REVIEW

CURRENT DEVELOPMENTS IN NUTRITION

Vitamins and Minerals

Implications of Vitamin D Research in Chickens can Advance Human Nutrition and Perspectives for the Future

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ABSTRACT

The risk of vitamin D insufficiency in humans is a global problem that requires improving ways to increase vitamin D intake. Supplements are a primary means for increasing vitamin D intake, but without a clear consensus on what constitutes vitamin D sufficiency, there is toxicity risk with taking supplements. Chickens have been used in many vitamin-D-related research studies, especially studies involving vitamin D supplementation. Our state-of-the-art review evaluates vitamin D metabolism and how the different hydroxylated forms are synthesized. We provide an overview of how vitamin D is absorbed, transported, excreted, and what tissues in the body store vitamin D metabolites. We also discuss a number of studies involving vitamin D supplementation with broilers and laying hens. Vitamin D deficiency and toxicity are also described and how they can be caused. The vitamin D receptor (VDR) is important for vitamin D metabolism; however, there is much more to understand about VDR in chickens. Potential research aims involving vitamin D and chickens should explore VDR mechanisms that could lead to newer insights into VDR. Utilizing chickens in future research to help elucidate vitamin D mechanisms has great potential to advance human nutrition. Finding ways to increase vitamin D intake will be necessary because the coronavirus disease 2019 (COVID-19) pandemic is leading to increased risk of vitamin D deficiency in many populations. Chickens can provide a dual purpose with addressing pandemic-caused vitamin D deficiency: *1*) vitamin D supplementation gives chickens added-value with the possibility of leading to vitamin-D-enriched meat and egg products; and *2*) using chickens in research provides data for translational research. We believe expanding vitamin-D-related research in chickens to include more nutritional aims in vitamin D status has great implications for developing better strategies to improve human health. *Curr Dev Nutr* 2021;5:nzab018.

Keywords: vitamin D, chicken, broiler, laying hen, VDR, vitamin D toxicity, human nutrition, vitamin D supplementation, egg, tibial dyschondroplasia

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Abbreviations used: COVID-19, coronavirus disease 2019; DBP, vitamin D binding protein; PTH, parathyroid hormone; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TD, tibial dyschondroplasia; vitamin D_2, ergocalciferol; vitamin D_3, cholecalciferol; VDR, vitamin D receptor; 1α-OH-D_3, 1-α-hydroxycholecalciferol; 1α-OHase, 1α-hydroxylase; 1,25-$(OH)_2$-D_3, 1,25-dihydroxycholecalciferol; 7-DHC, 7-dehydrocholesterol; 24,25-$(OH)_2$-D_3, 24,25-dihydroxycholecalciferol; 24-OHase, 24-hydroxylase; 25-OH-D_2, 25-hydroxyergocalciferol; 25-OH-D_3, 25-hydroxycholecalciferol; 25-OHase, 25-hydroxylase.

Introduction

Globally, many humans are at risk of vitamin D insufficiency because they lack sufficient sunlight exposure or dietary intake of vitamin D (1, 2). Vitamin D is an essential nutrient due to its metabolic impact of increasing calcium (Ca) absorption in animals (3). Vitamin D is also a fascinating nutrient because its most active form acts as a hormone to exert its biological effect (4). Although vitamin D was traditionally associated with bone formation, the effects of vitamin D on the body are broad as it also affects various physiological systems, such as the immune or reproductive systems (5). Even though vitamin D research is one of the most funded in the USA (6), there are gaps in knowledge of understanding vitamin D in relation to excessive intake in humans that could be explored using animal models.

Domesticated poultry species like chicken (*Gallus gallus*) or turkey (*Meleagris gallopavo*) are becoming increasingly important as a food source for addressing world population demands (7). Chickens are a powerful research model because of their quick generation time, they can be housed in large numbers, and photoperiod affects their feeding behavior and hormonal regulation (8–10). Chickens are widely used for testing feed additive effects because of their quick response time that can be measured by growth performance (11–13). Chickens also have a preference to use cholecalciferol (vitamin D_3) over ergocalciferol (vitamin D_2) which has implications for examining pharmacological effects (14). Chickens provide a dimension of not only being important for research to understand metabolic mechanisms, but they are also a food source via meat and egg production. Laying hens are frequently used as an ovarian cancer model and have importance as a preclinical model for

human ovarian cancer (10, 15, 16). Rodents and swine are mammalian models that have similar digestive tracts to humans, but there are factors in which chickens are superior for vitamin-D-related research, such as their preference for vitamin D_3 (14). Rats exhibit a preference for vitamin D_2 when fed both D_2 and D_3 as they were observed to circulate higher serum concentrations of 25-hydroxyergocalciferol (25-OH-D_2) compared to 25-hydroxycholecalciferol (25-OH-D_3) (17). Swine have a simple hindgut like humans, but it takes ~6 mo to reach sexual maturity, a problem that is important when comparing nutritional impacts at adult stage (18).

The first half of this review focuses on an overview of vitamin D mechanisms with regards to vitamin D metabolism and its metabolites. The second half of this review surveys how vitamin D research in the chicken model can be a means of understanding vitamin D insufficiency in humans and managing it. Vitamin D toxicity is an area of research that has been experimentally observed in chickens. There needs to be a better understanding of how vitamin D supplementation concentrations are administered to avoid potential complications. We also discuss the significance of incorporating the vitamin D receptor (VDR) into future vitamin D research with chickens to focus on vitamin D mechanisms. The last section of this review is dedicated to describing how future research with vitamin D and chickens can help address high-impact areas of human nutrition. As a food source and research model, chickens provide a means to respond to vitamin D deficiency as a result of the coronavirus disease 2019 (COVID-19) pandemic. Our state-of-the-art review aims to highlight the research and added-value that chickens can bring to further understanding vitamin D.

Search strategy and methodology

We searched the literature, for studies published in the English language, using PubMed and Google Scholar. We searched from 1 January, 1960 to 4 February, 2021 to ensure we included some of the foundational studies involving vitamin D. Some of our references go back to 1928 and they were used because they were referenced in some of the published research articles that are referenced in our review. We included searches up to 4 February, 2021 to ensure any newly published clinical trials involving vitamin D in relation to COVID-19 were included in our review. The main search terms used were: vitamin D, chicken, broiler, laying hen, vitamin D metabolism, human vitamin D, plasma vitamin D, egg yolk vitamin, vitamin D and muscle, vitamin D and COVID-19. We did not set any exclusion criteria for studies when searching; however, we focused primarily on searching for studies involving chickens. We included studies that used different animal models (e.g. mouse, rat, pig, etc.) because it was important to include lab animal models as well as production animals with regards to this review's scope. We also included in vitro studies while searching because they could have an experimental approach of giving vitamin D to the animal before collecting their cells for in vitro experiments.

Vitamin D metabolites and mechanisms

There are 2 major classes of vitamin D which are further subdivided into various forms that exert hormonal and physiological effects or inactive forms that are excreted. Cholecalciferol (D_3) is a major class of vitamin D and is synthesized de novo by animals (19, 20) (**Figure 1**). Ergocalciferol (D_2) is another major class of vitamin D that is primarily synthesized by microalgae and fungi (21, 22). Animals can use both

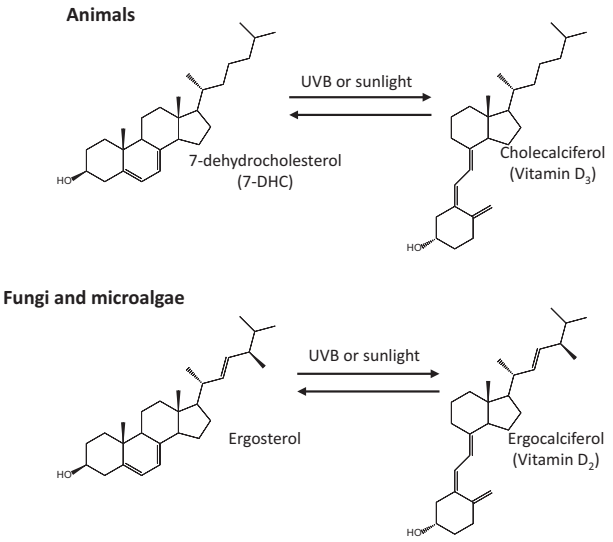

FIGURE 1 Comparison of vitamin D precursors and their nutritionally relevant forms vitamin D_3 and D_2. In animals, when ultraviolet B rays (UVB) or sunlight hits 7-dehydrocholesterol (7-DHC) on the skin, then 7-DHC will undergo multiple reactions and be converted to cholecalciferol (vitamin D_3). In fungi and microalgae, ergosterol undergoes the same pathway as 7-DHC to become ergocalciferol (vitamin D_2).

