MINISTRY OF AGRICULTURE, FISHERIES AND FOOD **CSG 15**

Research and Development

Final Project Report

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Project title	Optimising the efficiency of energy, calcium and phosphorus utilisation in laying hens fed a diet containing only vegetable sources of protein
MAFF project code	**LS0905**
Contractor organisation and location	ADAS Gleadthorpe, Meden Vale, Mansfield, Nottinghamshire, NG20 9PF & University of Leeds, Leeds, LS2 9JT
Total MAFF project costs	£ 95,466
Project start date	01/04/97
Project end date	31/03/00

Executive summary (maximum 2 sides A4)

Objectives There were three objectives, the first was to identify whether modern brown egg layers over-consumed feed during the late afternoon in an attempt to meet their requirements for calcium during egg shell formation, and whether this could be avoided by feeding limestone granules. The second objective was to determine the effects of dietary calcium and phosphorus intakes on calcium, phosphorus and nitrogen balances in laying hens, and to see how they were affected by feeding limestone granules and dietary microbial phytase supplementation. The third objective was to determine the effects of feeding four levels of dicalcium phosphate (DCP, an inorganic source of phosphorus), with or without dietary phytase supplementation (an enzyme which acts on phytate complexes and increases the availability of organic phosphorus), and with or without feeding limestone granules on egg production, egg shell density and tibial breaking strength at the end of lay.

Methodology There was one experiment to address each objective. Experiments 1 and 2 were carried out at Leeds University, and they were for a duration of eight weeks, this included a four week acclimatisation period and a four week monitoring period. Experiment 3 was carried out at ADAS Gleadthorpe and it was in large scale facilities and for a full laying year. In Experiment 1, two feeds, one containing optimal (according to published requirements) and one containing less than optimal calcium and available phosphorus concentrations were fed with, or without limestone granules to individually caged laying hens. This approach aimed to exacerbate differences in the putative over consumption of feed energy during the late afternoon. Feed and limestone intakes were recorded at intervals of 60 seconds using balances and an automated recording system. Time of egg laying was determined by recording hen behaviour using a video camera. In Experiment 2, there were two concentrations of calcium and available phosphorus (as for Experiment 1) and these were fed with, or without phytase supplementation, and with, or without access to limestone granules to individually caged laying hens. Balances of intakes and outputs of calcium, phosphorus and nitrogen were determined. In Experiment 3, there were four concentrations of DCP and these were fed with, or without

phytase supplementation. The range of DCP concentrations used differed depending on whether the diets were positively supplemented with phytase, or not. For example, the minimum and maximum DCP concentrations were lower when phytase was used as this took into account the putative effects of phytase on phosphorus availability, than when phytase was omitted. The eight diets were fed with, or without access to limestone granules, and when granules were provided they were either small (range 0.50 to 2.75 mm diameter) or large (range 2.00 to 4.00 mm diameter).

Results In Experiment 1, hens consumed feed at a constant rate throughout the light period and there was no suggestion of a peak in feed intake during the late afternoon. This invalidated the hypothesis that modern brown egg layers over-consume feed at this time of day in order to meet their calcium requirements for egg shell formation. Limestone intake occurred only during the late afternoon, and by observing the time of egg laying this was calculated to be proximate to the start of shell calcification. In Experiment 2, calcium and phosphorus balances were negative when hens were fed a ration having low calcium and available phosphorus contents. Thus, the dietary intake of calcium was not sufficient to support egg shell formation, and so egg shell formation occurred at the expense of bone calcium and phosphorus reserves. This was not the case when hens were fed the ration having optimal concentrations of calcium and available phosphorus, when they had small positive calcium and phosphorus balances. Phytase supplementation allowed the diet to support egg shell production without bone resorption, whereas without phytase supplementation the hens calcium balances were negative. Phosphorus balances were positive irrespective of phytase supplementation. Limestone granule supplementation produced large positive calcium and phosphorus balances, whereas when they were not on offer the balances were negative. Nitrogen balances were positive irrespective of dietary calcium and phosphorus contents, phytase supplementation, or access to limestone granules, but faecal nitrogen excretion was reduced by feeding optimal dietary calcium and available phosphorus contents. The findings reiterate the importance of feeding optimal calcium and available phosphorus concentrations to high yielding modern brown egg layers, and the benefits of phytase supplementation and supplementary calcium intake for maintaining bone calcium reserves. In Experiment 3, feed intakes were reduced throughout the laying year when hens were fed phytase supplemented diets and limestone granules, but this was associated with reduced rates of lay between 20 and 48 weeks of age. Dietary crude protein and essential amino acid contents may have been less than optimal for maintaining high rates of lay at a low feed intake. Hens consumed limestone granules and intakes increased to a maximum of approximately 15 g/bird.day by 51 weeks of age, but at 52 weeks of age intakes suddenly fell to only 4 g/bird.day. Limestone granule intakes then remained low for the remainder of the laying year (5 to 7 g/bird.day). The sudden reduction in limestone granule intake at 51 weeks of age occurred without a sudden reduction in feed intake, and without a sudden reduction in egg mass output. The findings suggest that high limestone granule intakes over a prolonged period may have lead to calcium toxicity, but hens were able to reduce their intake of supplementary calcium so as to avoid toxicity. There were no effects of dietary DCP concentrations, phytase supplementation, or access to limestone granules on egg weights, or egg mass outputs throughout the laying year. Egg shell densities and tibial breaking strengths at the end of lay were increased by phytase supplementation and by feeding limestone granules. Phytase supplementation was thought to have improved the availability of nutrients and minerals, and this is supported by the results of balance studies carried out in Experiment 2. Limestone granule supplementation produced calcium intakes that were greater than published optimal intakes for calcium, and the findings provide further evidence that higher intakes are required for egg shell quality than for production, and that higher intakes are required for bone strength than for egg shell quality.

Implications, future work and policy relevance The findings suggest that modern brown egg layers are able to tolerate a relatively wide range of calcium to available phosphorus intakes, in terms of egg production and egg shell density. Dietary calcium intakes should be set taking into account feed intakes, and producers should aim to provide a calcium intake of 5.0 g/bird.day during early lay, perhaps increasing to 5.5 g/bird.day during mid lay. There are additional benefits to egg shell densities and tibial breaking strengths at the end of lay from feeding a supplementary source of calcium, yet this is not typical commercial practice for caged layers, or for most free range layers. It would not be possible when feeding caged layers during the late afternoon to distribute limestone granules, or other coarse sources of calcium, using existing feeder equipment, as abrasion causes damage to the chain or auger. Furthermore, this technique would rely on hens selecting limestone grit from within the mash. Major changes to the design of laying cages are being dictated by the Laying Hens Directive (EC Council Directive 1999/74), and it would be opportune to consider approaches for incorporating choice feeding equipment when developing new styles of cages. The work has shown that poultry producers are not reliant on incorporating a high concentration of DCP within the ration, and this may help to reduce phosphorus excretion, and if phytase is used to replace some of the dietary inorganic phosphorus supply then further benefits are likely. Work is needed to quantify the range of effects microbial phytase has on nutrient and mineral availabilities as this would allow formulators better to match nutrient and mineral supply to the hens' requirements. There appears to be scope to further reduce the dietary supply of available phosphorus, but this would not be advisable without first establishing the hens' phosphorus requirements for optimising medullary bone volume at the end of lay. Faecal nitrogen contents were reduced by the separate feeding of limestone granules, but further work is required if egg production, shell quality and bone breaking strength are to be optimised in addition to reducing faecal nitrogen.

The project addressed MAFF's policy objectives of matching mineral composition and supply in modern genotypes, and optimising the efficiency of feed energy and protein utilisation. By feeding hens limestone granules they were able to meet their calcium requirements for egg shell formation without resorbing bone, and this improved tibial bone strength at the end of lay. Positive calcium balances in hens fed limestone granules meant that faecal phosphorus concentrations were reduced. Phytase supplementation of diets, combined with the separate feeding of limestone granules optimised feed conversion efficiencies.

Scientific report (maximum 20 sides A4)

OBJECTIVES

1. To monitor the pattern of feed and calcium intake throughout a 24 hour period in individually caged laying hens fed a mash containing two inclusion levels of calcium (optimum and 36% less than optimum according to the published calcium requirements of laying hens), and with or without access to limestone granules during the late afternoon.

2. To determine the total phosphorus, nitrogen and calcium balance of laying hens fed a mash containing two inclusion levels of dicalcium phosphate (optimum and less than optimum in terms of dietary calcium and available phosphorus levels) x two levels of phytase (with and without phytase) each determined with and without access to separate calcium (*ad libitum* or controlled).

3. To determine the effect of feeding four levels of dicalcium phosphate x two levels of phytase (with and without phytase) when provided with or without *ad libitum* access to separate calcium of two particle sizes (particle size 6, ranging in diameter from 2.00 to 4.00 mm, and particle size 7 range, 0.50 to 2.75 mm diameter) on feed usage, egg mass output, feed conversion efficiency, mortality, egg shell strength, bone breaking strength and feeding/husbandry techniques.

Objectives 1 and 2 were the responsibility of Leeds University and Objective 3 was the responsibility of ADAS.

EXTENT TO WHICH THE OBJECTIVES HAVE BEEN MET

The objectives have been fully met.

METHODOLOGY

Three experiments were carried out, the first two at Leeds University and the third at ADAS Gleadthorpe Poultry Research Centre. Experiment 1 was designed to measure the daily patterns of mash and limestone granule intake in laying hens. The objective was to see if laying hens consumed limestone granules, and if so, at what time of day and did limestone granule intake reduce mash intake. Laying hens are purported to have a high calcium appetite during the late afternoon, and mash intake may peak as the hens attempt to meet their calcium requirement for egg shell formation from external sources.

