

*Nataliia Pakholkiv,*

Ph.D. (Veterinary Sciences), Senior Researcher, Laboratory of Physiology, Biochemistry and Nutrition of Poultry Institute of Animal Biology of National Academy of Agrarian Sciences of Ukraine, Ukraine  
orcid.org/0000-0002-5774-2940, e-mail: talokha@gmail.com

*Iryna Nevostruyeva,*

Ph.D. (Agricultural Sciences), Senior Researcher, S. Gzhytskyi Laboratory of Metabolism Institute of Animal Biology of National Academy of Agrarian Sciences of Ukraine, Ukraine  
orcid.org/0000-0002-5797-5592, e-mail: iryna.nevostruyeva@gmail.com

*Volodumura Hudyma,*

Ph.D. (agricultural sciences), Chief Specialist of the Department of Food Safety and Veterinary Medicine,

Main Office of the State Production and Consumer Service in Lviv region, Ukraine  
orcid.org/0000-0003-1234-1948, e-mail: vlada\_bdzilka@ukr.net

## EFFECT OF VITAMIN D<sub>3</sub> ON HEMATOLOGIC INDICES AND RESISTANCE OF LAYING HENS

**Abstract.** The content of vitamin D<sub>3</sub> in standard fodder for laying hens varies within 2,5-3,500 IU / kg. It is known that vitamin D is involved in bone metabolism and regulates calcium homeostasis in the body. Experiment was carried out in three groups of laying hens of the cross-breed Hayesks brown, which for 30 days fed fodder, the content of vitamin D<sub>3</sub> in which was respectively 2,5; 5,0 and 10,0 thousand IU / kg.

An increase in the content of vitamin D<sub>3</sub> from 2.5 to 10.0 thousand IU / kg increased the concentration of 25-OH D<sub>3</sub> in blood plasma by half (from 15.10 to 27.25 ng / ml). The dose of 5.0 ths. IU / kg did not affect the concentration of 25-OH D<sub>3</sub> (16.56 ng / ml). Increased vitamin D in the diet increased the number of red blood cell and, consequently, hemoglobin concentration in the blood ( $p < 0.05$ ) and decreased the number of white blood cell ( $p < 0.05-0.01$ ). In the blood of chickens that received high levels of vitamin D in the diet, more monocytes and neutrophils were detected. Particularly significant effects were observed for monocytes, the proportion of which in the blood of laying hens in group 2 increased by 1.2; and in laying hens of the third group - 1.6 times ( $p < 0.01$ ), compared to the laying hens of the 1st group. With an increase in the amount of vitamin D<sub>3</sub> in the diet of leucocytes, the proportion of lymphocytes decreased, the content of which in laying hens of 1-, 2- and 3rd groups was 58.95, respectively; 54.57 and 48.26%. Under the influence of feeding 10.0 thousand IU / kg of vitamin D<sub>3</sub> in the blood of hens, the phagocytic activity increased ( $p < 0.05$ ), which is consistent with the increase in the number of monocytes and neutrophils. The bactericidal activity of blood serum of laying hens of groups 2 and 3 (5.0 and 10.0 ths. IU / kg D<sub>3</sub>) was 20% higher ( $p < 0.05$ ) than in laying hens of group 1 (2, 5 thousand IU / kg D<sub>3</sub>). Thus, an increase in the diet of laying hens in vitamin D<sub>3</sub> increases the innate immunity and does not stimulate a specific link.

**Key words:** Laying hens, vitamin D<sub>3</sub>, 25-OH D<sub>3</sub>, red blood cells, hemoglobin, white blood cells contain, innate immunity.

## INTRODUCTION

Recently, the idea of the biological function of vitamin D has undergone significant changes related to the detection of 1- $\alpha$ -hydroxylase and calcitriol receptor in many cell types [1-3],

including in the cells of the immune system: lymphocytes, monocytes, antigen-presenting cells [4]. It has been established that vitamin D regulates the immune response, that is, the spectrum of its action is wider than previously thought [3-5, 9].

The receptor for vitamin D and 1- $\alpha$ -hydroxylase are available in T- and B-lymphocytes [12]. Vitamin D3 reduces the proliferation and differentiation of T-helper cells and regulates the production of cytokines by them [4, 6]. The inhibition of the effect of calcitriol on the proliferation of B-lymphocytes is mainly associated with its action on T-helper [5]. Vitamin D reduces the risk of autoimmune diseases, but the mechanisms of this action are inadequate and need to be further studied [5, 7, 8].

Vitamin D has an effect on leukopoiesis and erythropoiesis [9-11]. High levels of vitamin D3 receptor expression have been detected in cystic cells [11].

