

Effects of vitamin D3 injection in close-up period on insulin resistance and energy balance in transition dairy cows

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Abstract

Background: Many studies in dairy cows are towards calcium homeostasis and there is a lack of knowledge about the effect of vitamin D in preventing insulin resistance and improving energy balance in the transition period of dairy cows.

Methods: The trial was conducted in a commercial dairy farm with about 1500 lactating cows in Tehran province, Iran. Twenty-four Holstein cows had been randomly selected and divided into control and treatment groups. In the treatment group, 12 cows, received a single dose of 8,000,000 IU vitamin D3 intramuscularly and in the control group, 12 cows were injected placebo (distilled water) 2–8 days before the expected calving time. Blood samples were collected between 8 and 10 AM 2 h after feeding on 21 and 7 days before calving and 1,3,7,15 and 30 days after calving. 25(OH)vitamin D, insulin-like growth factor 1 (IGF-1), insulin, nonesterified fatty acid (NEFA), β -hydroxybutyric acid (BHBA), albumin, total protein, glucose, urea, triglyceride, cholesterol and aspartate amino transferase (AST) were measured by commercially available kits. The insulin resistance index was calculated.

Results: Vitamin D3 injection significantly affected the amounts of 25(OH) vitamin D, urea, insulin and insulin resistance index ($p \leq 0.05$). On the other hand, the amounts of glucose, NEFA, BHBA concentration and AST activity were higher in control group ($p \leq 0.05$). Time had a significant effect on the amounts of most measured variables except IGF-1 and insulin. There were no group and time interactions for measured variables.

Conclusion: It seems that injection of vitamin D3 in close up period influenced lipolysis potentially modifying energy metabolism and resulted in reducing insulin resistance.

KEYWORDS

dairy cow, insulin resistance, transition period, vitamin D

1 | INTRODUCTION

For many years vitamin D has been known for its classical effect on calcium (Ca) metabolism and skeletal condition. Initial evidence for the non-classical effects of the active form of vitamin D (1, 25-dihydroxy

vitamin D) arose from studies in the early 1980s. Recent studies suggested that beyond the skeletal condition and calcium metabolism, vitamin D may have preventive and therapeutic effects for several diseases such as cardiovascular disease, autoimmune disease, cancer and types 1 and 2 diabetes (Adams & Hewison, 2008; Mathieu et al., 2005).

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Most vitamin D studies in cattle are implemented to better understand calcium haemostasis linked to the onset of lactation and hypocalcaemia after parturition (Goff et al., 1988; Horst et al., 1994).

The classical target organs of vitamin D include bones, intestine, kidney and parathyroid glands. Vitamin D promotes the active uptake of calcium to regulate calcium concentration of this element in normal limits. The epithelial calcium channel, transient receptor potential vanilloid 6 (TRPV6) and calbindin which transport calcium into the cells, are upregulated by vitamin D. (Hoenderop et al., 2005). Vitamin D induces bone remodelling in association with parathyroid hormone (PTH) to maintain serum calcium concentrations in a narrow normal limit. Vitamin D and calcium are thought to be necessary for growth plate development (Goltzman et al., 2004). Transition period management is an important step of preventing various diseases in dairy cows and maintaining calcium in a normal range is necessary. It should be noted that after parturition most cows undergo negative calcium balance (De Garis & Lean, 2008).

Vitamin D receptors (VDRs) are widespread in many organs such as the heart, stomach, pancreas, brain, skin and gonads. In addition, activated T and B lymphocytes and macrophages have nuclear receptors for Vitamin D. A variety of autoimmune diseases including type 1 diabetes, rheumatoid arthritis and multiple sclerosis have all been successfully prevented in mouse models receiving 1,25(OH)₂D₃ in their early life (Holick, 2004).

Studies have shown that a high dose of vitamin D can prevent diabetes type 1 by immune regulation. It is demonstrated that vitamin D is a great inhibitor of dendritic cell differentiation that directly blocks IL-12 secretion (Mathieu et al., 2005; Pittas et al., 2007). Vitamin D deficiency has long been linked to glucose intolerance in humans. Many studies in humans have shown that vitamin D plays a role in the pathogenesis of type 2 diabetes, by affecting either insulin sensitivity or β cell function, or both (Chiu et al., 2004; Pittas et al., 2007). The effect of vitamin D on rising cytosolic calcium level may act on insulin secretion by β cells (Pittas et al., 2007).

