Relationship between expression pattern of vitamin D receptor, 1 alpha-hydroxylase enzyme, and chemokine RANTES genes and selected serum parameters during transition period in Holstein dairy cows

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ABSTRACT

Objectives The objective of the present study was to evaluate the pattern of genetic expression of vitamin D receptor (VDR), 1 alpha-hydroxylase (1α-OHase) enzyme and chemokine regulated on activation normal T-cell expressed and secreted (RANTES) in peripheral blood of Holstein dairy cows during transition period.

Methods Blood samples were collected from 16 Holstein dairy cows at 3 weeks prior expected date of delivery (EDD), at the day of parturition, and 3 weeks post-partum for assessment of expression profile of studied genes using real-time PCR and measurement of glucose, ionized calcium (Ca), parathyroid hormone (PTH), inorganic phosphorous (P), sodium (Na), potassium (K), chloride (Cl), and magnesium (Mg) levels.

Results Compared with 3 weeks prior EDD, VDR gene expression decreased significantly at the day of parturition then increased significantly at 3 weeks post-partum. The genetic expression of 1α-OHase enzyme as well as PTH, K, Na and Cl levels increased significantly at the day of parturition. The Ca level decreased significantly at the day of parturition then increased significantly at 3 weeks post-partum. The P level increased significantly at the day of parturition then decreased significantly at 3 weeks post-partum. Glucose level decreased significantly at the day of parturition and at 3 weeks post-partum. RANTES gene expression showed non-significant changes among the three different time points. The expression of VDR gene had a negative correlation with the expression of 1α-OHase enzyme gene, and serum levels of glucose, PTH, P and K, but had a positive correlation with the serum Ca level. The expression of 1α-OHase enzyme gene had a positive correlation with serum levels of PTH, P and K, but had a negative correlation with the serum Ca level.

Conclusions Results of the current study indicate the importance of monitoring the genetic expression of VDR and 1α-OHase enzyme as indicators of metabolic changes during transition period, suggesting that they are candidate genes to judge the health status of dairy cows during such period.

INTRODUCTION

Transition period, period from the last 3 weeks prior parturition till 3 weeks after parturition, is the most critical period during the dairy cattle production life. During such period, dairy cows start extensive metabolic alterations of glucose, fatty acids, vitamins and minerals metabolism to support both the coming fetus and the onset of lactation. Several elements are implicated in building up the final picture of this period, either to pass smoothly or to fall in multifaceted metabolic and inflammatory disorders. In this regard, cows endure several endogenous homeostatic mechanisms displayed by minor and major elements, side by side to hormonal mechanisms aiming to protect transition cows from the risk of several disorders.

Vitamin D3, an essential pro-hormone produced in the skin by ultraviolet irradiation of 7-dehydrocholesterol, is rapidly re-bilitated in the liver to 25-hydroxyvitamin D (25-(OH)D3) by the aid of several cytochrome P450 enzyme systems, mainly the sterol 27-hydroxylase (CYP27A1). 1,2 1,25-Dihydroxyvitamin D3 (1,25-(OH)2D3), an active form of vitamin D, is formed primarily in the kidney by enzymatic hydroxylation of (25-(OH)D3) with the aid of cytochrome P450 27B1 enzyme (CYP27B1) which is simply well known as the 1 alpha-hydroxylase (1α-OHase) enzyme. Numerous cells and tissues other than renal tissue were found to share 1α-OHase enzyme expression. Extra renal activation of vitamin D3 by mononuclear cells in the peripheral blood has been documented in response to infectious and inflammatory disorders. Therefore, synthesis of
were mated naturally and pregnancy status was assessed located in Damietta governorate, Egypt. All selected cows were raised at a farm, randomly for this study. Their age ranged from 1.8 to 2.2 years, and their body weight ranged from 430 to 500 kg.

