25-OH-D$_3$[1] in poultry nutrition: what we have learned from a scientific and practical perspective

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Vitamin D serves several functions, the most recognized being the maintenance of calcium (Ca) and phosphorus (P) homeostasis (Whitehead, 1996). Skeletal development and upkeep, as well as eggshell strength, are vitamin D-dependent. The same holds true for cell growth and differentiation (Brown et al., 1999) and the immune system (Reinhardt and Hustmyer, 1987). Elevated blood glucose in chicks parallels marginal vitamin D status because insulin release is dependent on this vitamin (Hunt and Nielsen, 1987), as are numerous vitamin D-dependent genes that encode proteins to regulate cell function (Normal et al., 2006).

Because of its association with Ca and P, insufficient vitamin D manifests itself into readily discernible deformities of the skeleton, beak, and eggshells. Gross deficiency impairs egg production, and brings about late-stage embryonic death in hatching eggs. Egg yolk links the carryover effect of vitamin D from the hen to the chick, and is instrumental in the young chick's rapid bone growth via Ca mobilization (Coto et al., 2010).

Hydroxylation of Vitamin D

Today, we recognize two naturally occurring pre-vitamin D parent compounds – vitamin D$_2$ and vitamin D$_3$ – and neither is biologically active prior to hydroxylation. Ergocalciferol (D$_2$) is synthesized from ergosterol in many plants, whereas cholecalciferol (D$_3$) comes from 7-dehydrocholesterol in the skin of animals exposed to ultraviolet light. Cholecalciferol is twice hydroxylated – first, to 25-OH D$_3$ in the liver, and then to 1,25-diOH vitamin D$_3$ in the kidneys – to become the widely recognized potent form.

Poultry require 30 times more vitamin D$_2$ than vitamin D$_3$ for equivalent biopotency (Soares, 1982), presumably because vitamin D binding proteins have a greater affinity for vitamin D$_3$ (Haddad, 1980), and because of the rapid clearance rates from the body of 3H-labeled vitamin D$_2$ (Hoy et al., 1988).

Commercial Vitamin D Supplementation

Broiler growth rates climb 3-4% every year with less feed being consumed/lb live gain (Zuidhof et al., 2014). The boost in skeleton and body weight directly impacts requirements, and that includes higher vitamin levels (Jiang et al., 2011) to provide greater economic returns (Mejia et al., 2013). Nutrition is also increasingly scrutinized for pullets for the upcoming lay period,

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[1]25-OH D$_3$ refers to HyD® registered trademark of DSM Nutritional Products LLC, and represents the source of 25-OH D$_3$ discussed herein in poultry trials

especially in consideration of "long life" egg layers geared to produce 500 eggs in 100 weeks (Bain et al., 2016).

Modernday enclosed poultry housing necessitates vitamin D supplementation in the feed. Defensive vitamin supplementation – a low-cost strategy designed to avoid visual symptoms of deficiency – inadvertently places upper limits on economic returns. Marginal supplementation restrains live performance without obvious signs of deficiency (Ward, 2009). This represents the costliest supplementation level because borderline vitamin D triggers suboptimal feed conversion, breast blisters, late-stage embryonic mortality, and broken bones during processing, all of which can erroneously be attributed to other factors.

Vitamin D is included in commercial vitamin premixes in the form of cholecalciferol or vitamin D_3. Table 1 shows the U.S. vitamin D_3 supplementation rates for broilers and broiler breeders. That commercial rates are markedly higher than the NRC (1993) of 200 IU/kg feed is no surprise when considering that 1993 recommendations are a poor match with today's broilers that grow considerably faster with less feed.

Still, current supplementation levels are only 30% higher than reported in 1993 for the U.S. broiler industry (Ward, 1993), arguably little more than a subtle change after nearly 25 years of remarkable improvements in broiler genetics. One factor that has influenced vitamin D levels has been the rapid adoption of 25-OH D_3 (HyD®) in most poultry feeds during this period.

