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Effect of Parenteral Vitamin D3 Supplementation in Several Doses During a Six-day Period on Total Antioxidant Capacity in Healthy Holstein Bulls

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Abstract

BACKGROUND: Vitamin D3 is one of the most important vitamins that can be used either as an injection or as an oral supplement, alone or in combination with other vitamins in cows. Vitamin D3 has known effects on calcium regulation and bone health, and also has several non-calcium effects, including improving immune function and therapeutic and preventive effects on many chronic diseases.

OBJECTIVES: Many of these non-classical effects of vitamin D appear to be due to the effect of vitamin D on improving the body's antioxidant system. This positive effect on the antioxidant status can be due to the effect of vitamin D on the expression of many genes, including genes related to proteins involved in the antioxidant system such as Superoxide dismutase (SOD) and Catalase.

METHODS: In the present study, 15 bulls were divided into 3 groups and by intramuscular injection of 3 doses of vitamin D3, their serum levels of total antioxidant capacity (TAC) were calculated before injection and in two, four and six days after injection. The amounts of vitamins used in groups A, B and C were 3300000, 6600000 and 9900000 units, respectively.

RESULTS: The results of the study showed that TAC levels increase in groups depending on the dose. Total anti-oxidant capacity levels will also increase in the following days.

CONCLUSIONS: Therefore, it can be concluded that TAC in cattle is time-dependent and dose-dependent, and regardless of the therapeutic dose of vitamin D3 the use of higher doses of vitamin D3 can improve the antioxidant status.

KEYWORDS: Cattle, Parenteral, Six days, Total Antioxidant Capacity, Vitamin D_3

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Introduction

There is ample evidence that oxidative stress plays an important role in the development of chronic diseases such as diabetes and cardiovascular diseases. Because oxidative stress is the product of an imbalance between the reactive oxygen species (ROS) production and antioxidant responses, and any increase in antioxidant responses can ultimately lead to a reduction in oxidative stress.

Total antioxidant capacity (TAC) is used as an appropriate tool in determining the oxidative properties of cells (Zhou *et al*., 2018).

To improve the antioxidant status and consequently the antioxidant capacity, antioxidant compounds and substances can be provided to the animals as supplements. For this purpose, various vitamins are used in the diet or by injection, during the transition period of pregnant cows or the first days of calf birth. Vitamins A, E, and D are commonly used in cows (Mattioli *et al.*, 2020), either individually or in combination (Aktas *et al*., 2011).

One of the most important vitamins with antioxidant properties is vitamin D, which is related to the non-calcium function (Hochberg and Hochberg, 2019; Maalouf, 2008). Vitamin D plays a key role in the regulation and homeostasis of calcium in the body and is produced through the skin and liver as well as the kidneys.

When ultraviolet (UV) rays shine on the surface of the skin, the compound 7-dehydroxycholesterol is converted to pro-vitamin D, which acts on the skin. Then, pro-vitamin is transferred to the liver, during the process of hydroxylation, calcidiol is produced, which is eventually converted to 1,25-Dihydroxycholecalciferol in the kidney, which is the biologically active form of vitamin D (O'Brien and Jackson, 2012).

Vitamin D receptors (VDR) are found in almost every vertebrate cell in addition to the tissues such as bone, kidney, and intestine (Uwitonze and Razzaque, 2018). These receptors are important in the function of vitamin D for the bone health as well as the non-calcium function of vitamin D.

These receptors, both in the nucleus and in the cell membrane, increase the transcription and expression of many genes under the influence of vitamin D. Various non-calcium roles of vitamin D, such as reducing the risk of chronic and autoimmune diseases and type 2 diabetes, are due to the effect of vitamin D on the wide expression of genes (Mokhtari *et al., 2016)*.

Various studies have shown that the use of vitamin D supplements in humans, causes extensive regulatory activities of the genes in white blood cells, which are dependent on the epigenetic modification and function of the immune system (Hossein-Nezhad *et al.*, 2013).

Studies showed that different doses of vitamin D have different effects on parathyroid hormone and gene expression.

