Unlocking the Power of Vitamin D: Enhancing Immunity and Overcoming Broiler Challenges

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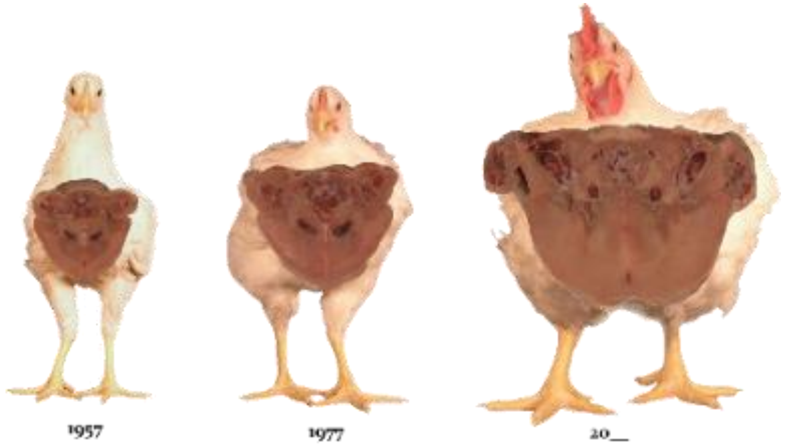
The modern broiler

- Modern broilers have a **growth rate almost double** than 30 years ago

- **The rate of bone development does not match** the fast growth rates of modern commercial broilers.
- Bone maturity and functional potentials (e.g. tibia weight, length, density) are **not reached until 25 to 35 wks of age in broilers**, long after the birds are typically marketed (Rath et al,. 2000*).

- The occurrence of lameness and bone fractures increases raising health, welfare and economic problems.
- If the problem is sub-clinical the broilers will reach the slaughterhouse but with increased condemnations, more carcasses being culled because of defects **including "black bone syndrome**".



* *N.C.Rath et al., Poultry Science, 2000;79:1024-1032*

Physiology

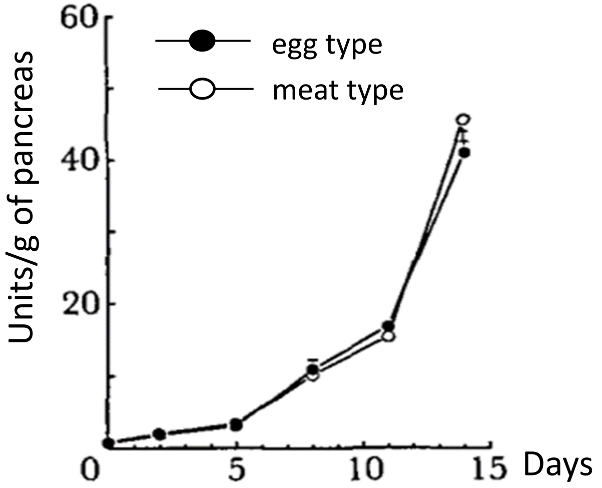
Development of the digestive enzymes after hatch

Development of digestive tract in the early weeks of life

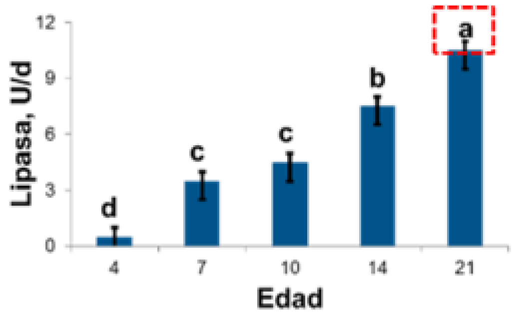
- Enzyme deficiencies
- Enteric challenges
- Enteritis and/or malabsorption
- Vaccination against coccidiosis

Ward, 2004

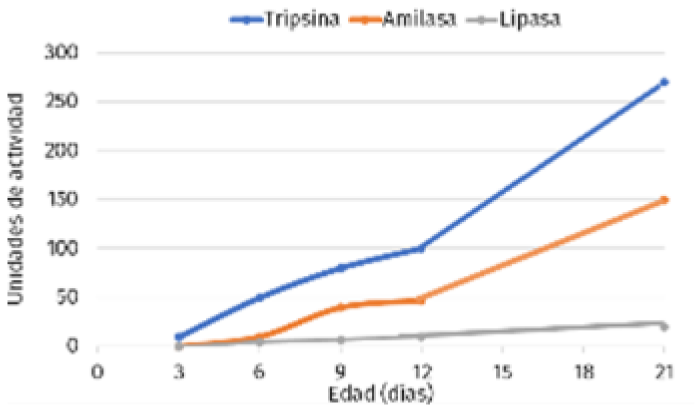
- Early weeks: low enzyme production:
  – Lipase, *Noy and Sklan, 1995.*
  – Liver: *Saunder-Blades, 2008*
  – Bile: *Noy and Sklan, 1995.dm*

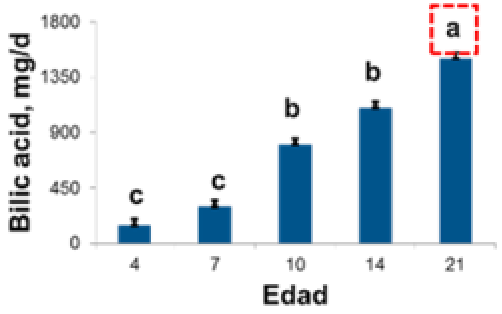


Nir et al., 1993



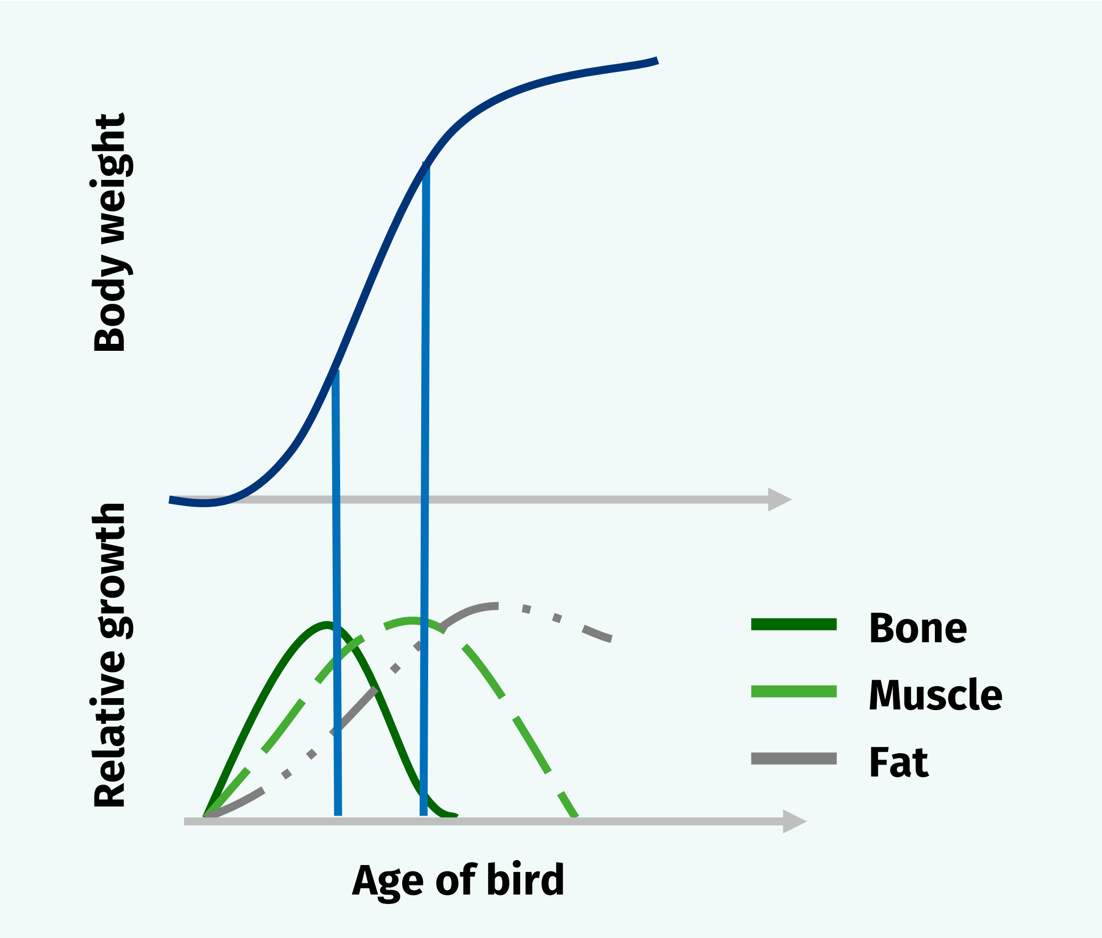
Adapted from: Noy and Sklan, 1995





Physiology

Allometric development of broiler growth



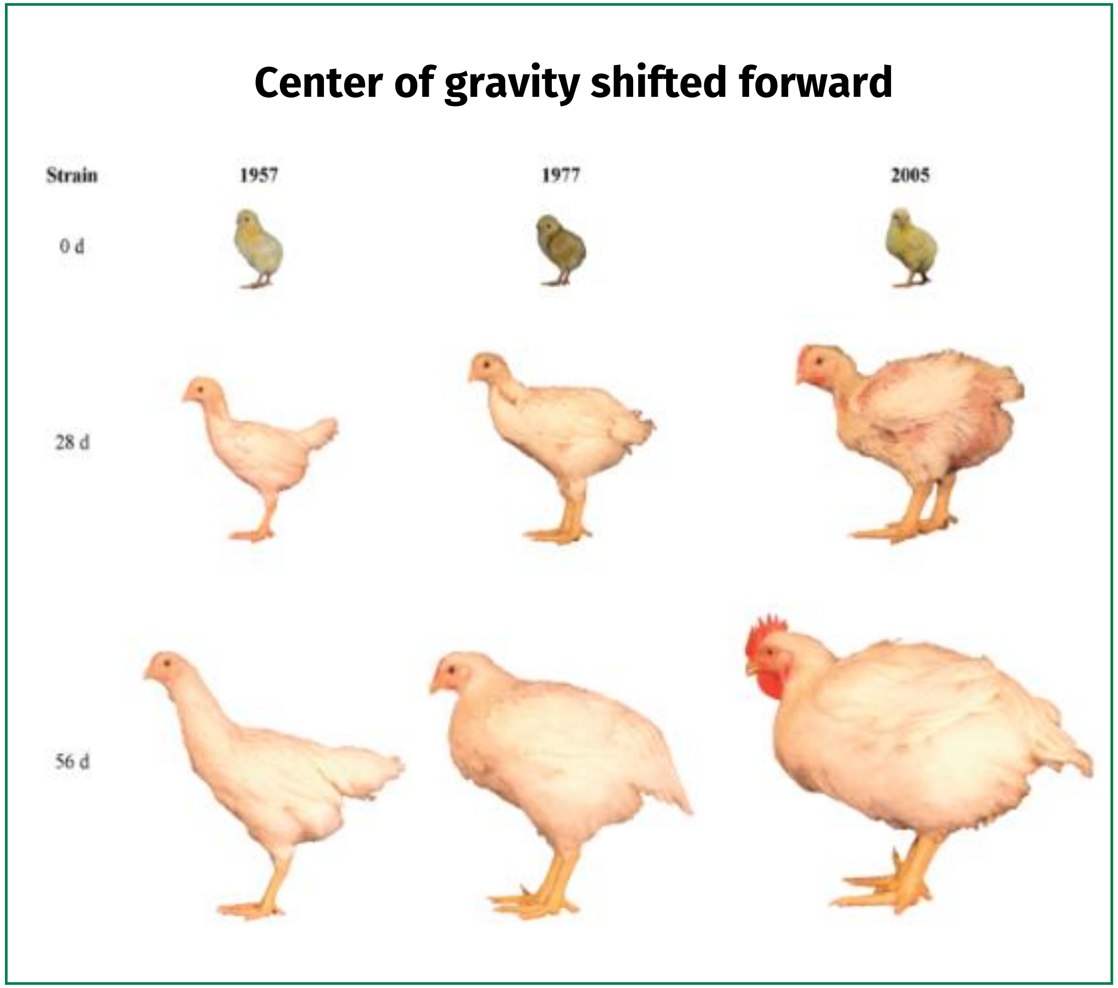
Adapted from Gonzales and Sartori, 2002

The bone tissue develops first. Then, during the rapid growth phase, the muscle is developed.