Experiment 2 was designed to examine the interactive effects of dietary calcium and available phosphorus contents with and without microbial phytase supplementation, and with or without access to limestone granules, on calcium, phosphorus and nitrogen balances in laying hens. The total phosphorus content of vegetable-based hen rations is usually greater than that required for maintenance and production, but not all of the phosphorus is available. Phytates present in plant material are complexed with phosphorus, and as hens are unable to degrade phytates, phytate-phosphorus is only partially available for absorption in the gut. Supplementing vegetable-based rations with microbial phytase, which is active in a specific pH environment, may be one method of increasing phosphorus availability. As phytates also complex with various proteins, phytase supplementation of hens rations may also increase protein digestibility. Balance studies were needed to examine the effects of dietary phytase supplementation at optimum calcium and available phosphorus contents, and at less than optimum calcium and available phosphorus contents, on dietary phosphorus and nitrogen utilisation. The effects of allowing or not allowing access to limestone granules on calcium balances were also examined.

Experiment 3 was designed to examine the interactive effects of dietary microbial phytase supplementation, dicalcium phosphate concentration and access to limestone granules on laying hen performance, mortality and tibial breaking strength. The removal of meat and bone meal from rations for laying hens, due to the BSE crisis, meant that there was an increased reliance on the use of inorganic calcium and phosphorus, and this is usually supplied as dicalcium phosphate. However, dicalcium phosphate dilutes the protein content and energy value of the diet, whereas meat and bone meal was a valuable source of highly digestible protein. Diets containing dicalcium phosphate are dusty and dicalcium phosphate may separate from other feed ingredients during feed handling and distribution. If separation does occur, then some birds will receive feed that is deficient in calcium and available phosphorus, whereas others will receive feed having high calcium and available phosphorus contents. This may increase the variability of egg mass output, egg shell quality and bone fragility within a flock. It would be desirable for producers to be less dependent on dicalcium phosphate as a dietary supply of calcium and inorganic phosphorus. One way of achieving this would be to supplement the diet with microbial phytase, so as to break down phytate phosphorus and increase phosphorus availability from plant material, and to provide the hens with a separate source of calcium so that they are able to select calcium according to their requirements.

Experiments 1 and 2 were carried out in small room facilities at Leeds University, and the rooms contained wire cages that were suited for housing laying hens individually. By comparison, the facilities used at ADAS Gleadthorpe enabled the effects of dietary treatment on laying hen performance to be examined under commercial housing conditions and for a large flock size.

EXPERIMENT 1 PATTERNS OF FEED AND LIMESTONE INTAKE IN LAYING HENS

Experiment treatments

There were two mash treatments formulated to have optimal dietary calcium and available phosphorus contents according to published literature (38.0 g/kg calcium and 3.60 g/kg available phosphorus) and less than optimal dietary calcium and available phosphorus contents (24.0 g/kg calcium and 2.0 g/kg available phosphorus). The diets were isoenergetic (metabolisable energy 11.7 MJ/kg) and isonitrogenous (180 g/kg crude protein). Each of the diets was fed with or without access to supplementary limestone granules particle size 6 (range 2.00 to 4.00 mm diameter). Limestone granules when fed, were available either *ad libitum* throughout the day, or in a measured quantity, this being 4.3 g limestone granules/bird, and given in the middle of the afternoon. The granules were fed in a container and this was placed adjacent to the food container. There was a two x three factorial arrangement of treatments.

The dietary treatments were fed to 48 Hisex brown laying hens for a four week acclimatisation period, followed by a monitoring period that lasted for four weeks (6 hens/dietary treatment). The hens were 30 weeks of age at the start of the monitoring period. The monitoring period was designed to coincide with peak rate of lay. All hens were housed individually in 48 ‘home cages’, but in addition there were six ‘balance cages’. The ‘balance cages’ contained either one or two digital balances depending on whether mash, or mash plus limestone granules were on offer to the hen. The balances were connected to a computer via a multiplexer and the weights of the mash or limestone on the balance trays were recorded at intervals of 60 seconds. This method allowed cumulative intakes of mash and limestone grit to be plotted against time of day.

The approach used was to divide the four week monitoring period into four five day monitoring periods. At the start of a five-day monitoring period six hens (one from each dietary treatment) were randomly allocated to the ‘balance cages’ and mash intakes, and where appropriate limestone granule intakes, were measured. The numbers of eggs laid per day were recorded, and when an egg was laid it was weighed. In addition to this, the time of lay was recorded using a time lapse video recorder. The tape was visually inspected and when a hen laid an egg the time-of-day was recorded. At the end of the five day recording period the hens were re-housed in their ‘home cages’. A further six hens were then randomly allocated to the ‘balance cages’ and the monitoring procedure was repeated. At the end of the four week monitoring period 24 of the 48 hens had been studied. Hens were fed daily and mash was available *ad libitum* throughout the study. A 14 h photoperiod was used.

EXPERIMENT 1b (RE-RUN) - PATTERNS OF FEED AND LIMESTONE INTAKE IN LAYING HENS

Experiment treatments

The results of Experiment 1 were disappointing and inconclusive as hens failed to lay at the expected rate. Feed usage was also much lower than expected. So as to avoid a delay in the achievement of project milestones Experiment 2 proceeded according to the planned time schedule, but at the end of Experiment 2 an additional study was carried out. The dietary treatments used were as for Experiment 2 (see below) and they were fed to 24 Lohmann brown hens aged 27 weeks, for a four week period. In this case, there was no acclimatisation period as hens were maintained on the same dietary treatment as used in Experiment 2. There were three replicate birds for each of the eight dietary treatments.

The approach used for measuring mash intake and limestone granule intake was as for Experiment 1, and similarly, measurements of egg production and time-of-lay were carried out as described for the earlier study.

Hens were fed each morning, and feed, and where applicable limestone granules, were provided *ad libitum*. The photoperiod was 15.5 h at 27 weeks of age and this was increased to 16 h at 28 weeks of age. A 16 h photoperiod was then used for the remaining three week monitoring period.

EXPERIMENT 2 TOTAL PHOSPHORUS, NITROGEN AND CALCIUM BALANCES OF LAYING HENS

<u>Experiment treatments</u>

There were eight dietary treatments, and these comprised four mash treatments fed with or without access to separate limestone granules particle size 6. The four mash treatments were optimal calcium and available phosphorus contents and less than optimal calcium and available phosphorus contents (38.0 g/kg calcium and 3.60 g/kg available phosphorus, and 24.0 g/kg calcium and 2.3 g/kg available phosphorus, respectively), both with and without microbial phytase (0.00 g/kg and 0.08 g/kg Natuphos, respectively). Natuphos (BASF Ltd) is a phytase preparation from a genetically modified *Aspergillus niger* strain. The diets were isoenergetic (metabolisable energy 11.9 MJ/kg) and isonitrogenous (180 g/kg crude protein). The experiment design was a two x two x two factorial arrangement of treatments.

The dietary treatments were fed to 48 Lohmann brown hens for a four week acclimatisation period, followed by a four week period during which measurements of feed intake, limestone intake, egg mass output, excreta and body weight gain were made. The hens were 23 weeks of age at the start of the monitoring period. All of the hens were housed individually in cages in two rooms of the animal house, 24 cages per room. Thus, within a room there were three replicate hens for each dietary treatment, and in total six replicate hens per dietary treatment.

Mash intake, limestone granule intake, whether a hen had laid an egg, and if so, the weight of the egg, were all recorded daily during a four day period of each week of monitoring. Body weight gain and excreta were recorded at the end of each four day period.

Hens were fed each morning, and feed and where applicable limestone granules, were provided *ad libitum*. The photoperiod was 9 h at 19 weeks of age, and this was increased by 1 hour at weekly intervals until 24 weeks of age, followed by weekly increases of 30 minutes until the end of the monitoring period at 27 weeks of age.

EXPERIMENT 3 EFFECTS OF DIETARY PHYTASE AND DICALCIUM PHOSPHATE CONTENTS AND ACCESS TO SEPARATE LIMESTONE GRANULES ON LAYING HEN PERFORMANCE

<u>Experiment treatments</u>

There were 24 dietary treatments, and these comprised eight mash treatments that were fed either with or without access to limestone granules. When limestone granules were provided they were either particle size 6 (range 2.00 to 4.00 mm diameter), or particle size 7 (range 0.50 to 2.75 mm diameter). The eight mash treatments comprised two concentrations of microbial phytase (0.00 or 0.08 g/kg Natuphos) and four concentrations of dicalcium phosphate (DCP). The ranges of concentrations of DCP used were unequal at each concentration of microbial phytase. When there was zero addition of microbial phytase to the diet, the range of DCP used was 3.0 to 9.0 g/kg, whereas, when the diet was supplemented with microbial phytase, the range of DCP used was 1.0 to 7.0 g/kg. Diets were formulated to have the same calcium concentration (41.9 g/kg), crude protein concentration (169.0 g/kg) and metabolisable energy value (11.6 MJ/kg). The calculated available phosphorus contents of the diets ranged from 2.3 g/kg to 3.4 g/kg when containing zero microbial phytase, and from 3.0 g/kg to 4.4 g/kg when supplemented with microbial phytase. The mash treatments provided a wide range of calcium to available phosphorus ratios (i.e. from 18.2 to 12.2 in diets not supplemented with microbial phytase, and from 14.0 to 9.5 in diets supplemented with microbial phytase). In addition, hens provided with access to separate limestone granules were able to increase the dietary ratio of calcium to available phosphorus by consuming limestone.

The experiment design was a factorial plus split plot design. The main plot treatments were microbial phytase supplementation x access to separate limestone granules, and nested within the main plots were concentrations of DCP. A main plot consisted of 16 consecutive cages and this was subdivided into four sub-plots, each sub-plot comprising four consecutive cages. All of the cages housed four 18-week pullets and this provided 48 birds per treatment combination. The total flock size was 1 152.

Mash treatments were applied at 18 weeks of age and access to separate limestone granules was provided from the start of the experiment at 20 weeks of age. Mash was provided in the feed trough, but in addition, when access to limestone granules was provided the feed trough housed a ‘U’ shaped tube that was fitted onto the external side of the trough using brackets. The ‘U’ shaped tube contained and separated the limestone granules from the mash.

The photoperiod was eight hours at 18 weeks of age and this was increased by one hour at both 19 and 20 weeks of age, followed by weekly increments of 15 minutes until a maximum photoperiod of 16 hours was reached at 43 weeks of age. The photoperiod was then maintained at 16 hours until the end of the experiment at 72 weeks of age.