D_2 and D_3, but D_3 has been reported to have a higher binding affinity to VDR (23). Vitamin D has intact A, B, and D steroid rings due to photolysis of the B ring of 7-dehydrocholesterol (7-DHC or provitamin D_3) when compared with generic steroids (24). The structure of 7-DHC allows the A ring to have the conformational capacity to undergo interconversion between two chair configurations (25). The structure of D_3 comprises a saturated 8-carbon side chain, which is metabolically produced by photolysis of 7-DHC on the skin surface exposed to ultraviolet light irradiation (26). This review will focus on D_3 because of its bioavailability and involvement in humans and chickens.

Vitamin D_3 synthesis in animals is a quick process dependent on exposure of the skin to ultraviolet B rays (UVB, 290–315 nm) (1). Vitamin D_3 synthesis begins when cholesterol is converted to 7-DHC in the skin (**Figure 2**). UVB interacts with 7-DHC by opening the electrolytic ring between carbons 9 and 10 of the B ring as a result of light absorption, converting 7-DHC to previtamin D_3 (25, 27). Previtamin D_3 can photochemically convert to lumisterol, tachysterol, or D_3 via thermal isomerization. D_3 is a major product of thermal isomerization as it requires the least energy for thermal rearrangement. D_3 on skin that is exposed too long to sunlight will be degraded to 5,6-trans-vitamin D_3 which has no calcemic effects like lumisterol or tachysterol (28). Thermal rearrangement gives previtamin D_3 and D_3 a state of equilibrium and reversibility; although this equilibrium favors D_3. De novo synthesis in humans converts 10–15% of available 7-DHC to D_3 (26). The physical "sun-screen" properties of the skin, such as melanin resulting in darker skin, reduces yield; environmental factors such as time of day, season, and latitude also affect yield (20, 29). The biological half-life of D_3 was suggested to be 50 h in human plasma, but radioisotopically labeled D_3 was observed to last 4 d (30, 31). Vitamin D_3 can subsequently convert into different metabolite forms as illustrated in **Figure 3**.

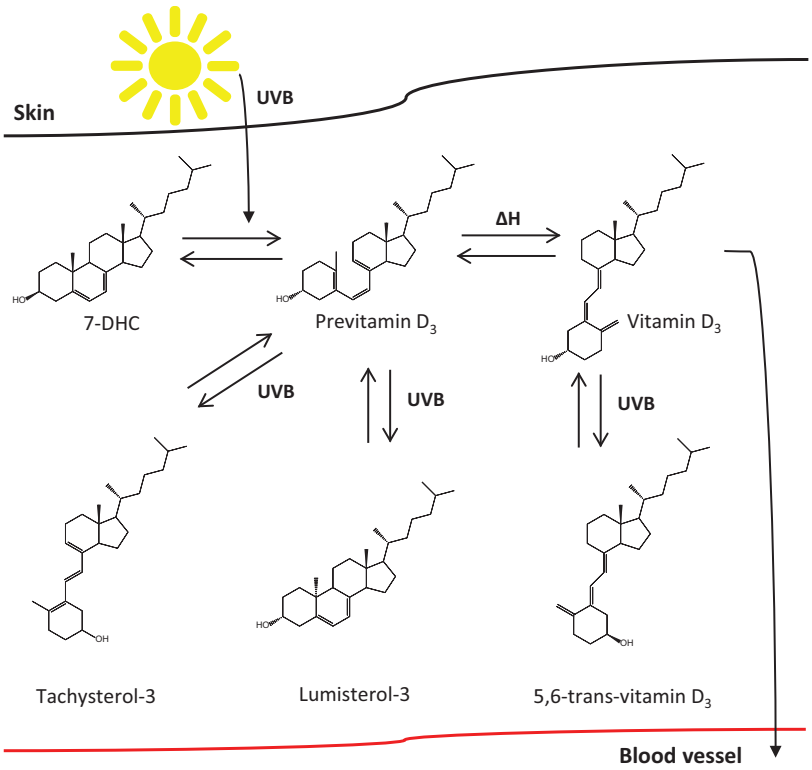

FIGURE 2 Biochemical reactions of 7-dehydrocholesterol (7-DHC) that leads to synthesis of vitamin D_3 and potential noncalcemic metabolites. When ultraviolet B rays (UVB) or sunlight hits 7-dehydrocholesterol (7-DHC) on the skin, 7-DHC is converted to previtamin D_3 which is then converted to vitamin D_3, lumisterol-3, or tachysterol-3 by thermal isomerization. Vitamin D_3 enters the blood circulation to be hydroxylated to its more active forms. Vitamin D_3 in the skin can be converted to 5,6-trans-vitamin D_3 by UVB if it does not go into the circulation. Adapted with permission from (27) and (32).

Feathers cover the skin on birds and also reflect UVB (33), which likely prevents 7-DHC on the skin from being converted to previtamin D_3. The earliest research on biogenic vitamin D in chickens was in the 1920s when Hou (34) described how surgically removing the preen (or uropygial) gland can cause rickets in chicks that were exposed to UVB or sunlight. The preen gland produces preen oil, which is a lipid compound that birds rub onto feathers to waterproof them (35, 36). It was proposed back then that preen oil contained vitamin D precursors and birds rubbing the oil onto feathers is what led to vitamin D synthesis (34, 37). In 1953, Knowles et al. (37) published findings that 10-d-old chicks were able to grow properly even after their preen glands were removed, suggesting that the preen gland was not needed for calcium metabolism. Rosenberg (38) published findings on how the preen glands in ducks, geese, and chickens had no 7-DHC and concluded that preening feathers was not how birds acquired vitamin D_3. A previous study described how laying hens fed a vitamin-D_3-deficient diet and exposed to UVB were able to lay eggs fortified with vitamin D_3 (39). Therefore, chickens would not be limited in vitamin D research with approaches involving UVB.

25-Hydroxylation

Most vitamin D is taken up by the liver and is hydroxylated at side chain C-25 to yield 25-OH-D_3. 25-OH-D_3 is the major circulating form of vitamin D_3, and it is synthesized in the liver of mammals (40–42) and the intestine, liver, and kidneys of birds (43, 44). Hydroxylation of C-25 is facilitated by the enzyme 25-hydroxylase (25-OHase), which consists of ≥6 cytochrome P-450-dependent mixed-function oxygenases of varying binding affinities and capacities (45). The vitamin D binding protein (DBP) binds to circulating 25-OH-D_3 in the blood, which is why 25-OH-D_3 is the major circulating form of vitamin D_3 (46). Circulating concentrations of 25-OH-D_3 along with DBP are valuable for vitamin D status to determine vitamin D deficiency or toxicity (46, 47). There is no consensus on what circulating concentration of 25-OH-D_3 is normal in humans, but it appears to be ~32–100 ng/mL with intoxication beginning above 150 ng/mL (1, 48). The 25-OH-D_3 concentration in an adult chicken is ~25.3 ng/mL, whereas in an adult turkey it is lower at 18.2 ng/mL (49). Although 25-OHase and 25-hydroxylation is predominant in the liver, there have been studies that observed 25-OHase expression in the small intestine (44, 50). The half-life of 25-OH-D_3 in humans is ≥18 d and is much greater than D_3 (50 h) (51, 52). 25-OH-D_3 is used as a vitamin D_3 status indicator in humans, and clinical trials normally administer D_3 or 25-OH-D_3 as intravenous, intramuscular, or oral doses to examine how vitamin D_3 status is affected (53–58).