In all, 16 primiparous Holstein dairy cows were selected for the present study. The chemokine regulated on activation normal T-cell expressed and secreted (RANTES), CCL5 chemokine, is expressed by many cells including blood lymphocytes in response to inflammatory signals. 12 It regulates the activation and trafficking of both inflammatory and non-inflammatory cells13,14 and is involved in the acute phase response.15

Cattle production systems and their nutritional stress are examples for several cascades that have a dramatic effect on the immune system. It was thought previously that general health issues during transition period only had a subsidiary effect on immune function. Nevertheless, with the clarification of more and more cellular and molecular immune pathways, these issues and their impacts on the immune system started to have a more strong reality at the molecular level. Stress of transition period in cattle leads to release of inflammatory cytokines that affect the expression of proteins responsible for immune cell trafficking and the initiation of an acute phase response.16,17

The aim of the present study was to evaluate the pattern of expression of VDR, 1α-OHase enzyme and the chemokine RANTES genes during normal transition period in peripheral blood of Holstein dairy cows. Likewise, the existing study will provide correlation data between the pattern of expression profile of such genes and selected serum parameters supposed to play a crucial role during the transition period.

**Materials and Methods**

**Animals**

In all, 16 primiparous Holstein dairy cows were selected randomly for this study. Their age ranged from 1.8 to 2.2 years, and their body weight ranged from 430 to 500 kg. The selected Holstein dairy cows were raised at a farm, located in Damietta governorate, Egypt. All selected cows were mated naturally and pregnancy status was assessed 45 days later using abdominal ultrasonography. All procedures were performed in accordance with the guidelines of Mansoura University, Mansoura, Egypt and approved by the Animal Welfare and Ethical Committee, Faculty of Veterinary Medicine, Mansoura University, code No. R/14.

The dairy cows under investigation were housed in semi-open shaded pens and fed on green fodder plus mixed ration which was formulated for both pre-calving heifers (2 weeks before calving) and dairy cows according to National Research Council (NRC).18 Based on clinical examination and haematological findings at 2 months of pregnancy, all studied cows were approved to be healthy and free from any previous diseases. The investigated cows were subjected to thorough clinical examination at the selected time points during transition period according to the standard protocols.19 If any of the selected dairy cows exhibited any form of transition period disorder or received any type of therapy, they were excluded to ensure accuracy of the data.

**Blood samples**

Two blood samples (5 mL each) were collected via jugular vein puncture from each of the 16 investigated cows at each of the four different time points; 2 months of pregnancy, 3 weeks prior to the expected date of delivery (EDD), at the day of parturition and 3 weeks post-partum. The first blood sample was collected in a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) as anti-coagulant for immediate analysis of total and differential leukocyte count then kept frozen at −80°C till molecular analysis. The second blood sample was collected into a plain tube without anticoagulant and immediately centrifuged at 3000 rpm for 15 min for separation of serum which was kept frozen at −80°C for subsequent biochemical analysis.

**RNA extraction from peripheral blood cells and cDNA synthesis**

RNA was extracted from peripheral blood cells using GENEzol RNA extraction reagent (Puregene, Genetix brands). The RNA pellet was eluted with 50 µL of RNase-free water and incubated for 10 min at 55°C to be dissolved completely. The extracted RNA was reverse transcribed to cDNA in 20 µL reaction using SensiFAST cDNA synthesis kit (Bioline, London, U.K.), where 5 µL of the RNA sample was added to 4 µL of 5 TransAmp Buffer, 1 µL of reverse transcriptase enzyme and 10 µL of UltraPure DNase/RNase-free water. The reaction mixture was incubated at 25°C for 10 min, then 42°C for 15 min and heated to 85°C for 5 min in a thermal cycler. Finally, the cDNA samples were diluted 1:10 in sterile DNase free water and stored at −20°C.

**Real-time PCR**

Quantitative real-time PCR, with a Bio-Rad real-time PCR system, was performed for all samples collected at the four different investigated periods to assess the genetic
expression profile of the VDR and 1α-OHase enzyme using primer sets described by Nelson and others and Aalberts and others, respectively. Furthermore, the expression of the chemokine RANTES gene was assessed using the primer set described by Aalberts and others (Table 1).