## MATERIALS AND METHODS

The experiment was conducted on three groups of hens cross "Hayseks brown." Laying hens received a standard full-fodder feed PC 1-18. Vitamin D3 in the diet of laying hens 1st (control) group was 2.5 thousand IU / kg. The diet of laying hens 2nd and 3rd groups were injected additional feed additive Romiviks D3 500, bringing vitamin D3 in the diet to 5.0 and 10.0 thousand IU / kg.

At the end of the experiment, in the 10 hens of each group, samples of venous blood were taken. The blood serum was determined by the content of 25-OH vitamin D by ELISA immunoassay using a set of reagents from Immundiagnostik (Germany). The number of red blood cell and white blood cell was counted in the chamber Goryaev. Concentration of hemoglobin in blood was determined by hemoglobinsin method. Phagocytic blood activity was determined by VM Mityushnikov (1985) using the daily Culture of Escherichia coli (strain VKM-125). Bactericidal activity of blood serum (according to P. A. Emelianenko (1980) using weakly pathogenic strain Escherichia coli VKM-125) and lysozyme activity of blood serum (according to A.G. Dorofeichuk (1983) using Micrococcus lysodeikticus daily strain VKM-109) were determined by nephelometric method [12].

## RESULTS

An increase in the diet of laying hens vitamin D3 led to an increase in serum concentrations hydroxylated form 25-OH D3, and these changes are not proportional to the dose of vitamin zhodovanoho (Table. 1). Thus, with increasing content in the diet of vitamin D3 from 2.5 to 5.0 thousand IU / kg concentration of 25-OH D3 serum changed slightly, while its further increase to 10.0 thousand. IU / kg increased its concentration almost twice ( $p < 0.001$ ).

Table 1

**The content of 25-hydroxyvitamin D3 in serum of hens ( $M \pm m$ ,  $n = 5$ )**

Indicator	The content of vitamin D3 in the diet		
	2,5 thousand IU/ kg	5,0 thousand IU / kg	10,0 thousand IU / kg
25-OH D3 ng / mL	15,12 $\pm$ 0,49	16,56 $\pm$ 0,44	27,25 $\pm$ 0,51*** ###

Note: In this and the following tables likelihood of differences between the control (1) and research (2 and 3) groups into account \* -  $p < 0,05$ ; \*\* -  $p < 0,01$ ; \*\*\* -  $p < 0,001$ ; the likelihood of differences between research (2) and research (3) groups # -  $p < 0,05$ ; ## -  $p < 0,01$ ; ### -  $p < 0,001$ .

Obviously, at a dose of 5.0 thousand IU / kg of feed 25-OH D3 almost completely translates into an active form of 1.25 (OH) 2, whereas at a dose of 10,000 IU / kg a significant portion of it continues to circulate in the blood channels

The increasing of vitamin D3 from 2.5 to 5.0 and 10.0 ths. IU / kg increased the number of red blood cell and, consequently, the concentration of hemoglobin in the blood ( $p < 0.05$ ) (Table 2). The number of red blood cell grew gradually in proportion to the increase in the amount of vitamin D. A slightly different pattern was found for hemoglobin. An increase in the content of vitamin D in the diet from 2.5 to 5.0 thousand IU / kg by one third increased its concentration in red blood cell. Instead, a further dose increasing of up to 10.0 ths. IU / kg did not significantly affect the hemoglobin concentration, which in the red blood cell of laying hens of the 2nd and 3rd groups did not differ significantly.

Table 2

**Hematological parameters of hens ( $M \pm m$ ,  $n = 10$ )**

Indicators	The content of vitamin D3 in the diet		
	2,5 thousand IU / kg	5,0 thousand IU / kg	10,0 thousand IU / kg
Red blood cells, T / L	2,89±0,14	3,23±0,30	3,49±0,23* #
Hemoglobin, g / l	100,68±8,18	127,69±8,19*	132,59±5,27* #
White blood cells, g/l	39,06±1,56	31,15±1,47**	34,33±1,77*

With the increase in the diet of vitamin D3 from 2.5 to 5.0 thousand IU / kg in the blood of hens, the number of white blood cell decreased ( $p < 0,05-0,01$ ). With further increase of vitamin D to 10.0 thousand IU / kg white blood cell count remained at the same level as that of a dose of 5.0 thousand IU / kg.

This assumption is consistent with changes in the number of lymphocytes in the blood (Table 3). Although the relative proportion of lymphocytes in the white blood cell formula of laying hens in the 2nd experimental group decreased insignificantly, the absolute number of lymphocytes in this group, taking into account the lower total number of white blood cell, was significantly lower than in the control. Consequently, a greater number of white blood cells in laying hens of group 1 is due to an increase in the number of lymphocytes that are responsible for a specific immune response. In the blood group's white blood cell hens, the third group observed a further decrease in the percentage of lymphocytes ( $p < 0.05$ ), however taking into account a slightly larger total number of white blood cell the absolute number of lymphocytes in the laying hens of the 2nd and 3rd experimental groups did not differ significantly.

