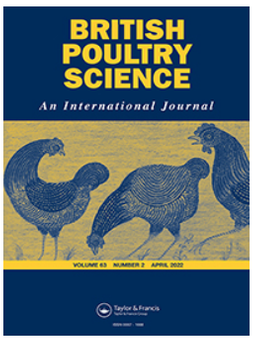

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Integument, mortality, and skeletal strength in extended production cycles for laying hens – effects of genotype and dietary zinc source

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ABSTRACT

1. This study on long-life layers, covering the period 20–100 weeks of age, investigated longitudinal effects on mortality, layer integument, and skeletal properties in Bovans White (BoW) and Lohmann Selected Leghorn Classic (LSL), with or without supplementation with dietary organic zinc (Zn).
2. Two experiments, using 1440 layers in furnished small group cages (FC) and 1836 layers in a traditional floor housing system (Floor), were run in parallel. Each replicate consisted of five adjacent cages containing eight hens in each FC, or a pen with 102 layers in the Floor group.
3. Mortality was recorded daily. Integument and keel bone condition were scored at 35, 55, 85, and 100 weeks of age on 20% of the layers. Tibial strength was recorded from 933 layers at 100 weeks. Statistical analyses were performed on replicate means, with four to five and nine replicates per combination of hybrid and diet in Floor and FC groups, respectively.
4. Cumulative mortality was 9.6% and 16.3% in FC and Floor, respectively, and increased in the latter part of the production cycle, particularly in the Floor group.
5. In FC, LSL had inferior feather cover, less keel bone deviation, and shorter claws than BoW. In Floor, LSL had superior feather cover, less severe vent wounds, more bumble foot, and cleaner plumage than BoW. In both production systems, claws grew longer and keel bone deviation became more severe with age.
6. In FC, layers fed organic Zn had lower body weight and less keel bone deviation at 100 weeks of age.
7. In conclusion, keel bone integrity, claw length, and mortality rate are potential threats to welfare in long-life layers. Feather pecking is a problem that needs addressing at an early stage in the production period. On the whole, organic Zn did not improve welfare conditions in long-life layers.

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Feather cover; keel bone damage; bone strength; long life layer; organic zinc; hybrid

Introduction

Interest in extended production cycles is increasing in Sweden and other European countries, and keeping layers in production until 100 weeks of age is a commonly mentioned goal (Pottgütter, 2016). The main reasons for conventional replacement of flocks at around 72 weeks of age in Europe include a decline in egg production and reduced shell quality (Bain et al. 2016). Besides persistence in lay and egg quality, conditions related to the welfare of long-life layers must be addressed. In order to maintain calcium (Ca) supply for egg shell production during the laying cycle, structural bone is gradually replaced with weaker medullary bone (Whitehead and Fleming 2000), as Ca is depleted to support the demand in shell formation. Osteoporosis is a major welfare challenge in commercial egg production, due to the associated increased risk of fractures. By the end of lay, a large proportion of layers show healed and/or acute fractures. Incidence is high, particularly in the keel bone, with a reported 48–97% affected birds per flock in non-cage systems and somewhat lower frequency (25–62%) in cage systems (Petrik et al. 2015; Rodenburg et al. 2008; Wilkins et al. 2011). Keel bone deviation is common, particularly among layers in housing systems providing access to perches (Abrahamsson and Tauson 1993). Käppeli et al. (2011) observed keel bone deviation in 20–83% of layers in flocks housed in non-cage systems, while Vits et al. (2005) reported incidence at 33% of layers in furnished cages. With a longer production cycle, depletion of calcium from the bones will continue for an extended period, potentially increasing the risk of osteoporosis and skeletal damage.

The feather cover of layers usually deteriorates to varying degrees during the laying cycle, often through a combination of feather pecking and abrasion (Kjaer and Sørensen 2002; Tauson et al. 2005). Feather pecking often leads to reduced welfare for a considerable number of birds, particularly in non-cage systems housing large groups of layers (Nicol et al. 1999; Bilčík and Keeling 2000), where the number of potential pecking victims is higher. The resulting poor insulation leads to greater energy demand and higher feed intake (Peguri and Coon 1993). With longer production cycles, maintaining adequate feather cover is critical in order to increase sustainability.

The trace mineral zinc (Zn) is essential for a wide range of biological functions, and deficiency impairs feed intake, growth, feed conversion ratio (FCR), immune function, and development of feathers, leading to skeletal and skin issues (Underwood, and Suttle 1999). Zinc is a component of the enzyme carbonic anhydrase, which supports egg shell formation in layers through the supply of carbonate ions (*e.g.* Zhang et al. 2017). It is thereby crucial to supplement high-

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producing layers, and Zn and other trace minerals are routinely added as a part of a premix in feed. The most common sources are inorganic mineral salts such as zinc oxide (ZnO) or sulphate ($ZnSO_4$), due to their low cost. These mineral salts tend to dissociate and form highly insoluble complexes with other dietary molecules, which reduces their uptake in the small intestine. In organic metal complexes, the trace mineral is bound to an organic carrier such as an amino acid, carbohydrate or lipid. This makes it more stable and less prone to react with other compounds and promotes more efficient absorption in the intestinal lumen (Underwood, and Suttle 1999; as reviewed by Świątkiewicz et al. (2014); Vinus (2017)).

The objective of the present study was to investigate the longitudinal effects of an extended production cycle on mortality, bird integument and skeletal strength in two layer genotypes, and to assess the effects of replacing dietary ZnO with organic Zn. The study lasted until 100 weeks of age and comprised two 80 week long experiments performed in parallel, one with furnished cages and one with a traditional floor housing system.