The numbers of eggs laid per sub-plot were recorded daily. On one day per four week monitoring period, the start of the first period being at 20 weeks of age, the weights of all eggs laid were measured. Feed usage and, where appropriate, limestone granule usage, were measured for each sub-plot at intervals of four weeks from 20 weeks of age. Body weights of four hens per sub-plot (i.e. one cage of hens, the same hens being weighed on each occasion) were measured at 18, 26, 48 and 72 weeks of age. Egg shell breaking strengths were measured in a sample of 10 eggs taken from each sub-plot at 27, 36, 45, 54, 63 and 72 weeks of age using an EQM supplied by TSS Ltd. Tibial breaking strengths were measured at 72 weeks of age in a sample of 1 hen per plot using a Stable Microsystems TA.XT2 Texture Analyser fitted with a three point jig.

RESULTS

EXPERIMENT 1 - DAILY PATTERNS OF FEED AND LIMESTONE INTAKE IN LAYING HENS

Feed intakes and rates of lay between 30 and 33 weeks of age were very low. Hens fed mash containing optimum calcium and available phosphorus had a mean feed usage of 80 g/bird.day and a mean rate of lay of 62%, whereas hens fed mash containing low calcium and available phosphorus had a mean feed usage of only 66 g/bird.day and a mean rate of lay of only 57%. At these ages, feed intakes are usually between 120 and 125 g/bird.day, and rates of lay are usually at least 90%. The reasons for such low feed intakes and rates of lay in this study are not known, as *post mortem* examinations did not identify any disease challenges.

Daily patterns of feed usage and limestone granule usage have not been included as feed intakes were atypical and limestone granule intakes were very low. Due to the infrequency of oviposition and the very low daily intake of limestone granules, it was not possible to establish a relationship between the time of oviposition and the time of limestone granule intake. This was re-examined in an additional experiment at Leeds University (Experiment 1b) using hens with very high rates of egg production.

EXPERIMENT 1b - DAILY PATTERNS OF FEED AND LIMESTONE INTAKE IN LAYING HENS

The hens used were those used in Experiment 2. Their feed intakes during the monitoring period were as expected (i.e. 125 g/bird.day during week four of monitoring), rates of lay continued to be high (i.e. 93% during week four of monitoring), and egg weight was good (i.e. 65 g/egg).

Feeding patterns were similar irrespective of whether birds were provided with access to separate limestone granules, or not. An example of a typical daily feeding pattern is shown in Figure 1.

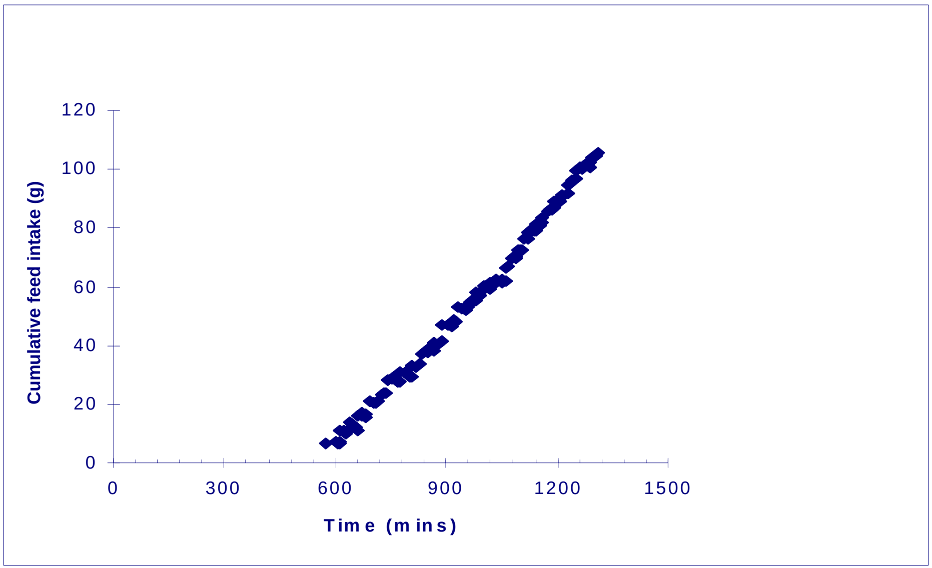

Figure 1 Cumulative feed intake for an individual bird against time-of-day (g)

Feed intake occurred almost exclusively during the photoperiod, but surprisingly there was a constant rate of feed intake throughout the photoperiod. Linear regressions of cumulative feed intake against time of day for the duration of the photoperiod, yielded correlation coefficients of between 0.97 and 1.00. It was surprising that there was no evidence of accelerated eating, either at the start of the photoperiod, or during the late afternoon, with or without access to limestone.

Limestone granule intakes were extremely variable between hens, and this is reflected in the large standard deviation, the mean intake was 12.5 g/bird.day±4.5g. Limestone granule intakes of 0.6 g/h were unreliable because of the sensitivity of the recording equipment, and so the only period of significant limestone granule intake was during the last 4 h of the photoperiod (Figure 2). Over this period hens consumed up to 3 g limestone/h. The timing of limestone granule intake supports the hypothesis that laying hens have calcium appetite during the late afternoon.

There was a trend towards a positive relationship between the time of oviposition and the time of limestone granule intake (p=0.26). The mean time of oviposition was 10.00 h, and the range was between 08.30 h and 13.30 h.

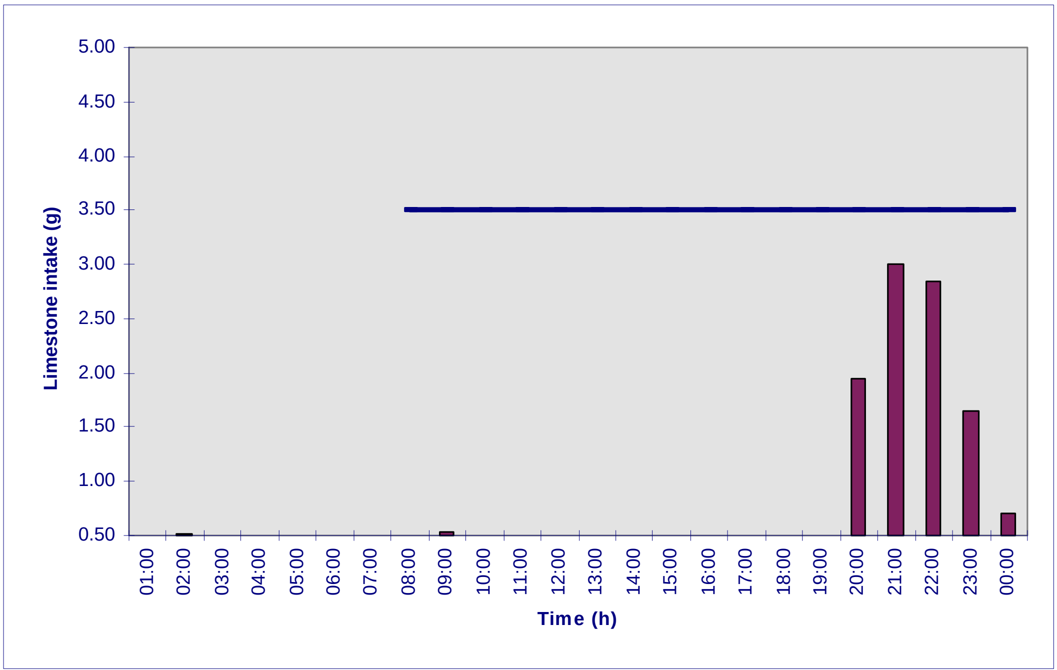

Figure 2 Mean daily pattern of limestone granule intake (duration of the photoperiod is illustrated by the horizontal line)

EXPERIMENT 2 - TOTAL PHOSPHORUS, NITROGEN AND CALCIUM BALANCES OF LAYING HENS
Feed usage between 23 and 26 weeks of age was 126 g/bird.day, this was typical of high performing brown egg layers. Rate of lay between 23 and 26 weeks of age was 99%. This was exceptionally good, and in addition, the eggs laid were large, the mean weight being 62 g. Thus, egg mass output during the monitoring period was an exceptional 61 g/bird.day.

Feed usage and limestone granule usage
There were no interactive effects of dietary calcium and available phosphorus contents, dietary phytase contents and limestone granule treatments on feed usage between 23 and 26 weeks of age. Feed intakes between 23 and 26 weeks of age tended to be lower in hens fed diets containing low calcium and available phosphorus contents, than in hens fed diets containing optimum calcium and available phosphorus contents (123 g/bird.day versus 129 g/bird.day, respectively, p=0.24). This may have been due to either a dietary calcium deficiency, and the effects of this on bone resorption and plasma phosphorus concentrations, as feed intakes may be reduced under these circumstances, or due to a phosphorus deficiency as feed intakes are reduced at low dietary phosphorus intakes. However, the latter is unlikely as feed usage between 23 and 26 was not increased by phytase supplementation. There was no evidence for the putative over-consumption of mash being prevented by feeding limestone granules, as feed intakes between 23 and 26 weeks of age were similar irrespective of limestone granule access (e.g. feed usage at 23 weeks of age was 125 g/bird.day in hens not offered limestone granules, and 124 g/bird.day in hens fed limestone granules).

As hens consumed limestone when it was on offer to them, their limestone intake was significantly higher than in hens not offered limestone granules (mean limestone intake between 23 and 26 weeks of age was 14 g/bird.day, p<0.05). Neither dietary calcium and available phosphorus contents, nor dietary phytase contents affected limestone granule intakes between 23 and 26 weeks of age, and this suggests that the hens either had a similar calcium appetite across all dietary treatments, or that they were unable to adjust their limestone granule intake so as to take into account the dietary supply of calcium and available phosphorus.

