3	10.7	10.5	10.2	11.3	11.0	10.2	9.8	10.5	10.2	10.0	10.1	10.0	11.4	10.9
		+	10.2	10.3	10.3	9.6	11.3	11.3	11.1	10.0	10.6	10.1	9.8	9.8	9.9	11.6	11.0
2.5	40	-	10.8	11.0	11.5	11.1	11.9	11.2	10.6	10.4	11.4	11.5	11.5	11.1	11.4	11.7	11.6
		+	10.5	10.7	10.6	9.0	12.0	12.1	11.5	10.6	11.3	11.6	11.3	11.3	11.2	11.8	11.3
2.5	48	-	11.4	11.5	11.5	11.2	12.0	11.5	11.0	10.7	11.7	12.0	11.8	11.8	11.8	12.0	11.5
		+	11.0	10.8	11.0	10.1	12.3	12.2	11.6	11.3	11.6	11.5	11.6	11.6	11.5	11.7	11.8
Main effect																	
AP	Pooled SEM		0.07	0.05	0.05	0.06	0.05	0.06	0.061	0.06	0.06	0.06	0.06	0.07	0.06	0.08	0.06
	$LSD_{0.05}$		0.18	0.14	0.15	0.18	0.13	0.16	0.169	0.16	0.16	0.16	0.16	0.18	0.16	0.21	0.17
	P value		0.446	0.029	0.667	0.383	0.000	0.000	0.000	0.347	0.624	0.051	0.000	0.003	0.000	0.759	0.001
Ca	Pooled SEM		0.08	0.06	0.07	0.08	0.06	0.07	0.074	0.07	0.07	0.07	0.07	0.08	0.07	0.09	0.08
	$LSD_{0.05}$		0.22	0.18	0.19	0.21	0.15	0.20	0.207	0.20	0.19	0.20	0.20	0.22	0.20	0.26	0.21
	P value		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
Phytase	Pooled SEM		0.07	0.05	0.05	0.06	0.05	0.06	0.061	0.06	0.06	0.06	0.06	0.07	0.06	0.08	0.06
	$LSD_{0.05}$		0.18	0.14	0.15	0.18	0.13	0.16	0.169	0.16	0.16	0.16	0.16	0.18	0.16	0.21	0.17
	P value		0.051	0.000	0.000	0.000	0.727	0.000	0.000	0.001	0.944	0.121	0.685	0.904	0.590	0.838	0.849

Table 10-9: Effect of dietary AP and Ca concentrations and supplemental phytase on egg shell weight (g) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	5.0	5.5	5.6	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.8	5.9	5.8	5.8	5.6
		+	5.1	5.5	5.5	5.4	5.6	5.6	5.8	5.8	5.7	5.6	5.6	5.7	5.8	5.5	5.6
1.5	40	-	5.5	5.7	5.8	5.6	5.8	6.0	6.1	6.0	5.7	5.9	5.7	6.0	5.8	5.8	5.8
		+	5.3	5.5	5.5	5.6	5.7	5.8	5.8	5.8	5.6	5.6	5.7	5.6	5.7	5.7	5.6
1.5	48	-	5.4	5.6	5.7	5.8	5.8	5.9	6.0	5.9	5.9	5.7	5.9	5.8	5.8	5.8	5.7
		+	5.4	5.6	5.7	5.7	5.8	6.0	5.9	5.9	5.8	5.8	5.8	5.8	5.8	5.7	5.5
2.5	32	-	5.1	5.3	5.3	5.4	5.5	5.7	5.8	5.7	5.7	5.6	5.7	5.7	5.7	5.8	5.6
		+	5.2	5.4	5.5	5.3	5.5	5.6	5.7	5.5	5.7	5.7	5.7	5.6	5.7	5.5	5.4
2.5	40	-	5.2	5.5	5.6	5.7	5.7	5.9	5.9	5.9	5.8	5.8	5.9	5.8	5.7	5.9	5.5
		+	5.2	5.5	5.7	5.7	5.9	6.0	5.9	6.0	5.9	5.9	6.1	6.0	5.9	5.8	5.8
2.5	48	-	5.5	5.7	5.7	5.8	5.8	6.0	5.9	6.0	5.8	6.0	5.7	5.7	5.8	5.8	5.6
		+	5.3	5.6	5.6	5.7	5.8	6.0	6.0	5.9	5.8	5.9	6.0	5.8	5.6	5.9	5.9
Main effect																	
AP	Pooled SEM		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05
	$LSD_{0.05}$		0.09	0.09	0.08	0.09	0.09	0.10	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.12	0.13
	P value		0.561	0.486	0.112	0.477	0.464	0.750	0.977	0.573	0.495	0.065	0.032	0.310	0.403	0.309	0.736