Materials and methods

Layers, housing and management

This study was conducted in research facilities at the Swedish University of Agricultural Sciences, with ethical approval from the Uppsala Local Ethics Committee.

The study included a total of 3276 white layers. Of these, 720 Lohmann Selected Leghorn Classic (LSL) and 720 Bovans White (BoW) were housed in furnished cages (FC) and 918 of each genotype were housed in a one-tier floor housing system (Floor). All birds were reared on the same farm and fed the same diet during rearing. Pullets intended for the Floor group were reared in an aviary with full access to all areas, whereas pullets intended for FC were fenced in on one of the tiers of the aviary rearing system, to resemble rearing in a conventional rearing cage. In accordance with Swedish regulations, no beak-trimming was performed. At 15 weeks of age, the pullets were transferred to the experimental facility and housed in one of two rooms in the same building, equipped with either FC (Experiment 1) or Floor housing (Experiment 2).

The cages used in Experiment 1 comprised 180 FC (Victorsson Industrier AB, Frillesås, Sweden) in three tiers. Each FC housed eight layers and provided 600 cm^2 cage area, 150 cm^2 nest area and 15 cm perch per bird, in accordance with the Swedish Animal Welfare Directive. The cage is described in detail in Wall and Tauson (2013), where it is referred to as a T8 cage. In the present study, the perch was a plastic cylinder with diameter of 35 mm, flattened on the top and bottom. The nest was lined with plastic netting (Netlon®). The litter facility, located on top of the nest, was replenished with sawdust twice a week. A wire gate restricted access to the litter box at night and during the egg laying period. From 23 weeks of age to the end of the study, when the light was on between 02.00 and 16.00 h, layers could enter the litter box between 10.00 and 15.00 h. The cage floor slope was 12% and manure belts were run twice a week. Water was provided from nipple drinkers and feed was manually distributed in feed troughs at the front of the cage. When placing the pullets in the FC on arrival at the research facility, two pullets in each cage were randomly chosen as focal birds and banded with coloured plastic leg rings.

The Floor group room used in Experiment 2 had 18 identical pens comprising 13.4 m^2 equipped with a Vencomatic® one-tier system (Vencomatic Group, Eersel, The Netherlands) with a classic raised slatted area and littered floor and the dimensions of different areas in the pen can be found in Alm et al. (2015). Colony nests lined with artificial turf were attached to the slatted area. From the start of lay, nests were available from one hour before lights-on and closed one hour before lights-out. Five rows of plastic mushroom-shaped perches were integrated into the slats. Wood shavings were used as litter material in the littered floor area (35% of total area) and replenished when needed. Each pen housed 102 layers. Manure under the slatted area was removed with scrapers twice a week. Each pen had one bell drinker and four conventional circular feed hoppers, into which feed was automatically distributed.

Dead birds or those with low body weight (BW) or other abnormalities were replaced with healthy birds until 20 weeks of age. The same lighting schedule was applied in both experiments, with nine hours of light per day on arrival, followed by a gradual increase to 14 hours at 23 weeks.

Diets and replicates

During the five-week adaption period, all layers were fed the same diets, *viz*. a standard pre-lay diet for three weeks, followed by a basal diet for two weeks. At 20 weeks, when the experimental period started, layers in replicate FC cages and Floor pens were randomly assigned to one of two dietary treatments. Half the birds remained on the basal diet (control), whereas the others received an experimental diet (EXP), the latter formulated as per the basal diet but with added ZnO replaced by an organic amino acid complex (Availa®Zn, Zinpro Corporation, Eden Prairie, MN). During the two experiments, ending at 100 weeks of age, the following three dietary phases were applied: phase 1 (20–40 weeks) with 40 mg of 78 mg added Zn being organic in EXP; phase II (41–60 weeks) and phase III (61–100 weeks) with all Zn added (60 mg) being organic in the EXP (see Table 1 for diet composition and Table 2 for chemical composition). Diets were provided for *ad libitum* consumption.

In the FC group, five adjacent cages, *i.e*. 40 layers in total, represented one experimental unit, resulting in nine replicates per combination of hybrid and dietary treatment. In the Floor group, each pen was an experimental unit, and the EXP was fed to five LSL pens and four BoW, and the control diet to four LSL and five BoW. Hence, the number of replicates per combination of hybrid and dietary treatment in the Floor group was either four or five.

Measurements

During the experiments, comprising the period from 20 to 100 weeks of age, deaths were recorded daily per replicate and dead birds were not replaced. At 35, 55, 85, and 100 weeks of age, a sample of layers were weighed and had their exterior appearance scored by the same person according to a standardised method (Tauson et al. 2005). The traits scored were condition of

Table 1. Diet composition (g/kg as fed).

