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# Phosphorus Requirement in Laying Hens

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*Research for development*

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# Phosphorus Requirement in Laying Hens

(Experiment PHF-52, Project Code PA12-51)

The study is performed on request of the Dutch Ministry of Economic Affairs and subsidised by the Dutch Product Board for Poultry and Eggs

Key words: Phosphorus, breed, laying hen, efficiency, performance, requirement

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**PREFACE**

On request of the Dutch Ministry of Economic Affairs, as well as of the Dutch Product Board Poultry and Eggs, this study was performed to investigate the retainable phosphorus requirement and utilisation in laying hens. The modern laying hen has a high egg number and laying persistence, and probably a different P-requirement than the current estimated requirements. Furthermore, due to higher levels of locomotion, laying hens housed in non-cage housing systems might have a better bone development. It is hypothesized that P supply by feed in such systems can be lowered without negative effects on bone quality and production performance. Determination of the P-requirement, -utilisation, and -excretion is important to support the lump phosphorus excretion and to reduce the phosphorus supply by the feed. The study comprises a literature review (Lambert et al., 2014) and an animal experiment. The results of the animal experiment are described in this report. The study is a collaborative project between Schothorst Feed Research and Wageningen Livestock Research.

Laura Star and Marinus van Krimpen
Project leaders

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## 1. Introduction

Phosphorus (P) is a very important mineral in poultry nutrition and is involved in many functions such as bone formation, energy metabolism, cellular structure and egg formation (Ahmadi & Rodehutscord, 2012). However, the majority of the P present in cereal grains and seeds is bound to phytic acid which is highly unavailable to non-ruminant species. As a consequence, dietary inorganic P needs to be added to the diet to meet the hens' P requirements (Boling et al., 2000; Ahmadi & Rodehutscord, 2012). However, due to the growing scarcity of mined phosphate rock, the P supplementation prices are already high and will increase in the future (Neset & Cordell, 2012). Furthermore, excess dietary P is also pointed out to be one of the major causes of eutrophication (Sharpley, 1999; Boling et al., 2000; Ahmadi & Rodehutscord, 2012).

One of the solutions to these issues consists in more accurately meeting the P requirements of the modern laying hens (Snow et al., 2004). The modern laying hens shows high levels of egg production and persistency, and are nowadays mostly housed in non-caged systems. Age and housing system are two important factors whose influence on P requirement and utilization is not clear yet.

Egg-day production and egg quality are altered by age (Bar & Hurwitz, 1987; Bar et al., 1999) and aged hens show a reduced ability to respond to Ca deficiency which might induce a higher depletion of Ca and P from the skeleton (Bar & Hurwitz, 1987). However, the influence of age on P utilization and excretion is subject of discussion and needs to be further explored.

Laying hens housed in alternative systems have a better bone development (Abrahamsson & Tauson, 1995), egg quality (Van den Brand et al., 2004) and seem to have a better P utilization (Neijat et al., 2011). Besides, birds in alternative systems have a higher feed intake. They might therefore be able to support lower dietary P levels without adverse effects on bone and egg quality. A reduction of dietary P levels can be considered as an option to reduce dependency of poultry farmers on inorganic P and to reduce P excretion by laying hens.

## 2. Objective

It was hypothesized that P supply by feed in alternative housing systems can be lowered without negative effects on bone quality and production performance. Therefore, the objectives of the current study were 1) to update the retainable phosphorus (rP) needs of two modern laying hen breeds from 36 to 90 weeks of age housed in an aviary system, 2) to investigating the influence of dietary rP levels on Ca and P content in eggs, manure, carcasses and bones.

## 3. Materials and Methods

Two experiments were conducted from September 2012 to October 2013 in the facilities of Schothorst Feed Research (Lelystad, The Netherlands). All animal procedures were approved by the Institutional Animal Care and Use Committee. The first experiment was designed to determine the P requirements of modern laying hens. The second experiment aimed at determining the P efficiency of laying hens as affected by age, breed and dietary P levels.

## *3.1. Experiment 1*

### *3.1.1. Birds and housing*

The experiment was performed with 12,700 Dekalb White and LSL Classic laying hens (6,350 hens per breed; hatchery Het Anker, Heteren, The Netherlands) that were housed from 17 to 90 weeks of age in a ventilated aviary layer building (Vencomatic, Eersel, The Netherlands) in which a daily photoperiod of 16L:8D was applied (layer facility of Schothorst Feed Research; house 4.5). The hens were randomly placed in 36 experimental units of 330 individuals per pen with chopped straw as litter (20.0 m² floor space; 36.8 m² living area).The hens were randomly assigned to 1 of 6 experimental diets. Three replicates were used for each dietary treatment × breed combination. The trial started when hens were 36 weeks of age. Feed and water were provided ad libitum. Daily records were kept of all routine study activities, health disorders and of mortality.

### *3.1.2. Treatments*

The experiment consisted of six dietary treatments differing in level of retainable phosphorus (rP) levels. The six wheat-based experimental diets were similar except for the phosphorus level, which was adjusted by replacing monocalcium phosphate at the expense of diamol. The dietary rP level was decreased over the five periods (Period 1: 36-45 wk; Period 2: 46-54 wk; Period 3: 55-65 wk; Period 4: 66-75 wk; Period 5: 76-90 wk) as presented in Table 1. The dietary rP levels investigated were based on current practice. Treatment 1 was the highest level observed and treatment 4 was the lowest level fed by Dutch feed mills. Treatment 5 and 6 received lower rP levels than in current practice. All other nutrients were comparable between treatments and between periods. Only the Ca level was increased with increasing age. No phytase was added to the diets.

Table 1. Completely randomized block design for retainable phosphorus levels (g rP/kg) fed to laying hens from 36 to 90 weeks of age (Experiment 1)

| Dietary treatment | Period 1<br>36-45 wk | Period 2<br>46-54 wk | Period 3<br>55-65 wk | Period 4<br>66-75 wk | Period 5<br>76-90 wk |
|---|---|---|---|---|---|
| 1 | 3.2 | 3.0 | 3.0 | 2.8 | 2.8 |
| 2 | 3.0 | 2.8 | 2.8 | 2.6 | 2.6 |
| 3 | 2.8 | 2.8 | 2.8 | 2.6 | 2.6 |
| 4 | 2.8 | 2.6 | 2.6 | 2.4 | 2.4 |
| 5 | 2.6 | 2.4[1] | 2.6 | 2.4 | 2.4 |
| 6 | 2.6 | 2.4 | 2.4 | 2.4 | 2.4 |

[1] Treatment 5 received feed containing 2.4 g rP/kg instead of 2.6 g rP/kg from 46-54 wk of age. The next period was therefore adapted and the birds received a diet with 2.6 g rP/kg instead of 2.4 g rP/kg.

*3.1.3. Experimental diets*

The composition of the diets with the highest and lowest rP level are given in Annex 1. Experimental diets for Experiment 1 were produced in the feed mill of ABZ Diervoeding (Nijkerk, The Netherlands) and stored in silos at the facility of SFR. The diets with the highest and lowest rP level were delivered. The levels in-between were mixed in the right dose at every feed supply by an automated weighing/mixing unit (Table 2). A new batch of each feed was delivered approximately every three weeks.

Table 2. Diet codes and mixing schedule (Experiment 1)

| Dietary treatment | Period 1<br>36-45 wk | Period 2<br>46-54 wk | Period 3<br>55-65 wk | Period 4<br>66-75 wk | Period 5<br>76-90 wk |
|---|---|---|---|---|---|
| 1 | A | C | C | E | E |
| 2 | 67% A+33% B | 67% C+33% D | 67% C+33% D | 50% E +50% F | 50% E +50% F |
| 3 | 33% A+67% B | 67% C+33% D | 67% C+33% D | 50% E +50% F | 50% E +50% F |
| 4 | 33% A+67% B | 33% C+67% D | 33% C+67% D | F | F |
| 5 | B | D | 33% C+67% D | F | F |
| 6 | B | D | D | F | F |

*3.1.4. Diet analysis*

A sample of each batch of each diet (500 g) was collected from the silo and pooled per diet from 36-45, 46-65, 66-90 weeks of age. Pooled samples were used to determine moisture, ash, crude protein, crude fat, crude fibre, P and Ca.

*3.1.5. Measurements*

Laying rate and mortality were recorded daily, while feed intake and egg weight were recorded weekly. Feed conversion rate and egg mass were calculated weekly based on measured parameters. Twenty birds per pen were weighed at 34, 45, 65, 75 and 90 weeks of age. Twenty eggs per pen were collected at 35, 45, 65, 75 and 90 weeks of age. Eggs collected at 35 weeks of age were sent to Kwetters (Veen, The Netherlands) and eggs collected at 45, 65, 75 and 90 weeks of age were sent to Controlebureau Pluimvee, Eieren en Eiproducten (CPE, Barneveld, The Netherlands) to be analysed for eggshell breaking strength and Haugh units.

## ***3.2. Experiment 2***

*3.2.1. Birds and housing*

Additionally, 36, 120, 120, 50, 90 and 90 laying hens were removed from layer facility 4.5 and placed in digestibility cages at, respectively, 35, 45, 61, 61, 75 and 90 weeks of age. The size of the digestibility cages was 0.88×0.70 m. Each round was lasting 10 days with 7 days of adaptation and 3 days of excreta collection. During this period, feed and water were provided ad libitum.

### 3.2.2. Treatments

The design of the experiment is presented in Table 3. At 35 weeks of age, 6 hens per cage and 1 replicate were used while at 45, 61, 75 and 90 weeks of age, 5 hens per cage and 3 replicates were used. The treatments corresponded to the treatments in Experiment 1.

Table 3. Completely randomized block design for retainable phosphorus levels (g rP/kg) fed to laying hens housed in digestibility cages at different ages (Experiment 2)

| Dietary treatment[1] | Round 1 35 wk | Round 2 45 wk | Round 3 61 wk | Round 3bis 61 wk[2] | Round 4 75 wk | Round 5 90 wk |
|---|---|---|---|---|---|---|
| 1 | Standard feed | 3.2 | 3.0 | 2.4 | 2.8 | 2.8 |
| 2 | Standard feed | 3.0 | 2.8 | --- | 2.6 | 2.6 |
| 4 | Standard feed | 2.8 | 2.6 | --- | --- | --- |
| 6 | Standard feed | 2.6 | 2.4 | 3.0 | 2.4 | 2.4 |

[1] Treatments corresponded with the treatments given in Table 1. Treatments 3 and 5 were not included.
[2] Round 2 was incorrectly implemented: hens at high rP level in the layer facility were assigned to low rP level in the digestibility cages and vice et versa (see Annex 4). In order to correct this, round 3bis was performed with 4 treatments and replicates not evenly distributed between the breeds. For Treatment 1, three replicates of Dekalb and two replicates of LSL were applied, for Treatment 6, three replicates of LSL and two replicates of Dekalb were applied.

### 3.2.3. Experimental diets

Experimental diets for Experiment 2 were produced by a specialized feed mill of Research Diet Services (RDS, Wijk bij Duurstede, The Netherlands). In order to assure a similar diet composition per round, apart from P, one basal mixture of feed was produced. This batch was sufficiently large to obtain all experimental diets. A sub-batch of this mixture was used for the production of the experimental diets with added mono calcium phosphate (MCP[1]) and diamol[2]. The remaining mixture was supplemented only with diamol to obtain the low P diet. Diets were bagged and labelled before sending to SFR.

### 3.2.4. Diet analysis

During bagging of the diets a composite sample was taken per diet. From this composite sample, two sub-samples (500 g) were taken. One sample was used by SFR for proximate analysis. The remaining sample was stored frozen (-20°C) at SFR. Samples were used to determine moisture, P and Ca.

### 3.2.5. Measurements

Excreta were collected per cage in the last three days of each experimental round. Each day excreta were collected semi-quantitatively during a 24-hour period. Excreta samples were freeze-dried, grounded and analysed for moisture, Ca, P and water-soluble P.

[1] Aliphos® monocal, produced by Tessenderlo, Brussels, Belgium.
[2] Diamol, produced by Damolin A/s, Fur, Denmark.

At the end of each round laying hens were euthanized by an intracardial injection with an euthanasia solution (T61). Subsequently, the middle toes of each bird were removed and excised between the second and third tarsal bones. Toes were collected per cage, autoclaved and cleaned. Clean bones were dried and ashed, followed by wet-ashing of bone ash with $HNO_3$ and $H_2O_2$ before being analysed for Ca and P content. This was performed for each round.

Subsequently, the abdominal cavity was opened and the contents of 20 cm of the ileum, beginning 1 cm proximal of the ileo-caecal junction, were collected and pooled per cage. Ileal chyme samples were freeze-dried, grinded and analysed for moisture, P and water-soluble P. Ileal chyme collection was only performed at 35 weeks of age.

The remaining carcasses of the birds were stored frozen (-20°C) at SFR until further processing. Laying hens carcasses of three birds per pen were pooled and grounded with a meat mixer in order to obtain a homogenous sample. The ground sample was mixed with 300 g of celite 545. After freeze-drying, samples were analysed for protein, fat and ash content. This was followed by wet-ashing of bone ash with $HNO_3$ and $H_2O_2$ before being analysed for Ca and P content. This was performed for each round.

Ten eggs per cage were collected. Yolk, albumen and egg shell were separated and pooled per cage. After freeze-drying, samples were analysed for moisture, P and Ca content. This was performed for each round. To calculate the P content in the fresh egg (g/kg) the following equation was used:

$$P_{egg} = 0.11 * P_{shell} * \frac{1000 - Moist_{Shell}}{1000} + 0.32 * P_{yolk} * \frac{1000}{1000 - MoistT_{yolk}} * \frac{1000 - Moist_{yolk}}{1000} + 0.57 * P_{alb} * \frac{1000}{1000 - MoistT_{alb}} * \frac{1000 - Moist_{alb}}{1000}$$

With: 0.11, 0.32 and 0.57 = percentage of, respectively, shell, yolk and albumen in an egg (Johnson, 2000).

$P_{shell}$, $P_{yolk}$ and $P_{alb}$ = P content (g/kg DM) in, respectively, the shell, the yolk and the albumen.

$Moist_{shell}$, $Moist_{yolk}$, $Moist_{albumen}$ = Moisture content (g/kg) of, respectively, the shell, the yolk and the albumen after freeze-drying.

$MoistT_{yolk}$, $MoistT_{alb}$ = Moisture content (g/kg) of, respectively, the yolk and the albumen before freeze-drying

All analyses were performed in the lab of Schothorst Feed Research.

Blood serum was collected from each bird and stored frozen (-20°C) at SFR until further processing. No financial support was obtained to analyse these samples.

### *3.3. Statistical analysis*

The experimental data were statistically analysed by analysis of variance (ANOVA) using Genstat statistical software. The following model was used for analyses of the effect of retainable P and differences between treatments for Experiment 1 (6 dietary treatments) and Experiment 2 (3 to 4 dietary treatments):

$$Y_{ijk} = \mu + Breed_i + Diet_j + interaction_{ij} + e_{ijk}$$

in which:

| | |
|---|---|
| $Y_{ij}$ | = dependent variable |
| $\mu$ | = overall mean |
| $Breed_i$ | = effect of breed (i = Dekalb White, LSL Classic) |
| $Diet_j$ | = effect of rP (j = 1…4) or (j = 1...3) and of all treatments (j= 1…6) |
| $e_{ijk}$ | = residual error |

For Experiment 1, some treatments were supplied the same rP level within a sub-period and could be used as replicates. The same model as above was used, but with inclusion of testing for linearity.

Treatment means were compared by least significant difference (LSD). Values with $P \leq 0.05$ were considered statistically significant, whereas $0.05 < P \leq 0.10$ was considered a near-significant trend. Statistics were based on two-sided tests.

## 4. Results of Experiment 1 – production performance

### *4.1. Feed analysis*

The results for the chemical analyses of the layer diets in Experiment 1 (bulk; based on the pooled samples of all batches of 36-45, 46-65 and 66-90 weeks of age) and Experiment 2 (bags) are presented in Tables 4a and 4b, respectively. In general, analysed crude protein levels were lower than the calculated values (max. 0.8 percentage points). The analysed crude fat, crude fibre and P levels were comparable to the calculated values. The analysed Ca levels were in general higher than the calculated values. For diet A and B and diet E and F the Ca levels were in line with each other. For diet C and D the difference in Ca level was totally different, with a too low level in diet D. However, both duplicate values were similar in diet D. Also the analysed ash level is lower in diet D, an indirect indication of the low Ca level. The difference in Ca level is probably caused by an analytical inaccuracy likely related to homogeneity of the sampled diets and coarse limestone.

Table 4a. Analysed nutrient composition (g/kg) of the experimental diets (pooled samples; Experiment 1)

| **Diets Exp.1[1,2]** | Moisture | Ash | Crude protein | Crude fath | Crude fibre | P | Ca |
|---|---|---|---|---|---|---|---|
| A | 114 (110) | 125 (112) | 154 (162) | 37 (35) | 38 (35) | 6.0 (5.8) | 38.6 (34.0) |
| B | 115 (110) | 124 (113) | 158 (162) | 36 (35) | 37 (35) | 5.1 (4.7) | 36.9 (34.1) |
| C | 113 (114) | 128 (126) | 152 (159) | 34 (33) | 37 (37) | 5.4 (5.5) | 42.1 (37.9) |
| D | 117 (114) | 110 (126) | 151 (159) | 34 (33) | 38 (37) | 4.6 (4.6) | 31.8 (37.9) |
| E | 110 (114) | 144 (130) | 151 (158) | 35 (33) | 36 (37) | 5.1 (5.2) | 45.5 (39.7) |
| F | 111 (114) | 142 (130) | 159 (158) | 35 (33) | 36 (37) | 4.8 (4.6) | 44.9 (39.6) |

[1] Diet A and B were fed from 36-45 weeks of age, diet C and D were fed from 46-65 weeks of age and diet E and F were fed from 66-90 weeks of age.
[2] Calculated values between brackets.