It was reported that vitamin D deficiency is linked to rising body mass index and vitamin D can improve insulin sensitivity in humans (Al-Shoumer & Al-essa, 2015). Many studies also demonstrated that cows in gestation and early lactation have insulin resistance (De Koster & Opsomer, 2013; Pires et al., 2007). The mechanism of insulin action in ruminants is similar to other species. Serum free fatty acid participates in the pathogenesis of insulin resistance in cows. Sinclair (2010) has shown that non-esterified fatty acids (NEFA) level is correlated with insulin resistance in dairy cows (Sinclair, 2010). On the other hand, chronic elevation of β -hydroxybutyric acid (BHBA) in cows with ketonemia results in higher insulin resistance of tissues (De Koster & Opsomer, 2013). It is believed that adipose tissue plays an endocrine role in dairy cows. mRNA expression of tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), leptin, adiponectin, haptoglobin, visfatin and resistin are confirmed in adipose tissue (De Koster & Opsomer, 2013). A study indicated that TNF- α plays an important role in the pathogenesis of insulin resistance, fatty change and necrosis of hepatocytes (Ohtsuka et al., 2001). Over-conditioned pre-parturient cows are more insulin

resistant than lean cows and have higher amounts of free fatty acid in serum than others (Sinclair, 2010). For dairy cows, the most important pathways that have an altered insulin resistance during the transition period are glucose uptake by skeletal muscle and adipose tissue; lipogenesis and lipolysis in adipose tissue; gluconeogenesis in the liver; and protein metabolism of skeletal muscle. Most recently, it is generally suggested that dairy cows are insulin resistant at the end of gestation and in early lactation. These homeostatic adaptations are necessary to ensure a sufficient glucose supply for the gravid uterus and lactating mammary gland in support of the growing offspring, both pre-natally and post-natally. The adaptation towards an insulin-resistant state seems to be conserved in mammals among different species (De Koster & Opsomer, 2013).

Recommended vitamin D₃ requirement for cattle health is not well-defined. The NRC (2001) recommends 21,000 IU supplemental vitamin D₃/day (~800–1000 IU/kg of DM) for lactating Holstein cows (calculated for 680 kg of BW). Dairy producers instead typically provide lactating cows with 30,000–50,000 IU of vitamin D₃ (Nelson et al. 2016). Nelson et al. clarifies that 22% of cows supplemented with 20,000 IU/day vitamin D (NRC recommendation) had serum 25(OH)D below 30 ng/ml whereas, 95% of cows receiving 30,000 IU/day or more had serum 25(OH)D above 40 ng/ml (Nelson et al. 2016). Nelson et al. (2016) showed that in the majority of dairy cows receiving 1.5–2.5 times the NRC recommendation and vitamin D average serum concentration was 60–70 ng/ml, ranging between 40 and 100 ng/ml. Lean and colleagues suggested 40,000 IU vitamin D for appropriate vitamin D functional role but considering optimal time supplementation before calving is a vital step (Lean et al., 2013).

New studies have shown that dairy cows have reduced serum concentration of vitamin D (25(OH)D) in critical periods just after calving (Holcombe et al., 2018; Nelson et al., 2016). In early lactation, cows are vulnerable to metabolic diseases and oxidative stress and there is a chance that reduced serum vitamin D plays a role in this condition. The effects of vitamin D on immune system regulation and energy balance are well known in human medicine but there is still a lack of knowledge in veterinary research in this field (O'Brien & Jackson, 2012).

Our objective was to assess the effect of a single vitamin D injection on the insulin status and energy balance of transition cows with a sufficient nutritional vitamin D supply. We assumed that after vitamin D injection in the treatment group, serum vitamin D amount will rise, and insulin resistance reduces and better energy balance after calving have resulted in injected dairy cows.

2 | MATERIALS AND METHODS

2.1 | Cows, experimental design and feed

The trial was conducted in a commercial dairy farm with about 1500 lactating cows in Tehran province, Iran. The rolling herd average for milk production was about 10,920 Kg per 305 days of milk production. The herd used mix loose pens with adjacent outside yards and free-stall facilities with sand bedding. The animals had free access to water

TABLE 1 Ingredients, chemical composition and nutritive value of diets fed to close-up cows