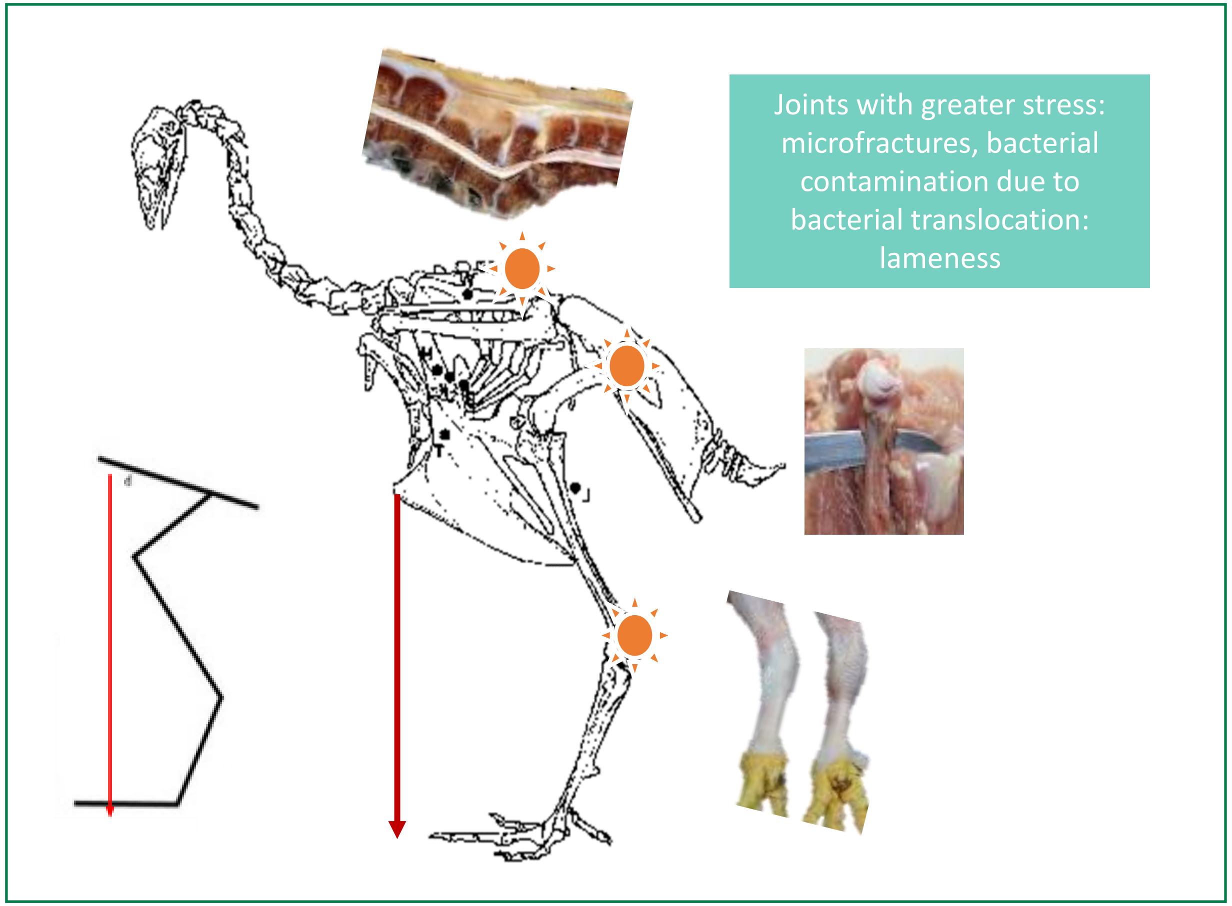
In modern broilers, muscle mass deposition begins almost at the same time as bone tissue formation.

Physiology

Allometric development of broiler growth

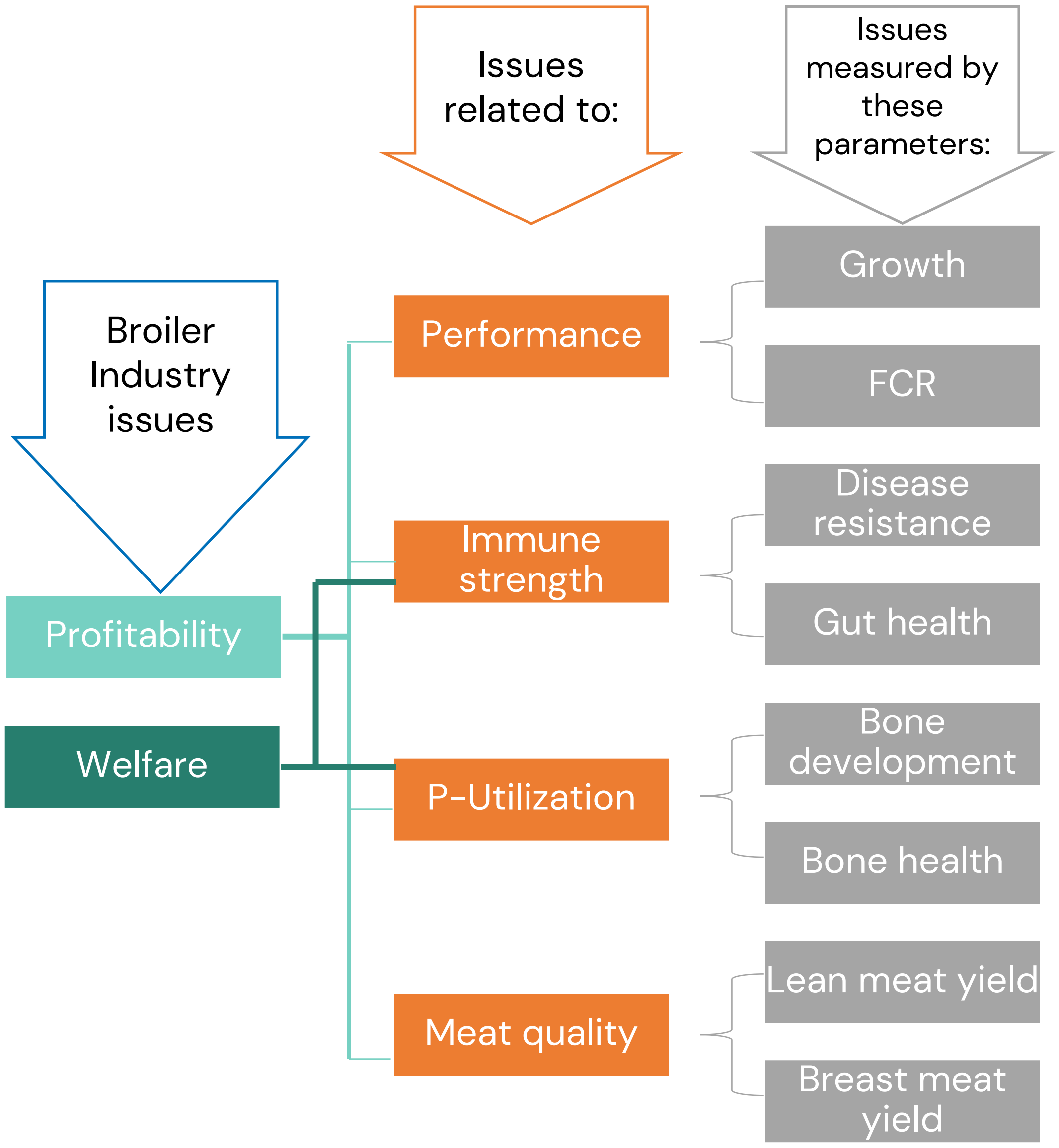


Zuidhof et al., 2014



Abourachid, 1993

The Main Challenges of the Poultry Industry



….Is there a common thread ?

Vitamin D is involved in all these mechanisms

Mineral homeostasis and bone metabolism

Regulator of immune function

Support skeletal muscle development and growth

Fertility

BIOLOGICAL ACTIONS OF VITAMIN D

animals MDPI

Article

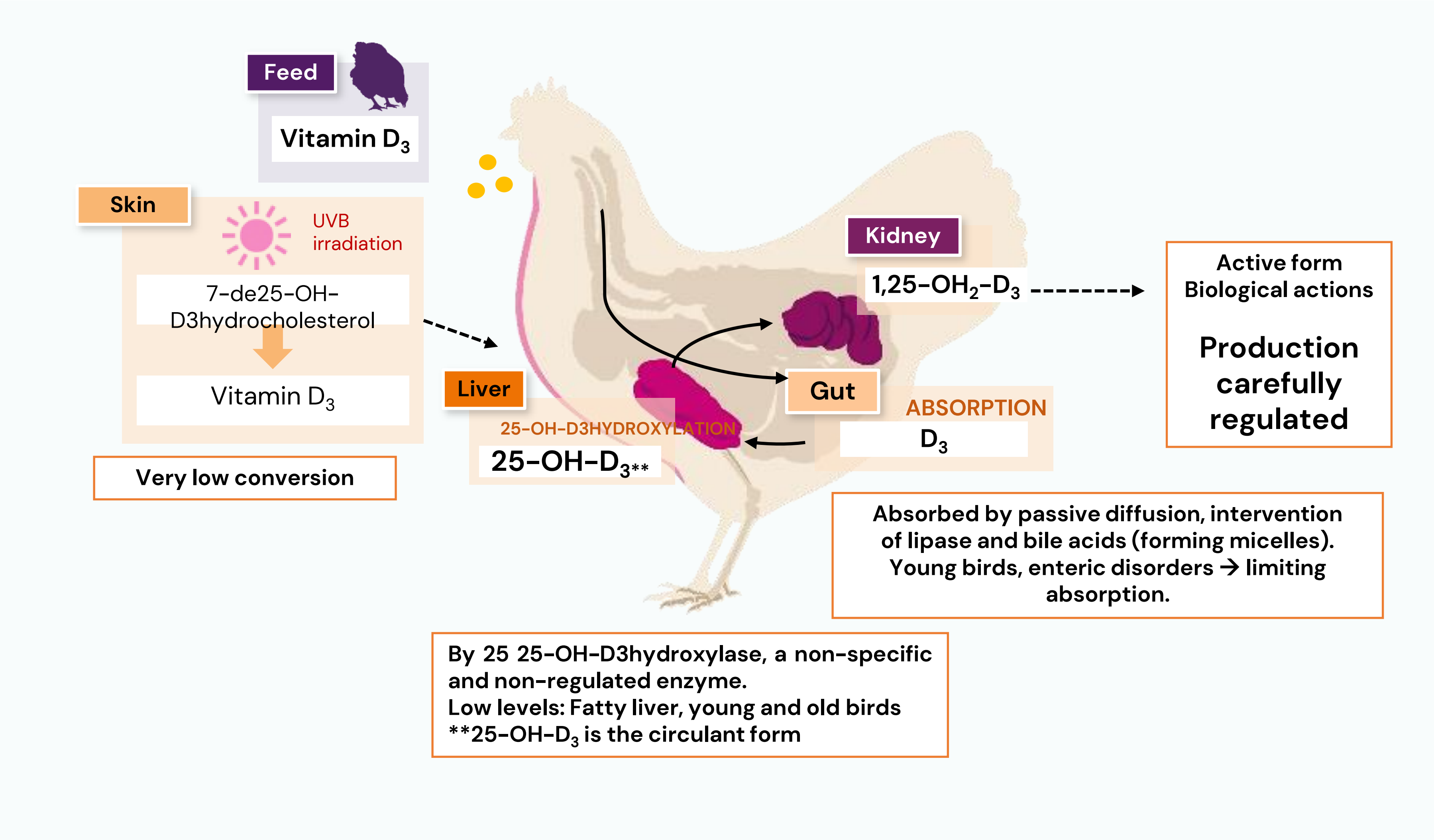
Classic and Non-Classic Effects of the Duration of Supplementation of 25-Hydroxicholecalciferol in Broiler Chicken Diets

Karen Prokoski [1], Leticia C. Bittencourt [2], Levy V. Teixeira [2,*], Cristiano Bortoluzzi [2], Elisangela Vanroo [1], Sabrina Palma [1] and Jovanir I. M. Fernandes [1]

Animals2021,11,2971.https://doi.org/10.3390/ani11102971

Vitamin D & 25OHD$_3$

Metabolism summary



Main limitations

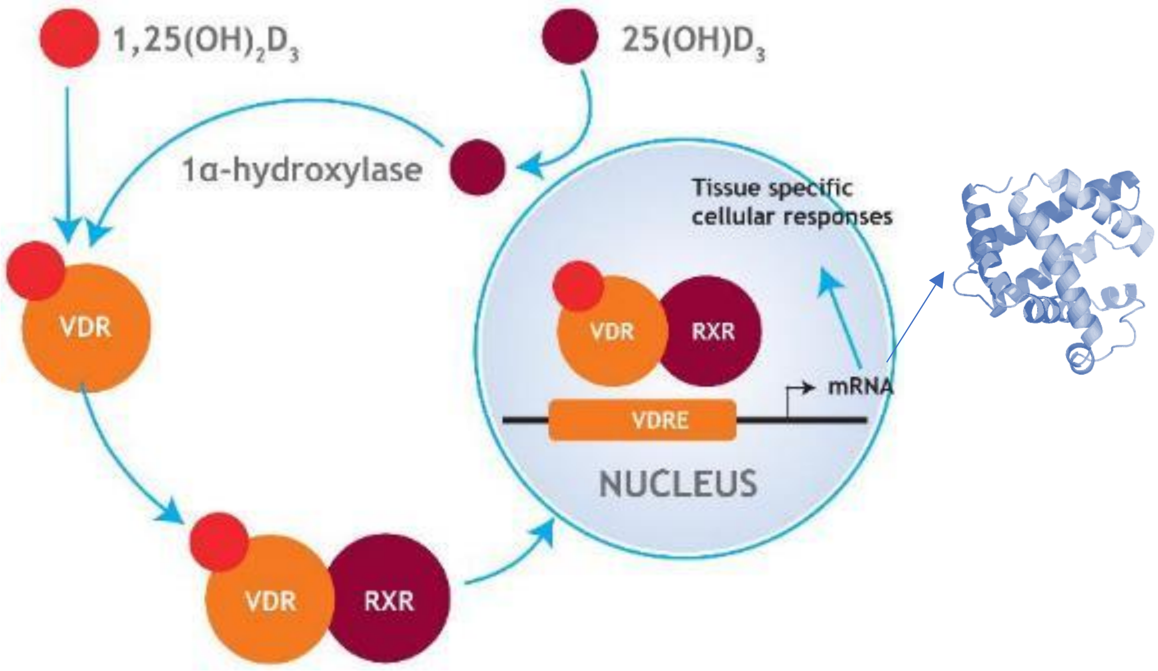
- Any gut challenge will compromise the absorption of Vit D$_3$;
- Any liver challenge will compromise the 25-OH-D3 hydroxylation of the Vit D$_3$ into 25-OH-D$_3$;
- Up to 10-14 days the lipases and the 25-25-OH-D3 hydroxylase are not totally efficient.