The analysed Ca level in the diets for Experiment 2 differ also, especially for Round 3. This difference has probably the same cause as the difference in Ca level found in the diets for Experiment 1.

Table 4b. Nutrient composition (g/kg) of the experimental diets (Experiment 2)

| **Diets Exp.2** | Moisture | P | Ca |
|---|---|---|---|
| Round 2[1] | | | |
| A | 111 | 4.9 | 32.1 |
| B | 106 | 5.1 | 31.8 |
| C | 108 | 5.4 | 33.5 |
| D | 110 | 5.4 | 36.0 |
| Round 3 | | | |
| A | 105 | 5.7 | 39.7 |
| B | 106 | 5.6 | 35.9 |
| C | 105 | 5.2 | 43.2 |
| D | 107 | 5.2 | 32.7 |
| Round 4 | | | |
| A | 105 | 4.9 | 40.0 |
| B | 104 | 4.8 | 40.2 |
| C | 107 | 4.5 | 40.6 |
| Round 5 | | | |
| A | 107 | 4.8 | 38.7 |
| B | 109 | 4.3 | 35.3 |
| C | 106 | 4.2 | 42.5 |

[1] Round 2 was incorrectly implemented: diet A should have the highest P level and diet D the lowest level, but this was opposite. Therefore, hens at high rP level in the layer facility were assigned to low rP level in the digestibility cages and vice et versa (see Annex 4).

### *4.2. Effect of retainable P level on production performance*

The results for production performance (feed intake, laying rate, egg weight, egg mass and feed conversion ratio (FCR)) for hens fed diets differing in retainable P level in the period 36-90 weeks of age are given in Table 5. In this experiment, six treatments were considered over the whole production period (36-90 weeks of age). The results for production performance in the sub-periods 36-45, 46-54, 55-65, 66-75 and 76-90 weeks of age are given in Annex 2. Within each sub-period, some treatments were supplied the same rP level and could be used as replicates. For each period, a table with statistical analysis of the six treatments and of the combined treatments is given. Only a few interactions were observed in the experiment and are described in paragraph 4.4. Breed effects on production performance are presented in paragraph 4.3.

Table 5. Production performance of laying hens fed diets differing in retainable phosphorus levels from 36-90 weeks of age (Experiment 1)

| **36-90 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Dietary treatment | | | | | | |
| 1. 3.2-3.0-3.0-2.8 | 118 | 91.5 | 62.2 | 57.0 | 2.064 | 7.2 |
| 2. 3.0-2.8-2.8-2.6 | 118 | 91.4 | 62.5 | 57.1 | 2.070 | 6.8 |
| 3. 2.8-2.8-2.8-2.6 | 119 | 91.6 | 62.5 | 57.3 | 2.065 | 6.1 |
| 4. 2.8-2.6-2.6-2.4 | 118 | 91.7 | 62.3 | 57.1 | 2.055 | 6.2 |
| 5. 2.6-2.4-2.6-2.4 | 118 | 92.4 | 62.2 | 57.5 | 2.052 | 6.3 |
| 6. 2.6-2.4-2.4-2.4 | 117 | 91.4 | 62.3 | 57.0 | 2.060 | 7.1 |
| *P-value* | *0.430* | *0.865* | *0.383* | *0.942* | *0.700* | *0.829* |
| *LSD* | *2.0* | *1.66* | *0.33* | *1.17* | *0.026* | *2.18* |

### *4.2.1. Feed intake*

The dietary rP level did not affect feed intake. Laying hens fed the lowest dietary rP level had a similar feed intake compared to hens fed higher dietary rP levels. This was observed for the overall experimental period and each sub-period.

### *4.2.2. Laying rate*

When comparing the six dietary treatments, dietary rP level did not affect laying rate. This was observed for the overall experimental period and each sub-period. However, at weeks 36-45, when treatments 3 and 4 (2.8 g rP/kg) were combined and treatments 5 and 6 (2.6 g rP/kg), there was a significant difference (P<0.05) observed (Annex 2). Hens fed the lowest rP level (2.6 g rP/kg) showed a higher laying rate than hens fed the highest rP level (+1.4%).

### *4.2.3. Egg weight*

Dietary rP level had a minor effect on egg weight. At 36-45 weeks of age and 55-65 weeks of age, a trend was observed (P=0.070 and P=0.065, respectively). After combining the treatments with the same rP level, the difference was significant (P<0.05 for both periods; Annex 2). At weeks 36-45, hens fed the highest rP level (3.2 g rP/kg) had a lower egg weight compared to hens fed 2.8 g rP/kg or 2.6 g rP/kg (-0.5 g compared to 2.6 g rP/kg). At 55-65 weeks of age, egg weight of hens fed 2.8 g rP/kg was significantly higher (+0.3 g) than hens fed 2.6 g rP/kg, but was similar compared to hens fed the lowest rP level (2.4 g rP/kg).

### *4.2.4. Egg mass*

When comparing the six dietary treatments, dietary rP level did not affect egg mass. This was observed for the overall experimental period and each sub-period. However, at weeks 36-45, when treatments 3 and 4 (2.8 g rP/kg) were combined and treatments 5 and 6 (2.6 g rP/kg), there was a significant difference (P<0.05) observed (Annex 2). Hens fed the lowest rP level (2.6 g rP/kg) showed a higher egg mass (+1.1 g/d) than hens fed the highest rP level (3.2 g rP/kg).

*4.2.5. Feed conversion ratio*

The dietary rP level did not affect FCR. Laying hens fed the lowest dietary rP level showed a similar FCR compared to hens fed higher dietary rP levels. This was observed for the overall experimental period and each sub-period.

*4.2.6. Mortality*

The dietary rP level did not affect mortality. The average mortality rate was 6.6% from 36-90 weeks of age (range 6.1-7.2%).

Treatments 1 to 4 were based on rP levels used in current practise, i.e. the highest and lowest levels fed by Dutch feed mills. Treatments 5 and 6 were fed lower levels and it was expected to induce a small P deficiency in the birds. However, from 36 to 90 weeks of age, birds responded well to the lowest rP levels in the diet. Therefore, a rP level of 2.6 g/kg seems to be sufficient for maximal egg number, egg weight, egg mass and feed conversion ratio from 36-45 weeks of age. From 45-90 weeks of age, a rP level of 2.4 g/kg seems to be sufficient for maximal egg number, egg weight, egg mass and feed conversion ratio.

## *4.3. Breed differences on production performance*

Two breeds were used in the experiment; Dekalb White and LSL Classic. The results for production performance (feed intake, laying rate, egg weight, egg mass and feed conversion ratio (FCR)) for each breed in the period 36-90 weeks of age are given in Table 6. The results for production performance for each breed in the sub-periods 36-45, 46-54, 55-65, 66-75 and 76-90 weeks of age are given in Annex 3.

Table 6. Production performance of Dekalb White and LSL Classic laying hens from 36-90 weeks of age (Experiment 1)

| **36-90 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Breed | | | | | | |
| Dekalb | 120[b] | 92.1 | 62.34 | 57.4 | 2.078[b] | 6.7 |
| LSL | 116[a] | 91.3 | 62.36 | 56.9 | 2.044[a] | 6.5 |
| *P-value* | *<0.001* | *0.082* | *0.868* | *0.133* | *<0.001* | *0.728* |
| *LSD* | *1.2* | *0.96* | *0.19* | *0.68* | *0.015* | *1.26* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

*4.3.1. Feed intake*

Feed intake was significantly higher for Dekalb White than LSL Classic laying hens at each age ($P<0.001$). On average the feed intake of the Dekalb White laying hens was 4 g/d higher compared to the LSL Classic hens.

*4.3.2. Laying rate*

Breeds differed in laying rate in the first two periods (36-45 and 46-54). Dekalb White hens produced significantly more eggs (+1.4% and +1.6%, respectively) during the first two periods ($P<0.05$) than LSL Classic hens. After 55 weeks of age, the Dekalb White hens produced numerically more eggs, where differences between breeds became smaller with aging of the hens. Over the entire production period, Dekalb White hens had a tendency to produce more eggs ($P=0.082$) than LSL hens.

*4.3.3. Egg weight*

Dekalb White hens laid significantly heavier eggs compared to LSL Classic hens from 36-45 weeks of age (+0.6 g) and 46-54 weeks of age (+0.3 g). However, after 55 weeks of age, the difference was not significant and at 76-90 weeks of age, LSL Classic hens had a significantly higher egg weight (+0.5 g). Over the entire production period, egg weight did not differ between the breeds.

*4.3.4. Egg mass*

During the first two periods (36-45 and 46-54 weeks of age), laying rate and egg weight were significantly higher for Dekalb White hens, resulting in a significantly higher egg mass (+1.3 g/d for both periods). At 55-65 weeks of age, a trend was observed, where Dekalb White hens had a higher egg mass ($P=0.095$) compared to LSL Classic hens. After 66 weeks of age, the difference was not significant and over the entire production period (36-90 weeks of age), both breeds showed a comparable egg mass.

*4.3.5. Feed conversion ratio*

As stated previously, feed intake was higher for Dekalb White hens for each period and over the entire production period. During the first two periods, egg mass was also significantly higher resulting in a comparable FCR between breeds. However, for the other periods, Dekalb White hens had a higher feed intake but not a higher egg mass anymore, resulting in a higher FCR for Dekalb White hens compared to LSL Classic hens. Over the entire production period (36-90 weeks of age), Dekalb White hens had a higher FCR (+0.034; $P<0.001$) compared to LSL Classic hens.

*4.3.6. Mortality*

Breeds did not differ in mortality rate. For period 36-90 weeks of age, mortality was 6.7% for Dekalb White hens comparable to 6.5% for LSL Classic hens.

Dekalb White hens showed higher egg performance levels from 36-54 weeks of age with a higher laying rate, egg weight and egg mass. However, feed intake was also higher, resulting in a comparable FCR. After 55 weeks of age, LSL Classic hens had a lower feed intake and a similar laying rate, egg weight and egg mass compared to Dekalb White hens. As a consequence, from 36-90 weeks of age, Dekalb White hens had higher feed intake, similar egg mass and therefore higher FCR. No difference in mortality was observed between breeds.

## 4.4. *Breed × retainable phosphorus level interactions*

### *4.4.1. Laying rate*

A breed × rP level interaction was found for laying rate at 46-54 weeks of age ($P<0.05$; Figure 1). Although there was no significant difference in laying rate when hens were fed different rP levels at 46-54 weeks of age (Annex 2), laying rate of each breed was affected by dietary rP level. Dekalb White hens had a lower laying rate at 2.6 g rP/kg compared to 3.0 g rP/kg, whereas LSL Classic hens had a higher laying rate at 2.4 and 2.6 g rP/kg compared to 3.0 g rP/kg.

### *4.4.2. Egg mass*

A breed × rP level interaction was also observed for egg mass from 46-54 weeks of age ($P<0.05$; Figure 2). Egg mass of Dekalb white laying hens was not affected by the rP level, but a 3.0 g rP/kg level reduced egg mass of LSL Classic hens compared to 2.4 and 2.6 g rP/kg levels.

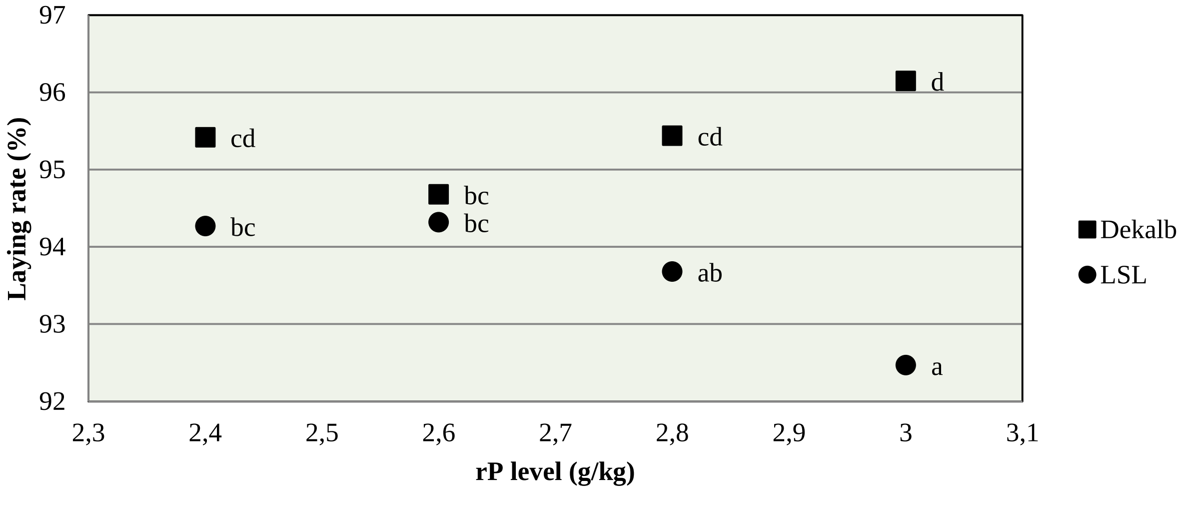


Figure 1. Laying rate (%) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels from 46-54 weeks of age (Experiment 1). [a-d] Values without a common superscript within a column differ significantly ($P\leq0.05$).

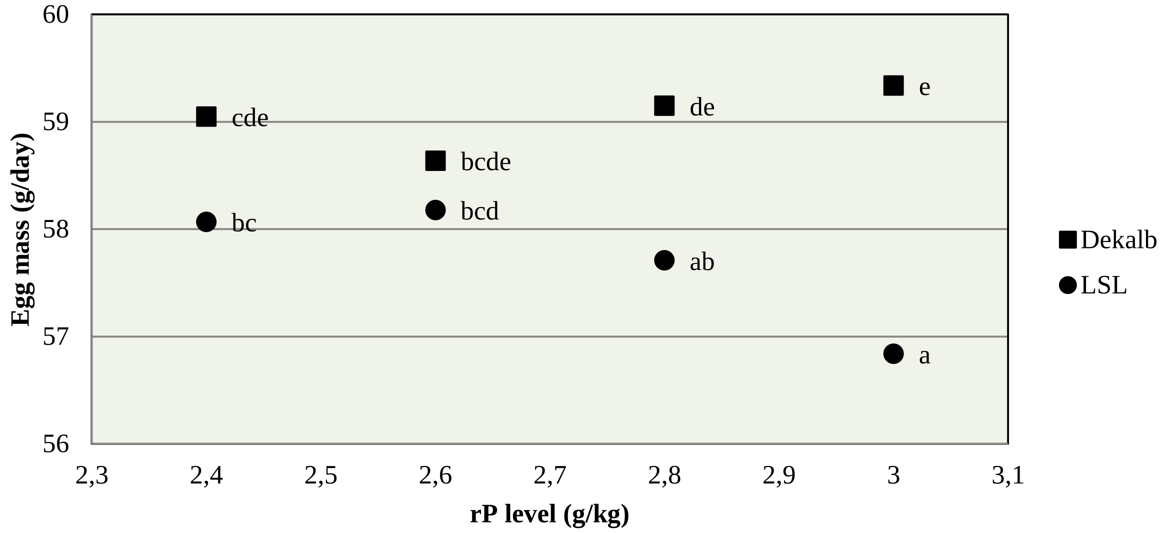


Figure 2. Egg mass (g/d) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels from 46-54 weeks of age (Experiment 1). ). $^{a-e}$ Values without a common superscript within a column differ significantly ($P \leq 0.05$).

## *4.5. Effect of retainable P level on egg and shell quality*

The results for egg and egg shell quality at 35, 45, 65, 75 and 90 weeks of age are presented in Table 7 (shell breaking strength) and Table 8 (Haugh units). No interactions between breed and dietary treatment were found. Therefore, only main effects are presented.

Eggs at 35 weeks of age were analysed by Kwetters, and eggs at 45, 61, 75 and 90 weeks of age were analysed by CPE.

### *4.5.1. Breaking strength*

Breaking strength was not affected by dietary rP level at any age. LSL Classic laying hens produced eggs with a significantly higher breaking strength at 35 weeks of age (+1.77 N; $P<0.05$) compared to Dekalb White hens. The overall higher breaking strength observed at 45 weeks of age compared to 35 weeks of age, was not as expected and was probably due a difference between the analysing companies.

### *4.5.2. Haugh units*

Haugh units were not affected by dietary rP level. At week 75, Dekalb White laying hens laid eggs with significantly higher Haugh units (+1.94; $P<0.05$). At week 90, a trend was observed, where Dekalb White hens produced eggs with higher Haugh units (+1.36: $P=0.100$). The overall lower Haugh unit observed at 45 weeks of age compared to 35 weeks of age, was not as expected and can be partly explained by a difference between the analysing companies.

