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Use of phytase in layer diets

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by X. Li and W. L. Bryden

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Researcher Contact Details
Name: Dr X. Li
Address: School of Animal Studies, University of Queensland, Gatton Qld 4343

Phone: (07) 5460 1567
Fax: (07) 5460 1444
Email: x.li1@uq.edu.au

In submitting this report, the researcher has agreed to AECL publishing this material in its edited form.

AECLContact Details:
Australian Egg Corporation Limited
A.B.N: 6610 2859 585
Suite 4.02, Level 4, 107 Mount St
North Sydney NSW 2060

Phone: 02 9409 6908
Fax: 02 9954 3133
Email: david@aecl,org
Website: http://www.aecl.org

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Foreword

Economic constraints and public concerns will compel the poultry industry to increasingly use a range of cheaper, alternative plant-derived feedstuffs in feed formulations and eliminate the use of animal by-product meals. However, use of such ingredients will also increase the dietary levels of phytic acid which can have wide ranging ramifications on performance.

Phytic acid is the major storage form of P in plant ingredients. Phosphorus bound in phytic acid constitutes about 60-80% of the total P in these ingredients. This phytate-bound P is generally unavailable to poultry. In addition, phytic acid can bind several biologically important minerals (Ca, Mg, Zn, Mn, Cu and Fe) and protein and lower their availability to birds.

This publication details studies which examined the effectiveness of supplemental phytase in improving the availability of P, amino acids and energy from plant feed ingredients for laying hens.

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

This report is an addition to AECL's range of research publications and forms part 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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Program Manager R&D
Australian Egg Corporation Limited

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About the Author

Dr. Xiuhua Li is a research scientist in the School of Animal Studies at the University of Queensland. Dr. Li graduated from Jilin Agricultural University in China and completed a Masters degree at the University of Minnesota before undertaking a PhD at the University of Sydney. She was previously employed at the University of Sydney as a postdoctoral fellow working in various aspects of poultry nutrition.

Professor Wayne Bryden is Professor of Animal Science and Head of the School of Animal Studies at the University of Queensland. Prior to taking up that appointment in 2002 he had been an Associate Professor in the Faculty of Veterinary Science and Director of the Poultry Research Foundation at the University of Sydney. He has degrees from both the University of New England and the University of Sydney and has worked in various aspects of poultry science, especially nutrition and toxicology for many years.

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Executive Summary

Supply of phosphorus in poultry diets is an important aspect of diet formulation. In Australia much of the P in diets is supplied by the inclusion of meat and bone meal. With increasing public pressure to reduce the use of meat and bone meals it will be important for the industry to find other strategies for the supply of P. A possible approach is the use of supplemental phytase in diets to release P bound within dietary ingredients as phytate.

In this year long project entitled, "Reducing the use of animal by-product meals in layer diets", two studies were undertaken to examine the influence of supplementing diets with phytase to reduce the need for the use of meat and bone meal in layer diets. Two experiments were conducted, one with older hens from 77 – 89 weeks of age and younger birds from 23 – 47 weeks of age.

Body weight, egg production and feed conversion of the hens from both experiments remained similar throughout the respective experimental periods irrespective of the treatment. The use of phytase and the removal of meat and bone meal from the diets did not affect production. The results indicate that the P requirements of laying hens were met throughout the production period even at the lowest level of P supplementation. This suggests that the P requirement of the modern layer should be evaluated along with the optimum Ca:P ratio of layer diets. Interestingly, at lower levels of available P there was a notable decrease in the ileal digestibility of amino acids, an area that requires further investigation.

Chapter 1

Introduction

Since the commercial expansion of the Australian Poultry industry, meat by-products meals have been a significant source of protein and P. Over the years, reliance on meat and bone meal products has declined as alternate plant protein meals have become available. Nevertheless, animal by-product meals are still an important component of a layer diet, especially as a source of P. There is increasing community concern, resulting largely from the occurrence of Bovine Spongiform Encephalopathy (BSE) or mad cow disease in Europe, as to the advisability of feeding animal by-product meals to animals. In Europe, the European Economic Community (EEC) has banned the use of such products in animal feeding.

With this background, there is increasing need for the Poultry industry to develop strategies to reduce their reliance on animal by-product meals. A major strategy involves a greater use of ingredients of plant origins in diet formulation. The P of a typical cereal grain diet is poorly utilized by laying hens. This is not unexpected considering that about two-thirds of the total P in plant feedstuffs is in the form of phytate P (Common, 1940. Nelson et al., 1968, Reddy et al 1982). Phytate is a hexaphosphoric acid myo-inositol salt of divalent cations of Ca, Cu, Mg, Mn, Zn, etc. Phytate also has the potential of binding with proteins at low and neutral pH (Cosgrove, 1980; Anderson, 1985), and complexing with proteases, such as pepsin and trypsin (Camus and Laporte. 1976; Singh and Krikorian, 1982). Due to low amounts of phytase in the digestive system of monogastric animals, the use of phytate P by these animals is negligible. Some feed ingredients have very small quantities of phytase, but the contribution of this phytase to the utilization of phytate P in feed ingredients is very small and inconsequential. Formation of complexes of phytic acid with divalent cations and amino acids not only reduces the availability of P, but also reduces the digestibility and availability of amino acids and cations to monogastric animals, including poultry.

The early studies of Nelson et al. (1968) clearly indicated that the availability of phytate P to chickens can be increased considerably by the addition of microbial phytase to diets. However, the high cost of production and the low stability of the enzyme in feed prevented commercial use of microbial phytase until recently. With commercial availability of microbial phytase and public concerns regarding P pollution, investigations on the use of microbial phytase in the diets of broilers (Biehl, et al., 1995; Mitchell and Edwards, 1996), turkeys (Yi et al, 1996), and laying hens has been revived in recent years. There has been much interest in the use phytase in poultry diets to increase the availability of P, and also to reduce environmental pollution from P in excreta (Kornegay, 2001). A number of studies have demonstrated, conclusively, that phytase improves phytate P utilization in layer diets (Gordon and Roland, 1997; Carlos and Edwards, 1998; Um and Paik, 1999; Bowland *et al*, 2000). Invariably, the studies that have been conducted have used corn/soybean meal diets that do not reflect diets used in the Australian layer industry.