25-OH Vitamin D_3 Supplementation

25-OH vitamin D_3, as HyD®, gained self-affirmed GRAS status in the late 1990s for U.S. broilers, turkeys, and laying hens, and today, includes minor poultry species such as ducks, pheasants, and quail. In 2017, swine was added to the list.

Not all sources of commercial 25-OH D_3 are equivalent. When comparing manufacturer's recommendation for 25-OH D3, Brand X resulted in about 50% as much increase in plasma levels of 25-OH D3 as HyD in broiler chicks (14.0 ng/ml for Brand X vs 29.2 ng/ml for HyD). Twice as much of Brand X was equivalent to HyD in this & other studies.

Since the year 2,000, the use of 25-OH D_3 has shown continuous year-to-year growth in poultry feeds. In comparison to the recommended 62.5 mg/ton feed, data accounting for >90% of the broiler industry in 2014 find that starter feeds contain an average of 50.4 mg/ton (Figure 1). Addition rates decline into the grower and finisher feeds, while breeder feeds average 42.6 mg/ton.

In poultry, 25-OH D_3 is the major vitamin D metabolite in the blood (Haussler and Rasmussen, 1972) and probably contributes as much as 80% of the circulating vitamin D activity in serum (Ovesen et al., 2003). The relationship between circulating 25-OH D_3 and Ca is significant, whereas that for 1,25-diOH D_3 and Ca is not (Barger-Lux et al., 1995; Litta, 2016).

Plasma levels of 25-OH D_3 closely follow dietary levels. Chick plasma contained 10 to 30 ng 25-OH D_3/ml serum when fed no 25-OH D_3, but this rose to 55 to 80 ng 25-OH D_3/ml serum 69 ug HyD/kg feed was added to the diet (Yarger et al., 1995). Tissue concentrations of 25-OH D_3 were lower by several-fold in birds fed vitamin D_3. Circulating and tissue levels are also eminently dependent on dietary levels of 25-OH D_3 for turkeys (Lanenga et al., 1999) or laying hens (Terry et al., 1999).

25-OH Vitamin D_3 in Commercial Practice – Why So?

When 25-OH D_3 was introduced, it was generally assumed to be a super-potent source of vitamin D_3 because data indicated that 25-OH D_3 is 2.5 to 4.5 times as active as vitamin D_3 for chicks (Soares et al., 1978). But over time, field observations aligned with research to find 25-OH D_3 exerted effects beyond those of vitamin D_3 (Soares et al., 1995; Litta, 2016), and that tissues can have a requirement specifically for 25-OH D_3 (Vieth, 1999).

Today, there are 4 areas where 25-OH D_3 is recognized to offer important advantages under poultry production scenarios –

- Absorption of 25-OH D_3 is more effective than for vitamin D_3
- Skeletal development is enhanced and better developed
- Growth and meat yield are improved significantly
- Modulates and enhances immune system

Absorption of 25-Hydroxy Vitamin D_3

An important advantage exists with 25-OH D_3 for absorption (Ward, 2004). Vitamin D_3 requires the formation of fatty acid-laced micelles in the intestinal lumen. And it needs bile acids to facilitate absorption. On the other hand, the uptake of 25-OH D_3 occurs by a different mechanism, completely independent of fat absorption or without the need for bile acids (Sitrin et al., 1987). In rats, for example, ligation of the bile ducts essentially halted the absorption of vitamin D_3, but had no impact on 25-OH D_3.

Young birds have a poorly developed pancreas (for lipase to generate of fatty acids from fat) and lack sufficient bile during the initial 10-18 days of life (Jon et al., 1998). At a time critical for vitamin D to facilitate P and Ca uptake, 25-OH D_3 is not dependent on factors limited in supply.

The more rapid absorption of 25-OH D_3 is favored by intestinal binding proteins in intestinal cells (Nechama et al., 1977). These proteins have an affinity for 25-OH D_3 more than 1,000 times greater than for other D_3 metabolites (Teegarden et al., 2000). This actively facilitates absorption of 25-OH D_3 by a completely different mechanism than by vitamin D_3.