	Phase 1	Phase I	Phase II	Phase II	Phase III	Phase III
Ingredient	Control	Exp	Control	Exp	Control	Exp
Wheat	393	389	411	403	448	440
Soy meal	196	197	157	159	134	135
Oats	150	150	150	150	150	150
Barley	50	50	50	50	-	
Rapeseed	30	30	30	30	30	30
Rapeseed meal	13.9	13.9	34.4	34.4	64.6	64.6
Soy oil	13	14.5	-	-	-	
Maize gluten meal	-	-	7.5	7.5	7.5	7.5
Vegetable fatty acids	29.4	28.4	37.0	38.5	40.6	42.7
Limestone coarse (0.5–2.5 mm)	92.7	89.4	92.2	89.0	99.0	95.7
Limestone fine (0–0.2 mm)	5.0	5.0	10	10	10	10
Monocalcium phosphate	10.5	10.5	5.5	8.7	1.9	5.1
Sodium chloride	2.8	2.8	2.4	2.4	2.3	2.3
Sodium bicarbonate	1.6	1.6	1.5	1.5	1.5	1.5
Feed additives[1, 2, 3]	11.9	18.1	11.6	16.4	11.1	15.9
Total	1000	1000	1000	1000	1000	1000

[1]The phase 1 premix provided (per kg diet as is): retinyl acetate: 13,000 IU; cholecalciferol: 3,000 IU; dl-α-tocopheryl acetate E: 50 mg; biotin: 0.30 mg; Fe as in iron sulphate: 40 mg; Cu as in copper sulphate: 11.4 mg; Mn as in manganese oxide: 86 mg; iodine: 1.4 mg; selenium: 0.31 mg; Zn as in zinc oxide: 78 mg in control diet and 38 mg in experimental diet; organic Zn: 40 mg in experimental diet.

[2]The phase 2 and 3 premix provided (per kg diet as is): retinyl acetate: 10,000 IU; cholecalciferol: 3,000 IU; dl-α-tocopheryl acetate: 35 mg; biotin: 0.30 mg; Fe as in iron sulphate: 25 mg; Cu as in copper sulphate: 6 mg; Mn as in manganese oxide: 79 mg; iodine: 0.5 mg; selenium: 0.25 mg; Zn as in zinc oxide: 60 mg in control diet; organic Zn: 60 mg in experimental diet.

[3]Xylanase, phytase, synthetic amino acids (methionine and lysine), and a premix of natural pigments were included in all phases.

plumage (neck, breast, back, wings, tail, and cloaca), feather hygiene, cleanliness of feet, wounds on the comb and rear body (vent), claw length, incidence of toe pad hyperkeratosis and bumble foot and keel bone deformities. The scoring system was according to a 1–4 range for each trait, with the higher score indicating better condition. The six parameters for plumage condition were summarised, giving a total score ranging from six to 24 points. In the FC group (Experiment 1), eight of 10 focal birds that received leg rings on arrival to the facility were scored for external appearance. If eight focal birds could not be identified, due to either mortality or loss of leg rings, a replacement hen was randomly chosen. In the Floor group (Experiment 2), 20 hens were chosen at random from different sections of each pen for scoring of exterior appearance. On each occasion, 288 and 360 layers were scored in the FC and Floor groups, respectively, representing 20% of the layers in each production system.

At the end of both experiments (100 weeks), material for subsequent analyses of bone breaking strength was collected from a total of 492 layers in the FC (12–15 layers per replicate) and 441 layers in the Floor group (21–27 layers per replicate). The layers were killed by an intravenous injection of pentobarbital sodium (100 mg/ml), and BW was recorded. After being euthanised, each bird was necropsied in order to select only birds still in lay for analyses of skeletal strength. For the relevant birds, the right leg was removed and frozen. Prior to analysis of bone strength, specimens were thawed at room temperature and skin, ligaments and muscles were removed. The tibial bone was subjected to a three-point bending test at room temperature using an electromechanical testing device (Avalon Technologies, Rochester, MN, USA). The loading speed was 1 mm/s and the span length was 50 mm. The load was applied in an anterior-posterior direction while data were collected at 50 Hz until failure, using software provided with the testing device (Testware II, Avalon technologies, Rochester, MN, USA).

Statistical analyses

Experiment 1, the FC group, and Experiment 2, the Floor group, were considered as two separate trials. Effect of housing system was therefore not statistically evaluated.

All data without apparent deviations from normality and homoscedasticity, according to diagnostic plots of residuals, were analysed using mixed linear models in SAS statistical software (release 9.4, SAS Institute Inc., Cary, NC). The data from scoring of exterior appearance were of a repeated-measures nature (Littell et al. 2006), and the fixed part of the models included hybrid (two levels), diet (two levels), age (four levels), and all interactions. Each replicate, *i.e.* five adjacent FC (Experiment 1) and one Floor pen (Experiment 2), was regarded as random, and the relationship between time points within each replicate was modelled using an autoregressive AR(1) covariance structure.

For keel bone deviation, BW was included as a covariate in the model. For traits in scoring of exterior appearance with apparent deviations from normality or homoscedasticity

Table 2. Calculated and analysed chemical composition of diets (g/kg DM feed).

	Phase I (n = 4)	Phase I (n = 4)	Phase II (n = 3)	Phase II (n = 3)	Phase III (n = 6)	Phase III (n = 6)
	Control	Exp	Control	Exp	Control	Exp
Metabolisable energy MJ/kg feed[1]						
DM	878	878	889	888	887	888
CP[2]	186	188	182	181	182	183
Ash[2]	141	141	139	140	152	154
EU Fat[1]	88	89	83	84	85	86
Linolenic acid C18:2[1]	28	28	21	21	21	22
Methionine[1]	4.8	4.7	4.5	4.5	4.3	4.3
Methionine+cysteine[1]	8.3	8.3	7.9	7.9	7.9	7.9
Ca[2]	40.5	40.0	43.0	45.3	46.1	49.3
K[2]	9.2	9.1	8.0	8.4	7.4	7.4
P[2]	6.5	6.5	5.8	6.7	4.8	5.1
Mg[2]	2.2	2.3	2.2	2.4	2.3	2.4
S[2]	3.0	3.0	3.2	3.2	3.2	3.3
Na[2]	2.1	2.1	1.9	1.9	1.9	1.9
Zn mg/kg DM feed	143[3]	117[3]	-	-	111[4]	113.6[4]
Xanthophyll mg/kg [1]	2.5	2.6			2.5	2.5

[1]Calculated.
[2]Analysed in all feed batches.
[3]Analysed in one batch of feed.
[4]Analysed in two batches of feed.

