Effects of Parenteral Vitamin D3 Supplementation on Hematological Parameters of Healthy Holstein bulls


1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran
2. Department of Health Food Education, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
3. Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.

Corresponding author: mkeywanloo@semnan.ac.ir

Abstract

Vitamin D has been shown to play physiological functions beyond calcium and phosphorus homeostasis and control of bone metabolism in the body, as its cellular receptors are present in numerous tissues. Twenty healthy bulls were divided into four groups to evaluate the effect of different doses of vitamin D3 on the number of bovine blood cells. Group A received 11,000 units/kg of vitamin D3, group B received 22,000 units/kg of vitamin D3, group C received 33,000 units/kg of vitamin D3, and group D received 44,000 units/kg of vitamin D3. The control group was injected with 10 ml of physiological saline intramuscularly. Blood samples were taken before the injection and 2, 4 and 6 days after the injection and the WBC counts (including granulocytes and lymphocytes), hematocrit, hemoglobin and platelets were examined by a cell counter. The results showed that vitamin D can cause leukopenia (such as neutropenia and lymphopenia) and thrombocytopenia and an increase in hematocrit and hemoglobin levels in the blood. Although the increase or decrease mentioned is largely dose- and time-dependent, first, the best group to indicate this is group B, and second, perhaps to study the long-term effects of injections, especially High doses require tests to be performed with larger groups over a longer period.

Keywords: Vitamin D3, White blood cell, Hematocrit, Hemoglobin, Platelet, Cattle.

Introduction

The function of vitamin D is classically known to be a factor in regulating calcium metabolism and bone health. However, different physiological roles of vitamin D have been shown in recent years and it has been observed that in different cell types, genes related to vitamin D receptors are expressed (Titmarsh et al. 2017). Vitamin D is a steroid-like hormone that works by binding to its receptors (VDR). The calcium function of vitamin D is related to calcium metabolism, but its non-calcium function is related to the role of vitamin D in diseases such as hypertension, diabetes mellitus, cardiovascular disease, cancer, and autoimmune diseases. These roles are very broad because vitamin D plays a role in the widespread expression of many genes in cells. The role of vitamin D in regulating the function of immune cells, as well as its role in the proliferation,
differentiation and differentiation of hematopoietic cells, is one of those non-calcium roles of vitamin D. A good example is that vitamin D plays a role in the treatment of blood malignancies (Medrano et al. 2018).

Vitamin D is involved in the regulation and proliferation of many cells in the body, such as the normal growth of breast cells and liver cells, as well as the maturation of pneumocytes (Medrano et al. 2018). As mentioned earlier, the immunomodulatory function of vitamin D has been demonstrated in numerous studies (Luong, Nguyen, and Pham Nguyen 2005). This role, which affects the function of the immune system, can be due to the effect of vitamin D on innate immunity. For example, macrophages and monocytes play a key role in initiating nonspecific responses to pathogens or tissue damage, and vitamin D can differentiate monocyte precursors into adult macrophages (Tanaka et al. 1983). Because vitamin D is a potent regulator of monocyte activity and, for example, following a tissue transplant, inflammatory responses can themselves cause severe tissue damage, vitamin D can reduce the severe tissue damage associated with inflammation (Sadeghi et al. 2006). Besides, vitamin D inhibits the maturation of monocyte dendritic cells derived from monocytes and suppresses their ability to present antigens to T cells (Griffin et al. 2000). Increased expression of vitamin D receptors also occurs with activation of T cells. It is possible that vitamin D exerts its effects on inflammation and autoimmune diseases through its regulatory effect on T helper 17 cell and ultimately affects regulatory T cells (Boonstra et al. 2001). As seen in T cells, activated B cells also increase the expression of Vitamin D Receptor (VDR) genes, so activated B cells can metabolize vitamin D and respond to vitamin D (Chen et al. 2007).

Other studies show that vitamin D is also effective in erythropoiesis because VDR is expressed in the bone marrow by certain cellular subtypes such as stromal and accessory cells (Zhou, LeBoff, and Glowacki 2010). Elevated haemoglobin and HTC levels are seen following vitamin D treatment (Aucella et al. 2003).