The absorption of 25-OH D_3 was 83% as opposed to 66% for vitamin D_3, and occurred mainly in the upper jejunum (Bar et al., 1980). Net losses back into the intestinal tract was 20% for vitamin D_3 and 7% for 25-OH D_3. Along with more being absorbed, 25-OH D_3 retention in the body occurs with greater economy.

Early work (Yarger et al., 1995), along with more recent studies (Saunders-Blades et al., 2004; Bray, 2011; Vignale et al., 2015), verified the 1970s and 1980s work. For example, absorption was tested in broilers fed vitamin D_3 at 2760 and 5520 IU/kg as treatments #1 and #2, while 25-OH D_3 (69 mg/kg HyD) was added on top of those to give respective treatments #3 and #4 (Vignale et al., 2015). 21- and 42-day blood levels of 25-OH D_3 was more the 2-fold higher than the corresponding vitamin D_3-only diets.

Intestinal Malabsorption is a Consideration. Enteric disturbances are especially detrimental to nutrient absorption. A decrease in shank weight and length was commensurate with lower plasma 25-OH D_3 in turkey poults with diarrhea, malabsorption, and subsequent stunting (Perry et al., 1991). In commercial poults diagnosed with leg problems in the field (Bar et al., 1982), plasma 25-OH D_3 was less than half that found in normal poults.

A recent malabsorption model confirmed the advantage of 25-OH D_3 in broiler chicks (Rebel and Weber, 2009). Chicks were inoculated on day of hatch with either saline solution or with homogenates from malabsorption syndrome tissues. Plasma levels of 25-OH D_3 of the infected chicks fed 25-OH D_3 (69 ug/kg) and non-infected chicks fed vitamin D_3 (2760 IU/kg) were similar (Figure 2). But plasma 25-OH D_3 of infected chicks fed vitamin D_3 was low on day 3, and declined to "undetectable" by day 8. This simply illustrates that intestinal disruptions can completely hinder vitamin D_3 absorption, but have minimal impact on 25-OH D_3.

Enteric malabsorption is a growing concern with the removal of growth promotants from diets, since these help control bacterial overgrowth and related issues. Within the first 10-15 days of life, impaired absorption can severely affect birds already struggling to get off to a good start, and absorbing sufficient Ca and P is part of that scenario.

Skeletal Development Improved with 25-OH D_3

Skeletal disorders and mobility are high on the list of concerns for animal welfare and bird performance. Taken with the greater absorption of 25-OH D_3, it's no surprise that bone strength is boosted. Across a wide range of conditions and genetics, bone strength improved by more than 10% (Figure 3).

In a recent trial (Wideman et al., 2015) broilers were supplemented with either 5,500 IU vitamin D_3/kg diet or 5,550 IU vitamin D_3/kg diet + 33.9 ug 25(OH)D_3/l water. In a 'mobility model' to facilitate stress, lameness was evident by day 28, and the cumulative incidence of lameness was higher (P<0.05) in the vitamin D_3 group by day 53. This demonstrated 25-OH D_3's ability to lower outbreaks of lameness. In laying hens, problems with cage layer fatigue and poor eggshell quality can be similarly addressed.

New studies from the University of Georgia (Kim et al., 2017) determined that mineral apposition rate was more pronounced 25-OH D_3-fed HyLine W36 pullets. Three treatments were compared – 2760 and 5520 IU/kg of vitamin D_3 alone, and 2760 IU/kg D_3 + 25-OH D_3 (69 ug/kg feed). At 10 weeks, the advantage of 25-OH D_3 was clearly evident (Figure 4). The 25-OH

D$_3$ diet also improved overall (18-60 weeks) hen-egg production, as well as FCR during the 36- to 48-week period.