In all of the layer studies, the release of P by phytase has been the focus of research but phytic acid and inorganic P, both interact with non-P components of the feed as well; including cations, protein and energy (Ravindran *et al,* 1995*;* Selle *et al,* 2000). The demonstration of the anti-nutritive effect of phytic acid in relation to both energy and amino acid availability has been shown in broilers but no comparable studies have been undertaken with laying hens.

In this year long project, two experiments were conducted to determine the efficacy of microbial phytase in laying hen diets with the objective of reducing the use of animal by-product meals.

Animal Experimentation

Experimental procedures described in this report which involved the use of birds were approved by the University of Queensland Animal Ethics Committee and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Chapter 2

Material and methods

Experiment 1, experimental diets that contained varying amounts of meat meal, available P and phytase were fed to laying hens from 77 – 89 weeks of age (old birds). In addition to production and egg parameters the ileal digestibility of energy and amino acids were determined. The same experimental diets and protocol was used in experiment 2, but in this experiment hens from 23 – 47 weeks of age (young birds) were used.

Birds and housing

For both experiments 19 week old Isa Brown layer pullets were obtained from the same hatchery. The birds used in the two experiments were from different hatches and after arrival at the University the birds in experiment 1 were maintained in the University's layer flock until 77 weeks of age. In contrast, the birds in experiment 2 were placed in experimental cages upon arrival at the University. Birds in experiment 1 were fed experimental diets for 12 weeks (77-89 weeks of age) and birds in experiment 2 were fed experimental diets for 24 weeks (23-47 weeks of age). Birds in both experiments were housed in conventional Australian cages that were located in an open sided shed at 3 birds/cage (450 cm^2 per bird). In each experiment 270 layers were selected and allocated to 90 cages. Each cage was treated as a replicate and each experimental diet was fed to 9 cages. The length of the light period in both experiments was 16 hrs and birds had free access to feed and water.

Feed ingredients

The main ingredients used in the experiments including soybean meal, cottonseed meal, canola meal, sorghum and rice pollard were analysed for phytase activity and phytate P content (Table 1) prior to formulating diets by BRI Australia Limited using methods of Engelen *et al*. (1994) and AOAC (2000), respectively (Selle *et al*., 2003). Wheat was not included due to its high concentration of phytase activity.

Table 1. Phytase activity and phytate P content of feed ingredients

Ingredient	Phytase activity (FTU/kg)	Phytate P (mg/100g)
Soybean meal	40	405
Cottonseed meal	10	705
Canola meal	<10	515
Sorghum	40	225
Rice pollard	170	1080

Experimental diets

The experimental diets contained varying amounts of meat and bone meal, available P and phytase. The diets were formulated to contain: a) standard level of available P (4.0 g/kg) with (Diet 1) or without meat and bone meal (Diet 2), Diet 3 was derived from Diet 2 and supplemented with phytase; b) a medium level of available P (2.9g/kg) without meat and bone meal (Diet 4), Diet 5 was derived from Diet 4 and supplemented with phytase; c) a low level of available P (1.8 g/kg) without meat and bone meal (Diet 6), Diet 7 was derived from Diet 6 and supplemented with phytase (as summarised in Table 2). Phytate-P contents (3.0 g/kg) were similar across all diets. All diets contained recommended levels of crude protein, calcium, lysine and sulphur-containing amino acids. The limestone used in the diets consisted of 60% chips and 40% powder. Phyzyme Phytase was kindly supplied by Feed Works and supplemented to the diet at the level of 90g/tonne which gave

450 FTU/kg of diet. The detailed composition of the experimental diets, fed as mash, is shown in Table 3.

Table 2. Summary of experimental treatments

Diet 1	Avail P 4 g/kg + MBM
Diet 2	Avail P 4 g/kg -- MBM
Diet 3	Diet 2 + Phytase
Diet 4	Avail P 2.9 g/kg -- MBM
Diet 5	Diet 4 + Phytase
Diet 6	Avail P 1.8 g/kg -- MBM
Diet 7	Diet 6 + Phytase

Table 3. Diet formulation (g/kg) for Experiments 1 and 2

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Sorghum	603.7	576.6	576.6	582.3	582.3	587.1	587.1
Canola	54	70	70	70	70	70	70
Cotton meal	10.2	30	30	30	30	30	30
Rice pollard	46	18	18	17	17	17	17
Meat and bone meal	59.6	-	-	-	-	-	-
Soybean meal	122.2	168.3	168.3	167.3	167.3	166.2	166.2
Corn oil	10.2	21.7	21.7	20.2	20.2	18.7	18.7
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate		15	15	9.1	9.1	3.1	3.1
Limestone	87.3	93.3	93.3	97	97	100.8	100.8
Salt	1.637	2.1	2.1	2.1	2.1	2.1	2.1
Methionine	1.44	1.44	1.44	1.44	1.44	1.44	1.44
Sodium bicarbonate	2.21	2.03	2.03	2.04	2.04	2.04	2.04
Premix*	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lysine	0.11	-	-	-	-	-	-
Phytase (g/ton)	-	-	90	-	90	-	90
Analysis (g/kg)							
AME (MJ/kg)	11.7	11.7	11.7	11.7	11.7	11.7	11.7
Protein	188	186	186	186	186	186	186
Calcium	39.0	39.0	39.0	39.0	39.0	39.0	39.0
P	7.0	7.0	7.0	5.9	5.9	4.8	4.8
Avail. P	4.0	4.0	4.0	2.9	2.9	18	1.8
Phytate-P	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Lysine	8.8	8.9	8.9	8.9	8.9	8.9	8.9
Methionine	4.3	4.3	4.3	4.3	4.3	4.3	4.3