1-Hydroxylation

25-OH-D_3 undergoes an additional hydroxylation step at the 1-C position by 1α-hydroxylase (1α-OHase) to yield 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$-D_3] in the kidney of mammals

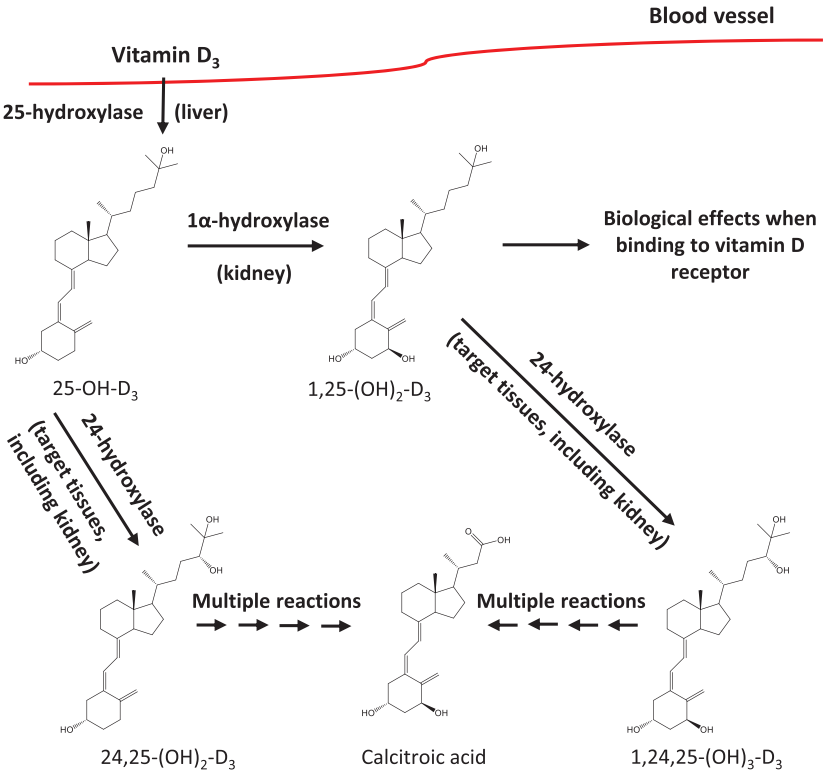

FIGURE 3 Metabolic pathway of vitamin D_3 to its subsequent metabolite forms. Vitamin D_3 in circulation goes to the liver to be converted to 25-hydroxycholecalciferol (25-OH-D_3). 25-OH-D_3 can be further hydroxylated to either 24,25-dihydroxycholecalciferol [24,25-$(OH)_2$-D_3] or 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$-D_3]. When 1,25-$(OH)_2$-D_3 binds to the vitamin D receptor, then biological effects are exerted through gene transcription. 1,25-$(OH)_2$-D_3 can also be further hydroxylated by 24-hydroxylase to 1,24,25-trihydroxycholecalciferol and undergoes a series of reactions to ultimately become calcitroic acid, a water-soluble metabolite that is safely excreted in urine. Adapted with permission from (27) and (32).

and chickens (59, 60). This is also why vitamin D is considered a hormone because 25-OH-D_3 has to be transported via circulation to the kidney for further hydroxylation (4, 61). Although most 1-hydroxylation occurs in the kidney, there are extrarenal tissues such as lymph nodes that express 1α-OHase to convert 25-OH-D_3 to 1,25-$(OH)_2$-D_3, denoting its possible function as a modulator for vitamin D activities in those tissues (62, 63). Given the strict regulation (64) and the fast turnover rate (65–67), 1,25-$(OH)_2$-D_3 is a poor biomarker for vitamin D status (68).

Considering 1,25-$(OH)_2$-D_3 is the vitamin D form that exerts biological effects as a ligand for VDR, 1α-OHase activity is tightly regulated to avoid homeostatic imbalance (69). 1α-OHase is primarily regulated by the parathyroid hormone (PTH), fibroblast growth factor 23, Ca, phosphate, and 1,25-$(OH)_2$-D_3 (70–73). Administering PTH stimulates the renal production of 1,25-$(OH)_2$-D_3 (74, 75), though this PTH effect can be mirrored using cAMP, which indicates that a part of the effect of PTH on 1,25-$(OH)_2$-D_3 production is mediated by adenylate cyclase (70). However, it is not clear how PTH mediates 1α-OHase gene expression (76).

24-Hydroxylation

Hydroxylation of C-24 can occur with 25-OH-D_3 or 1,25-$(OH)_2$-D_3, which results in 24,25-dihydroxycholecalciferol [24,25-$(OH)_2$-D_3] and 1,24,25-trihydroxycholecalciferol, respectively (77, 78). 24,25-$(OH)_2$-D_3 is considered an inactive form of vitamin D because studies have shown that 24,25-$(OH)_2$-D_3 inhibits signaling cascades involved with Ca and phosphorus (P) absorption which also inhibits bone mineralization (79). The 24-hydroxylase (24-OHase) enzyme is responsible for 24-hydroxylation (80). Studies with rats reported that 24-OHase activity occurs in the kidney and intestine (74, 81). It was also noted that the intestinal 24-OHase K_m value for 1,25-$(OH)_2$-D_3 is similar to kidney 24-OHase, leading to speculation that the intestinal role of 24-OHase was to initiate degradation of 1,25-$(OH)_2$-D_3 (74). In chickens, 24-OHase expression was highest in the kidney, then thymus and bursa, then intestine, lower in various muscle tissues, and little to no expression in the liver and was not statistically relevant (59).

An in vitro study reported how rat kidney 24-OHase expression increased in the absence of PTH (60). Infants diagnosed with idiopathic infantile hypercalcemia had severe hypercalcemia and a genetic mutation of 24-OHase (82). Hypercalcemia is caused by unregulated Ca absorption as an effect of increased sensitivity to vitamin D without 24-OHase. Reduced 24,25-$(OH)_2$-D serum concentration can also be indicative of chronic kidney disease due to the negative correlation of 24,25-$(OH)_2$-D with increasing PTH concentrations (83). 24-OHase-null mice without 24-OHase activity are unable to convert 1,25-$(OH)_2$-D_3 to its 24-hydroxylated forms (79). The mice had 49% mortality rate at weaning and impaired bone mineralization (84). Even though 24-OHase is tightly regulated by 1,25-$(OH)_2$-D_3, it is important to

maintain Ca homeostasis by preventing hypercalcemia (85). The half-life of serum 24,25-$(OH)_2$-D_3 may not be as long as 25-OH-D_3 but it can last ≥15 d (86, 87).

1-α-Hydroxycholecalciferol

First synthesized and described in 1973 (88), 1-α-hydroxycholecalciferol (1α-OH-D_3) is a synthetic form of vitamin D_3 that has a similar efficacy to 1,25-$(OH)_2$-D_3, but has an added benefit of being cheaper to synthesize for dietary uses (89). It is assumed that the 1-α-hydroxyl group on 1α-OH-D_3 is structurally required for the hormonal activity of vitamin D (89). 1α-OH-D_3 is readily used in humans with renal cancer due to vitamin D deficiency caused by the lack of 1α-OHase activity to synthesize 1,25-$(OH)_2$-D_3 in the kidneys (90, 91). However, 1α-OHase is a critical regulatory enzyme that prevents the overproduction of 1,25-$(OH)_2$-D_3 because 24-OHase works to regulate serum 1,25-$(OH)_2$-D_3 concentrations (92, 93). Therefore, it is possible that 1α-OH-D_3 can result in hypercalcemia. The mode of action of 1α-OH-D_3 indicates it is able to avoid the critical regulation necessary to maintain homeostatic concentrations of 1,25-$(OH)_2$-D_3 and bypass the negative feedback mechanism caused by 1α-OHase activity (89). It is not clear what the half-life of 1α-OH-D_3 is in serum, but its biological activity from intravenous administration can last ∼12–16 h (89, 94).

Vitamin D absorption, transportation, excretion, and tissue distribution

Vitamin D absorption occurs in the small intestine by passive diffusion via micelle solubilization and cholesterol transporters (95–97). As a fat-soluble vitamin, vitamin D is dependent on fats and bile salts to be absorbed; otherwise, its absorption is greatly decreased, similar to how lipids are metabolized (98). Rats given pharmacological doses of D_3 absorbed a greater amount in the proximal part of the small intestine than the distal part without any signs of saturation (99). However, the dietary vitamin D absorption efficacy differs between animals, whereby young chicks and turkey poults absorb ∼84% intestinal D_3 (100), whereas humans absorb almost 20% (101), and rats absorb 42% (102). The high level of absorption in chicks and poults also highlights why they are good models for vitamin D research. Poultry are highly susceptible to vitamin D deficiency due to malabsorption caused by infectious or noninfectious factors. Vitamin D also lacks an active transport mechanism unlike vitamin K_1 (103) or A (104), which suggests that absorption could lead to toxic concentrations if there is excessive intake (99). Chickens are able to absorb dietary vitamin D_2; however, their capacity to absorb vitamin D_2 is low as chicks had to be given a daily dose of 1000 IU D_2 to have the same body weight as chicks given 100 IU D_3/d (14). The reason for the reduced absorptive efficacy of D_2 in chickens is a result of a faster clearance rate because D_2 is outcompeted in the process of binding to DBP compared to D_3 (105). Once absorbed, dietary vitamin D will go into lymphatic circulation in chylomicra. Polar metabolite forms of vitamin D_3 (25-OH-D_3; 1,25-$(OH)_2$-D_3) are transported by DBP (46).