In the blood of laying hens that received high levels of vitamin D3 in the diet, more monocytes and neutrophils were detected, that is, cells responsible for phagocytosis. Particularly significant effects were found for monocytes, the proportion of which in laying hens of the 2 nd group increased by 1.2; and in laying hens of the third group - 1.6 times ( $p < 0.01$ ), compared to the laying hens of the 1st group.

The bactericidal activity of the blood serum is approximately the same in laying hens of groups 2 and 3 (Table 4), which received in the diet of 5.0 and 10.0 thousand IU / kg of vitamin D3, while in the first group hens receiving 2.5 Thousands of IU / kg of vitamin D3, this figure was 20% lower ( $p < 0.05$ ).

Table 3

**White blood cells formula, % (M ± m, n = 10)**

Indicators	The content of vitamin D3 in the diet		
	2,5 thousand IU / kg	5,0 thousand IU / kg	10,0 thousand IU / kg
Lymphocytes, %	58,95±2,49	54,57±1,59**	48,26±1,97*#
Neutrophils, %	27,42±2,52	31,43±1,41	35,34±1,71*
Monocytes, %	5,17±0,66	6,30±0,36	8,52±0,53**##
Basophils, %	2,51±0,14	2,34±0,11	2,44±0,22
Eosinophils, %	5,95±0,48	5,36±0,63	5,44±0,40

Table 4

**Indicators of innate imunité (M ± m, n = 10)**

Indicators	The content of vitamin D3 in the diet		
	2,5 thousand IU / kg	5,0 thousand IU / kg	10,0 thousand IU / kg
Bactericidal act., %	62,24±0,97	74,98±2,40**	72,07±2,56**
Lysozyme Act, %	23,81±2,28	18,22±2,24	26,62±3,28
Phagocytosis %	31,18±1,92	33,59±2,11	38,42±1,97*

Thus, the increase in the diet of laying vitamin D3 enhances innate immunity, and it does not stimulate a specific link. Moreover, the number of lymphocytes in this case decreases. Obviously, the more effective prevention of penetration into the body of antigenic factors reduces the need of proliferation of white blood cells.

**CONCLUSIONS**

1. Increased vitamin D3 in the diet increases the number of red blood cell and hemoglobin concentration and reduces the amount of white blood cell in the blood of laying hens.

2. For a greater amount of vitamin D3 in the diet in the blood of hens, the number of monocytes and neutrophils increases and phagocytic and bactericidal activity increases.

**PROSPECTS FOR FURTHER RESEARCH**

In the future, it is necessary to investigate the effect of high doses of vitamin D3 on parameters specific resistance in laying hens.

**REFERENCES**

1. Ceglia L. Vitamin D and its role in skeletal muscle. *Curr. Opin. Clin. Nutr. Metab. Care*, 2009, vol. 12(6), pp. 628–633.
2. Bikle D. D. Vitamin D and bone *Curr Osteoporos Rep.* 2012; 10(2), pp. 151–159.
3. Kamen D. L., Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *J. Mol. Med. (Berl)*, 2010, vol. 88(5), pp. 441–450.
4. Aranow C. Vitamin D and the Immune System. *J. Investig. Med.*, 2011, vol. 59(6), pp. 881–886.
5. Prietl B., Treiber G., Pieber T. R., Amrein K. Vitamin D and immune function. *Nutrients*, 2013, no 5, pp. 2502–2521.

6. Adams J. S., Hewison M. Update in vitamin D. *J. Clin. Endocrinol. Metab.*, 2010, vol. 95, pp. 471–478.
7. Holick M. F. Vitamin D deficiency. *N. Engl. J. Med.*, 2007, vol. 357, pp. 266–281.
8. Battault S., Whiting S. J., Peltier S. L., Sadrin S., Gerber G., Maixent J. M. Vitamin D metabolism, functions and needs: From science to health claims. *Eur. J. Nutr.*, 2013, vol. 52, pp. 429–441.
9. Hewison M. An update on vitamin D and human immunity. *Clin. Endocrinol*, 2012, vol. 76, pp. 315–325.
10. Baeke F., Takiishi T., Korf H., Gysemans C., Mathieu C. Vitamin D: Modulator of the immune system. *Curr. Opin. Pharmacol*, 2010, vol. 10, pp. 482–496.
11. White J.H. Vitamin D metabolism and signaling in the immune system. *Rev. Endocr. Metab. Disord.*, 2012, vol. 13, pp. 21–29.
12. Ferreira G. B., van Etten E., Verstuyf A., Waer M., Overbergh L., Gysemans C., Mathieu C. 1,25-Dihydroxyvitamin D<sub>3</sub> alters murine dendritic cell behaviour in vitro and in vivo. *Diabetes Metab. Res. Rev.*, 2011, vol. 27, pp. 933–941.