Vitamin D metabolites in pullets are essential for a successful start of lay

Eggshell formation is a fascinating process and is suggested to be the most rapid form of biomineralisation in the animal kingdom. Every day, a laying hen deposits about 2.2g of calcium (Ca) in the eggshell, which represents about 10% of her body stores, without including Ca absorbed in the gut.

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At the end of her production cycle, the hen has deposited an amount of Ca that equals 50x her body stores into eggshells. To be able to deposit such a high amount of Ca, laying hens, egg producing animals in general, developed special features. There are two main players involved in Ca deposition in eggshell: vitamin D metabolism and medullary bone.

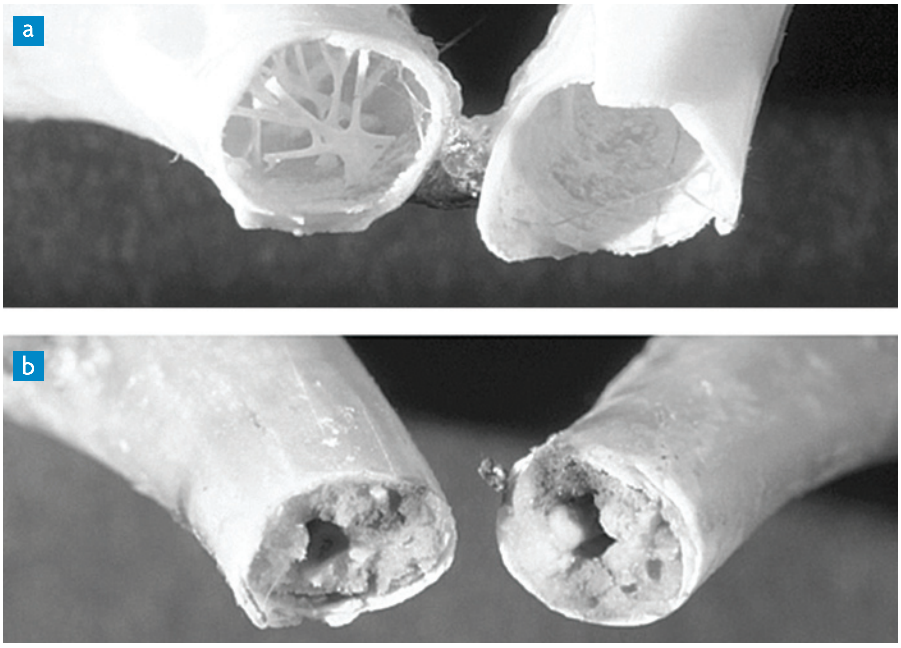

Fig. 1. Humerus of laying hen showing (a) normal pneumatised internal cavity and (b) cavity filled with medullary bone (Whitehead, 2004).

Vitamin D in eggshell formation

The main source of vitamin D in livestock is supply via feed. In the liver, vitamin D is converted to 25-hydroxyvitamin D (25(OH)D), which is an intermediate metabolite. In the kidney, 25(OH)D needs to be converted to become the metabolically active form 1,25-dihydroxyvitamin D (1,25(OH)$_2$D). The major role of 1,25(OH)$_2$D is to maintain serum Ca homeostasis.

1,25(OH)$_2$D increases Ca absorption in the gut, reduces Ca excretion via the kidneys and facilitates Ca resorption from bone. The crucial role of 1,25(OH)$_2$D during eggshell formation becomes evident by the fact that its serum concentration at the peak of shell formation is significantly elevated to maintain blood Ca concentration (Table 1).

Looking at this connection, it becomes clear that any disruption in the vitamin D metabolism has a huge effect on eggshell formation and/or bone quality. These disruptions can have various reasons such as reduced gut absorption of calcium and vitamin D due to gastrointestinal disorders, metabolic stress on the liver at onset of egg production, or reduced vitamin D metabolism in liver and kidney due to high levels of dietary mycotoxins. However, the largest effects are caused by reduced 1,25(OH)$_2$D concentrations originating in the limited activity of 1α-hydroxylase, which occurs in ageing laying hens.

Medullary bone as Ca reservoir

The second element in eggshell formation is the bone. In laying hens, bones consist of two distinct types of bone: structural bone and medullary bone (Fig. 1). Structural bone, cortical and trabecular bone, gives stability and flexibility to the skeleton. The formation of cortical bone in birds starts during embryonic development and increases after hatch, reaching its peak at around 6-11 weeks when also daily body weight gain is at its maximum.

Structural bone formation stops when the pullet enters puberty. At this time, the formation of medullary bone starts (Fig. 2).

In contrast to cortical bone, medullary bone does not have structural properties but is acting as a dynamic Ca store. Eggshell formation mostly happens during the night when the hen does not have access to feed. The required Ca can thus only come from solubilised coarse limestone in the gut or

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Table 1. Changes in blood 1,25(OH)$_2$D and Ca concentrations during eggshell formation (adapted from Gloux et al. (2020)).

