

# Understanding the lack of 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub> in aged laying hens

During the last decade, great progress has been made on the laying persistency of white and brown laying hens. As a result, there is an increasing number of flocks that pass the '500 eggs milestone' in many countries. This milestone is better known as '500 eggs per hen housed without moulting'.

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However, the challenge remains to produce eggs with a good shell quality until the end of the production period without compromising the hen's bone strength.

Understanding the calcium metabolism in laying hens is crucial to design feeding strategies that ensure good eggshell quality on the one hand and that prevent osteoporosis on the other. Simply increasing the dietary calcium level or feeding additional coarse calcium limestone sources to older birds to compensate for the reduced efficiency of intestinal calcium absorption with age, is probably not an ideal strategy. Excess calcium in the intestinal lumen will merely compromise the digestibility of energy and protein, when we need to focus on maximising nutrient utilisation for reasons of feedstuff costs, carbon footprint and environmental impact.

## Compromised activation of 25(OH)D<sub>3</sub> into 1,25(OH)<sub>2</sub>D<sub>3</sub>

A few very interesting studies in aged laying hens, focusing on the vitamin D metabolism related to eggshell formation, were done. In 1982, Abe et al. found that the increased number of cracked and soft-shelled eggs in aged White Leghorns was associated with disorders in vitamin D metabolism.

They showed that the 1- $\alpha$ -hydroxylase activity in renal homogenates of 72-week-old hens was significantly reduced compared to homogenates of 38-week-old hens.

This enzyme is crucial to activate 25-hydroxycholecalciferol (25(OH)D<sub>3</sub>), the circulating form of vitamin D, into 1,25-dihydroxycholecalciferol (1,25(OH)<sub>2</sub>D<sub>3</sub>), the bioactive form.

Consequently, the levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> in aged laying hens were significantly reduced in plasma and various target tissues, like the small intestines, cortical and medullary bone.

This adversely affects calcium absorption from the intestinal tract and mobilisation from bone and reduces the amount of calcium available for calcium carbonate deposition in the eggshell.

In the end, this reduced 1- $\alpha$ -hydroxylase activity with age limits the efficacy of vitamin D and its metabolites – except for sources of 1,25(OH)<sub>2</sub>D<sub>3</sub> – to support the calcium metabolism in older laying hens.



## Fibroblast growth factor 23 and 1,25(OH)<sub>2</sub>D<sub>3</sub>

Recently, Gloux et al. (2020) confirmed these observations, showing that the plasma levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> in 90-week-old hens were significantly lower than in 23-week-old hens, despite similar plasma contents of the precursor molecule 25(OH)D<sub>3</sub>. Apparently, the circulating 25(OH)D<sub>3</sub> could not be adequately activated in aged laying hens.

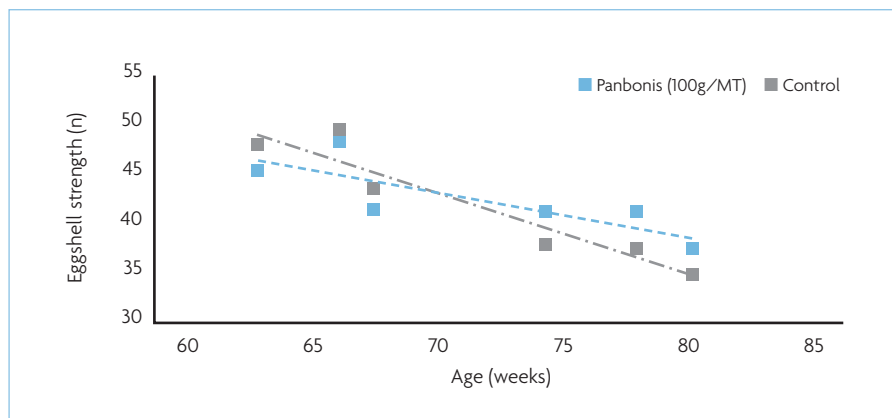
In their study, Gloux et al. (2020) quantified the expression of relevant candidate genes for the regulation of calcium metabolism and eggshell mineralisation. They took samples of tissues that are important for calcium homeostasis, like the parathyroid gland, jejunum, liver, kidney, and medullary bone from laying hens at 23 and 90 weeks of age.