(FC: toe pad hyperkeratosis, bumble foot, foot hygiene, plumage hygiene, wounds on comb, wounds in vent; Floor: toe pad hyperkeratosis, wounds on the comb and wounds in the vent), score 4 was converted to 1 and scores 1, 2, and 3 to 0, in order to enable logistic regression analysis with Proc Glimmix. The mixed linear models for cumulative mortality and skeletal strength included the fixed effects of hybrid (n = 2), diet (n = 2), and their interaction.

For all analyses, a value of $P < 0.05$ after Tukey-Kramer adjustment for multiple comparisons was considered statistically significant. All statistical analyses were conducted on replicate means.

Results

Mortality and skeletal strength

Cumulative mortality from 20 to 100 weeks of age and tibial strength at 100 weeks are presented in Table 3. Mortality was not affected by hybrid, diet, or hybrid×diet in either of the experiments. Cumulative mortality over time is illustrated in Figure 1.

In FC, tibial strength was significantly higher in LSL than in BoW (Table 3). There was no effect of diet, or interaction between diet and hybrid. In the Floor group, skeletal strength was not affected by either hybrid or diet, and there were no interactions.

BW and integument

Experiment 1 – Furnished cages

Results from bird BW and scoring of integument in FC birds are presented in Table 4. The LSL layers in this group had inferior feather cover compared with BoW, and the condition was worst when fed EXP, resulting in a hybrid×diet interaction. Differences in feather cover at the four ages for BoW and LSL when fed the control or EXP are shown in Figure 2(a). At 35 and 55 weeks of age, feather cover was superior in BoW, whereas there was no difference at 85 and 100 weeks, resulting in a hybrid×age interaction. The LSL had less keel bone deviation and shorter claws than BoW. There were no hybrid differences in toe pad hyperkeratosis or BW.

Table 3. Cumulative mortality from 20 to 100 weeks and skeletal strength of tibia at 100 weeks in Lohmann Selected Leghorn (LSL) and Bovans White (BoW) layers, as affected by diet in two experiments performed in different production systems.

	Hybrid		Diet		Pooled SD[6]		P-value		
Experiment 1 Furnished cages	LSL n = 18	BoW n = 18	E[1] n = 18	C[2] n = 18	H[3]	D[4]	H[3]	D[4]	H× D[5]
Tibial strength, N	152.8[7]	147.6	147.0	153.3	10.4	10.2	0.047	0.193	0.176
Cumulative mortality, %	9.6	9.6	8.5	10.7	5.8	5.5	1.000	0.246	0.661
Experiment 2 Floor system	LSL n = 9	BoW n = 9	E n = 9	C n = 9	H	D	H	D	H × D
Tibial strength, N	208.6	212.8	212.5	208.9	15.1	15.2	0.864	0.227	0.922
Cumulative mortality, %	14.6	18.0	15.6	17.0	7.1	7.2	0.367	0.767	0.534

[1]E = experimental diet
[2]C = control diet
[3]H = hybrid effect
[4]D = diet effect
[5]H×D = hybrid×diet effect
[6]SD = standard deviation
[7]values are means.

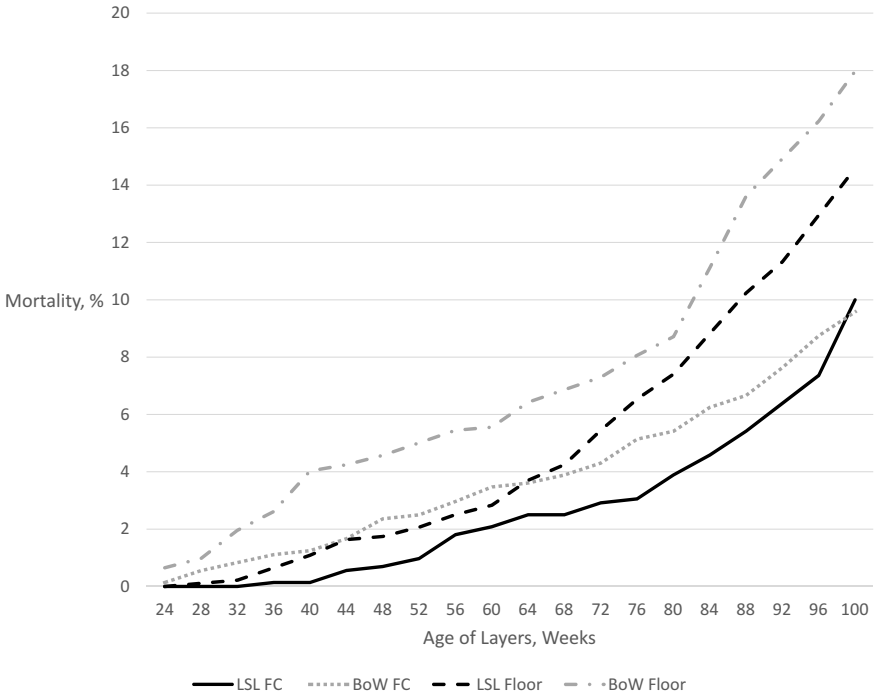

Figure 1. Cumulative mortality in Lohmann Selected Leghorn (LSL) and Bovans White (BoW) from 20 to 100 weeks when housed in furnished 8-hen cages (FC) or in traditional floor housing system (Floor).