Egg production
There were no interactive effects of dietary calcium and available phosphorus contents, dietary phytase contents, or access to limestone granules on rates of lay, egg weights, or egg mass outputs between 23 and 26 weeks of age. Neither dietary calcium and available phosphorus contents, phytase contents, nor access to

limestone granules had an effect on rate of lay between 23 and 26 weeks of age. Egg weights between 23 and 26 weeks of age were not affected by dietary calcium and available phosphorus contents, or by dietary phytase contents at 23 and 24 weeks of age, but there were effects of phytase supplementation on egg weights at 25 and 26 weeks of age. Eggs were heavier from hens fed phytase supplemented diets (e.g. at 25 weeks of age, mean egg weights were 65 g, versus 61 g, respectively (p<0.05). There was a consistent effect of limestone granule intake on egg weight between 23 and 26 weeks of age. Eggs were heavier when hens consumed limestone granules mean egg weights were 61 g, versus 59 g, respectively (p<0.05). Egg mass outputs between 23 and 26 weeks of age were not affected by dietary calcium and available phosphorus contents, or by dietary phytase contents. Although dietary phytase supplementation improved egg weights at 25 and 26 weeks of age, this was negated by hens having numerically lower rates of lay. There was a trend for egg mass outputs at 23 and 26 weeks of age to be greater in hens allowed access to limestone granules, and this was due to hens laying heavier eggs when fed limestone granules. Differences in egg mass outputs between hens allowed access, or not allowed access to limestone granules, were significant at 24 and 25 weeks of age (64 g egg/bird.day, versus 61 g egg/bird.day at 24 weeks of age, respectively, p<0.05).

Total calcium and available phosphorus intakes

The effects of dietary treatments on total calcium (feed calcium plus limestone calcium), available phosphorus and nitrogen intakes, and the calculated ratios of total calcium to phosphorus intakes are shown in Table 1. Feed calcium intakes at 23 and 24 weeks of age, and feed available phosphorus intakes between 23 and 26 weeks of age, were higher in hens fed diets containing optimum calcium and available phosphorus contents, than in hens fed diets containing low calcium and available phosphorus contents (e.g. at 23 weeks of age, 4.6 g feed calcium/bird.day and 0.41 g feed available phosphorus/bird.day, versus 2.8 g feed calcium/bird.day and 0.25 g feed available phosphorus/bird.day, respectively, p<0.05). This was due to the optimum calcium and available phosphorus diet having higher concentrations of calcium and available phosphorus, and hens tended to eat more than when fed the low calcium and available phosphorus ration. There were similar trends for feed calcium intakes at 25 and 26 weeks of age to be higher in hens fed diets containing optimum calcium and available phosphorus contents. However, allowing hen's access to limestone granules had the greatest effect on total daily calcium intake. Hens fed limestone granules had an additional calcium intake between 23 and 26 weeks of age of 5.6 g/bird.day, and this significantly increased their total daily calcium intake at 25 and 26 weeks of age (p<0.05). There were similar trends for increased total daily calcium intakes in hens fed limestone granules at 23 and 24 weeks of age. Microbial phytase supplementation increased phytate-phosphorus availability, and this was equated with increasing feed available phosphorus intakes between 23 and 26 weeks of age, compared with hens fed rations containing zero microbial phytase (p<0.05).

It was not surprising that the calculated ratios of total calcium to available phosphorus intakes were increased between 23 and 26 weeks of age by allowing hens access to limestone granules (p<0.05), and there was a trend for the ratio to be higher in hens fed diets containing optimum calcium and available phosphorus contents. This was significant at 24 weeks only (p<0.05). Microbial phytase supplementation tended to reduce the ratio of total calcium to available phosphorus intake, and this was significant at 24 weeks of age (p<0.05).

Table 1 Effect of dietary treatment on total calcium, available phosphorus and nitrogen intakes (g/bird.day) and the ratio of total calcium to available phosphorus intakes

Age (weeks)	Dietary treatment	Total calcium	Available phosphorus	Nitrogen	Total calcium:phosphorus ratio
23	Low Ca and P	5.6	0.32[b]	3.5	18
	Optimum Ca and P	7.1	0.48[a]	3.7	15
	No phytase	5.6	0.33[b]	3.6	17
	Phytase	7.2	0.47[a]	3.6	15
	No limestone	3.8	0.40	3.6	10[b]
	Limestone	8.9	0.40	3.6	22[a]
	sem	0.59	0.01	0.09	1.5
24	Low Ca and P	6.2	0.33[b]	3.6	19[a]
	Optimum Ca and P	7.2	0.50[a]	3.8	14[b]
	No phytase	6.2	0.33[b]	3.6	19[a]
	Phytase	7.2	0.49[a]	3.8	15[a]
	No limestone	3.9	0.41	3.7	10[b]
	Limestone	9.5	0.42	3.7	23[a]
	sem	0.52	0.01	0.10	1.4
25	Low Ca and P	6.9	0.32[b]	3.5	22
	Optimum Ca and P	6.9	0.47[a]	3.6	15
	No phytase	6.8	0.33[b]	3.5	21
	Phytase	7.0	0.46[a]	3.6	15
	No limestone	3.7[b]	0.39	3.6	9[b]
	Limestone	10.0[a]	0.40	3.5	25[a]
	sem	0.82	0.01	0.08	3.3
26	Low Ca and P	5.9	0.32[b]	3.6	18
	Optimum Ca and P	7.2	0.49[a]	3.7	15
	No phytase	6.4	0.34[b]	3.7	19
	Phytase	6.7	0.48[a]	3.6	14
	No limestone	3.9[b]	0.41	3.7	10[b]
	Limestone	9.2[a]	0.40	3.6	23[a]
	sem	0.67	0.01	0.09	2.1

Values having differing superscripts are significantly different at $p<0.05$

<u>Body weights</u>

The mean body weight at the start of the monitoring period was 1.833 kg, and there were no significant differences in starting body weight between hens allocated to the different treatments. There were no interactive effects of dietary treatments on body weights at 23, 24, 25 or 26 weeks of age. Hens fed diets containing optimum calcium and available phosphorus contents were heavier from 24 weeks of age than hens fed diets containing low calcium and available phosphorus contents, and this is likely to have been due to their tendency for a higher feed usage (e.g. at 24 weeks of age body weights were 1.86 kg, versus 1.80 kg, respectively ($p<0.05$). There were no effects of dietary phytase supplementation, or access to limestone granules on body weights between 23 and 26 weeks of age.

<u>Faecal calcium, nitrogen and phosphorus concentrations between 23 and 26 weeks of age</u>

Faecal mass outputs between 23 and 26 weeks of age were similar across all dietary treatments, the mean was 109 g fresh weight/bird. There were dietary effects on the concentrations of calcium, phosphorus and nitrogen in the excreta. Faecal calcium concentrations were lower in hens fed diets containing low calcium and available phosphorus contents than in hens fed diets containing optimum calcium and available phosphorus contents when hens were not fed limestone granules, whereas when hens were fed limestone granules the faecal concentrations of calcium were similar irrespective of dietary calcium and available phosphorus contents (e.g. mean faecal calcium concentrations were 6.0 mmol/l, versus 8.9 mmol/l when comparing hens fed low calcium and available phosphorus with hens fed optimal calcium and available phosphorus and no limestone granule access, $p<0.001$). A lower faecal calcium excretion in hens fed diets containing low dietary calcium and available phosphorus concentrations and no access to limestone granules, may have been due to lower calcium intakes than in hens fed diets containing optimum calcium and available phosphorus contents (3.0 g calcium/bird.day, compared with 4.9 g calcium/bird.day, respectively). Total calcium intakes were also lower when hens were fed a diet containing low calcium and available phosphorus contents and access to limestone granules was provided, than in hens fed a diet containing optimum calcium and available phosphorus contents and limestone granules (8.6 g total calcium/bird.day, compared with 10.5 g total calcium/bird.day), yet faecal calcium concentrations were similar. This suggests that even at very high calcium intakes, the levels of calcium absorption and retention were similar to those at lower calcium intakes.

Faecal phosphorus concentrations were reduced in hens fed diets containing low calcium and available phosphorus contents, compared with hens fed diets containing optimal calcium and available phosphorus contents (2.7 mmol phosphorus/l, compared with 3.4 mmol phosphorus/l, respectively, $p<0.001$). A reduction in faecal phosphorus excretion at low dietary calcium and available phosphorus intakes was anticipated, as the ration was formulated to have been deficient in available phosphorus. Limestone granule intake reduced faecal phosphorus excretion (2.7 mmol phosphorus/l when fed limestone granules, compared with 3.4 mmol phosphorus/l when not fed limestone granules, $p<0.001$). This suggests that when hens were not fed a supplementary source of calcium they resorbed bone in order to meet their calcium requirements for eggshell formation. Bone contains a lower ratio of calcium to phosphorus than the egg shell, and bone resorption would have increased plasma phosphorus concentrations above that needed for egg shell formation. The hen would have needed to increase phosphorus excretion in order to maintain a physiological balance.

There were interactive effects of dietary calcium and available phosphorus contents and limestone granule treatment on faecal nitrogen concentrations. Faecal nitrogen concentrations were higher in hens fed diets containing low calcium and available phosphorus contents, than in hens when diets containing optimum calcium and available phosphorus contents when they were not fed limestone granules, but this was not the case when hens were fed limestone granules (51 gN/kgDM when fed a diet containing low calcium and available phosphorus contents and no limestone granules, compared with 37 gN/kgDM when fed a diet containing optimum calcium and available phosphorus contents and no limestone granules, $p<0.01$). Total nitrogen intakes tended to be lower in hens fed diets containing low calcium and available phosphorus contents than in hens fed diets containing optimal calcium and available phosphorus contents, due to a trend for lower feed intakes, as diets were formulated to be isonitrogenous. A low calcium and available phosphorus ration, fed in the absence of limestone granules, either reduced dietary protein efficiency, or hens were losing body nitrogen.

Neither faecal nitrogen, phosphorus, nor calcium concentrations were affected by dietary phytase supplementation.

<u>Egg calcium, phosphorus and nitrogen concentrations between 23 and 26 weeks of age</u>
Egg calcium concentrations were not affected by dietary treatment, but there were effects of limestone supplementation on egg phosphorus concentrations. Egg phosphorus concentrations were reduced by feeding limestone granules (0.86 mmol P/l, compared with 0.97 mmol P/l, $p<0.05$), and there were also reductions in faecal phosphorus concentrations when hens were fed limestone granules. The findings suggest that hens fed supplementary calcium did not mobilise bone calcium reserves in order to support egg shell formation, whereas bone was mobilised when limestone granules were unavailable, and this resulted in increased phosphorus excretion.

