Long-term effects of dietary calcium and phosphorus level, and feed form during rearing on egg production, eggshell quality, and bone traits in brown laying hens from 30 to 89 wk of age

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ABSTRACT The effects of feeding strategies during rearing (0−16 wk) of brown laying hens on mid and end laying performance (30−89 wk) were studied. The rearing feeding strategies followed a 3 × 2 factorial arrangement with feed form; mash with inclusion of 3% finely ground wheat straw (**MWS**), crumbles with inclusion of 3% finely ground wheat straw (**CWS**), and crumbles with inclusion of 3% unground oat hulls as fiber sources (**COH**) at 2 dietary Ca and P levels (high or low Ca−P). Feed conversion ratio improved with COH and MWS compared with CWS from 30 to 59 wk. Rate of lay and egg mass production showed a feed form × Ca−P interaction from 60 to 89 wk. Low Ca−P led to a higher egg production, but only when COH and MWS were fed. BW at 89 wk was higher with CWS compared to COH and MWS. BW uniformity was better with COH compared to MWS at 51 wk and both CWS and MWS at 67 wk. Tibia characteristics were not clearly affected by treatment, although there was a feed form × Ca−P interaction on compression at 89 wk, where compression was lower with MWS and low vs. high Ca−P. Low Ca−P during rearing led to higher eggshell thickness, compared to high Ca−P at 45 wk of age, but breaking strength was lower with low vs. high Ca−P at 75 wk. Although eggshell quality was affected by Ca−P and there were some interactions with feed form at some ages, the effect was not consistent. There was no clear relationship between eggshell quality and tibia characteristics. It was concluded that feeding low Ca−P in combination with COH and MWS during the rearing period positively affects egg production during late lay. Also, dietary Ca−P levels, compared to commercial practice, can be lowered during rearing, as this will not affect eggshell quality and bone mineralization at later ages.

Key words: egg-type pullet, phosphorus, calcium, feed form, egg production

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INTRODUCTION

Feeding strategies used in poultry reared for egg production may influence not only the growth of the pullet itself, but also the subsequent performance of the future laying hen. A variety of different nutritional aspects have been studied in the rearing of layer type pullets such as calcium (**Ca**) and/or phosphorus (**P**) levels (Keshavarz, 2003; Jing et al., 2018; Dijkslag et al., 2019), fiber levels and sources (Guzmán et al., 2016) and feed form (Gous and Morris, 2001; Frikha et al., 2009a; Saldaña et al., 2015a; Bozkurt et al., 2019). However, to the authors' knowledge, longitudinal studies covering the complete cycle, from rearing till end of lay, are not available. Better understanding and development of nutritional strategies during rearing that confer improvements or help to optimize performance or improve eggshell quality in the subsequent laying period are beneficial.

Moderate amounts of fiber (2−4%) in the diet of egg-type pullets improve energy efficiency, ADG, ADFI, and gizzard development, although the effect of different fiber sources varies (Guzmán et al., 2015a,b). Structural components, such as oat hulls (**OH**), improve not only gizzard musculature tone, grinding activity, and digesta retention time in the proventriculus and gizzard, but also decrease digesta pH in the gizzard thereby improving nutrient utilization (Svihus, 2011, 2014) and phytate P solubility (Nahashon et al., 1994). Feed form offered to egg-type pullets also has a strong effect on pullet development. Several trials showed that, compared to mash, pelleted (Deaton and May, 1988; Frikha et al.,

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2009a,b) or crumbled feed (Guzmán et al., 2015a; Saldaña et al., 2015a,b) resulted in an increased ADG due to a higher ADFI. Since feed form and dietary fiber affect pullet development, it is likely that these factors could affect the birds' capability for an efficient egg production in the long term.

Eggshell quality decreases with hen age (Park and Sohn, 2018; Wistedt et al., 2019; Benavides-Reyes et al., 2021). The decline in eggshell quality as bird's age is a multifactorial issue where hormonal and enzymatic factors play an important role (Wistedt et al., 2019). From a nutritional point of view, both Ca and P are critical nutrients for ensuring good eggshell quality (Summers, 1995).

P is an essential nutrient, involved in numerous body functions for laying hens including bone formation, energy storage, cellular structure, and egg formation (Snow et al., 2004; Kebreab et al., 2009). A P deficiency will negatively affect rate of lay, as it results in irregular laying cycles caused by a random regression of the ovum (Harms and Sloan, 1999). In broilers, it is known that lower dietary Ca and P at the start of life results in a more efficient repletion of these minerals in later stage (Rousseau et al., 2016). It can be hypothesized that the same mechanism is applicable for laying-type poultry as well.

Previous research from our group showed that reducing dietary nonphytate P from 0.33 to 0.17% during rearing and start of lay from 16 to 27 wk of age did not affect any of the egg production and bone strength parameters (Dijkslag et al., 2019). Keshavarz (2003) studied the effect of feeding low P, approximately 0.1% nonphytate P, to rearing pullets from day-old till 26 wk of age and its effect on growth and laying performance. This study showed a reduced growth and egg production when birds were fed with low dietary P. Due to the short period in which this strategy was applied after rearing, the potential long-term effects of low dietary Ca and P (**Ca−P**) during rearing on subsequent egg production remain unknown. To the authors' knowledge this effect has not been studied previously.

Recently, an experiment carried out by our group, was published in which the effect of reduced Ca−P in 3 different feed forms was studied in layer type pullets till 16 wk of age (Dijkslag et al., 2021). This study covered the rearing period and the effect on layer performance, eggshell quality, and bone traits till 32 wk of age. We concluded from this work that feeding egg-type pullets with mash compared to crumbled diets negatively affected BW gain and feed conversion ratio (**FCR**) during rearing, but this did not clearly affect subsequent early egg production. The addition of oat hulls as coarse fiber source did not affect growth performance during rearing, but some improvement was observed on FCR during lay. Feeding a low-compared with a high Ca−P level during rearing did not affect pullet development and egg production, but some improvements on eggshell quality were identified at 32 wk of age with low Ca−P. Also, low Ca−P led to a decreased bone mineralization at 11 and 16 wk of age, although bone breaking strength was unaffected till 32 wk of age. The current paper is a sequel of the previous trial (Dijkslag et al., 2021) and covers the mid and end laying period from 30 till 89 wk of age. The aim of the current study was to elucidate the effects of the rearing strategies used (feed form and Ca−P) on layer performance and eggshell quality for this given period, where it is expected that feeding structural components and low Ca−P during rearing will affect layer performance at later age.

MATERIALS AND METHODS

The protocol for the experiment conformed to the standards for animal experiments and was approved by the Trouw Nutrition Animal Care Committee and followed recommendations of the Junta de Castilla-La Mancha (Spain) Animal Welfare Department as stated in the royal decree RD 53/2013 (Boletín Oficial del Estado, 2013).

Birds and Husbandry

A total number of 1,380-day-old female Bovans Brown egg-type pullets with intact beaks were reared in a semicommercial rearing facility (Granja Agas S.A., Cuenca, Spain). Birds were assigned to 6 different dietary treatments in 10 replicate groups in adjacent cages, with 23 pullets in each cage. As this experiment was a continuation of a previously published study (Dijkslag et al., 2021), further details of birds and husbandry during the rearing period can be found there.

At 16 wk of age, birds were transferred to an experimental layer house facility (Trouw Nutrition Poultry Research Centre, Casarrubios del Monte, Spain) with 2 separate, but identical rooms. Due to capacity limits, the number of replicates was decreased from 10 to 8 and selection was based on lowest mortality rate during rearing. Remaining birds of the 2 replicates were not culled, but sold as laying hen. Each room contained a 2-level battery with 24 enriched cages where 20 birds per cage were placed. The animals from each cage in the rearing facility remained together as experimental unit when transferred to the layer facility. The assignment of cages in the layer facility followed a complete randomized experimental block design based on room, battery level and location in the room. The dimensions of each cage were 241 × 62.5 × 45 cm (length × width × height) and contained a scratch pad, perches, a laying nest and 4 drinking nipples. After arrival at the layer facility, light was increased with 1 h/wk from 12 h/d till 15 h/d at 19 wk of age. Light intensity was initially set at 20 lux and gradually reduced to 8 lux until the end of the experiment to avoid feather pecking behavior, as birds were restless and showed signs of aggression. Rooms were climate controlled, and temperature was set at 20°C.

Dietary Treatments

Dietary treatments during rearing were arranged in a 3 × 2 factorial arrangement with 2 factors: feed form

Table 1. Overview dietary treatments in the rearing phase from 0 to 16 wk of age.