* Each kg of premix contained the following : vitamin A, 2,200 IU.; vitamin D_3, 700 IU.; vitamin E, 4 g; vitamin K_3, 0.4 g; riboflavin (vitamin B_2) 1.6 g; pyridoxine HCl (vitamin B_6) 1 g; cyanocobalamin (vitamin B_{12}), 3 g; biotin, 0.02 g; niacin, 6 g; thiamine (vitamin B_1), 0.3 g; calcium pantothenate, 3 g; folic acid, 0.4 g; antioxidant, 25 g; manganese (MnO), 15g; zinc (ZnO), 10 g; iron ($FeSO_4.H_2O$), 4 g; copper ($CuSO_4.H_2O$), 1 g; iodine ($Ca(IO_3)_2$) 0.2 g; cobalt ($CoCO_3$), 0.06 g; selenium (Na_2SeO_3), 0.02 g; molybdenum (Na_2MoO_4), 0.32 g. Choline chloride and salt were obtained locally.

Measurements

Egg production was recorded daily. Feed intake, egg weight and body weight were measured monthly in both Experiments 1 and 2. Eggs were collected for 3 days and egg weight, egg shell breaking strength, shell thickness, yolk colour and Haugh unit were measured (Balnave *et al*., 1992) when layers were 89 weeks of age (12 weeks on treatment) in Experiment 1 and 47 (24 weeks on treatment) in Experiment 2, respectively.

Apparent metabolisable energy assay

The AME was determined using the method described (Li et al 2001). Celite (a source of acid-insoluable ash; AIA) was added (20g/kg) to the experimental diets as an indigestible marker when hens were 88 weeks of age in Experiment 1, 46 weeks of age in Experiment 2, respectively. After an adaptation period of 4 days, feed intake was monitored, and the excreta were collected daily at 09:00 h for 3 days, dried for 24 h at 80 °C in a forced-air oven and pooled within a pen for analysis. Care was taken to avoid contamination of excreta from feathers, scales and debris. The dried excreta were allowed to equilibrate to atmosphere conditions before being weighed.

Amino acid digestibility assay

At the end of excreta collection, 4 replicates of 3 layers per replicate were euthanised by an intracardial injection of pentobarbitone sodium. Right-foot middle toe of each layer were collected for determination of the ash content. The contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum is defined as that portion of the small intestine extending from vitelline diverticulum (formerly Meckel's diverticulum; McLelland, 1979) to a point 40 mm proximal to the ileo-caecal junction. The digesta from the 3 birds in the same cage were pooled and stored at −20 °C in airtight containers before freeze drying. In Experiment 2, when the layers were 47 weeks of age (24 weeks on treatment), toe, excreta and ileal samples were collected using the same protocol as for experiment 1.

Chemical analysis

Sample preparation

The ileal digesta and excreta samples were freeze-dried. Dietary ingredients, diets, excreta and ileal digesta were ground to pass through a 0.5 mm sieve and representative samples were taken and stored at -20 °C in airtight plastic containers until analysed.

Dry matter

Duplicate samples (approximately 3 g) were placed in an oven for 24 h at 105 °C and cooled in a desiccator to room temperature before weighing.

Gross energy

Gross energy of diets, ileal digesta and excreta samples was determined by combustion in an IKA Calorimeter (System C 2000 Basic; IKA®–Wereke GMBH & CO. Germany) which had been standardised using benzoic acid.

Amino acids

Amino acid content of diets and ileal digesta was analysed with a Shimadzu LC-10A amino acid analyser (Shimadzu Corp, Kyoto, Japan), using standard ion-exchange column chromatographic separation techniques and fluorimetric detection of amino acids after reaction with *O*-phthaldialdehyde. A finely ground sample containing approximately 80 mg protein was hydrolysed under nitrogen with 8 M hydrochloric acid containing phenol (3g/ L) at 121^{o}C and 16 psi for 16 hr. The hydrolysate was made up to 100 ml with purified water. An aliquot of hydrolysate containing about 8 mg protein and 1 ml of 4 mM DL-norleucine (as the internal standard) were evaporated to dryness with rotary evaporator at 65^{o}C of water bath to remove hydrochloric acid. The dried mixture was dissolved in 8 ml 0.2N sodium citrate diluents and adjusted pH value to 2.20. After removing fat with chloroform the sample was collected in a syringe and filtered through a 0.22 μm pore nylon filter membrane (Alltech, Baulkam Hills, NSW) into injection vials.

Amino acids were eluted with a gradient system of two sodium citrate buffers at different time intervals and separated using a single, electrically heated (60^{o}C), stainless steel column (Shim-Pack® Amino-Na, I.D. 6.0 mm X 10 cm) packed with a cation exchange resin (sodium form). A standard amino acid mixture (Standard H, Pierce Chemical Co, USA) was used to determine elution times of individual amino acids. A sequenced external standard with known concentrations of amino acids was used to determine the recovery factors for correction of amino acid losses during hydrolysis (Li *et al*., 2006).

Acid insoluble ash

The AIA contents were determined in diet, ileal digesta and excreta samples. Briefly samples (1.5g for diets; 1.0 - 1.2 g for ileal digesta) were weighed into sintered-glass crucibles (Pyrex) and dried for 24 h at 105 °C in a drying oven and weighed for dry matter. The dried samples were ashed at 500°C for 8 h and boiled with 4 N HCI in a crystallising dish. Hydrochloric acid was removed under suction and the residue was rinsed with purified water. The procedure was repeated until the sample appeared white (Mollah *et al.,* 1983). The crucibles were then oven-dried (105 °C) and re-weighed.

Ca and P

Ca and P contents were determined using an Inductive-Coupled Plasma Emission Spectrophotometer after digesting 0.25-0.5 g samples with 4 ml nitric acid in a water bath at 100°C for 2 hrs.

Toe ash

Toe samples were dried at 105^{o}C for 48hr and then ashed at 480^{o}C for 8hr according to the procedure of Potter (1988) for toe ash determination.