There were interactive effects of phytase and limestone granule treatment on egg nitrogen concentrations. Egg nitrogen concentrations were similar between hens fed diets supplemented with phytase, and hens fed diets containing zero microbial phytase, when limestone granules were not fed, but phytase supplementation increased egg nitrogen concentrations when hens were fed limestone granules (e.g. 51 gN/kgDM in hens fed diets containing zero microbial phytase and limestone granules, versus 57 gN/kgDM in hens fed diets supplemented with phytase and limestone granules, $p<0.05$).

Calcium, phosphorus and nitrogen balances (expressed as a proportion of intakes)

Hens fed diets containing low calcium and phosphorus contents maintained a high egg mass output by resorbing bone (5% of body calcium and 4% of body phosphorus were lost by the bird). By comparison, hens fed diets containing optimal calcium and available phosphorus contents were able to maintain a high egg mass output without resorbing bone. They had small positive calcium and phosphorus balances (4% of calcium and 7% of phosphorus were retained by the bird). Microbial phytase supplementation allowed egg mass output to occur without bone resorption. Calcium balances were positive when diets contained phytase, whereas when diets did not contain phytase, the hens' calcium balances were negative (+7% body calcium and -7% body calcium, respectively). Phosphorus balances were positive irrespective of phytase treatment. It was not surprising that hens fed limestone granules had a large positive calcium balance (24% of calcium was retained by the bird), and phosphorus balances were also positive (12% of phosphorus intake was retained by the bird). By comparison, when limestone granules were not offered, bone resorption occurred and this led to negative calcium and phosphorus balances (55% of body calcium and 7% of body phosphorus were lost by the bird). Nitrogen balances were positive irrespective of dietary calcium and available phosphorus contents, microbial phytase supplementation, or access to limestone granules (19% and 14% of nitrogen intakes were retained when hens were fed diets containing optimal or low calcium and available phosphorus contents, respectively; 18% and 15% of nitrogen intakes were retained when hens were fed diets containing phytase, or zero phytase, respectively, and; 25% and 6% of nitrogen intakes were retained when hens were allowed, or not allowed access to limestone granules, respectively).

EXPERIMENT 3 - EFFECTS OF DIETARY PHYTASE AND DICALCIUM PHOSPHATE CONTENTS (DCP) AND ACCESS TO SEPARATE LIMESTONE GRANULES ON LAYING HEN PERFORMANCE

Overview of feed usage and bird performance between 20 and 72 weeks of age

Feed usage between 20 and 72 weeks of age was 124 g/bird.day, which was higher than the breed standard but not atypical of intakes for caged brown egg layers at Gleadthorpe. When hens were offered limestone granules they had a mean intake of 8 g/bird.day and this provided an extra 3 g of calcium/bird.day. Rate of lay was 86% (313 eggs/hen housed) and mean egg weight was 65.6 g; these were good, and so egg mass output was better than expected (56.5 g/bird.day).

Effects of dietary treatments on feed usage and limestone granule usage

There were interactive effects of microbial phytase supplementation and limestone granule treatment on feed usage between 20 and 48 weeks of age, and between 20 and 72 weeks of age (Table 2). Feed intakes were similar between hens fed diets with, or without phytase supplementation when limestone granules were not on offer, but when limestone granules particle size 6 were on offer, phytase supplementation reduced feed intakes ($p<0.05$). There were similar trends for phytase supplementation to reduce feed intakes in hens offered limestone granules size 7. Hens eating limestone granules had an increased ratio of calcium to available phosphorus intake (e.g. limestone intake increased the ratio of calcium to available phosphorus intake from 15.9:1 to 25.1:1 in hens fed diets containing zero phytase), and this may have increased the hens' phosphorus requirement as phosphorus is needed for calcium metabolism. There was no supplementary source of available phosphorus, and so phosphorus intake could be increased only by eating more mash. Supplementing rations with phytase increased feed phosphorus availability and this reduced the ratio of calcium to available phosphorus intake (e.g. reduced from 15.9:1 to 11.2:1 in hens fed diets containing phytase when not offered limestone), and when hens fed diets containing phytase were offered limestone the ratio of calcium to available phosphorus intake was still within the putative optimal range (calcium to available phosphorus intakes of between 18.2:1 and 18.5:1, against a putative optimal range of 10:1 to 20:1). Thus, the combination of phytase supplementation and feeding limestone granules appears to have enabled the hens' requirements for calcium and phosphorus to be met at a lower mash intake.

Table 2 Feed usage between 20 and 72 weeks of age (g/bird.day)

	Limestone granule access			Mean
	None	Particle size 6	Particle size 7	
0 Phytase	126	125	124	125
+ Phytase	127	118	122	122
Mean	127	122	123	
sed±	Phytase.limestone granules		1.669	$p<0.05$
	Phytase		0.963	$p<0.05$
	Limestone granules		2.047	$p<0.01$

There were interactive effects of microbial phytase supplementation and DCP content on feed usage between 20 and 48 weeks of age ($p<0.05$), between 48 and 72 weeks of age ($p<0.05$), and between 20 and 72 weeks of age ($p<0.01$)(illustrated in Table 3). Feed intakes for all periods were lower in hens fed diets supplemented with phytase when the DCP content was 3 kg/tonne, or 7 kg/tonne, but there were no effects of phytase supplementation in diets containing 5 kg/t DCP. The reasons for this are not known.

Table 3 Feed usage between 20 and 72 weeks of age (g/bird.day)

	Dicalcium phosphate concentration (kg/tonne)			Mean
	3	5	7	
0 Phytase	126	122	127	125
+ Phytase	121	124	122	122
Mean	124	123	125	
sed±	Phytase.dicalcium phosphate		1.672	$p<0.01$
	Phytase		0.963	$p<0.05$
	Dicalcium phosphate		1.183	NS

There were interactive effects of microbial phytase supplementation and limestone granule access on limestone grit usage between 20 and 72 weeks, and between 20 and 48 weeks of age (illustrated for 20 to 72 weeks in Table 4), but not between 48 and 72 weeks of age. Phytase supplementation increased the intake of limestone granules particle size 6 between 20 and 72 weeks of age, and between 20 and 48 weeks of age, but the converse was found for limestone granules particle size 7 ($p<0.05$). The interactive effects of phytase supplementation and limestone granule particle size on limestone granule intake may have been due to differences in phosphorus and calcium availability between treatments, and the hens attempting to balance their supply of these nutrients by adjusting limestone grit intake. Phytase supplementation increases the availability of feed organic phosphorus, and possibly calcium, whereas, limestone granule size may have affected calcium availability through time.

Table 4 Limestone granule intake between 20 and 72 weeks of age (g/bird.day)

	Limestone granule access			Mean
	None	Particle size 6	Particle size 7	
0 Phytase	0	7.9	8.9	8.4
+ Phytase	0	8.5	8.4	8.5
Mean	0	8.2	8.7	
sed±	Phytase.limestone granules		0.212	$p<0.05$
	Phytase		0.122	NS
	Limestone granules		0.150	$p<0.001$

Limestone granule intakes between 48 and 72 weeks of age were similar irrespective of particle size and, as hens ate limestone when on offer, their limestone intake was higher than in hens not offered limestone granules (7 g/bird.day for birds offered limestone granules particle size 6, or particle size 7, compared with 0 g/bird.day for birds not offered limestone granules, $p<0.001$). Limestone granule intakes tended to be lower between 48 and 72 weeks of age, than between 20 and 48 weeks of age, and when examining patterns of limestone intakes with age there were some interesting findings (Figure 3). Limestone granule intakes increased between 20 and 51 weeks of age, up to a maximum intake of approximately 15 g/bird.day (5.6 g calcium/bird.day), followed by abrupt falls in intake at 52 weeks of age to approximately 4 g limestone/bird.day (1.6 g calcium/bird.day), and intakes then remained at this level until 59 weeks of age. At 60 weeks of age, limestone granule intakes increased, and were approximately 7 g/bird.day between 60 and 67 weeks of age (2.6 g calcium/bird.day), but intakes fell to approximately 5 g/bird.day between 68 and 71 weeks of age (1.9 g calcium/bird.day). Mash intakes did not follow a similar age related pattern. Increases in limestone granule intakes between 20 and 40 weeks of age were not unexpected as this coincided with increasing egg mass outputs, and therefore, increasing calcium requirements for egg shell formation. However, very high limestone intakes over a prolonged period were surprising, and the sudden and large reductions in limestone intakes at 52 weeks of age suggests that calcium intakes may have become toxic. Although egg mass outputs were falling at 52 weeks of age, the reduction in egg mass outputs were small, and therefore, the hens' calcium requirements for egg shell formation were only marginally reduced (e.g. mean egg mass output between 48 and 51 weeks of age was 57.7 g/bird.day, compared with a mean of 56.8 g/bird.day between 52 and 55 weeks of age).