Cortical is the hard-outer surface of bone, while trabecular is less dense and supports structure with cortical. Medullary serves as a Ca reserve and can provide 40% of Ca for eggshell formation (Mueller et al., 1969). Upon commencement of lay, structural bone formation ceases but continues to be resorbed. Vitamin D & Ca deficiency hastens this loss of cortical and trabecular to cause 30-35% birds exhibiting broken bones (Fleming, 2000).

Today, 25-OH D$_3$ is used widely in pullet programs, largely because of the consistency of field reports that keel bone and body weight are improved with 25-OH D$_3$ supplementation. This is especially critical as egg production continues to start sooner and last longer. 25-OH D$_3$ in the diet improved Ca resorption with no adverse effects on bone quality (Saunders-Blades and Korver, 2008). Hens were simply better able to mobilize medullary bone Ca, and maintain structural bone integrity, while reducing the number eggs with defective shells. This is consistent with research and field reports for laying hens fed 25-OH D$_3$ (Figure 5).

Meat yield improvements with 25-OH D$_3$

Breast meat is a premium economic contribution for integrators. Initial research with 25-OH D$_3$ suggested carcass and breast yield improvements when feeding 25-OH D$_3$, and subsequent investigations and field work in North America reinforced these findings. Expanded globally across nearly 25 different studies with various conditions and broiler genetics, 25-OH D$_3$ provided 0.5 – 0.6% more breast yield (Figure 6).

To further understand the process by which 25-OH D$_3$ increases breast yield, research zeroed in at the molecular level with emphasis on the satellite cells (Berri et al., 2013; Hutton et al., 2013). Satellites are the precursors to muscle cells, and assist in regenerating muscle after injury or disease. The stimulation of satellite cells can differentiate and cause existing muscle fibers to form new fibers. In this manner, satellite cells become muscle cells that grow and expand to be expressed as additional breast meat.

Hutton et al. (2013) demonstrated that dietary 25-OH D$_3$ significantly increased the number of satellite cells and muscle fiber cross-sectional area of the *Pectoralis major* in 49-day old broilers. More satellite cells were present in breast tissue, along with more vitamin D receptors (VDR), in birds fed 25-OH D$_3$ + vitamin D$_3$, as opposed to vitamin D$_3$ alone (Berri et al., 2013). Other muscle-stimulating myogenic factors were invigorated by 25-OH D$_3$ (Berri et al., 2013).

Through a complicated sequence of testing at cellular level, the University of Arkansas (Vignale et al., 2015) found 25-OH D$_3$ to have important ramifications on protein synthesis rate. Corroborating findings for more breast yield, fractional protein synthesis increased by nearly 4-fold with dietary 25-OH D$_3$ (Table 3). These investigations also noted that 25-OH D$_3$ exerted the greatest increase in breast meat when fed throughout the entire period, as opposed to 21 days.

Skeletal muscles harbor VDR and 1-α hydroxylase that converts 25-OH D_3 to the active 1,25-diOH D_3 form (this enzyme has no effect on vitamin D_3). Ohio State University OARDC research found breast and thigh muscles to contain 1-α hydroxylase at concentrations second only to kidneys (Shanmugasundarum and Selvaraj, 2012). That 25-OH D_3 overexpressed VDR in skeletal muscle is intriguing in this process (Berri et al., 2013; Morris, 2014; Vognale et al., 2015).

Immune System Modulation Benefits from 25-OH D_3

The association between 25-OH D_3 and the immune system occurs through VDRs located in virtually all immune-related cells (Provvedini et al., 1983). Initial work with poultry showed 25-OH D_3 to improve the immune response to Newcastle vaccinations (Mireles, 1997), and increase titres to IBD and coccidiosis (Mireles et al., 1999). More recently, Morris (2014) reported a series of experiments to conclude that 25-OH D_3 exerts benefits over vitamin D_3 during disease and vaccine challenge while improving bird performance –