Although many studies have examined the relationship between serum vitamin D concentrations and inflammation, there are not many studies that have examined the relationship between serum vitamin D concentrations and leukocyte populations in humans or animals. In a study done in smokers, was shown that there was an inverse relationship between vitamin D concentration and white blood cell count (Lee et al. 2015). Another study by Aronson found that there was a negative correlation between vitamin D content and WBC counts and a negative correlation between vitamin D and haemoglobin (Aronson et al. 2012). Another study of cats referred to a veterinary hospital (Hospital for Small Animals, Royal (Dick) School of Veterinary Studies) found that cats with neutrophilia had lower levels of vitamin D than cats with lower neutrophils in the normal range (Titmarsh et al. 2017).

However, no study has been done on the relationship between vitamin D and the count and indices of blood cells in cattle. In the present study, we aimed to investigate the effect and relationship between different doses of vitamin D on changes in white blood cells (including lymphocytes and granulocytes), hematocrit, haemoglobin and platelets in cattle.

As in previous studies, it has been concluded that the expression of genes affected by vitamin D, is dose and time-dependent (Zhong et al. 2014), The aim of this study was the understanding of
changes in the count of blood cells, following the change in the dose of vitamin D supplemented? And if a change occurs, are these changes dose and time-dependent?

Although the results of the effect of sex hormones on the function and number of blood cells are different, the results of research based on a meta-analysis study showed that testosterone has a moderate immune suppressant effect on immune function. Testosterone suppresses immune function, and the effect of estrogen varies depending on the measurement of immunity used. On the other hand, the effect of estrogen depends on the level of immunity used. Estrogen suppresses cell-mediated immune function while reducing the parasitic load. These results suggest that these correlation studies have a limited value for studying the effects of sex hormones on immune function. Finally, it can be concluded that the use of bulls, due to less stress than female bulls (including milking and pregnancy) and due to the possibility of homogeneity of experimental groups, is a good model for generalizing the results to Bovine (Foo et al. 2017).

Material and method

Twenty Holstein bulls (Yue et al. 2018), weighing approximately 300 kg, were randomly divided into five groups. The health of all cows was ensured. The bulls were fed with TMR diet consisting of 1 kg of hay, 1 kg of wheat straw, 6 kg of corn silage, and 4.5 kg of grain concentrate (per head). Group A was the group that received 3,300,000 units of vitamin D3 injected intramuscularly (with the same therapeutic dose for cows) (Constable et al. 2016). In group B, 6,600,000 units (twice the therapeutic dose), in group C, 9,900,000 units (three times the therapeutic dose) and in group D, 132,000,000 units (four times the therapeutic dose) were injected intramuscularly into cows. The other group is the control group and received 10 ml of physiological saline by intramuscular injection. Blood sampling was performed intravenously, just before the vitamin D and physiological serum injection, two days after the injection, four days after the injection, and 6 days after the injection. This study was conducted by the Faculty of Veterinary Medicine of Semnan University at the Deimeh Farm (50 km from Garmsar-Semnan road) in the fall of 2018. Whole blood samples were taken immediately using the Kinhoden Model Cell Counter (Made in Japan: The device is made for human and animal purposes and can be used for different animal species by setting up the device), and the values of White Blood cell (WBC), Hemoglobin (HGB), Hematocrit (HCT), Platelet (PLT), lymphocytes and neutrophils were measured. These tests were performed for all groups and after each blood sampling.

To assess the normal distribution of data, Shapiro–Wilk test was used and most parts of the data were non-parametric. Moreover, due to the few data in each group, non-parametric analyses of data was considered. Friedman test was used to compare the differences between measured values over time. In order to make a comparison between different groups at each time point, Kruskal-Wallis was implemented. Statistical analysis of the samples was performed using SPSS 20 software.