Ca	Pooled SEM		0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.05	0.05	0.05	0.06
	$LSD_{0.05}$		0.11	0.11	0.10	0.11	0.11	0.12	0.11	0.13	0.12	0.13	0.12	0.13	0.14	0.15	0.16
	P value		0.000	0.002	0.000	0.000	0.000	0.000	0.003	0.000	0.296	0.008	0.001	0.210	0.721	0.061	0.409
Phytase	Pooled SEM		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05
	$LSD_{0.05}$		0.09	0.09	0.08	0.09	0.09	0.10	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.12	0.13
	P value		0.676	0.251	0.386	0.107	0.693	0.586	0.197	0.330	0.447	0.479	0.664	0.155	0.941	0.116	0.693

Table 10-10: Effect of dietary AP and Ca concentrations and supplemental phytase on shell percentage (% of egg) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	9.3	9.5	9.3	9.4	9.4	9.2	9.2	9.3	9.5	9.6	9.6	9.4	9.2	9.3	9.1
		+	9.3	9.6	9.5	9.0	9.2	9.1	9.2	9.3	9.2	9.1	9.1	9.1	9.4	9.0	8.9
1.5	40	-	9.6	9.8	9.6	9.4	9.4	9.3	9.6	9.4	9.2	9.6	9.2	9.5	9.2	9.2	9.1
		+	9.8	9.7	9.6	9.6	9.7	9.5	9.5	9.5	9.3	9.4	9.5	9.2	9.2	9.2	9.0
1.5	48	-	9.6	9.5	9.6	9.5	9.4	9.4	9.4	9.4	9.4	9.2	9.6	9.3	9.0	9.1	9.1
		+	9.8	9.8	9.8	9.5	9.6	9.5	9.6	9.5	9.5	9.5	9.5	9.6	9.4	9.1	9.0
2.5	32	-	9.6	9.6	9.2	9.3	9.4	9.5	9.6	9.3	9.5	9.3	9.5	9.4	9.3	9.5	9.2
		+	9.5	9.5	9.5	9.1	9.3	9.2	9.4	9.0	9.4	9.4	9.2	9.2	9.3	9.1	9.0
2.5	40	-	9.6	9.6	9.6	9.4	9.5	9.4	9.5	9.5	9.4	9.3	9.5	9.5	9.1	9.3	8.7
		+	9.7	9.7	9.5	9.6	9.7	9.4	9.3	9.5	9.4	9.5	9.7	9.4	9.3	9.3	9.2
2.5	48	-	9.9	9.8	9.6	9.6	9.6	9.6	9.4	9.4	9.2	9.6	9.3	9.3	9.0	9.3	9.0
		+	9.6	9.8	9.6	9.6	9.7	9.5	9.6	9.7	9.5	9.7	9.5	9.2	8.9	9.4	9.1
Main effect																	
AP	Pooled SEM		0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.06	0.06
	$LSD_{0.05}$		0.13	0.13	0.11	0.12	0.13	0.14	0.13	0.14	0.18	0.14	0.15	0.14	0.16	0.17	0.18
	P value		0.162	0.750	0.144	0.404	0.198	0.084	0.609	0.895	0.854	0.638	0.640	0.724	0.408	0.067	0.812
Ca	Pooled SEM		0.06	0.06	0.05	0.05	0.06	0.06	0.06	0.06	0.08	0.06	0.06	0.06	0.07	0.08	0.08
	$LSD_{0.05}$		0.16	0.16	0.13	0.15	0.15	0.17	0.16	0.18	0.22	0.17	0.18	0.18	0.19	0.21	0.22
	P value		0.000	0.055	0.00	0.000	0.005	0.034	0.109	0.005	0.705	0.215	0.253	0.378	0.217	0.968	0.882
Phytase	Pooled SEM		0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.06	0.06
	$LSD_{0.05}$		0.13	0.13	0.11	0.12	0.13	0.14	0.13	0.14	0.18	0.14	0.15	0.14	0.16	0.17	0.18
	P value		0.792	0.492	0.139	0.612	0.221	0.429	0.992	0.811	0.791	0.801	0.743	0.157	0.261	0.282	0.940

Table 10-11: Effect of dietary AP and Ca concentrations and supplemental phytase on shell thickness (mm) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	0.37	0.40	0.39	0.40	0.39	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.38	0.37	0.36