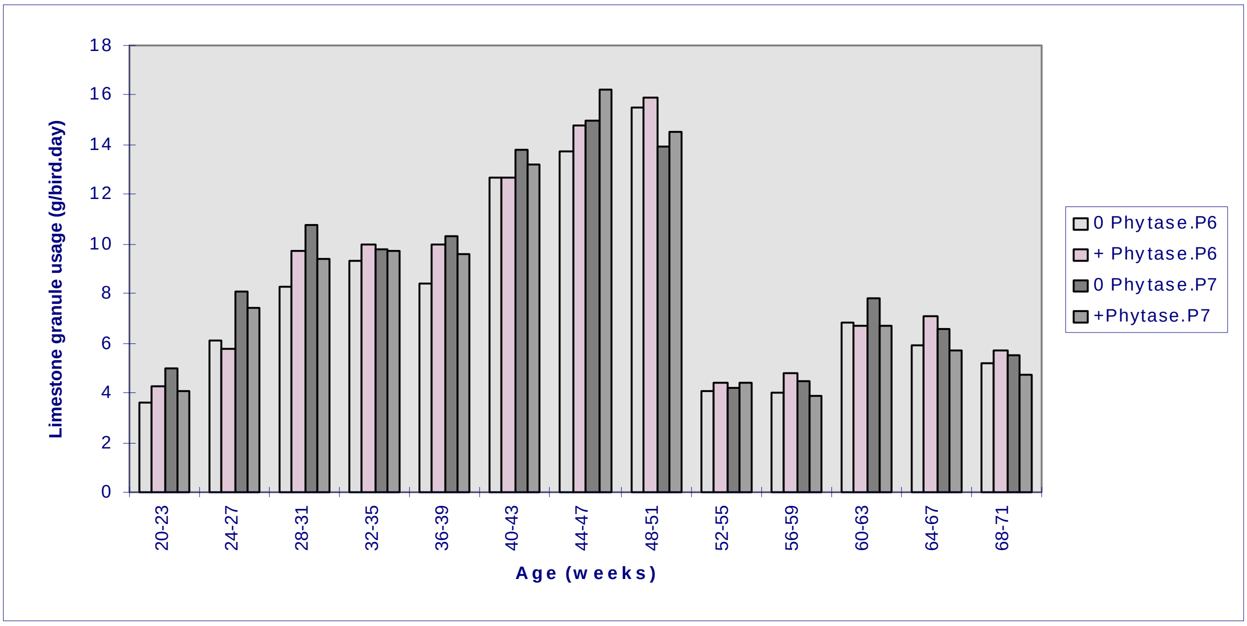

Figure 3 Age-related patterns in limestone granule intakes (g/bird.day)

There were interactive effects of dietary phytase supplementation, limestone granule access and DCP contents on the calculated intakes of calcium to available phosphorus between 20 and 72 weeks of age ($p<0.05$)(Table 5). The ratios of calcium to available phosphorus intakes were lower when hens were fed diets supplemented with phytase (note: comparing the same limestone granule treatment and the same dietary concentration of DCP). This was due to microbial phytase increasing feed organic phosphorus availability. Limestone granule intake increased the ratio of calcium to available phosphorus intake, but this was greater in hens' fed diets not containing phytase and when the dietary DCP contents were lower (i.e. when the ratio of calcium to available phosphorus intake was already high). Increasing dietary DCP concentrations reduced the ratio of calcium to available phosphorus intakes, this was because DCP is a source of inorganic phosphorus.

Table 5 Calculated ratios of intake of calcium to available phosphorus between 20 and 72 weeks of age

		Dietary treatment		
Phytase	Limestone granules		DCP (kg/t)	
		3	5	7
0 Phytase	Zero	18.2	15.5	14.0
	Particle size 6	28.8	24.5	21.9
	Particle size 7	30.2	26.0	22.5
+ Phytase	Zero	12.3	11.0	10.2
	Particle size 6	20.7	18.5	16.4
	Particle size 7	20.1	17.6	16.9
sed±	Phytase.limestone granule.DCP	0.404	*p*<0.05	
	Phytase.limestone	0.208	*p*<0.001	
	Phytase.DCP	0.233	*p*<0.001	
	Limestone.DCP	0.286	*p*<0.001	
	Phytase	0.120	*p*<0.001	
	Limestone	0.147	*p*<0.001	
	DCP	0.173	*p*<0.001	

There was a trend for similar interactive effects between microbial phytase supplementation, limestone granule access and DCP contents on the calculated intakes of calcium to available phosphorus between 20 and 48 weeks of age, and between 48 and 72 weeks of age (*p*=0.18 and *p*=0.15, respectively). Interactive effects between microbial phytase and limestone access, between microbial phytase and DCP, and between limestone access and DCP, on the calculated ratios of calcium to available phosphorus intakes for the periods 20 to 48 weeks of age, and 48 to 72 weeks of age were identified (*p*<0.001 in all cases except for limestone access and DCP which was *p*<0.01). Phytase supplementation reduced the ratio of calcium to available phosphorus intake, but the effect was greater when hens were offered limestone granules, and when dietary DCP contents were lower. Limestone granule access increased the ratio of calcium to available phosphorus intake, but the effect was greater when dietary DCP contents were lower.

<u>Rates of lay, egg weights and egg mass outputs</u>
There were interactive effects of microbial phytase supplementation and limestone granule treatment on rates of lay between 20 and 48 weeks of age (Table 6), but not between 48 and 72 weeks, or between 20 and 72 weeks of age.

Rates of lay between 20 and 48 weeks of age were similar between hens fed diets with or without phytase when limestone granules were not on offer, whereas phytase supplementation reduced rates of lay when hens were offered a source of large limestone granules (particle size 6), and improved rates of lay when birds were offered a more variable source of limestone granules (particle size 7, *p*<0.05). This may have been due to lower mash intakes in hens fed diets containing phytase when offered limestone granules size 6, as a low protein intake reduces rates of lay (e.g. feed usage was 115 g/bird.day between 20 and 48 weeks of age in hens fed diets containing phytase x access to limestone granules particle size 6, compared with 122 g/bird.day in hens fed diets containing zero phytase x zero access to limestone granules). Although mash intakes were reduced by phytase supplementation in birds offered limestone granules size 7, this was to a lesser extent than when offered larger granules, and phytase supplementation may have improved the availability of phytate-nitrogen and other phytate-nutrients (e.g. feed usage was 118 g/bird.day between 20 and 48 weeks of age in hens fed diets containing phytase x access to limestone particles size 7, compared with 120 g/bird.day in hens fed diets containing zero phytase x zero access to limestone granules). Total calcium intakes between 20 and 48 weeks of age were similar across all phytase (with and without phytase supplementation) and all positive limestone granule access (i.e. granules size 6 and granules size 7) treatment combinations and so calcium intake was not thought to have been responsible for the reported differences in rates of lay.

Table 6 Rates of lay between 20 and 48 weeks of age (eggs/bird.day)

	Limestone granules	Mean

	None	Particle size 6	Particle size 7	
0 Phytase	0.91	0.90	0.87	0.89
+ Phytase	0.92	0.88	0.90	0.90
Mean	0.92	0.89	0.89	
sed±	Phytase.limestone granules		0.0099	$p<0.05$
	Phytase		0.0057	NS
	Limestone granules		0.0070	$p<0.01$

Increasing the dietary concentration of DCP from 3 to 9 kg/t (when diets contained zero phytase), or from 1 to 7 kg/t (when diets contained phytase) did not affect rates of lay between 20 and 72, between 20 and 48, or between 48 and 72 weeks of age.

There were no interactive effects of phytase supplementation and limestone granule treatment on egg weights between 20 and 48 weeks of age, but there were interactive effects of these treatments on egg mass outputs for the same period. Egg mass outputs between 20 and 48 weeks of age were similar irrespective of phytase supplementation when hens were not offered limestone granules, but phytase supplementation lowered egg mass outputs when hens were offered limestone granules particle size 6, whereas the opposite was found when hens were offered limestone granules particle size 7 ($p<0.05$). This was largely due to the interactive effects of these treatments on rates of lay between 20 and 48 weeks of age.

Neither phytase supplementation, limestone granule access, nor DCP concentration affected egg weights, or egg mass outputs between 20 and 72, or between 48 and 72 weeks of age.

Feed conversion efficiency

There were interactive effects of phytase supplementation, limestone granule access and DCP on feed conversion efficiencies between 20 and 48 weeks of age and between 20 and 72 weeks of age (illustrated for 20 to 72 weeks of age in Table 7).

Feed conversion efficiencies between 20 and 48 weeks of age were improved by phytase supplementation when hens were either denied access to limestone granules, or when offered limestone granules particle size 6 and a DCP concentration of 3 kg/t was used ($p<0.05$), but there were no effects of phytase supplementation when hens were fed limestone granules particle size 7. There were similar findings for the period 20 to 72 weeks of age except that hens fed phytase supplemented diets had similar feed conversion efficiencies to hens fed diets containing zero phytase when they were denied access to limestone granules. When DCP concentrations of 5 or 7 kg/t were used, phytase supplementation improved feed conversion efficiencies between 20 and 48 weeks of age in hens offered limestone granules particle size 7, but not when limestone granules particle size 6 were offered, or when access to limestone granules was denied. There were no effects of phytase supplementation on feed conversion efficiencies between 20 and 72 weeks of age across any of the limestone granule treatments when the DCP concentration was 5 kg/t. When the DCP concentration was 7 kg/t, phytase supplementation improved feed conversion efficiencies between 20 and 72 weeks of age when hens were allowed access to limestone granules particle size 7, but not when limestone granule access was either denied, or granules of particle size 6 were fed. The interactive effects of phytase, limestone granule access and DCP content on feed conversion efficiencies between 20 and 48 weeks of age, and between 20 and 72 weeks of age, were largely due to treatment effects on feed usage as egg mass outputs were not significantly affected.

Table 7 Feed conversion efficiencies between 20 and 72 weeks of age

Dietary treatment

Phytase	Limestone granules	DCP (kg/t) 3	5	7
0 Phytase	Zero	0.452	0.455	0.438
	Particle size 6	0.440	0.461	0.466
	Particle size 7	0.464	0.451	0.443
+ Phytase	Zero	0.466	0.461	0.455
	Particle size 6	0.485	0.448	0.484
	Particle size 7	0.457	0.472	0.481

sed±	Phytase.limestone granule.DCP	0.01273	*p*<0.05
	Phytase.limestone	0.00712	NS
	Phytase.DCP	0.00735	*p*=0.19
	Limestone.DCP	0.00900	*p*=0.17
	Phytase	0.00411	*p*<0.01
	Limestone	0.00503	*p*=0.19
	DCP	0.00528	NS

Body weight

Body weights at 20 weeks of age, when dietary treatments were first applied, were similar between all treatments. There were no effects of phytase supplementation, or DCP concentration on body weights at 26 or 48 weeks of age, and DCP concentrations did not affect body weights at 72 weeks of age. Phytase supplementation reduced body weights at 72 weeks of age, compared with hens fed diets containing zero phytase (1.96 kg in hens fed diets containing phytase and 2.05 kg in birds fed diets containing zero phytase, *p*<0.05). Hens fed limestone granules had lower body weights at 26 and 48 weeks of age than hens not fed limestone granules, but body weights were lowest when birds were offered limestone granules particle size 6 (Table 8). Although not significant, there was a trend for body weights at 72 weeks of age to be lower when hens were fed limestone granules particle size 6. Body weight differences were thought to have been due to treatment effects on feed usage, as feed usage tended to be lowest when hens were fed limestone granules particle size 6.

