Vitamin D and its Metabolites in Animal Health

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19. DERZSY NAPOK 2011
Havenstein et al. Dept. of Poultry Science, North Carolina State University, USA

<table>
<thead>
<tr>
<th>Breed</th>
<th>Diet</th>
<th>Weight [g]</th>
<th>Feed Conv.</th>
<th>M[%]</th>
<th>TD [%] (1991diet)</th>
<th>TD [%] (1957diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 42</td>
<td>Day 84</td>
<td></td>
<td></td>
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<tr>
<td>1957</td>
<td>1957</td>
<td>508</td>
<td>1400</td>
<td>3.00</td>
<td>3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>1991</td>
<td>1991</td>
<td>2132</td>
<td>4498</td>
<td>2.04</td>
<td>9.1</td>
<td>48.6</td>
</tr>
</tbody>
</table>

1957 Athens-Canadien Random breed (a typical 1957 breed)
1991 Arbor Acres (a typical 1991 breed)
M Mortality
TD Tibial Dyschondroplasia
Calcium and its importance in bone quality

- Cortical and trabecular bone: Support function
- Medullar Bone: Mineral-pool with high turnover

Leg weaknesses

- different causes (genetics, feed, environment)
  1. Bone growth disorders in young fast growing poultry, tibial dyschondroplasia as example
  2. Osteoarthrosis (bone and joint disorders caused by degenerative processes)
  3. Fractures because of osteoporosis in laying hens
leg problems in poultry rearing

A Normal
B Ca/D deficiency rickets
C P deficiency rickets
D Tibial dyschondroplasia

Courtesy by Prof. C. Whitehead
calcium turnover in the laying hen

- Calcium ++ concentration: 2.5 mmol/L
- Weight: 55 - 70 g
- Shell: 5 g
- Organic: 4%
- Inorganic: 95%
  (calcite CaCO₃)

Hydroxylapatite: 3*Ca₃(PO)₄*Ca(OH)₂

Ca/P: 2.5:1
95% of body Ca

Uptake:
Ca 4 - 5 g/d
P 0.4 g/d

10⁻⁷ mol/L > 10⁻⁶ toxic
Activity:

- **Cholecalciferol (Vitamin D₃)**
  - Official mass – activity relation: 1 IU = 25 ng
  - (2x more active, depending on biosassay)

- **25-Hydroxyvitamin D₃**
  - Official mass – activity relation: 1 IU = 12 ng
  - (2x more active, depending on biosassay)

- **1,25-Dihydroxyvitamin D₃**
  - Official mass – activity relation: 1 IU = 5 ng
  - (5x more active, depending on biosassay)

- **Ergocalciferol (Vitamin D₂)**
  - (10x less active in birds)
metabolism of vitamin D

Regulation:
- PTH, CT, GH, E₂
- 1,25(OH)₂D
- circulating Ca, PO₄

Concentrations in plasma:
- Cholecalciferol: n.d. (pg-level)
- 25(OH)D: 10 - 50 ng/mL
- 1,25(OH)₂D: 17 - 80 pg/mL

Vitamin D → 25(OH)D → 24,25(OH)₂D → 1α,25(OH)₂D → 1α,24,25(OH)₃D → Calcitroic acid

Ca intestine → CaBP → Kidney

Ca urine

Ca circulating

Ca bone

VDR in bone cells
Vitamin D3

- prevents and cures rickets
- but not tibial dyschondroplasia

Plasma vitamin D-metabolites after feeding dietary cholecalciferol in a curative chicken model (Data after Goff and Horst 1995)

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Herbonis
25-Hydroxyvitamin D₃ (storage form)

- prevents and cures rickets
- improves performance
- tibial dyschondroplasia only partially
- Published experiments in peer reviewed journals
  - 75µg/kg is equipotent to 2µg/kg 1,25(OH)₂D₃ (lowers incidence from 64% to 10%)
  - trials without effect

Fig. 3: Plasma levels of 25-hydroxy vitamin D (trial C0195)

Fig. 4: Plasma levels of 1,25-dihydroxy vitamin D (trial C0195)
**1,25-Dihydroxyvitamin D₃ (synthetic Calcitriol)**

most active VDM, strictly controlled formation
genomic action through VD-Receptor in intestine
non-genomic (“fast“) action in muscle and other tissue

- Cures rickets
- Most active compound in curing and preventing TD
  - Edwards et al. 10 µg/kg prevents TD
  - Whitehead et al. 2 µg/kg lowers TD from 25% -> 0%

- Active in improving egg shell strength
- Active in layer fatigue and osteoporosis (Whitehead et al.)