- 25-OH D_3 birds gained more weight than did vitamin D_3 birds when injected with LPS (an inflammatory agent) by 2.5% (P=0.03) at 21 days and 3.8% (P<0.01) at 35 days
- 25-OH D_3 birds had 3-7 times less inflammatory agent, cytokine IL-1B mRNA
- Starter-only 25-OH D_3 supplementation was less effective as going beyond starter feeds

The modulatory effect of 25-OH D_3 also extends to breeders and their progeny (Saunders-Blades and Korver, 2008). *In vitro* killing by E. coli leucocytes from immune cells was significantly improved from chicks of dams fed 69 ug 25-OH D_3/kg feed. This coincided with lower embryonic mortality and increased body weight of the progeny, and demonstrated a carryover effect by dietary 25-OH D_3 in breeders.
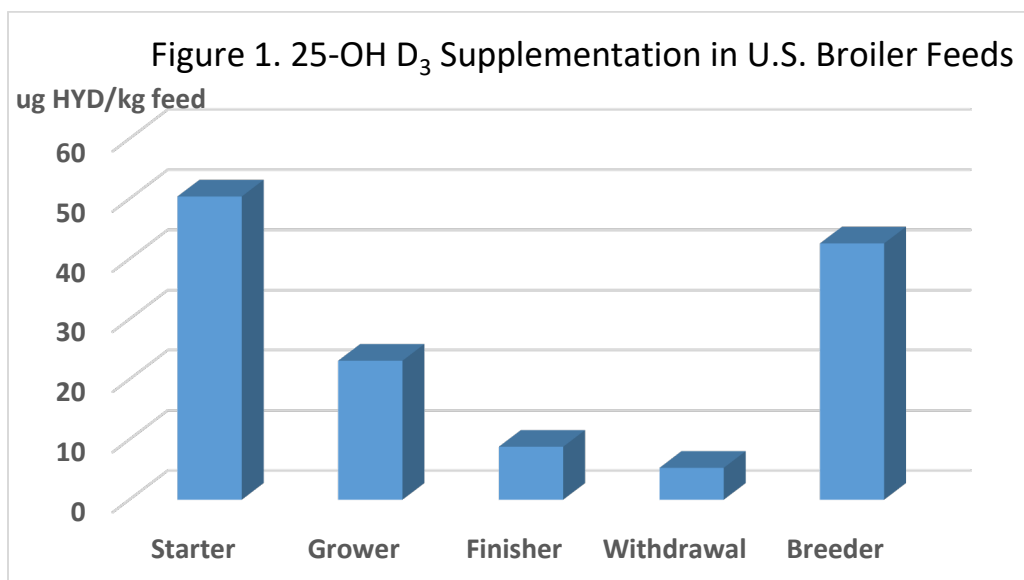
This is What We Know

25-OH D_3 represents one of the most exciting contributions to animal nutrition and health in recent decades. Research findings and field observations during the past 15 years currently recognize 4 primary benefits provided by this metabolite – more dependable absorption, a stronger skeletal structure, more breast and meat yield, and a more functional and economic immune system.

References upon request.

Table 1. Commercial Vitamin D$_3$ Fortification in Broiler Feeds

	High 25%	Average	Low 25%
		MIU/ton feed	
Starter	4.87	3.35	2.20
Grower	4.25	2.80	1.68
Finisher	3.61	2.32	1.28
Withdrawal	3.42	2.12	1.27
Breeder	5.40	3.68	2.48

Source – DSM Nutritional Products, Inc., 2014

Figure 1. 25-OH D$_3$ Supplementation in U.S. Broiler Feeds

Source – DSM Nutritional Products, Inc., 2014

Figure 2. Plasma 25-OH in Broiler Chicks Fed Vitamin D₃ or 25-OH D₃ as Affected by Malabsorption