		+	0.37	0.41	0.39	0.40	0.39	0.37	0.38	0.38	0.38	0.38	0.38	0.39	0.39	0.35	0.36
1.5	40	-	0.38	0.41	0.40	0.40	0.40	0.39	0.40	0.39	0.39	0.39	0.38	0.40	0.39	0.36	0.37
		+	0.38	0.41	0.39	0.41	0.40	0.39	0.39	0.40	0.39	0.39	0.40	0.38	0.39	0.36	0.36
1.5	48	-	0.38	0.41	0.40	0.41	0.40	0.39	0.39	0.38	0.39	0.39	0.40	0.39	0.38	0.37	0.37
		+	0.39	0.41	0.41	0.40	0.40	0.40	0.40	0.39	0.40	0.39	0.40	0.40	0.40	0.37	0.36
2.5	32	-	0.38	0.40	0.38	0.39	0.39	0.39	0.39	0.38	0.39	0.38	0.39	0.39	0.39	0.36	0.36
		+	0.38	0.40	0.39	0.40	0.39	0.38	0.38	0.37	0.38	0.39	0.38	0.38	0.39	0.36	0.36
2.5	40	-	0.38	0.41	0.39	0.40	0.39	0.39	0.39	0.39	0.39	0.39	0.40	0.39	0.38	0.37	0.35
		+	0.39	0.41	0.39	0.42	0.40	0.39	0.38	0.39	0.39	0.40	0.40	0.39	0.40	0.37	0.37
2.5	48	-	0.39	0.41	0.40	0.41	0.40	0.39	0.39	0.39	0.39	0.40	0.39	0.38	0.38	0.36	0.36
		+	0.38	0.40	0.39	0.41	0.41	0.39	0.39	0.40	0.39	0.40	0.40	0.38	0.37	0.37	0.37
Main effect																	
AP	Pooled SEM		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003
	$LSD_{0.05}$		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.007	0.007
	P value		0.407	0.144	0.115	0.513	0.716	0.362	0.129	0.952	0.855	0.259	0.647	0.060	0.247	0.423	0.463
Ca	Pooled SEM		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.003	0.003
	$LSD_{0.05}$		0.007	0.006	0.006	0.006	0.006	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.008	0.009
	P value		0.000	0.008	0.003	0.000	0.000	0.002	0.054	0.000	0.166	0.055	0.021	0.432	0.212	0.248	0.636
Phytase	Pooled SEM		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003
	$LSD_{0.05}$		0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.007	0.007
	P value		0.385	0.480	0.503	0.541	0.129	0.430	0.175	0.171	0.855	0.735	0.849	0.650	0.119	0.593	0.376

Table 10-12: Effect of dietary AP and Ca concentrations and supplemental phytase on shell weight per surface area (mg/cm2) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)														
			22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1.5	32	-	75.8	79.0	78.1	79.0	78.9	78.1	78.0	78.9	79.8	80.5	80.3	79.9	78.0	78.7	77.1
		+	75.8	79.4	78.7	75.7	77.4	76.8	78.2	78.5	78.3	77.0	76.7	77.6	79.1	76.0	75.8
1.5	40	-	79.4	81.4	80.6	78.9	79.5	79.5	81.9	80.3	78.0	81.1	77.8	80.9	78.0	78.5	77.5
		+	79.7	80.3	79.4	79.7	81.0	80.4	79.9	80.0	78.3	79.0	79.6	77.8	77.8	77.5	75.9
1.5	48	-	79.1	79.6	80.6	80.0	79.2	79.6	80.1	79.9	80.2	78.3	81.3	78.8	77.3	77.4	77.4
		+	79.8	81.1	81.6	79.5	80.4	80.8	81.5	80.5	79.8	80.2	80.0	80.8	79.6	77.6	75.9
2.5	32	-	77.4	78.5	76.5	77.0	78.5	79.9	80.7	78.3	79.6	78.2	79.5	79.4	78.4	79.6	77.4