Results

There was no statistically significant difference in group A and C between the WBC counts at different times (p > 0.05) and there was a significant difference in groups D and B (p < 0.05) but...
according to the results of the count of WBC and shown in graph 1, the trend of decreasing the amount of WBC from day 0 (exactly the moment before the injection of vitamin D) to the sixth day after the injection is quite evident and this change is observed in all groups. However, this decrease was the lowest in group A and the highest in group D. There was a statistically significant difference in groups A, B and D between Hematocrit (HCT) values at different times but there was no significant difference in group C. As can be seen from the results for hematocrit (HTC), as shown in Graph 2, the trend of increasing HTC values from day 0 to day 6 after injection is evident, and this change is observed in all groups, although this increase was the lowest in group A and the highest in group D. Although significant differences was detected in Mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), values in just group B over time, Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values showed no significant differences over time in all groups (Table 1). There was a statistically significant difference in all groups between the levels of Haemoglobin (Hb) at different times (p < 0.05). Haemoglobin (Hb) levels increase in different groups with a slight slope during the days after vitamin D administration, but this upward trend peaks on the sixth day. Interestingly, the growth rate of haemoglobin during these six days is lower in group A than in the other groups and higher in group D than in the other groups (Graph 3). There was a significant difference in groups A, B and C between platelet (PLT) values at different times but there was no significant difference in group D. The measurement of platelets during the six days after vitamin D administration is shown in Graph 4, and as shown, the values decrease in all groups and on the sixth day are less than day 0 value in the same group. However, the severity of the decrease in group D is not higher than the other groups. Thus, the change in platelet count in group C shows the greatest decrease, and in the next rank is group B. In groups A and D, the platelet count decreases until the sixth day, and this decrease in groups A and D goes through a similar process. Regarding platelet distribution width (PDW), no significant differences was detected from day 0 to day 6 in all groups (Table 2). There was a significant difference in groups A and C between the counts of lymphocytes at different times, but there was no significant difference in groups D and B. There was a significant difference in all groups between the counts of granulocytes at different times. As mentioned in the case of decreasing changes in WBC values, there is a similar pattern in the number of lymphocytes and granulocytes, and as shown in graphs 6 and 7, the lymphocyte and granulocyte counts decrease over 6 days after injection of vitamin D and just like the WBC graph, the reduction is lowest in Group A and highest in Group D.

**Discussion**

The results of the present study show that vitamin D cannot reduce the counts of WBC in therapeutic doses and increasing the dose of vitamin D supplement to cattle to four times the usual dose, causes a sharp decrease in the counts of WBC (Leucopenia). Although there are overall declining changes in WBC values in both Group A and Group C, these changes are not statistically significant. White Blood Cell counts are within the normal range of bovine species (4900–12,000 cells/µl) (Constable et al. 2016), both before and up to 6 days after vitamin D injection. As Vanham showed in 1988, vitamin D has distinct antiproliferative effects (Vanham et al. 1988). These results may confirm the results of our study, but Icardi in 2013 showed that vitamin D can regulate systemic cytokines and increase the WBC (Icardi et al. 2013). Despite these conflicting results
with our research, it is accepted that vitamin D plays a very important role in the immunomodulation of the immune system (Luong, Nguyen, and Pham Nguyen 2005). In many cases, serum vitamin D levels can be changed following inflammation, and even vitamin D can act as a negative acute-phase protein (Waldron et al. 2013), and hypovitaminosis D may be a consequence of chronic inflammatory disease rather than the cause of it. This means that vitamin D supplementation can be a beneficial treatment for many inflammatory diseases and in many inflammatory diseases, such as tissue transplant complications, reducing the number of white blood cells helps to prevent progression of the disease (He et al. 2014). Evidence suggests that hypovitaminosis D may predispose to an acute inflammatory response (Bertoldo et al. 2010). To confirm the observations confirming the anti-proliferative effects of vitamin D, studies have been conducted on the effectiveness of vitamin D in the form of a chemotherapy drug for cancer (Ma, Johnson, and Trump 2016) and it has been proven that vitamin D deficiency in chronic leukaemia has been associated with poor prognosis and decreased patient survival (Aref, Ibrahim, and Azmy 2013). However, the results showed that vitamin D reduced the WBC, but the question is, does an increase in vitamin D dosage further reduce the counts of WBC? The answer is yes. Soliman in 2012 showed that the administration of a megadose of vitamin D in children led to an increase in white blood cell counts, but this increase was not statistically significant (Soliman et al. 2012). Titmarsh hypothesized in 2016 that there may be a negative correlation between vitamin D levels and WBC counts (Titmarsh et al. 2017). The change in the leukocyte counts is more affected by the changes in lymphocytes and neutrophils. Increasing the expression of vitamin D receptors by activating T cells makes it possible for vitamin D to exert its effects on inflammation and autoimmune diseases through its regulatory effect on Th17 cells (Weaver et al. 2007).