Soares et al., 1995; Atencio et al., 2005; Khan et al. 2010

Vitamin D & 25OHD$_3$

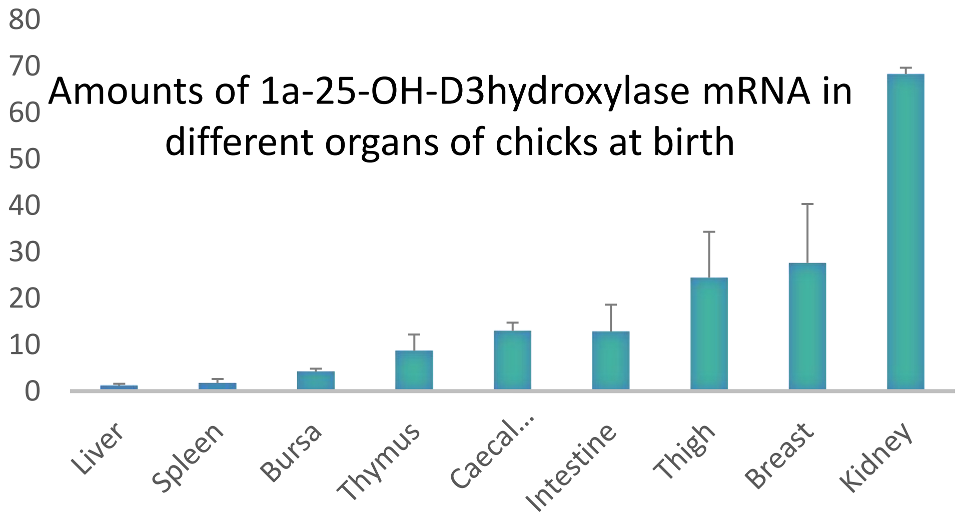
Mode of action

Vitamin D_3 (Calcitriol form) acts as a hormone via activation of its receptor (VDR), which regulates and modulates gene expression in target cells.



Vitamin $_{D3}$ (25-OH D3$_{or}$ 1,25-$(OH)_2$D3) enters the target cell and 1,25-$(OH)_2$D3binds to its receptor, VDR, which is a transcription factor. The vitamin-receptor complex enters the nucleus and induces the synthesis of messenger ribonucleic acid (mRNA).

This mRNA codes for a protein responsible for a biological effect, such as *Calcium Binding Protein* or CaBP.

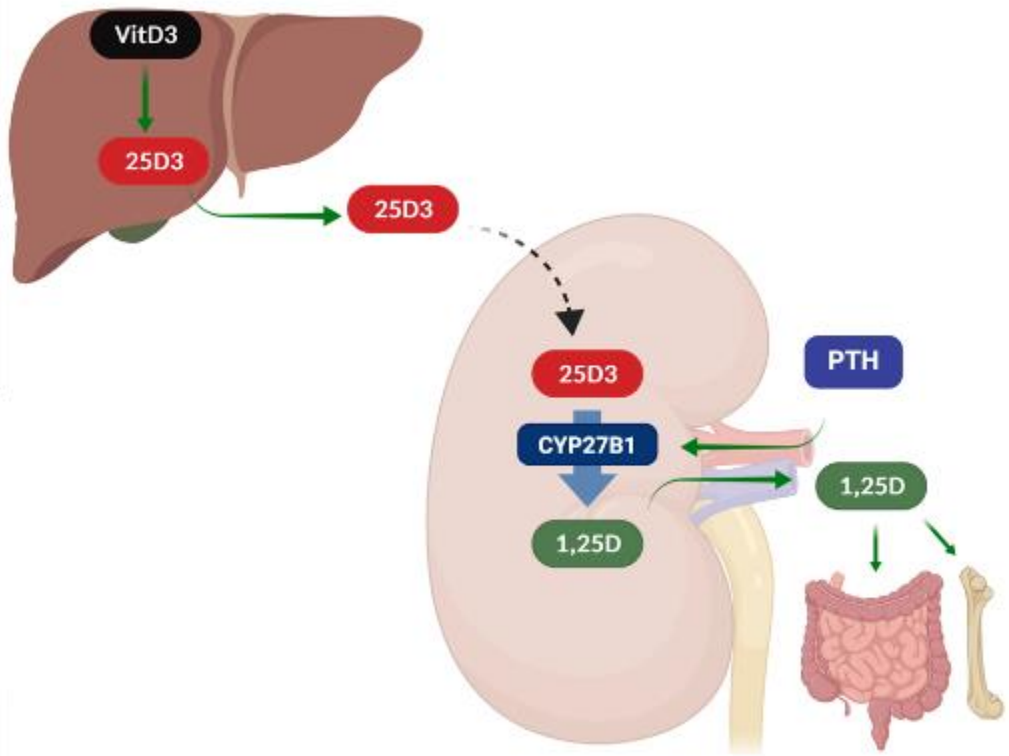


ex: Between 200 and 600 genes per cell type are the primary targets of vitamin D , (Carlberg, 2014)

Vitamin D's "Classic" Role: Ca/P homeostasis

In the kidney

- $1,25(OH)_2D_3$ enhances the expression of genes for basolateral calcium transporters (**resorption from urine to blood**)



In the intestine

- $1,25(OH)_2D_3$ stimulates **Ca absorption** through the epithelium by increasing the permeability of tight junctions

- Enhances the **absorption of phosphate** by an upregulation of the Na-Pi-transporter

In the bone

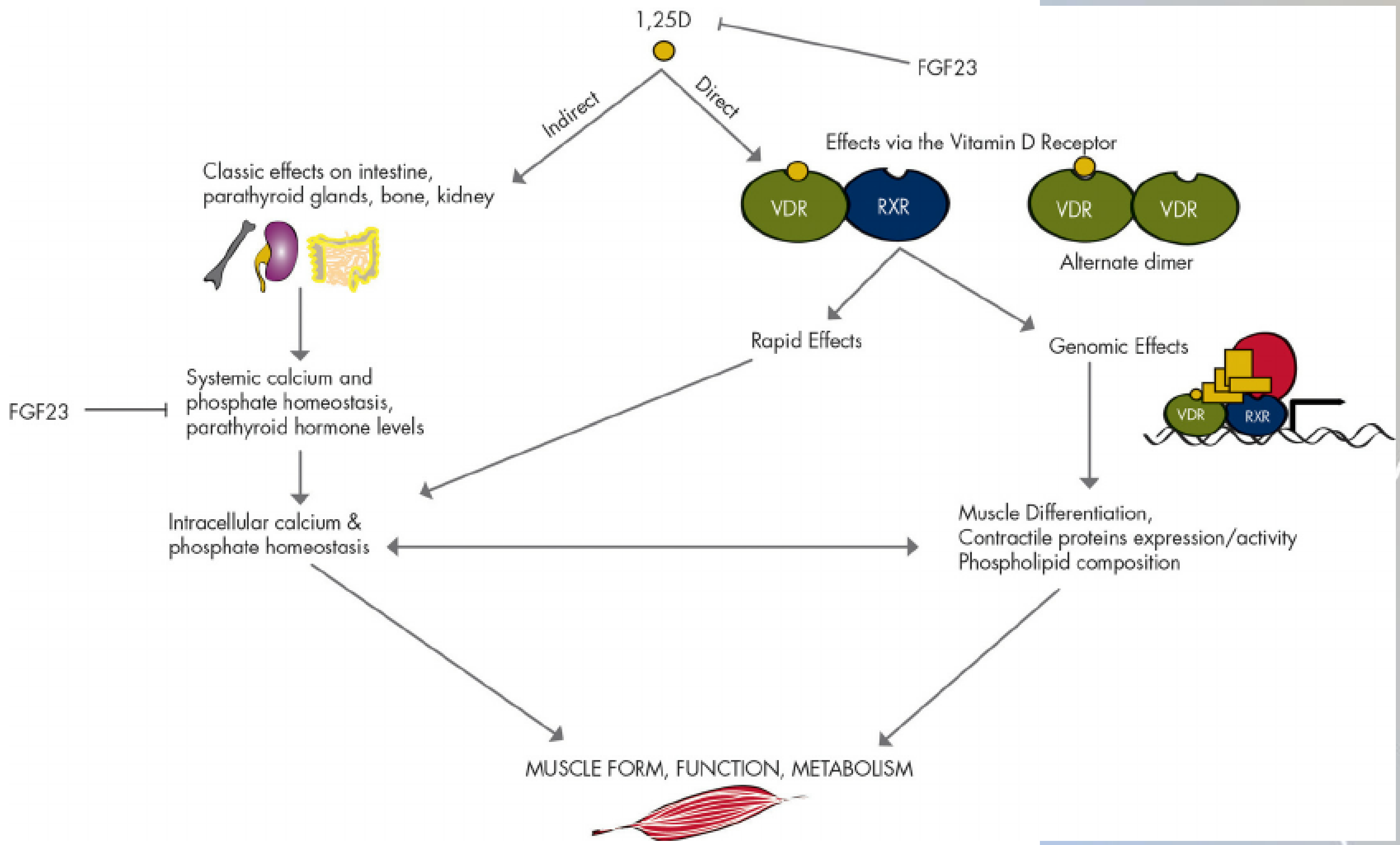
Under hypocaelcemia:
$1,25(OH)_2D_3$ + PTH stimulates **mobilization of Ca from bones** to increase ionized Ca

Under positive calcium balance:
$1,25(OH)_2D_3$ **stimulates bone growth and mineralization** by:

- increasing plasma Ca and P
- direct effects exerted in osteoblasts

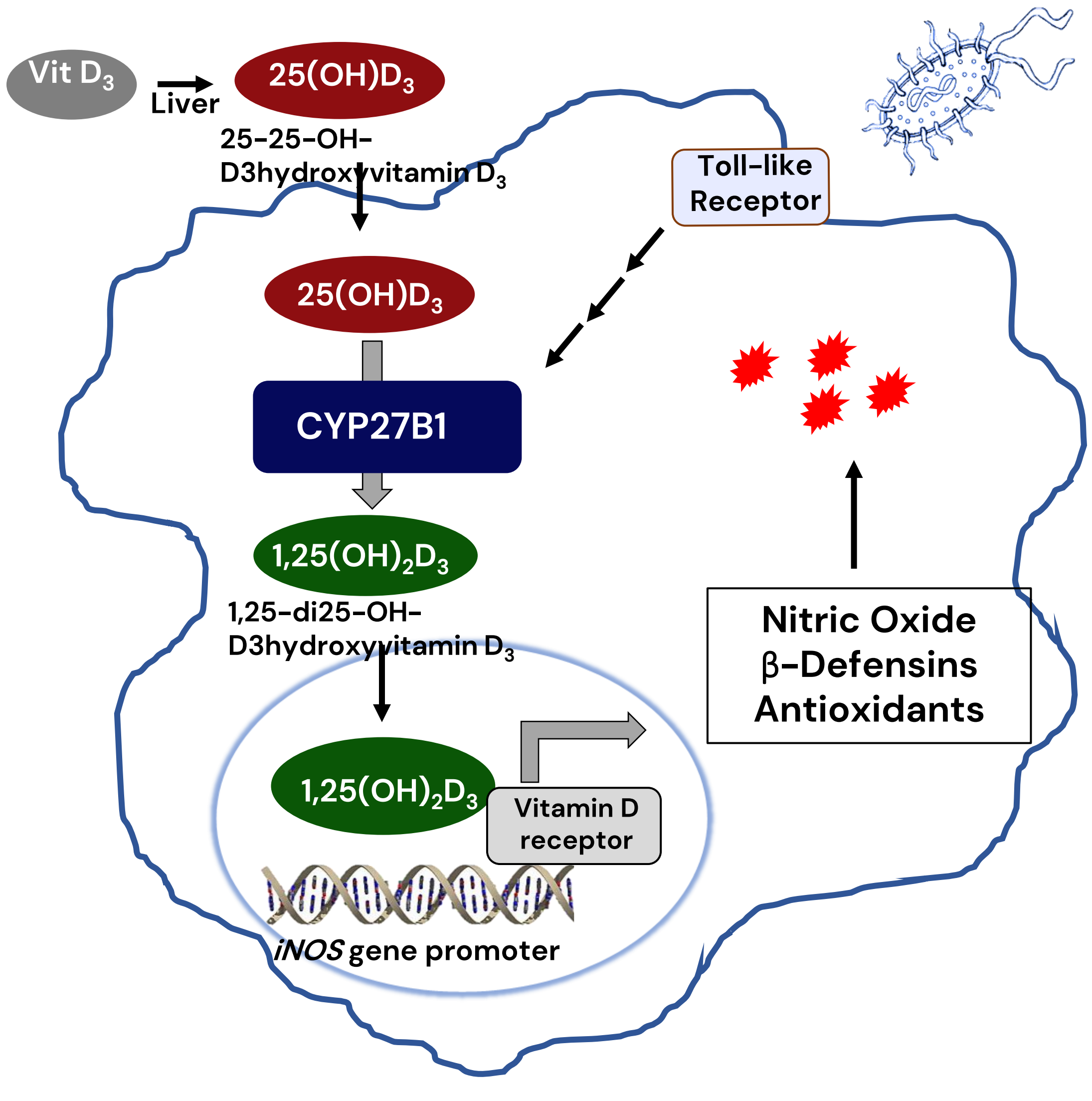
(Image from Nelson et al., 2020)

Vitamin D's "Increasingly Popular" Role: Muscle development and growth



Christian M., et al, Endocrine Reviews, February 2013, 34(1):33– 83

Vitamin D's "Increasingly Popular" Role: The Immune System



Macrophages

$1,25(OH)_2D_3$ stimulates gene expression involved in innate immunity, including toll-like receptors 2 (TLR2) and -4 (TLR4) associated with the defense against infectious agents such as lipopolysaccharides (LPS).