Label treatment	Feed form	Fiber source (3%)[1]	GMD (μm)[2]	Ca (g/kg)[3]	rP (g/kg)[4]
1. MWS—High Ca−P	Mash	WS	955/876/1105	7.5/6.7/7.3	3.6/3.2/3.0
2. MWS—Low Ca−P	Mash	WS		5.8/4.7/5.0	3.2/2.6/2.4
3. CWS—High Ca−P	Crumbles	WS	640/591/663	7.5/6.7/7.3	3.6/3.2/3.0
4. CWS—Low Ca−P	Crumbles	WS		5.8/4.7/5.0	3.2/2.6/2.4
5. COH—High Ca−P	Crumbles	OH	650/676/695	7.5/ 6.7/7.3	3.6/3.2/3.0
6. COH—Low Ca−P	Crumbles	OH		5.8/4.7/5.0	3.2/2.6/2.4

[1]WS = fine wheat straw; OH = coarse oat hulls.
[2]Geometric mean diameter from, respectively, 0 to 6, 6 to 11, and 11 to 16 wk of age (only determined in high Ca−P).
[3]Dietary Ca content from, respectively, 0 to 6, 6 to 11, and 11 to 16 wk of age.
[4]Dietary retainable P content from, respectively, 0 to 6, 6 to 11, and 11 to 16 wk of age.

and dietary Ca−P level. The tested feed forms were mash with inclusion of 3% finely ground wheat straw (**MWS**), crumbles with inclusion of 3% finely ground wheat straw (**CWS**), and crumbles with inclusion of 3% unground oat hulls as fiber source (**COH**). The tested dietary Ca−P levels were high and low. High Ca was set at 7.5, 6.7, and 7.3 g/kg and low Ca at 5.8, 4.7, and 5.0 g/kg for 0 to 6 wk, 6 to 11 wk, and 11 to 16 wk of age, respectively. High P was set at 3.6, 3.2, and 3.0 g/kg and low P at 3.2, 2.6, and 2.4 g/kg for 0 to 6 wk, 6 to 11 wk, and 11 to 16 wk of age, respectively, all expressed as retainable P (CVB, 2016). All treatments are presented in Table 1 in more detail. The experimental diets were only fed during the rearing period (0−16 wk of age) and details regarding feed composition are presented in Table 2. During the laying period all birds received a commercial crumbled layer diet (Nanta, Griñón, Spain) formulated according to the CVB (2016) recommendations with adequate dietary Ca−P. The ingredient composition of the experimental layer diets with the calculated and analyzed compositions is presented in Table 3. Samples of experimental diets were analyzed according to AOAC International (2005), for DM content by oven-drying (934.01), total ash (942.05), nitrogen by combustion (990.03) using a LECO analyzer and ether extract (960.39). Samples

Table 2. Composition, calculated, and determined analysis (g/kg, as-fed) of experimental diets fed to pullets from 0 to 16 wk of age.

	0−6 wk				6−11 wk				11−16 wk			
Treatment[1]	1 and 3	2 and 4	5	6	1 and 3	2 and 4	5	6	1 and 3	2 and 4	5	6
Ingredient												
Corn	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Wheat	351.8	351.8	351.8	351.8	404.0	404.0	404.0	404.0	423.8	423.8	423.8	423.8
Soybean meal	262.7	262.7	262.7	262.7	217.9	217.9	217.9	217.9	199.3	199.3	199.3	199.3
Soya oil	22.3	22.3	22.3	22.3	17.1	17.1	17.1	17.1	12.0	12.0	12.0	12.0
Sodium bicarbonate	2.8	2.8	2.8	2.8	2.9	2.9	2.9	2.9	2.8	2.6	2.6	2.6
Methionine Hydroxy analog	2.7	2.7	2.7	2.7	2.2	2.2	2.2	2.2	1.3	1.3	1.3	1.3
Sodium chloride	2.1	2.1	2.1	2.1	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Lysine (Biolys 70)	1.0	1.0	1.0	1.0	1.4	1.4	1.4	1.4	0.2	0.2	0.2	0.2
L-Threonine 98%	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5	0.1	0.1	0.1	0.1
Calcium carbonate	11.3	7.9	11.3	7.8	10.3	6.5	10.3	6.5	11.5	7.1	11.5	7.1
Monocalcium phosphate	6.7	4.8	6.7	4.8	4.9	1.6	4.9	1.6	4.1	0.9	4.1	0.9
Sepiolite 15/30		5.3		5.3	0.5	7.7	0.6	7.8	2.0	9.3	2.0	9.3
Premix[3]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Premix phytase[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Wheat straw fine[4]	30.0	30.0			30.0	30.0			30.0	30.0		
Oat hulls coarse[5]			30.0	30.0			30.0	30.0			30.0	30.0
Calculated analysis												
AMEn, kcal/kg[6]	2,698	2,698	2,698	2,698	2,697	2,697	2,697	2,697	2,699	2,699	2,699	2,699
CP	185.3	185.3	185.3	185.3	165.3	165.3	165.0	165.0	157.0	157.0	156.7	156.7
Ca	7.5	5.8	7.5	5.8	6.7	4.7	6.7	4.7	7.3	5.0	7.3	5.0
P	5.1	4.7	5.1	4.7	4.6	3.8	4.6	3.9	4.3	3.6	4.3	3.6
rP[2]	3.6	3.2	3.6	3.2	3.2	2.6	3.2	2.6	3.0	2.4	3.0	2.4
Determined analysis[7]												
Ca	8.7/7.8	7.2/7.3	8.0	6.2	11.9/9.3	8.4/6.7	8.9	6.7	8.6/7.7	5.9/5.7	7.8	5.7
P	5.1/4.9	4.8/4.8	5.2	4.8	4.9/4.6	3.8/3.6	4.5	3.6	4.0/3.8	3.3/3.3	4.0	3.2

[1]Treatment 1: mash, high Ca and P; Treatment 2: mash, low Ca and P; Treatment 3: crumbles, high Ca and P; Treatment 4: crumbles, low Ca and P; Treatment 5: crumbles with 3% oat hulls, high Ca and P; Treatment 6: crumbles with 3% oat hulls, low Ca and P.
[2]rP = retainable phosphorus (CVB, 2016).
[3]Provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 50 IU; vitamin K3, 2.0 mg; vitamin B1, 2.0 mg; vitamin B2, 2.0 mg; vitamin B6, 4.0 mg; vitamin B12, 0.03 mg; niacinamide, 40 mg; D-pantothenic acid, 10 mg; folic acid, 1.0 mg; biotin, 0.15 mg; choline, 260 mg; iron, 67.7 mg (as $FeSO_4 \cdot 7H_2O$); copper, 15 mg (as $CuSO_4 \cdot 5H_2O$); manganese, 90 mg (as $MnSO_4 \cdot H_2O$); zinc, 80 mg (as ZnO); iodine, 1.0 mg (as CaI); selenium, 0.25 mg (as $Na_2SeO_3 \cdot 5H_2O$); Phytase, 500 FTU (supplied by Trouw Nutrition Spain).
[4]Calculated analysis: CP 30 g/kg, ether extract 14 g/kg, crude fiber 377 g/kg.
[5]Calculated analysis: CP 32 g/kg, ether extract 15 g/kg, crude fiber 315 g/kg.
[6]AMEn is based on calculation methods from CVB (2016) for chickens.
[7]For treatment 1 to 4, 2 figures are displayed. The first was determined in mash, the second in crumbles.

Table 3. Composition, calculated, and determined analysis (g/kg) of the experimental diets during the laying period (30−89 wk of age).

Period	30−34 wk	34−40 wk	40−60 wk	60−89 wk
Ingredient				
Barley	50.0			50.0
Corn	577.5	441.3	607.2	353.5
Wheat		190.2		250.0
Rapeseed meal	80.0	80.0	80.0	80.0
Soybean meal	134.1	129.3	145.4	96.8
Soybean oil	7.3	8.0	9.6	11.1
Corn DDGS	50.0	50.0	50.0	50.0
Calcium carbonate	90.2	90.4	98.5	99.0
Monocalcium phosphate	2.0	1.8	0.9	
Sodium chloride	2.8	2.9	2.9	2.8
Methionine hydroxy analog	1.4	1.4	1.3	1.2
Lysine (Biolys 70)	0.5	0.7	0.2	
Lysine sulfate				1.4
L-Tryptophan	0.1			
Premix Canthaxanthin[1]	0.8	0.7	0.7	0.8
Choline chloride (75%)	0.4	0.3	0.4	0.4
Premix[2]	3.0	3.0	3.0	3.0
Calculated analysis				
AMEn, kcal/kg[3]	2800	2800	2775	2750
CP	160.0	160.0	155.0	150.0
Ca	37.0	37.0	40.0	40.0
P	4.0	4.1	3.9	3.7
rP[4]	3.1	3.1	3.0	2.9
Determined analysis				
CP	165.5	164.0	156.8	155.0
Ca	32.4	30.9	41.3	36.6
P	3.6	3.4	3.4	3.3

[1]Provided per kilogram of complete diet: 3.4 mg canthaxanthin (supplied by Trouw Nutrition Spain).