Table 8 Body weights at 26, 48 and 72 weeks of age (kg)

Age (weeks)	Limestone granule treatment None	Particle size 6	Particle size 7	Mean	sed	p
26	1.85	1.79	1.83	1.83	0.019	<0.05
48	2.01	1.87	1.94	1.94	0.031	<0.01
72	2.02	1.98	2.03	2.01	0.040	NS

Mortality

Mortalities between 20 and 48, between 48 and 72, and between 20 and 72 weeks of age were not affected by dietary treatments. Mean mortality between 20 and 72 weeks of age was 11.9% and this was higher than usual, however *post mortem* examinations did not identify any disease challenges.

Egg shell weight and density

Egg shell weights and densities were not affected by dietary treatment until the end of the laying period at 72 weeks of age; at this age egg shell weights were higher when hens were fed phytase supplemented diets, or when fed limestone granules particle size 6 (*p*<0.05 and *p*<0.01, respectively, Tables 9 and 10). Egg shell densities at 72 weeks of age were higher when fed phytase supplemented diets, or when fed limestone granules (*p*<0.01).

Table 9 Egg shell weights (g) and densities (mg/cm^2) at 72 weeks of age

	Dietary phytase supplementation 0 Phytase	+ Phytase	Mean	sed	*p*

Shell weights	6.4	6.6	6.5	0.047	<0.05
Shell densities	80.8	82.5	81.7	0.535	<0.01

Table 10 Egg shell weights (g) and densities (mg/cm^2) at 72 weeks of age

	Limestone granule access			Mean	sed	*p*
	None	Particle size 6	Particle size 7			
Shell weights	6.3	6.6	6.5	6.5	0.146	<0.01
Shell densities	79.9	82.7	82.3	81.7	0.656	<0.01

<u>Tibial weight, length and breaking strength</u>
Body weights at 72 weeks of age of hens culled for measurements of tibial breaking strength were similar across all treatments. There were no effects of phytase supplementation, access to limestone granules, or DCP concentrations on tibial length at 72 weeks of age, but tibial weights tended to be heavier in hens fed diets containing phytase, and with access to limestone granules (13.2 g/bone in hens fed phytase supplemented diets, compared with 12.7 g/bone in hens fed diets containing zero phytase, p=0.06, and 13.2 g/bone when offered limestone particles, sizes 6 or 7, compared with 12.5 g/bone when not offered limestone granules, p=0.07). Tibial breaking strengths at 72 weeks of age were increased by phytase supplementation, and by offering access to limestone granules (19.9 kg maximum breaking strength in hens fed diets containing zero phytase, compared with a maximum breaking strength of 22 kg in hens fed phytase supplemented diets, p<0.05, and a maximum breaking strength of 18.8 kg in hens not offered limestone granules, compared with a maximum breaking strength of 22 kg in hens fed limestone granules, particle sizes 6 or 7, p<0.01).

DISCUSSION
<u>Feed intake and limestone intake</u>
The diurnal patterns of mash intake recorded in Experiment 1b for modern brown egg layers differed from those reported in earlier strains of layers, and to those reported for modern white egg layers (Duncan *et al.,* 1970; Wood-Gush and Horne, 1970; Keshavarz, 1998). In most studies, laying hens had a period of high food intake in the first hour of the light period, and a second peak in high food intake during the last 2 or 3 h of the light period. The late afternoon peak in food intake was associated with hens' attempting to meet their calcium requirements for egg shell formation (Taylor, 1970; Hughes, 1972). In Experiment 1b, there was a consistent feeding rate throughout the light period, even in hens fed limestone granules. Thus, modern brown egg layers did not overeat during the late afternoon in order to meet their calcium requirements for egg shell formation. In, both modern and earlier strains of layers feed intakes were negligible during darkness.

Limestone grit intakes in Experiment 1b varied between hens, but overall the findings for modern brown egg layers, laying on most days, were not dissimilar to those reported for earlier hybrids on days when eggs were formed and laid, or formed but not laid (Hughes, 1972; Mongin and Sauver, 1974). The daily patterns of limestone grit intake in Experiment 1b differed from those of Hughes (1972), in that limestone grit intake occurred over a shorter period of the late afternoon in modern brown egg layers. Limestone grit intake was thought to occur proximate to the start of egg shell formation, based on a 9.25 h period from ovulation to the start of calcification in the shell gland, whereas in earlier hybrids, supplementary calcium intake occurred prior to egg shell formation (Hughes, 1972).

In Experiment 2, neither limestone granule supplementation, nor phytase supplementation affected feed usage during early lay, but this was contradictory to the findings of Experiment 3, where interactive effects of dietary phytase supplementation and limestone granule access on feed usage between 20 and 48 weeks of age, and between 20 and 72 weeks of age, were identified. In Experiment 2, rates of lay were similar irrespective of limestone granule treatment, but egg weights and egg mass outputs were increased by feeding limestone granules. Thus, feed saving benefits associated with offering a supplementary calcium source may have been prevented by the hens' requirement for protein when supporting a very high egg mass output.

Limestone granule intakes were much higher in Experiment 2, than in Experiment 3, when comparing hens of a similar age (14 g/bird.day between 23 and 26 weeks of age in Experiment 2, compared with 8 g/bird.day between 23 and 26 weeks of age in Experiment 3), but limestone granule intakes increased up to 51 weeks of age in Experiment 3, and at this age intakes were similar between studies. Differences in housing and feeder equipment between studies may have accounted for this. In Experiment 2, hens were housed individually and the limestone granules were placed in a pot, whereas in the subsequent study, hens were housed in groups of four and limestone granules were presented to the hens in a ‘U’ shaped tube fastened on to the outer side of the feeder trough.

The sudden reduction in limestone intakes at 52 weeks of age in Experiment 3 may have been due to calcium toxicity. Although calcium absorption is generally considered to be poorer as dietary calcium intake increases, a linear relationship between calcium intake and calcium retention, for intakes of between 0.6 and 3.6 g Ca/day was reported by Hurwitz and Bar (1969). Clunies *et al.,* (1992) reported similar findings for a wider range of calcium intakes (1.2 to 6.1 g/day). In Experiment 3, hens fed limestone had a calcium intake of approximately 11 g/bird.day at 51 weeks of age, against a calcium requirement of approximately 5 g/bird.day for eggshell formation (based on a calcium content of 373 mg/g shell and a calcium utilisation efficiency of 0.5, Charles *et al.,* 1997, citing Simons 1986). Although calcium retention was not measured in Experiment 3, an increase in tibial breaking strength at 72 weeks of age in hens fed limestone granules, compared with hens not offered limestone granules, suggests that calcium retention was greater when fed limestone. Furthermore, faecal calcium concentrations measured in Experiment 2, were similar between hens fed low, or high calcium diets when provided with access to limestone granules. For calcium retention to remain high at very high calcium intakes, there would have been a need to reduce limestone granule intake in order to avoid calcium toxicity, and as feed intake did not follow a similar age-related pattern this provides further evidence that the laying hen is able to independently control calcium intake from two different sources.

The trend for lower feed intakes in hens fed a diet containing low calcium and available phosphorus contents (24.0 g/kg calcium and 2.3 g/kg available phosphorus, Experiment 2) was similar to the findings of Roush *et al.,* (1986), where hens fed diets low in calcium (25 g/kg calcium) had depressed feed intakes irrespective of dietary available phosphorus content (3.5 to 6.5 g/kg available phosphorus). The low calcium and available phosphorus diet used in Experiment 2 was formulated to have been phosphorus deficient, and feed intake is usually reduced in hens fed phosphorus-deficient diets (e.g. Hartel, 1990), yet this was not the case, as feed intake was not increased by phytase supplementation. Clunies *et al.,* (1992) suggested that reduced feed intakes in hens fed diets low in calcium may be related to increased plasma phosphorus concentrations following bone resorption. The ratio of calcium to phosphorus in the bone is much higher than in the egg shell, since calcium is largely stored in the bone as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), whereas calcium carbonate is the main form of egg shell calcium. Thus, mobilising bone calcium reserves in order to support egg shell formation causes an increase plasma phosphorus concentrations, and this leads to increased phosphorus excretion (Boorman *et al.,* 1989). In Experiment 2, negative calcium and phosphorus balances for hens fed the low calcium and available phosphorus diet, suggests that bone calcium was mobilised in order to support egg shell formation.

<u>Rates of lay, egg weights and egg mass outputs</u>
In Experiment 2 there were no effects of calcium and available phosphorus contents, phytase supplementation, or limestone granule access on rates of lay, whereas in Experiment 3 there were interactive effects of phytase supplementation and limestone granule access on rates of lay between 20 and 48 weeks of age. Other workers have failed to identify interactions between calcium and phosphorus levels in feed as affecting egg production variables (Keshavarz, 1986). Gordon and Roland (1997) reported similar rates of lay when feeding diets having available phosphorus contents of between 2.0 and 5.0 g/kg, and dietary supplementation with phytase provided no improvement. It is likely that the depressions in rates of lay observed in Experiment 3 for hens fed phytase supplemented diets when provided with a source of large limestone granules, were due to low feed intakes during this period. Egg production is reduced at low protein intakes, and a reduction in daily feed intake of 7 g/bird.day, as experienced in Experiment 3, represents a considerable reduction in protein intake.

Egg weights and egg mass outputs were increased by limestone granule supplementation, and dietary phytase supplementation in Experiment 2, but there were no effects of these treatments, or dietary DCP concentration on egg weights, or egg mass outputs between 20 and 72 weeks of age in Experiment 3. Other workers have reported improvements in egg weights when feeding phytase supplemented rations (e.g. Um and Paik, 1999). Differences in treatment effects on feed intakes between experiments may partly account for a lack of conformity between experiments, but other workers have also failed to identify interactive effects of calcium and available phosphorus levels on egg production variables (Keshavarz, 1986). However, the findings of Experiment 3 indicate that hens are able to maintain high egg mass outputs across a very wide range of calcium and available phosphorus intakes.