	DMI	%		
Legume hay immature	4.17	32.61	Nel 3X (Mcal)	19.45
Corn silage immature	2.42	18.97	Nel 3X (Mcal/kg)	1.53
Wheat straw	0.46	3.63	RUP Kg	0.74
Corn gluten meal	0.13	1.01	RUP % of DM	5.84
Extruded linseed (full-fat flaxseed) [†]	0.41	3.21	RDP kg	0.74
Barley grain rolled	1.35	10.57	RDP % of DMI	1.12
Corn grain ground dry	1.50	11.71	CP Kg	1.86
Wheat bran	0.13	1.05	CP % DM	14.6
Canola meal	0.45	3.53	NDF %	37.31
Cotton seed meal (solvent extracted)	0.32	2.49	ADF %	23.01
Soybean meal, expellers	0.36	2.79	NFC %	39.81
Full-fat soy, roasted	0.19	1.46	EE %	4.73
Fish meal, anchovy	0.18	1.44	Ca/P	3.48
Vegetable oil	0.15	1.17	Absorbable Ca (kg/day)	0.16
Anionic supplement [*]	0.50	3.91	Dietary Ca %	1.24
Limestone	0.02	0.15	Absorbable P (kg/day)	0.05
Antimycotoxin [†]	0.02	0.12	Dietary P %	0.36
Availa chromium ¹	0.01	0.06	Mg %	0.57
Yeast [†]	0.02	0.12	Cl%	0.87
Total	12.78	100.0	K%	1.44
Forage % of DMI	55.33		Na %	0.04
Concentrate % of DMI	43.67		S%	0.35
			DCAD (mEq/Kg Dm)	-81.46

¹Zinpro, Eden Prairie, USA.

^{*}refer to Table 3.

[†]refer to Table 2.

throughout the experiment. The predominant forages used in this farm were alfalfa hay and corn silage, and the main concentrates consisted of corn, barley, soybean meal, canola meal, linseed meal, cottonseed meal, wheat bran, gluten feed and sugarcane bagasse. Feed composition details for both close-up and early lactation cows are depicted in Tables 1 and 2 and formulated based on NRC (2001). Total mixed ration (TMR) was mixed by a mechanized feeder and were offered to cattle twice a day. It was estimated that total diet includes between 20,000 and 30,000 IU of VitD3 per day/head. The farm held a policy of drying off the cattle 8 weeks before the expected calving day. The expected calving day was based on the date of artificial insemination and pregnancy diagnosis by ultrasonography. All the cows in the close-up period were placed on a diet with anionic salts based on producer recommendation (500 g/head/day, Aniomix, Tehran, Iran). The ingredients of anionic salt were presented in Table 3. DCAD of diet was calculated as about -81.46 mEq/Kg of DM (Calculated as $[Na^+ \times 435mEq + K^+ \times 256mEq] - [Cl^- \times 282mEq + S^{2-} \times 624mEq]$). Cattle urine pH was measured twice weekly using a digital pH meter (Jenway, Model 3040, England) from 2 days after the anionic salts were added until

calving to make sure that the value was never less than 5.8 or more than 6.8.

Twenty-four Holstein cows had been randomly selected and put in control and treatment groups. Blood sampling was conducted in the same season and time in treatment and control groups. The average daily milk production during the previous lactation at first, second and third months after parturition in treatment and control groups were 47.3, 48.7, 49.1, and 44.4, 46.6, 47.9, respectively. In the treatment group, 12 cows, received a single dose of 8,000,000 IU vitamin D3 (cholecalciferol, Darou Pakhsh Co., Iran) intramuscularly and in the control group, 12 cows have injected placebo (sterile injectable distilled water, Darou Pakhsh Co., Tehran, Iran) 2–8 days before expected calving time. However, if selected cows did not calve during the expected time (based on the date of artificial insemination and pregnancy diagnosis by ultrasonography) or any diseases during trial, they were excluded from the study. In our study, the selected dose of vitamin D3 was based on previously published studies (Julien et al., 1977; Littledike & Horst, 1982). Elimination was also done for any cow that developed health-related issues for any reason during the study period.