[2]Provided per kilogram of complete diet: vitamin A, 7,500 IU; vitamin D3, 1,500 IU; vitamin E, 6 IU; vitamin K3, 2.0 mg; vitamin B1, 2.0 mg; vitamin B2, 3.0 mg; vitamin B6, 3.0 mg; vitamin B12, 0.03 mg; niacinamide, 20 mg; D-pantothenic acid, 6.5 mg; folic acid, 0.5 mg; biotin, 0.1 mg; choline, 295 mg; iron, 40 mg (as $FeSO_4{\cdot}7H_2O$); copper, 12 mg (as $CuSO_4{\cdot}5H_2O$); manganese, 90 mg (as $MnSO_4{\cdot}H_2O$); zinc, 60 mg (as ZnO); iodine, 1.0 mg (as CaI); selenium, 0.20 mg (as $Na_2SeO_3{\cdot}5H_2O$); Phytase, 600 FTU (supplied by Trouw Nutrition Spain).

[3]AMEn is based on calculation methods from CVB (2016) for laying hens.

[4]rP (retainable phosphorus) is based on calculation methods from CVB (2016) for laying hens.

were also analyzed for starch by the α-amylase glucosidase method (996.11), crude fiber by sequential extraction with diluted acid and alkali (962.09), and Ca and P by flame absorption spectrophotometry (965.17).

Observations

Performance Parameters Performance parameters under evaluation of the current study were BW, ADFI, egg production, FCR, and flock uniformity. In order to evaluate these, individual BW was determined at 51, 67, and 89 wk of age. FCR was calculated as the ratio between ADFI and average daily egg mass production (rate of lay × egg weight). Flock uniformity was determined by calculating the percentage of birds within the range mean ±10% average BW per experimental unit.

Bone Parameters At the age of 67 and 89 wk, 1 hen per experimental unit (within the range mean ±5% average BW in that unit) was euthanized by cervical dislocation. Right tibias were removed for analysis of breaking strength and ash content. Tibia breaking strength was recorded by using a TA.XT plus100C texture analyzer (Stable Micro Systems, Godalming, UK), after removal of meat and other tissue adhered to the bone. The clean tibia was placed on its support positioned horizontally (perpendicularly in relation to the probe) and the minimum force needed to cause failure was measured at a constant velocity of the measuring head shift equal to 3 mm/s and a force of 15 g in the middle part of the tibia. To determine ash contents, tibias were weighed, placed in preweighed crucibles and dried for 18 h at 103°C. After dry matter weight was recorded, bones were ashed in a muffle oven at 550°C for 720 min. Crucibles were cooled down to room temperature in silica desiccators and weighed to record ash content.

Egg Parameters At 38, 45, 51, 59, 67, 75, 84, and 89 wk of age, 4 intact, clean, and normal-shaped eggs per pen were randomly taken to evaluate eggshell quality (breaking strength, shell weight, shell thickness). Eggshell breaking strength and eggshell thickness were recorded by using a TA.XT plus100C texture analyzer (Stable Micro Systems, Godalming, UK). The whole egg was placed on its support positioned vertically in relation to the probe and egg weight was recorded. After that the breaking strength was measured at a constant velocity of the measuring head shift equal to 2 mm/s and a force of 2 g. Eggshell thickness was measured with the specific function of the texture analyzer at a constant velocity of the measuring head shift equal to 0.5 mm/s in the middle region of the egg (including membrane), after rinsing the shell to remove any adhering albumen and shells were dry and weighed. Shell weight per unit surface area (**SWUSA**), in mg/cm^2, was calculated as eggshell weight divided by eggshell surface area, where surface area was calculated by the equation (Phirinyane et al., 2011):

$$SA = 3.9782 \times \left(W^{0.7056}\right)$$

where SA is the surface area in cm^2 and W is egg weight in g.

Statistical Analysis

The experimental treatments were arranged in a 3 × 2 factorial arrangement with 2 factors: feed form and dietary Ca−P level. Pen was the experimental unit for performance and eggshell quality data, while hen was the experimental unit for tibia related data. Measurements were evaluated by analysis of variance using GenStat (21st edition, VSNI, Hemel Hempstead, UK) statistical software according to the following general model:

$$Y_{ijk} = \mu + \text{Feed form}_i + \text{Ca} - \text{P level} + \text{Feed form}_i \times \text{Ca} - \text{P level}_j + \text{Block}_k + e_{ijk}$$

where Y_{ijk} is the measured response, μ is the overall mean effect, Feed $form_i$ is the fixed feed form during rearing effect (i = MWS, CWS, or COH) and Ca−P $level_j$ is the fixed dietary Ca−P level during rearing (j = high or low). Room number was added as factor into the model only when its effect was significant ($P < 0.05$). All interactions between Feed $form_i$ and Ca−P $level_j$ were included and e_{ijk} is the error associated with the ith feed form and the jth Ca−P level. The P value of the statistical model is given per response parameter. Treatment means were compared by least significant difference (**LSD**) after significant effects were confirmed by ANOVA. Values with $P \leq 0.05$ were considered to be statistically significant.

RESULTS

Egg Production

Livability was 88% during the mid and end laying period (30−89 wk of age) and was not related to any of the treatments (data not shown). During the trial period, the average rate of lay was similar to or higher than the breeder companies' manual standard (Bovans Brown Product Guide, www.bovans.com) and therefore considered adequate (Figure 1). The effects of the rearing dietary treatments on egg production from 30 to 89 wk of age are presented as means per 30 wk period (2 periods) and over the total trial period (Table 4).

From 30 to 59 wk of age, feed form during rearing affected FCR. FCR was 0.07 and 0.10 g/g lower for COH and MWS as compared to CWS ($P = 0.008$). An interaction was observed for feed form × Ca−P level on rate of lay ($P = 0.015$) from 60 to 89 wk of age. When fed CWS or MWS, rate of lay was unaffected by Ca−P level. However, when fed COH, rate of lay was 4.9% higher with low compared to high dietary Ca−P level. An interaction was observed for feed form × Ca−P level on egg mass production ($P = 0.032$) from 60 to 89 wk of age. When fed CWS, egg mass production was unaffected by Ca−P level. However, egg mass production was 3.5 and 1.9 g/d higher when low compared to high Ca−P level was fed in combination with respectively COH and MWS. No effects of dietary treatments during rearing were observed on any of the egg production parameters during the entire period from 30 to 89 wk of age.

BW and BW Uniformity

The effects of the dietary treatments during the rearing period on BW and BW uniformity are presented in Table 5. Feed form affected BW and BW uniformity. At 89 wk of age, birds fed CWS had 60 g higher BW compared to MWS where COH was intermediate ($P = 0.050$). At 51 wk of age, BW uniformity was 12.0% points higher when COH was fed compared to MWS, where CWS was intermediate ($P = 0.008$). At 67 wk of age, BW uniformity was 10.6 and 9.2% points higher when COH was fed compared to CWS and MWS respectively ($P = 0.014$), but at 89 wk of age these differences were diminished.

Tibia Characteristics

The effects of the dietary treatments during the rearing period on tibia characteristics at 67 and 89 wk of age are presented in Table 6. A feed form × Ca−P level interaction was observed on relative tibia weight at 67 wk of age ($P = 0.017$). When CWS was fed, relative tibia to BW weight was 0.07% points higher with low compared to high Ca−P level. Relative tibia weight was not affected by Ca−P level when COH or MWS were fed. A feed form × Ca−P level interaction was observed on compression at 89 wk of age ($P = 0.047$). When fed MWS, compression was 0.28 mm higher with high compared to low Ca−P. Compression was not affected by Ca−P level when CWS or COH were fed.