Table 4. Live weight and integument scores in Lohmann Selected Leghorn (LSL) and Bovans White (BoW) layers, as affected by diet and age in furnished 8-hen cages. Scores for feather cover ranged from 24 to 6 points, all other traits from 4 to 1. The higher the score, the better the condition.

	Hybrid		Diet		Age (weeks)				Pooled SD[10]			P-value						
Item	LSL n = 72	BoW n = 72	E[1] n = 72	C[2] n = 72	35 n = 36	55 n = 36	85 n = 36	100 n = 36	H[3]	D[4]	A[5]	H[3]	D[4]	A[5]	H× D[6]	H× A[7]	D× A[8]	H × D× A[9]
Live weight, g[11]	1850[13]	1873	1843	1880	1806[b]	1869[a]	1874[a]	1896[a]	87	85	81	0.086	0.005	<0.001	0.040	0.926	0.914	0.903
Feather cover[11]	14.6	16.3	15.3	15.6	19.9[a]	15.6[b]	13.2[c]	13.1[c]	3.3	3.4	2.0	<0.001	0.344	<0.001	0.011	0.004	0.852	0.116
Keel bone deviation[11]	3.5	3.3	3.5	3.3	3.8[a]	3.5[b]	3.3[c]	3.0[d]	0.4	0.4	0.4	<0.001	<0.001	<0.001	0.988	0.207	0.939	0.437
Claw length[11]	3.1	2.8	3.0	2.9	3.6[a]	3.2[b]	2.8[c]	2.3[d]	0.6	0.6	0.3	<0.001	0.161	<0.001	0.368	0.007	0.646	0.421
Toe pad hyperkeratosis[12]	3.9	3.9	3.9	3.9	3.8[bc]	3.8[b]	3.9[ab]	3.9[a]	0.1	0.1	0.1	0.708	0.294	0.004	0.049	0.946	0.904	0.592
Bumble foot[12]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.0	0.0	0.0	x	x	x	x	x	x	x
Foot hygiene[12]	3.9	3.9	3.9	3.9	3.8	4.0	4.0	4.0	0.1	0.1	0.1	x	x	x	x	x	x	x
Plumage hygiene[12]	3.4	3.1	3.3	3.2	3.2	3.2	3.2	3.3	0.2	0.2	0.2	x	x	x	x	x	x	x
Wounds comb[12]	3.2	3.2	3.2	3.2	3.6	3.1	3.2	3.1	0.2	0.2	0.2	x	x	x	x	x	x	x
Wounds vent[12]	3.6	3.7	3.6	3.6	3.8	3.6	3.7	3.5	0.2	0.2	0.2	x	x	x	x	x	x	x

[1]E = experimental diet
[2]C = control diet
[3]H = hybrid effect
[4]D = diet effect
[5]A = age effect
[6]H×D = hybrid×diet effect
[7]H × A = hybrid×age effect
[8]D×A = diet×age effect
[9]H×D × A = hybrid×diet×age effect
[10]SD = standard deviation
[11]Statistically analysed by mixed linear models in SAS
[12]statistically analysed with binary logistic regression by Glimmix procedure in SAS
[13]All values shown are means.
x – no statistical results generated in logistic regression.
[a-d]Means within the same row without any common superscript are significantly different ($P < 0.05$).