Calculations

Apparent digestibility coefficients of ileal energy and amino acids were calculated using AIA as the marker. Celite was added to diets to increase the AIA fraction and to improve the precision of the measurement. The following is the example of apparent ileal nutrient digestibility calculated using AIA. The calculations were based on the assumption that the marker is 100% recoverable.

$$\text{Nutrient digestibility coefficient} = \frac{(N / AIA)_d - (N / AIA)_i}{(N / AIA)_d}$$

where, $(N / AIA)_d$ = ratio of nutrient to acid-insoluble ash in diet

and $(N / AIA)_i$ = ratio of nutrient to acid-insoluble ash in ileal digesta.

Digestibility values for methionine are not reliable since it may be destroyed to some extent when acid hydrolysis is carried out in the presence of carbohydrates (Blackburn, 1978).

Statistical analyses

All the data were analysed according to the method of Steel *et al*. (1997) using the Minitab program version 11.0 (Minitab, 1996). The comparisons were made between all treatment diets; diets with or without meat and bone meal; diets containing different available P levels with or without phytase (excluding the diet with meat and bone meal); diets containing the same level of available P with or without phytase. Tables summarising statistical analyses are given in this report.

Chapter 3

Results

Experiment 1

Egg production, feed intake, feed efficiency, egg weight and egg quality parameters of the layers from 77 to 89 weeks of age are shown in Tables 4A and 4B. As expected with older hens, there were large variations in egg production. Therefore, the small increase in egg parameters with supplemental phytase did not show statistical significant differences except for shell thickness which was improved ($P<0.05$). There were no differences in egg mass, feed efficiency and egg quality parameters between the diet with meat and bone meal and those without meat and bone meal supplemented with phytase. Layers fed on diets containing meat and bone meal had slightly higher egg production, but significantly higher ($P<0.05$) feed intake than those fed on a diet without meat and bone meal. Interestingly, dietary available P level did not affect the production parameters except for yolk colour which was increased ($P<0.05$) in layers fed diets containing a low level of P.

The digestibility coefficients of ileal energy and amino acids, along with toe ash and excreta ash, Ca, P and AME results are shown in Tables 5A and 5B. Apparent ileal digestibilities of aspartic acid and histidine were significantly lower in layers fed diets with meat and bone meal than in the diet without meat and bone meal ($P<0.05$). The same trend was observed for other amino acids although the differences were not statistically significant. In contrast, ileal digestible energy was higher ($P<0.05$) in the diet with meat and bone meal than those without meat and bone meal.

There was a highly significant ($P<0.05$) effect of dietary available P level on apparent ileal amino acid digestibility. As dietary available P level decreased, apparent ileal amino acid digestibility decreased (Table 5B). The effect of P on amino acid digestibility was more pronounced ($P<0.01$, except for methionine $P=0.02$) when comparisons were made between diets with different levels of P (Diets 2, 4 and 6) without supplemented phytase. However, when the statistical analysis and comparisons were made between diets with different levels of available P and supplemented phytase (Diets 3, 5 and 7), there were no significant differences in digestibility of all amino acids tested in this study ($P>0.05$). Comparing layers fed diets containing the same P level with or without supplementing phytase, the digestibility of apparent ileal amino acids was similar between diets containing 4.0 g/kg of available P with or without supplemented phytase. Phytase improved amino acid digestibility by 1-4 and 2-5 % units in layers fed diets containing 2.9 and 1.8 g/kg available P, respectively. The amino acid digestibility of diets containing 2.9 g/kg P with supplementing phytase was comparable ($P>0.05$) with the diet containing 4.0 g/kg available P. However layers fed diets containing 1.8 g/kg available P showed significant lower amino acid digestibility than those fed diet containing 4.0 g/kg available P ($P<0.05$).

Toe ash content and AME were not affected by phytase or dietary meat and bone meal inclusion. As expected excreta P decreased as the dietary available P level declined. Excreta Ca contents varied inconsistently among treatments and may reflect the form in which Ca was fed.

Experiment 2

Egg production, feed intake, feed efficiency, egg weight and egg quality parameters of the layers from 23 to 47 weeks of age are shown in Tables 6A and 6B. There were no significant differences in egg production, egg mass and feed intake among the treatments. There were significant differences ($P<0.05$) in feed conversion, yolk colour and shell thickness between diets, but no clear pattern or no significant differences were found when the main effects were examined.

The digestibility coefficients of ileal energy and amino acids along with toe ash and excreta ash, Ca, P and AME results are shown in Tables 7A and 7B. Diets with or without meat and bone meal and different available P levels did not affect amino acid digestibility of these young layers as was observed with old layers in Experiment 1. However, phytase significantly ($P<0.01$) increased ileal amino acid digestibility in this experiment (3-11 %units except for histidine $P=0.024$) in pullets fed diet containing the lowest level of available P (1.8 g/kg of diet) compared to those fed diets containing the same level of P without supplementing phytase. Moreover, the amino acid digestibility of diets containing 1.8 g/kg P with phytase was comparable with diets containing 4.0 g/kg available P. Excreta P content decreased as the dietary available P levels decreased ($P<0.05$). Layers fed diet with meat and bone meal excreted more P than those on diets without meat and bone meal. Excreta Ca content varied inconsistently among treatments as in Experiment 1.