		+	77.3	78.7	78.7	76.0	77.5	77.6	79.0	76.1	79.1	79.1	77.8	77.2	78.3	76.7	75.6
2.5	40	-	78.5	79.8	79.9	79.2	79.7	80.1	80.4	80.5	79.5	78.7	80.4	80.1	77.6	79.2	74.2
		+	78.9	79.8	79.4	80.6	81.4	80.1	79.6	80.6	79.5	80.6	82.5	80.2	79.0	79.0	78.2
2.5	48	-	81.3	81.6	80.0	80.7	80.5	81.7	79.8	80.0	78.1	81.2	78.6	78.4	77.2	78.8	76.0
		+	78.8	80.9	79.9	80.3	80.9	80.6	81.3	81.6	80.0	82.0	80.9	78.3	75.8	79.7	77.9
Main effect																	
AP	Pooled SEM		0.38	0.37	0.32	0.36	0.37	0.41	0.38	0.42	0.52	0.42	0.42	0.43	0.46	0.51	0.54
	$LSD_{0.05}$		1.05	1.03	0.89	0.99	1.02	1.14	1.06	1.16	1.43	1.16	1.18	1.21	1.28	1.42	1.49
	P value		0.429	0.631	0.09	0.743	0.505	0.163	0.695	0.768	0.740	0.309	0.275	0.541	0.378	0.091	0.961
Ca	Pooled SEM		0.46	0.45	0.391	0.43	0.45	0.41	0.47	0.51	0.63	0.51	0.52	0.53	0.56	0.62	0.66
	$LSD_{0.05}$		1.29	1.26	1.09	1.21	1.25	1.14	1.30	1.42	1.76	1.42	1.45	1.48	1.57	1.74	1.83
	P value		0.000	0.009	0.00	0.000	0.000	0.001	0.024	0.000	0.740	0.056	0.048	0.269	0.484	0.644	0.920
Phytase	Pooled SEM		0.38	0.37	0.32	0.36	0.37	0.41	0.38	0.42	0.52	0.42	0.42	0.43	0.46	0.51	0.54
	$LSD_{0.05}$		1.05	1.03	0.89	0.99	1.02	1.14	1.06	1.16	1.43	1.16	1.18	1.21	1.28	1.42	1.49
	P value		0.737	0.935	0.450	0.328	0.441	0.445	0.647	0.861	0.985	0.950	0.922	0.129	0.392	0.188	0.931

Table 10-13: Effect of dietary AP and Ca concentrations and supplemental phytase on shell breaking strength (kg) of hens from 22 to 80 weeks of age in Experiment 2

AP (g/kg)	Ca (g/kg)	Phytase	Age (week)								
			22	50	54	58	62	66	70	74	78
1.5	32	-	3.7	3.4	3.5	3.6	3.4	4.1	3.1	3.1	3.0
		+	3.6	3.6	3.6	3.1	3.3	3.3	3.1	3.1	3.2
1.5	40	-	4.0	3.5	3.6	3.5	3.3	3.4	3.4	3.3	3.2
		+	4.2	3.7	3.6	3.4	3.5	3.3	3.2	3.1	3.0
1.5	48	-	4.2	3.7	3.7	3.5	3.7	3.5	3.1	3.1	3.2
		+	4.3	3.7	3.5	3.6	3.7	3.7	3.2	3.1	2.9
2.5	32	-	3.9	3.6	3.5	3.4	3.4	3.5	3.2	3.2	3.2
		+	4.0	3.3	3.5	3.4	3.2	3.0	3.0	3.0	2.9
2.5	40	-	4.1	3.7	3.7	3.4	3.6	3.4	3.1	3.2	3.0
		+	4.2	3.8	3.8	3.5	3.7	3.4	3.2	3.2	3.1
2.5	48	-	4.4	3.6	3.4	3.6	3.4	3.3	3.2	3.3	2.8
		+	4.1	3.9	3.5	3.8	3.6	3.3	3.2	3.2	3.2
Main Effect											
AP	Pooled SEM		0.05	0.05	0.05	0.05	0.05	0.12	0.05	0.05	0.05
	$LSD_{0.05}$		0.14	0.15	0.14	0.15	0.14	0.33	0.15	0.14	0.15
	P value		0.18	0.61	0.82	0.30	0.97	0.14	0.61	0.90	0.71
Ca	Pooled SEM		0.06	0.06	0.06	0.07	0.06	0.15	0.06	0.06	0.07
	$LSD_{0.05}$		0.17	0.18	0.18	0.18	0.18	0.41	0.18	0.18	0.19
	P value		0.00	0.01	0.15	0.03	0.02	0.92	0.58	0.77	0.93
Phytase	Pooled SEM		0.05	0.05	0.05	0.05	0.05	0.12	0.05	0.05	0.05
	$LSD_{0.05}$		0.14	0.15	0.14	0.15	0.14	0.33	0.15	0.14	0.15
	P value		0.85	0.48	0.86	0.61	0.77	0.23	0.60	0.33	0.86