Translocation of de novo synthesized vitamin D_3 from the skin is facilitated by DBP (106). Almost all circulating vitamin D_3 is bound to protein (107). In rats, DBP contains 458 amino acid residues and has a molecular weight of 54–58 kDa (108). Chickens have 2 DBPs, 54 and 60 kDa, both of which preferentially bind to 25-OH-D_3 over 25-OH-D_2, which also explains why D_2 has a lower bioavailability in chickens (109). In well-nourished humans, DBP binds ∼88% of the 25-OH-D_3 in serum with a much greater affinity than 1,25-$(OH)_2$-D_3, signifying the binding affinity of DBP is dependent on stochiometric aspects of vitamin D which differs based on the hydroxyl grouping (110). DBP is also maintained at a significant concentration in plasma that greatly exceeds the concentration of 25-OH-D_3 (111). Serum DBP is lower in Black Americans compared with white Americans, which suggests that the lower concentrations of serum 25-OH-D_3 in Black Americans is connected to their DBP concentration (112) (**Table 1**). Also, a study exploring 25-OH-D_3 in trans individuals receiving exogenous hormonal treatment reported no differences in serum 25-OH-D and transwomen trending towards an increase in DBP (113) (Table 1). Plasma DBP has a short turnover time (111) (1–3 d) compared to plasma 25-OH-D_3 which is ≥18 d (52). There are alleles of DBP polymorphisms that are inversely related to circulating 25-OH-D_3 which is dependent on vitamin D intake (114).

When the 25-OH-D_3 blood concentration needs to be decreased, the body can convert 25-OH-D_3 to 24,25-$(OH)_2$-D_3 (78). 24-Hydroxylation generates biologically inactive forms of vitamin D that are bound to bile and excreted in feces (115). Ultimately, this pathway results in a water-soluble molecule called calcitroic acid that will be safely excreted (115) (Figure 3). Esvelt and DeLuca (116) observed calcitroic acid had a much lower binding affinity to VDR from chick intestines with a similar binding affinity to 24,25-$(OH)_2$-D_3 compared with 1,25-$(OH)_2$-D_3, which indicated calcitroic acid and 24,25-$(OH)_2$-D_3 were inactive vitamin D_3 forms.

Vitamin D_3 is found and stored in multiple tissues, such as adipose tissue, skeletal muscle tissue, bone, liver, intestinal mucosa, (117, 118), brain (119), and skin (26). Adipose tissue contains the highest concentrations of vitamin D_3 with skeletal muscles being the second highest storage site for vitamin D_3 (117). Vitamin D_3 is found in the mammalian liver, but the liver contains high concentrations of 25-OH-D_3 as the liver is a transient organ for hydroxylating vitamin D_3 to 25-OH-D_3 (120, 121). Plasma vitamin D is mostly 25-OH-D_3 and then 24,25-$(OH)_2$-D_3 (49). Variable concentrations of distributed vitamin D_3 and its multiple metabolite forms denote differences in tissue lipid content and DBP associated with tissues.

Addressing vitamin D insufficiency in humans by understanding vitamin D metabolism in chickens

It has recently been speculated that vitamin D deficiency in humans will be a global issue for addressing health due to implications related to osteomalacia (122–124). Clinical studies are important for applying research findings to advance human health. Although there are many more clinical studies in the literature as reviewed by Plum and DeLuca (125), we highlight 2 clinical study examples that examined vitamin D supplementation in humans. One study noted how vitamin D_3 supplementation given during pregnancy could reduce adverse outcomes related to excessive inflammation (126) (Table 1). Another study observed how vitamin D_3 supplementation improved vitamin D status in overweight and obese African Americans (127) (Table 1). One drawback with clinical trials is controlling the variation of vitamin D intake, whether it is caused by sunlight exposure between participants or their diet, outside of the experimental study, can affect vitamin D status (128). A secondary drawback is when participants fail to adhere to their daily supplementation as part of the study design (129).

TABLE 1 Summary of selected studies involving humans, vitamin D supplementation, and their outcomes

Sex	Objective	Treatment regimen	Research findings	Refs
Vitamin D supplementation and disease				
♂ + ♀	Determined the efficacy of 1α-OH-D_3 as a replacement for 1,25-$(OH)_2$-D_3 in 3 patients with chronic renal failure	• 3 patients were given 1α-OH-D_3 and 1,25-$(OH)_2$-D_3 intravenously • Intestinal absorption of radioactive calcium was measured	1α-OH-D_3 was effective in improving calcium absorption in the patients; 1α-OH-D_3 required a higher dose to have a similar effect to 1,25-$(OH)_2$-D_3	(90)
♂ + ♀	Compared D_3 and 25-OH-D_3 absorption in the intestine in 9 patients with chronic liver disease	• Patients were given a dose of 250 mL radiolabeled D_3 or 25-OH-D_3 of liquid formula diet • Serum samples were collected at 0, 4, 8, 12, and 24 h after the dose	Control subjects had peak absorption of ∼21% for D_3 at 12 h and over 40% for 25-OH-D_3 at 8 h; subjects with severe cholestatic liver disease had peak absorption of ∼1% for D_3 at 4 h and ∼12% for 25-OH-D_3 at 4 h	(101)
♂ + ♀	Assessed if vitamin D supplementation given to overweight or obese African Americans improved their vitamin D status and insulin sensitivity	• Participants were given 4000 IU D_3/d (100 μg/d) or placebo and supplemental calcium of 600 mg/d for 12 wk • Participants had their measurements collected on days 0, 42, and 84 and underwent a 2-h glucose tolerance test	Vitamin D supplementation did not show evidence of reducing diabetes risk; vitamin D supplementation increased serum 25-OH-D_3 over time	(127)
♂ + ♀	Evaluated vitamin D and calcium supplementation programs involving elderly men and women in northern European regions	• Community-dwelling residents of age 66 y old or older were given 2 daily supplements • Calcium supplement was 1000 mg elemental calcium as calcium carbonate • Vitamin D_3 supplement was 400 μg	Active participants in the programs had a reduction in bone fracture incidence; vitamin D and calcium supplementation could reduce osteoporotic fractures in elderly people in vitamin-D-deficient regions	(130)
Vitamin D supplementation effects on biomarkers				
♂	Explored if circulating DBP and DBP genotypes were different between Black Americans and white Americans	• Patients between the ages of 30 and 64 y old were recruited • Dietary calcium and vitamin D, serum 25-OH-D_2 and 25-OH-D_3, and patients' genotypes for DBP single-nucleotide polymorphisms were measured	Black Americans had lower concentrations of total 25-OH-D_2 and 25-OH-D_3 and DBP compared to white Americans; there is some genetic variation in DBP between Black Americans and white Americans	(112)
♀	Assessed vitamin D supplementation effects during pregnancy on vitamin D status and immune markers associated with adverse pregnancy outcomes	• Pregnant women <20 wk to delivery were given 2 doses of D_3 as supplement capsules • Treatment doses: 10 μg/d or 50 μg/d D_3 (400 or 2000 IU D_3/d)	Higher vitamin D_3 supplementation caused an increase in serum 25-OH-D_3 concentration; increased the peripheral blood IL-10 regulatory $CD4^+$ T cell percentage; vitamin D supplement impacts on increasing regulatory T cell concentrations could reduce inflammation associated with pregnancy	(126)
Vitamin D measurements				
♂ + ♀	Examined exogenous HT effects on 25-OH-D and DBP in trans individuals	• Transwomen and transmen received exogenous HT for 3 mo • Baseline and 3 mo serum samples were collected and total 25-OH-D, free 25-OH-D, albumin, and DBP were measured	HT did not affect total 25-OH-D concentration in transwomen and transmen; transwomen trended towards an increase in DBP; trans individuals receiving HT does not affect vitamin D status assessments	(113)[1]
♂	Quantified the relation with D_3 input and serum 25-OH-D_3 concentration during winter months in men	• Men from Omaha, NE, were randomly assigned a treatment group and given a daily supplement in tablet form • Treatments were: placebo, supplemental D_3 (25 μg), and 1 or 2 tablets containing 125 μg D_3	Serum 25-OH-D_3 was increased by D_3 dose amount; data suggests that average daily vitamin D intake should be ∼5000 IU/d or 125 μg/d to maintain serum 25-OH-D_3 during winter months	(54)
Vitamin D receptor				
♂	Determined VDR and 1α-OHase were expressed in human brain tissue	• Human brain tissue from 5 men, free of any psychopathology or substance abuse • Tissues were sectioned and stained with primary antibody for VDR and secondary antibodies for VDR or 1α-OHase	VDR is widespread throughout brain tissue except in layers in the cerebellum; 1α-OHase is expressed in the same regions as VDR, including the cerebellar layers that VDR was not expressed in	(131)

[1]Trans individuals in this study are on exogenous hormonal treatment.
♂, male; ♀, female; 1α-OH-D_3, 1-α-hydroxycholecalciferol; 1α-OHase, 1α-hydroxylase; 1,25-$(OH)_2$-D_3, 1,25-dihydroxycholecalciferol; 25-OH-D_3, 25-hydroxycholecalciferol; DBP, vitamin D binding protein; HT, hormonal treatment; VDR, vitamin D receptor.