The reaction was consisted of 2 µL of cDNA template, 10 µL SYBR Green PCR Master mix (SensiFAST SYBR NO-ROX kit, Bioline, London, UK), 0.8 µL of 10 µM of each forward and reverse primers (Vivantis Technologies Sdn Bhd., Malaysia) with adding 6.4 µL of sterilised UltraPure DNase-free water to bring the total volume to 20 µL. The reaction mixtures were subjected to the following programme: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, 72°C for 15 s. The specificity of each primer was assessed by gel electrophoresis and melting curve analysis. In addition, the efficiency of each primer was calculated via the equation ‘Efficiency = −1+10(−1/slope)’. Relative quantification of mRNA transcripts was determined using the 2−ΔΔCt method described by Livak and Schmittgen, where β-actin gene was used as the house keeping gene. In the studied cows, the relative expression of each studied gene at 2 months of pregnancy served as a control value that was used for quantitative assessment of the fold change variation in its expression profile during transition period relative to this control value.

Biochemical analysis

Biochemical analysis of all the selected serum parameters was conducted directly after separation of serum. Parathyroid hormone (PTH) was estimated by electrochemiluminescence assay using COBAS apparatus e411 kits (Roche Diagnostics, Germany). The serum ionized calcium (Ca), inorganic phosphorous (P), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) were measured spectrophotometrically using commercial test Kits (Human Gesellschaft Fur Biochemica und Diagnostica mbH, Germany). Glucose was assessed by colorimetric method using Spinreact kits (SPINREACT, S.A., Girona, Spain). For all of the measured parameters, detection methodology was conducted according to the information supplied by the manufacturers.

Statistical analysis

Data analyses were performed using a statistical software program (SPSS for windows V.21, SPSS, Chicago, USA). Data were tested for normal distribution using Kolmogorov–Smirnov test. The data were normally distributed; therefore, mean and SD for each variable were calculated. Data were subjected to repeated measures analysis of variance (ANOVA), in which Wilks’ Lambda test was selected to evaluate the effect of time. Where Wilks’ Lambda test indicated a statistically significant difference, one-way ANOVA was used to identify which time (3 weeks prior EDD, at the day of parturition and 3 weeks post-partum) was statistically different. Correlation between different biochemical parameters was analysed using Spearman correlation. Differences between means at a p-value of <0.05 were considered to be significant.

RESULTS

Clinically, the investigated dairy cows did not express any detectable clinical alterations throughout the study period and remain clinically healthy. All cows demonstrated normal labouring and delivered a single calf without obvious clinical illness. All the assessed serum parameters, including Ca, P, Mg, PTH, glucose, K, Na and Cl levels at the four different time points were within the normal range values and the recorded changes during transition period were only transitory in response to the physiological requirement of the animals at each time point.

Time had a significant effect on the total leucocyte count (TLC) as well as neutrophils, lymphocytes and monocytes counts during transition period in the examined cows (Wilk’s Lambda test, p<0.05). At the day of parturition, the TLC and neutrophils count were significantly (p<0.05) increased, while the lymphocytes count and monocytes count were significantly (p<0.05) decreased when compared with 3 weeks prior EDD and

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Table 1  Quantitative real-time PCR primer sequences for evaluation of genetic expression of vitamin D receptor, 1 alpha-hydroxylase enzyme, and chemokine regulated on activation normal T-cell expressed and secreted during transition period in Holstein dairy cows

| Gene     | Accession No # | Strand | Primer sequence 5'−3'   | Reference               |
|----------|----------------|--------|-------------------------|-------------------------|
| VDR      | NM_001167932   | F      | AGCCACCGGCCTCCATTCA     | Nelson and others20      |
| 1α-OHase | XM_588481      | R      | AACAGCGCCTTCCGCTTCAT    | Aalberts and others31    |
|          |                | F      | TGGGACACAGATGTGCACTTCGC |                         |
|          |                | R      | TCTCAGACTGTTCTCATGAGT   |                         |
| RANTES   | NM_175827      | F      | CACCCACGTCAGGAGTATT     | Aalberts and others31    |
|          |                | R      | CTCGCACCCACTTCTCTCT     |                         |
| β-actin  | NM_173979.3    | F      | GGATCCTGACCTCAAAGTA     | Nelson and others20      |
|          |                | R      | CACACGGAGCTCGTTTAGA     |                         |

1α-OHase, 1 alpha-hydroxylase enzyme; RANTES, regulated on activation normal T-cell expressed and secreted; VDR, vitamin D receptor.
3 weeks post-partum. Eosinophil and band cell counts showed non-significant changes among all tested time points (table 2).