⇒ no product available for animal nutrition
Prevention of tibial dyschondroplasia or osteoporosis, Egg shell quality

Slow processes may not be recognized by the calcium homeostatic regulation

$1\alpha,25(OH)_2D_3$, the active metabolite has a different quality of action
Vitamin D from natural sources:

Sparse, fish liver oil is one of the richest source, because vitamin D is produced under the influence of sunlight by zoo- and phyto-plankton, the basis of the marine food chain.

Others (found in the 1980ties):

<table>
<thead>
<tr>
<th>Solanum glaucophyllum</th>
<th>Cestrum diurnum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotiana sp.</td>
<td></td>
</tr>
<tr>
<td>Nierembergia veichtii</td>
<td>Solanum lycopersicum</td>
</tr>
<tr>
<td>Trisetum flavescens</td>
<td>alfaalfa</td>
</tr>
</tbody>
</table>

most 10-50 times less traces
not in textbook: the natural alternative

*Solanum glaucophyllum* from the wild
cultivation
**Solanum glaucophyllum**

Wild forms and a cultivated variety, selected for high and uniform active content: Hervit® (non-GMO)

Active content:
- Wild: VDM 0–20 ppm (as 1,25-Dihydroxyvitamin D$_3$)
- Cultivation: VDM 25-30 ppm
  - >90% in glycosidic bound form
  - <5% free 1,25-Dihydroxyvitamin D$_3$
  - <5% 25(OH)D$_3$; vitamin D$_3$

Inactive content:
- Primary plant metabolites, flavonoids

Toxic components: Jain et al.: alkaloid Solasodine

☞ Hervit is controlled for a low alkaloid content and pesticides, heavy metals, microbial purity
Product characterization:

Active content: 1,25-dihydroxyvitamin D₃ specs; CoA

Inactive content: Weende analysis specs; CoA

Specific components: pesticide
residues by HPLC
heavy metals by AA
microbial contamination bacteriol.
alcaloids HPTLC,
HPLC/MS others HPTLC
Panbonis – Herbal Vitamin D₃
Standardized, formulated product for adding to feed
Content 10 ppm (as 1,25-Dihydroxyvitamin D₃)

Product properties:
Light green powder, particle size 100-500 µm (92%)
Good miscibility with feed
Storage stability (0 / 20 / 40 °C): 36mt >90%
Thermo stability at extruding, pelleting: >90%
Non-GMO product, controlled for heavy metals, pesticide residues and microbial contamination

Legal status:
CH Registered as FA ‘Herbal Vitamin D’
EU Notified as under Regulation EC767/2009 as SG standardized leaves
Asia (Taiwan, Thailand, Malaysia, Japan) as ‘herbal FA’
SA Brazil, Argentina, Chile, Mexico as ‘herbal FA’
Solbone-A-cws

A standardized, formulated extract with good cold water solubility

Content: 50 ppm (as 1,25-Dihydroxyvitamin D₃)

Application: via drinking water in poultry and swine rearing

Properties: Light brown powder with a malty taste
particle size 60-300 µm (90%)
Freely soluble in water to maximal 30% w/v

Storage stability: 0/20/40 °C: 24mt >90%
Thermo stability: 100°C >90%
Stability in water: 20°C (5 %), 3 days >90%
Thermo stability solution 1g/L 120°C/3 min >80%

Registration: CH registration as FA in progress
EU registration as FA in preparation
Asia registered in Japan, Thailand others in progress
SA registered in Brazil
Biological effects
Product characterization

Herbal vitamin D₃ (1,25(OH)₂D₃-glycosides)
- Stable (storage, temperature)
- Water soluble (Solbone-A-cws)

In the intestinal tract - ‘slow release’
By digestive enzymes - broader tolerance

Free active Vitamin D - natural active VDM
- Fast onset of action

Resorption without overloading liver and kidneys

Direct action on bones - better Ca + P utilization
mineral-related conditions

**Poultry**
- leg weaknesses in fast growing broiler chickens
- eggshell quality and osteoporosis of old laying hens

**Swine**
- calcium-related problems at farrowing
  - lactating sow
  - piglet survival

**Ruminants**
- calcium-related problems during calving
  - milk fever
  - meat quality

**Others, pets, horses** (bone growth, osteoporosis, kidney insufficiency)
Bioassay for Vitamin D activity:
Japanese quail egg shell assay