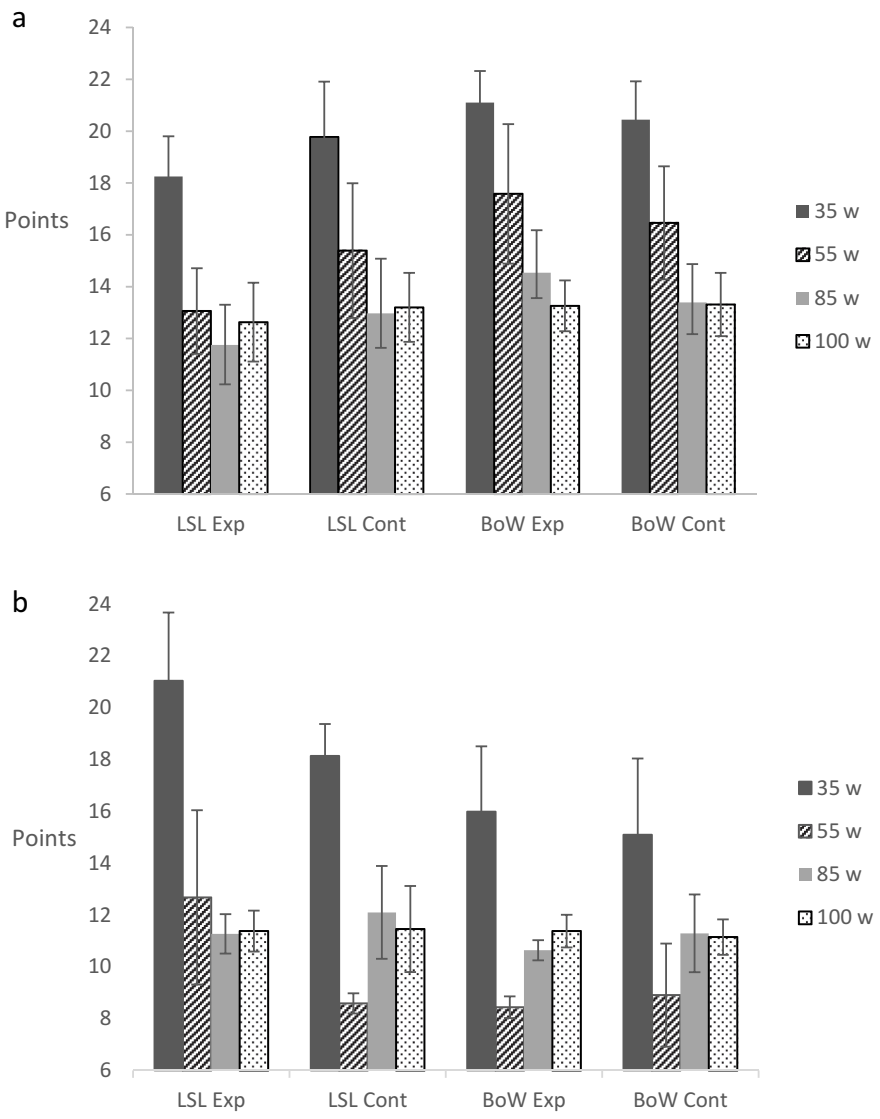

Figure 2 (a) Feather cover in Lohmann Selected Leghorn (LSL) and Bovans White (BoW) layers at four ages when housed in furnished 8-hen cages (Experiment 1) or (b) a traditional floor housing system (Experiment 2). All layers were fed either a control diet (Cont) or a diet with organic Zn (Exp). A higher score indicates better feather cover. Bars indicate mean±standard deviation.

In the FC group, birds fed EXP had lower BW, and there was an interaction between hybrid and diet because the LSL birds fed the EXP had lower BW than all other combinations of hybrid and diet. Birds fed EXP had less keel bone deviation than birds fed the control diet. At 100 weeks of age, 201 out of 280 (72%) palpated layers had keel bone deviation (data not shown), and, of these, 69 had score 1 or 2. Feather cover, claw length and toe pad hyperkeratosis were not affected by diet. BW increased and feather cover, keel bone deviation, and claw length deteriorated with increasing age, whereas toe pad hyperkeratosis status improved.

For incidence of bumble foot, foot and plumage hygiene and wound on the comb or vent, the procedure did not converge and P-values were therefore not generated.

Experiment 2 – Floor system

Results of bird BW and scoring of integument in the Floor group are presented in Table 5. There were no effects of diet on either BW or integument traits in the Floor group, but there was a diet×age interaction for feather cover. This interaction occurred because feather score for layers fed the experimental diet, which was 18.5 points at 35 weeks, was lowest at 55 weeks (8.7 points) and improved thereafter (11.7 and 11.3 points at 85 and 100 weeks respectively). For layers fed the control diet, with an initial feather score of 16.6 at 35 weeks, there were no differences between the scores at 35, 85 and 100 weeks (10.6, 11.0 and 11.4 points respectively). The BoW layers had inferior feather cover compared to LSL. However, this was inferior only at 35 weeks of age, and not at 55, 85, and 100 weeks, resulting in a hybrid×age interaction. Differences in feather cover at the four ages for BoW and LSL when fed the control or EXP are shown in Figure 2(b). The BoW layers had a superior score for bumble foot, but inferior scores for plumage hygiene and vent wounds compared to LSL. The BW increased with age from 85 to 100 weeks of age, but feather cover condition decreased with age, with the worst condition observed at 55 weeks. Claw length increased with age, while bumble foot was worst at 35 weeks and improved thereafter. Foot hygiene improved towards the end of the study, whereas plumage hygiene was worst at 35 and 100 weeks. At 100 weeks, 180 out of 360 (50%) palpated layers had keel bone deviation (data not shown), and of these 28 had a score 2 and no bird received a score 1.

Table 5. Live weight and integument scores in Lohmann Selected Leghorn (LSL) and Bovans White (BoW) layers, as affected by diet and age when housed in a traditional floor system. Scores for feather cover ranged from 24 to 6 points, all other traits from 4 to 1. The higher the score, the better the condition.