The relative gene expression of VDR, 1α-OHase enzyme and RANTES (mean±SD) in the studied cows at 2 months of pregnancy was 1.0±0.0 for each. Time had a significant effect on the genetic expression of VDR and 1α-OHase enzyme as well as levels of Ca, P and PTH during transition period in the studied cows (Wilks’s Lambda test, p<0.05). Compared with 3 weeks prior EDD, the VDR gene expression decreased significantly at the day of parturition (p<0.05), and then increased significantly at 3 weeks post-partum (p<0.05). The genetic expression of 1α-OHase enzyme was significantly greater on the day of parturition compared with other time points (p<0.05). The genetic expression of RANTES showed non-significant changes among the three different time points. The Ca level decreased significantly at the day of parturition (p<0.05), and then increased significantly at 3 weeks post-partum (p<0.05) when compared with 3 weeks prior EDD. In contrast, the P level was significantly (p<0.05) increased at the day of parturition, and then was significantly (p<0.05) decreased at 3 weeks post-partum in comparison with 3 weeks prior EDD. The PTH level was significantly higher at the day of parturition and 3 weeks post-partum than that of 3 weeks prior EDD (p<0.05). However, the K, Na and Cl levels were significantly higher at the day of parturition than that of other time points (p<0.05) (table 3).

There was a negative correlation between VDR gene expression and 1α-OHase enzyme gene expression (r = −0.634; p=0.001). Moreover, there was a negative correlation between VDR gene expression and levels of glucose (r = −0.538; p=0.002), PTH (r = −0.447; p=0.013), P (r = −0.912; p=0.001) and K (r = −0.646; p=0.001). However, there was a positive correlation between VDR gene expression and Ca level (r = 0.717; p=0.001).

There was a positive correlation between 1α-OHase enzyme gene expression and levels of PTH (r = 0.680; p=0.001), P (r = 0.620; p=0.001) and K (r = 0.738; p=0.001). Meanwhile, there was a negative correlation between 1α-OHase enzyme gene expression and Ca level (r = 0.738; p=0.001).

**DISCUSSION**

Vitamin D, the sunlight hormone, plays a crucial role in the production cycle of dairy cattle. It was discussed widely that the major role of the renal active metabolite of vitamin D₃ is concise to normal Ca homeostasis in an endocrine pattern shared with PTH.²³ In human studies, Stoffels and others²⁴ and Stoffels and others²⁵ reported that white blood cells are able to express VDR and

### Table 2 Total and differential leukocyte counts in Holstein dairy cows during transition period

|                  | Total white blood cell count, / μL | Neutrophils count, / μL | Lymphocytes count, / μL | Monocytes count, / μL | Eosinophils count, / μL | Band cells count, / μL |
|------------------|-----------------------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|
| 3 weeks prior EDD| 7495±853ᵃ | 2504±682ᵃ | 4456±734ᵃ | 273±115ᵃ | 111±115ᵃ | 149±44ᵃ |
| At the day of parturition | 9405±861ᵇ | 6225±976ᵇ | 2883±627ᵇ | 117±27ᵇ | 90±60ᵇ | 90±23ᵇ |
| 3 weeks post-partum | 6960±611ᵃ | 2780±608ᵃ | 3501±281ᵃ | 287±101ᵃ | 287±101ᵃ | 105±131ᵇ |

Wilks’ Lambda test for time: p=0.001

|                  | p=0.001 | p=0.001 | p=0.001 | p=0.001 | p=0.818 | p=0.072 |

ᵃᵇ: Variables with different superscript letter in the same column are significantly different at p < 0.05.

EDD, expected date of delivery.