Clinical trials are powerful for ascertaining treatment effects in humans and complement animal studies that can validate effects in controlled environments.

Value of dietary vitamin D_3 with broiler chicken production

Broiler chickens (grown for meat) have been thoroughly used for vitamin-D-related studies to elucidate specific impacts of vitamin D intake on physiology and metabolism. Studies with broilers can also be designed to control for sunlight exposure or monitoring feed intake (**Table 2**). Researchers can examine nutrient effects on metabolic relations in broilers by measuring growth performance parameters like breast muscle yield. Despite having low utilization of D_2 (14, 132), there are significant implications for researching vitamin D effects using chickens. The poultry industry is interested in reducing tibial dyschondroplasia (TD) in fast-growing broilers. TD occurs in fast-growing avian species and is a lesion in which the growth plate of the tibia head is avascular and is not mineralized causing bowing of the tibiotarsus and lameness of the bird (133, 134). Increasing dietary vitamin D_3 with increased dietary Ca has been observed to reduce the incidence and severity of TD in young broilers (135–137) (Table 2). There are studies that have examined vitamin D_3 supplementation with other nutrients: strontium supplementation reduced body weight gain (138) and adding D_3 to diets with increased P and microbial phytase improved P and Ca utilization (139) (Table 2).

TABLE 2 Summary of selected studies involving broiler chickens, vitamin D, and their outcomes

Chicken strain and sex	Objective	Treatment regimen	Research findings	Refs
Tibial dyschondroplasia (TD)				
Peterson × Arbor Acre ♂	Compared and contrasted different D_3 metabolite supplementation with preventing TD	• 7 treatments involving different vitamin D isoforms • 0.1–10.0 μg vitamin D isoform/kg of diet	None of the diets were effective at 0.1 or 1.0 μg/kg at reducing TD severity or incidence; diets effective at higher concentrations, except diets with D_3 and 24,25-$(OH)_2$-D_3 supplementation	(135)
Ross 308 ♂	Examined how different concentrations of dietary vitamin D_3 with different concentrations of calcium, available phosphorus, and vitamin A, would affect TD incidence	• Dietary D_3 concentrations: 5, 20, 125, and 250 μg D_3/kg of diet	Diets with higher concentrations of vitamin D_3 and optimal dietary calcium and phosphorus concentrations can significantly reduce TD incidence	(136)
Cobb 500 ♂	Compared and contrasted different D_3 metabolite supplementation with preventing TD	• Dietary vitamin D_3 or 25-OH-D_3 concentrations ranged from 3.13, 6.25, 12.5, 25.0, 50.0, and 100.0 μg vitamin D isoform/kg of diet	25-D_3 reduced incidence and severity of TD; increasing concentrations of vitamin D also reduced incidence	(137)
Ross × Ross ♀	Examined maternal vitamin D_3 effects on performance, leg pathology, and bone quality of broiler chicks	• Broiler breeder hens fed diets containing 6.25 or 50.0 μg D_3/kg of diet	Hens fed the 50.0 μg D_3 diet, resulted in chicks that had lower TD incidence scores; chicks from later hatches, regardless of maternal diet, had reduced TD scores	(140)
Genetic lines from Auburn University ♂ + ♀	Determined the response of 25-OH-D_3 in broiler chicks between 2 lines selected for high and low incidence of TD	• Diets comprised basal D_3 (69 μg/kg) plus 25-OH-D_3 (0, 69, and 345 μg/kg)	Supplemented 25-OH-D_3 had no effect on high TD line, but decreased TD incidence in low TD line	(141)[1]
Peterson × Arbor Acre ♂	Determined how disulfuram affected vitamin D_3, 25-OH-D_3, and 1,25-$(OH)_2$-D_3 on TD development in broiler chicks	• Basal dietary D_3 concentration: 27.5 μg/kg • 25-OH-D_3 and 1,25-$(OH)_2$-D_3 supplementation concentrations: 10 μg/kg	Chicks fed diets containing D_3, 25-OH-D_3, or 1,25-$(OH)_2$-D_3 supplementation, and disulfiram, had reduced body weight and increased TD incidence	(142)
The influence of dietary vitamin D on phosphorus utilization				
Peterson × Arbor Acre ♂	Examined how supplemental phytase, vitamin D_3, and dietary Ca:tP would affect utilization of phytate phosphorus and calcium	• Dietary vitamin D_3 concentrations: 66 and 660 μg/kg; 6600 μg/kg was also used without phytase	Diets containing 600 μg/kg of D_3, phytase and between 1.1:1 and 1.4:1 Ca:tP ratio were most optimal for growth performance and calcium and phosphorus utilization	(139)
Commercial, not stated, ♂ + ♀	Determined effects of multiple Ca:P ratios in addition to different concentrations of vitamin D_3 on phosphorus utilization in broiler chicks	• Dietary vitamin D_3 concentrations: 2.5, 5.0, and 10.0 μg/kg for Exp. 1 • Dietary vitamin D_3 concentrations: 5.0, 10.0, and 20.0 μg/kg for Exp. 2	Increased dietary vitamin D_3 concentrations led to increased body weight and bone ash; however, the Ca:P ratio was more correlated to body weight and bone ash	(143)
No UV light/exposure				
Commercial, not stated, ♂	Determined vitamin D_3 requirement of broiler chicks housed in UV-less environment	• Exp. 2 D_3 concentration: 0, 1.25, 2.50, and 5.0 μg/kg • Exp. 3 D_3 concentration: 0, 5.0, 10.0, and 20.0 μg/kg	Without UV light, there was 77% incidence of rickets in chicks fed diets containing 5.0 μg D_3 compared to 20% when chicks received UV light	(144)[2]
Peterson x Arbor Acre ♂	Determined if dietary 1,25-$(OH)_2$-D_3 can reduce vitamin D deficiency effects in broiler chicks if there is no UV light	• Dietary D_3 concentrations ranged from 0, 5.0, 27.5, and 50.0 μg/kg • Dietary 1,25-$(OH)_2$-D_3 concentrations were 0 and 10.0 μg/kg	Dietary D_3 supplementation concentration of 27.5 μg/kg led to increased tibia bone ash in chicks without UV light; adding 10 μg 1,25-$(OH)_2$-D_3/kg of diet, was more effective than D_3 in reducing TD incidence and severity	(145)
Dietary vitamin D interaction with other nutrients				
Ross 308 ♂	Examined strontium and vitamin D_3 interactive effects in broilers	• Dietary vitamin D_3 concentrations: 125 and 250 μg/kg • Dietary strontium concentrations: 0, 400, 800, and 1200 μg/kg	Higher concentrations of strontium caused a decrease in body weight gain and feed conversion ratio; however, the negative effect is reduced by higher concentrations of vitamin D_3	(138)
Ross (strain # not stated) ♂	Examined dietary effects of different concentrations of D_3, 1α-OH-D_3, 25-OH-D_3, 1,25-$(OH)_2$-D_3, and vitamin C on TD incidence and severity in broiler chicks	• 75 μg D_3/kg of diet was control diet • 25-OH-D_3 diets: 75 or 250 μg/kg • 5 μg 1α-OH-D_3 + control	Chicks fed 25-OH-D_3 had much lower TD incidence (10%) compared to chicks fed D_3 (65%); adding ascorbic acid had no effect on TD incidence	(146)
Ross × Ross ♂	Determined interactive dietary effects of vitamin A with D_3, 25-OH-D_3, and 1,25-$(OH)_2$-D_3 and if high concentrations of vitamin A can prevent vitamin D toxicity in broiler chicks	• Vitamin A supplementation concentrations: 450 and 13,500 μg/kg of diet • D_3 and 25-OH-D_3 supplementation concentrations: 0, 5, 10, 20, 40, and 80 μg/kg of diet	Chicks fed diets containing 13,500 μg vitamin A, had a linear decrease in rickets incidence when dietary D_3 concentration increased; 25-OH-D_3 and 1,25-$(OH)_2$-D_3 were more effective in reducing rickets	(147)

(*Continued*)

TABLE 2 (*Continued*)