Resulting in

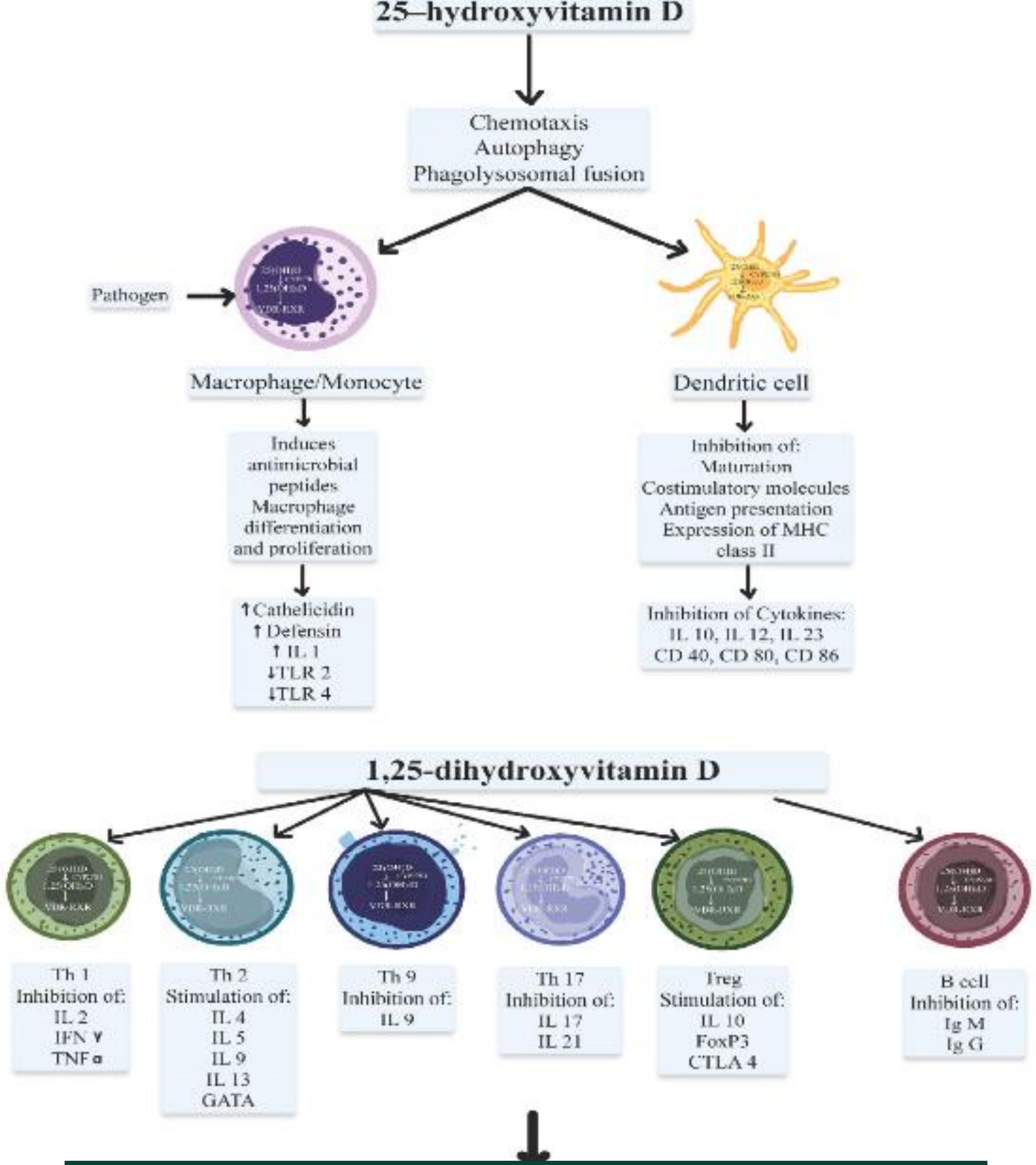
- Increased bactericidal activity
- Reduction of ROS
- Reduction in inflammation

Source: Poindexter

Norman, 2008; Shanmugasundaram & Selvaraj, 2012; Morris et al., 2014, Shojadoost et al., 2015

Vitamin D modulates the Innate Immune System

The bacterial infection triggers the activation of toll-like receptors (TLRs) that regulates VDRs expression and 25(OH)D3, 1-25-OH-D3hydroxylase activity

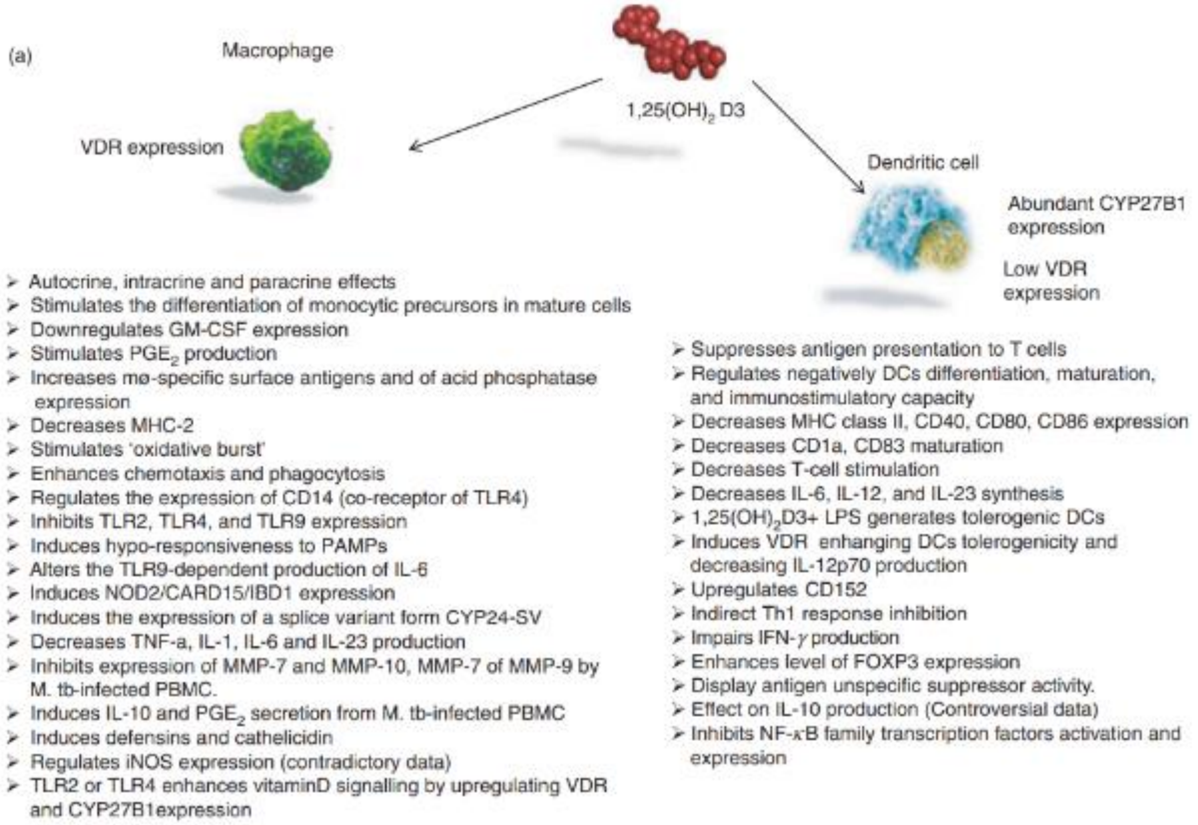


Bacterial killing
Decrease inflammation
Decrease autoimmunity

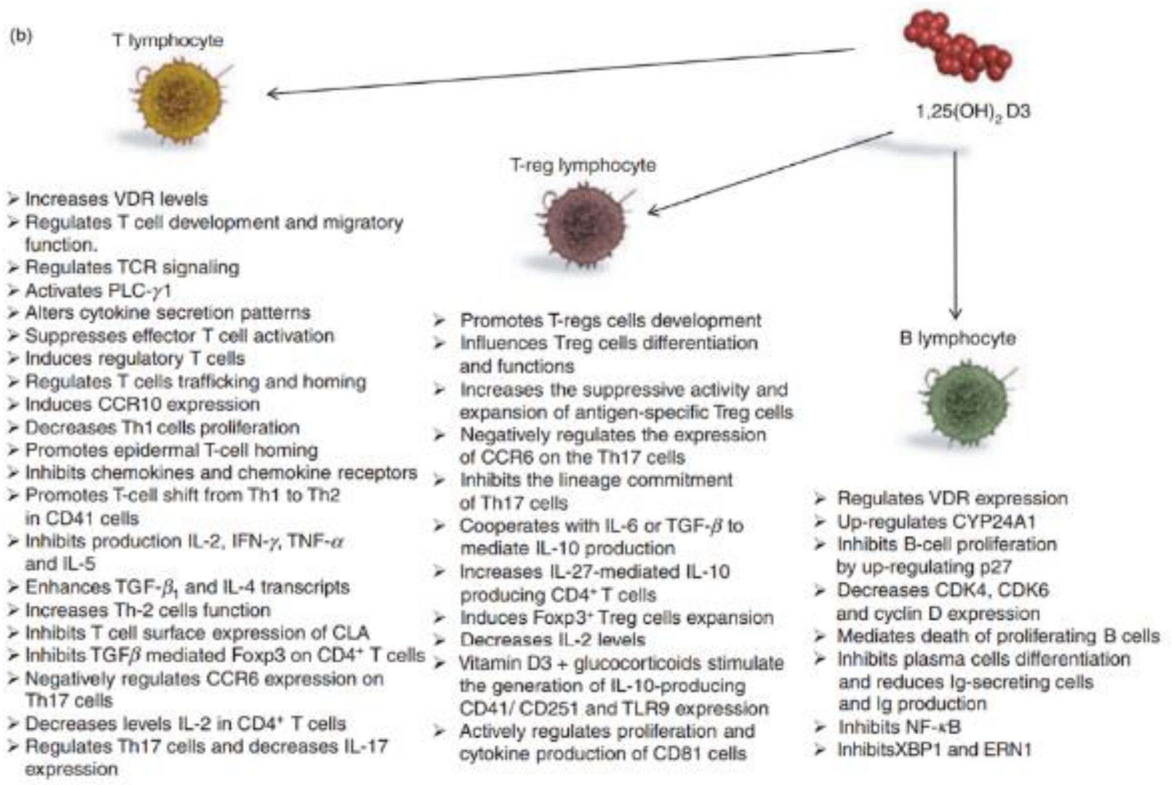
Claudia Sirbe abd coll, International Journal of molecular Sciences, 2022

Vitamin D and 25-OH-D3 boosts immune function and health

The main immune cells have VDR (Vit D receptor) and have the ability to convert calcidiol to calcitriol (enzyme 1-α-25-OH-D3hydroxylase CYP27B1)



Passive & Innate Immunity



Adaptive Immunity

Michelino Di Rosa and coll, Immunology 2011

Skeletal Muscle-Immune System Interplay: A Two-Way Route

In humans and animals, **vitamin D and VDR expression are necessary for muscle development, myocyte differentiation, muscular volume, and function maintenance**