Eggshell Quality

The effects of the dietary treatments during the rearing period on eggshell quality, measured at 38, 45, 51, 59, 67, 75, 84, and 89 wk of age are presented in Tables 7A and 7B. Ca−P level affected eggshell thickness only at 45 wk of age. Eggshell thickness was 0.017 mm larger ($P = 0.029$) with low compared to high Ca−P level. A feed form × Ca−P level interaction ($P = 0.019$) was observed for eggshell thickness at 51 wk of age. When MWS was fed, eggshell thickness was 0.019 mm larger with high compared to low Ca−P level, where Ca−P level did not affect eggshell thickness with CWS or COH. A feed form × Ca−P level interaction ($P = 0.003$) was found on eggshell thickness at 59 wk of age. When MWS or CWS was fed, eggshell thickness was 0.022 mm larger or 0.017 mm less, respectively, with high compared to low Ca−P level, but Ca−P level did not affect eggshell thickness with COH. A feed form × Ca−P level interaction ($P = 0.029$) was observed on eggshell thickness at 67 wk of age. With high Ca−P level, eggshell thickness was 0.018 mm larger with CWS compared to COH, where MWS was intermediate. Feed form did not affect eggshell thickness at low Ca−P level. A feed form × Ca−P level interaction ($P = 0.003$) was observed on SWUSA at 59 wk of age. When MWS or CWS was fed, SWUSA was 3.4 mg/cm^2 higher or 2.9 mg/cm^2 lower, respectively, with high compared to low Ca−P level, but Ca−P level did not affect SWUSA with COH. A feed form × Ca−P level interaction ($P = 0.013$) was observed on SWUSA at 67 wk of age. When COH was fed, SWUSA was 4.7 mg/cm^2 higher with low compared to high Ca−P level, but Ca−P level did not affect SWUSA with CWS or MWS. Ca−P level affected

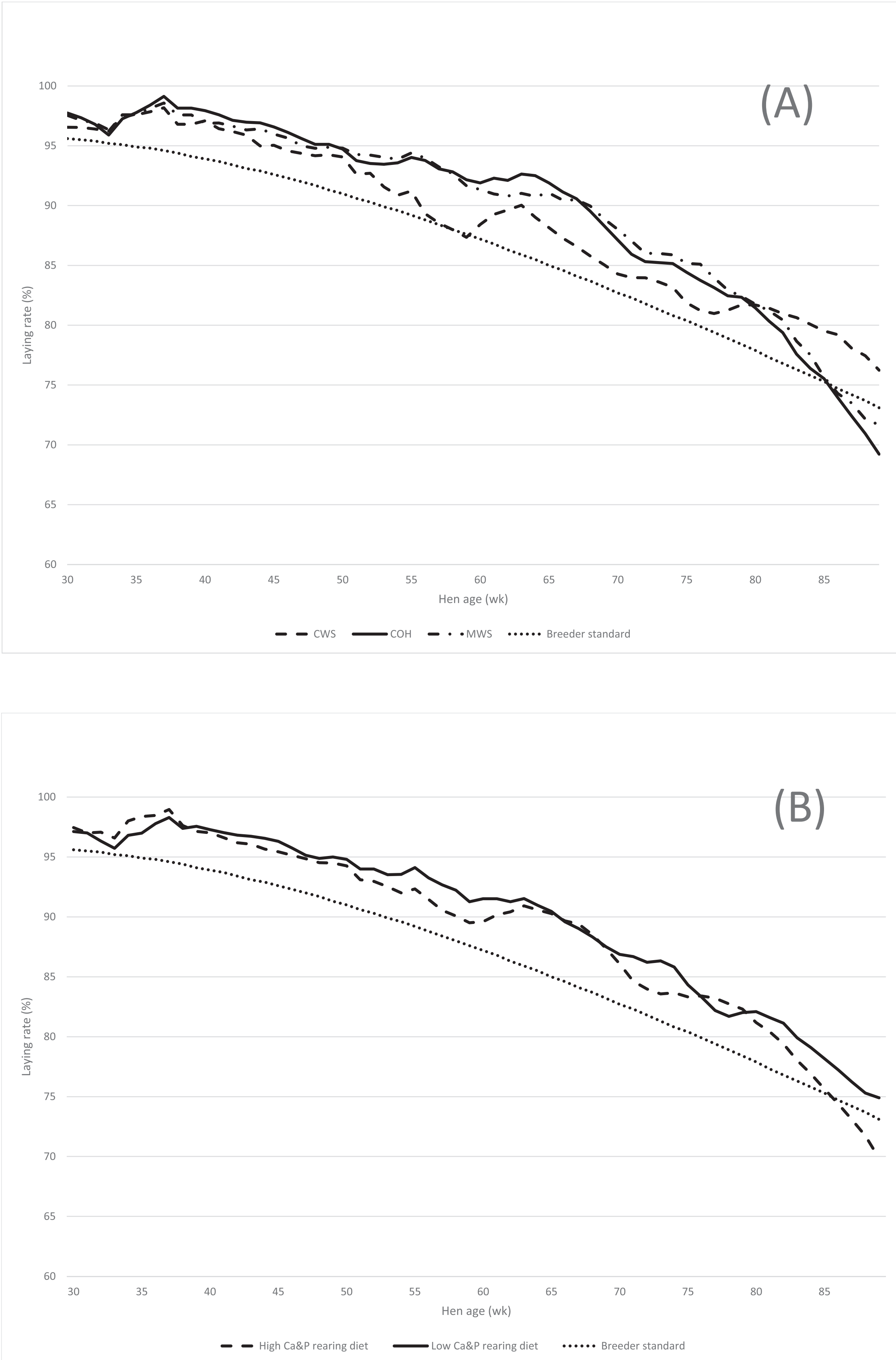

Figure 1. Rate of lay of Bovans Brown laying hens aged 30 to 89 wk, fed rearing diets from 0 to 16 wk of age as mash (MWS), crumbles (CWS), or crumbles with 3% coarse oat hulls (COH) feed form (A) and high or low dietary Ca and P (B), compared to breeder company manual standard rate of lay.

Table 4. Egg production parameters of laying hens in 30 wk periods (30−59 and 60−89 wk of age) and from 30 to 89 wk of age, fed diets during rearing (0−16 wk of age) as crumbles (CWS), crumbles with 3% coarse oat hulls (COH), or mash (MWS) feed form, and high or low calcium and phosphorus (Ca−P) level.

	30−59 wk					60−89 wk					30−89 wk				
Effect[1]	Rate of lay (%)	ADFI (g/d)	Egg weight (g)	Egg mass production (g/d)	FCR[2] (g/g)	Rate of lay (%)	ADFI (g/d)	Egg weight (g)	Egg mass production (g/d)	FCR[2] (g/g)	Rate of lay (%)	ADFI (g/d)	Egg weight (g)	Egg mass production (g/d)	FCR[2] (g/g)
Feed form															
CWS	93.8	132.3	62.6	58.7	2.26[a]	82.5	140.6	64.5	53.2	2.65	88.3	136.4	63.5	56.0	2.44
COH	95.5	131.1	62.8	59.9	2.19[b]	82.4	142.1	65.0	53.5	2.66	89.1	136.2	63.7	56.8	2.40
MWS	95.4	127.9	62.2	59.4	2.16[b]	83.2	138.0	64.3	53.5	2.59	89.2	132.9	63.2	56.3	2.36
SEM ($n = 16$)	0.58	1.35	0.22	0.37	0.022	1.03	1.78	0.25	0.65	0.040	0.71	1.48	0.22	0.44	0.028
Ca−P level															
High	94.6	130.2	62.5	59.1	2.21	81.8	140.7	64.5	52.8	2.67	88.2	135.4	63.4	55.9	2.42
Low	95.2	130.7	62.6	59.6	2.20	83.5	139.8	64.7	54.1	2.59	89.5	134.9	63.5	56.8	2.38
SEM ($n = 24$)	0.47	1.10	0.18	0.31	0.018	0.84	1.45	0.20	0.53	0.033	0.58	1.21	0.18	0.36	0.023
Feed form × Ca−P level															
CWS—High	93.6	131.2	62.5	58.5	2.24	84.3[a]	141.6	64.0	54.0[ab]	2.63	88.9	136.4	63.2	56.2	2.43
CWS—Low	94.0	133.4	62.7	58.9	2.27	80.8[ab]	139.7	64.9	52.5[b]	2.66	87.6	136.4	63.7	55.8	2.45
COH—High	94.7	130.9	62.5	59.2	2.21	79.9[b]	140.6	64.8	51.8[b]	2.73	87.5	135.7	63.5	55.6	2.44
COH—Low	96.4	131.3	63.0	60.7	2.16	84.8[a]	143.5	65.2	55.3[a]	2.60	90.7	136.7	64.0	58.0	2.36
MWS—High	95.5	128.4	62.4	59.6	2.16	81.4[ab]	139.8	64.5	52.5[b]	2.67	88.3	134.1	63.4	56.0	2.40
MWS—Low	95.4	127.4	62.1	59.2	2.15	85.0[a]	136.2	64.1	54.4[a]	2.51	90.2	131.7	62.9	56.7	2.33
SEM ($n = 8$)	0.82	1.90	0.32	0.53	0.030	1.45	2.52	0.35	0.92	0.057	1.00	2.09	0.31	0.62	0.039
Source of variation	*P* values														
Feed form	0.072	0.072	0.268	0.071	0.008	0.822	0.280	0.107	0.939	0.420	0.556	0.185	0.172	0.433	0.181
Ca−P level	0.329	0.732	0.684	0.247	0.697	0.174	0.683	0.316	0.092	0.086	0.125	0.786	0.582	0.077	0.144
Feed form × Ca−P level	0.540	0.702	0.369	0.185	0.476	0.015	0.412	0.179	0.032	0.210	0.090	0.703	0.259	0.092	0.361

[a−b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Treatments were applied during rearing (0−16 wk of age). All diets were similar in composition and fed as crumbles after 16 wk of age.
[2]Feed conversion ratio for egg production (ADFI/egg mass production).

