Vitamin D3 metabolite 1,25(OH)2D3 and immune response in broilers

As antibiotic-free broiler production, often even including ionophore coccidiostats, is becoming more common, the immunocompetence of birds becomes increasingly important. One way to achieve this is to modulate and support the immune system to increase disease resistance of the animals.

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For optimal performance, the immune system of animals needs to be in balance: it needs to be active enough to keep the animal resilient against (potential) pathogens, but not too active to cause allergies or a loss in performance. A major component is the control of the inflammatory reaction to prevent an overreaction of the immune response.

High stocking densities, (heat) stress, and intestinal infections can cause inflammatory reactions, and impair productive performance. During inflammation, nutrients are used to support the immune response while inflammatory mediators can suppress appetite, which further reduces nutrient availability for production.

Vitamin D3 metabolism

Vitamin D3 requires two metabolic steps to become biologically active. Initially, vitamin D3 is converted to 25-hydroxyvitamin D3 [25(OH)D3] in the liver. In a second step, 25(OH)D3 is metabolised to 1,25(OH)2D3, the metabolic active form of vitamin D3.

This last step is mediated by the enzyme 1α-hydroxylase and mainly takes place in the kidney. However, it has been discovered that also some extrarenal tissues show 1α-hydroxylase expression. 1,25(OH)2D3 then binds to the vitamin D3 receptor (VDR) to exert its biological functions.

Vitamin D3 is well-known for its function on calcium and phosphorus homeostasis. Only in the last decades the knowledge on additional effects such as fertility, muscle repair, cognitive functions and immunity became widely accepted. This was connected to the discovery of VDR in tissues and cell types that are not directly related to mineral metabolism, such as immune cells.

Vitamin D3 and immunity

Although some beneficial effects of vitamin D3 on the immune system have been observed for a long time, it has recently gained a lot of attention, especially in relation to the Covid-19 pandemic.

Vitamin D3 metabolites have been shown to act on different parts of the immune system, both on the innate and adaptive immunity.

Evidence gathered so far indicates that 1,25(OH)2D3 upregulates immune reactions,

while preventing excessive responses at the same time.

One of the key findings is a modulatory effect on inflammation, especially on chronic inflammation. In many studies, 1,25(OH)2D3 has been shown to increase the secretion of anti-inflammatory cytokines and decrease the production of pro-inflammatory cytokines.

Other studies showed that 1,25(OH)2D3 is important in immunomodulation. It increases 'self-tolerance' (reduced auto-immune reaction) and tolerance to non-dangerous molecules (for example allergens, embryonic cells).

Finally, 1,25(OH)2D3 also seems to play a role in the direct defence against pathogens by increasing the production of

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Fig. 1. Interleukin-10 level in the jejunum at day 21, four hours after the first LPS-injection.

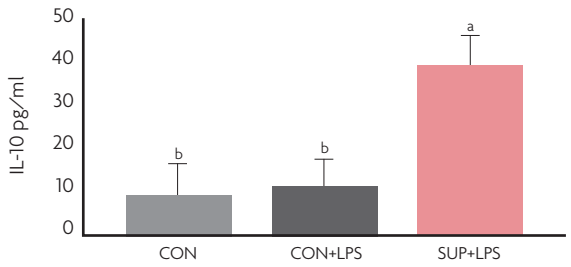
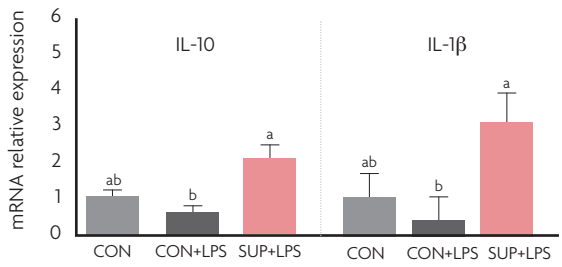


Fig. 2. mRNA expression of IL-10 and IL-1β in the spleen at day 21, four hours after the first LPS injection.



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antimicrobial peptides, which – among other functions – act as endogenous antimicrobials.