Human studies have shown that gene expression is directly dependent on the dose of consumed vitamin D; Thus, when the dose of vitamin D3 is increased up to 10,000 units/day, the amount of gene expression continues, while the decrease in parathyroid hormone (PTH) at a dose of 4,000 units/day remains in a plateau state and does not change followed by an increase in Vitamin D3 doses (Shirvani *et al*., 2019).

The expression of many genes involved in improving antioxidant capacity is increased following vitamin D supplementation, which, as noted, is dependent on increasing the dose of vitamin D supplemented (Sepidarkish et al., 2019).

The expression of several molecules involved in the antioxidant defense system including glutathione, glutathione peroxidase and superoxide dismutase (SOD) improves the antioxidant capacity. Besides, the suppression of NADPH gene expression occurs as an important source of ROS (Mokhtari *et al.*, 2016).

A daily intake of 7 to 12 units/kg is a good amount for a cow, especially if the cow does not have enough access to the sunlight. The recommended injectable dose of vitamin D for cattle is 11,000 units/kg, which can meet the needs for the vitamin D in the caws for 3 to 6 months. Injections of 15 to 17 million units of vitamin D can cause poisoning (Constable *et al*., 2016).

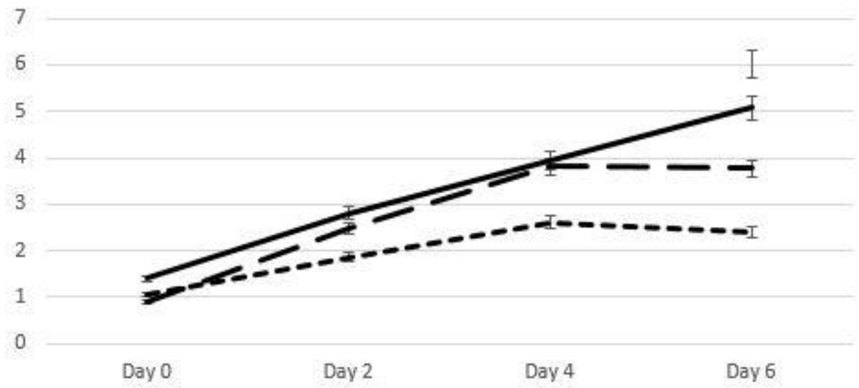

Figure 1. TAC levels during day 0 (the day before injection), day 2, day 4 and day 6 after vitamin D injection in groups A, B and C

The use of recommended amounts of vitamin D in the cattle makes us confident that we have prevented bone disorders, but as noted, human studies have shown that increased expression of various genes, including genes related to the antioxidant compounds, is dose-dependent and increasing the vitamin D supplement will increase the expression of these genes and thus improves TAC.

In this study, different doses of vitamin D were injected into the cattle to investigate its effect on the changes in TAC.

Materials and Methods

Fifteen Holstein bulls, weighing approximately 300 kg, were randomly divided into 3 groups of five cows. The health of all cows was ensured.

The bulls were fed with TMR diet consisting of 1 kg of alfalfa hay, 1 kg of wheat straw, 6 kg of corn silage, and 4.5 kg of grain concentrate (per head). Group A received 3,300,000 units of vitamin D3 injected intramuscularly (with the same therapeutic dose for cows). In group B and group C, 6,600,000 units (twice the therapeutic dose) and 9,900,000 units were injected intramuscularly (VITAMIN D3 300,000 U/1ML AMP- OSVEH PHARMACEUTICAL CO.)

Blood sampling was performed intravenously, just before the vitamin D injection, and two, four, and 6 days after the injection (10 ml of blood was taken from the tail vein by a vacuum tube). Serum samples were used to measure TAC.

The serum TAC levels were determined using a novel automated measurement method, developed by Erel. In this method, the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, was measured. The results are expressed as μmol Trolox Eq/L (Erel, 2004).

The statistical analysis of the samples was performed using SPSS 20.0 software. Since the statistical dispersion of the samples was normal, the comparison of TAC values in each group at different times was performed using the Repeated Measure method. One-way ANOVA test was used to compare TAC values in each period between different groups. The P-value less than 0.05 was considered as significant.