Table 7. Effect of dietary retainable phosphorus (P) level on egg shell breaking strength (N) in eggs from Dekalb White and LSL Classic laying hens at 35, 45, 65, 75 and 90 weeks of age (Experiment 1)

| **Treatment** | Age in weeks | | | | |
|---|---|---|---|---|---|
| | 35 | 45 | 65 | 75 | 90 |
| Dietary treatment | | | | | |
| 1. 3.2-3.0-3.0-2.8 | 45.6 | 52.6 | 45.9 | 40.5 | 35.3 |
| 2. 3.0-2.8-2.8-2.6 | 47.6 | 51.3 | 43.5 | 40.2 | 34.4 |
| 3. 2.8-2.8-2.8-2.6 | 46.3 | 51.8 | 44.3 | 40.3 | 35.5 |
| 4. 2.8-2.6-2.6-2.4 | 46.9 | 51.7 | 44.2 | 41.1 | 35.3 |
| 5. 2.6-2.4-2.6-2.4 | 46.7 | 51.2 | 43.5 | 40.9 | 35.6 |
| 6. 2.6-2.4-2.4-2.4 | 44.6 | 50.2 | 44.7 | 42.2 | 35.8 |
| *P-value* | *0.170* | *0.925* | *0.499* | *0.524* | *0.903* |
| *LSD* | *2.40* | *4.59* | *2.77* | *2.38* | *2.52* |
| Breed | | | | | |
| Dekalb | 45.4[b] | 50.6 | 44.3 | 41.2 | 35.5 |
| LSL | 47.2[a] | 52.3 | 44.3 | 40.5 | 35.2 |
| *P-value* | *0.015* | *0.212* | *0.962* | *0.336* | *0.667* |
| *LSD* | *1.39* | *2.65* | *1.60* | *1.38* | *1.45* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).

Table 8. Effect of dietary retainable phosphorus (P) levels on Haugh units in eggs from Dekalb White and LSL Classic laying hens at 35, 45, 65, 75 and 90 weeks of age (Experiment 1)

| **Treatment** | Age in weeks | | | | |
|---|---|---|---|---|---|
| | 35 | 45 | 65 | 75 | 90 |
| Dietary treatment | | | | | |
| 1. 3.2-3.0-3.0-2.8 | 74.0 | 70.8 | 83.5 | 78.4 | 78.2 |
| 2. 3.0-2.8-2.8-2.6 | 74.9 | 70.9 | 81.8 | 80.1 | 75.1 |
| 3. 2.8-2.8-2.8-2.6 | 75.0 | 71.1 | 83.2 | 77.6 | 74.7 |
| 4. 2.8-2.6-2.6-2.4 | 72.8 | 71.6 | 82.7 | 78.7 | 76.3 |
| 5. 2.6-2.4-2.6-2.4 | 73.0 | 70.8 | 83.0 | 77.9 | 76.8 |
| 6. 2.6-2.4-2.4-2.4 | 75.1 | 70.8 | 81.7 | 80.2 | 77.2 |
| *P-value* | *0.118* | *0.896* | *0.939* | *0.376* | *0.151* |
| *LSD* | *2.17* | *1.57* | *2.16* | *3.06* | *2.85* |
| Breed | | | | | |
| Dekalb | 73.8 | 70.7 | 83.0 | 79.8[a] | 77.1 |
| LSL | 74.4 | 71.3 | 82.3 | 77.8[b] | 75.7 |
| *P-value* | *0.340* | *0.171* | *0.539* | *0.032* | *0.100* |
| *LSD* | *1.25* | *0.90* | *1.24* | *1.77* | *1.64* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).

### 4.6. *Body weight*

The results for body weight at 34, 45, 65, 75 and 90 weeks of age are presented in Table 9. No interaction effects between breed and dietary treatment were found. Therefore, only the main effects are presented.

Dietary rP level did not affect body weight of laying hens at any age. At 65, 75 and 90 weeks of age, Dekalb White hens had a significantly higher body weight than LSL Classic hens (respectively, +65 g, +73 g and +43 g; P<0.001, P<0.01 and P<0.05).

Table 9. Body weight (kg) of Dekalb White and LSL Classic laying hens at 34, 45, 65, 75 and 90 weeks of age (Experiment 1)

| **Treatment** | Age in weeks | | | | |
|---|---|---|---|---|---|
| | 34 | 45 | 65 | 75 | 90 |
| Dietary treatment | | | | | |
| 1. 3.2-3.0-3.0-2.8 | 1.596 | 1.613 | 1.629 | 1.720 | 1.761 |
| 2. 3.0-2.8-2.8-2.6 | 1.603 | 1.673 | 1.617 | 1.719 | 1.720 |
| 3. 2.8-2.8-2.8-2.6 | 1.602 | 1.647 | 1.628 | 1.778 | 1.739 |
| 4. 2.8-2.6-2.6-2.4 | 1.566 | 1.632 | 1.628 | 1.743 | 1.758 |
| 5. 2.6-2.4-2.6-2.4 | 1.587 | 1.645 | 1.631 | 1.725 | 1.722 |
| 6. 2.6-2.4-2.4-2.4 | 1.582 | 1.629 | 1.629 | 1.717 | 1.708 |
| *P-value* | *0.397* | *0.290* | *0.990* | *0.551* | *0.433* |
| *LSD* | *0.04* | *0.05* | *0.04* | *0.08* | *0.06* |
| Breed | | | | | |
| Dekalb | 1.590 | 1.649 | 1.660[a] | 1.770[a] | 1.756[a] |
| LSL | 1.588 | 1.630 | 1.595[b] | 1.697[b] | 1.713[b] |
| *P-value* | *0.891* | *0.211* | *<0.001* | *0.003* | *0.023* |
| *LSD* | *0.02* | *0.03* | *0.03* | *0.04* | *0.04* |

[a-c] Values without a common superscript within a column differ significantly (P≤0.05).

## 5. Results of Experiment 2 – phosphorus in eggs, manure and carcasses

### 5.1. *Round 1 – 35 weeks of age*

At 35 weeks of age, Round 1 for P in eggs, manure and carcasses was performed. This included only six digestibility cages in the experiment, because the birds did not receive any dietary treatment yet. This measurement was only performed to have a reference value, therefore no statistical analysis was performed. However, the analysis of ileum digesta was only performed at Round 1 and the results are shown in Table 10.

Difference in total P between ileal and faecal digesta is on average 4.15 g/kg. This difference is due to the large amount of soluble P in faecal digesta. The soluble P fraction is related to the resorption of medullary bone and is excreted by the uric acid fraction in the faeces.[3] The results of the P content not related to egg shell formation (=P–soluble P) is similar between ileal and faecal digesta. Furthermore, this indicates that 33% of the P content of the faeces is soluble P, and related to egg shell formation.

Table 10. Phosphorus (P) and soluble phosphorus (P) in the ileal and faecal digesta from Dekalb White and LSL Classic laying hens at 35 weeks of age (Experiment 2)

| **Round 1** | Ileal | | Faecal | |
|---|---|---|---|---|
| **35 weeks of age** | P (g/kg DM) | Soluble P (g/kg DM) | P (g/kg DM) | Soluble P (g/kg DM) |
| Average | 9.53 | 0.75 | 13.68 | 4.55 |
| Dekalb | 9.53 | 0.00 | 13.79 | 4.63 |
| LSL | 9.52 | 1.57 | 13.56 | 4.47 |

### *5.2. Round 2 – 45 weeks of age*

Dietary rP levels had no significant effect on egg characteristics at 45 weeks of age (Table 11). The P content of the egg was mainly found in the yolk, and for this egg component a near-significant trend was observed for P content (P=0.081). The P content of the yolk increased with a decrease in dietary rP levels. This was not as expected, and was an indication that birds had received the wrong diets. The treatments that should have received a diet with low rP received the high rP diet and vice et versa (see Annex 4).

Dietary rP level had an effect on faecal characteristics at 45 weeks of age (Table 12). Hens fed by mistake the lowest rP levels (treatment with 3.2 and 3.0 g rP/kg) had significantly lower P content in manure than hens fed the highest rP levels (treatment with 2.6 and 2.8 g rP/kg; P<0.001). Hens fed 2.8 g rP/kg (treatment with 3.0 g rP/kg) had a significantly lower soluble P content in manure than hens fed other treatments (P<0.05). A near-significant trend (P=0.060) was observed for Ca content in manure, with a higher Ca content for hens fed 3.0 g rP/kg (treatment with 2.8 g rP/kg) compared to the other treatments.

[3] There is increased demand for calcium during the period of egg shell formation in the shell gland. Because this usually occurs during the night when supply of calcium from the digestive system is low, a high proportion of shell calcium comes from resorbed medullary bone (Whitehead, 2004). Calcium is stored in bones as calcium phosphate, so both Ca and P are resorbed at the same time from the medullary bone. Calcium is used for egg shell formation and P is excreted by uric acid.

Table 11. Phosphorus (P) and calcium (Ca) content (g/kg DM) in egg shell, egg yolk, egg albumen and total egg (g/kg) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 45 weeks of age (Experiment 2)

| **Round 2** | Egg shell | | Egg yolk | | Egg Albumen | | Total egg | |
|---|---|---|---|---|---|---|---|---|
| **45 weeks of age** | P | Ca | P | Ca | P | Ca | P | Ca |
| Dietary treatment[1] | | | | | | | | |
| 1. 3.2 | 1.19 | 359 | 10.63 | 2.87 | 0.76 | 0.45 | 1.91 | 39.63 |
| 2. 3.0 | 1.11 | 360 | 11.13 | 2.85 | 0.72 | 0.40 | 1.99 | 39.63 |
| 4. 2.8 | 1.04 | 358 | 11.17 | 2.88 | 0.71 | 0.41 | 1.99 | 39.56 |
| 6. 2.6 | 1.12 | 360 | 11.24 | 2.82 | 0.72 | 0.42 | 2.01 | 39.75 |
| *P-value* | *0.404* | *0.814* | *0.081* | *0.485* | *0.388* | *0.676* | *0.265* | *0.883* |
| *LSD* | *0.18* | *4.5* | *0.59* | *0.09* | *0.06* | *0.09* | *0.11* | *0.49* |
| Breed | | | | | | | | |
| Dekalb | 1.10 | 360 | 11.19 | 2.86 | 0.73 | 0.41 | 2.00 | 39.63 |
| LSL | 1.12 | 359 | 10.89 | 2.85 | 0.73 | 0.43 | 1.95 | 39.66 |
| *P-value* | *0.704* | *0.639* | *0.940* | *0.790* | *0.812* | *0.480* | *0.250* | *0.866* |
| *LSD* | *0.13* | *3.2* | *0.42* | *0.07* | *0.04* | *0.06* | *0.07* | *0.36* |

[1] The diets of round 2 were incorrectly implemented: hens at high rP level in the layer facility were assigned to low rP level in the digestibility cages and vice et versa (see Annex 4).

Dietary rP levels had an effect on bone ash (P<0.01) at 45 weeks of age (Table 13). Hens fed 2.8 g rP/kg (treatment with 3.0 g rP/kg) had a higher bone ash content (+9.2 g/kg DM when compared to hens fed 2.6 g rP/kg (treatment with 3.2 g rP/kg)). Dietary rP levels had no effect on other bone characteristics or on carcass characteristics.

No significant breed differences were observed regarding egg and faecal characteristics. Only a tendency was observed for soluble P content of the faeces (P=0.067), which was higher for Dekalb White hens. Furthermore, breed differences were observed for bone ash (P<0.001) at 45 weeks of age. Dekalb White laying hens had a significantly lower bone ash content (-8.4 g/kg DM) than LSL Classic laying hens. A near-significant trend was observed for bone Ca (P=0.087), which was slightly higher for LSL Classic hens.

Table 12. Phosphorus (P), calcium (Ca) and soluble phosphorus content in manure (g/kg DM) in Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 45 weeks of age (Experiment 2)

| **Round 2** | Manure | | |
|---|---|---|---|
| **45 weeks of age** | P | Ca | Soluble P |
| Dietary treatment[1] | | | |
| 1. 3.2 | 14.03[a] | 57.68 | 3.76[b] |
| 2. 3.0 | 13.58[a] | 56.77 | 2.98[a] |
| 4. 2.8 | 15.72[b] | 66.07 | 3.87[b] |
| 6. 2.6 | 16.42[b] | 58.75 | 3.72[b] |
| | | | |
| *P-value* | *<0.001* | *0.060* | *0.016* |
| *LSD* | *1.21* | *7.29* | *0.55* |
| | | | |
| Breed | | | |
| Dekalb | 15.14 | 58.16 | 3.76 |
| LSL | 14.73 | 61.48 | 3.40 |
| | | | |
| *P-value* | *0.317* | *0.189* | *0.067* |
| *LSD* | *0.86* | *5.16* | *0.39* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).
[1] The diets of round 2 were incorrectly implemented: hens at high rP level in the layer facility were assigned to low rP level in the digestibility cages and vice et versa (see Annex 4).

Table 13. Ash, phosphorus (P) and calcium (Ca) content of the carcasses and bones (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 45 weeks of age (Experiment 2)

| **Round 2** | Carcass | | | Bone | | |
|---|---|---|---|---|---|---|
| **45 weeks of age** | Ash | P | Ca | Ash | P | Ca |
| Dietary treatment[1] | | | | | | |
| 1. 3.2 | 82.5 | 6.07 | 12.10 | 532[a] | 176.1 | 385 |
| 2. 3.0 | 81.9 | 6.24 | 12.55 | 541[b] | 176.0 | 387 |
| 4. 2.8 | 83.3 | 6.21 | 12.57 | 530[a] | 176.3 | 388 |
| 6. 2.6 | 81.2 | 6.40 | 13.28 | 527[a] | 176.3 | 388 |
| *P-value* | *0.773* | *0.560* | *0.233* | *0.003* | *0.986* | *0.295* |
| *LSD* | *4.39* | *0.49* | *1.17* | *6.7* | *2.00* | *3.0* |
| Breed | | | | | | |
| Dekalb | 81.0 | 6.11 | 12.55 | 526[a] | 175.9 | 386 |
| LSL | 83.4 | 6.35 | 12.70 | 535[b] | 176.3 | 388 |
| *P-value* | *0.122* | *0.159* | *0.704* | *<0.001* | *0.543* | *0.087* |
| *LSD* | *3.11* | *0.35* | *0.83* | *4.7* | *1.41* | *2.1* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).
[1] The diets of round 2 were incorrectly implemented: hens at high rP level in the layer facility were assigned to low rP level in the digestibility cages and vice et versa (see Annex 4).

At 45 weeks of age, a breed × rP level interaction was observed for faecal digesta soluble P content (P<0.05; Figure 4) and for bone ash (P<0.01; Figure 5). Dekalb White and LSL Classic laying hens had similar soluble P content at 2.8 and 2.6 dietary rP levels but when hens were fed higher dietary rP levels (3.2 and 3.0 g rP/kg), LSL Classic hens excreted less soluble P in manure than Dekalb White laying hens.

Dekalb White and LSL Classic laying hens had similar bone ash when fed 3.2 g rP/kg, but when hens were fed lower dietary rP levels (3.0, 2.8 and 2.6 g rP/kg), LSL Classic hens showed higher bone ash than Dekalb White laying hens.

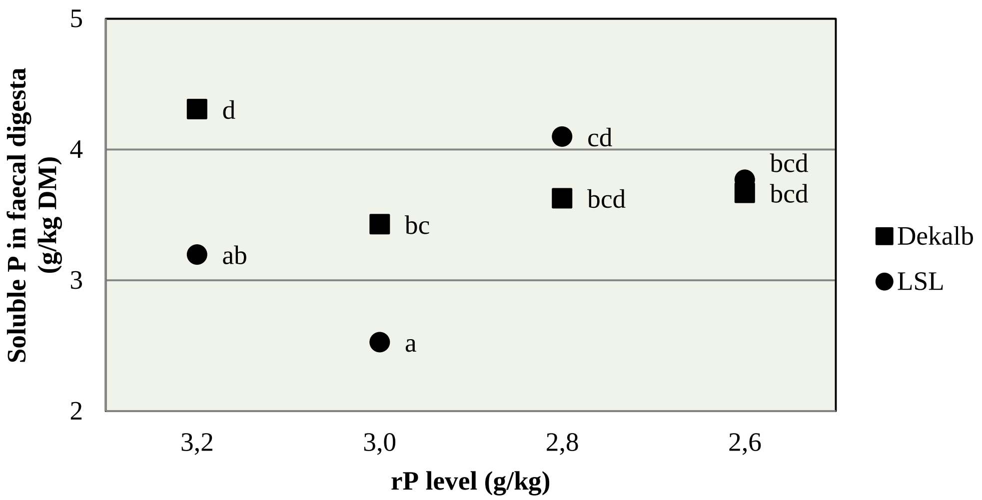


Figure 3. Faecal digesta soluble P content (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 45 weeks of age (Experiment 2).