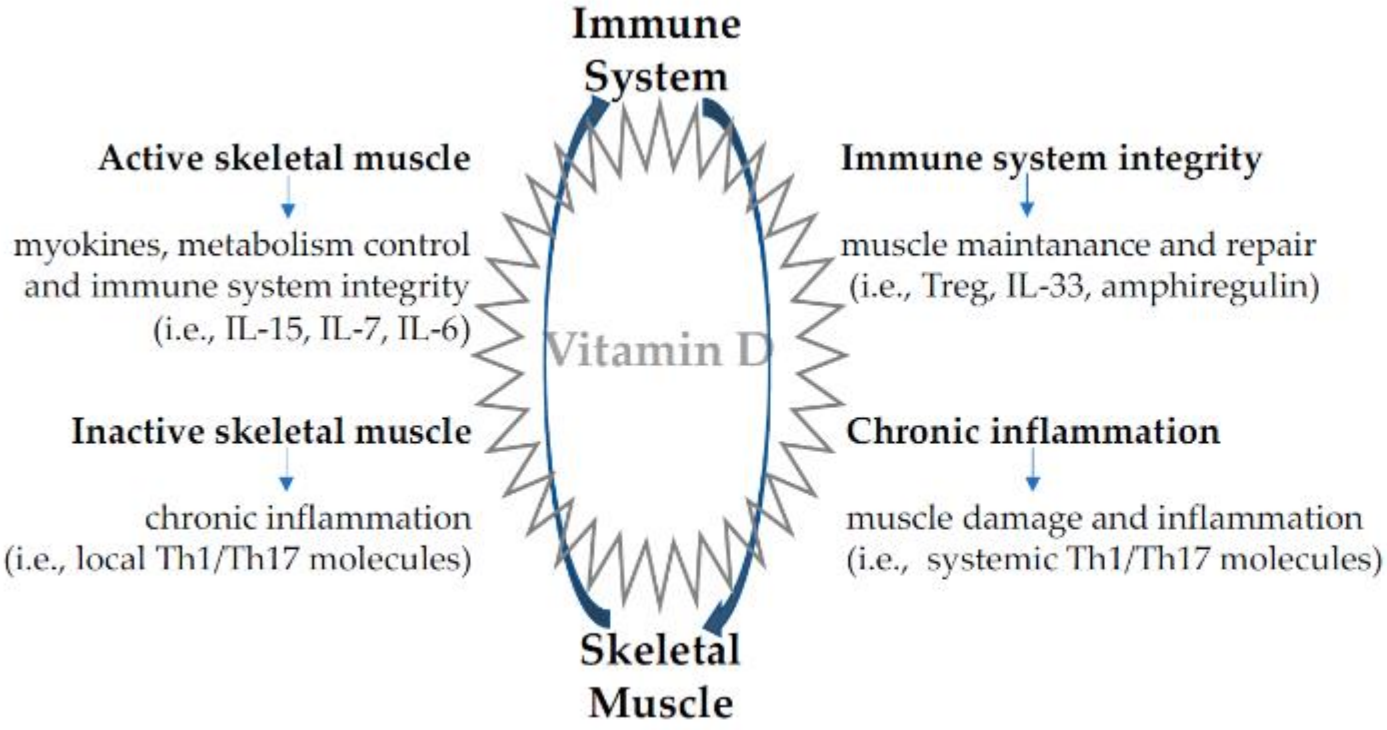


Figure 2. The three-side network relationship connecting the Immune System-Skeletal Muscle Network and vitamin D. Vitamin D acts within the immune-muscle axis, exerting regulatory protective effects.

Skeletal Muscle-Immune System Interplay: A Two-Way Route

The review provides the evidence **that deficiency of vitamin D through oxidative stress and disruption of mitochondrial function may affect the development of skeletal muscle atrophy.**

European Journal of Applied Physiology (2019) 119:825–839
https://doi.org/10.1007/s00421-019-04104-x

INVITED REVIEW

Mechanisms of vitamin D on skeletal muscle function: oxidative energy metabolism and anabolic state

Katarzyna Patrycja Dzik[1] · Jan Jacek Kaczor[1]

Received: 9 December 2018 / Accepted: 13 February 2019 / Published online: 4 March 2019

Vitamin D Deficiency

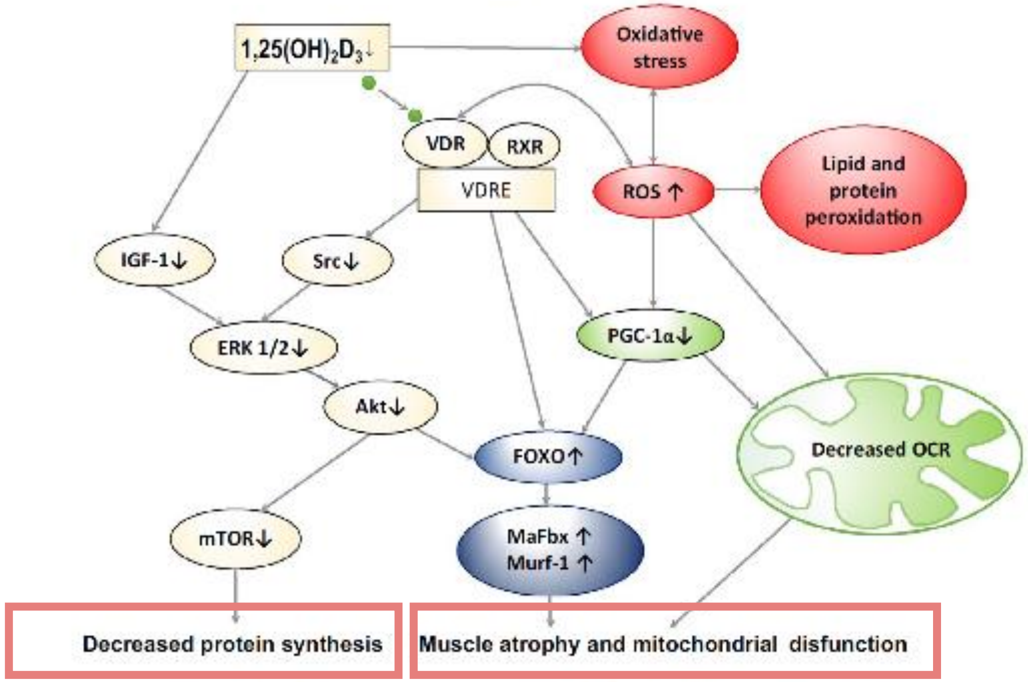


Fig. 2 The graphical abstract of the vitamin D action in the skeletal muscle in vitamin D deficiency conditions. Vitamin D deficiency decreases IGF-1 and PGC-1α via VDR—the nuclear receptor. Src/ERK1/2/Akt/FOXO3a signalling cascade triggers the muscle atrophy through Murf-1 and MaFbx. Vitamin D deficiency increases oxidative stress and attenuates mitochondrial biogenesis and function. *Akt* serine/threonine-specific protein kinase, *ERK 1/2* extracellular signal-regulated kinases 1 and 2, *FOXO* forkhead box protein, *IGF-1* insulin-like growth factor 1, *MaFbx* muscle atrophy F-box protein, *mTOR* mammalian target of rapamycin kinase, *MuRF1* muscle ring finger protein, *OCR* oxygen consumption rate, *PGC-1α* peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *ROS* reactive oxygen species, *RXR* retinoid X receptor, *Src* steroid receptor coactivator complex, *VDR* vitamin D receptor, *VDRE* vitamin D response elements

25-OH-D3 is THE Biomarker to assess the vitamin D status in humans and animals
=> 25OHD3 is the **circulating form**

$25OHD_3$ — Safe, efficient, reliable form (circulating form)

↓

$1,25\text{-}(OH)_2\text{-}D_3$ — Active Form, 'hormonal' Form

↓

VDR

VDR – found in cells and tissues

$1,25\text{-}(OH)_2\text{-}D_3$ **VDR**

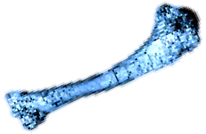
$1,25\text{-}(OH)_2\text{-}D_3$ **VDR**

$1,25\text{-}(OH)_2\text{-}D_3$ **VDR**

Mineral metabolism

GUT
Ca & P absorption

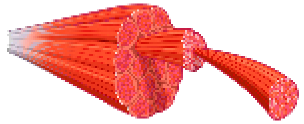
BONE
Bone turnover



KIDNEY
Ca and P reabsorption

IMMUNE SYSTEM
Stimulation

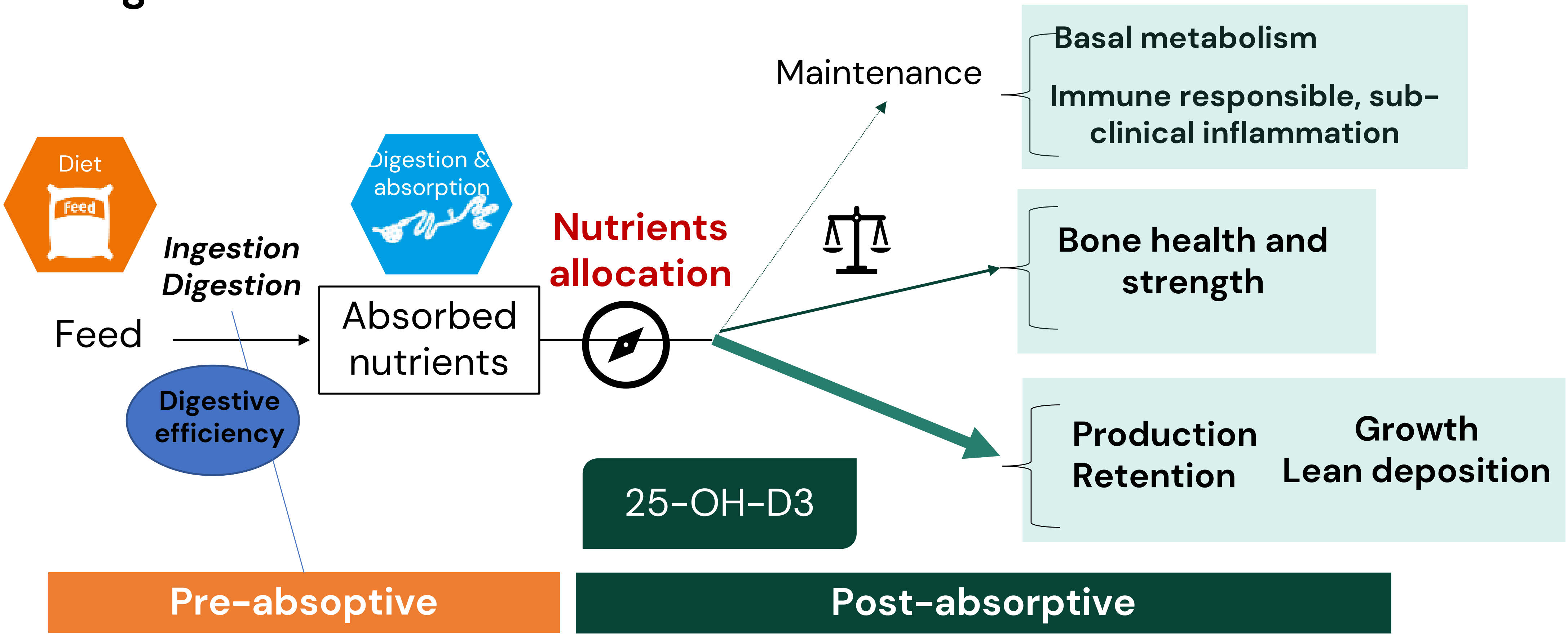
MUSCLE CELLS
Growth and differentiation



$1,25\text{-}(OH)_2\text{-}D_3$ binds to **V**itamin $\mathbf{D_3}$ **R**eceptors **(VDR)** → triggering their functions