As seen in T cells, active B cells increase the expression of Vitamin D Receptor (VDR) genes. Therefore, with the activation of cells, the expression of mRNA genes related to Vitamin D Receptor (VDR) increases. Some studies suggest that Vitamin D Receptor (VDR) expression in leukocyte subsets can increase leukocyte migration from tissue to blood (Brennan et al. 1987). In another study in 1984, the results showed that vitamin D had an inhibitory effect on lymphocyte proliferation (Rigby, Stacy, and Fanger 1984). On the effect of vitamin D on neutrophil populations, a 2016 study by Titmarsh found that cats with neutrophils had lower serum vitamin D than cats with normal neutrophil populations (Titmarsh et al. 2017). A human study in 2011 showed a negative relationship between neutrophil count and vitamin D (Hollams et al. 2011). Surveys in 2013 have confirmed such results (Gorman et al. 2013).

The results of our study also show that the levels of lymphocytes and neutrophils decrease (Neutropenia and lymphopenia) within six days after vitamin D injection, and this decrease follows a steep slope in group D. Decreases in lymphocyte and granulocyte counts (lymphopenia) were not observed in all groups, but in groups B and D related to lymphocytes, this decrease was not statistically significant. This negative relationship between vitamin D and Granulocytes has been in line with the results of previous studies.

Studies have been performed on the relationship between vitamin D and hematocrit as well as haemoglobin, and these findings indicate that vitamin D affects erythropoiesis (Zhou, LeBoff, and Glowacki 2010) and increases in haemoglobin and hematocrit occur after treatment with vitamin D (Aucella et al. 2003). Vitamin D can stimulate erythrocyte precursor cell receptors, which enhance the maturation and proliferation of congenital erythroid cells. Vitamin D may increase the production of erythropoietin (Icardi et al. 2013). Another study found that administering vitamin
D to patients with inflammation could improve anaemia (Arabi et al. 2020). Marwah showed in 2012 that there was a positive association between vitamin D and haemoglobin and red blood cell counts (Marwah, Walls, and Blann 2012). On the other hand, Doudin in 2018 showed that vitamin D has an inhibitory role in regulating hematopoiesis in adolescents and there is an indirect relationship between haemoglobin parameters including haemoglobin and RBC and serum levels of vitamin D. The author attributes this indirect link to the possible role of this circulating steroid hormone (vitamin D) in the formation of RBC from homocytoblasts (Doudin et al. 2018).

The results of our study show that vitamin D at higher doses (four times the usual dose) can increase the concentration of haemoglobin on days 4 to 6 after injection. At normal doses, vitamin D injections do not affect increasing or decreasing haemoglobin levels (The changes were statistically significant in all groups).

As the results of the present study show, increasing the dose of vitamin D can lead to an increase in hematocrit in all groups that received vitamin D, but this increase in group C does not show a statistically significant difference. Among these, the highest increase in hematocrit is from group D (The group that received the most vitamin D). Based on this, it can be said that vitamin D has a direct and dose-dependent relationship with the amount of hematocrit and haemoglobin.

Another blood cell whose effectiveness in injecting vitamin D has been studied is platelet count. Platelets are active cell structures that do not have a nucleus that is made up of megakaryocytes in the bone marrow and play an important role in blood flow as well as to inflammation (Wang et al. 2012). Akbas in 2016 showed that there is a negative relationship between vitamin D deficiency and platelet count (Akbas et al. 2016). Park (2017) showed that there is a negative relationship between vitamin D levels and blood platelet count (Park et al. 2017). Yildirim in 2016 showed that vitamin D deficiency may play a role in increasing MPV as an indicator of platelet size (Yildirim et al. 2016). Kara (2019) also showed a clear negative relationship between serum levels of vitamin D and platelet counts (Kara and Soylu 2019). According to studies, vitamin D has anti-thrombogenic, anti-inflammatory and anticoagulant activity. Aihara in 2004 showed that the vitamin D-VDR system may play an important role in anti-thrombogenic function in vivo samples. The vitamin D-VDR system appears to increase the expression of anti-thrombogenic factor genes and inhibit the expression of thrombogenic genes (Aihara et al. 2004).