- **Table 3** Relative gene expression profile of vitamin D receptor, 1 alpha-hydroxylase enzyme, and chemokine regulated on activation normal T-cell expressed and secreted and serum levels of calcium, phosphorus, magnesium, and parathyroid hormone in Holstein dairy cows during transition period

|                  | VDR | 1 α-OHase | RANTES | Calcium (mmol/L) | Phosphorous (mmol/L) | Magnesium (mmol/L) | PTH (pg/mL) |
|------------------|-----|-----------|--------|------------------|---------------------|--------------------|-------------|
| 3 weeks prior EDD| 2.51±0.54ᵃ | 0.034±0.01ᵃ | 0.058±0.15ᵃ | 2.04±0.11ᵃ | 2.39±0.10ᵃ | 1.18±0.31ᵃ | 16.07±2.01ᵃ |
| At the day of parturition | 0.19±0.11ᵇ | 1.71±0.76ᵇ | 0.016±0.04ᵇ | 1.87±0.32ᵇ | 2.61±0.12ᵇ | 0.79±0.24ᵇ | 27.20±7.99ᵇ |
| 3 weeks post-partum | 7.29±1.59ᵇ | 0.05±0.03ᵃ | 0.001±0.003ᵇ | 2.50±0.12ᶜ | 1.61±0.25ᶜ | 0.92±0.20ᵃ | 17.35±2.67ᵃ |

Wilks’ Lambda test for time: p=0.001

ᵃᵇᶜ: Variables with different superscript letter in the same column are significantly different at p < 0.05.

EDD, expected date of delivery; 1α-OHase, 1 alpha-hydroxylase enzyme; PTH, parathyroid hormone; RANTES, regulated on activation normal T-cell expressed and secreted; VDR, vitamin D receptor.
1α-OHase enzyme genes that affect immune cells proliferation and differentiation in an autocrine and paracrine manner. However, in bovine medicine, knowledge of vitamin D in inflammatory modulations still lags behind what is known about Ca homeostasis. Since the transition period for dairy cows resembles a period of disturbed immune status, it was interesting to study the impact of such critical period on the pattern of expression profile of VDR and 1α-OHase enzyme genes in peripheral blood cells.

In the studied cows, the levels of Ca, P, Mg, PTH, glucose, K, Na, and Cl at the four time points were within the normal range values. The normal reference values for the estimated serum parameters needed for interpretation were as follows: Ca (2.0–2.5 mmol/L), P (1.3–2.6 mmol/L), Mg (0.75–1.0 mmol/L), PTH (18.34–40.82 pg/mL), glucose (2.5–4.2 mmol/L), K (3.9–5.8 mmol/L), Na (132–152 mmol/L) and Cl (95–110 mmol/L). All the recorded changes in these parameters during transition period were only transitory in response to the physiological requirements of the animals at each time point.

In the current study, the TLC as well as neutrophils count was increased, while lymphocytes count was decreased at the day of parturition indicating altered immune status of the examined cows during the transition period. The higher neutrophils count and lower lymphocytes count are, the more severe the inflammatory response and the stronger the immune suppression. The elevated level of corticosteroids at calving was found to induce neutrophilia through increasing neutrophils output from bone marrow, and/or demargination of neutrophils from the blood vessel wall with a subsequent leukocytosis.

In the present study, the decrease in VDR gene expression at the day of parturition was attributed to the increased PTH level and/or decreased Ca level. In studies conducted on rats, Goff and others and Reinhardt and others suggested that the increased PTH secondary to dietary Ca deficiency may be responsible for the lack of 1,25-(OH)2D3 mediated upregulation and thus downregulates the expression of VDR. Meanwhile, at 3 weeks post-partum, the increased VDR gene expression suggests genetic adaptation to meet the challenge against potential microbial infection in the disturbed immune body system that occurs as a result of metabolic stress during this critical period. The expression of 1α-OHase enzyme gene was increased at the day of parturition. The high PTH level and the low Ca level at this time may serve as the stimulus for the upregulation and production of 1α-OHase enzyme, which converts 25-(OH)D3, the major circulating form of inactive vitamin D, into 1,25-(OH)2D3, the active form of vitamin D.