Table 5. BW and BW uniformity of laying hens at 51, 67, and 89 wk of age, fed diets during rearing (0−16 wk of age) as crumbles (CWS), crumbles with 3% coarse oat hulls (COH), or mash (MWS) feed form, and high or low calcium and phosphorus (Ca−P) level.

Effect[1]	BW (g)			BW uniformity[2] (%)		
	wk 51	wk 67	wk 89	wk 51	wk 67	wk 89
Feed form						
CWS	2009	1985	2019[a]	75.4[ab]	70.0[b]	67.9
COH	1988	1976	1972[ab]	82.0[a]	79.2[a]	69.0
MWS	1981	1969	1959[b]	70.0[b]	68.6[b]	64.2
SEM ($n = 16$)	13.2	14.7	17.4	2.50	2.56	3.54
Ca−P level						
High	2003	1989	1981	73.3	72.1	69.2
Low	1982	1964	1986	78.3	73.1	64.9
SEM ($n = 24$)	10.8	12.0	14.2	2.04	2.09	2.89
Feed form × Ca −P level						
CWS—High	2043	2022	2010	70.3	69.1	70.9
CWS—Low	1974	1949	2028	80.6	71.0	65.0
COH—High	1980	1969	1979	80.8	77.9	73.0
COH—Low	1997	1983	1965	83.2	80.4	65.0
MWS—High	1987	1977	1954	69.0	69.4	63.8
MWS—Low	1974	1961	1965	71.1	67.9	65.6
SEM ($n = 8$)	18.6	20.8	24.6	3.54	3.62	5.01
Source of variation	*P* values					
Feed form	0.312	0.735	0.050	0.008	0.014	0.604
Ca−P level	0.167	0.151	0.796	0.096	0.753	0.296
Feed form × Ca −P level	0.078	0.120	0.784	0.437	0.836	0.659

[a−b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Treatments were applied during rearing (0−16 wk of age). All diets were similar in composition and fed as crumbles after 16 wk of age.
[2]Evaluated as the percentage of birds within ±10% of the average BW.

Table 6. Tibia parameters of laying hens at 67 and 89 wk of age, fed diets during rearing (0−16 wk of age) as crumbles (CWS), crumbles with 3% coarse oat hulls (COH), or mash (MWS) feed form, and high or low calcium and phosphorus (Ca−P) level.

Effect[1]	67 wk				89 wk			
	Relative weight (% of BW)	Ash (% of DM)	Breaking strength (kg)	Compression (mm)	Relative weight (% of BW)	Ash (% of DM)	Breaking strength (kg)	Compression (mm)
Feed form								
CWS	0.60	46.0	29.24	2.43	0.61	47.5	24.22	2.09
COH	0.60	46.1	27.80	2.71	0.62	46.0	22.29	2.08
MWS	0.58	44.5	26.02	2.43	0.64	48.2	23.76	2.02
SEM ($n = 16$)	0.012	0.86	1.474	0.258	0.012	1.24	1.275	0.053
Ca−P level								
High	0.59	45.6	28.16	2.53	0.62	47.5	24.07	2.09
Low	0.60	45.5	27.21	2.52	0.62	46.9	22.87	2.03
SEM ($n = 24$)	0.010	0.70	1.204	0.211	0.010	1.01	1.041	0.043
Feed form × Ca−P level								
CWS—High	0.56[b]	46.5	31.00	2.51	0.60	45.8	23.02	2.04[ab]
CWS—Low	0.63[a]	45.5	27.47	2.35	0.62	49.1	25.42	2.13[a]
COH—High	0.60[ab]	46.2	29.11	2.61	0.62	46.9	22.75	2.06[ab]
COH—Low	0.60[ab]	46.1	26.50	2.81	0.62	45.0	21.83	2.09[ab]
MWS—High	0.60[ab]	44.1	24.37	2.48	0.65	49.8	26.42	2.16[a]
MWS—Low	0.56[b]	44.9	27.66	2.39	0.63	46.5	21.11	1.88[b]
SEM ($n = 8$)	0.017	1.22	2.085	0.365	0.017	1.75	1.803	0.075
Source of variation	*P* values							
Feed form	0.414	0.353	0.316	0.685	0.189	0.451	0.541	0.369
Ca−P level	0.472	0.914	0.582	0.952	0.813	0.659	0.391	0.594
Feed form × Ca−P level	0.017	0.763	0.224	0.876	0.435	0.161	0.118	0.047

[a−b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Treatments were applied during rearing (0−16 wk of age). All diets were similar in composition and fed as crumbles after 16 wk of age.

Table 7A. Eggshell quality parameters at 38, 45, 51, and 59 wk of age from laying hens fed diets during rearing (0−16 wk of age) as crumbles (CWS), crumbles with 3% coarse oat hulls (COH), or mash (MWS) feed form, and high or low calcium and phosphorus (Ca−P) level.

Effect[1]	38 wk			45 wk			51 wk			59 wk		
	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)
Feed form												
CWS	6493	0.437	93.6	5858	0.407	87.9	5469	0.429	87.9	5569	0.397	88.8
COH	5846	0.438	92.1	5533	0.423	88.0	5266	0.431	88.0	5393	0.395	88.4
MWS	5853	0.433	92.4	5710	0.418	89.4	5502	0.430	88.5	5289	0.398	88.1
SEM ($n = 16$)	211.8	0.0052	0.95	113.7	0.0064	0.70	167.1	0.0031	0.45	161.7	0.0030	0.58
Ca−P level												
High	6137	0.432	92.8	5713	0.408[b]	88.2	5488	0.432	88.7	5432	0.398	88.5
Low	5991	0.440	92.7	5687	0.425[a]	88.6	5337	0.428	87.5	5402	0.395	88.3
SEM ($n = 24$)	173.0	0.0043	0.78	92.8	0.0052	0.57	136.5	0.0025	0.55	132.0	0.0037	0.48
Feed form × Ca−P level												
CWS—High	6507	0.433	93.6	5990	0.402	87.6	5769	0.431[ab]	88.2	5601	0.388[b]	87.3[b]
CWS—Low	6478	0.441	93.6	5726	0.412	88.2	5168	0.427[ab]	87.6	5536	0.405[a]	90.2[a]
COH—High	5946	0.427	92.1	5544	0.413	87.5	5160	0.427[ab]	88.2	5543	0.398[ab]	88.5[ab]
COH—Low	5747	0.449	92.0	5522	0.433	88.5	5373	0.435[a]	87.8	5243	0.393[b]	88.3[ab]
MWS—High	5958	0.436	92.5	5605	0.408	89.5	5534	0.439[a]	89.7	5152	0.409[a]	89.8[a]
MWS—Low	5748	0.429	92.3	5814	0.428	89.3	5470	0.420[b]	87.2	5427	0.387[b]	86.4[b]
SEM ($n = 8$)	299.6	0.0074	1.35	160.8	0.0090	0.99	236.4	0.0043	0.78	228.7	0.0052	0.82
Source of variation	*P* values											
Feed form	0.061	0.748	0.498	0.146	0.205	0.243	0.565	0.897	0.757	0.476	0.841	0.747
Ca−P level	0.555	0.212	0.934	0.847	0.029	0.609	0.441	0.186	0.072	0.871	0.418	0.753
Feed form × Ca−P level	0.944	0.188	0.995	0.352	0.835	0.819	0.231	0.019	0.374	0.459	0.003	0.003

[a−b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Treatments were applied during rearing (0−16 wk of age). All diets were similar in composition and fed as crumbles after 16 wk of age.
[2]SWUSA = shell weight per unit surface area.