Vedi Castamagna

Supporting nutrient allocation and metabolism



Support resilience via **digestibility enhancement** and gut functionality

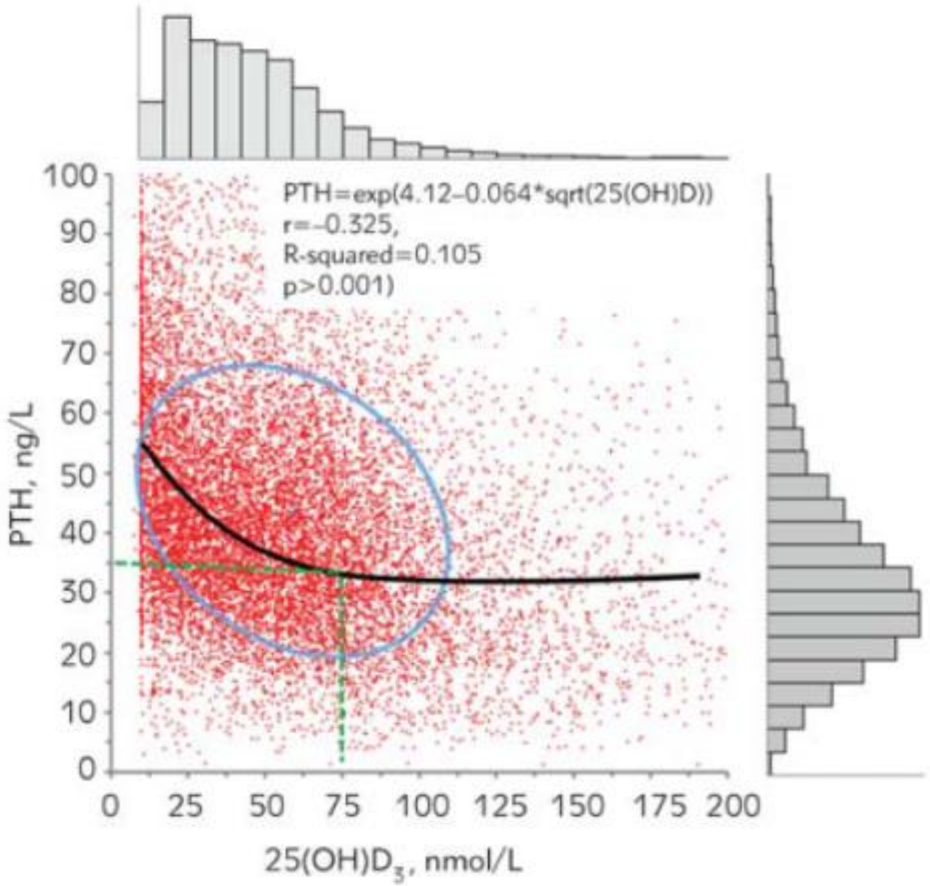
Support resilience via post-absorptive effects, supporting immune competency, mitigating oxidative stress, protecting cells, and mitigation inflammation

Rauw (2009), Doeschl-Wilson, (2011)

References values and interpretation of vitamin D status in humans

Optimum 25-OH-D_3 level in humans for endocrine/skeletal homeostasis function

*Laboratory results of **9890 female** and **2723 male individuals** aged **38.8±22.1 years** who had simultaneous measurements of 25(OH)D3 and PTH*



Source: Serdar et al., 2017

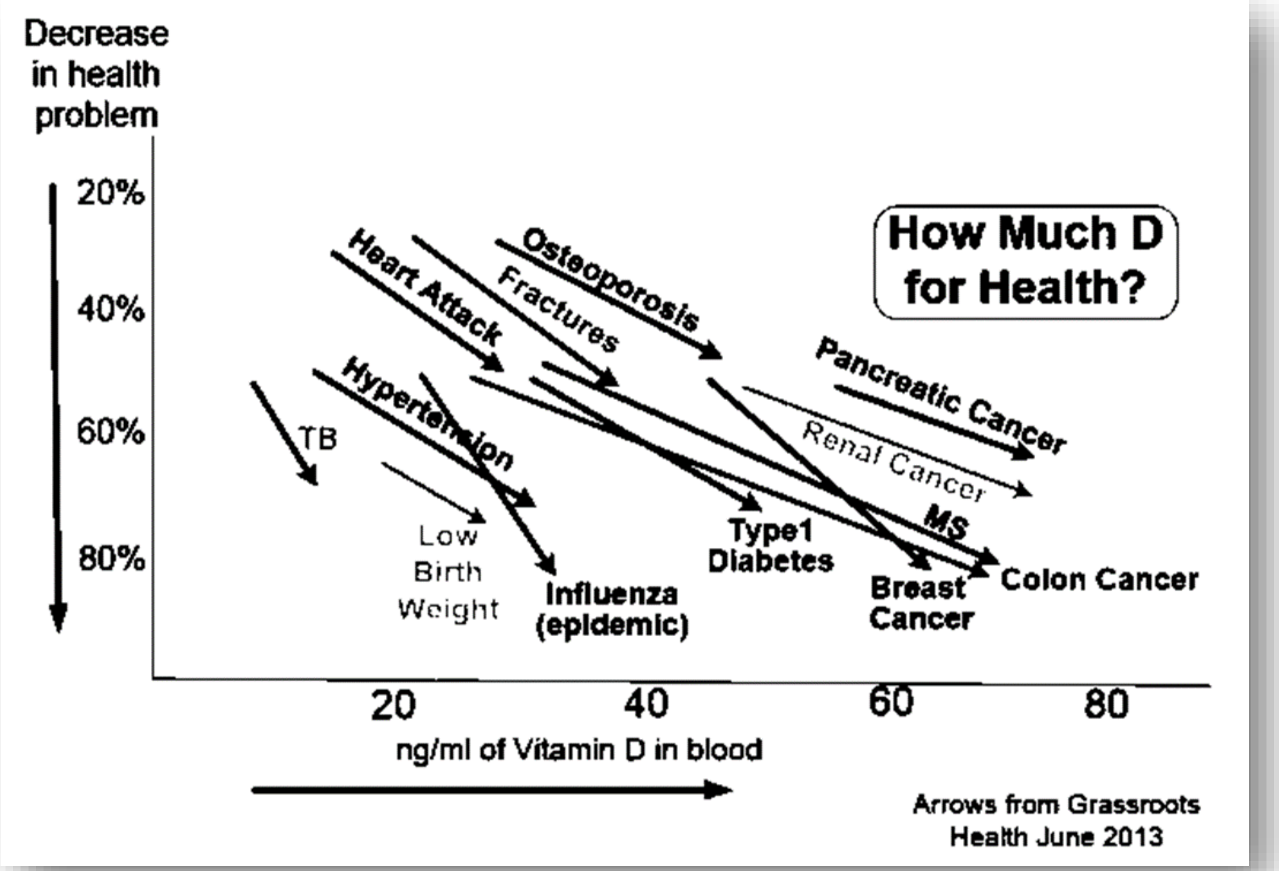
- There is an inverse relationship between PTH and 25-OH-D_3
- PTH levels reach **a stable plateau** (35 ng/l) above 25-OH-D_3 levels of **75 nmol/l (30 ng/ml)**
- The value of 30 ng/ml is suggested to be the **clinical decision threshold for 25-OH-D_3** for both male and female adults

25OHD_3 ng/ml	Reference
30 - 31	Chapuy et al., 1997
20	Malabanet et al., 1998
32 - 36	Heaney et al., 2003
30 - 31	Holick et al., 2005
20 - **30**	Balland et a., 2007, 2008*
30	Serdar et al., 2017

** seasonal influence (winter-summer)*

References values and interpretation of vitamin D status in humans

Optimum 25-OH-D3 level in humans for paracrine/autocrine functions



Outcome	Type of Evidence	Optimal 25OHD	Reference
All-cause mortality rate	Observational study of 25(OH)D concentration due to vitamin D supplementation	>30 ng/mL	[8]
Alzheimer's disease and dementia	Meta-analysis of observational studies	>25 ng/ml	[93]
Breast cancer	Observational study of 25(OH)D concentration due to vitamin D supplementation	>60 ng/mL	[33]
Colorectal cancer	Meta-analysis of observational studies	30–40 ng/mL	[34]
Cardiovascular disease	Observational study of the CVD mortality rate for CVD patients	>30 ng/mL	[9]
Myocardial infarction	Observational study of 25(OH)D concentration due to vitamin D supplementation'1n	>30 ng/mL	[8]
SARS-CoV-2 infection	Retrospective observational study	>50 ng/mL	[75]
COVID-19 mortality	Retrospective cohort study	>60 ng/mL	[82]
Diabetes mellitus type 2	RCT with an analysis of intratrial 25(OH)D for prediabetes patients	>50 ng/mL	[70]
Gene expression	Clinical trial	>40 ng/mL	[45]
Hypertension	Observational study of 25(OH)D concentration due to vitamin D supplementation	>40 ng/mL	[16]
Preterm delivery	Observational study of 25(OH)D concentration due to vitamin D supplementation	>40 ng/mL	[106]

Grant et al., 2022

Interpretation of vitamin D status in humans

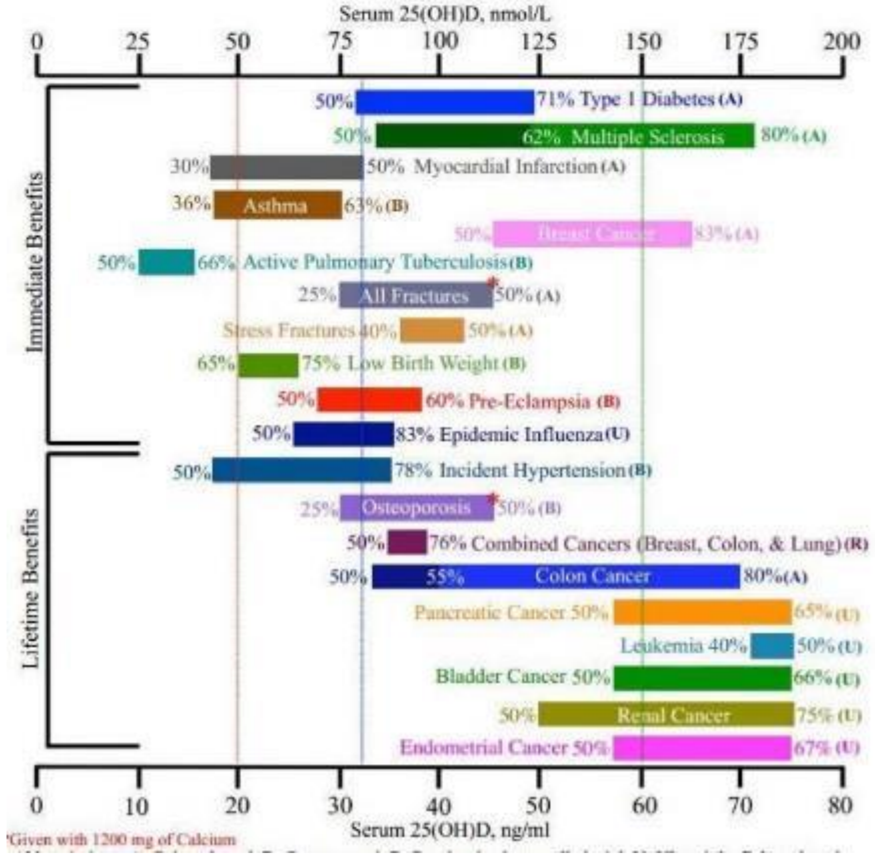
Optimum 25-OH-D3 level in humans for different functions and deficiencies

- *Reference Baeke et al., **2010** (adaptation):*

$25OHD_3$ level (ng/ml)	Status
30 - 50	Healthy individuals
<30	Insufficiency
<15	Deficiency (rickets, osteomalacia possible)
<5	Severe deficiency (rickets, osteomalacia frequent)

- *Reference Holick, **2023** (adaptation):*

$25OHD_3$ level (ng/ml)	Status
>30 (40 – 60?)	Associated with non-calcemic health benefits (e.g. severity of COVID-19- lung injury and mortality)=> **Paracrine/autocrine functions**
30	Adequate for optimum bone health=> **Endocrine functions**
20 - 30	Insufficiency
<20	Deficiency; risk of bone disorders