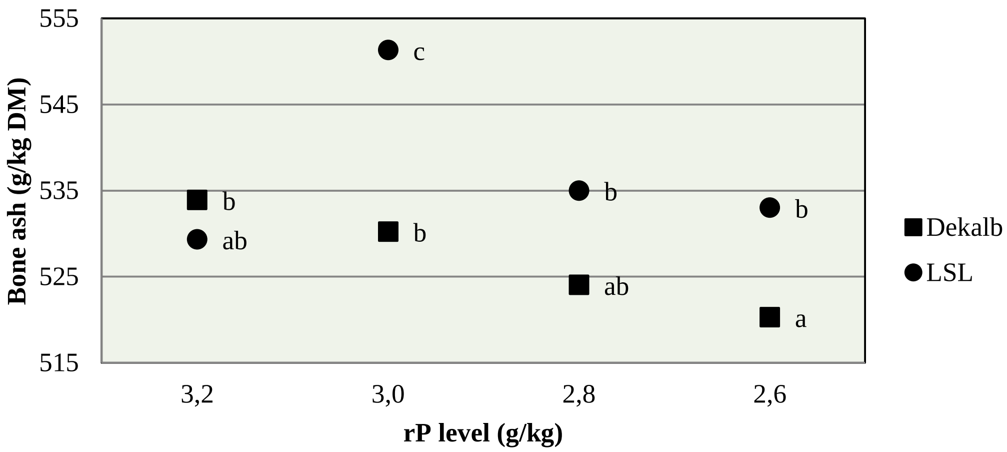


Figure 4. Bone ash content (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 45 weeks of age (Experiment 2).

### *5.3. Round 3 – 61 weeks of age*

Dietary rP level had no effect on egg or bone characteristics at 61 weeks of age (Table 14 and Table 16). However, dietary rP level had an effect on carcass P content (P<0.05; Table 16). Hens fed the lowest dietary treatment (2.4 g rP/kg) had a significantly lower carcass P content compared to hens fed 2.8 and 3.0 g rP/kg (-0.64 and -0.47 g P/kg DM, respectively). Dietary rP level had an effect on faecal P content (P<0.01; Table 15). Hens fed 3.0 g rP/kg had a significantly higher faecal P content than hens fed other treatments (+1.25 g P/kg DM when compared to hens fed 2.8 g rP/kg).

Significant breed differences were only observed for carcass P and Ca content (P<0.01 and P<0.05, respectively; Table 16). Dekalb White laying hens showed lower carcass P and Ca content (-0.45 g P/kg and -0.9 g Ca/kg DM, respectively) at 61 weeks of age.

Table 14. Phosphorus (P) and calcium (Ca) content (g/kg DM) in egg shell, egg yolk, egg albumen and total egg (g/kg) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3** | Egg shell | | Egg yolk | | Egg Albumen | | Total egg | |
|---|---|---|---|---|---|---|---|---|
| **61 weeks of age** | P | Ca | P | Ca | P | Ca | P | Ca |
| Dietary treatment | | | | | | | | |
| 1. 3.0 | 0.91 | 358 | 10.78 | 2.82 | 0.75 | 0.48 | 1.93 | 39.54 |
| 2. 2.8 | 0.84 | 358 | 10.78 | 2.80 | 0.75 | 0.53 | 1.93 | 39.62 |
| 4. 2.6 | 0.90 | 359 | 10.83 | 2.78 | 0.76 | 0.50 | 1.93 | 39.71 |
| 6. 2.4 | 0.97 | 358 | 10.82 | 2.78 | 0.75 | 0.50 | 1.94 | 39.61 |
| *P-value* | *0.185* | *0.801* | *0.947* | *0.873* | *0.389* | *0.682* | *0.894* | *0.794* |
| *LSD* | *0.12* | *3.2* | *0.26* | *0.10* | *0.01* | *0.09* | *0.05* | *0.36* |
| Breed | | | | | | | | |
| Dekalb | 0.90 | 358 | 10.76 | 2.78 | 0.75 | 0.49 | 1.92 | 39.62 |
| LSL | 0.91 | 358 | 10.84 | 2.81 | 0.75 | 0.52 | 1.94 | 39.62 |
| *P-value* | *0.681* | *0.920* | *0.328* | *0.464* | *0.316* | *0.409* | *0.198* | *0.984* |
| *LSD* | *0.09* | *2.3* | *0.18* | *0.07* | *0.01* | *0.06* | *0.04* | *0.26* |

Table 15. Phosphorus (P), calcium (Ca) and soluble phosphorus content in manure (g/kg DM) in Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3** | Manure | | |
|---|---|---|---|
| **61 weeks of age** | P | Ca | Soluble P |
| Dietary treatment | | | |
| 1. 3.0 | 14.38[b] | 67.2 | 1.78 |
| 2. 2.8 | 13.25[a] | 58.3 | 1.77 |
| 4. 2.6 | 12.69[a] | 61.6 | 1.58 |
| 6. 2.4 | 12.49[a] | 62.0 | 1.55 |
| *P-value* | *0.001* | *0.258* | *0.531* |
| *LSD* | *0.83* | *9.03* | *0.42* |
| Breed | | | |
| Dekalb | 13.15 | 60.2 | 1.68 |
| LSL | 13.26 | 64.3 | 1.67 |
| *P-value* | *0.701* | *0.191* | *0.953* |
| *LSD* | *0.59* | *6.39* | *0.30* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).

Table 16. Ash, phosphorus (P) and calcium (Ca) content of the carcasses and bones (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3** | Carcass | | | Bone | | |
|---|---|---|---|---|---|---|
| **61 weeks of age** | Ash | P | Ca | Ash | P | Ca |
| Dietary treatment | | | | | | |
| 1. 3.0 | 83.8 | 6.37[bc] | 12.53 | 525 | 175.4 | 384 |
| 2. 2.8 | 88.3 | 6.54[c] | 12.83 | 535 | 175.3 | 384 |
| 4. 2.6 | 84.3 | 6.05[ab] | 12.10 | 529 | 175.7 | 384 |
| 6. 2.4 | 82.8 | 5.90[a] | 11.97 | 525 | 176.0 | 382 |
| *P-value* | *0.382* | *0.028* | *0.240* | *0.149* | *0.895* | *0.442* |
| *LSD* | *6.89* | *0.44* | *0.96* | *9.6* | *1.96* | *3.6* |
| Breed | | | | | | |
| Dekalb | 82.6 | 5.99[a] | 11.91[a] | 527 | 175.4 | 383 |
| LSL | 87.0 | 6.44[b] | 12.81[b] | 530 | 175.8 | 383 |
| *P-value* | *0.070* | *0.007* | *0.013* | *0.472* | *0.467* | *0.631* |
| *LSD* | *4.87* | *0.31* | *0.68* | *6.8* | *1.38* | *2.5* |

[a-c] Values without a common superscript within a column differ significantly (P≤0.05).

At 61weeks of age, a breed × rP level interaction was observed for faecal digesta Ca content (P<0.05; Figure 5), faecal digesta soluble P (P<0.05; Figure 6) and for carcass Ca content (P<0.05; Figure 8).

Dekalb White and LSL Classic laying hens had similar faecal digesta Ca content at 2.6, 2.8 and 3.0 dietary rP levels, but when hens were fed lower dietary rP level (2.4 g rP/kg), LSL Classic hens excreted more Ca in manure than Dekalb White laying hens (Figure 5).

Dekalb White and LSL Classic laying hens had similar faecal digesta soluble P content at 2.4 and 2.6 dietary rP levels but when hens were fed higher dietary rP levels (2.8 and 3.0 g rP/kg), LSL Classic hens excreted less soluble P in manure than Dekalb White laying hens (Figure 6).

Dekalb White and LSL Classic laying hens had similar carcass Ca content at 2.6, 2.8 and 3.0 dietary rP levels but when hens were fed lower dietary rP level (2.4 g rP/kg), LSL Classic hens deposited more Ca in the bones than Dekalb White laying hens (Figure 7).

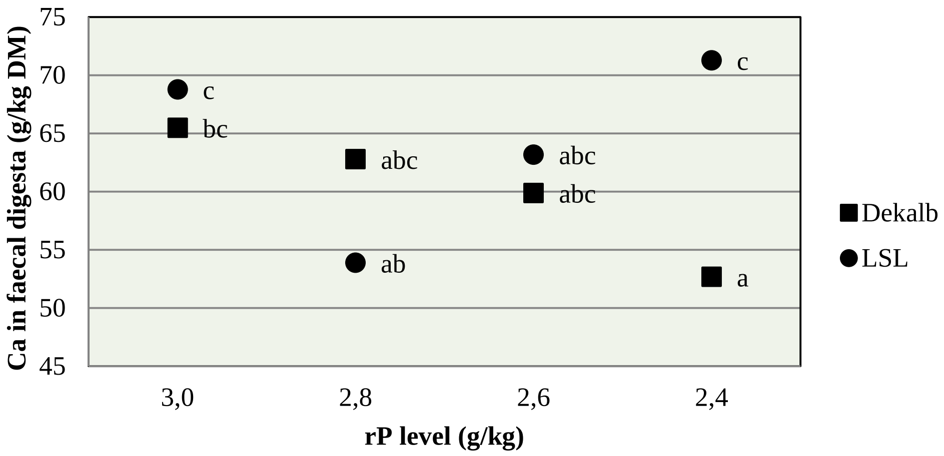


Figure 5. Ca in faecal digesta (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 61 weeks of age (Experiment 2).

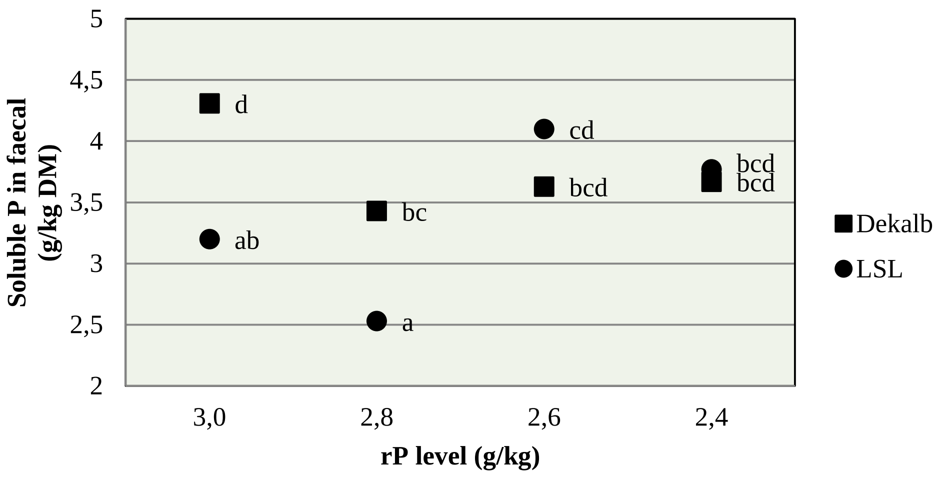


Figure 6. Soluble P in faecal digesta (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 61 weeks of age (Experiment 2).

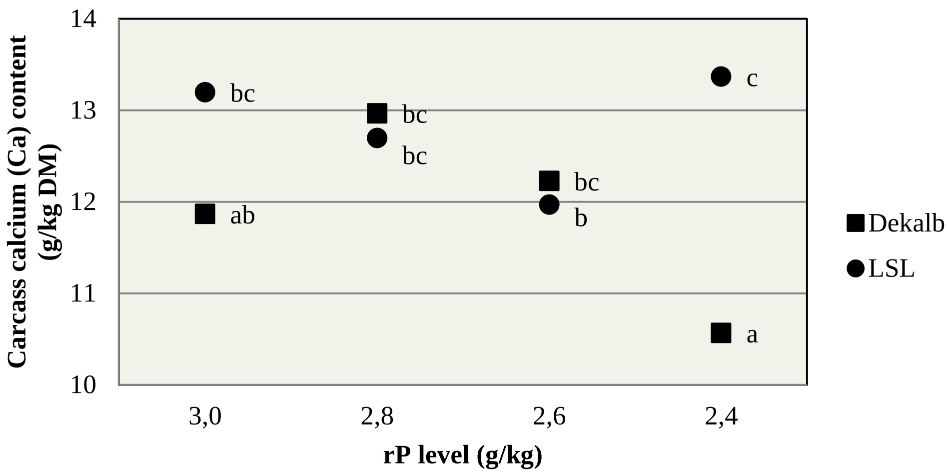


Figure 7. Carcass Ca content (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 61 weeks of age (Experiment 2).

### *5.4. Round 4 – 75 weeks of age*

Dietary rP level did not affect any of the egg, manure, carcass or bone at 75 weeks of age. Breed differences were observed only for carcass ash (P<0.05; Table 19), Dekalb White laying hens had a significantly lower carcass ash content (-5.9 g/kg DM) at 75 weeks of age. A near-significant trend was observed for carcass P content (P=0.056).

Table 17. Phosphorus (P) and calcium (Ca) content (g/kg DM) in egg shell, egg yolk, egg albumen and total egg (g/kg) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 75 weeks of age (Experiment 2)

| **Round 4** | Egg shell | | Egg yolk | | Egg Albumen | | Total egg | |
|---|---|---|---|---|---|---|---|---|
| **75 weeks of age** | P | Ca | P | Ca | P | Ca | P | Ca |
| Dietary treatment | | | | | | | | |
| 1. 2.8 | 0.95 | 373 | 11.18 | 2.70 | 0.75 | 0.33 | 1.98 | 41.16 |
| 2. 2.6 | 0.95 | 371 | 11.17 | 2.72 | 0.74 | 0.32 | 1.98 | 40.86 |
| 6. 2.4 | 0.94 | 371 | 11.17 | 2.68 | 0.74 | 0.33 | 1.97 | 40.93 |
| *P-value* | *0.970* | *0.852* | *0.945* | *0.739* | *0.571* | *0.808* | *0.564* | *0.850* |
| *LSD* | *0.06* | *11.31* | *0.07* | *0.09* | *0.04* | *0.07* | *0.01* | *1.23* |
| Breed | | | | | | | | |
| Dekalb | 0.93 | 371 | 11.16 | 2.69 | 0.75 | 0.36 | 1.97 | 40.91 |
| LSL | 0.96 | 372 | 11.18 | 2.71 | 0.73 | 0.30 | 1.98 | 41.05 |
| *P-value* | *0.142* | *0.760* | *0.323* | *0.533* | *0.177* | *0.042* | *0.151* | *0.768* |
| *LSD* | *0.05* | *9.23* | *0.05* | *0.08* | *0.03* | *0.05* | *0.01* | *1.01* |

Table 18. Phosphorus (P), calcium (Ca) and soluble phosphorus content in manure (g/kg DM) in Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 75 weeks of age (Experiment 2)

| **Round 4** | Manure | | |
|---|---|---|---|
| **75 weeks of age** | P | Ca | Soluble P |
| Dietary treatment | | | |
| 1. 2.8 | 13.49 | 75.6 | 2.87 |
| 2. 2.6 | 12.67 | 68.4 | 2.93 |
| 6. 2.4 | 12.52 | 71.3 | 2.55 |
| *P-value* | *0.443* | *0.161* | *0.287* |
| *LSD* | *1.76* | *7.74* | *0.54* |
| Breed | | | |
| Dekalb | 12.87 | 73.6 | 2.88 |
| LSL | 12.92 | 69.9 | 2.69 |
| *P-value* | *0.932* | *0.219* | *0.364* |
| *LSD* | *1.44* | *6.32* | *0.44* |

Table 19. Ash, phosphorus (P) and calcium (Ca) content of the carcasses and bones (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 75 weeks of age (Experiment 2)

| **Round 4** | Carcass | | | Bone | | |
|---|---|---|---|---|---|---|
| **75 weeks of age** | Ash | P | Ca | Ash | P | Ca |
| Dietary treatment | | | | | | |
| 1. 2.8 | 86.7 | 6.54 | 12.13 | 537 | 175.3 | 388 |
| 2. 2.6 | 89.0 | 6.38 | 12.45 | 537 | 176.0 | 386 |
| 6. 2.4 | 85.7 | 6.46 | 12.78 | 547 | 175.5 | 386 |
| *P-value* | *0.578* | *0.702* | *0.436* | *0.191* | *0.285* | *0.339* |
| *LSD* | *6.92* | *0.42* | *1.08* | *12.49* | *0.98* | *2.86* |
| Breed | | | | | | |
| Dekalb | 84.2[a] | 6.29 | 12.21 | 535 | 175.6 | 386 |
| LSL | 90.1[b] | 6.63 | 12.70 | 546 | 175.5 | 387 |
| *P-value* | *0.040* | *0.056* | *0.244* | *0.033* | *0.811* | *0.161* |
| *LSD* | *5.65* | *0.34* | *0.88* | *10.20* | *0.80* | *2.34* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

At 75 weeks of age, a breed × rP level interaction was observed for eggshell P ($P<0.05$; Figure 8). Dekalb White and LSL Classic laying hens had similar eggshell P content at 2.6 and 2.8 dietary rP levels, but when hens were fed a lower dietary rP level (2.4 g rP/kg), LSL Classic hens deposited more P in the eggshell than Dekalb White laying hens.

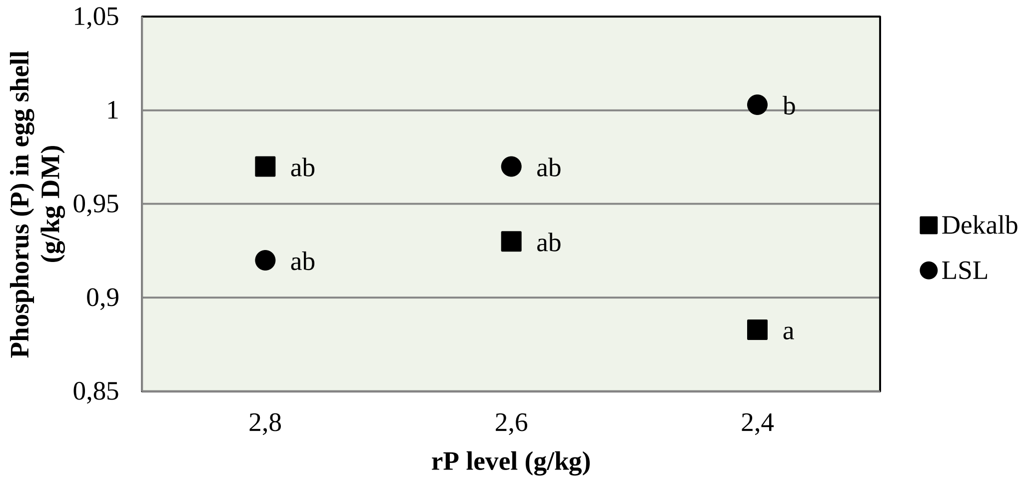


Figure 8. Phosphorus (P) in eggshell (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 75 weeks of age (Experiment 2).