Table 4A. Performance of ISA Brown laying hens fed experimental diets from 77-89 weeks of age with or without Phytase (Experiment 1)

Measurements	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	P value
Production performance								
Egg production (HD) (%)	70.18	66.90	66.18	67.50	69.83	60.97	69.21	>0.05
Egg weight (g/egg)	66.95	63.56	64.68	65.55	63.82	66.53	67.09	>0.05
Egg mass (g/hen/day)	47.40	42.31	43.55	44.71	44.57	40.66	45.14	>0.05
Feed intake (g/hen/day)	102.91	93.22	97.05	96.50	95.10	95.50	97.76	>0.05
FCR (g feed/g egg)	2.33	2.26	2.56	2.21	2.20	2.56	2.22	>0.05
Egg quality parameters								
Yolk colour (Roche scale)	10.85	10.16	10.2	11.12	10.66	11.12	11.11	>0.05
Haugh unit	68.7	68.16	72.80	59.87	69.14	67.12	65.00	>0.05
Shell thickness (mm)	0.30	0.30	0.32	0.30	0.32	0.29	0.316	>0.05

Table 4B. Statistical summary of the main effects of the data in Table 4A (Experiment 1)

Treatments	Egg Prod. (%)	Egg Wt. (g/egg)	Egg Mass (g/h/d)	Feed Intake (g/h/d)	FCR (g feed/g egg)	Yolk Colour	Haugh Unit	Shell thickness (mm)
MBM								
+MBM	70.18	66.95	47.4	102.9[a]	2.33	10.85	68.71	0.305
--MBM	66.77	65.20	43.50	95.86[b]	2.34	10.80	66.68	0.311
P value	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
Avail. P (g/kg)								
4.0	67.75	65.06	44.42	97.73	2.38	10.44[b]	69.66	0.312
2.9	68.67	64.68	44.64	95.80	2.21	10.88[ab]	65.06	0.314
1.8	65.09	66.81	42.90	96.63	2.39	11.11[a]	66.00	0.304
P value	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05
Phytase								
+Phytase	68.41	65.19	44.42	96.64	2.33	68.52	10.73	0.32[a]
--Phytase	66.39	65.65	43.77	97.03	2.34	65.72	10.86	0.30[b]
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05

[ab], means within the same column with different superscripts differ (P<0.05)

Table 5A. Toe ash (%DM), apparent digestibility coefficients of ileal amino acids and DE, excreta DE, excreta ash (%), Ca (mg/g) and P (mg/g) with or without Phytase at 89 weeks of age (Experiment 1)

	Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	SEM	P value
Toe ash	(%DM)	13.2	13.3	13.2	13.5	13.7	14.1	13.7	0.414	0.678
Apparent ileal digestibility coefficients	Asp	0.72^{bcd}	0.77 ^{a}	0.75 ^{abc}	0.71 ^{cd}	0.75^{ab}	0.69^{d}	0.71 ^{cd}	0.016	0.004
	Thr	0.68^{ab}	0.69^{a}	0.67^{ab}	0.64^{bc}	0.68^{ab}	0.59^{c}	0.63^{bc}	0.016	0.005
	Ser	0.73^{a}	0.74^{a}	0.73^{a}	0.70^{a}	0.73^{a}	0.67^{b}	0.71^{a}	0.016	0.047
	Glu	0.82^{bc}	0.85^{a}	0.83^{abc}	0.80^{c}	0.83^{ab}	0.78^{d}	0.81^{bc}	0.007	<0.001
	Gly	0.72^{ab}	0.74^{a}	0.71^{abc}	0.69^{bc}	0.71^{abc}	0.64^{d}	0.68^{cd}	0.016	0.002
	Ala	0.79^{ab}	0.80^{a}	0.77^{abc}	0.75^{c}	0.79^{ab}	0.71^{d}	0.76^{bc}	0.009	<0.001
	Val	0.75^{a}	0.76^{a}	0.74^{ab}	0.70^{bc}	0.74^{ab}	0.67^{c}	0.71^{b}	0.016	0.001
	Met	0.90^{ab}	0.91^{a}	0.83^{d}	0.86^{cbd}	0.87^{abcd}	0.83^{d}	0.85^{cbd}	0.016	0.016
	Ile	0.76^{a}	0.78^{a}	0.74^{ab}	0.71^{b}	0.74^{ab}	0.67^{c}	0.71^{b}	0.016	<0.001
	Leu	0.79^{ab}	0.80^{a}	0.78^{abc}	0.75^{c}	0.78^{ab}	0.71^{d}	0.76^{bc}	0.009	<0.001
	Tyr	0.77^{d}	0.79^{a}	0.76^{ab}	0.73^{b}	0.76^{ab}	0.67^{c}	0.72^{b}	0.016	<0.001
	Phe	0.79^{ab}	0.81^{a}	0.79^{ab}	0.75^{cd}	0.78^{ab}	0.72^{d}	0.76^{bc}	0.010	<0.001
	His	0.74^{bc}	0.78^{a}	0.75^{bc}	0.76^{abc}	0.77^{ab}	0.70^{d}	0.73^{c}	0.009	<0.001
	Lys	0.76^{ab}	0.81^{a}	0.78^{ab}	0.72^{bcd}	0.74^{bc}	0.65^{d}	0.70^{cd}	0.022	0.002
	Arg	0.80^{b}	0.84^{a}	0.83^{ab}	0.80^{b}	0.79^{b}	0.74^{c}	0.79^{b}	0.016	0.002
	Mean AA	0.77^{ab}	0.79^{a}	0.76^{ab}	0.74^{b}	0.77^{ab}	0.70^{c}	0.74^{b}	0.012	<0.001
	DE	0.75	0.73	0.72	0.72	0.75	0.72	0.74	0.016	0.707
Excreta	AME (MJ/kg DM)	12.18^{a}	11.36^{b}	11.44^{ab}	11.77^{ab}	11.57^{ab}	11.77^{ab}	11.89^{ab}	1.237	0.317
	Ash (%)	25.6^{b}	27.9^{ab}	29.1^{a}	27.7^{ab}	30.6^{a}	27.1^{ab}	24.2^{b}	1.55	0.129
	Ca (mg/g)	81.2^{b}	90.7^{ab}	96.6^{ab}	93.7^{ab}	107.8^{a}	91.9^{ab}	86.0^{ab}	7.51	0.315
	P (mg/g)	19.7^{a}	18.1^{a}	18.2^{a}	14.5^{b}	13.5^{b}	12.5^{b}	12.7^{b}	0.823	<0.001

^{abcd}, means within the same rows with different superscripts differ (P<0.05)

Table 5B. Statistical summary of the main effects of the data in Table 5A (Experiment 1)