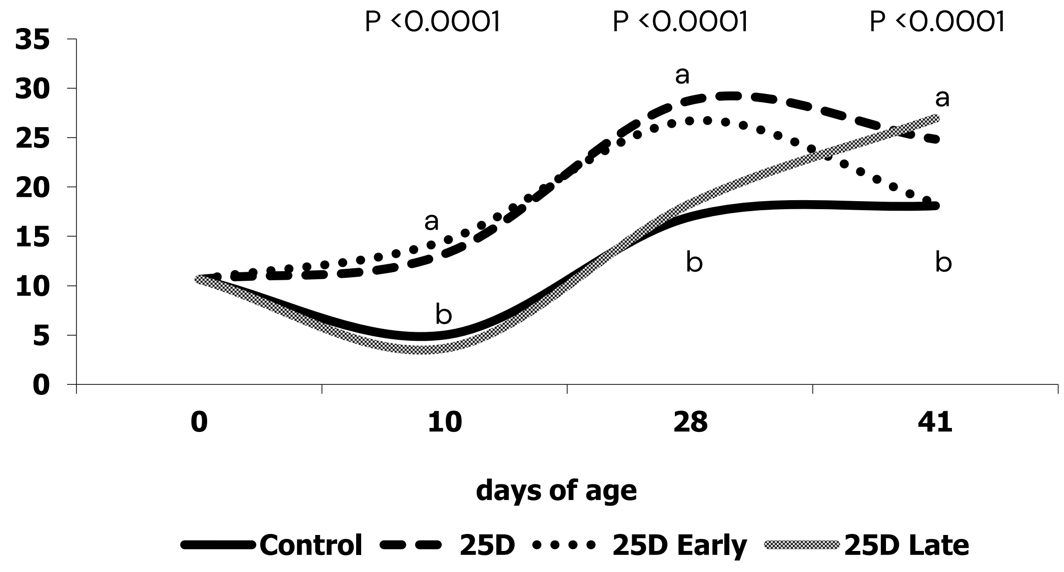


Baggerly C et al , 2013

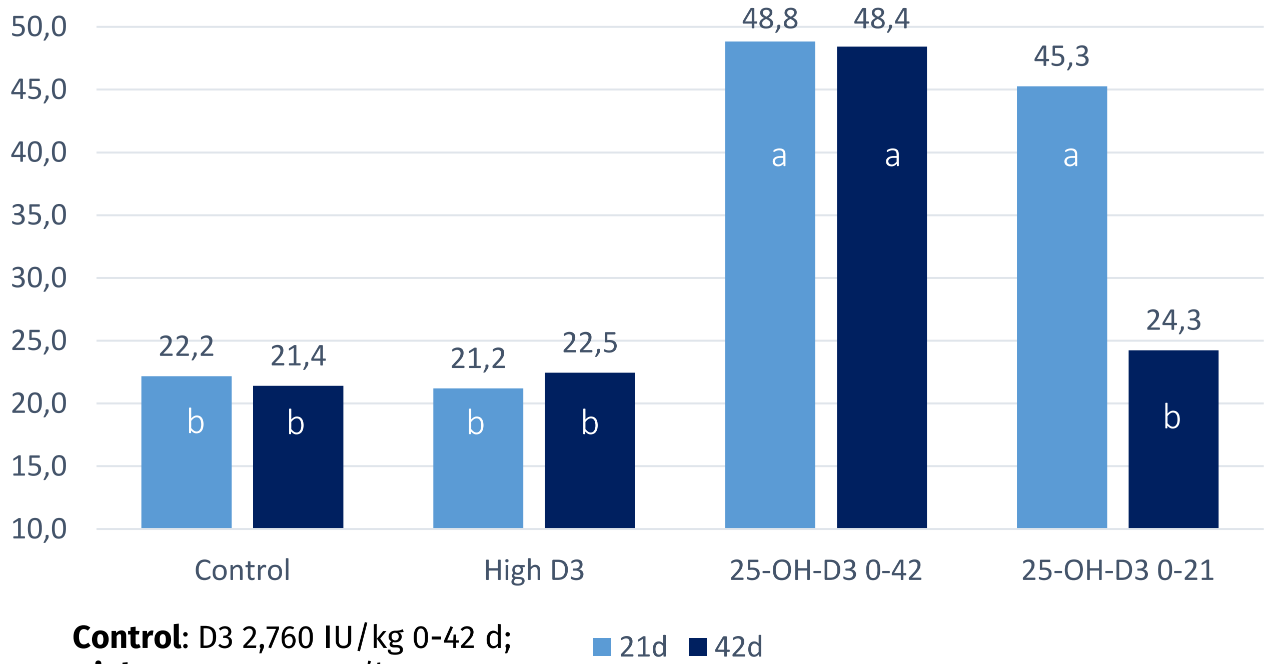
Vitamin D_3 nutritional status

Confrontation of dietary vitamin D_3 and $25OHD_3$

- **Providing $25OHD_3$ in the diet significantly improves $25OHD_3$ plasma level**
- The absorption of $25OHD_3$ being faster, more efficient and independent from fat metabolism ensures higher $25OHD_3$ plasma level even when gut functionality is impaired (e.g., malabsorption syndrome)



Saunders-Blades & Korver, 2014



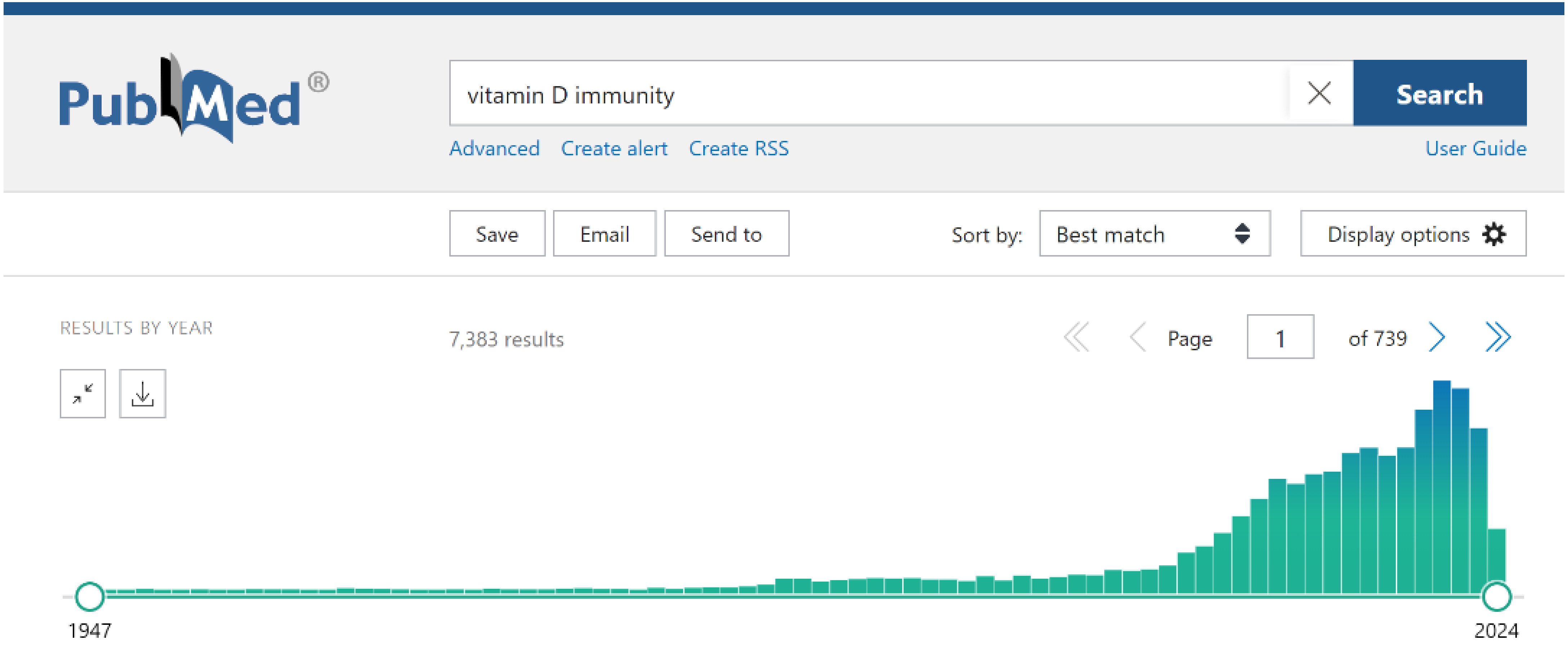
Control: D3 2,760 IU/kg 0-42 d;
High D3: D3 5,520 IU/kg 0-42;
25-OH-D3 0-42: 25-OH-D3 69mg/kg + D3 2,760 IU/kg 0-42;
25-OH-D3 0-21: 25-OH-D3 69mg/kg + D3 2,760 IU/kg 0-21 and D3 2,760 IU/kg 22-42

Source: Vignale,K., J. of Nutrition, 2015

How 25-OH-D3 enhancing Immunity In modern Broiler production

Why focussing on vitamin D as an immunomodulator

Number of publications / year, including in the title vitamin D and immunity



Scopus, "vitamin D" and immunty

Vitamin D Improve resilience and health

Vitamin D_3 and $25OHD_3$ show significant improvements in immune response

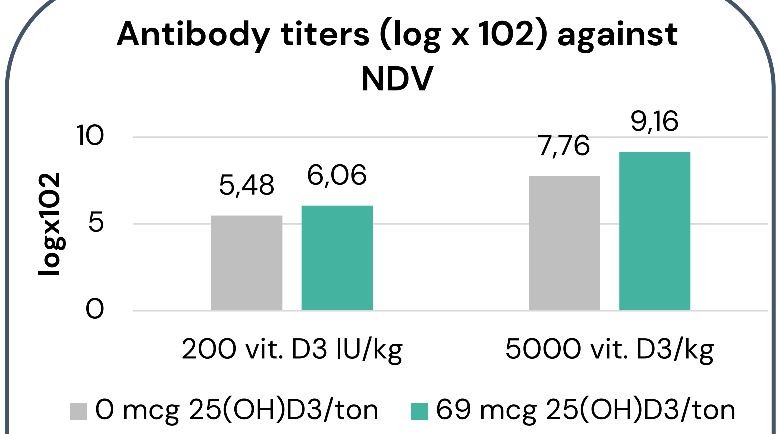
Effect on the immune system and microbial pathogens	Dose	Host	Reference
Increased anti-NDV antibodies and total and nonspecific intestinal IgA levels, increased peripheral blood $CD3^+CD8^+$ T cells	5,000 IU/kg combined with 69 µg/kg of 25(OH)D3	Broiler chickens	Vazquez et al., (2018)
Higher antibody titers to NDV vaccine antigens	2,000 IU/kg	Broiler chickens	Gómez-Verduzco et al., (2013)
Enhanced DTH reactions to phytohemagglutinin	69 mg/kg of 25(OH)D3	Broiler chickens	Gómez-Verduzco et al., (2013)
Reduced cellular responses to antigens in chickens challenged with SRBC. Reduced thymic weight and quantities of abdominal macrophages	Not supplemented with vitamin D_3	Broiler chickens	Aslam et al., (1998)
Higher levels of Salmonella-specific IgG	69 µg/kg of 25(OH)D3	Broiler chickens	Chou et al., (2009)
Upregulation of IL-10 gene expression	100 µg/kg of 25(OH)D3	Broiler chickens	Morris et al., (2015)
Upregulation in expression of TLR2 and TLR4 in addition to Th2 genes.	69 and 275 µg/kg of 25(OH)D3	Broiler chickens	Rodriguez-Lecompte et al., (2016)

Abbreviations: DTH, delayed-type hypersensitivity; IL-10, interleukin-10; SRBC, sheep red blood cells; TLR, Toll-like receptor; Th2, T helper 2.

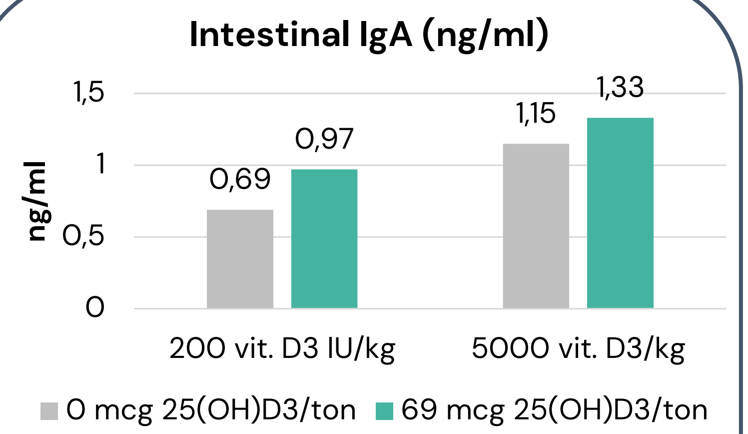
Source: Shajadoost. et al., 2021

Vitamin D Improve resilience and health

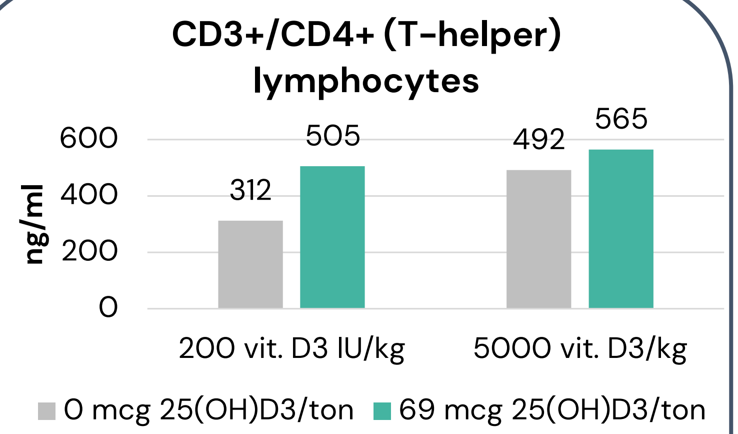
Vitamin D_3 at 5,000 IU/kg feed and the addition of 25OHD_3 at 69 mg/kg significantly increased the immunocompetence of broilers



- Vitamin D average effect: 5.77 vs 8.46 P<0.05
- 25-OH-D3 average effect: 6.62 vs 7.61 P<0.05



- Vitamin D average effect: 0.83 vs 1.24 P<0.05
- 25-OH-D3 average effect: 0.92 vs 1.15 P<0.05



- Vitamin D average effect: 408 vs 528 P<0.05
- 25-OH-D3 average effect: 402 vs 535 P<0.05