In cows under investigation, the Ca level was decreased and the PTH level was dramatically increased at early lactation period. Although there is a mild decrease in serum Ca level at the day of parturition, the dairy cows under investigation did not express any clinical abnormalities suggesting a case of subclinical hypocalcaemia which appears to be present in 25% of dairy cows in the first lactation period without alteration of the physiological function as stated previously by Reinhardt and others. Such findings could be explained by the presence of an adequate level of PTH for mobilization of Ca from the skeletal reserve and from the gastrointestinal tract. Therefore, the low serum Ca level at calving period is closely related to the increased PTH level, and the return of Ca to its normal level is associated with a decrease in the concentration of the hormone. Moreover, Doković and others attributed the decreased blood Ca level at calving period to the onset of lactation and the increased mammary gland activity. Likewise, the higher K and P levels adversely affect the Ca homeostasis and have been found to reduce the Ca level in blood and stimulate the release of PTH.

The decreased serum glucose level in the studied cows at the day of parturition and at 3 weeks post-partum indicates a high demand for glucose at early lactation. Such a hypoglycaemic state is advantageous as it lowers the levels of both insulin and insulin-like growth factor-1 with subsequent increased growth hormone secretion. Increased growth hormone secretion stimulates hepatic gluconeogenesis and increases glucose supply to meet milk production during early lactation period. In the existing study, transient mild increase in the serum K level at the day of parturition may be synchronized with metabolic acidosis that occurs at such time. During such period, the maternal acid–base system gradually increases oxygen tension, decreases arterial CO2 tension, and a parallel reduction in plasma bicarbonate concentration with a resultant secondary and compensatory metabolic acidosis. However, the mild

**Table 4** Serum levels of glucose, potassium, sodium and chloride in Holstein dairy cows during transition period

|                      | Glucose (mmol/L) | Potassium (mmol/L) | Sodium (mmol/L) | Chloride (mmol/L) |
|----------------------|------------------|--------------------|-----------------|------------------|
| 3 weeks prior EDD    | 2.75±0.12a       | 3.73±0.31b         | 137.44±12.01a   | 93.10±6.17b      |
| At the day of parturition | 2.51±0.16b     | 5.74±0.76b         | 175.96±15.51b   | 119.60±5.79b     |
| 3 weeks post-partum  | 2.26±0.14c       | 3.61±0.84d         | 156.17±10.55a   | 99.5±5.15a       |

Wilks’ Lambda test for time

- p=0.004
- p=0.001
- p=0.602
- p=0.001

*a,b,c*: Variables with different superscript letter in the same column are significantly different at p < 0.05.

EDD, expected date of delivery.
increase in Na and Cl levels in cows under investigation were recorded at the day of parturition. Such changes in these electrolytes may be resulted from the high activity of rennin–angiotensin–aldosterone system, which occurs during this period, as well as from synergistic cooperation with vasopressin, resulting in a positive Na balance with increased extracellular fluid volume. Furthermore, increased Na concentration at the day of parturition may be related to the increased aldosterone concentration which is observed at calving as well as during first week of lactation.

In Holstein dairy cows under investigation, there was a negative correlation between the genetic expression of VDR and glucose level. Several human studies have documented that vitamin D and its receptor protein are essential components in maintaining glucose tolerance and normal insulin release, where pancreatic B cells possess VDR. Thus, any deviation in the normal function of VDR complex can affect pancreatic B cells and insulin sensitivity that causes impaired insulin production and/or release affecting the level of glucose.

In the examined Holstein dairy cows, there was a positive correlation between VDR gene expression and Ca level during transition period. The upregulation of VDR gene is an essential step in maintaining the Ca level in plasma within a normal range. Thus, in the existing study, the level of Ca decreased significantly at the onset of parturition, a result that could be due to the significant downregulation of VDR gene during this period.