	Toe Ash	Apparent Ileal digestibility coefficients																	Excreta			
Treatment	%DM	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Mean AA	DE	AME (MJ/kg DM)	Ash (%)	Ca (mg/g)	P (mg/g)
MBM																						
+ MBM	13.2	0.72[b]	0.38	0.73	0.82	0.72	0.79	0.75	0.90	0.76	0.79	0.77	0.79	0.74[b]	0.76	0.80	0.77	0.75[a]	12.12	25.6	81.2	19.7
- MBM	13.3	0.77[a]	0.69	0.74	0.85	0.74	0.80	0.76	0.91	0.78	0.80	0.79	0.81	0.78[a]	0.81	0.84	0.79	0.73[b]	11.36	27.9	90.7	18.1
SEM	0.33	0.016	0.016	0.016	0.010	0.011	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.009	0.022	0.016	0.016	0.004	0.322	1.60	8.53	0.75
P value	0.794	0.05	0.61	0.51	0.08	0.20	0.51	0.55	0.65	0.44	0.55	0.32	0.31	0.02	0.16	0.07	0.22	0.033	0.145	0.348	0.458	0.18
Avail. P (g/kg)																						
4.0	13.2	0.76[a]	0.68[a]	0.74[a]	0.84[a]	0.73[a]	0.79[a]	0.75	0.88	0.76[a]	0.79[a]	0.78[a]	0.80[a]	0.77[a]	0.80[a]	0.84	0.78[a]	0.73	11.40	28.5	93.6	18.1[a]
2.9	13.6	0.74[a]	0.66[a]	0.72[a][b]	0.82[a]	0.70[a]	0.77[a][b]	0.72	0.87	0.73[b]	0.77[a][b]	0.74[b]	0.77[b]	0.77[a]	0.73[b]	0.80	0.75[a]	0.73	11.67	29.1	100.8	14.0[b]
1.8	13.9	0.70[b]	0.61[b]	0.69b	0.80[b]	0.66[b]	0.74[b]	0.69	0.84	0.69[c]	0.74[b]	0.70[c]	0.74[c]	0.72[b]	0.68[c]	0.77	0.72[b]	0.73	11.83	25.6	88.9	12.6[b]
SEM	0.29	0.013	0.013	0.013	0.008	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.009	0.013	0.013	0.013	0.011	0.147	1.13	5.06	0.544
P value	0.273	0.002	0.0025	0.017	0.005	0.001	0.013	0.002	0.221	<0.001	0.005	<0.001	0.001	0.001	<0.001	<0.001	0.001	0.898	0.135	0.089	0.272	<0.001
Phytase																						
+Phytase	13.5	0.74	0.66	0.72	0.82	0.70	0.77	0.73	0.85	0.73	0.77	0.75	0.78	0.75	0.74	0.81	0.76	0.74	11.63	28.0	96.8	14.8
- Phytase	13.6	0.73	0.65	0.71	0.81	0.70	0.76	0.72	0.87	0.73	0.76	0.74	0.76	0.75	0.74	0.80	0.75	0.73	11.63	27.6	92.1	15.0
SEM	0.25	0.011	0.015	0.011	0.011	0.015	0.011	0.011	0.015	0.015	0.011	0.015	0.011	0.011	0.021	0.015	0.011	0.010	0.129	1.01	4.24	0.825
P value	0.761	0.581	0.495	0.346	0.386	0.719	0.336	0.471	0.427	0.698	0.307	0.661	0.381	0.969	0.968	0.720	0.636	0.376	0.990	0.77	0.440	0.842

[abcde], means within the same column with different superscripts differ (P<0.05)

Table 6A. Performance of ISA Brown laying hens fed experimental diets from 23-47 weeks of age with or without Phytase (Experiment 2)

Measurements	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	P value
Production performance								
Egg production (HD) (%)	94.1	95.3	98.1	95.9	95.8	93.9	94.6	>0.05
Egg weight (g/egg)	60.7[b]	62.6[a]	61.5[ab]	62.2[ab]	61.5[ab]	61.7[ab]	61.1[ab]	<0.05
Egg mass (g/hen/day)	57.2	59.6	60.4	59.7	58.9	57.9	57.9	>0.05
Feed intake (g/hen/day)	109.2	110.5	111.2	107.5	109.4	108.8	105.7	>0.05
FCR (g feed/g egg)	1.87[a]	1.82[ab]	1.82[ab]	1.78[b]	1.85[ab]	1.84[ab]	1.82[ab]	<0.05
Egg quality parameters								
Yolk colour (Roche scale)	11.8[a]	11.1[bc]	11.0[bc]	10.8[bc]	11.4[ab]	10.5[c]	11[bc]	<0.05
Haugh unit	72.0	72.0	71.6	71.4	75.2	77.3	72.4	>0.05
Shell thickness (mm)	0.316[a]	0.298[b]	0.318[a]	0.314[ab]	0.311[ab]	0.323[a]	0.324[a]	<0.05

[abc], means within the same row with different superscripts differ (P<0.05)

Table 6B. Statistical summary of the main effects of the data in Table 6A (Experiment 2)

Treatments	Egg Prod. (%)	Egg Wt. (g/egg)	Egg Mass (g/h/d)	Feed Intake (g/h/d)	FCR (g feed/g egg)	Yolk Colour	Haugh Unit	Shell thickness (mm)
MBM								
+MBM	94.1	60.7	57.2	109.2	1.87	11.8[a]	72.0	0.316
--MBM	95.6	61.8	59.10	108.8	1.82	11.1[b]	73.3	0.315
P value	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05
Avail. P (g/kg)								
4.0	95.8	61.6	59.1	110.3	1.84	11.30	71.8	0.31
2.9	95.8	61.8	59.2	108.0	1.81	11.00	71.9	0.31
1.8	94.5	61.5	58.1	107.7	1.83	10.90	75.6	0.32
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Phytase								
+ Phytase	96.2	61.4	59.1	108.8	1.83	11.14	73.1	0.32
-- Phytase	94.8	61.8	58.6	109.0	1.83	11.11	73.1	0.31
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Table 7A. Toe ash (%DM), apparent digestibility coefficients of ileal amino acids and DE, excreta DE, excreta ash (%), a (mg/g) and P (mg/g) with or without Phytase at 47 weeks of age (Experiment 2)

	Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	SEM	P value
Toe ash	%DM	14.4	14.1	14.0	14.7	13.9	14.7	14.1	0.37	0.580
Apparent ileal digestibility coefficients	Asp	0.70^{b}	0.72^{ab}	0.76^{ab}	0.73^{ab}	0.72^{ab}	0.69^{b}	0.78^{a}	0.022	0.206
	Thr	0.63^{ab}	0.63^{ab}	0.64^{ab}	0.63^{ab}	0.61^{ab}	0.59^{b}	0.70^{a}	0.032	0.427
	Ser	0.68^{ab}	0.70^{ab}	0.71^{ab}	0.69^{ab}	0.68^{ab}	0.65^{b}	0.76^{a}	0.027	0.294
	Glu	0.79^{bc}	0.81^{abc}	0.84^{ab}	0.81^{abc}	0.82^{abc}	0.78^{c}	0.86^{a}	0.016	0.063
	Gly	0.67	0.69	0.71	0.68	0.67	0.66	0.75	0.027	0.411
	Ala	0.75^{abc}	0.75^{abc}	0.78^{ab}	0.74^{bc}	0.76^{abc}	0.70^{c}	0.81^{a}	0.022	0.122
	Val	0.70^{ab}	0.71^{ab}	0.72^{ab}	0.70^{ab}	0.71^{ab}	0.67^{b}	0.76^{a}	0.022	0.376
	Met	0.84	0.84	0.85	0.84	0.85	0.84	0.88	0.022	0.850
	Ile	0.72^{ab}	0.72^{ab}	0.75^{ab}	0.73^{ab}	0.72^{ab}	0.69^{b}	0.79^{a}	0.027	0.372
	Leu	0.75^{ab}	0.75^{ab}	0.78^{ab}	0.75^{ab}	0.76^{ab}	0.71^{b}	0.81^{a}	0.022	0.194
	Tyr	0.72^{ab}	0.73^{ab}	0.75^{ab}	0.72^{ab}	0.72^{ab}	0.69^{b}	0.78^{a}	0.027	0.353
	Phe	0.76^{ab}	0.76^{ab}	0.79^{ab}	0.76^{ab}	0.75^{ab}	0.73^{b}	0.81^{a}	0.022	0.389
	His	0.69^{b}	0.75^{a}	0.76^{a}	0.74^{ab}	0.74^{ab}	0.72^{ab}	0.77^{a}	0.016	0.064
	Lys	0.71^{b}	0.73^{ab}	0.77^{ab}	0.75^{ab}	0.72^{ab}	0.75^{ab}	0.82^{a}	0.035	0.431
	Arg	0.78	0.80	0.82	0.80	0.79	0.80	0.85	0.022	0.684
	Mean AA	0.73^{ab}	0.74^{ab}	0.76^{ab}	0.74^{ab}	0.74^{ab}	0.71^{b}	0.79^{a}	0.022	0.359
	DE	0.72^{bc}	0.73^{ab}	0.72^{abc}	0.72^{abc}	0.71^{bc}	0.68^{c}	0.77^{a}	0.016	0.054
Excreta	AME (MJ/kg DM)	13.23	12.98	13.16	13.06	12.94	13.09	12.93	0.106	0.355
	Ash (%)	22.6^{ab}	23.0^{ab}	24.3^{ab}	23.8^{ab}	23.7^{ab}	25.4^{a}	20.7^{b}	1.31	0.297
	Ca (mg/g)	65.2	70.3	68.8	70.6	71.8	77.7	59.6	7.10	0.701
	P (mg/g)	22.0^{a}	18.1^{bc}	19.4^{b}	16.2^{cd}	14.3^{d}	11.7^{e}	12.0^{e}	0.75	<0.001

^{abcde}, means within the same row with different superscripts differ (P<0.05)

Table 7B. Statistical summary of the main effects of the data in Table 7A (Experiment 2)

	Toe Ash	Apparent ileal diegetibility coefficeients																Excreta			
Main Effects	(%DM)	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Mean AA	DE	AME (MJ/kg DM)	Ash (%)	Ca (mg/g)	P (mg/g)
Meat &bone meal																						
+MBM	14.41	0.70	0.63	0.68	0.79	0.67	0.75	0.70	0.84	0.72	0.75	0.72	0.76	0.69	0.71	0.78	0.73	0.72	13.23	22.6	65.2	22.0[a]
-MBM	14.11	0.72	0.63	0.70	0.81	0.69	0.75	0.71	0.84	0.72	0.75	0.73	0.76	0.75	0.72	0.80	0.74	0.73	12.98	23.0	70.3	18.1[b]
SEM	0.506	0.027	0.035	0.027	0.016	0.032	0.022	0.022	0.016	0.027	0.022	0.027	0.022	0.022	0.039	0.022	0.022	0.022	0.106	1.20	7.30	0.91
P value	0.699	0.532	0.961	0.784	0.450	0.776	0.978	0.798	0.955	0.995	0.966	0.854	0.972	0.075	0.719	0.598	0.725	0.677	0.144	0.810	0.636	0.024
Avail. P (g/kg)																						
4.0	14.07	0.74	0.63	0.71	0.82	0.70	0.76	0.71	0.85	0.74	0.77	0.74	0.77	0.76	0.75	0.81	0.75	0.73	13.07	22.9	69.9	18.4[a]
2.9	14.27	0.72	0.62	0.69	0.81	0.68	0.75	0.71	0.84	0.73	0.75	0.72	0.76	0.74	0.74	0.80	0.74	0.72	13.00	23.0	68.6	15.6[b]
1.8	14.38	0.74	0.65	0.71	0.82	0.71	0.76	0.72	0.86	0.75	0.77	0.74	0.77	0.75	0.79	0.83	0.76	0.73	13.01	23.9	71.4	11.9[c]
SEM	0.283	0.019	0.025	0.022	0.016	0.022	0.019	0.019	0.016	0.019	0.019	0.022	0.019	0.011	0.027	0.019	0.019	0.011	0.074	0.86	4.79	0.50
P value	0.743	0.701	0.684	0.677	0.832	0.592	0.861	0.821	0.670	0.774	0.828	0.697	0.761	0.612	0.442	0.544	0.714	0.822	0.772	0.682	0.915	<0.001
Phytase																						
+ phytase	13.98	0.75	0.65	0.72	0.84[a]	0.71	0.78[a]	0.73	0.86	0.75	0.78[a]	0.75	0.78	0.76	0.77	0.82	0.76	0.73	13.01	22.5	67.0	15.27
- phytase	14.50	0.72	0.62	0.68	0.80[b]	0.68	0.73[b]	0.70	0.84	0.72	0.74[b]	0.71	0.75	0.74	0.74	0.80	0.73	0.71	13.05	24.1	72.9	15.32
SEM	0.215	0.017	0.021	0.017	0.01	0.019	0.013	0.017	0.013	0.017	0.013	0.017	0.013	0.01	0.023	0.017	0.017	0.009	0.060	0.66	3.74	0.902
P value	0.107	0.110	0.313	0.131	0.021	0.223	0.023	0.129	0.258	0.108	0.034	0.094	0.105	0.237	0.338	0.485	0.132	0.155	0.660	0.100	0.279	0.969