TABLE 2 Ingredients, chemical composition and nutritive value of diets fed to fresh cows

	DMI	%		
Alfalfa hay	3.704	17.02	NEL 3X (Mcal)	36.09
Corn silage	2.83	12.99	NEL 3X (Mcal/kg DM)	1.85
Wheat straw	0.185	0.85	RUP (kg)	1.75
Corn gluten meal	0.518	2.38	RUP % of DMI	8.06
Extruded linseed (full-fat flaxseed) ¹	0.446	2.05	RDP (kg)	1.83
Barley grain rolled	2.71	12.41	RDP % of DMI	8.39
Corn grain ground dry	2.64	12.13	CP kg	3.58
Wheat bran	0.21	0.94	CP % of DM	16.45
Canola meal	0.155	5.31	NDF % of DMI	33.73
Cotton seed W lint	0.531	2.44	ADF % of DMI	19.33
Soybean meal, expellers	1.682	7.73	NFC % of DM	43.05
Soybean seeds, whole roasted	0.344	1.58	EE % of DM	4.68
Fish meal, anchovy	0.165	0.76	Absorbable Ca (kg/day)	0.18
Vegetable oil	0.21	0.96	Dietary Ca % of DMI	0.82
Beet Pulp, dried	3.532	16.23	absorbable P (kg/day)	0.11
Calcium carbonate	0.089	0.41	Dietary P % of DMI	0.51
DCP (Di-Calcium Hydrogen Phosphate)	0.048	0.22	Ca/P ratio	1.62
Sodium Bicarbonate	0.181	0.83	Mg % DMI	0.34
Salt	0.039	0.18	Cl % of DMI	0.36
Magnesium oxide	0.051	0.23	K % of DMI	1.64
Vitamin/Mineral supplement ⁶	0.161	0.74	Na % of DMI	0.42
Bentonite	0.061	0.28	S % of DMI	0.25
Propylene glycol ²	0.250	1.15	DCAD (mEq/kg DM)	+345.3688
Antimycotoxin ³	0.015	0.07		
Yeast ⁴	0.015	0.07		
Rumen protected methionine ⁵	0.009	0.04		
Total	20.78	100		
Forage% of DM	30.87			
Concentrate % of DM	66.75			

¹Shayflax, Tehran, Iran.

²Glycoline, Vitalac Co, France.

³Mycosorb, Alltech Co, USA.

⁴levucell, (*Saccharomyces cerevisiae* CNCM I-1077), Nutritech Co, USA.

⁵Mepron, Evonik industries, Wien, Austria.

⁶Vitamin/mineral supplement contain: Ca 13.4%, P 1.08%, Mg 3.4%, Na 10.35%, Cl 8.11%, Co 20 mg/kg, Cu 1500 mg/kg, I 70 mg/kg, Fe 3000 mg/kg, Mn 4000 mg/kg, Se 370 mg/kg, Zn 5000 mg/kg, Vit A 700000 IU/kg, Vit D 200000 IU/kg, Vit E 2000 IU/kg.

The parity of the cows ranged from 3 to 7, with parity groups numbered as 3 for third lactations, 4 for fourth and 5 for more. The body conditions of all cows were scored in three different stages based on a five-point scale and an increment of 0.25: far off, day of calving and 30 ± 2 post-partum. All scorings were performed by a single evaluator. All the cows in both groups were clinically healthy based on Duffield et al. and had a body condition score (BCS) between 3.25 and 4 initially in the far-off period. The cows were also categorized into two groups based on BCS; BCS of ≥ 3.7 were enrolled as fat cow (control, *n* = 5 and treatment, *n* = 4), and a BCS of < 3.7 as non-fat cow (control, *n* = 7 and treatment, *n* = 8) for statistical purposes.

2.2 | Blood sampling and variables measurements

Blood samples were collected via jugular vein between 8 and 10 AM 2 h after feeding on 21 and 7 days before calving and 1, 3, 7, 15 and 30 days after calving. We considered 21 days before calving as a covariate for other times. Blood samples were taken by disposable syringe on the plain tube and chilled immediately after collection and serum was harvested immediately after centrifugation at 2000 × *g* for 10 min. Serum was frozen at −20°C until delivery to the laboratory for further analysis. 25(OH)D (assay range: 1–350 ng/ml and analytical sensitivity: 0.53 ng/ml), insulin-like growth factor 1 (IGF-1, assay

TABLE 3 Ingredients of Anionic salt used in diet of cows in close-up period (ANIOMIX®)