Time post ovulation (hours)	Stage of eggshell formation	1,25(OH)$_2$D (pg/mL)	Ca (mg/L)
0-1	None	275^{b}	229
9-10	Start	343^{ab}	240
18-19	Peak	375^{a}	234

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from medullary bone, which has to be replenished during the following day. It is inevitable that during mobilisation of medullary bone also some cortical bone will be resorbed by osteoclasts.

These cells cannot distinguish between cortical and medullary bone. An increase in bone volume and cortical thickness leaves more space for medullary bone deposition, which increases the margin until structural integrity of the skeleton is impaired. Therefore, the better the bone development during the pullet phase, the better the bone strength of the laying hen, especially during extended production cycles.

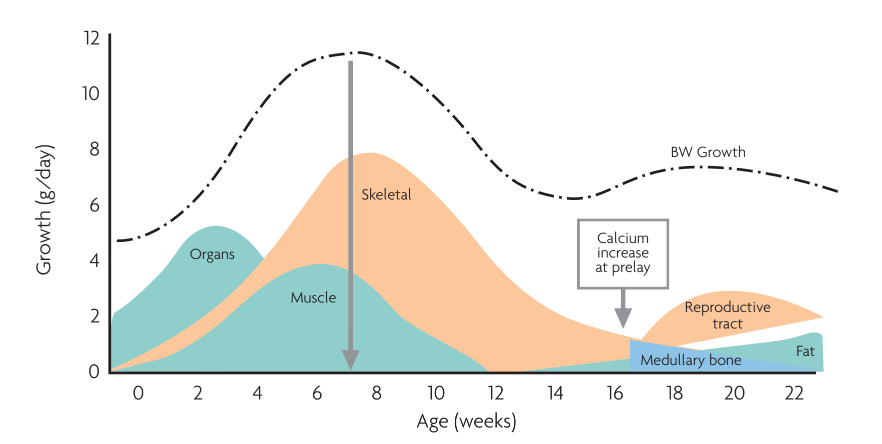

Fig. 2. Growth rate of different tissues in laying hens (reproduced with courtesy of Hendrix Genetics).

Supporting pullets means better laying start

It has been shown in several studies that bone formation in pullets is supported not only by adequate dietary Ca and P contents but also by the supplementation of vitamin D (-metabolites). We tested the supplementation of a complementary feed based on Solanum glaucophyllum meal (SG, waxy leaf nightshade meal) on bone development in pullets and its effect on production performance during the start of egg production. SG contains secondary plant metabolites which support the vitamin D metabolism of the animals.

A total of 1,296 ISA-brown pullets (12 birds/cage) either received a corn-wheat-soybean meal control diet (CON) as such or supplemented with 80g/T Panbonis10 (PAN80) from 5-17 weeks of age. Phase 1 diet from 5-8 weeks contained 10.6g Ca, 4.4g avP and 3,000 IU vitamin D/kg feed, while the Phase 2 diet contained 12.0g Ca, 4.0g avP and 2,000 IU vitamin D/kg feed. At week 16 and week 20, bone ash and breaking strength in the supplemented group was higher than in the control group (Table 2).

At 18 weeks of age, the pullets were transferred to the layer barn and the experiment continued until week 34. The birds received either the control diet CON or the supplemented diet (PAN80), resulting in four treatments for the layer phase: Panbonis in pullet phase (Y/N) x Panbonis in layer phase (Y/N). During lay, diets contained 38.0g Ca, 4.0g avP and 3,000 IU vitamin D/kg feed.

PAN80 only given during the layer phase improved production performance but the effects were larger for the birds fed PAN80 during the pullet and layer period (Table 3).

T3 is reported at the end, as this is not a treatment that is likely or recommended in practice.

These results show that the supplementation of Solanum glaucophyllum in the pullet diet has beneficial effect also on the early laying hens. The higher bone ash suggests a better mineralisation of the bone, thus optimally preparing the young hen for egg production at start of lay. ■

References are available from the authors on request

Table 2. Femur breaking strength and ash content in pullets (16 weeks) and laying hens (20 weeks).

Treatment	Breaking strength (N)		Bone ash (%)	
Age (weeks)	16	20	16	20
CON	9.2	11.7	40.7	44.3
Pan80	9.3	13.4	41.4	45.2

Table 3. Performance of pullets and laying hens receiving either a control diet (CON) or a diet supplemented with Solanum glaucophyllum (PAN80).

Treatment	Diet pullet	Diet layer	Feed intake (g/d)	Laying rate (%)	Egg mass (g)	Eggshell strength (N)
T1	CON	CON	100.1	71.4	40.2	45.1
T2	CON	PAN80	97.5	73.4	40.6	46.1
T4	PAN80	PAN80	97.5	75.4	41.7	48.1
T3	PAN80	CON	96.8	72.4	39.5	46.1