Table 7B. Eggshell quality parameters at 67, 75, 84, and 89 wk of age from laying hens fed diets during rearing (0−16 wk of age) as crumbles (CWS), crumbles with 3% coarse oat hulls (COH), or mash (MWS) feed form, and high or low calcium and phosphorus (Ca−P) level.

Effect[1]	67 wk			75 wk			84 wk			89 wk		
	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)	Eggshell breaking strength (g)	Eggshell thickness (mm)	SWUSA[2] (mg/cm^2)
Feed form												
CWS	5549	0.415	88.4	4742	0.412	87.2	4993	0.412	87.7	5272	0.4399	86.7
COH	5160	0.411	86.2	4831	0.415	86.4	4755	0.416	88.8	4955	0.4362	86.3
MWS	4964	0.413	87.0	4699	0.410	85.0	4990	0.410	87.7	4698	0.4296	84.8
SEM ($n = 16$)	181.9	0.0041	0.73	108.5	0.0039	0.77	149.7	0.0135	0.78	226.0	0.0053	1.11
Ca−P level												
High	5136	0.414	86.7	4900^{a}	0.415	87.2^{a}	4953	0.411	88.2	4856	0.438	86.2
Low	5313	0.411	87.7	4614^{b}	0.410	85.2^{b}	4873	0.414	88.0	5094	0.433	85.7
SEM ($n = 24$)	148.5	0.0034	0.59	88.6	0.0032	0.63	122.2	0.0110	0.64	184.5	0.0043	0.91
Feed form × Ca−P level												
CWS—High	5669	0.421^{a}	89.1^{a}	4898	0.412	87.8	5034	0.406	86.9	4988	0.441	85.9
CWS—Low	5429	0.409^{ab}	87.6^{a}	4586	0.411	86.6	4951	0.417	88.4	5556	0.439	87.5
COH—High	4878	0.403^{b}	83.8^{b}	4917	0.415	86.9	4828	0.414	88.5	4752	0.430	85.3
COH—Low	5443	0.419^{ab}	88.5^{a}	4745	0.415	85.8	4683	0.419	89.2	5158	0.442	87.3
MWS—High	4862	0.419^{ab}	87.1^{a}	4886	0.418	86.8	4996	0.415	89.0	4827	0.441	87.3
MWS—Low	5066	0.406^{ab}	87.0^{a}	4511	0.403	83.2	4984	0.405	86.3	4568	0.418	82.3
SEM ($n = 8$)	257.2	0.0058	1.03	153.4	0.0055	1.09	211.7	0.0066	1.10	319.6	0.0075	1.57
Source of variation	*P* values											
Feed form	0.085	0.737	0.111	0.684	0.667	0.142	0.447	0.623	0.834	0.215	0.389	0.466
Ca−P level	0.408	0.493	0.224	0.030	0.242	0.038	0.646	0.657	0.495	0.368	0.469	0.715
Feed form × Ca−P level	0.307	0.029	0.013	0.798	0.361	0.427	0.952	0.292	0.141	0.402	0.092	0.056

[a−b]Means within a column and within a source without a common superscript differ significantly ($P < 0.05$).
[1]Treatments were applied during rearing (0−16 wk of age). All diets were similar in composition and fed as crumbles after 16 wk of age.
[2]SWUSA = shell weight per unit surface area.

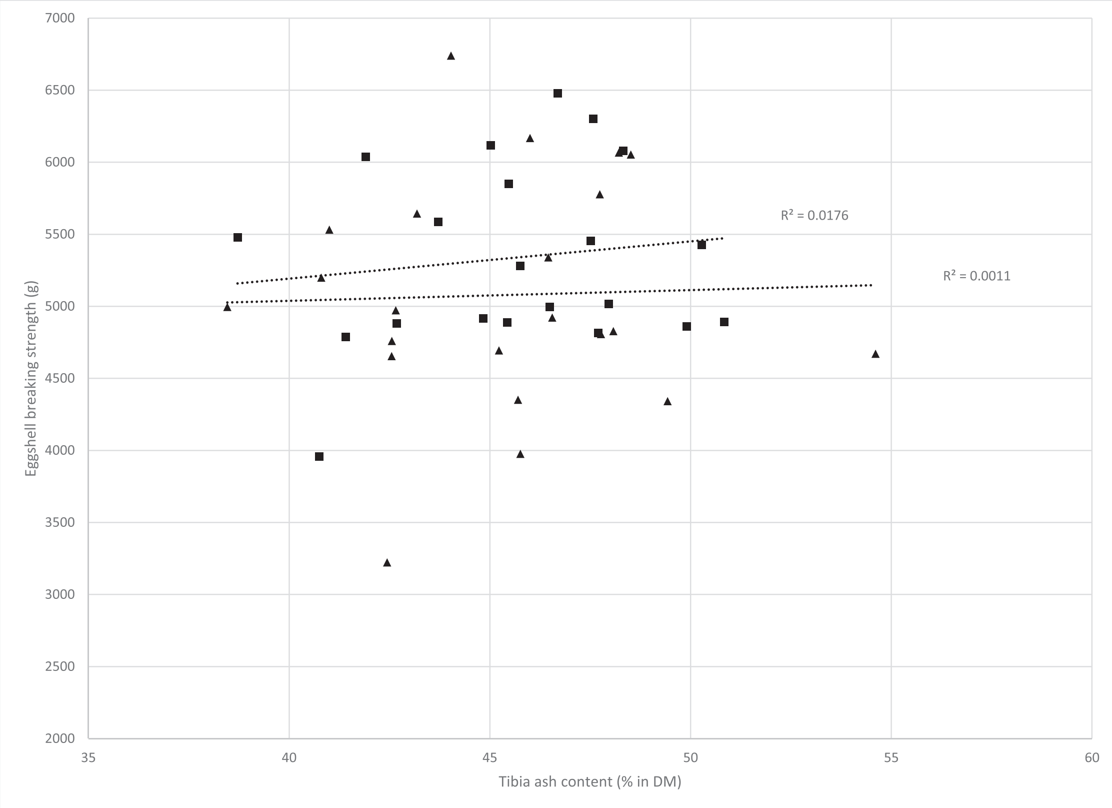

Figure 2. Relation of eggshell breaking strength and tibia ash content in Bovans Brown laying hens at 67 wk of age fed rearing diets from 0 to 16 wk of age with high (▲) or low (□) dietary Ca and P.

eggshell breaking strength and SWUSA at 75 wk of age. Eggshell breaking strength was 286 g higher ($P = 0.030$) and SWUSA was 2.0 mg/cm^2 higher ($P = 0.038$) with high compared to low Ca−P level. Egg exterior quality, like broken shells, dirty shells, shell less eggs, and other abnormalities, was not affected by any of the dietary treatments (data not shown).

DISCUSSION

Egg Production

Livability (88%) was lower compared to the breeder companies' manual standard (94%) (Bovans Brown Product Guide, www.bovans.com). The relative low livability was due to restless and aggressive behavior after transition of the nonbeak-trimmed pullets from the rearing to the layer facility. The intact beaks likely exacerbated this problem, increasing fatalities since, as birds remained nervous throughout the trial period. Rate of lay and egg mass production showed a feed form × Ca−P level interaction effect from 60 to 89 wk of age. The rearing diets with a larger particle size distribution (COH and MWS, Table 1) had an increased rate of lay and seemed to benefit from a lower Ca−P level compared to CWS. Rate of lay is affected by dietary P supply and suboptimal levels have been reported to decrease egg production (Summers, 1995; Boling et al., 2000; Snow et al., 2004; Bello et al., 2020), probably caused by a random regression of the ovum (Harms and Sloan, 1999). However, we need to be careful with this hypothesis, as an amino acid deficiency causes similar effects (Harms and Sloan, 1999). As P supply to the hens in the present study has been the same for all treatment groups since 16 wk of age and tibia mineralization was not affected by treatments, a P deficiency seems unlikely. Although the explanation of such interaction remains unexplained, the combined use of coarse particles (i.e., mash or coarse oat hulls) with low Ca−P in rearing diets used in this study seemed beneficial for egg production of older laying hens. Punna and Roland (1999) found no negative effects of feeding low dietary P levels of 0.2% from d 1 on laying performance even when this level was maintained in the laying period till 48 wk of age. The present study showed that feeding a much lower rearing dietary Ca−P level, as compared to commercial practice, resulted in no detrimental effects on egg production parameters in laying hens till 89 wk of age. This observation is in line with statements of Ren et al. (2020) and Li et al. (2017), who suggested that current commercial diets for both broilers and layers contain excessive amounts of Ca and P and where the content could be reduced without affecting production or welfare. The present study shows that this seems to account for rearing pullets as well.