Ingredient	Amount
Vitamin A	300,000 IU
Vitamin D3	45,000 IU
Vitamin E	3000 IU
Calcium (Ca)	150 gr
Chlorine (CL)	150 gr
Magnesium (Mg)	25 gr
Sulfur (S)	35 gr
Zinc (Zn)	1500 mg
Manganese (Mn)	1200 mg
Copper (CU)	500 mg
Selenium (Se)	8 mg
Cobalt (Co)	9 mg
Iodine (I)	12 mg
Chrome (Cr)	14 mg
Monensin	400 mg
Niacin (B3)	4 gr
Antioxidant	1000 gr
DCAD	-6473.21 mEq/kg

range: 1–400 ng/ml and analytical sensitivity: 0.53 ng/ml) and insulin (0.2–60 mIU/L and analytical sensitivity: 0.11 mIU/L) were measured by enzyme-linked immunosorbent assay with species-specific kits for cow (Shanghai Crystal Day Biotech Co., LTD, Shanghai, China). Albumin, total protein, glucose, urea, triglyceride, cholesterol and aspartate aminotransferase (AST) were measured by commercially available kits (Pars azmoon, Tehran, Iran). The NEFA and β -hydroxybutyric acid (BHBA) were measured with commercial kits based on enzymatic reactions (Randox Laboratories Ltd., Ardmore, UK). All measurements were performed by a biochemical auto-analyzer (Biotechnica, BT 1500, Rome, Italy). Control serum (Randox Laboratories Ltd., Ardmore, UK) was used for controlling measurement accuracy. We used a mathematical calculation to predict insulin resistance index ($RQUICKI_{BHB} = 1/[\log \text{glucose (mg/dl)} + \log \text{insulin } (\mu\text{U/ml)} + \log \text{NEFA (mmol/l)} + \log \text{BHB (mmol/l)}]$). RQUICKI index may be interpreted the way that lower values are indicative of higher insulin resistance of an individual (De Koster & Opsomer 2013).

2.3 | Data management and statistical analysis

Six cows were excluded from the study due to health problems (four cows due to metritis and mastitis in the treatment group) or not calving in the desired time window (two cows in the control group, 2–8 days after injection). The excluded cows substituted by proper cows regarding parity, milk production and BCS.

Normality of variables was evaluated by PROC UNIVARIATE of SAS software, version 9.2 (SAS Inst. Inc., Cary, NC). Variables with Shapiro-

Wilk values $p > 0.05$ were considered normal (25(OH)D, IGF-1, insulin, albumin, total protein, cholesterol, triglyceride, urea, glucose, insulin resistance index) while all other variables were normalized using the natural logarithm.

Data of serum profile was analyzed using repeated-measures ANOVA (Mixed procedure in SAS, version 9.2). The model for all serum metabolites contained the effects of treatment (treatment and control), time of sampling (-7, 1, 3, 7, 15, 30 days after calving), BCS category (BCS of ≥ 3.7 were enrolled as fat cow and a BCS of < 3.7 as non-fat cow) and parity groups (third lactations, fourth lactation and fifth or more) for statistical purposes. All variables were offered to each model and then removed in a backward stepwise elimination approach. Treatment, time and interaction between treatment and time were included in the final model for all variables. In addition, interactions between treatment and the significant covariates were tested and included in the final model if significant. Only first sampling time was added to basic models for all variables as significant covariate. Cows that had a BCS of ≥ 3.7 were enrolled as fat cow and a BCS of < 3.7 as non-fat cow. Parity was classified into three groups: cow with third parity (control, $n = 3$; test, $n = 5$), fourth parity (control, $n = 5$; test, $n = 5$) and fifth or more parity (control, $n = 4$; test, $n = 2$). The average age of cows in the control and treatment groups was 6.35 and 6.0 years, respectively. Interactions between the time of sampling and BCS were tested to see if the BCS effect was significant. In addition, the interaction of BCS and significant covariates (parity and health situation) were tested and included in the final model if significant. Differences with $p \leq 0.05$ were considered as significant and $0.05 < p \leq 0.10$ were considered as a tendency. Least square means (LSM) and standard errors (SE) are presented.

3 | RESULTS

BCS and parity were not associated with the blood variables and, therefore, were not included in the final models. Results from the first sampling moment (data not shown) were used as covariate in the models, which had an effect on the outcome of the treatment comparison in this study. This study shows that injection of vitamin D3 was resulted in higher amounts of 25(OH)D, urea, insulin and insulin resistance index in the treatment group than in the control group ($p \leq 0.05$). On the other hand, the amounts of glucose, NEFA, BHBA concentrations and AST activity were higher in the control group than in the treatment group ($p \leq 0.05$, Table 4).

Time has a significant effect on serum glucose, BHBA, NEFA, albumin, total protein, AST, cholesterol, triglyceride, urea concentrations and insulin resistance index ($p \leq 0.05$, Table 4). There were not any group \times time interactions for measured variables (Table 4).