### *5.5. Round 5 – 90 weeks of age*

Dietary rP level affected egg yolk P (P<0.05) at 90 weeks of age (Table 20). Surprisingly, hens fed the middle treatment (2.6 g rP/kg) had a lower egg yolk P content compared to 2.4 and 2.8 g rP/kg (-0.52 and -0.44 g P/kg DM, respectively). The same trend was observed for P in eggs and bone P and Ca content (P<0.05, P<0.001 and P <0.001, respectively; Tables 20 and 22) with hens fed 2.6 g rP/kg having a lower bone P and Ca and a lower egg P content compared to hens fed 2.4 and 2.8 g rP/kg. Dietary rP level also affected the carcass P and Ca content (P<0.01 and P<0.05, respectively; Table 22). Hens fed the lowest dietary rP level (2.4 g rP/kg) had a significantly lower carcass P and Ca content compared to hens fed 2.8 g rP/kg (-0.59 g P/kg DM and -1.23 g Ca/kg DM).
Breed differences were only observed for bone ash, P and Ca content (P<0.05, P<0.01, P<0.01, respectively; Table 22) at 90 weeks of age. LSL Classic laying hens had significantly higher bone ash content (+7 g/kg DM), bone P content (+3.6 g P/kg DM) and bone Ca content (+14 g Ca/kg DM) at 90 weeks of age.

Table 20. Phosphorus (P) and calcium (Ca) content (g/kg DM) in egg shell, egg yolk, egg albumen and total egg (g/kg) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 90 weeks of age (Experiment 2)

| **Round 5** | Egg shell | | Egg yolk | | Egg Albumen | | Total egg | |
|---|---|---|---|---|---|---|---|---|
| **90 weeks of age** | P | Ca | P | Ca | P | Ca | P | Ca |
| Dietary treatment | | | | | | | | |
| 2.8 | 0.91 | 352 | 10.81[b] | 2.55 | 0.76 | 0.53 | 1.91[b] | 38.87 |
| 2.6 | 0.88 | 355 | 10.37[a] | 2.60 | 0.74 | 0.48 | 1.84[a] | 39.15 |
| 2.4 | 0.87 | 354 | 10.89[b] | 2.50 | 0.75 | 0.62 | 1.92[b] | 39.03 |
| *P-value* | *0.313* | *0.844* | *0.035* | *0.371* | *0.834* | *0.309* | *0.043* | *0.842* |
| *LSD* | *0.06* | *9.91* | *0.40* | *0.15* | *0.07* | *0.18* | *0.07* | *1.07* |
| Breed | | | | | | | | |
| Dekalb | 0.89 | 354 | 10.66 | 2.50 | 0.74 | 0.57 | 1.89 | 39.01 |
| LSL | 0.88 | 354 | 10.72 | 2.60 | 0.76 | 0.52 | 1.89 | 39.02 |
| *P-value* | *0.854* | *0.983* | *0.697* | *0.100* | *0.436* | *0.526* | *0.980* | *0.993* |
| *LSD* | *0.05* | *8.09* | *0.33* | *0.12* | *0.05* | *0.15* | *0.06* | *0.88* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

Table 21. Phosphorus (P), calcium (Ca) and soluble phosphorus content in manure (g/kg DM) in Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 90 weeks of age (Experiment 2)

| **Round 5** | Manure | | |
|---|---|---|---|
| **90 weeks of age** | P | Ca | Soluble P |
| Dietary treatment | | | |
| 2.8 | 13.92 | 73.2 | 3.20 |
| 2.6 | 13.93 | 13.8 | 3.20 |
| 2.4 | 13.87 | 72.9 | 3.22 |
| *P-value* | *0.998* | *0.786* | *0.999* |
| *LSD* | *2.05* | *3.016* | *0.89* |
| Breed | | | |
| Dekalb | 13.83 | 72.9 | 3.28 |
| LSL | 13.98 | 73.7 | 3.13 |
| *P-value* | *0.846* | *0.493* | *0.669* |
| *LSD* | *1.68* | *2.463* | *0.73* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

Table 22. Ash, phosphorus (P) and calcium (Ca) content of the carcasses and bones (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 90 weeks of age (Experiment 2)

| **Round 5** | Carcass | | | Bone | | |
|---|---|---|---|---|---|---|
| **90 weeks of age** | Ash | P | Ca | Ash | P | Ca |
| Dietary treatment | | | | | | |
| 2.8 | 87.9 | 6.84$^{b}$ | 12.75$^{b}$ | 537 | 176.8$^{b}$ | 395$^{b}$ |
| 2.6 | 85.7 | 6.71$^{b}$ | 12.65$^{b}$ | 540 | 167.9$^{a}$ | 364$^{a}$ |
| 2.4 | 87.2 | 6.28$^{a}$ | 11.53$^{a}$ | 541 | 175.2$^{b}$ | 393$^{b}$ |
| *P-value* | *0.473* | *0.008* | *0.020* | *0.412* | *<0.001* | *<0.001* |
| *LSD* | *4.08* | *0.32* | *0.88* | *7.20* | *2.56* | *11.29* |
| Breed | | | | | | |
| Dekalb | 86.1 | 6.65 | 12.37 | 536$^{a}$ | 171.5$^{a}$ | 377$^{a}$ |
| LSL | 87.8 | 6.56 | 12.26 | 543$^{b}$ | 175.1$^{b}$ | 391$^{b}$ |
| *P-value* | *0.270* | *0.497* | *0.737* | *0.025* | *0.005* | *0.007* |
| *LSD* | *0.33* | *0.26* | *0.72* | *5.88* | *2.09* | *9.22* |

$^{a,b}$ Values without a common superscript within a column differ significantly (P≤0.05).

At 90 weeks of age, a breed × rP level interaction was observed for faecal digesta Ca content (P<0.01; Figure 9), carcass P content (P<0.01; Figure 10), carcass Ca content (P<0.05; Figure 11), bone ash (P<0.05; Figure 12), bone P (P<0.05; Figure 13) and bone Ca (P<0.05; Figure 14).

Dekalb White and LSL Classic laying hens had similar faecal digesta Ca content at 2.6 and 2.8 g rP/kg, but when LSL Classic hens were fed lower dietary rP level (2.4 g rP/kg), they excreted more Ca in manure than Dekalb White laying hens (Figure 9).

Dekalb White laying hens had higher P in carcass when fed 2.6 and 2.8 g rP/kg compared to LSL Classic laying hens, but when they were fed 2.4 g rP/kg, LSL Classic laying hens showed higher P in carcass (Figure 10).

Dekalb White laying hens had higher Ca in carcass when fed 2.8 g rP/kg compared to LSL Classic laying hens, but when they were fed 2.4 g rP/kg, LSL Classic laying hens showed higher Ca in carcass (Figure 11).

Dekalb White and LSL Classic laying hens had similar bone ash when fed 2.8 g rP/kg, but when LSL Classic hens were fed lower dietary rP level (2.4 and 2.6 g rP/kg), they showed higher bone ash than Dekalb White laying hens (Figure 12).

Dekalb White and LSL Classic laying hens had similar bone P and bone Ca content when fed 2.4 and 2.8 g rP/kg, but when they were fed the middle dietary rP level (2.6 g rP/kg), LSL Classic laying hens showed a higher bone P and Ca content than Dekalb White laying hens (Figure 13 and 14).

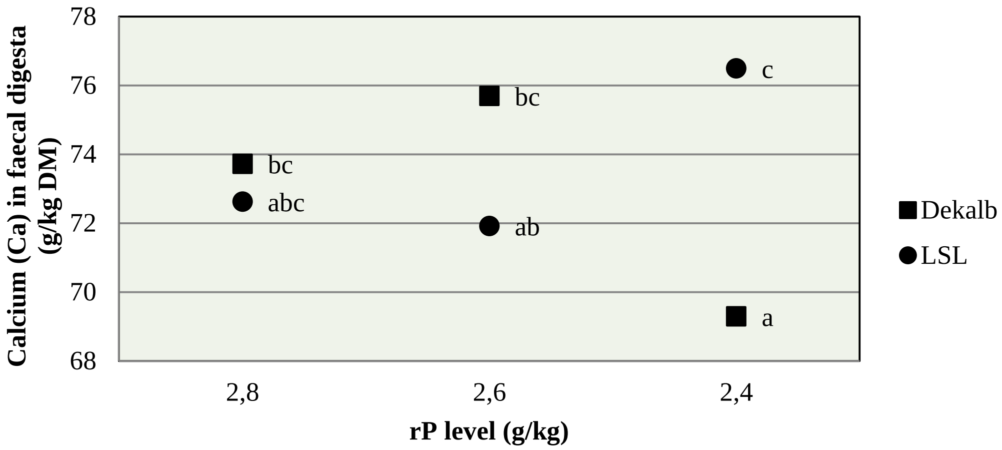


Figure 9. Ca in faecal digesta (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

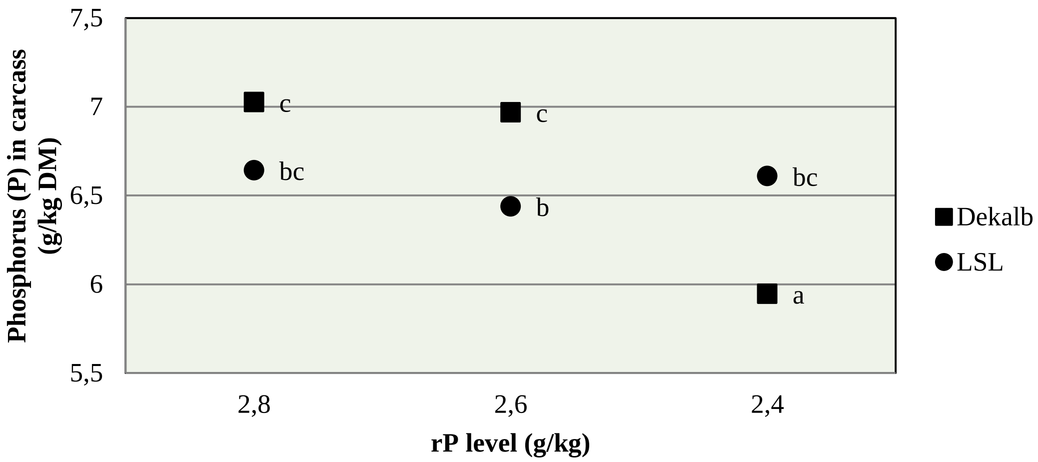


Figure 10. Phosphorus (P) in carcass (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

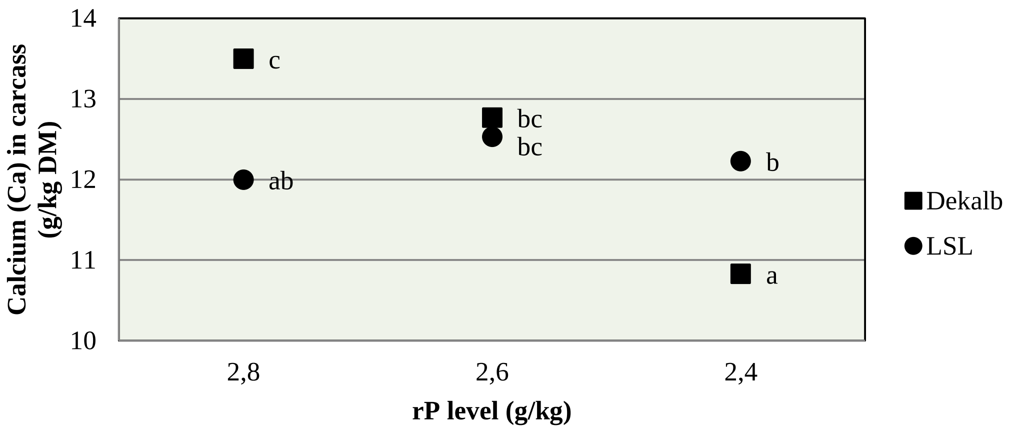


Figure 11. Calcium (Ca) in carcass (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

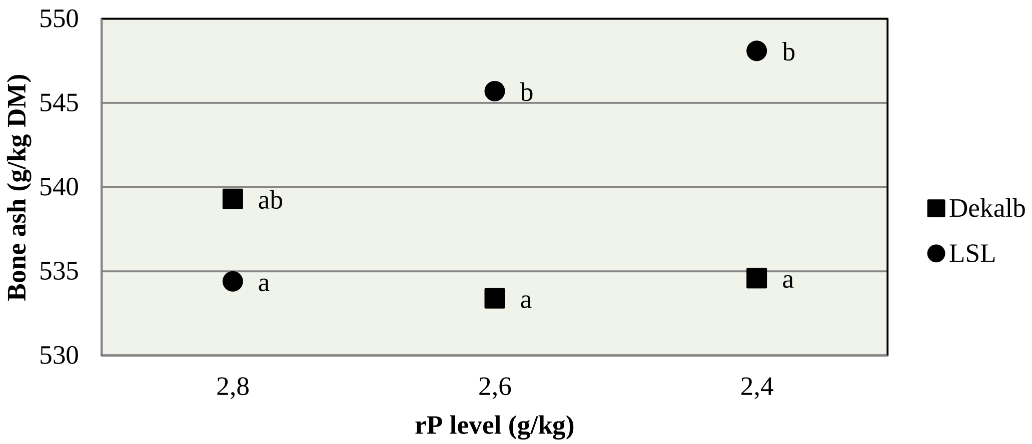


Figure 12. Bone ash (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

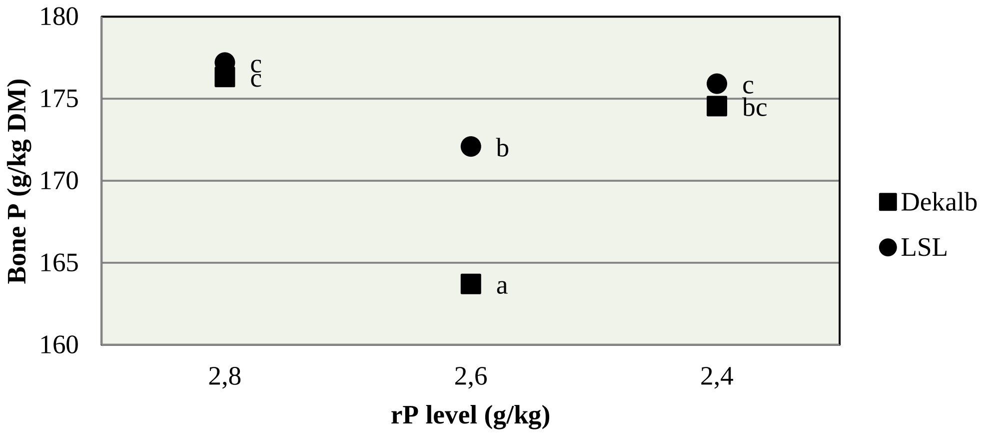


Figure 13. Bone phosphorus (P; g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

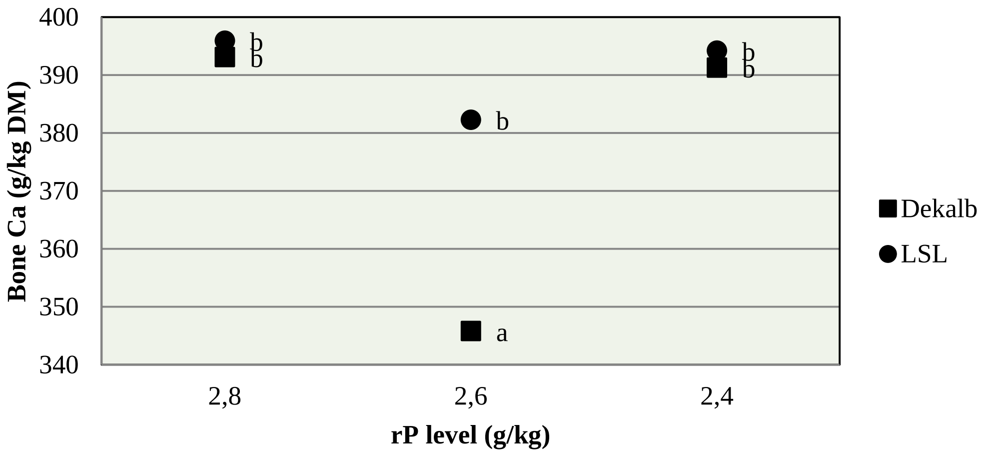


Figure 14. Bone calcium (Ca; g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus (rP) levels at 90 weeks of age (Experiment 2).