Figure 3. Summary of Experimental and Field Trials
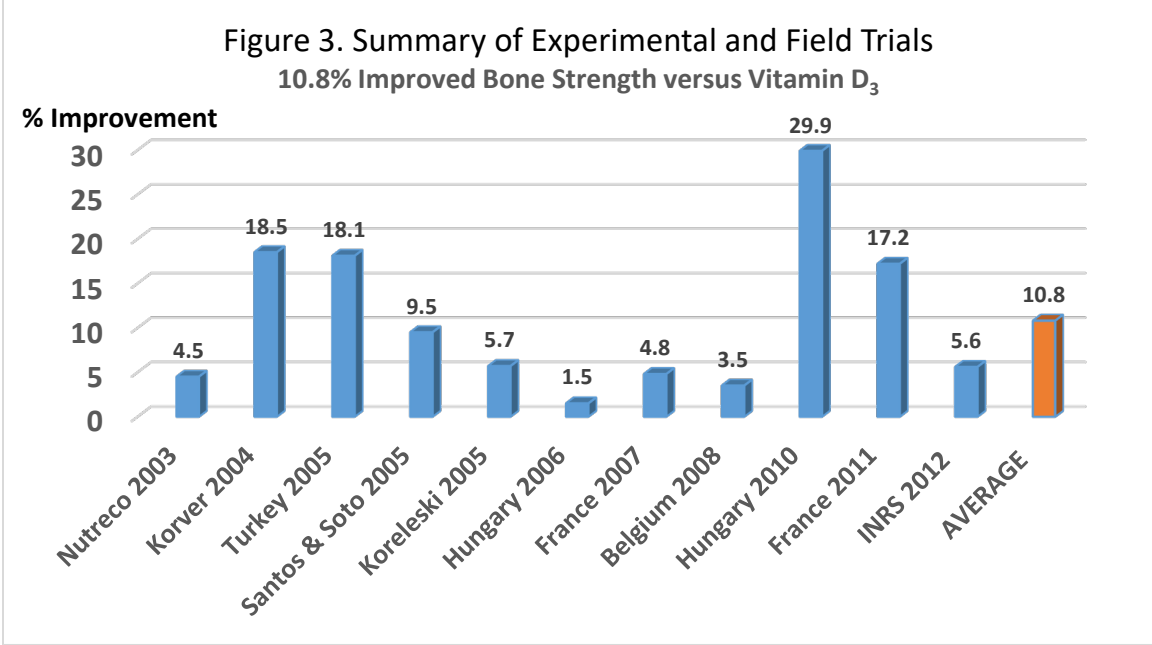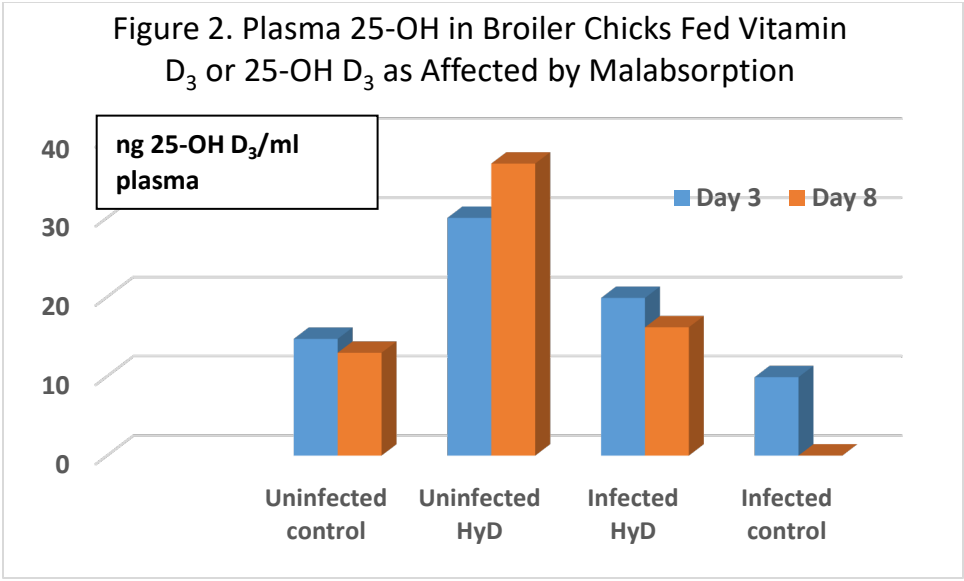**10.8% Improved Bone Strength versus Vitamin D₃**