Since the serum Ca level is inversely proportional to serum PTH, the PTH may suppress VDR expression. Based on this, the results of the present study revealed a significant negative correlation between VDR gene expression and level of PTH in Holstein dairy cows during transition period. In rats, both a high PTH level, which occurs secondary to Ca deficiency, and PTH administration partially blocks a 1,25(OH)2D3-mediated increase in renal VDR suggesting that PTH may have suppressive actions on VDR expression.

Based on the negative correlation between Ca and P levels and between Ca and K levels, and the positive correlation between VDR gene expression and Ca level, our data analysis revealed a significant negative correlation between the genetic expression of VDR and the levels of P and K which is well suited to these facts. In rats, Sriussadaporn and others found that low P diet rapidly decreases serum P level that upregulates intestinal VDR through increasing VDR gene expression. Therefore, increased P level exerts a broad modulating effect on VDR-mediated gene expression that suppresses VDR gene expression.

Results of the present study recorded a significant negative correlation between 1α-OHase enzyme gene expression and serum Ca level. Such results seem to be similar to that occur in the kidney cells in response to reversible hypocalcaemic episodes. In case of low ionized plasma Ca level, there is upregulation of the renal 1α-OHase enzyme gene to augment the production of the 1,25(OH)2D3. In turn, this active metabolite acts synergistically with PTH to initiate a rapid homeostatic mechanism aimed to raise plasma Ca level through enhancing Ca absorption from intestine, Ca re-absorption by the kidneys, and Ca resorption from the bony skeleton. Moreover, blood monocytes in hypocalcaemic cows showed a muted intracellular Ca response as compared to those with normal plasma Ca level. Thus, low circulating Ca level sensitizes immune cells particularly mononuclear cells to upregulate the 1α-OHase enzyme gene expression.

There was a significant positive correlation between 1α-OHase enzyme gene expression and PTH level as well as between 1α-OHase enzyme gene expression and P level in Holstein dairy cows under investigation during transition period. During episodes of hypocalcaemia, the elevated level of serum P and sensitization of Ca sensors in parathyroid gland stimulate PTH secretion to upregulate the expression of 1α-OHase enzyme gene. Based on the negative correlation between 1α-OHase enzyme gene expression and serum Ca level and between K and Ca levels, our data analysis revealed a significant positive correlation between 1α-OHase enzyme gene expression and K level in Holstein dairy cows during transition period.

In the present study, decreased expression of VDR gene in dairy cows during transition period led to a decrease in the serum Ca level and an increase in PTH with subsequent increased expression of 1α-OHase enzyme gene. The recorded alterations in Ca and PTH levels during this critical period explain the significant negative correlation between VDR gene expression and 1α-OHase enzyme gene expression. Healy and others stated that PTH acts to upregulate 1α-OHase enzyme expression and downregulate VDR expression in mice, but the mechanism by which PTH downregulates VDR expression is not clear. Furthermore, lack of vitamin D3 is accompanied with a lack in VDR protein and thus down regulates the VDR gene coincidental with upregulation of the 1α-OHase enzyme gene to activate vitamin D3 in different tissues.

**LIMITATIONS**

This study was carried out on primiparous cows, but it will also be interesting to evaluate the effect of multiple parities as well as various metabolic disorders on the expression profile of the studied genes. Besides, the expression of the studied genes was assessed at fairly long intervals. Hence, more studies that will monitor their expression profile at short intervals may definitely provide a more significant and clear data. Additionally, in vitro studies are needed to confirm the possible correlation between the selected serum parameters and expression profile of the selected genes in mononuclear cells in peripheral blood. Further in vitro investigations are also required to verify the effect of variable Ca concentrations on the expression of VDR and 1α-OHase genes in peripheral mononuclear cells.
CONCLUSIONS

The results of the current study indicate that the relative expression of vitamin D receptor (VDR) and 1 alpha-hydroxylase (1α-OHase) enzyme genes is an indicator of metabolic changes during transition period. Genetic expression of such genes can add to the metabolic profile test in diagnosis of metabolic disorders in dairy cattle before any of their clinical manifestations are detected, suggesting that they are candidate genes to judge the health status of dairy cows and the profitability of dairy herds during such period.

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