Supplementation of 1,25(OH)$_2$D$_3$ in lipopolysaccharide challenged broilers

Lipopolysaccharides (LPS) are large molecules found in the cell wall of Gram-negative bacteria such as E. coli or Salmonella spp. Injections with LPS are used to trigger and examine the inflammatory response.

A trial was performed to evaluate the effects of a plant-based source of the active metabolite of vitamin D$_3$ (1,25(OH)$_2$D$_3$-glycosides) on performance and inflammatory responses in LPS challenged broilers. 1,25(OH)$_2$D$_3$-glycosides predominantly occur in the plant Solanum glaucophyllum or waxy-leaf nightshade.

The glycosides have to be cleaved enzymatically in the gut, before the bioactive component (free 1,25(OH)$_2$D$_3$) can be absorbed. After absorption it does not need additional activation in the liver nor the kidney, and can immediately interact with the VDR in the body.

A total of 144, 20-day-old male Cobb 500 broilers, were divided into three treatments: a basal diet without LPS administration (CON); a basal diet with LPS administration (CON + LPS), and a basal diet supplemented with 5µg of 1,25(OH)$_2$D$_3$ from 1,25(OH)$_2$D$_3$-glycosides/kg of diet with LPS administration (SUP + LPS).

The LPS challenge was administered as intraperitoneal application at 21 and 23 days of age (1.0 and 1.12mg/kg BW, respectively). The performance of broilers was evaluated at 25 days of age, when all birds were slaughtered.

	CON	CON + LPS	SUP + LPS	Difference LPS (SUP - CON)
Weight gain (g/bird)	415.4[a]	377.1[b]	413.4[a]	+ 36.3g
Feed intake (g/bird)	566.1	548.5	560.9	+ 12.4g
FCR	1.36	1.45	1.36	-9 points

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 1. Performance of broilers from 20-25 days of age.

The values at day 25 were compared to the values of the birds at day 20.

At day 21, four hours after the LPS challenge, one bird per pen was selected for tissue sampling.

No antibiotic growth promoters or anticoccidials were used.

The supplementation of 1,25(OH)$_2$D$_3$-glycoside in LPS challenged birds improved performance to values similar to the unchallenged control (Table 1).

This effect may be related to the modulation of the inflammatory response caused by the active metabolite and less diversion of nutrients to support the immune response.

Jejunal interleukin-10

Levels of anti-inflammatory interleukin-10 (IL-10) in the jejunum of broilers were also measured. IL-10 is an important anti-inflammatory cytokine and important for developing immunotolerance.

In this trial the level of IL-10 in the jejunum four hours after the first LPS-injection was significantly higher (38.2pg/mL; P = 0.011) in birds challenged with LPS that received 1,25(OH)$_2$D$_3$-glycosides compared to those that received a control diet only, independent of the LPS-challenge (9.7pg/mL, on average) (Fig. 1).

mRNA expression of IL-10 and IL-1β

Among birds challenged with LPS, those supplemented with 1,25(OH)$_2$D$_3$-glycosides showed greater mRNA expression of IL-10 (P = 0.014) and IL-1β (P = 0.045) in the spleen four hours after the first LPS-injection (Fig. 2). IL-1β is a pro-inflammatory cytokine whose secretion is triggered by inflammatory agents such as LPS.

The increase in IL-1β can be seen as an indication of a quick reaction of the immune system to the LPS challenge, where the increase in IL-10 acts as a negative feedback to keep the inflammatory reaction under control.

In conclusion, dietary supplementation with 1,25(OH)$_2$D$_3$-glycosides preserves performance and modulates the inflammatory response by increasing the production of IL-10 in the jejunum and the mRNA expression of IL-10 and IL-1β in the spleen of broilers challenged with LPS, thus supporting the birds to better cope with the inflammatory challenge. ■

Nunes, R. A., et al. (2020). Active vitamin D3-glycoside preserves weight gain and modulates the inflammatory response in broiler chickens challenged with lipopolysaccharide. Animal Feed Science and Technology, 270