Egg P or Ca content where not affected by diet or breed except at 90 weeks of age. Hens fed 2.6 g rP/kg had a lower egg yolk P and egg total P compared to diets 2.8 and 2.4 g rP/kg. Faecal digesta P content was affected by diet, but only at 45 and 61 weeks of age. Faecal Ca was not affected by diet or breed. Carcass Ca was not affected by diet. However, carcass P was higher with a higher dietary rP level, but only at 61 and 90 weeks of age. Dekalb White hens showed higher carcass P at 61 weeks of age. Bone P and Ca content were not affected by diet nor breed at 45, 61 and 75 weeks of age. However, bone P and Ca were lower in hens fed 2.6 g rP/kg at 90 weeks of age. This effect might be due to the low bone P and Ca of Dekalb hens when fed 2.6 g rP/kg (Figures 13 and 14). Average P content in egg and carcass for treatment 1 and treatment 6 are detailed in Table 23.

### *5.6. Phosphorus in fresh eggs and carcasses*

In the Netherlands, the reference values for phosphorus (P) in eggs and carcasses were calculated by Versteegh & Jongbloed (2000) and reach, respectively, 1.7 and 5.6 g/kg. To evaluate if the results of the current study were similar to those of Versteegh & Jongbloed (2000) P content in egg and in carcass must be expressed in g/kg of fresh material. Therefore, the following equation was used to calculate P content in the fresh egg (g/kg):

Equation 1:

$$P_{egg} = 0.11 * P_{shell} * \frac{1000 - Moist_{Shell}}{1000} + 0.32 * P_{yolk} * \frac{1000}{1000 - MoistT_{yolk}} * \frac{1000 - Moist_{yolk}}{1000} + 0.57 * P_{alb} * \frac{1000}{1000 - MoistT_{alb}} * \frac{1000 - Moist_{alb}}{1000}$$

With: 0.11, 0.32 and 0.57 = percentage of, respectively, shell, yolk and albumen in an egg (Johnson, 2000).

$P_{shell}$, $P_{yolk}$ and $P_{alb}$ = P content (g/kg DM) in, respectively, the shell, the yolk and the albumen.

$Moist_{shell}$, $Moist_{yolk}$, $Moist_{albumen}$ = Moisture content (g/kg) of, respectively, the shell, the yolk and the albumen after freeze-drying.

$MoistT_{yolk}$, $MoistT_{alb}$ = Moisture content (g/kg) of, respectively, the yolk and the albumen before freeze-drying.

A similar formula was used for calcium Ca content in eggs (g/kg egg). Results on dry matter base were shown in Tables 11, 14, 17 and 20.

To correct for celite addition and moisture of P content in the fresh carcass (g/kg) the following equation was used

$$P_{carcass,\ FM} = \left(300 + \left(700 * \frac{1000 - MoistT_{carcass}}{1000}\right)\right) * \left(\frac{P_{carcass,\ DM}}{1000 - Moist_{carcass}}\right) * \left(\frac{1000}{700}\right)$$

With: $P_{carcass,\ FM}$ = P content (g/kg of fresh material) in the carcass.

$P_{carcass,\ DM}$ = P content (g/kg of dry matter) in the carcass.

$Moist_{carcass}$ = Moisture content (g/kg) of the carcass after freeze-drying.

$MoistT_{carcass}$ = Moisture content (g/kg) of the carcass before freeze-drying.

A similar formula was used for Ca content in carcasses (g/kg carcass). Results on dry matter base were shown in Tables 13, 16, 19 and 22.

The results of P content in fresh material are shown in Table 23. The P content in eggs was not affected by dietary P treatment, and was relatively constant over time. The P content in eggs was about 1.95 g/kg egg, which is higher than the reference of 1.7 g P/kg egg by Versteegh & Jongbloed (2000).

Phosphorus content in carcass was affected by treatment at 61 and 90 weeks of age ($P<0.05$). Laying hens at a high dietary P level had a higher carcass P content. The P content in carcass was also affected by age. A significant correlation ($P<0.05$) was found for the age effect of P content in carcass (Figure 15). As carcass P content is correlated to age, it makes it possible to extrapolate to younger and older birds (Table 24). For P in carcass the reference value of 5.6 g P/kg carcass is comparable to the results of the currents study if laying hens are 75 weeks of age or older. When laying hens are slaughtered before 75 weeks of age a reference value of 5.6 g P/kg carcass seems to be high compared to the results of the current study.

Table 23. Phosphorus (P; g/kg) content in eggs and carcasses of laying hens at 35, 45, 61, 75 and 90 weeks of age. Laying hens were fed diets high or low in phosphorus level from 36 to 90 weeks of age

| **P content** | Egg | | | | | | Carcass | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Weeks** | 35 | 45[1] | 61 | 75 | 90 | *$R^2$* | 35 | 45[1] | 61 | 75 | 90 | *$R^2$* |
| Dietary treatment | | | | | | | | | | | | |
| A: 3.2-3.0-2.8-2.8 | 1.99 | 1.91 | 1.93 | 1.98 | 1.91 | | 5.15 | 5.28 | 5.57[b] | 5.61 | 5.90[b] | |
| B: 2.6-2.4-2.4-2.4 | 1.98 | 2.01 | 1.94 | 1.97 | 1.92 | | 5.20 | 5.62 | 5.16[a] | 5.50 | 5.33[a] | |
| **Average** | **1.99** | **1.96** | **1.94** | **1.98** | **1.92** | ***0.46***[2] | **5.18** | **5.45** | **5.37** | **5.56** | **5.62** | ***0.77***[3] |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

[1] Diets were not correctly fed to the birds. Birds of treatment A received a diet with a rP level of 2.4 g rP/kg diet instead of 3.2 g/kg, whereas birds of treatment B received a diet with a rP level of 3.2 g rP/kg diet instead of 2.4 g/kg.
[2] Correlation was not significant.
[3] Correlation was significant with $P < 0.05$.

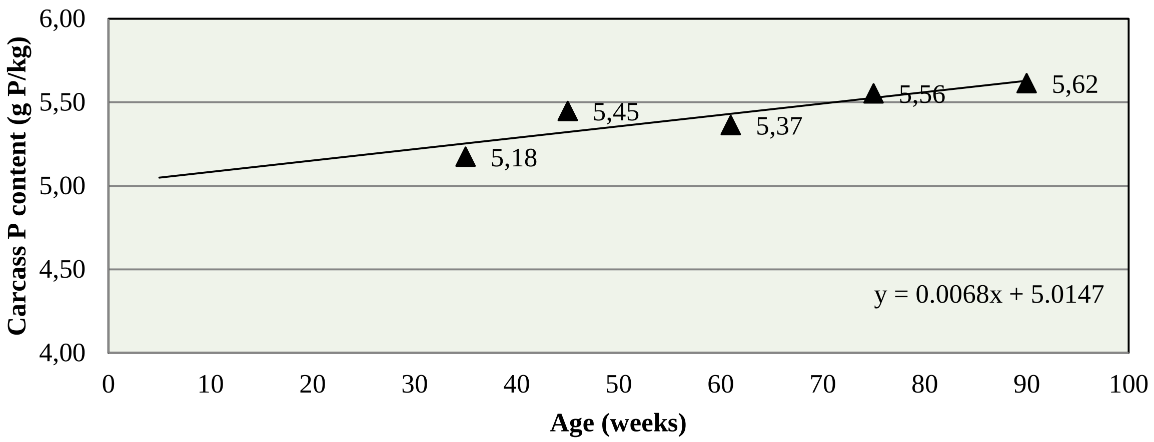


Figure 15. Linear regression of carcass phosphorus content in laying hens (Experiment 2).

Table 24. Extrapolation of carcass phosphorus content (g/kg) for laying hens aging 18, 25, 35, 45, 61, 75, 90 and 100 weeks (Experiment 2)

| Age (weeks) | 18 | 25 | 35 | 45 | 61 | 75 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|
| Carcass P content | 5.14 | 5.18 | 5.25 (5.18)[1] | 5.32 (5.45)[1] | 5.43 (5.37)[1] | 5.52 (5.56)[1] | 5.63 (5.62)[1] | 5.69 |

[1] Values between brackets are experimental values.

# 6. Discussion

## 6.1. *Effect of dietary retainable phosphorus levels on production performance*

### *6.1.1. Retainable phosphorus requirement*

The aim of the first experiment was to update the rP needs of two modern laying hen breeds from 36 to 90 weeks of age. Treatments 1 to 4 were based on rP levels used in current practise, i.e. the highest and lowest levels fed by Dutch feed mills. Treatments 5 and 6 were fed lower levels and it was expected to induce a small P deficiency in the birds. However, from 36 to 90 weeks of age, birds responded well to the lowest rP levels in the diet. Furthermore, birds did not differ in body weight, and egg and shell quality (breaking strength and Haugh units) were similar among treatments. The lowest dietary rP level could therefore significantly ensure the same production performance than higher rP levels in the feed. Therefore, it cannot be concluded that the minimum requirement was reached.

### *6.1.2. Breed*

Breeds did differ in production performance. Dekalb White hens showed higher performance from 36-54 weeks of age with a higher laying rate, egg weight and egg mass. However, feed intake was also higher, resulting in a comparable FCR. After 55 weeks of age, LSL Classic hens had a lower feed intake and a similar laying rate, egg weight and egg mass compared to Dekalb White hens. As a consequence, from 36-90 weeks of age, Dekalb White hens had higher feed intake, similar egg mass and therefore higher FCR. No difference in mortality was observed between breeds.

Production performance was affected by high dietary rP levels in young LSL Classic hens, but not in Dekalb White hens. High dietary P levels can depress egg production and feed efficiency in laying hens because P forms complexes with other minerals (Ca, Mg, Al) which decreases digestibility of those nutrients (Rama Rao et al., 2006). Ahmadi & Rodehutscord (2012) showed that dietary P levels higher than 3.5 g aP/kg decreased production performance when hens were fed diets without phytase inclusion. The current study was then the first experiment showing that different layer breeds can have different sensitivities to dietary rP level. Different breeds can show specific needs which raises the question of precision livestock nutrition for breeds.

### *6.1.3. Age*

Many investigations have been conducted to determine the exact P requirements of laying hens (Keshavarz & Nakajima, 1993; Gordon & Roland, 1997; Van der Klis et al., 1997; Punna & Roland, 1999; Boling et al., 2000; Boorman & Gunaratne, 2001; Bar et al., 2002; Sohail & Roland, 2002; Snow et al., 2004; Musapuor et al., 2006; Ahmadi & Rodehutscord, 2012). However, prior to this study, the P needs of first-cycle laying hens aged up to 90 weeks had never been investigated. From 36 to 90 weeks of age, birds responded well to the lowest rP levels in the diet. The lowest dietary rP level could therefore significantly ensure the same production performance than higher rP levels in the feed. This indicate that the modern laying hens can be fed 2.6 g rP/kg from 36 to 45 weeks of age (312 mg rP/d; feed intake of 120 g/h/d) and 2.4 g rP/kg from 46 to 90 weeks of age (288 mg rP/d; feed intake of 120 g/h/d). In the Dutch feeding tables system, the P needs are expressed in retainable phosphorus (rP) while available phosphorus (aP) is often preferred in literature. This makes it complicated to compare our study with literature.

*6.1.4. Housing system*

It is widely accepted that new housing systems have improved hen welfare. Birds can explore more active behaviour and seem to have a better bone development (Abrahamsson & Tauson, 1995). However, recently is was shown that high number of birds has fractions on the keel bone, especially in the second part of the laying cycle (Kempen, 2013). These type of bone fractions, however, are mainly related to the design of the house, and in particular with the position of the perches, The current study did not focus on the welfare of the birds or on the incidence of bone fractions, but mainly on the production performance. It is comforting that most studies show comparable or even higher performances in alternative housing systems (egg production, eggshell quality). The current trial was performed in an aviary system. As discussed before, laying hens performed well at the lowest dietary rP levels. However, if this would be different for (enriched) cage systems was not tested in the current study. Besides, birds in alternative systems generally have a higher feed intake compared to cage-housed birds, thereby partly compensating for the reduced rP levels per kg of diet. Overall, the rP intake in the current study was reduced by ~10% compared to the recommended rP intake levels for cage-housed hens (Van der Klis and Blok, 1997) without adverse effects on production performance, and bone and egg quality.

*6.1.5. Phytase*

In practise it is common to use the enzyme phytase in the diet to improve the availability of P that is bound to phytic acid. Phytase was not added to the diets in the current study, so that the availability of phytic bound P was not affected by the use of phytase.

## ***6.2. Effect of dietary retainable phosphorus on P level in eggs, manure and carcasses***

*6.2.1. Trial set-up*

The second experiment aimed at investigating the influence of dietary rP levels on Ca and P content in eggs, manure, carcasses and bones.

Round 2 of Experiment 2 was incorrectly implemented. Laying hens at a high rP level in the practical layer facility (Experiment 1) were assigned to a low rP level in the digestibility cages and vice versa. In order to test the effect, Round 3bis was implemented to study the responsiveness of laying hens fed new diets during 10 days. Results of Round 3bis are given in Annex 4. If results from Round 3bis were compared with the results of Round 3, it was shown that hens fed high dietary rP in the barn and low dietary rP in digestibility cages (Round 3bis) did not show any difference for faecal P content and carcass P content, whereas hens fed high dietary rP in the barn and in the digestibility cages (Round 3) showed higher contents for both parameters (Tables 15 and 16). Furthermore, P in eggs was significantly higher for hens fed 3.0 g rP/kg in digestibility cages (hens were fed 2.4 g rP/kg in the practical scale facility). This indicates that a 10-days feeding period can overcome the effects caused by feeding diets differing in retainable P levels. Therefore, results of Round 2 should be interpreted with care.

### *6.2.2. Phosphorus level in the egg*

Egg P or Ca were not affected by dietary P except at 90 weeks of age. Hens fed 2.6 g rP/kg had a lower egg yolk P and egg total P compared to hens fed diets with 2.8 and 2.4 g rP/kg. The P content in eggs constituted a very constant level over time even when laying hens were fed diets differing in P levels.

### *6.2.3. Phosphorus level in manure*

In general, the P and Ca content in faecal digesta was found to be lower when hens were fed a low dietary rP level, but only when hens were younger than 75 weeks of age. Snow et al. (2004), Rama Rao et al. (2006) and Zweifel et al. (2009) also found a lower P content in manure when hens were fed lower dietary P levels. However, it is not known why the faecal P content of older hens is not affected by dietary P levels.

*Ileal and faecal phosphorus content*

Difference in total P between ileal and faecal digesta were on average 4.15 g/kg. This difference was due to the large amount of soluble P in faecal digesta. The soluble P fraction is related to the resorption of medullary bone (Whitehead, 2004) and is excreted by the uric acid fraction in the faeces. The results of the P content not related to egg shell formation (=P–soluble P) is similar between ileal and faecal digesta. Furthermore, this indicates that 33% of the P content of the faeces is soluble P, and related to egg shell formation.

### *6.2.4. Phosphorus level in carcass and bone*

The P and Ca content in carcass were found to be lower at a low dietary rP level, but this was significant only at 61 and 90 weeks of age. However, this is an indication that birds fed the lowest rP level were fed deficient amounts of rP. Interestingly, no sign of P-deficiency in lower egg production or a lower P content in the toe bones was observed. This indicates that P for production performance has a higher priority than P for carcass.

The P and Ca content in bones were only affected at 90 weeks of age and were not linearly decreasing with decreasing dietary rP levels. The effect of dietary P levels on bone quality is controversial in literature. Lei et al. (2011) and Snow et al. (2004) did not find any effect of dietary rP levels on bone P content which is in line with our findings. However, Punna & Roland (1999) and Sohail & Roland (2002) found lower bone mineral density (not measured in our experiment) with lower dietary rP levels. At 90 weeks of age, hens fed 2.6 g rP/kg had a significantly lower P and Ca content in bone compared to hens at a level of 2.8 or 2.4 g rP/kg. This finding is due to the extremely low P and Ca content in bone of Dekalb White laying hens at a level of 2.6 g rP/kg. The explanation for the latter is not known yet.

*6.2.5. Reference values for lump phosphorus excretion*

In the Dutch system the reference values for phosphorus in eggs and carcasses were calculated by Versteegh & Jongbloed (2000) and reach, respectively, 1.7 and 5.6 g/kg. Based on the results of the current study, the value for P in egg is with 1.95 g/kg higher than the 1.7 g/kg found by Versteegh & Jongbloed (2000), but lower than the value used in the CORPEN system (2.15 g P/kg) by INRA in France (Perrot et al., 2006). For the current study, the P level in fresh egg was calculated based on the weight of the different egg components as described by Johnson (2000). Johnson stated that an egg contains 32% yolk, 57% albumen and 11% egg shell. Most of the P in egg is found in the yolk, so the percentage of yolk will affect the P level. Other studies observed that the amount of yolk varies between 25% to 32% (Cotterill, 1977; Suk & Park, 2001; Wu et al., 2005). For calculation of P in eggs it might have been better to use the average yolk percentage of the different studies. This means a yolk percentage of 28 to 29%, and this will result in an egg P content of 1.8 g/kg.

For P in carcass values of and 5.2 g P/kg carcass from 18 to 35 weeks of age, 5.4 g P/kg carcass from 35 to 75 weeks of age and 5.6 g P/ kg carcass from 75 to 100 weeks of age were observed. For laying hens at age of slaughter (>75 weeks of age) the Dutch reference value for the carcass is similar to the value found in the current study.