	Hybrid		Diet		Age (weeks)				Pooled SD[10]			P-value						
Item	LSL n = 36	BoW n = 36	E[1] n = 36	C[2] n = 36	35 n = 18	55 n = 18	85 n = 18	100 n = 18	H[3]	D[4]	A[5]	H[3]	D[4]	A[5]	H× D[6]	H× A[7]	D× A[8]	H × D× A[9]
Live weight, g[11]	[13]1827	1849	1839	1836	1817[a]	1825[b]	1838[b]	1870[a]	51	52	49	0.137	0.705	0.003	0.981	0.063	0.010	0.068
Feather cover[11]	13.4	11.6	13.0	12.0	17.6[a]	9.8[c]	11.3[b]	11.3[b]	3.7	3.8	2.3	0.013	0.226	<0.001	0.232	0.011	0.044	0.056
Keel bone deviation[11]	3.8	3.7	3.7	3.7	4.0[a]	3.8[b]	3.8[b]	3.4[c]	0.2	0.2	0.1	0.246	0.785	<0.001	0.367	0.657	0.715	0.861
Claw length[11]	3.5	3.6	3.5	3.5	3.8[a]	3.6[b]	3.5[b]	3.3[c]	0.3	0.3	0.2	0.231	0.982	<0.001	0.799	0.017	0.478	0.801
Toe pad hyperkeratosis[12]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	0.0	0.0	0.0	0.772	0.207	0.400	0.784	x	x	x
Bumble foot[11]	3.7	3.9	3.8	3.8	3.7[b]	3.8[a]	3.9[a]	3.8[a]	0.2	0.2	0.2	0.009	0.735	<0.001	0.501	0.159	0.571	0.867
Foot hygiene[11]	3.1	3.2	3.2	3.2	2.9[c]	3.1[bc]	3.2[b]	3.5[a]	0.3	0.3	0.2	0.172	0.831	<0.001	0.954	0.857	0.840	0.676
Plumage hygiene[11]	3.5	3.2	3.3	3.4	3.2[bd]	3.4[ace]	3.4[abc]	3.3[de]	0.2	0.3	0.3	<0.001	0.411	0.003	0.884	0.082	0.817	0.079
Wounds comb[12]	2.8	2.7	2.7	2.8	2.6	2.9	2.7	2.8	0.2	0.2	0.2	0.392	0.789	0.606	0.305	0.976	x	x
Wounds vent[12]	3.5	3.3	3.4	3.3	3.4[a]	3.1[b]	3.8[a]	3.5[a]	0.3	0.3	0.3	<0.001	0.176	<0.001	0.304	<0.001	x	x

[1]E = experimental diet
[2]C = control diet
[3]H = hybrid effect
[4]D = diet effect
[5]A = age effect
[6]H×D = hybrid×diet effect
[7]H × A = hybrid×age effect
[8]D×A = diet×age effect
[9]H×D × A = hybrid×diet×age effect
[10]SD = standard deviation
[11]Statistically analysed by mixed linear models in SAS
[12]Statistically analysed with binary logistic regression by Glimmix procedure in SAS
[13]Values are means.
x – no statistical results generated in logistic regression.
[a-e]Means within the same row without any common superscript are significantly different ($P < 0.05$).

Discussion

The hypothesis tested in this study was that feeding higher bioavailability organic Zn, compared with ZnO, improved skeletal integrity, integument health and viability in long-life layers. However, the only effects observed for the organic Zn supplement were lower BW and less keel bone deviation in the FC group. As BW was included as a covariate in the statistical analyses, the difference in keel bone deviation was not a consequence of differences in BW. The reason for this lack of general improvement on replacing inorganic Zn with organic was not identified, although one possibility was that Zn was not limited, thereby masking possible higher bioavailability of the organic Zn.

Problems with poor air quality and inferior litter condition were observed in the Floor group room. Investigations at the end of the experiments revealed that the litter area farthest away from the air inlets was not ventilated as intended, due to equipment (feed augers *etc.*) preventing air-flow. Therefore, the Floor group birds were challenged by an inferior indoor climate, which might have influenced their behaviour and physical well-being. High humidity and elevated ammonia levels are problems associated particularly with low-density floor systems, such as the conventional floor housing used in the present study. Problems have been reported particularly in buildings without heating and during periods with low outdoor temperatures. Thus, the climate in the research facility was probably similar to prevailing conditions in some commercial flocks.

There is always a risk of bias due to unintended subjectivity when visual scoring is performed by the human eye. One strength of the present study design was that all integument scoring was performed by the same trained individual. During long-term experiments, feather cover deteriorated with age in both production systems due to feather pecking, which is a common finding (Wahlström et al. 2001; Hinrichsen et al. 2016). In the FC group, feather cover deteriorated until 85 weeks of age and then remained stable until 100 weeks. In the Floor group, feather cover was worst at 55 weeks of age and then showed a slight, but significant, improvement. This was likely because some birds assessed at 85 and 100 weeks in the Floor group had gone through a moult, and hence replaced their plumage.

There were differences between the two genotypes in feather cover in both production systems, with BoW having superior feather cover compared with LSL in FC, but this was inferior compared with LSL in the Floor group. However, these genotype differences were significant only at 35 weeks of age for the Floor group, and until 55 weeks in the FC group. The lack of effect of organic Zn on feather cover was in agreement with Martin (2016), who evaluated the same organic Zn source (Availa®Zn) used in the present study.

Mortality increased in both experiments in the latter part of the production period, as indicated by the steeper slope of the mortality curves in Figure 1, confirming the findings of Sherwin et al. (2010). This increase was more prominent in the Floor group (Experiment 2), where average mortality rates were considerably higher than in the FC group (Experiment 1). Higher mortality in non-cage systems compared with FC is a common finding. Besides a higher risk of deaths due to cannibalism (Rodenburg et al. 2008), there is an increased risk of infectious diseases in non-cage systems, due to the contact with litter and manure and impaired air quality (Lay et al. 2011). As dead birds were not subjected to autopsy, the reason for the increased mortality in the latter part of the production period was not determined.