In our study, the effect of vitamin D administration on reducing platelet count is seen. Platelet counts decreased in all groups (except the control group) from the day of injection until the sixth day after injection, but these changes in group D are not statistically significant.

According to all the information extracted from the present study, it can be said that vitamin D can increase the count of white blood cells by increasing the dose (this decrease is due to a decrease in the count of lymphocytes and neutrophils). It also reduces platelet counts. An increase in the amount of haemoglobin and hematocrit also results from an increase in the dose of vitamin D. But to answer the question of how much the increase in vitamin D3 dose intensifies these reductions or increases, it may be said that the increase or decrease caused by group B is more specific than other groups and is statistically significant (except for lymphocyte count test).
Due to the risk of poisoning at high doses of vitamin D3, if we want favourable changes in blood cells, it may be reasonable to say that increasing the dose of vitamin D to twice the usual dose (recommended for best calcemic effects) is reasonable.

In conclusion, the results of our research show that the use of vitamin D causes certain effects on blood cells, which show their effects better with increasing dose and over time. Since it was impossible to follow the cows for blood sampling and examination in the following weeks and months, further studies over longer periods may have shown better and more accurate results, especially regarding the effect of high doses of vitamin D on blood cell counts. Because the expression of VDR-dependent genes in leukocyte subsets can increase leukocyte migration from tissue to blood (Brennan et al. 1987). Erythroid progenitor cells in the bone marrow undergo a series of regulated processes related to cell proliferation and differentiation. Given the longer lifespan of red blood cells compared to white blood cells (which are more susceptible to removal and isolation by the spleen) (Doudin et al. 2018), it is likely that changes in red blood cells due to vitamin D administration may require a longer time.

**Conflict of interest:** The authors declare that there is no conflict of interest regarding the publication of this article

**References**


Boonstra, André, Franck J Barrat, Chad Crain, Victoria L Heath, Huub FJ Savelkoul, and Anne O’Garra. 2001. ‘1α, 25-Dihydroxyvitamin D3 has a direct effect on naive CD4+ T cells to enhance the development of Th2 cells’, The Journal of Immunology, 167: 4974-80.


Gorman, S, CE Weeden, DHW Tan, NM Scott, and J Hart. 2013. ‘Reversible Control by Vitamin D of Granulocytes and Bacteria in the Lungs of Mice: An’.


He, Xiyue, Juan Yan, Xiaotong Zhu, Qinghui Wang, Wei Pang, Zanmei Qi, Meilian Wang, Enjie Luo, Daniel M Parker, and Margherita T Cantorna. 2014. ‘Vitamin D inhibits the occurrence of experimental cerebral malaria in mice by suppressing the host inflammatory response’, The Journal of Immunology, 193: 1314-23.


Ma, Yingyu, Candace S Johnson, and Donald L Trump. 2016. ‘Mechanistic insights of vitamin D anticancer effects.’ in, Vitamins & Hormones (Elsevier).


Wang, Yiming, Marc Andrews, Yan Yang, Sean Lang, Joseph W Jin, Alison Cameron-Vendrig, Guangheng Zhu, Adili Reheman, and Heyu Ni. 2012. 'Platelets in thrombosis and hemostasis: old topic with new mechanisms', Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders), 12: 126-32.


Yildirim, Tulay, Dilek Solmaz, Gurkan Akgoł, and Yuksel Ersoy. 2016. 'Relationship between mean platelet volume and vitamin D deficiency in fibromyalgia'.


Graph 1. White blood cell (WBC) counts on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control.

Graph 2. Hematocrit (HTC) percents on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control
Graph 3. Hemoglobin (HGB) values on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control.