## 7. Conclusion

The aim of the first experiment was to update the rP needs of two modern laying hen breeds from 36 to 90 weeks of age. The aim of the second experiment was to investigate the influence of dietary rP levels on Ca and P content in eggs, manure, carcasses and bones. Based on the first experiment, it is not certain that the minimum requirement was reached. The low rP diets did not negatively affect production performance, egg quality and body weight. However, in the second experiment, the carcasses P levels indicate that a dietary rP level of 2.4 g/kg was too low. It is therefore for sure that the lowest levels were close to the minimum level. Lowering dietary P levels in the feed is a way to reduce feed costs for farmers and to mitigate environmental pollution from laying hen farms.

Furthermore, based on the results of the second experiment it can be concluded that the Dutch reference values for of 5.6 g P/kg carcass is still valid at the end of the laying period, whereas the reference value of 1.7 g P/kg egg is in general too low and should be increased to at least 1.8 g P/kg egg.

## 8. Recommendations

For the governmental organisations:

- It is recommended to update the Dutch reference values for P in egg to at least 1.8 g P/kg egg. The Dutch reference value of 5.6 g P/kg carcass can be used unchanged.
- The lowest level tested in the current trial will probably not be used in practise, because the level is close to deficiency and feed mills will not take this risk.

For the feed mills:

- We recommend to feed hens with diets containing a minimum of 2.7 g rP/kg (2.6 g rP/kg + 5% safety margin) from 18 to 45 weeks of age and a minimum of 2.5 g rP/kg (2.4 g rP/kg + 5% safety margin) from 46 to 90 weeks of age. This is for laying hens with an average feed intake of 120 g/h/d with a daily rP requirement of 327 mg/h/d and 302 mg/h/d from 18-45 and 46-90 weeks of age, respectively. When feed intake of the laying hens is lower than 120 g/h/d, rP levels in the diet should be adjusted to meet the needs of the birds.
- Phytase was not used in the current trial. However, it is strongly recommended to use phyatse in the diets. Feed costs will be reduced and P excretion by the birds will be lowered.

For further research:

- It is of interest to test the effect of phytase (e.g. dose-levels, different phytase products) in laying hens of different ages.
- In the current study, blood serum was collected from each bird in Experiment 2 and stored frozen (-20°C) at SFR until further processing. No financial support was obtained to analyse these samples, but it is still of interest to analyse these samples to have an idea about the P and Ca levels in blood.

## 9. Summary

The modern laying hen has a high rate of lay and laying persistence, and probably a different P-requirement than the current estimated requirements. Furthermore, due to higher levels of locomotion, laying hens housed in non-cage housing systems might have a better bone development. It was hypothesized that P supply by feed in such systems can be lowered without negative effects on bone quality and production performance.

Two experiment were performed. The aim of the first experiment was to update the retainable phosphorus (rP) needs of two modern laying hen breeds from 36 to 90 weeks of age. The aim of the second experiment was to investigate the influence of dietary rP levels on Ca and P content in eggs, manure and carcasses to validate the current reference P-contents in eggs and carcass of laying hens. The first experiment consisted of six dietary treatments differing in rP level. The trial was divided in five phases in which the following diets were supplied:

| Dietary treatment | Period 1<br>36-45 wk | Period 2<br>46-54 wk | Period 3<br>55-65 wk | Period 4<br>66-75 wk | Period 5<br>76-90 wk |
|---|---|---|---|---|---|
| 1 | 3.2* | 3.0 | 3.0* | 2.8* | 2.8* |
| 2 | 3.0* | 2.8 | 2.8* | 2.6* | 2.6* |
| 3 | 2.8 | 2.8 | 2.8 | 2.6 | 2.6 |
| 4 | 2.8* | 2.6 | 2.6* | 2.4 | 2.4 |
| 5 | 2.6 | 2.4[1] | 2.6 | 2.4 | 2.4 |
| 6 | 2.6* | 2.4 | 2.4* | 2.4* | 2.4* |

[1] Treatment 5 received feed containing 2.4 g rP/kg instead of 2.6 g rP/kg from 46-54 wk of age. The next period was therefore adapted and the birds received a diet with 2.6 g rP/kg instead of 2.4 g rP/kg.
* Treatments used for Experiment 2 at respectively 45, 61, 75 and 90 weeks of age.

The treatments used in Experiment 2 corresponded to the treatments in Experiment 1 (marked with * in the table above). Effects were evaluated with two layer breeds, LSL Classic and Dekalb White, housed in a semi-commercial multi-tier aviary system. Each diet × breed treatment was performed with three replicates (330 birds per replicate). Production was measured per phase. For Experiment 2, a small number of birds was removed from the aviary system to cages at 35, 45, 60, 75 and 90 weeks of age. In these birds the P content in manure, carcass, and eggs was determined.

Based on the first experiment, it is not certain that the minimum P requirement was reached. The low rP diets did not negatively affect production performance, egg quality and body weight. Therefore, a rP level of 2.6 g/kg and 2.4 g/kg seems to be sufficient for maximal egg number, egg weight, egg mass and feed conversion ratio from 36-45 and 45-90 weeks of age, respectively. However, in the second experiment, the carcasses P levels indicate that a dietary rP level of 2.4 g/kg was too low. It is therefore for sure that the lowest levels were close to the minimum level. Part of the reduction in recommendation of dietary rP-contents is related to the increased feed intake levels of free-range housed hens compared to cage-housed hens. Nevertheless, the adapted requirements result in an overall decrease rP intake of about 10%.

Furthermore, based on the results of the second experiment it can be concluded that the Dutch reference values for of 5.6 g P/kg carcass is still valid at the end of the laying period, whereas the reference value of 1.7 g P/kg egg is in general too low and should be increased to at least 1.8 g P/kg egg.

## 10. Samenvatting

De moderne leghen heeft een hoge eiproductie en goede legpersistentie en hierdoor waarschijnlijk een andere P behoefte dan de huidige geschatte behoefte. Daarnaast zijn leghennen tegenwoordig niet meer gehuisvest in traditionele batterijkooien en hebben ze meer bewegingsvrijheid, wat gepaard gaat met een betere botontwikkeling. De P verstrekking in niet-kooisystemen kan waarschijnlijk verlaagd worden zonder dat dit ten koste gaat van botkwaliteit en productieprestaties.
Om de P-behoefte van leghennen in scharrelsystemen te testen zijn twee experimenten uitgevoerd. Het doel van het eerste experiment was het vaststellen van de opneembaar fosforbehoefte (oP) van twee huidige leghenrassen van 36 tot 90 weken leeftijd. Het doel van het tweede experiment was om het effect van het oP-gehalte in voer op P- en Ca-gehalte in eieren, mest en karkassen vast te stellen, waarmee nagegaan kan worden of er aanleiding is om de huidige referentiewaarden voor P in eieren en uitgelegde hennen aan te passen. Het eerste experiment bestond uit zes voerbehandelingen die verschilden in oP-gehalte. Het experiment was verdeeld in vijf perioden waarin de volgende oP-gehalten zijn verstrekt:

| Voerbehandeling | Periode 1<br>36-45 wk | Periode 2<br>46-54 wk | Periode 3<br>55-65 wk | Periode 4<br>66-75 wk | Periode 5<br>76-90 wk |
|---|---|---|---|---|---|
| 1 | 3,2* | 3,0 | 3,0* | 2,8* | 2,8* |
| 2 | 3,0* | 2,8 | 2,8* | 2,6* | 2,6* |
| 3 | 2,8 | 2,8 | 2,8 | 2,6 | 2,6 |
| 4 | 2,8* | 2,6 | 2,6* | 2,4 | 2,4 |
| 5 | 2,6 | 2,4[1] | 2,6 | 2,4 | 2,4 |
| 6 | 2,6* | 2,4 | 2,4* | 2,4* | 2,4* |

[1] Behandeling 5 heeft voer met 2,4 g oP/kg gehad in plaats van 2,6 g oP/kg van 46-54 wk leeftijd. De periode daarna is daarom aangepast en de hennen hebben in deze periode voer met 2,6 g oP/kg gehad.
* Behandelingen gebruikt voor Experiment 2 op respectievelijk 45, 61, 75 en 90 weken leeftijd.

De behandelingen gebruikt voor Experiment 2 zijn vergelijkbaar met de behandelingen van Experiment 1 (gemarkeerd met * in bovenstaande tabel). De behandelingen zijn uitgevoerd met twee leghenrassen, LSL Classic en Dekalb White, die zijn gehuisvest in een semi-commercieel volièresysteem. Elk combinatie van voer × ras is uitgevoerd met drie herhalingen (330 hennen per herhaling). Productieprestaties zijn bepaald per periode. Voor Experiment 2 is een klein aantal hennen vanuit het volièresysteem geplaatst op kooien op een leeftijd van 35, 45, 61, 75 en 90 weken. Van deze hennen is het P-gehalte in mest, karkassen en eieren bepaald.

Uit het eerste experiment blijkt dat de minimale behoefte aan P waarschijnlijk niet bereikt is. Het voer met het laagste oP-gehalte had geen negatief effect op productieprestaties, eikwaliteit en lichaasmgewicht. Een oP-gehalte van 2,6 g/kg en 2,4 g/kg van respectievelijk 36-45 en 45-90 weken leeftijd lijkt dan ook voldoende voor een optimaal aantal eieren, eigewicht, eimassa en voederconversie. Echter, uit het tweede experiment komt naar voren dat een oP-gehalte van 2,4 g/kg voer het gehalte aan P in het karkas verlaagde. Dit geeft aan dat het laagste oP-gehalte dicht tegen de minimale behoefte aan ligt. Een deel van de verlaging in oP-gehalte hangt samen met de hogere voeropname van leghennen in scharrelsystemen ten opzichte van kooisystemen. Desondanks resulteren de nieuwe oP-aanbevelingen in een circa 10% lagere oP-opname.

Daarnaast kan op basis van de resultaten van het tweede experiment geconcludeerd worden dat de huidige Nederlandse referentiewaarde van 5,6 g P/kg karkas nog steeds voldoet, ook in oude leghennen. De referentiewaarde van 1,7 g P/kg ei is echter aan de lage kant en zou voor de gehele legperiode verhoogd moeten worden tot minimaal 1,8 g P/kg ei.

## 11. References


Abrahamsson, P. & Tauson, R. (1995). Aviary systems and conventional cages for laying hens: effects on production, egg quality, health and bird location in three hybrids. *Acta Agriculturae Scandinavica, Section A – Animal Science* 45 (3): 191–203

Ahmadi, H. & Rodehutscord, M. (2012). A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. *Poultry Science* 91 (8): 2072–2078

Bar, A. & Hurwitz, S. (1987). Vitamin D metabolism and calbindin (calcium-binding protein) in aged laying hens. *The Journal of Nutrition*: 1775–1779

Bar, A., Razaphkovsky, V. & Vax, E. (2002). Re-evaluation of calcium and phosphorus requirements in aged laying hens. *British Poultry Science* 43 (2): 261–269

Bar, A., Vax, E. & Striem, S. (1999). Relationships among age , eggshell thickness and vitamin d metabolism and its expression in the laying hen. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 123: 147–154

Boling, S.D., Douglas, M.W., Johnson, M.L., Wang, X., Parsons, C.M. & Koelkebeck, K.W. (2000). The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poultry Science* 79 (2): 224–230

Boorman, K.N. & Gunaratne, S.P. (2001). Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. *British Poultry Science* 42 (1): 81–91

Van den Brand, H., Parmentier, H.K. & Kemp, B. (2004). Effects of housing system (outdoor vs cages) and age of laying hens on egg characteristics. *British Poultry Science* 45 (6): 745–752

Cotterill, O.J., Marion, W.W., Naber, E.C. (1977). A nutrient re-evaluation of shell eggs. *Poultry Science* 56 (6): 1927-1934

Gordon, R.W. & Roland, D.A. (1997). Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. *Poultry Science* 76 (8): 1172–1177

Jalal, M.A., Scheideler, S.E. (2001). Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poultry Science 80 (10): 1463-1471

Johnson, A.L. (2000). Reproduction in the female. In: Sturkie's Avian Physiology, fifth edition, Ed.: Whittow, G.C., Academic Press, San Diego, California, United States of America: pp.569–596

Kempen, I. (2013). Voorkomen van borstbeenafwijkingen bij leghennen [To prevent keel bone fractions in layinghens]. Mededeling 69, Proefbedrijf Pluimveehouderij, Geel, Belgium.

Keshavarz, K. & Nakajima, S. (1993). Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. *Poultry Science* 72: 144–153

Van der Klis, J.D., Versteegh, H.A, Simons, P.C. & Kies, A.K. (1997). The efficacy of phytase in corn-soybean meal-based diets for laying hens. *Poultry Science* 76 (11): 1535–1542

Van der Klis, J.D., Blok, M.C. (1997). Definitief systeem opneembaar fosfor pluimvee [Final retainable phosphorus system poultry]. CVB report nr. 20, Centraal Veevoederbureau, Lelystad, The Netherlands.

Lambert, W., De Lange, L., Van Krimpen, M.M., Van der Klis, J.D., Star, L. (2014). P-requirement in modern laying hens - a literature review. Report 1326, Schothorst Feed Research, Lelystad, The Netherlands.

Lei, Q.B., Shi, L.X., Zhang, K.Y., Ding, X.M., Bai, S.P. & Liu, Y.G. (2011). Effect of reduced energy, protein and entire substitution of inorganic phosphorus by phytase on performance and bone mineralisation of laying hens. *British Poultry Science* 52 (2): 202–213

Liu, N., Liu, G.H., Li, F.D., Sands, J.S., Zhang, S., Zheng, A.J., Ru, Y.J. (2007). Efficacy of phytases on egg production and nutrient digestibility in layers fed reduced phosphorus diets. *Poultry Science* 86 (11): 2337–2342

Musapuor, A., Afsharmanesh, M. & Shahrbabak Moradi, H. (2006). Use of microbial phytase for decrease of pollutant due to environmental poultry excreta phosphorus. *International Journal of Agriculture & Biology* 8 (1): 35–37

Neijat, M., House, J.D., Guenter, W. & Kebreab, E. (2011). Calcium and phosphorus dynamics in commercial laying hens housed in conventional or enriched cage systems. *Poultry Science* 90 (10): 2383–2396

Neset, T.S.S. & Cordell, D. (2012). Global phosphorus scarcity: identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture* 92 (1): 2–6

Perrot, P., Bouvarel, I. & Lescoat, P. (2006). Réactualisation des références relatives à la composition corporelle en protéines , phosphore et oligo-éléments des volailles : etat des lieux et impact sur les rejets avicoles. *Science et Techniques Avicoles* 56: 1–6

Punna, S. & Roland, D.A. (1999). Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus-deficient diets from day one of age. *Poultry Science* 78 (10): 1407–1411

Rama Rao, S.V., Raju, M.V.L.N., Reddy, M.R. & Pavani, P. (2006). Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. *Animal Feed Science and Technology* 131 (1-2): 135–150

Sharpley, A. (1999). Reducing the environmental impact of poultry production : focus on phosphorus agricultural phosphorus , water quality , and poultry production : are they compatible? *Poultry Science* 78: 660–673

Snow, J.L., Douglas, M.W., Koelkebeck, K.W., Batal, A.B., Persia, M.E., Biggs, P.E., Parsons, C.M. (2004). Minimum phosphorus requirement of one-cycle and two-cycle (molted) hens. *Poultry Science* 83 (6): 917–924

Sohail, S.S. & Roland, D.A. (2002). Influence of dietary phosphorus on performance of hy-line W36 hens. *Poultry Science* 81 (1): 75–83

Suk, Y.O., Park, C. (2001). Effect of breed and age of hens on the yolk to albumen ratio in two different genetic stocks. Poultry Science 80 (7): 855-858

Versteegh, H.A. & Jongbloed, A.W. (2000). De hoeveelheid droge stof, as, stikstof, calcium, magnesium, fosfor, natrium, kalium, koper, zink en ijzer in eieren en in vleeskuikenouderdieren op twee leeftijden [The level of dry matter, ash, nitrogen, calcium, magnesium. phosphorus, sodium, potassium, copper, zinc and iron in eggs and broiler breeders at two ages]. Wageningen Livestock Research, Lelystad, The Netherlands.