Results

The results of TAC measurement in group A showed that its value increased during days 2, 4 and 6. The pattern of increase in TAC activity in group B also increased as in group A, but the rate of increase was higher than group A. In group C, the pattern of increase was similar to the previous ones, but the amount of increase was greater than in group B. In general, it can be said that in group C, the TAC activity increased with a significant slope until the sixth day.

In group B, the amount of TAC increased until the fourth day, but decreased slightly by the sixth day. The pattern of changes in group A was similar to group B, but its values were lower than group B.

There was a statistically significant difference between the groups in TAC values on the second and fourth days of the experiment (*P*≤0.05).

There was no significant difference between the amount of TAC in groups B and C on the fourth day. Except for this period, there was a significant difference among TAC values between all groups at any time (Figure 1). These results indicated that TAC levels increased, either at higher doses or in the following days and that the total amount of TAC can be increased, dose- and time-dependently.

The animals were observed regarding the toxic signs of the high Vitamin D_3 dose administration during and up to 30 days after the study period and no suspicious signs of toxicity were detected.

Discussion

Antioxidant enzymes; superoxide dismutase and glutathione peroxidase play a key role in regulating and maintaining the proper and physiological level of free radicals and this action is achieved by removing peroxides (Pari and Latha, 2005).

A study on diabetic rats showed a marked decrease in the enzyme activity. Liver superoxide dismutase was observed in diabetic rats and there was a direct relationship between vitamin D deficiency with a decrease in TAC in rats (Ravi *et al.*, 2004). Also, a 2013 study found that vitamin D administration improved TAC status in the diabetic rats (Salum *et al*., 2013).

Glutathione peroxidase can be mentioned as an antioxidant marker in the liver that protects tissue from reactive hydroxyl radicals. A 2012 study by George on diabetic rats showed that treatment with vitamin D and insulin was able to balance ROS production with the activity of antioxidant enzymes. Therefore, treatment with vitamin D can reduce the production of free radicals and increase the production of antioxidant enzymes and prevent tissue damage (George *et al*., 2012).

Another study conducted by Masjedi et al., in 2019 showed that there was a clear positive association between vitamin D, decrease in TAC levels, the activity of SOD and glutathione peroxidase in female follicles, and the increase in ROS levels in women with the ovarian disease. They are more polycystic and have a negative association between ROS and vitamin D.

This study emphasized that vitamin D administration can have a positive effect on increasing TAC, SOD, and glutathione peroxidase activity (Masjedi *et al*., 2019). Similar results in a 2019 study on mosque showed that vitamin D administration played a very positive role in improving or preventing mice with polycystic ovaries, by improving either the function of steroidogenic enzymes or antioxidant function (Masjedi *et al*., 2020).

Other studies have all highlighted the positive association between TAC and vitamin D (Baser et al., 2015). The improvement in antioxidant status following the administration of vitamin D is due to the increased expression of proteins that produce effective proteins and enzymes in the antioxidant system. Also, increased expression of many genes (approximately nine hundred genes) that have different roles occurs following the administration of vitamin D. (Norman *et al*., 2001).

Increased expression of SOD and catalase genes (Lisse, 2020) and many other enzymes involved in improving the antioxidant status of copper on SOD occurs by the administration of vitamin D. Besides, increased expression of vitamin D receptor (VDR) genes occurs following the administration of vitamin D (Zhong *et al.*, 2014).

What has been found in the present study is that vitamin D has a very significant effect on increasing the TAC in cows and this increase can occur depending on the dose of vitamin D prescribed; as the dose of the vitamin is increased, TAC is also increased. A 2020 study by Ray et al., showed that administering vitamin D3 to the pork could increase the animal's antioxidant status (Rey *et al*., 2020).

Also, the results of our study showed that the amount of TAC increased in the days after vitamin D administration. Although this increase was different in different groups and at different times, what is clear is that the amount of TAC was higher in all groups on the sixth day than on the first day. The reasons for this increase may be the rise in serum levels of the vitamin continuously for weeks after vitamin administration (Gupta *et al*., 2017).