Chicken strain and sex	Objective	Treatment regimen	Research findings	Refs
1α-OH-D$_3$ efficacy				
Ross 308 ♂	Determined relative biological value of 1α-OH-D$_3$ to 25-OH-D$_3$ in broiler diet	• Dietary 25-OH-D$_3$ concentrations: 0, 1.25, 2.5, 5.0, and 10.0 μg/kg • Dietary 1α-OH-D$_3$ concentrations: 0.625, 1.25, 2.5, and 5.0 μg/kg	Relative biological value for 1α-OH-D$_3$ to 25-OH-D$_3$ is 202 to 267% for growth performance and bone mineralization	(148)
Ross × Ross ♂ + ♀	Evaluated 1α-OH-D$_3$ efficacy in relation to vitamin D$_3$ in broiler chicks' growth performance	• Dietary D$_3$ concentrations: 2.5, 5.0, 10.0, 20.0, and 40.0 μg/kg • Dietary 1α-OH-D$_3$ concentrations: 0.625, 1.25, 2.5, 5, and 10.0 μg/kg	1α-OH-D$_3$ can achieve vitamin D adequacy in broilers without UV light and is ∼8× as effective as D$_3$ on weight basis	(149)[3]

[1]Strain not described, but the lines were developed and described in (141).
[2]Experiment 1 of (144) utilized Arm-a-Lite® to determine if fluorescent light was blocked so vitamin D intake was solely from the diet.
[3]This study used 1α-OH-D$_3$ from 2 different suppliers that had different purity grades; however, they were diluted to equal dilutions when prepared for experimental diets.
♂, male; ♀, female; 1α-OH-D$_3$, 1-α-hydroxycholecalciferol; 1,25-(OH)$_2$-D$_3$, 1,25-dihydroxycholecalciferol; 24,25-(OH)$_2$-D$_3$, 24,25-dihydroxycholecalciferol; 25-OH-D$_3$, 25-hydroxycholecalciferol; Ca: P, calcium: phosphorus ratio; Ca: tP, calcium: total phosphorus ratio; UV, ultraviolet.

Exploring the dietary intake of vitamin D with other nutrients can cause variation, but it will also help address vitamin D insufficiency with regards to how food sources can affect vitamin D status. Many studies examining dietary D$_3$ effects in broilers have repeatedly shown improved bone health (135, 137, 140, 143, 144) (Table 2). Chicken feed can be supplemented with 25-OH-D$_3$ and has been very effective in reducing TD compared with broilers only fed D$_3$ (141, 146). Studies involving broilers being fed 1,25-(OH)$_2$-D$_3$ reported an increase in bone ash and plasma Ca, and reduced the incidence of rickets (142, 145, 147). 1α-OH-D$_3$ has been observed to have better bioefficacy than 25-OH-D$_3$ when used as an additive in broiler feed (148) and it is ∼8× more effective than D$_3$ (149) (Table 2). Broiler chicks fed diets with higher concentrations of Ca and supplemented with 1α-OH-D$_3$ had a lower plasma 25-OH-D$_3$ concentration (150). It has also been suggested that due to the high bioavailability of 1α-OH-D$_3$ in a synthetic mixed micelle with human intestinal cells (Caco-2), 1α-OH-D$_3$ can be used to treat severe vitamin D deficiency (151).

Effects of dietary vitamin D$_3$ with laying hens in production

Laying hens provide eggs for consumption and their production performance is an accessible trait to quantify dietary or supplementary effects (**Table 3**). There was improved bone structure in laying hens that were fed vitamin D$_3$, 25-OH-D$_3$ or 1,25-(OH)$_2$-D$_3$ (152, 153). However, Ca intake is more important for egg production and quality (154, 155). While most studies involving vitamin D$_3$ with laying hens focused on egg quality (Table 3), 1 study explored how vitamin D$_3$ affected the hen ovary during follicle development (156). Enriching laying hen diets with specific vitamins has a direct impact on vitamin content in the egg yolk.

Eggs from laying hens have a powerful value for addressing nutrient deficiencies, especially for lipid-related nutrients (157). The fatty acid profiles of egg yolk are directly related to carotenoids and the diet quality of a hen (158). When laying hens were fed layer-breeder diets with supplemented vitamin A, the vitamin A concentration in the egg yolk was greatly increased (159). Increasing concentrations of vitamin E in a laying hen diet also increases vitamin E content in the egg yolk (160) (Table 3). It should be noted that competitive antagonism for intestinal absorption can occur between fat-soluble vitamins when dietary supplements are provided to hens (161, 162). However, there are possibilities of creating specific value-added eggs to address specific vitamin needs by feeding hens with supplemental concentrations of a particular vitamin in their diet (157).

The egg yolk vitamin D$_3$ content can reach a concentration that will meet daily vitamin D demands if a hen is fed a diet with high concentrations of vitamin D$_3$ (163) (Table 3). Mattila et al. (163) reported how laying hens fed a diet with the highest vitamin D$_3$ content (216 μg D$_3$/kg of feed) resulted in an egg yolk containing 23 μg D$_3$/100 g and 1.5 μg 25-OH-D$_3$/100 g. Eating 1 egg from those laying hens could easily achieve the vitamin D RDA for adult men and women (15 μg/d or 600 IU/d) in the USA (164, 165). Although there has been some research examining value-added eggs, when hens were fed diets with high concentrations of dietary vitamin D$_3$ (163, 166–168) (Table 3), there are implications for exploring the feasibility of value-added eggs that can be a food supply to address areas of nutrient demands in locations that are geographically poor in sunlight and have little access to other dairy products or fortified foods. There are positive implications for exploring how vitamin-D-enriched eggs can improve nutritional status of at-risk populations such as the elderly.

Vitamin D deficiency and toxicity in humans and chickens

Even though vitamin D is stored in tissues, vitamin D deficiency can occur under specific conditions. An animal consuming a vitamin-D-poor diet and not exposed to sunlight will eventually express signs of deficiency such as rickets for young, growing animals and an increased risk of bone fractures or muscle weakness (169–171). Elderly men and women are most susceptible to vitamin D deficiency (172, 130) (Table 1); however, children and young adults can also be at risk of vitamin D deficiency if they have a vitamin-D-poor diet or are in geographical locations with poor sunlight exposure (173–175). Vitamin D deficiency can also occur if there is a genetic defect within the VDR (176). Lack of signaling from 1,25-(OH)$_2$-D binding to VDR also affects various genes responsible for cell proliferation and may influence cancer incidence (177–179).

Vitamin D toxicity is rare but can occur due to an excessive intake of vitamin D from supplements (1). Holick (1) noted how humans taking daily vitamin D doses >1250 μg/d (50,000 IU/d) increase their serum 25-OH-D$_3$ concentration to 150 ng/mL which is associated with

TABLE 3 Summary of selected studies involving layer pullets/hens, vitamin supplementation on egg properties, and their outcomes