Figure 4. Relative mineralization of pullet tibia between vitamin D₃ and vitamin D₃ + 25-OH vitamin D₃ (Kim et al., 2017)

Layer pullets
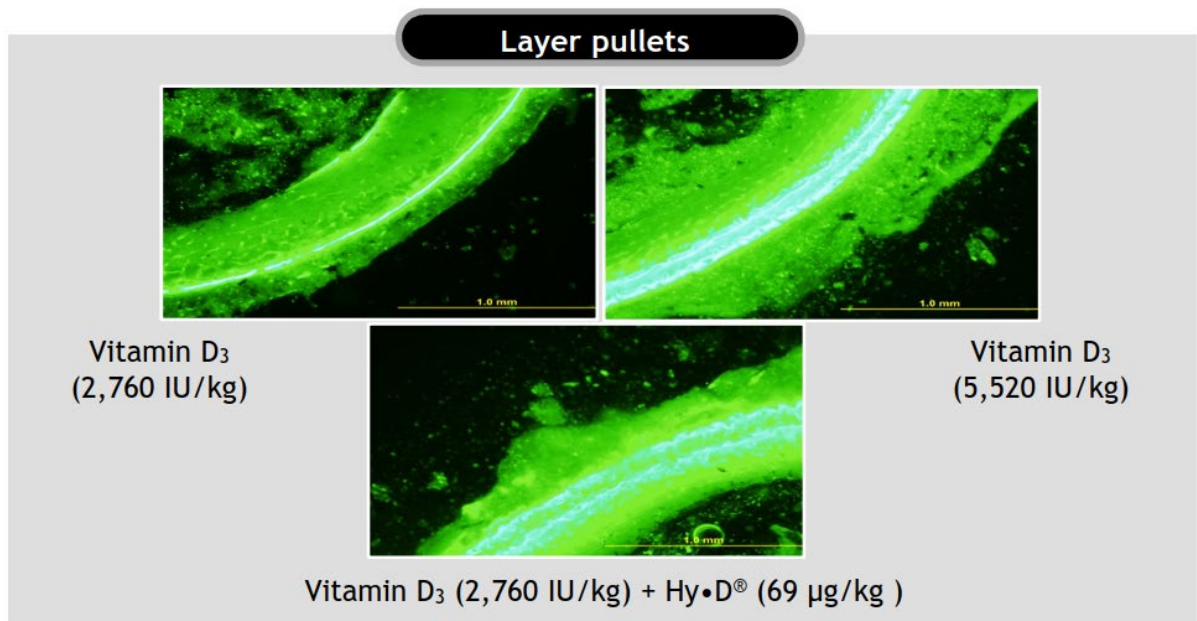
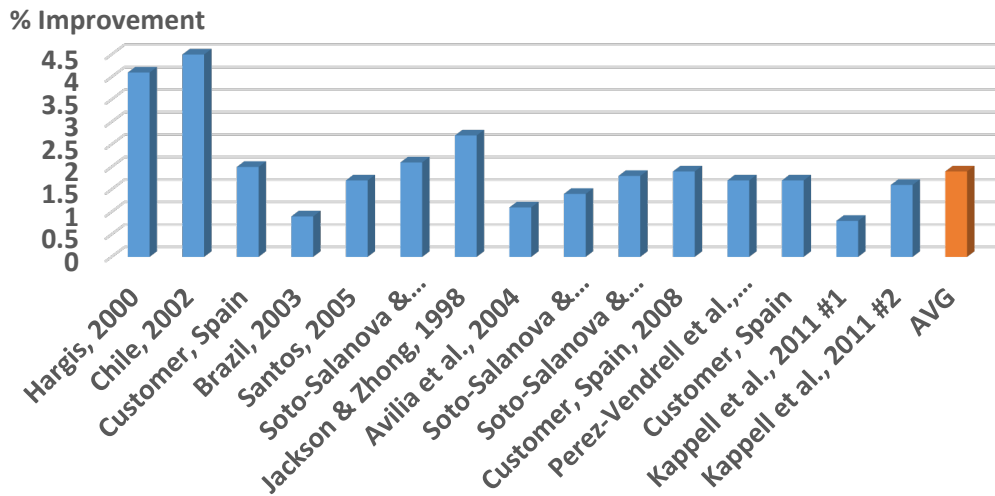Vitamin D₃ (2,760 IU/kg)