Rate of lay with CWS showed a tendency ($P = 0.072$) to be lower compared to COH and MWS. This was mainly due to a reduction in egg production between 50 and 60 wk in this treatment group (Figure 1A). Also, FCR was better from 30 to 59 wk of age when COH and MWS were fed during rearing. This could be a carryover effect of an improved stomach development during rearing, as relative gizzard + proventriculus weight at 16 wk of age was significantly higher in these treatments (Dijkslag et al., 2021). A better developed gizzard will result in an improvement in grinding capacity and contribute to an increased nutrient availability (Svihus, 2011). This effect seems to disappear at later ages (60−89 wk), which might be an effect of the gizzards adapting to a diet similar for all treatments during the laying period and without structural components (Svihus, 2011). In the present study gizzard size after 32 wk of age was not determined, but it seems likely that with COH and MWS it decreased, as avian gizzard size can change quickly and adapt to diet structure (Starck, 1999). In contrast to the present study, Guzmán et al. (2016) found no effect of structural components (wheat straw and sugar beet pulp) in rearing diets on egg production and FCR till 46 wk of age compared to the control, although wheat straw compared to sugar beet pulp improved rate of lay. This difference might be caused by the nature of the structural components used in both trials. Where in the present study finely ground wheat straw was used as control (i.e., filler) and unground oat hulls or mash instead of crumbled feed were used to provide dietary structure.

BW and BW Uniformity

Saldaña et al. (2016) found a higher BW at 17 wk of age in pullets fed crumbles compared to mash. However, this effect disappeared at 46 wk of age, after all birds received diets as mash from 17 wk onward. This finding is in contrast with the present study, where CWS fed birds had a higher BW at 89 wk of age compared to MWS. CWS also showed the highest BW of all feed form treatments throughout the trial period and also during rearing and the first 12 wk of the laying period (Dijkslag et al., 2021). So, these birds were able to maintain a higher BW relative to the other treatments throughout the laying period. CWS fed birds had numerically the highest ADFI and lowest egg mass production, and therefore the worst FCR of the feed form treatments, which might explain their ability to maintain a higher BW.

BW uniformity, as response to feed form during rearing, was not consistent throughout the rearing and layer period. As previously reported, till 11 wk of age MWS clearly showed the lowest BW uniformity compared to COH and CWS. When pullets were transferred to the laying facility at 16 wk of age and at 25 and 32wk of age, BW uniformity was not affected by feed form (Dijkslag et al., 2021). However, BW uniformity was better with COH compared to MWS at 51 wk and compared to CWS and MWS at 67 wk of age. At 89 wk of age BW uniformity with COH was still numerically best of the feed form treatments. Feed form affects BW uniformity in laying hens (Ege et al., 2019), but to the authors' knowledge the long-term effect of feed form for pullets on BW uniformity at later age with laying hens is not reported previously. Since the results are not consistent, the long-term effect of feed form during rearing on laying hen BW uniformity remains unclear.

Tibia Characteristics

Tibia breaking strength decreased from 67 to 89 wk of age, independent of treatment, although tibia ash contents increased slightly. This indicates loss of structural bone, as medullary bone lacks substantial intrinsic strength (Rath et al., 2000). A meta-analysis approach by Hervo et al. (2022), including 71 experiments, showed that tibia breaking strength in laying hens significantly decreases with age. In high-producing hens, the loss of structural bone over time is inevitable (Korver, 2020), as structural bone is catabolized to provide some of the Ca required for eggshell formation. The structural bone is not replaced as long as the hen remains in production (Korver et al., 2004). When tibia parameters at 67 and 89 wk are compared to data from these hens at 32 wk of age (Dijkslag et al., 2021), an increase of ash content, breaking strength and compression were observed at 67 compared to 32 wk. Alfonso-Carrillo et al. (2021) reported a significant positive correlation between medullary bone ash content and tibia breaking strength in laying hens of 105 wk of age. This correlation might also explain the increase in tibia breaking strength at 67 wk of age in the present study. In agreement with the present study, Rath et al. (2000) reported, in a comparison of bones from 25-, 75-, and 150-wk-old laying hens, that 75-wk-old birds have stronger bones than the 25-wk-old birds, and that this did not change significantly at 150 wk of age. Older laying hens lay less eggs compared to younger animals and need therefore less Ca for eggshell formation. This could explain the increase of ash content in the present study at 67 wk of age.

Eggshell Quality

Figure 2 shows the relation between eggshell breaking strength and tibia ash content at 67 of age for high and low Ca−P in the present study and indicates no correlation. In agreement to this, several authors, according to the meta-analysis of Hervo et al. (2022), concluded that the relationship between bone integrity and eggshell quality is absent or unclear. Also, Alfonso-Carillo et al. (2021) found that the relationship between tibia and eggshell quality is rather poor or not obvious. These authors found that hens with a good vs. poorer eggshell quality have a slightly larger uterus (shell gland) and a greater capacity to mobilize the calcium needed for eggshell formation and retain a lesser amount of medullary bone.

Eggshell thickness was affected by Ca−P level at several ages, but the relationship was not consistent. At 45 wk eggshell thickness was higher with low Ca−P level, but at other moments it was opposite and interacted with feed form (wk 51 and 67). At wk 59 it was lower with high Ca−P level in combination with CWS, but at the same time higher with high Ca−P level in combination with MWS. The fact that all hens received a similar diet with a sufficient Ca−P supply probably caused this inconsistency. This is also reflected in an absence of an effect on tibia mineralization. The long-term effect of dietary Ca−P supply during rearing on eggshell quality is probably too weak or not present at all. In fact, even when suboptimal P diets are fed during the laying period, there is no strong effect on eggshell quality parameters (Boorman and Gunaratne, 2001; Pongmanee et al., 2020). It was remarkable that eggshell thickness was at its lowest point at 59 wk of age and increased after that. Usually, eggshell thickness reduces as laying hens age and this has been reported by several authors (Al-Batshan et al., 1994; Benavides-Reyes et al., 2021). In contrast to these authors and in agreement with the current experiment, Park and Sohn (2018) found no decreasing eggshell thickness as hens aged till 78 wk of age. Eggshell breaking strength was only affected by treatments once and appeared to be improved at 75 wk with high Ca−P level. As this was observed at only one age and was also not consistently numerically improved at other ages, we cannot conclude that Ca−P level affected eggshell breaking strength. Also, SWUSA was affected by Ca−P level and like eggshell thickness, the effects were not consistent. At 59 wk SWUSA was higher with high Ca−P level in combination with MWS, but lower with CWS. SWUSA appeared to be higher with high Ca−P level at 75 wk of age. A decrease of eggshell breaking strength was also observed as hens aged. This decrease in breaking strength is probably due to a decrease of the mammillary knob density (Park and Sohn, 2018). These authors found a decreased mammillary knob density as hens aged, but eggshell thickness was not affected by age. The decrease in mammillary density reduces the attachment points of the eggshell mineral to the membranes and therefore should negatively impact eggshell mechanical properties (Benavides-Reyes et al., 2021).

CONCLUSIONS

From this experiment, we can conclude that feeding rearing pullets a diet lower in Ca−P compared to commercial practice does not have any negative long-term effects on egg production, eggshell quality and bone characteristics in laying hens up to 89 wk of age. Additionally, it can also be concluded that there is no clear relationship between bone mineralization and eggshell quality. The findings of the current study suggest that the use of rearing diets having adequate physical structure, (e.g., coarse mash or unground oat hulls) improve feed efficiency. Also, rearing diets with low dietary Ca −P strategies in combination with diets with adequate physical structure have positive effects on subsequent egg production.

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DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

Al-Batshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590–1596.

Alfonso-Carrillo, C., C. Benavides-Reyes, J. de los Mozos, N. Dominguez-Gasca, E. Sanchez-Rodríguez, A. I. Garcia-Ruiz, and A. B. Rodriguez-Navarro. 2021. Relationship between bone quality, egg production and eggshell quality in laying hens at the end of an extended production cycle (105 weeks). Animals 11:623.

AOAC International. 2005. Official Methods of Analysis of the AOAC International. 18th ed. AOAC Int, Gaithersburg, MD.

Bello, A., Y. Dersjant-Li, and D. R. Korver. 2020. Effects of dietary calcium and available phosphorus levels and phytase supplementation on performance, bone mineral density, and serum biochemical bone markers in aged white egg-laying hens. Poult. Sci. 99:5792–5801.

Benavides-Reyes, C., E. Folegatti, N. Dominguez-Gasca, G. Litta, E. Sanchez-Rodriguez, A. B. Rodriguez-Navarro, and M. Umar Faruk. 2021. Research note: Changes in eggshell quality and microstructure related to hen age during a production cycle. Poult. Sci. 100:101287.