Graph 4. Platelet levels (PLT) on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control.
Graph 5. Lymphocyte counts (L) on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control.

Graph 6. Granulocyte counts (Gr) on day 0 and on days two, four and six after vitamin D administration in groups A, B, C, D and Control.
### Table 1. Red Blood Cell indices including Mean Corpuscular Volume (MCV) (fL), Mean Corpuscular Hemoglobin (MCH)(pg), and Mean Corpuscular Hemoglobin Concentration (MCHC)(g/dL) in different groups during days 0 to 6.

<table>
<thead>
<tr>
<th>Group</th>
<th>MCV0</th>
<th>MCV2</th>
<th>MCV4</th>
<th>MCV6</th>
<th>MCH0</th>
<th>MCH2</th>
<th>MCH4</th>
<th>MCH6</th>
<th>MCHC0</th>
<th>MCHC2</th>
<th>MCHC4</th>
<th>MCHC6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>34.12</td>
<td>32.40</td>
<td>33.20</td>
<td>10.32</td>
<td>10.75</td>
<td>9.87</td>
<td>12.57</td>
<td>28.87</td>
<td>29.90</td>
<td>29.97</td>
<td>30.07</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>31.25</td>
<td>31.92</td>
<td>35.10</td>
<td>37.30</td>
<td>9.70</td>
<td>11.90</td>
<td>11.80</td>
<td>11.42</td>
<td>30.20</td>
<td>29.35</td>
<td>30.60</td>
<td>31.40</td>
</tr>
<tr>
<td>C</td>
<td>33.52</td>
<td>35.52</td>
<td>37.07</td>
<td>39.02</td>
<td>10.27</td>
<td>12.80</td>
<td>12.02</td>
<td>12.40</td>
<td>28.47</td>
<td>30.10</td>
<td>30.52</td>
<td>31.42</td>
</tr>
<tr>
<td>D</td>
<td>30.62</td>
<td>30.95</td>
<td>35.00</td>
<td>36.12</td>
<td>9.37</td>
<td>9.55</td>
<td>12.40</td>
<td>11.70</td>
<td>28.05</td>
<td>29.35</td>
<td>30.60</td>
<td>31.42</td>
</tr>
<tr>
<td>Control</td>
<td>30.40</td>
<td>33.20</td>
<td>29.10</td>
<td>10.68</td>
<td>12.10</td>
<td>10.50</td>
<td>10.35</td>
<td>29.85</td>
<td>30.05</td>
<td>29.90</td>
<td>28.70</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Platelet distribution width (PDW) (%) and Platelet levels (PLT) (10^9/L) on day 0 and on days two, four and six after vitamin D administration in groups A, B, C and D and control.

<table>
<thead>
<tr>
<th>Group</th>
<th>PLT0</th>
<th>PDW0</th>
<th>PLT2</th>
<th>PDW2</th>
<th>PLT4</th>
<th>PDW4</th>
<th>PLT6</th>
<th>PDW6</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>802.5</td>
<td>17.70</td>
<td>735.75</td>
<td>14.70</td>
<td>745.00</td>
<td>14.17</td>
<td>702.5</td>
<td>14.05</td>
</tr>
<tr>
<td>B</td>
<td>765.5</td>
<td>14.20</td>
<td>725.00</td>
<td>14.20</td>
<td>685.00</td>
<td>14.10</td>
<td>622.5</td>
<td>13.85</td>
</tr>
<tr>
<td>C</td>
<td>763.75</td>
<td>13.92</td>
<td>620.00</td>
<td>13.92</td>
<td>597.50</td>
<td>13.95</td>
<td>615.00</td>
<td>13.87</td>
</tr>
<tr>
<td>D</td>
<td>748.75</td>
<td>14.27</td>
<td>705.00</td>
<td>14.27</td>
<td>700.00</td>
<td>13.85</td>
<td>672.5</td>
<td>14.02</td>
</tr>
<tr>
<td>Control</td>
<td>770.85</td>
<td>13.80</td>
<td>770.55</td>
<td>12.80</td>
<td>770.85</td>
<td>13.25</td>
<td>770.75</td>
<td>13.90</td>
</tr>
</tbody>
</table>