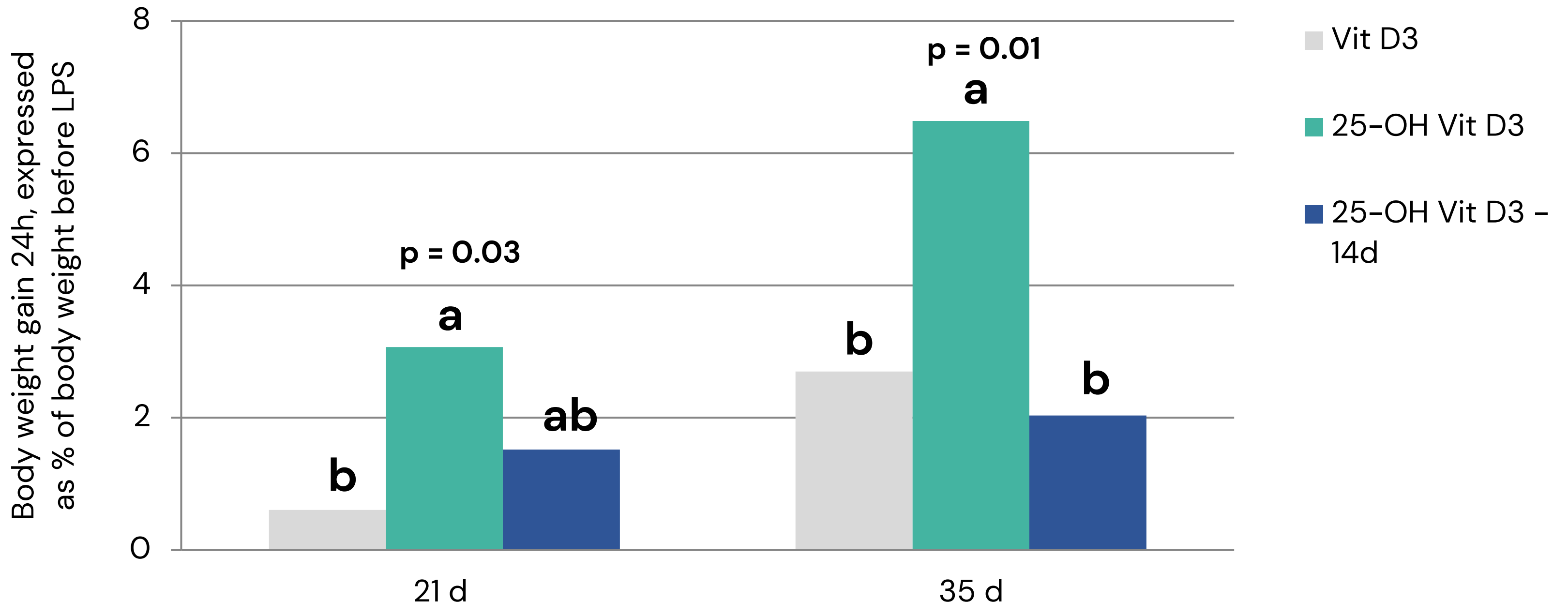
NDV: New Castle Disease Virus

Source: Vásquez et al., 2018

Vitamin D Improve resilience and health

Confrontation of dietary vitamin D_3 and $25OHD_3$

25-OH-D3 better modulation of the Immune Response



Treatments

- 1. Vit D3 3,000 IU/kg D3
- 2. *25OHD3 69mg/kg (2,760 IU/kg) 1-35d
- 3. *25OHD3 69mg/kg (2,760 IU/kg) 1-14d; control 15-35d
- 20 and 34 days of age, Salmonella typhimurium LPS

Regulation of the inflammatory response → Energy for performance

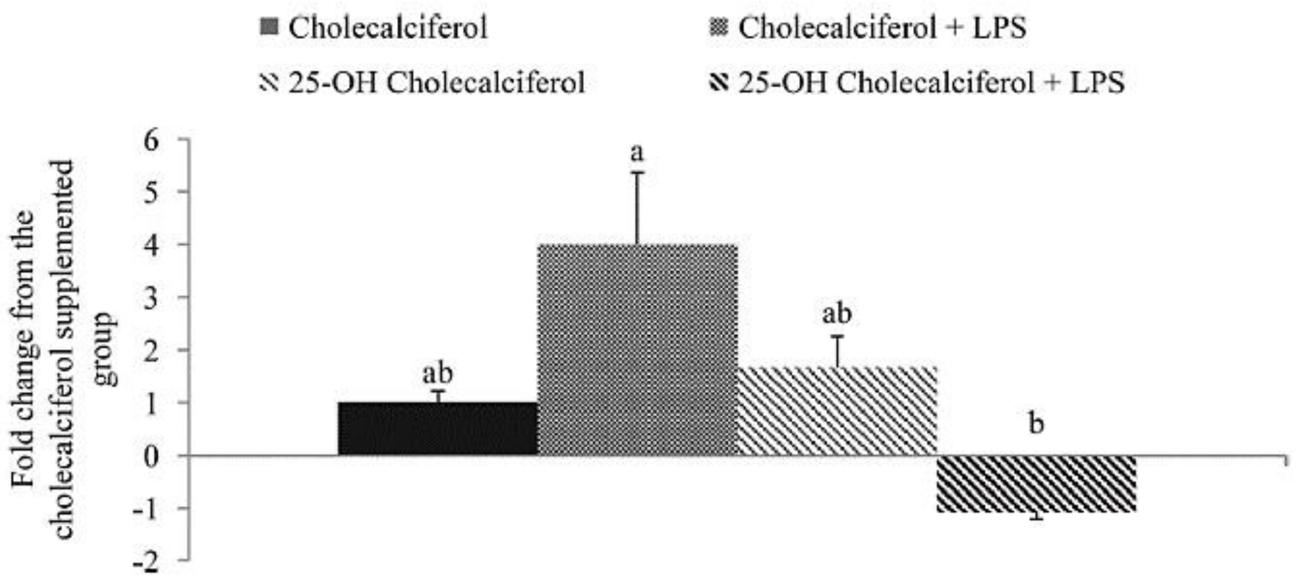
Morris A. et al., 2014

Vitamin D Improve resilience and health

Confrontation of dietary vitamin D_3 and 25OHD_3

25-OH-D3 better modulation of the Immune Response

Levels of IL-1β mRNA in the liver



Morris A. et al., 2014

The production of acute phase proteins requires a large amount of energy that is not directed to growth.

Treatments:

- 1. Control 3,000 IU/kg D3
- 2. 25-OH-D3; 69ug/kg (2,760 IU/kg) 1-28d

28 d – challenged or not (LPS).

24 h after challenge - measured IL-1β mRNA

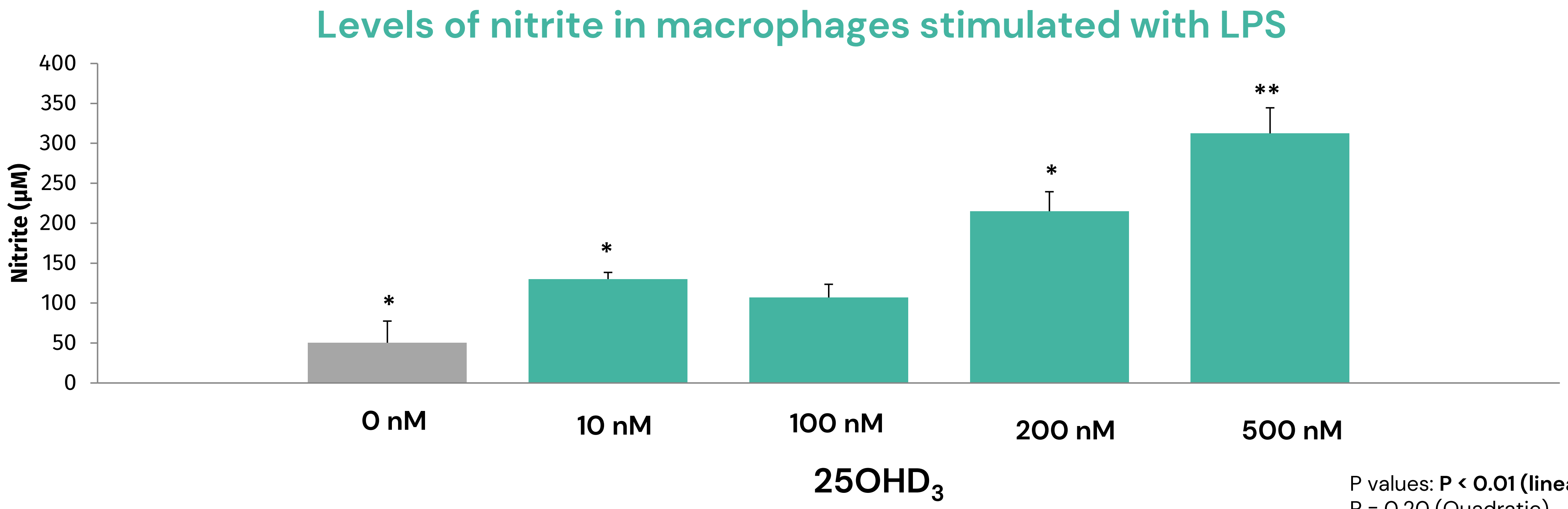
(Real time PCR).

IL-1β= pro-inflammatory mediator

Vitamin D Improve resilience and health

Confrontation of dietary vitamin D_3 and 25OHD$_3$

25-OH-D3 better modulation of the Immune Response



P values: **P < 0.01 (linear)**,
P = 0.20 (Quadratic)

ROS-ON: antimicrobial components responsible for eliminating bacteria

Morris A. and Selvaraj, 2015

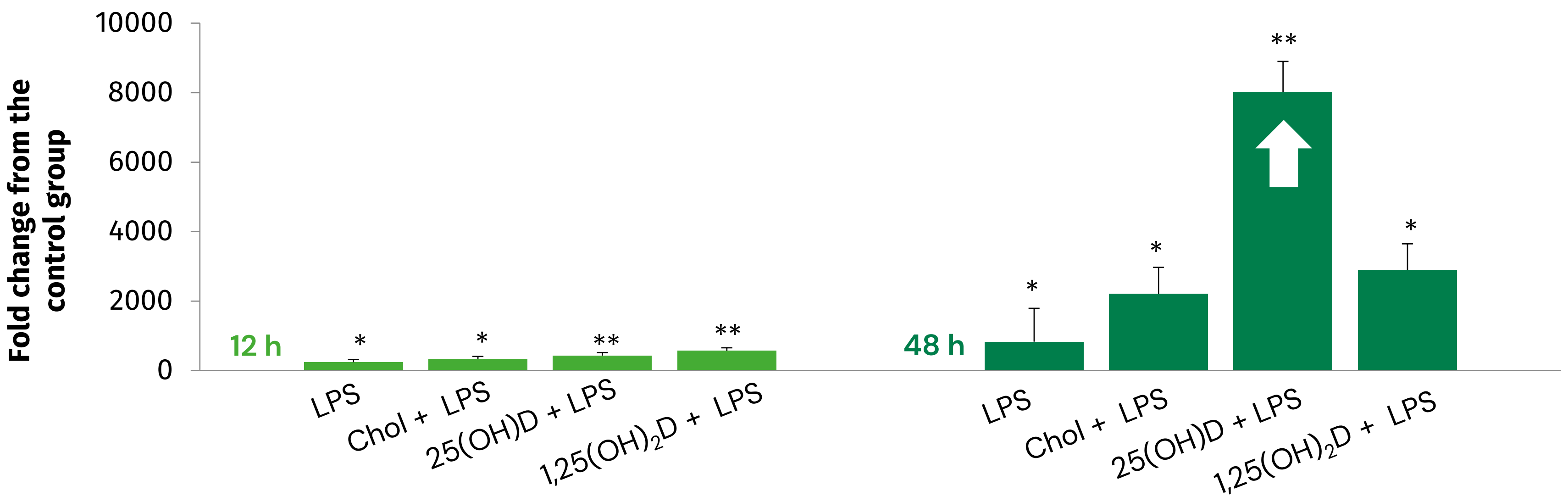
Vitamin D Improve resilience and health

Confrontation of dietary vitamin D_3 and $25OHD_3$

25-OH-D3 better modulation of the Immune Response

IL-10: Anti-inflammatory marker

Levels of IL-10 mRNA in macrophages stimulated with LPS



P values: 12 h: 25-25-OH-D3hydroxycholecalciferol, P = 0.17; LPS, P < 0.01; 25-25-OH-D3hydroxycholecalciferol *LPS, P = 0.25; 48 h: 25-25-OH-D3hydroxycholecalciferol, P < 0.01; LPS, P < 0.01; 25-25-OH-D3hydroxycholecalciferol *LPS, P < 0.01.

25OHD3 increases the production of IL-10 in macrophages stimulated with LPS

Morris A. and Selvaraj, 2015

25-OH-D3 and immune response

25-OH-D3 - anti-inflammatory activity:

- Limits excessive immune reactions that are costly in terms of energy.
- Avoids the impact on weight gain.
- Improves performance efficiency.

Antimicrobial effects:

- Improves the production of antimicrobial peptides
- Favors early maturation of monocytes and macrophages, improving the non-specific immune response.
- 25-OH-D3 **increases the immune response**, both humoral and cellular, at the time of infection, **when it is necessary.**

Conclusion and key home messages

Broilers are exposed to a number of challenges that affect **the post-absorptive metabolism, nutrients allocation and immune systems**

The adaptation in the metabolism may affect protein balance (Proteolysis – Proteosynthesis), amino-acids requirements (quantitatively and qualitatively), **which compromise muscle deposition and bones mineralization**

Vitamin D nutrition, and higher vitamin D blood levels thanks to 25(OH)D3 nutrition, **supports the immune competency, and helps to cope with the difference challenges** (mitigation of virus effect, mitigation of LPS effects, Muscle – Immune axis modulation….)

➔ **<u>maintain optimal performances</u>**