Vitamin D₃ (5,520 IU/kg)

Vitamin D₃ (2,760 IU/kg) + Hy•D® (69 μg/kg)

Figure 5. Impact of 25-OH D₃ on Egg Production in Breeders and Laying Hens

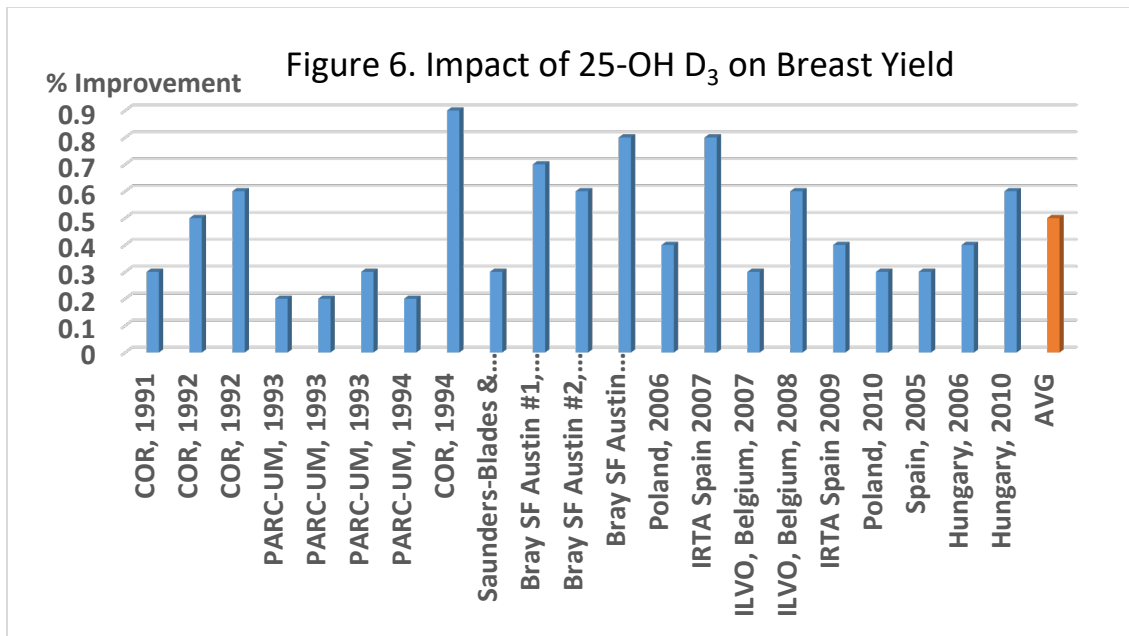Figure 6. Impact of 25-OH D$_3$ on Breast Yield

Table 3. Protein Synthesis Rate in Broiler Fed Vitamin D$_3$ and 25-OH D$_3$[1]
(Vignale et al., 2015)

Treatment	IU/kf feed	Fractional protein synthesis rate, % at 42 days
Control Vitamin D$_3$	2,760	4.44[b]
High Vitamin D$_3$	5,520	4.38[b]
Control + HyD (0-42 days)	5,520	16.27[a]
Control + HyD (1-21 days) + vitamin D3 only (22-42 days)	5,520	14.77[a]

[1]69 ug/kg feed