Chicken strain	Objective	Treatment regimen	Research findings	Refs
Dietary vitamin D supplementation impacts on vitamin D content in egg yolk				
Lohmann White	Examined how vitamin D_3 is effectively transferred from feed to egg yolk and how supplemental D_3 affects 25-OH-D_3 in the egg yolk	• Dietary vitamin D_3 concentrations: 26.6, 62.4, and 216.0 μg/kg	When D_3 content in feed was increased from 62.4 to 216 μg/kg, there was a 7-fold increase in egg yolk D_3 and 1.5-fold increase in 25-OH-D_3	(163)
Lohmann White	Examined how quickly and effectively, high concentrations of vitamin D_3 supplementation can transfer from feed to the egg yolk	• Basal dietary vitamin D_3 concentrations: 43 and 107 μg/kg • Experimental dietary vitamin D_3 concentrations: 280 and 300 μg/kg	Vitamin D content in egg yolk achieved a peak (30 μg D_3/100 g yolk weight) around 8–13 d from the start of the experimental high D_3 diets; feeding 1708.7 μg D_3/kg diet did not affect eggshell strength or harm the hens	(166)
Lohmann LSL White	Assessed dietary effects of vitamin D_2 and D_3 supplementation during entire egg-laying period	• Dietary D_3 concentrations: 62.5, 150.0, 375.0 μg/kg • Diets with D_2 contained 62.5 μg D_3 + 150.0 or 375.0 μg D_2	Dietary D_3 was more effective with increasing vitamin D content in the egg yolk compared to D_2; no negative effect of dietary supplementation of D_2 or D_3 on body weight or tibia strength	(167)
Hy-Line W-36	Determined the dietary effects of various concentrations of vitamin D_3 supplementation on laying hen production performance, bone health, and egg quality	• Dietary D_3 concentrations: 42.0, 208.7, 458.7, 875.35, and 1708.7 μg/kg	No dietary effect of D_3 supplementation on keel bone damage; increasing dietary vitamin D_3 supplementation concentrations led to increased vitamin D_3 content in the egg yolk	(168)
Dietary vitamin D supplementation effects on egg production				
Hy-Line W-36	Determined if dietary supplementation of vitamin D_3, 1,25-$(OH)_2$-D_3, and 1α-OH-D_3 improved eggshell quality, egg production, and tibia strength	• Dietary 1α-OH-D_3 and 1,25-$(OH)_2$-D_3 concentrations for Exp. 1: 0, 0.75, 1.5, 3.0, 4.5 μg/kg • Dietary vitamin D_3 concentrations: 0, 12.5, 25.0, 37.5 μg/kg	1α-OH-D_3 and 1,25-$(OH)_2$-D_3 did not affect eggshell quality or egg production; increased dietary D_3 concentrations led to quadratically increased serum calcium at oviposition	(152)
Lohmann White	Examined increased dosage of dietary vitamin D_2, D_3, and 25-OH-D_3 on bone mineralization and egg production	• Basal diet contains 75.0 μg D_3/kg of diet • Supplemented vitamin D_2, D_3, and 25-OH-D_3 concentrations added to basal diet: 75.0 and 225.0 μg/kg	No soft tissue calcification was observed in hens fed diets with supplemental vitamin D concentrations; supplement doses did not affect egg quality, egg production, or bone health	(153)
Dietary vitamin supplementation effects on egg yolk content				
Hy-Line W-36	Examine dietary effects of different sources of fatty acids and 2 concentrations of vitamin E on lipid profile in eggs	• Vitamin E concentrations: 10.9 and 90.9 mg/kg • Dietary D_3 concentration was 62.5 μg/kg	Fatty acid source influenced yolk lipid profile; interaction between fatty acid source and 90.9 mg of vitamin E/kg of diet, led to increased vitamin E content in egg	(158)[1]
Rhode Island Red	Evaluate dietary vitamin A supplementation on maternal hen on accumulation of vitamins A, E, C, and carotenoids in embryonic liver	• Dietary vitamin A supplementation concentrations: 0, 3, 30, and 120 μg retinol equivalent/g of diet	Egg yolk vitamin A content was increased as dietary vitamin A supplementation increased; however, vitamin E and carotenoid concentrations were reduced as a trade-off	(159)[2]
Hy-Line Brown	Determine if dietary vitamin E supplementation in laying hen diets can fortify eggs in dose-dependent response	• Diets contained 40 mg of vitamin E and 0, 50,100, and 200 mg were added supplement concentrations • Dietary D_3 concentration was 75.0 μg/kg	Vitamin E in the egg yolk increased linearly as vitamin E in diet increased	(160)[3]
Dietary calcium effects on egg production				
Hy-Line W-36 and Dekalb XL	Determine if increased dietary macronutrient concentrations, lacking calcium, leads to increased liver fat and affect egg size and production	• Dietary calcium concentrations ranged from 1.0 to 4.1%; there were multiple experiments with different concentrations within that range • Dietary D_3 concentration was 55.0 μg/kg	Lower calcium concentrations led to increased feed intake, body weight, liver weight, and fat pad; although, there was no effect on egg size or production	(154)
Hy-Line W-36	Does calcium and $NaHCO_3$ supplementation in diets with continuous feed access with 24-h light, lead to improved eggshell quality?	• $NaHCO_3$ supplementation: 0 or 0.5%; dietary calcium source: ground limestone or 1/3 ground limestone with 2/3 oyster shell • Dietary D_3 concentration was 41.3 μg/kg	Feeding supplementary $NaHCO_3$ increased egg elasticity and 24-h photoperiod improved eggshell quality	(155)

[1]Vitamin E form was not stated; therefore, we assumed the authors used DL-α-tocopherol.
[2]Article stated the hens were fed a proprietary breeders' wheat-barley-based diet so vitamin D content is not stated; vitamin A form used in the article was retinol-acetate.
[3]Vitamin E form used for dietary supplementation in the study was DL-α-tocopherol.
1α-OH-D_3, 1-α-hydroxycholecalciferol; 1,25-$(OH)_2$-D_3, 1,25-dihydroxycholecalciferol; 25-OH-D_3, 25-hydroxycholecalciferol; $NaHCO_3$, sodium bicarbonate.

hypercalcemia and hyperphosphatemia. Laying hens fed 375 μg D_3/kg of feed (15,000 IU D_3/kg of feed) for 48 wk were observed to not have any histopathological issues with their liver, kidney, heart, and brain (167). Plasma vitamin D_3 was not measured in those laying hens, but the vitamin D_3 content in the egg yolk of those hens was increased relative to dietary vitamin D_3. Broiler chicks are reported to have a high tolerance to excess dietary D_3 because chicks fed 1250 μg D_3/kg of feed (50,000 IU D_3/kg of feed) had similar body weight gain, feed intake, and tibia ash as chicks fed 2.5 μg D_3/kg of feed (180). Based on what Baker et al. (180) observed compared with what Morrissey et al. (181) observed with chicks, feeding high concentrations of D_3 is nowhere near as toxic as 25-OH-D_3. There was a clinical trial during winter in which men were given daily oral doses of 5000 IU D_3 for ∼20 wk which led to an increase in serum 25-OH-D_3 concentration to maintain vitamin D status (54) (Table 1). When taken into consideration with what was observed with rats and chickens, excessive vitamin D_3 intake may not be as toxic as suspected, especially with regulatory feedback mechanisms.

Increasing the intake of 25-OH-D_3 may be more effective in eliciting vitamin D pathways for increasing Ca absorption, but there are risks associated with toxicity. Chicks fed diets containing 100 mg of 25-OH-D_3/kg of feed led to emaciation and deaths as a result of vitamin D intoxication (181). In young chicks, toxicity caused mineralization and lesions in kidneys, and fragile bones (181). In rats, excessive concentrations of dietary 25-OH-D_3 led to a linear decrease in body weight and rats given 4600 nmol/d of 25-OH-D_3 had grayish-white kidney coloration as a sign of calcification (182). Laying hens fed 825 μg 25-OH-D_3/kg of feed (10×) exhibited toxic effects such as a decrease in body weight, reduction in feed efficiency, decreased eggshell thickness, egg production, and egg quality parameters such as egg and albumen weights (183). Broiler chicks fed diets with 25-OH-D_3 containing 10x concentration (690 μg/kg) higher than the basal diet (69 μg/kg) exhibited renal calcification and their serum 25-OH-D_3 concentration was 242 ng/mL (184), but renal calcification may be dependent on dietary Ca (181). It is important to note that in Yarger et al.'s (184) study, chicks fed the diet with the lowest 25-OH-D_3 level (69 μg/kg) had a 25-OH-D_3 serum concentration of 37 ng/mL, highlighting how much 25-OH-D_3 can accumulate in serum. In a clinical study with adult men and women given single oral doses of 25-OH-D_3 (5 or 10 μg/kg), serum 25-OH-D_3 increased over a course of 4 h and then dropped to near baseline after a week (185). 25-OH-D_2 may be a safer form of vitamin D supplementation because it is not likely to be as potent and toxic compared to 25-OH-D_3 (186). Dietary supplemental 25-OH-D_3 dosages should be taken with extra care because of its potency and potential cause of pathologies.

VDR research in chickens has great potential to elucidate vitamin D metabolism

VDR is a transcription factor which 1,25-$(OH)_2$-D_3 binds to and exerts the activity of vitamin D through gene expression (187). When 1,25-$(OH)_2$-D_3 binds to VDR, a conformational change transforms VDR to interact with other factors in gene transcription (188). VDR is classified as a nuclear transcription factor which requires a specific ligand [1,25-$(OH)_2$-D_3] and heterodimerizes with the retinoid X receptor (RXR) (189). Found in many animals with some form of calcified skeleton (190) and lamprey (191), the evolutionary origins of VDR are still unclear. One of the primary functions of VDR is regulating Ca and phosphate homeostasis for bone mineralization as was characterized in VDR-null mice (192, 193). Genetic defects in VDR cause vitamin-D-dependent rickets type II (176) because Ca absorption for bone mineralization was heavily reduced as VDR is necessary for expressing proteins that facilitate Ca absorption. Bouillon et al. (190) reviewed how the VDR-null mouse model was significant in illustrating the role of VDR as a transcription factor involved with multiple physiological systems. In mammals, VDR mutations can lead to baldness because of a corepressor that binds to VDR (194, 195). VDR has also been found in the human brain along with 1α-hydroxylase, suggesting that vitamin D has autocrine/paracrine potential in the brain (131) (Table 1). Aging is also connected to reducing VDR expression in human muscle tissues (196).