Boletín Oficial del Estado. 2013. Real decreto 53/2013 sobre protección de los animales utilizados para experimentación y otres fines científícos incluyendo de docencia. B. O. E. 34:11370–11471.

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult. Sci. 79:224–230.

Boorman, K. N., and S. P. Gunaratne. 2001. Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. Br. Poult. Sci. 42:81–91.

Bozkurt, M., B. Koçer, G. Ege, A. E. Tüzün, H. H. Bijik, and E. Poyrazoğlu. 2019. Influence of the particle size and form of feed on growth performance, digestive traits and nutrient digestibility of white egg-laying pullets from 1 to 112 D of age. Poult. Sci. 98:4016–4029.

CVB. 2016. CVB Booklet Feeding of Poultry. Federatie Nederlandse Diervoederketen. CVB series no 59, The Hague, The Netherlands.

Deaton, J. W., and J. D. May. 1988. Weight gain of summer-reared egg-type pullets fed pellets versus mash. Poult. Sci. 67:375–377.

Dijkslag, M. A., M. L. Elling-Staats, Y. Yen, L. M. Marchal, and R. P. Kwakkel. 2019. The effect of coarse and wet feeding on performance parameters, gastrointestinal tract and tibia traits, and digesta phytase activity in egg-type pullets, either fed a low or moderate phosphorus diet. Poult. Sci. 98:4729–4744.

Dijkslag, M. A., R. P. Kwakkel, E. Martin-Chaves, C. Alfonso-Carillo, C. Walvoort, and A. Navarro-Villa. 2021. The effects of dietary calcium and phosphorus level, and feed form during rearing on growth performance, bone traits and egg production in brown egg-type pullets from 0 to 32 weeks of age. Poult. Sci. 100:101130.

Ege, G., M. Bozkurt, B. Koçer, A. E. Tüzüm, A. Uygun, and G. Alkan. 2019. Influence of feed particle size and feed form on productive performance, egg quality, gastrointestinal tract traits, digestive enzymes, intestinal morphology, and nutrient digestibility of laying hens reared in enriched cages. Poult. Sci. 98:3787–3801.

Frikha, M., H. M. Safaa, E. Jiménez-Moreno, R. Lázaro, and G. G. Mateos. 2009a. Influence of energy concentration and feed form of the diet on growth performance and digestive traits of brown egg-laying pullets from 1 to 120 days of age. Anim. Feed Sci. Technol. 153:292–302.

Frikha, M., H. M. Safaa, M. P. Serrano, and X. Arbe. 2009b. Influence of the main cereal and feed form of the diet on performance and digestive tract traits of brown-egg laying pullets. Poult. Sci. 88:994–1002.

Gous, R. M., and T. R. Morris. 2001. The influence of pelleted feed on the response of growing pullets to photoperiods of less than ten hours. Br. Poult. Sci. 42:203–206.

Guzmán, P., B. Saldaña, O. Bouali, L. Cámara, and G. G. Mateos. 2016. Effect of fiber of the rearing phase diets on egg production, digestive tract traits, and body measurements of brown egg-laying hens fed diets differing in energy concentration. Poult. Sci. 95:1836–1847.

Guzmán, P., B. Saldaña, M. V. Kimiaeitalab, and J. García. 2015b. Inclusion of fiber in diets for brown-egg laying pullets: Effects on growth performance and digestive tract traits from hatching to 17 week of age. Poult. Sci. 94:2722–2733.

Guzmán, P., B. Saldaña, H. A. Mandalawi, A. Pérez-Bonilla, and R. Lázaro. 2015a. Productive performance of brown-egg laying pullets from hatching to 5 weeks of age as affected by fiber inclusion, feed form, and energy concentration of the diet. Poult. Sci. 94:249–261.

Harms, R. H., and D. R. Sloan. 1999. Changes in the laying cycle as a result of a phosphorus deficiency. J. Appl. Poult. Res. 8:191–194.

Hervo, F., A. Narcy, Y. Nys, and M.-P. Létourneau-Montminy. 2022. Effect of limestone particle size on performance, eggshell quality, bone strength, and in vitro/in vivo solubility in laying hens: a meta-analysis approach. Poult. Sci. 101:101686.

Jing, M., S. Zhao, A. Rogiewicz, B. A. Slominski, and J. D. House. 2018. Assessment of the minimal available phosphorus needs of pullets during the pre-laying period. Poult. Sci. 97:557–567.

Kebreab, E., J. France, R. P. Kwakkel, S. Leeson, H. D. Kuhni, and J. Dijkstra. 2009. Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. Poult. Sci. 88:680–689.

Keshavarz, K. 2003. Effects of continuous feeding of low-phosphorus diets with and without phytase during the growing and laying periods on performance of two strains of leghorns. Poult. Sci. 82:1444–1456.

Korver, D. R. 2020. Calcium nutrition, bone metabolism, and eggshell quality in longer-persisting layer flocks. Proc. Aust. Poult. Sci. Symp. 31:1–7.

Korver, D. R., J. L. Saunders-Blades, and K. L. Nadeau. 2004. Assessing bone mineral density in vivo: quantitative computed tomography. Poult. Sci. 83:222–229.

Li, X., D. Zhang, and W. L. Bryden. 2017. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? Anim. Prod. Sci. 57:2304–2310.

Nahashon, N. S., and H. S. Nakaue. 1994. Phytase activity, phosphorus and calcium retention, and performance of single comb white leghorn layers fed diets containing two levels of available phosphorus and supplemented with direct-fed microbials. Poult. Sci. 73:1552–1562.

Park, J. A., and S. H. Sohn. 2018. The influence of hen aging on eggshell ultrastructure and shell mineral components. Korean J. Food. Sci. An. 38:1080–1091.

Phirinyane, T. B., J. C. Moreki, H. J. van der Merwe, and J. P. Hayes. 2011. Effect of dietary different ratios of coarse and fine limestone particles on egg production and eggshell quality of laying hens at peak production. Res. Opin. Anim. Vet. Sci. 1:334–338.

Pongmanee, K., I. Kühnn, and D. R. Korver. 2020. Effects of phytase supplementation on eggshell and bone quality, and phosphorus and calcium digestibility in laying hens from 25 to 37 wk of age. Poult. Sci. 99:2595–2607.

Punna, S., and D. A. Roland Sr. 1999. Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus-deficient diets from day one of age. Poult. Sci. 78:1407–1411.

Rath, N. C., G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Factors regulating bone maturity and strength in poultry. Poult. Sci. 79:1024–1032.

Ren, Z., W. Sun, X. Cheng, Y. Liu, D. Han, J. Yan, C. Pau, Y. Duan, and X. Yang. 2020. The adaptability of Hy-Line brown laying hens to low-phosphorus diets supplemented with phytase. Poult. Sci. 99:3525–3531.

Rousseau, X., A. S. Valable, M. P. Létourneau-Montminy, N. Même, E. Godet, M. Magnin, Y. Nys, M. J. Duclos, and A. Narcy. 2016. Adaptive response of broilers to dietary phosphorus and calcium restrictions. Poult. Sci. 95:2849–2860.

Saldaña, B., C. E. Gewehr, P. Guzmán, J. Garcia, and G. G. Mateos. 2016. Influence of feed form and energy concentration of the rearing phase diets on productivity, digestive tract development and body measurements of brown-egg laying hens fed diets varying in energy concentration from 17 to 46 wk of age. Anim. Feed Sci. Technol. 221:87–100.

Saldaña, B., P. Guzmán, L. Cámara, J. García, and G. G. Mateos. 2015a. Feed form and energy concentration of the diet affect growth performance and digestive tract traits of brown-egg laying pullets from hatching to 17 weeks of age. Poult. Sci. 91:1879–1893.

Saldaña, B., P. Guzmán, H. M. Safaa, and R. Harzalli. 2015b. Influence of the main cereal and feed form of the rearing phase diets on performance, digestive tract, and body traits of brown-egg laying pullets from hatch to 17 weeks of age. Poult. Sci. 94:2650–2661.

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

Starck, J. M. 1999. Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fiber content. J. Exp. Biol. 202:3171–3179.

Summers, J. D. 1995. Reduced dietary phosphorus levels for layers. Poult. Sci. 74:1977–1983.

Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J. 67:207–223.

Svihus, B. 2014. Function of the digestive system. J. Appl. Poult. Res. 23:306–314.

Wistedt, A., Y. Ridderstråle, H. Wall, and L. Holm. 2019. Age-related changes in the shell gland and duodenum in relation to shell quality and bone strength in commercial laying hen hybrids. Acta Vet. Scand. 61:14.