<u>Egg shell quality and tibial breaking strength</u>

It was surprising that egg shell weights and densities were not affected by dietary treatment until the end of the laying period at 72 weeks of age (Experiment 3), although this is the age at which dietary treatments are likely to have a more pronounced effect as eggs are larger, and calcium homeostasis may be less efficient with age (e.g. Charles *et al.,* 1997, citing Bar and Hurwitz, 1987). Although egg shell weights and densities were increased by dietary phytase supplementation, and by feeding limestone granules, there were no effects of dietary DCP concentrations. As the diets were formulated to have an iso-calcium content as the DCP content increased, this means that egg shell weights and densities were not affected by feeding diets having a wide range in calcium to available phosphorus ratios (12.3 to 18.2 when feeding diets not containing microbial phytase, and 9.5 to 14.0 when feeding diets containing microbial phytase). This is similar to the findings of Charles *et al.,* (1997), when reviewing calcium and phosphorus requirements for laying hens, as optimal ratios of calcium to available phosphorus intakes were within the range of 7 to 26.5, though generally around 10 to 20. The authors suggested that at times when calcium supply is sufficient for shell calcium carbonate deposition then the concept of calcium to available phosphorus ratios may be less relevant. In Experiment 3, mean feed intake was 124 g/bird.day, and this provided a dietary calcium intake of 5.2 g/bird.day, which was thought to have been borderline-adequate for egg shell formation. At the end of lay when egg size was large, limestone granule supplementation improved egg shell density, and this was similar to the findings of other authors (Miller and Saunde, 1975). More recently, Keshavarz and Nakajima (1992) found that oyster shell supplementation improved egg shell quality in later lay, even on high calcium diets. Phytase supplementation may have improved egg shell density at the end-of-lay by improving the availability of other nutrients and minerals. Um and Paik (1999) reported higher retentions of calcium, phosphorus, iron, magnesium and zinc in laying hens fed phytase supplemented diets. Although high levels of dietary magnesium may be detrimental to egg shell quality, “reasonable levels” of magnesium are not detrimental and might be beneficial (Harms and Roland, 1975).

Charles *et al.,* (1997) when reviewing calcium and phosphorus requirements for laying hens, suggested that there were different requirements for fully satisfying the demands of production, shell deposition and bone strength. Reichmann and Connor (1977) found that levels of calcium and phosphorus which did not affect production traits were beneficial to bone breaking strength. Other authors have suggested that a daily intake of calcium much higher than the 3.75 g recommended by NRC (1984) was needed for maximal bone mineralisation (Cheng and Coon, 1990). The hens’ calcium requirement for bone maintenance is very small, and was estimated by Roland *et al.,* (1973) as being 0.02% of the diet or less. The problem of bone fragility in laying hens stems from the release of bone reserves for the needs of shell deposition. In Experiment 3, the separate feeding of limestone granules increased tibial breaking strength when measured at 72 weeks of age. Other workers have reported an increase in tibial breaking strength at high calcium intakes (El Boushy and Papadopolos, 1979), and the separate feeding of oyster shell improved medullary bone volumes (Rennie *et al.,* 1997), medullary bone forms the labile calcium reserves.

Phytase supplementation also improved tibial breaking strengths when measured in Experiment 3 at the end of lay. This may have been due to microbial phytase increasing nutrient and mineral availabilities as discussed previously for egg shell density. There are conflicting reports in the literature as to whether phosphorus requirements for bone mineralisation are greater than required for egg production and eggshell density. El Boushy (1979) reported an increase in tibial breaking strength as available phosphorus increased within the range of 2 to 4 g/kg, whereas Whitehead (1994) found no effect on bone composition when feeding

4.5 to 6.0 g total phosphorus/kg. In Experiment 3, there were no effects of increasing available phosphorus contents within the range of 2.3 to 3.4 g/kg (in diets not containing phytase) and within the range 3.0 to 4.4 g/kg, on tibial breaking strength.

CONCLUSIONS

1. High producing brown egg layers consume limestone granules only during the last few hours of the photoperiod. This is the time when hens have an increased calcium requirement for egg shell formation.
2. There were indications that the combination of dietary phytase supplementation and feeding limestone granules reduced feed intakes, and at low feed intakes the dietary crude protein concentration may need to be increased so as to support a high rate of lay.
3. Phytase supplementation of diets, combined with the separate feeding of limestone granules optimised feed conversion efficiencies.
4. Eggshell weights, eggshell densities and tibial breaking strengths at the end of lay were improved by the separate feeding of limestone granules, and by dietary phytase supplementation.
5. Faecal phosphorus concentrations were reduced by the separate feeding of limestone granules, and faecal nitrogen concentrations were reduced by feeding optimum dietary calcium and available phosphorus contents.

IMPLICATIONS OF FINDINGS

The findings suggest that modern brown egg layers are able to tolerate a relatively wide range of calcium to available phosphorus intakes, in terms of egg production and eggshell density. Dietary calcium concentrations should be set taking into account feed intakes, and producers should aim to provide a calcium intake of 5.0 g/bird.day during early lay perhaps increasing to 5.5 g/bird.day during mid-lay. There are additional benefits to egg shell densities and tibial breaking strengths at the end-of-lay from feeding a supplementary source of calcium, yet this is not typical commercial practice for caged layers, or for most free range layers. It would not be possible when feeding caged layers during the late afternoon to distribute limestone granules, or other coarse sources of calcium, using existing feeder equipment, as abrasion causes damage to the chain or auger. Furthermore, this technique would rely on hens selecting limestone grit from within the mash. Major changes in the design of laying cages are being dictated by the Laying Hens Directive (EC Council Directive 1999/74), and it would be opportune to consider approaches for incorporating choice feeding equipment when developing new styles of cages. The work has shown that poultry producers are not reliant on incorporating a high concentration of DCP within the ration, and this may help to reduce phosphorus excretion, and if phytase is used to replace some of the dietary inorganic phosphorus supply then further benefits are likely. Surprisingly, faecal nitrogen contents were reduced by the separate feeding of limestone granules, but further work is required if egg production, shell quality and bone breaking strength are to be optimised in addition to reducing faecal nitrogen excretion.

The project addressed MAFF's policy objectives of matching mineral composition and supply in modern genotypes, and optimising the efficiency of feed energy and protein utilisation. By feeding hens limestone granules they were able to meet their calcium requirements for egg shell formation without resorbing bone, and this improved tibial bone strength at the end of lay. Positive calcium balances in hens fed limestone granules meant that faecal phosphorus concentrations were reduced. Phytase supplementation of diets, combined with the separate feeding of limestone granules optimised feed conversion efficiencies.

POSSIBLE FUTURE WORK

1. To examine and quantify the effects of high calcium intakes on calcium absorption, retention and excretion at different stages of the laying year (beginning of lay, mid lay and end of lay), and to determine the effects on these variables of suddenly withdrawing a supplementary calcium source.
2. To determine whether choice feeding equipment could be successfully incorporated into new style laying cages (as determined by the Laying Hens Directive EC Council Directive 1999/74), and to assess the benefits of choice feeding calcium in these facilities.
3. To determine the hens minimum requirement for available phosphorus in terms of optimising medullary bone volume at the end of lay.

4. To determine the effects of phytase on nutrient and mineral availabilities, so that the dietary supply of nutrients and minerals can better match the hens' requirements for health, maintenance and egg production.

IP, TECHNOLOGY TRANSFER

Technology transfer has been limited to date. An abstract has been submitted for publication in the Proceedings of the Nutritional Society (in press) and a paper is being drafted for publication.

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Phytase supplementation of diets for laying hens. By G.R. BARKLEY, J.M. FORBES & H.M. MILLER, *Centre for Animal Sciences, Leeds Institute for Biotechnology and Agriculture, University of Leeds, Leeds LS2 9JT*

Phosphorus is an essential nutrient required for energy metabolism, bone development, and eggshell formation in the laying hen. Poultry diets largely consist of cereal grains and oil seed meals that contain high concentrations of phytic acid. Phytate phosphorus has previously been relatively unavailable to single stomached animals. Microbial phytase included in the feed increases the availability of phosphorus from phytic acid.

Individually caged Lohmann pullets (48) were fed *ad libitum* diets containing (per kg dry matter) 38g calcium and 3.6g available phosphorus (normal diet) or 24g Ca and 2.3g P (low diet) from 19 to 27 weeks of age. Each of the diets was fed with or without microbial phytase (0.08 g/kg), and with or without access to *ad libitum* particulate limestone, resulting in a 2*2*2 factorial arrangement of treatments.

Treatment	Feed intake (g/d)	Limestone mean intake (g/d)	Body mass (g)	Egg production (%)	Egg mass (g)	Feed conversion efficiency†
Low Ca P	122*	15	1830*	99	64	0.51
Normal Ca P	130*	13	1930*	98	64	0.49
No phytase	127	14	1880	101*	63*	0.50
Phytase	126	14	1880	96*	66*	0.50
No limestone	128	-	1880	99	62*	0.49
Limestone	125	14	1870	99	66*	0.52
SEM	3.2	3.4	30	1.7	0.9	0.013

Only main effects shown. * Pairs of means within columns with an asterisk differ significantly ($P<0.05$).

† Feed conversion efficiency = ((Egg production / 100) *Egg mass) / Feed intake.

The results presented refer to the final week of the trial. Birds fed low levels of calcium and phosphorus ate less feed ($P<0.05$) and weighed less ($P<0.05$) than those fed normal levels. Neither supplementation with phytase nor access to *ad libitum* limestone significantly affected feed intake or body mass. Birds fed phytase supplemented diets laid fewer eggs ($P<0.05$) but heavier eggs ($P<0.05$) resulting in a similar egg mass output to that from the non-supplemented birds. Birds with access to limestone laid larger ($P<0.05$) eggs. (Not shown in the table) Phytase supplementation of the low phosphorus diet increased egg mass ($P<0.05$) from 61 to 67 (SE 1.2) g but phytase supplementation of the normal phosphorus diet did not increase egg mass. Phytase supplementation of the low phosphorus diet increased feed conversion efficiency ($P<0.05$) from 0.48 to 0.53 (SE 0.014), whereas, supplementation of the normal diet reduced feed conversion efficiency ($P<0.05$) from 0.50 to 0.45 (SE 0.014).

Phytase supplementation of a low phosphorus diet would appear to be the optimal feeding regime in terms of egg mass and feed efficiency. Providing *ad libitum* limestone is recommended as this increased egg mass ($P<0.05$). These results highlight the importance of the ratio between calcium and available phosphorus.

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