Principle: Egg-laying quails are given vitamin D-depleted diet until laying performance drops to <10 %. Animals are then given the test diet for 3 weeks (LMU Munich, Germany)

Laying performance
During a control period are animals selected with a laying performance >80%. After one week on a vitamin D-deficient diet laying performance is <10%. The test article will replete laying performance according to their vitamin D content

50% effect
Liver 
Kidney 
Bone 

25(OH)D₃ 
1,25(OH)₂D₃ 

48 – 72 hours 
24 hours 

Vitamin D 
Solbone A
Broiler trial: Preventing leg anomalies

Location: Roslin Institute, Edinburgh, Scotland, Whitehead C et al.

Title: Effects of vitamin D metabolites on bone

Objective: Compare effectiveness of different vitamin D metabolite preparations on bone development

Procedure: 240 male day-old broilers (Ross 308). Basal (control) diet was set on all-vegetable broiler starter diet containing 8 g Ca, 6g available P and 1000 IU vitamin D/kg. Other diets obtained with appropriate supplements. Birds were fed on diets from one day old. At 14 days, proximal tibias were dissected for determination of TD incidence and severity and tibia breaking strength was measured.
Reduction of Leg Problems in Broiler Chickens

Model for tibial dyschondroplasia in unsexed Ross 308 chicks from day 1 to 14. Trial performed at Roslin Institute, Edinburgh, UK (C. Whitehead et al.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0%</th>
<th>2.5%</th>
<th>5%</th>
<th>10%</th>
<th>0%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Birds with other leg weaknesses</td>
<td>27%</td>
<td>7%</td>
<td>4%</td>
<td>0%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td>No Birds with tibial dyschondroplasia</td>
<td>31%</td>
<td>93%</td>
<td>93%</td>
<td>90%</td>
<td>93%</td>
<td>97%</td>
</tr>
<tr>
<td>No Birds with healthy animals</td>
<td>42%</td>
<td>93%</td>
<td>93%</td>
<td>90%</td>
<td>93%</td>
<td>97%</td>
</tr>
</tbody>
</table>

- control group
- synth. Calcitriol positive control [µg]
- PAN-HVD as [µg Ctl]
- Solbone-A as [µg Ctl]

All animals received 1000 IU vitamin D₃
Phosphorous utilization

P and Ca balance trial in broiler chickens. Days 12-17 / 17-22

<table>
<thead>
<tr>
<th>treatment</th>
<th>Ca % digest</th>
<th>P % digest</th>
<th>Ca % excret</th>
<th>P % excret</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal (P_{tot} = 0.66%)</td>
<td>0.51^a</td>
<td>0.43^a</td>
<td>0.47^a</td>
<td>0.40^a</td>
</tr>
<tr>
<td>Control P_{red} (P_{tot} = 0.50%)</td>
<td>0.37^b</td>
<td>0.47^b</td>
<td>0.46^a</td>
<td>0.22^b</td>
</tr>
<tr>
<td>P_{red} (P_{tot} = 0.50%) + Panbonis 75 g/ft</td>
<td>0.43^c</td>
<td>0.42^c</td>
<td>0.42^b</td>
<td>0.20^b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>treatment</th>
<th>Ca_{ret} g/kg f</th>
<th>P_{ret} g/kg f</th>
<th>Pyt-P_{ret} g/kg f</th>
<th>diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control normal (P_{tot} = 0.66%)</td>
<td>4.55^a</td>
<td>2.83^a</td>
<td>1.29^a</td>
<td></td>
</tr>
<tr>
<td>Control P_{red} (P_{tot} = 0.50%)</td>
<td>3.32^b</td>
<td>2.37^b</td>
<td>1.46^a</td>
<td>-</td>
</tr>
<tr>
<td>P_{red} (P_{tot} = 0.50%) + Panbonis 75 g/ft</td>
<td>3.87^c</td>
<td>2.62^c</td>
<td>1.52^b</td>
<td>11%</td>
</tr>
</tbody>
</table>

Different letters p<0.05

Same effect also published by Cheng et al. 1996
Other animal models

Herbal vitamin D$_3$ in a rat model for osteoporosis:

Normal (sham operated)  ovariectomized

Fosamax  Solbone
Safety
Herbal Vitamin D₃’ safety:

In broilers:
Recommended dose: on top of usual Vitamin D₃:
    <2’000 IUD/kg: 0.2 to 0.5 g/kg feed
    >2’000 IUD/kg: 0.1 to 0.2 g/kg feed

  5 g/kg  no adverse effect
  12 g/kg  adverse effect level
- after day 14: reduced weight gain in half of animals
- at day 25: hypercalcemia
  blood calcium +38%
  animal weight –36%
  mortality 1.4 ⇒ 5.7%
- at day 38: recovery, normal blood calcium and
  growth curve after change to normal feed
Plasma kinetics after a single dose of an extract of *Solanum glaucophyllum* to cows

1,25(OH)$_2$D$_3$  Ca

**Plasma 1,25(OH)$_2$D$_3$ in cows after a single application of an extract of *Solanum glaucophyllum***

```
Plasma 1,25(OH)$_2$D$_3$ in cows after a single application of an extract of *Solanum glaucophyllum*

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
<th>168</th>
<th>192</th>
<th>216</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg/ml</td>
<td></td>
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<tr>
<td>5 mg/kg bw</td>
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<tr>
<td>10 mg/kg bw</td>
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<td>control</td>
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**Plasma calcium in cows after a single application of an extract of *Solanum glaucophyllum***

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Plasma calcium in cows after a single application of an extract of *Solanum glaucophyllum*

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>24</th>
<th>72</th>
<th>120</th>
<th>168</th>
<th>216</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/l</td>
<td>2.4</td>
<td>2.2</td>
<td>2.6</td>
<td>2.4</td>
<td>2.2</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>5 mg/kg bw</td>
<td></td>
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<td>10 mg/kg bw</td>
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<td>control</td>
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</table>
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<table>
<thead>
<tr>
<th>Biological actions</th>
<th>Molecular properties</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>VD activity</td>
<td>R</td>
<td>TD(^1)</td>
</tr>
<tr>
<td>Vitamin D(_3)</td>
<td>full</td>
<td>Y</td>
</tr>
<tr>
<td>25(OH)D(_3)</td>
<td>full</td>
<td>Y</td>
</tr>
<tr>
<td>1,25(OH)(_2)D(_3)</td>
<td>full</td>
<td>Y</td>
</tr>
<tr>
<td>Herbal active VD</td>
<td>full</td>
<td>Y</td>
</tr>
</tbody>
</table>

1) TD: tibial dyschondroplasia  
2) OP: osteoporosis  
3) Lipophilicity: a measure for the fat-solubility - is the opposite of water-solubility.  
4) Tissue accumulation: the tendency of a lipophilic molecule to dissolve in fat-rich tissue and thus accumulates in the body.  
5) Half-life: time measure by which half of the applied compound is cleared from the body  
6) Chemical stability: determines the storage stability and reactivity with feed components and heat stability at feed processing  
7) Tolerance: the factor at which toxicity starts. Based on recommended doses of 2500 IU/kg for VD\(_3\), 69µg/kg for 25(OH)D\(_3\), 5µg/kg 1,25(OH)\(_2\)D\(_3\) and 250, resp. 100mg/kg feed for Panbonis.)
Herbal active Vitamin D₃

Active agent is 1,25-dihydroxyvitamin D₃, the most active natural VDM ¹) in man and animal with proven activity in:

Bone-related effects
- Cures vitamin D-deficiency (rickets) faster
- Prevents leg weakness (tibial dyschondroplasia)

Other benefits
- Improves phosphorous absorption
- Improves performance (1-8 %, weight gain and feed conversion)
- Improves meat tenderness (in beef, data by Foote et al. 2004)

The present glycosidic form of the active vitamin D₃ shows a better safety profile than free 1,25-dihydroxyvitamin D₃.

The standardized and formulated product has a good stability (up to 3 years)

¹) In glycosylated form, a naturally stabilized form
In conclusion, you have the choice of 3 different forms of vitamin D₃ each with it’s merits:

<table>
<thead>
<tr>
<th>Vitamin D₃</th>
<th>cheap; covers normal conditions, rickets</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxyvitamin D₃</td>
<td>advantage in specific conditions: performance, mineral supply, rickets</td>
</tr>
<tr>
<td>Herbal active vitamin D₃</td>
<td>advantage in specific conditions: calcium uptake, bone health, phosphorus utilization, rickets</td>
</tr>
</tbody>
</table>

Thank you