A downregulated vitamin D receptor (VDR) expression in the jejunum as well as of genes involved in transcellular and paracellular calcium transport were consistent with a decreased intestinal calcium absorption in aged laying hens. Gloux et al. also indicated an overexpression of VDR in medullary bone as well as increased osteoclast activity, which might point to increased bone mobilisation when hens age.

However, mobilisation of bone mass also liberates phosphate in excess to the demand for eggshell formation. This excessive phosphate must be excreted by the kidney. Phosphate excretion is stimulated by fibroblast growth factor 23 (FGF23), which is produced in medullary bone. Its production starts at initiation of eggshell formation and rises until completion of eggshell formation.

Gloux et al. showed that FGF23 genes were clearly upregulated in 90-week-old laying

**Fig. 1. The development of eggshell strength in H&N Brown Nick laying hens from 60-80 weeks of age.**



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 hens. As FGF23 also stimulates the degradation of 1,25(OH)<sub>2</sub>D<sub>3</sub> in the kidney, it further reduces calcium retention and increases bone loss in old hens. The authors of this study indicated FGF23 expression as a 'signature of a deteriorated Ca/P balance in ageing laying hens'.

**Solanum glaucophyllum – a plant-based source of 1,25(OH)<sub>2</sub>D<sub>3</sub>-glycosides**

Supplementation of laying hen diets with the vegetable source of the bioactive form of vitamin D as glycosides from *Solanum glaucophyllum*, known as Panbonis, may stimulate the intestinal absorption of calcium and overcome the negative effects of FGF23 on the circulating 1,25(OH)<sub>2</sub>D<sub>3</sub>, restoring the Ca/P balance and improving eggshell quality in ageing laying hens.

Twenty-eight producing 110-week-old Lohmann LSL-Lite laying hens were housed in individual cages for 10 weeks by UNESP, Brazil (Group of Prof. Edney Pereira da Silva). Hens were fed a corn/soy diet (3.8% Ca, 0.25% avP, 2700 IU vitamin D<sub>3</sub>/kg, 500 FTU phytase/kg), with and without 100g Panbonis 10/kg. Production performance parameters were recorded. Egg weight, shell weight, thickness and strength were measured six times during the experiment.

Parameter	Control	Panbonis	SEM	P
Daily feed intake (g/bird)	107.1	105.2	0.93	0.440
Egg production (%/hen day)	86.7 <sup>b</sup>	90.4 <sup>a</sup>	1.07	0.040
FCR (g/g egg)	1.85 <sup>a</sup>	1.77 <sup>b</sup>	0.02	0.036
Egg weight (g)	66.5	66.0	0.69	0.797
Eggshell weight (g)	5.48 <sup>b</sup>	6.06 <sup>a</sup>	0.08	0.005
Eggshell thickness (mm)	0.34 <sup>b</sup>	0.36 <sup>a</sup>	0.00	0.001
Eggshell strength (kgf)	2.53 <sup>b</sup>	3.74 <sup>a</sup>	0.13	<0.001

**Table 1. The effect of Panbonis supplementation (100mg/kg feed) in diets for laying hens between 116-126 weeks of age.**

Results are given in Table 1. Results indicated that eggshell quality was clearly improved during this 10-week period.

Moreover, laying rate was increased although fully productive hens randomly distributed over treatments based on body weight, were selected at the start of the experiment. It was therefore concluded that Panbonis stimulated calcium metabolism in aged hens resulting in better eggshell calcification.

Similar effects of Panbonis were shown in a field trial in the Netherlands, where 100g/MT was added on top of a commercial layer diet (3.6% Ca, 0.47% P, 2400 IU 50:50 vitamin D<sub>3</sub> and 25(OH)D<sub>3</sub>, 500 FTU phytase/kg), because the numbers of second

grade eggs were increasing in a flock of 60-week-old H&N Brown Nick hens. Hens were group-housed in two identical parallel barns on the same farm.

One barn of 6,500 hens was fed the control and one barn the supplemented test diet. Eggshell strength was measured six times between 60 and 80 weeks of age (Fig. 1).

It was shown that although the hens fed the Panbonis supplemented diet started at lower values, they ended at higher eggshell strength values than the control, as the reduction with age was clearly less pronounced for hens fed the Panbonis supplemented diet. Panbonis therefore fills the physiological gap in vitamin D<sub>3</sub> metabolism in ageing laying hens.