Since this increase in serum vitamin D levels can lead to increased expression of genes involved in the antioxidant system, it can be expected to increase the amount of TAC in the days after administration. As previously reported by Kamei et al., in 1993, increased expression of VDR genes occurs following vitamin D administration (Kamei *et al.*, 1993).

Besides, Zhang et al., in 2014 showed that the expression of VDR genes increased in the following days and VDR gene expression levels increased at 4, 8, and 24 hours after vitamin D administration. Therefore, it can be concluded that increase in the expression of these genes is dose- and time-dependent, which is increased by the time and dose (Zhong *et al*., 2014).

Similar results have been observed in women with the increased SOD expression up to 8-fold within 48 hours of vitamin D administration (Lisse, 2020).

Overall, it can be concluded that the administration of vitamin D3 in the cattle can lead to a significant increase in TAC, which is completely dose- and time-dependent. This means that as we increase the amount of vitamin D3 injected the amount of serum TC increases and the amount of TAC increases with each passing day of vitamin D administration.

Thus, similar to the expression of genes involved in the antioxidant system enzymes, the increase in TAC in the cattle is dependent to the dose and time of vitamin D administration.

Since measuring the total antioxidant capacity was performed in a short time in this study, it seems selecting a longer period of the similar study would be preferable and appropriate.

Conclusion

None.

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Animal Welfare and Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have adhered to international standards for the protection of animals used for scientific purposes.

Conflict of Interest

The authors declared no conflict of interest.

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تأثیر تزریق دزهای مختلف ویتامین D_3 بر میزان ظرفیت آنتی اکسیدانی تام در گاوهای نر نژاد هلشتاین

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چکیده

زمینه مطالعه: ویتامین D3 یکی از مهمترین ویتامین‌هایی است که می‌تواند به صورت تزریقی یا به شکل مکمل خوراکی، به تنهایی یا در ترکیب با سایر ویتامین‌ها در گاوها مورد استفاده قرار گیرد. ویتامین D3 اثرات شناخته شده‌ای بر تنظیم کلسیم و سلامت استخوان دارد و همچنین دارای اثراتی وَرای متابولیسم کلسیم است، از جمله بهبود عملکرد سیستم ایمنی بدن و اثرات درمانی و پیشگیرانه بر بسیاری از بیماری‌های مزمن.

هدف: بسیاری از این تأثیرات غیرکلسمیک ویتامین D به دلیل تأثیر ویتامین D در بهبود سیستم آنتی‌اکسیدانی بدن است. این تأثیر مثبت بر وضعیت آنتی اکسیدانی می‌تواند به دلیل تأثیر ویتامین D بر بیان بسیاری از ژن‌ها از جمله ژن‌های مرتبط با پروتئین‌های درگیر در سیستم آنتی‌اکسیدانی مانند سوپراکسید دیسموتاز (SOD) و کاتالاز باشد.

روش کار: در مطالعه حاضر، ۱۵ گاو نر به ۳ گروه تقسیم شدند و با تزریق عضلانی ۳ دوز ویتامین D3 ، سطح سرمی آنتی‌اکسیدان کل (TAC) آنها قبل از تزریق و در دو، چهار و شش روز پس از تزریق محاسبه شد. مقدار ویتامین‌های مورد استفاده در گروه‌های A، B و C به ترتیب ۳۳۰۰۰۰۰، ۶۶۰۰۰۰۰ و ۹۹۰۰۰۰۰ واحد بود.

نتایج: نتایج مطالعه نشان داد که سطح ظرفیت آنتی‌اکسیدانی تام (TAC) بسته به دوز در گروه افزایش می‌یابد. سطح ظرفیت آنتی‌اکسیدانی کل نیز در روزهای بعدی افزایش خواهد یافت.

نتیجه‌گیری نهایی: بنابراین، می‌توان نتیجه گرفت که TAC در گاوها وابسته به زمان و وابسته به دوز است و صرف نظر از دوز درمانی ویتامین D3 ، استفاده از دوزهای بالاتر ویتامین D3 می‌تواند وضعیت آنتی‌اکسیدانی را بهبود بخشد.

واژه‌های کلیدی: تزریق، شش روز، ظرفیت آنتی‌اکسیدانی تام، گاو، ویتامین D_3

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