An important element of VDR expression in most tissues in the body is its connection to immune function (197). Immune cells like macrophages express VDR (198) and when 1,25-$(OH)_2$-D_3 binds to VDR, then it causes signal transduction with immune activity (199). VDR is connected to anti-inflammatory elements and modulating anti-inflammatory cytokine activity while inhibiting proinflammatory cytokine production (200, 201). Research in mice has shown that lacking or having defective VDR led to increased inflammation (201, 202). Reduced or no signaling from VDR also highlights why vitamin D deficiency is correlated to increased inflammation (203). The connection of VDR to immune function will be a significant hotbed area of research in the future because of the global need to address vitamin D insufficiency.

There is a lack of research into the VDR in chickens that will be necessary for illustrating vitamin D mechanisms. However, the chicken genome has been sequenced (204, 205), so VDR has been annotated. There is some research that explored VDR as a biological candidate gene for improving production (growth rate for broilers, egg production and egg quality for laying hens) (206–208). VDR has also been shown to be a mediator for muscle tissue in broilers and in vitro (209). To our knowledge, there are currently no studies that have explored VDR and bone mineralization in the chicken model. Therefore, further characterizing VDR in chickens has significance for researchers and the poultry industry. Future research examining vitamin D using the chicken model should go a step further to examine how VDR expression is influenced, as such conditions can provide greater insights into vitamin D metabolism.

Hypothesis testing for vitamin D studies in chickens can increase their impact by considering VDR expression levels. VDR has been identified to be present in almost all tissues in mammalian models, but does the same principle apply to the avian model which has a distinctly different kidney structure (210) that is also very efficient? Birds evolved to mobilize Ca quickly from their bones for eggshell formation and this Ca may also be resorbed from the kidneys, which is also a site for VDR (211, 212). If chickens were fed exorbitant concentrations of 25-OH-D_3 and were at risk of renal calcification, then VDR expression with 1α-OHase should be greatly increased to explain that effect. However, the alternative hypothesis would be explained by a decrease in 24-OHase which can also explain why renal calcification would be possible because the chicken had a higher Ca absorption without feedback inhibition. By understanding how VDR is affected by experimental treatments in chickens, future researchers will be able to draw conclusions that can connect the biological effects of vitamin D. Such knowledge can be translated

towards human nutrition to identify ways of addressing vitamin D insufficiency while reducing the risk of vitamin D toxicity.

Future research with vitamin D in chickens can help elucidate vitamin D deficiency-related issues to help address high-impact areas of human nutrition

The COVID-19 pandemic has led to a significant reduction in sunlight exposure for many people, as a result of lockdown and stay-home orders, inevitably increasing the risk of vitamin D deficiency (213, 214). Vitamin D deficiency is linked to a higher risk of COVID-19 infection because 1,25-$(OH)_2$-D_3 needs to bind to VDR for signal transduction pathways related to innate immunity (215). Vitamin D deficiency leads to increased inflammatory mediation from immune cells like macrophages, which exacerbates respiratory problems from severe acute respiratory coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic (215). Higher-risk populations to COVID-19 such as elderly people are more likely to have a lower serum 25-OH-D_3 concentration and be vitamin D deficient from reduced sunlight exposure (213, 216). Giving high supplemental doses of vitamin D_2 or D_3 to geriatric humans can address the vitamin D deficiency issue (217, 218). However, as of February 2021, there have yet to be any reported clinical studies on vitamin D supplementation reducing symptoms for those infected with COVID-19. Such clinical studies are likely underway and will be reported in the near future. Grant et al. (213) suggested that a combination of randomized controlled trials and large population studies are needed to examine if vitamin D supplementation can quickly increase 25-OH-D_3 concentration in humans to reduce the risk of influenza or COVID-19. We recommend that chickens would be a stellar model to work with because they would serve a dual purpose: *1*) their added-value with production if vitamin D supplementation results in vitamin-D-enriched meat and egg products; and *2*) provide data for translational research.

There is research interest in exploring vitamin D content in muscle tissue with different production animals. There was a study that involved pigs and showed how increased dietary vitamin D_3 and 25-OH-D_3 in feed increased vitamin D_3 and 25-OH-D_3 content in adipose and white muscle tissue (219). A recent review covered how 25-OH-D accumulates in skeletal muscle cells to be utilized during winter months (220). Also, a recent study reported how Irish beef contained vitamin D_2 and 25-OH-D_2 and other Irish meats contained little or immeasurable concentrations of the 2 metabolites (221). The study had samples collected from each of the 4 seasons. Median total vitamin D activity was observed at higher concentrations from samples collected in the summer and autumn, which indicates how meat may be a possible food source of vitamin D.

Broiler chickens would be valuable for elucidating vitamin D storage mechanisms because they can be housed in controlled environments to prevent UVB exposure. Broilers could also be fed dietary 25-OH-D_3 during their growing phases and have their 25-OH-D_3 concentration measured in plasma and muscle tissue. Using fast- and slow-growing broiler strains could serve as an excellent model for addressing Mason et al.'s (220) conclusions of exploring endocrine mechanisms regulating 25-OH-D_3 uptake and release in muscles. A study using broilers demonstrated that replacing most dietary D_3 with 25-OH-D_3 can increase the number of mitotically active skeletal muscle satellite cells, highlighting that dietary 25-OH-D_3 is connected to skeletal muscle yield (222). Building from Hutton et al.'s (222) findings, if dietary vitamin D_3 supplementation can increase vitamin D_3 concentrations in broiler chicken muscles, then there is a possibility for the poultry industry to cultivate a "vitamin D broiler" that yields muscles that contain higher concentrations of vitamin D_3 for consumers. These vitamin-D-enriched broilers can be important, not only for their increased added-value production, but they can also help alleviate issues relating to vitamin D deficiency cases that arise from the COVID-19 pandemic to even post-COVID-19.

Eggs are a staple food source that can help address vitamin D deficiency when the hens are fed diets with high concentrations of vitamin D_3 (167). The possibility of vitamin-D-enriched eggs was discussed earlier in this review in the section "*Effects of dietary vitamin D_3 with laying hens in production.*" Exploring consumer aspects of vitamin-D-enriched eggs will be important for trying to address vitamin D deficiency because if the vitamin-D-enriched eggs lose a lot of their content from cooking, then their value is reduced. Therefore, it will be necessary to quantify how different processing methods affect vitamin D content in egg yolks, especially in vitamin-D-enriched eggs. Jakobsen and Knuthsen (223) published an analytical article that reported boiled or fried eggs had vitamin D retention that ranged from 82 to 88% and prolonged heat treatment in an oven resulted in 39–45% retention. Their article highlights that vitamin D content in eggs is influenced by cooking methods (223). Determination of the vitamin D content in egg yolks after cooking has significant implications for people who depend on dietary sources for vitamin D intake.

Chickens can be used as translational research models for experimental approaches involving vitamin D deficiency, inflammation, and respiratory diseases. *Mycoplasma gallisepticum* is a bacterial species that causes respiratory infections in chickens and other birds (224) that can be used as an infectious agent for studies involving chickens. Similar to the spike proteins of SARS-CoV-2 (225), *M. gallisepticum* also uses adhesins to attach to host cells for cell invasion (226). Considering COVID-19 elicits a proinflammatory response in the lungs, which can be more severe in people with vitamin D deficiency; *M. gallisepticum* can likely cause a similar response in vitamin-D-deficient chickens. A starting approach can use in vitro methods to characterize how chicken-derived macrophages and *M. gallisepticum* interactions are influenced with or without vitamin D treatment during incubation. Building from the in vitro study findings can help develop hypotheses using broilers or laying hens that are fed vitamin-D-deficient diets. They could be subsequently infected with *M. gallisepticum* to examine if there is an increase in proinflammatory immune marker expression. Characterizing inflammatory elements caused by respiratory disease agents in vitamin-D-deficient chickens could potentially identify markers that can help us develop alternate countermeasures against COVID-19 or other respiratory diseases.

Conclusions

Addressing global vitamin D insufficiency cannot be solely dependent on fortification methods or increasing sunlight exposure. Chickens are a valuable food source that have the potential to increase consumer vitamin D intake through egg and meat products that can be enriched with vitamin D. Research studies involving dietary vitamin D intake in

chickens can help researchers develop critical questions to address undiscovered ways we can improve vitamin D intake and/or metabolism. We recommend future research involving chickens and vitamin D should incorporate more molecular techniques. Exploring physiological dimensions with how the birds are affected by vitamin D can illustrate gaps in knowledge where researchers can improve chicken nutrition. By advancing chicken nutrition knowledge in vitamin D, researchers can compare such strategies to humans to target and develop preventative strategies to improve vitamin D status.

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