In many studies, the term 'keel bone damage' is used for both deviation and fractures, but the origin and consequences of these are quite different. Keel bone deviation in layers is associated with long-term pressure when resting on perches, and design is essential to limit severity (Tauson and Abrahamsson 1994; Pickel et al. 2011). Keel bone fractures are believed to be associated with collisions with the housing equipment and other hens (Freire et al. 2003; Sandilands et al. 2009; Stratmann et al. 2015). Published work indicates that keel bone fractures induce behavioural changes in layers (Nasr et al. 2012; Gebhardt-Henrich and Fröhlich 2015), most likely due to pain, but less is known about its influence on layer welfare (Riber et al. 2018). It has been suggested that birds with keel bone deviation may have impaired ability to perform complicated balance manoeuvres, *e.g.* in situations where they lose their foothold when positioned on raised perches or elevated tiers, increasing the risk of skeletal injuries (Harlander-Matauschek et al. 2015). Bone mass is highly correlated with mechanical loading, so birds in non-cage housing systems with better opportunities for exercise have stronger bones than birds in cages (Leyendecker et al. 2005). Based on a study investigating the incidence and pathology of keel bone deviation in layers, Fleming et al. (2004) concluded that hens with keel bone deviation are more likely to have weaker bones overall, and that lack of bone mass is likely the underlying cause of keel bone deviation in laying hens. This was confirmed by the poorer skeletal strength and deviation scores in the FC group (Experiment 1) compared with the Floor group (Experiment 2) in the present study. Layers in the FC group fed EXP had less keel bone deviation than layers fed the control diet. This indicated that, in a cage environment with restricted possibility for movement, organic Zn can be beneficial for bone integrity. According to measurements at 100 weeks of age in both FC and Floor groups, bone strength did not differ between birds fed different diets. Similarly, Swiatkiewicz and Koreleski (2008) found no effect of replacing inorganic Zn with organic sources (amino acid complexes) on bone properties at the end of lay at 70 weeks of age. However, it is possible that some regeneration of structural bone occurred in the latter part of the production period, when egg production declined and days out of lay became more frequent. An improvement in tibial bone breaking strength during the production cycle has been observed in other studies (Leyendecker et al. 2005; Wistedt et al. 2019).

Whitehead (2004) concluded that the length of time that birds are in a continuously reproductive state is likely to be significant for degree of osteoporosis, and that short periods out of lay may allow regeneration of structural bone. However, keel bone deformation is irreversible and will not improve even if regeneration of bone mass occurs later on in life. This could explain why the difference in keel bone deviation related to diet in the FC group in Experiment 1 at 100 weeks of age was not accompanied by a corresponding difference in tibial strength. The severity of keel bone deviation increased with layer age during the laying period, confirming findings by Wahlström et al. (2001).

Wounds on the comb as a consequence of aggressive pecking were observed in layers in both experiments, and there was no effect of genotype, dietary treatment or age. Wounds in the vent arise due to vent pecking, described as a separate form of cannibalism, which can lead to severe wounds and even death of the victim (Savory 1995). Vent wounds were observed in some layers in the integument, but cannibalistic pecking was not considered a problem in the present study.

Bumble foot is characterised by severe inflammation and swelling of the foot-pad, which is painful and may affect the ability to walk and use perches (Tauson and Abrahamsson 1996). In the present study, birds in the FC group (Experiment 1) had the highest average score for bumble foot (score 4) at all four ages, indicating that bumble foot was absent among those birds. Previous studies have shown that the incidence of bumble foot is largely affected by the presence of perches and that hardwood is better than plastic in this regard (Tauson and Abrahamsson 1996). In the present study, plastic perches were used in both FC and Floor groups, and the absence of bumble foot in the FC group was therefore somewhat surprising. The inferior litter condition in the Floor group may have contributed to the incidence of bumble foot, as wet litter is a factor associated with the condition (Wang et al. 1998). The higher incidence of bumble foot found in LSL birds compared with BoW in the Floor group was in agreement with the findings that LSL may be more susceptible to bumble foot than other genotypes (Tauson and Abrahamsson 1994, 1996).

Toe pad hyperkeratosis is associated mainly with cages, and sloping wire floors combined with brittle distal toe pads have been identified as important factors (Tauson and Abrahamsson 1996). Zinc is required for synthesis of collagen and keratin, both of which are essential for skin strength and integrity (Underwood, and Suttle 1999). Positive effects of organic zinc sources on skin quality and foot health in broilers have been reported (Saenmahayak et al. 2010). However, while some toe pad hyperkeratosis was observed in the FC group, it was minor, possibly due to a combination of moderate cage floor slope and robust skin properties.

Excessively long claws can break off more easily, leading to bleeding and higher vulnerability to infection (Lay et al. 2011). In non-cage systems, walking and scratching on different surfaces prevents excessive claw growth (Vits et al. 2005), whereas in the FC group, claw-shortening devices must be provided according to the EU Directive setting minimum standards for layers (CEC 1999). In the present study, the claw-shortening device in FC (Experiment 1) consisted of perforations in the manure deflector on the inside of the feed trough. Claw length increased with age in both housing systems, but most prominently in the FC group in the latter part of the production period, indicating that the device did not shorten the claws sufficiently. Abrasive tapes used as a claw-shortening device can have a more prominent effect in cages (Tauson 1986).

Regarding the welfare of long-life layers, it was concluded that feather cover remained unchanged during the latter part of the extended laying cycle, i.e. between 85 and 100 weeks of age. However, feather cover deteriorated in both production systems, and considerably in the Floor group at 55 weeks, so it was evident that feather pecking is a problem that needs to be addressed. The increase in mortality in the latter part of the production cycle, especially in the Floor group, indicated the need for studies on the causes of death in long-life layers. Incidence of keel bone deviation increased during the laying period in both production systems, as did claw length in the FC group. To avoid jeopardising the welfare of long-life layers, measures should be taken to support skeletal integrity and adequate measures for claw abrasion should be provided in FC systems. The reason for the lack of any effect of organic compared with inorganic Zn was not identified, but the positive effects from the higher bioavailability of organic Zn might have been masked by high Zn availability in the control diet.

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