Chapter 4

Discussion

Experiments described in this report were designed to reflect industry diets, both in terms of composition and the level of nutrients that are currently used by industry nutritionists when feeding Isa Brown birds. Two experiments were conducted to examine the influence of removing meat and bone meal from the diet, changing the level of available P in the diet and adding phytase to the diet on the performance of egg production, quality and nutrient digestibility. The diets so designed were fed in 2 experiments to birds either at the end of the production cycle (77-89 weeks of age) in Experiment 1 or at peak lay (23-47 weeks of age) in Experiment 2.

Meat and bone meal did not significantly affect egg production parameters in both old and young layers and this would suggest that it can be removed from layer diets in the future without a production penalty. Phytase slightly increased egg production in both young and old layers and improved egg shell thickness significantly in old layers (77-89 weeks), which is consistent with the report by Kornegay (2001). However, the variable nature of the responses with older birds possibly masked other effects.

Interestingly, in this study, at the lower levels of available P there was a notable reduction in the ileal digestibility of amino acids. Although there was a non-significant variation in feed conversion efficiency, the reasons for the reduction in ileal digestibility of amino acids is not clear. The observations may relate to changes in P and phytate dynamics in the gut and a corresponding reduction in amino acid digestibility through the formation of complexes with phytate. This is in the area in which there is much interest and discussion (Selle *et al*., 1990). Supplementation of diets with phytase increased amino acid digestibility by 1 to 5 % units in old layers fed 2.9 and 1.8 g/kg available P and 3 to 11% units in young layers fed 1.8 g/kg available P. These increases are similar to what have been observed with broilers (Selle et al., 2000; Selle et al., 2006), but unfortunately there is no comparable data for laying hens.

The study in Experiment 2 was conducted to include the maximum metabolic period of the laying hen ie. around peak lay and it must therefore be concluded that the dietary P concentrations met the birds requirement for P even at the lowest level especially with the high level of production recorded. Other studies reported by Mikaelian and Sell (1981), Miles *et al*., (1983), Hartel (1989), Simmons *et al.,* (1992) and Usayran and Balnave (1995) have reached similar conclusions. Our results (174 mg available P/day) indicate that the available P requirement of laying hens for egg production performance is lower than the NRC (1994) recommendation of 250 mg/day. This is in accord with the results of a number of recent and long term experiments with laying hens which have indicated that a diet with 0.10-0.13% available P (i.e., a typical corn-soybean diet without any 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 available P level of 0.40-0.45% that normally is used by industry (Gordon and Roland, 1998; Van der Klis et al., 1997; Parsons, 1999, Carlos and Edwards, 1998; Um and Paik, 1999).

In reviewing the literature Kornegay (2001) suggested that variability in the efficacy of phytase in different diets may relate to changes in the Ca:P ratio. He suggested that as the ratio becomes wider, a number of factors could influence the liberation of P from phytase. These factors include:

1. Phytate utilisation is influenced by calcium and P levels in the diet;
2. Additional calcium in the diet can bind with phytate to form insoluble complexes that are less accessible to phytase;

3. Extra calcium may directly suppress phytase by competing for the active sites of the enzymes.
4. The effect of the Ca:P ratio is greater at lower levels of available P or because less P would be released as a result of reduced phytase activity.

Diets without meat and bone meal showed higher amino acid digestibility coefficients and this effect was more pronounced in older layers. The results of this study would indicate that meat and bone meal can be removed from layer diets without penalty but further definition of the P requirements of the modern laying hen is required, especially in relation to dietary calcium and vitamin D levels and supplemental dietary phytase. Phytase improved apparent ileal amino acid digestibility but the extent depended on the age of the layers and other dietary factors.

Implications and suggestions

The available P content of layer diets used by the industry in the presence of phytase is considerably greater than recently reported values and alos the NRC (1994) recommendation. This suggests that current dietary usage of P is in excess of the layers' requirement. The more information that can be generated regarding the available P requirement of laying hens with and without phytase will assist in determining an appropriate P content of layer diets. In so doing, the cost of diets should be reduced along with a reduction of P excretion from poultry units into the environment. Moreover, there is a concern by industry that the widening of the Ca:P ratio due to increased use of phytase without Ca correction might produce a deleterious effect on performance and shell quality. This aspect of dietary phytase supplementation requires further delineation as most published reports do not include a dietary Ca correction. In further studies the influence of changes in dietary Ca:P ratio on amino acid digestibility should also be investigated.

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