Whitehead, C.C. (2004). Overview of bone biology in the egg-laying hen. *Poultry Science* 83 (2): 193–199

Wu, G. Bryant, W.W., Voitle, R.A., Roland, D.A., Sr. (2005). Effects of dietary energy on performance and egg composition of Bovans White and Dekalb White hens during phase I. Poultry Science 84 (10): 1610-1615

Zweifel, R., Schäublin, H. & Menzi, H. (2009). Evaluation of manure quantity and quality of laying hens held in different aviary systems and fed with different feeding strategies. *In*: *Aviforum*, Zollikofen, p.3052

## Annex 1. Diet compositions

Diets A and B were supplied in period 1 from 36-45 weeks of age; Diets C and D were supplied in period 2 and 3 from 46-54 and 55-65 weeks of age; Diets E and F were supplied in period 4 and 5 from 66-75 and 75-90 weeks of age.

| | | Diet code | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| | Ingredients | | | | | | |
| | Wheat + enzyme | 43.21 | 43.21 | 42.21 | 42.21 | 41.83 | 41.83 |
| | Maize | 25.23 | 25.23 | 25.23 | 25.23 | 25.23 | 25.23 |
| | Sunfl.s.meal.38%CP <16%CF | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| | Soyabean meal. inl. hipro | 4.45 | 4.45 | 4.45 | 4.45 | 4.45 | 4.45 |
| | Maisgl.meal >60%CP | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| | Rapeseedmeal W-E"00" | 1.24 | 1.24 | 1.24 | 1.24 | 1.24 | 1.24 |
| | Soyabean oil | 0.56 | 0.56 | 0.56 | 0.56 | 0.56 | 0.56 |
| | Animal blend. fat | 0.75 | 0.75 | --.-- | --.-- | --.-- | --.-- |
| | Poultry fat | --.-- | --.-- | 0.75 | 0.75 | 0.75 | 0.75 |
| | Limestone (coarse) | 7.49 | 7.70 | 8.54 | 8.70 | 9.05 | 9.16 |
| | Mono-Ca-Phos. TESS | 0.98 | 0.50 | 0.87 | 0.50 | 0.77 | 0.50 |
| | Salt | 0.14 | 0.14 | 0.14 | 0.14 | 0.17 | 0.17 |
| | Premix Lay | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 | 1.30 |
| | Lysin-HCl | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| | Glu-Xyl | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| | Termin-8 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| | Diamol | --.-- | 0.27 | 0.06 | 0.27 | --.-- | 0.16 |
| | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| | Nutrients | | | | | | |
| g/Kg | Moisture | 110 | 110 | 114 | 114 | 114 | 114 |
| g/Kg | Crud ash | 112 | 113 | 126 | 126 | 130 | 130 |
| g/Kg | Crude protein | 162 | 162 | 159 | 159 | 158 | 158 |
| g/Kg | Crude fat | 35 | 35 | 33 | 33 | 33 | 33 |
| g/Kg | Crude fiber | 35 | 35 | 37 | 37 | 37 | 37 |
| g/Kg | Starch | 450 | 450 | 432 | 432 | 430 | 430 |
| g/Kg | Ca | 34.0 | 34.1 | 37.9 | 37.9 | 39.7 | 39.6 |
| g/Kg | P | 5.8 | 4.7 | 5.5 | 4.6 | 5.2 | 4.6 |
| **g/Kg** | **Ret.P mild** | **3.2** | **2.6** | **3.0** | **2.4** | **2.8** | **2.4** |
| g/Kg | Na | 1.3 | 1.3 | 1.3 | 1.3 | 1.4 | 1.4 |
| g/Kg | Cl | 2.9 | 2.9 | 2.9 | 2.9 | 3.1 | 3.1 |
| g/Kg | K | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| g/Kg | d.LYS | 6.6 | 6.6 | 6.6 | 6.6 | 6.5 | 6.5 |
| g/Kg | d.MET | 3.7 | 3.7 | 3.6 | 3.6 | 3.6 | 3.6 |
| g/Kg | d.M+C | 6.2 | 6.2 | 6.0 | 6.0 | 6.0 | 6.0 |
| g/Kg | d.THR | 4.4 | 4.4 | 4.3 | 4.3 | 4.3 | 4.3 |
| g/Kg | d.TRP | 1.4 | 1.4 | 1.3 | 1.3 | 1.3 | 1.3 |
| KCal | E_Lay | 2944 | 2944 | 2912 | 2912 | 2900 | 2900 |

## Annex 2. Production performance for each diet per sub-period in Experiment 1

| 36-45 weeks of age | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) |
|---|---|---|---|---|---|
| Dietary treatment | | | | | |
| 1. 3.2 | 117 | 95.8 | 60.2 | 57.9 | 2.009 |
| 2. 3.0 | 117 | 97.0 | 60.7 | 58.8 | 1.993 |
| 3. 2.8 | 118 | 96.9 | 60.9 | 59.0 | 2.002 |
| 4. 2.8 | 117 | 96.7 | 60.9 | 58.8 | 1.983 |
| 5. 2.6 | 117 | 97.5 | 60.7 | 59.2 | 1.977 |
| 6. 2.6 | 116 | 96.8 | 60.6 | 58.7 | 1.979 |
| *P-value* | *0.726* | *0.279* | *0.070* | *0.240* | *0.548* |
| *LSD* | *2.4* | *1.42* | *0.44* | *1.09* | *0.042* |
| Retainable P level | | | | | |
| 3.2 | 117 | 95.8[b] | 60.2[b] | 57.9[b] | 2.009 |
| 3.0 | 117 | 97.0[ab] | 60.7[ab] | 58.8[ab] | 1.993 |
| 2.8 | 117 | 96.8[ab] | 60.9[a] | 58.9[ab] | 1.993 |
| 2.6 | 117 | 97.2[a] | 60.7[a] | 59.0[a] | 1.983 |
| *P-value* | *0.809* | *0.042* | *0.013* | *0.035* | *0.346* |
| *LSD* | *2.1* | *1.17* | *0.36* | *0.88* | *0.034* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

| 46-54 weeks of age | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) |
|---|---|---|---|---|---|
| Dietary treatment | | | | | |
| 1. 3.0 | 119 | 94.3 | 61.6 | 58.1 | 2.049 |
| 2. 2.8 | 120 | 95.0 | 61.7 | 58.6 | 2.042 |
| 3. 2.8 | 121 | 94.1 | 61.9 | 58.3 | 2.057 |
| 4. 2.6 | 118 | 94.5 | 61.8 | 58.4 | 2.019 |
| 5. 2.4 | 119 | 94.7 | 61.8 | 58.5 | 2.043 |
| 6. 2.4 | 119 | 95.0 | 61.7 | 58.7 | 2.022 |
| *P-value* | *0.426* | *0.449* | *0.714* | *0.773* | *0.177* |
| *LSD* | *2.9* | *1.08* | *0.40* | *0.88* | *0.034* |
| Retainable P level | | | | | |
| 3.0 | 119 | 94.3 | 61.6 | 58.1 | 2.049 |
| 2.8 | 120 | 94.6 | 61.8 | 58.4 | 2.050 |
| 2.6 | 118 | 94.5 | 61.8 | 58.4 | 2.019 |
| 2.4 | 119 | 94.8 | 61.7 | 58.6 | 2.033 |
| *P-value* | *0.263* | *0.699* | *0.612* | *0.639* | *0.126* |
| *LSD* | *2.5* | *0.97* | *0.33* | *0.74* | *0.028* |

| **55-65 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) |
|---|---|---|---|---|---|
| Dietary treatment | | | | | |
| 1. 3.0 | 118 | 92.8 | 62.3 | 57.8 | 2.044 |
| 2. 2.8 | 119 | 93.2 | 62.6 | 58.3 | 2.037 |
| 3. 2.8 | 119 | 93.1 | 62.6 | 58.3 | 2.020 |
| 4. 2.6 | 117 | 93.2 | 62.3 | 58.1 | 2.016 |
| 5. 2.6 | 118 | 93.5 | 62.2 | 58.2 | 2.030 |
| 6. 2.4 | 117 | 92.4 | 62.3 | 57.6 | 2.031 |
| *P-value* | *0.315* | *0.894* | *0.065* | *0.837* | *0.594* |
| *LSD* | *2.0* | *1.94* | *0.32* | *1.25* | *0.035* |
| Retainable P level | | | | | |
| 3.0 | 118 | 92.8 | 62.3[ab] | 57.8 | 2.044 |
| 2.8 | 119 | 93.1 | 62.6[a] | 58.3 | 2.029 |
| 2.6 | 118 | 93.4 | 62.3[b] | 58.1 | 2.023 |
| 2.4 | 117 | 92.4 | 62.3[ab] | 57.6 | 2.031 |
| *P-value* | *0.150* | *0.633* | *0.018* | *0.511* | *0.576* |
| *LSD* | *1.7* | *1.54* | *0.27* | *0.99* | *0.030* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

| **66-75 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) |
|---|---|---|---|---|---|
| Dietary treatment | | | | | |
| 1. 2.8 | 120 | 91.0 | 62.2 | 56.6 | 2.127 |
| 2. 2.6 | 120 | 90.1 | 62.5 | 56.3 | 2.136 |
| 3. 2.6 | 121 | 90.3 | 62.4 | 56.3 | 2.128 |
| 4. 2.4 | 120 | 90.7 | 62.2 | 56.4 | 2.129 |
| 5. 2.4 | 120 | 91.8 | 62.1 | 57.0 | 2.103 |
| 6. 2.4 | 120 | 90.2 | 62.2 | 56.1 | 2.134 |
| *P-value* | *0.844* | *0.588* | *0.382* | *0.878* | *0.390* |
| *LSD* | *2.3* | *2.18* | *0.41* | *1.51* | *0.033* |
| Retainable P level | | | | | |
| 2.8 | 120 | 91.0 | 62.2 | 56.6 | 2.13 |
| 2.6 | 121 | 90.2 | 62.4 | 56.3 | 2.13 |
| 2.4 | 120 | 90.9 | 62.1 | 56.5 | 2.12 |
| *P-value* | *0.479* | *0.527* | *0.085* | *0.883* | *0.566* |
| *LSD* | *1.7* | *1.65* | *0.31* | *1.12* | *0.027* |

| **76-90 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) |
|---|---|---|---|---|---|
| Dietary treatment | | | | | |
| 1. 2.8 | 115 | 85.8 | 64.0 | 55.0 | 2.091 |
| 2. 2.6 | 116 | 85.6 | 64.1 | 54.9 | 2.119 |
| 3. 2.6 | 118 | 86.0 | 64.1 | 55.1 | 2.142 |
| 4. 2.4 | 116 | 85.6 | 63.8 | 54.6 | 2.130 |
| 5. 2.4 | 116 | 86.5 | 63.8 | 55.1 | 2.106 |
| 6. 2.4 | 116 | 84.9 | 63.9 | 54.3 | 2.140 |
| *P-value* | *0.265* | *0.725* | *0.251* | *0.768* | *0.180* |
| *LSD* | *2.5* | *1.98* | *0.39* | *1.38* | *0.045* |
| Retainable P level | | | | | |
| 2.8 | 115 | 85.8 | 64.0[ab] | 55.0 | 2.091 |
| 2.6 | 117 | 85.7 | 64.1[b] | 55.0 | 2.134 |
| 2.4 | 116 | 85.7 | 63.8[a] | 54.7 | 2.125 |
| *P-value* | *0.096* | *0.977* | *0.039* | *0.751* | *0.088* |
| *LSD* | *1.9* | *1.61* | *0.30* | *1.12* | *0.037* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

## Annex 3. Production performance for each breed per sub-period in Experiment 1

| **36-45 weeks of age** | Feed intake (g/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Breed | | | | | | |
| Dekalb | $119^{b}$ | $97.5^{b}$ | $61.0^{b}$ | $59.4^{b}$ | 1.990 | 1.0 |
| LSL | $115^{a}$ | $96.1^{a}$ | $60.4^{a}$ | $58.1^{a}$ | 1.990 | 0.9 |
| | | | | | | |
| *P-value* | *<0.001* | *0.002* | *<0.001* | *<0.001* | *0.466* | *0.531* |
| *LSD* | *1.4* | *0.83* | *0.26* | *0.63* | *0.024* | *0.33* |

$^{a,b}$ Values without a common superscript within a column differ significantly (P ≤ 0.05).

| **46-54 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Breed | | | | | | |
| Dekalb | $121^{b}$ | $95.4^{b}$ | $61.9^{b}$ | $59.1^{b}$ | 2.045 | 0.6 |
| LSL | $118^{a}$ | $93.8^{a}$ | $61.6^{a}$ | $57.8^{a}$ | 2.033 | 0.6 |
| | | | | | | |
| *P-value* | *0.001* | *<0.001* | *0.012* | *<0.001* | *0.199* | *0.935* |
| *LSD* | *1.7* | *0.63* | *0.23* | *0.51* | *0.020* | *0.45* |

$^{a,b}$ Values without a common superscript within a column differ significantly (P ≤ 0.05).

| **55-65 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Breed | | | | | | |
| Dekalb | $120^{b}$ | 93.6 | 62.4 | 58.4 | $2.049^{b}$ | 1.3 |
| LSL | $116^{a}$ | 92.5 | 62.4 | 57.8 | $2.010^{a}$ | 1.2 |
| | | | | | | |
| P-value | <0.001 | 0.052 | 0.292 | 0.095 | <0.001 | 0.520 |
| LSD | 1.2 | 1.12 | 0.18 | 0.72 | 0.020 | 0.38 |

$^{a,b}$ Values without a common superscript within a column differ significantly (P ≤ 0.05).

| **66-75 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| Breed | | | | | | |
| Dekalb | $122^{b}$ | 91.1 | 62.2 | 56.6 | $2.148^{b}$ | 1.5 |
| LSL | $119^{a}$ | 90.3 | 62.3 | 56.3 | $2.105^{a}$ | 1.4 |
| | | | | | | |
| *P-value* | *<0.001* | *0.223* | *0.124* | *0.473* | *<0.001* | *0.493* |
| *LSD* | *1.3* | *1.26* | *0.24* | *0.87* | *0.019* | *0.41* |

$^{a,b}$ Values without a common superscript within a column differ significantly (P ≤ 0.05).

| **76-90 weeks of age** | Feed intake (g/h/d) | Laying rate (%) | Egg weight (g) | Egg mass (g/d) | FCR (g/g) | Mortality (%) |
|---|---|---|---|---|---|---|
| <u>Breed</u> | | | | | | |
| Dekalb | $118^{b}$ | 85.8 | $63.7^{a}$ | 54.7 | $2.155^{b}$ | 2.3 |
| LSL | $115^{a}$ | 85.7 | $64.2^{b}$ | 55.0 | $2.087^{a}$ | 2.5 |
| | | | | | | |
| *P-value* | *<0.001* | *0.953* | *<0.001* | *0.383* | *<0.001* | *0.624* |
| *LSD* | *1.4* | *1.14* | *0.23* | *0.80* | *0.026* | *0.71* |

$^{a,b}$ Values without a common superscript within a column differ significantly ($P \leq 0.05$).

## Annex 4. Result of Round 3bis – 61 weeks of age

Round 2 of Experiment 2 was incorrectly implemented. Laying hens at a high rP level in the practical layer facility (Experiment 1) were assigned to a low rP level in the digestibility cages and vice versa. In order to test the effect, Round 3bis was implemented to study the responsiveness of laying hens fed new diets during 10 days. Round 3bis was performed with 4 treatments and replicates not evenly distributed between the breeds. A one-way ANOVA was performed because the design of this round was unbalanced when breed was taken into account.

Set-up of Round 2, Round 3 and Round 3bis

| **Treatment** | Round 2 45 wk | | Round 3 61 wk | | Round 3bis 61 wk | |
|---|---|---|---|---|---|---|
| | Barn | Lab | Barn | Lab | Barn | Lab |
| 1 | 2.6 | 3.2 | 3.0 | 3.0 | 3.0 | 2.4 |
| 2 | 2.8 | 3.0 | 2.8 | 2.8 | --- | --- |
| 4 | 3.0 | 2.8 | 2.6 | 2.6 | --- | --- |
| 6 | 3.2 | 2.6 | 2.4 | 2.4 | 2.4 | 3.0 |

Phosphorus (P) and calcium (Ca) content (g/kg DM) in egg shell, egg yolk, egg albumen and total egg of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3bis** | Egg shell | | Egg yolk | | Egg Albumen | | Total egg | |
|---|---|---|---|---|---|---|---|---|
| **61 weeks of age** | P | Ca | P | Ca | P | Ca | P | Ca |
| Dietary treatment | | | | | | | | |
| 3.0 | 0.84 | 349 | 11.10 | 2.68 | 0.74 | 0.40 | 1.95[b] | 38.62 |
| 2.4 | 0.86 | 355 | 10.95 | 2.60 | 0.76 | 0.48 | 1.92[a] | 39.17 |
| *P-value* | *0.557* | *0.129* | *0.188* | *0.207* | *0.108* | *0.170* | *0.017* | *0.215* |
| *LSD* | *0.09* | *7.96* | *0.24* | *0.13* | *0.02* | *0.12* | *0.03* | *0.95* |

[a,b] Values without a common superscript within a column differ significantly ($P \leq 0.05$).

Phosphorus (P), calcium (Ca) and soluble phosphorus content in manure (g/kg DM) in Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3** | Manure | | |
|---|---|---|---|
| **61 weeks of age** | P | Ca | Soluble P |
| Dietary treatment | | | |
| 3.0 | 12.07 | 64.4[b] | 1.76 |
| 2.4 | 12.83 | 48.8[a] | 2.10 |
| | | | |
| *P-value* | *0.381* | *<0.001* | *0.532* |
| *LSD* | *1.89* | *6.26* | *1.20* |

[a,b] Values without a common superscript within a column differ significantly (P≤0.05).

Ash, phosphorus (P) and calcium (Ca) content of the carcasses and bones (g/kg DM) of Dekalb White and LSL Classic laying hens fed diets differing in retainable phosphorus levels at 61 weeks of age (Experiment 2)

| **Round 3bis** | Carcass | | | Bone | | |
|---|---|---|---|---|---|---|
| **61 weeks of age** | Ash | P | Ca | Ash | P | Ca |
| Dietary treatment | | | | | | |
| 3.0 | 85.6 | 6.20 | 12.84 | 528 | 175.0 | 383 |
| 2.4 | 84.6 | 6.15 | 11.89 | 529 | 175.9 | 382 |
| | | | | | | |
| *P-value* | *0.778* | *0.839* | *0.168* | *0.670* | *0.261* | *0.825* |
| *LSD* | *9.56* | *0.60* | *1.44* | *6.67* | *1.84* | *5.05* |