Pairwise comparisons for each sampling time showed that amount of insulin 30 days after calving was higher in the treatment group, NEFA value 7 days after calving was higher in the control group, triglyceride amount 7 days before calving was higher in the control group, urea 3 days after calving was higher in the treatment group, glucose 7 days after calving was higher in the control group and insulin resistance

TABLE 4 Least square mean and SE of measured variables and the effects of time and treatment in trial groups

Variables	Control	Treatment	Group	Time	Time*group
25(OH)Vit D(ng/ml)	93.21 ± 4.1	108.41 ± 3.77	S	S	NS
NEFA(mmol/l)	0.78 ± 0.05	0.63 ± 0.05	S	S	NS
BHBA(mmol/l)	0.52 ± 0.42	0.43 ± 0.42	S	S	NS
IGF-1(ng/ml)	98.14 ± 15.01	96.55 ± 15.1	NS	NS	NS
Insulin(mIU/l)	14.31 ± 0.46	15.04 ± 0.46	S	NS	NS
Insulin resistance index	0.318 ± 0.09	0.369 ± 0.09	S	S	NS
Glucose(mg/dl)	83.88 ± 16.41	78.45 ± 16.23	S	S	NS
Total protein(g/dl)	7.08 ± 0.62	7.14 ± 0.62	NS	S	NS
Albumin(g/dl)	4.25 ± 0.03	4.20 ± 0.02	NS	S	NS
Cholesterol(mg/dl)	100.36 ± 2.2	96.46 ± 2.2	NS	S	NS
Triglyceride(mg/dl)	4.76 ± 0.64	5.19 ± 0.63	NS	S	NS
Urea(mg/dl)	28.05 ± 0.51	28.29 ± 0.51	S	S	NS
AST(IU/l)	83.34 ± 2.74	76.84 ± 2.66	S	S	NS

Abbreviations: S: significant difference ($p \leq 0.05$), NS: not significant difference.

index was higher at 7 and 30 days after calving in the treatment group ($p \leq 0.05$, Figures 1–3).

4 | DISCUSSION

In the previous study, serum vitamin D concentrations were reduced in early lactation probably due to a raised demand in calcium homeostasis and consumption by the immune system. This condition may be associated with metabolic diseases after calving (Holcombe et al., 2018). In the present study injection of vitamin D3 prevented the reduction of vitamin D in early lactation and permitted to comprise control and treatment groups in important metabolic factors. The treatment group had significantly higher 25(OH)D concentrations after intervention than in the control group. Julien et al. (1977) conducted a trial that treated cows with 10 million IU vitamin D intramuscularly and studied milk fever incidents in the treatment group and also, a previous study recommended up to 10 million IU vitamin D3 injected intramuscularly in the prepartum period (Littledike & Horst, 1982). In our study, the selected dose of vitamin D3 was based on this recommendation. Although there is not any clear evidence suggesting injected vitamin D could reduce milk fever incidence in cattle (Julien et al., 1977).

Occurrence of metabolic and infectious diseases, stressful situations, trauma at calving time, energy excess or deficit and digestive upsets during transition period suggested the importance of this time in dairy cows (Bertoni et al., 2015). In late pregnancy and during early lactation, skeletal muscles and adipose tissues take up minimal amounts of glucose. The pancreas releases insulin into the bloodstream, which suppresses hepatic gluconeogenesis, glycogenolysis in the liver and skeletal muscles and adipose tissue lipolysis, whereas insulin stimulates glucose uptake in skeletal muscles and adipose tissues. Several studies have shown that cows are involved in negative energy balance and insulin resistance after parturition and onset of

lactation (Ji et al., 2012; Kerestes et al. 2009; Leblanc 2010). Many health disorders in dairy cattle were attributed to the process of uncontrolled lipid mobilization and raising NEFA in response to excessive NEB in early lactation and controlled lipid mobilization could prevent metabolic disease after calving (Leblanc, 2010). Elevated NEFA and BHBA are good indicators of negative energy balance in the transition period. Although NEFA could provide energy for many tissues but the excessive amounts in plasma could be toxic (Adewuyi et al., 2005). Excessive NEFA could convert to ketone bodies such as acetate, acetoacetate and β -hydroxybutyrate (BHBA). An early study showed a direct relationship between raising NEFA and insulin resistance in dairy cows (Oliveira et al., 2016). In dairy cows and other ruminants, adipose tissue's macrophage and lipolysis mechanism (adipose tissue remodelling) particularly in transitional period involved in the induction of IL-6, TNF- α and IL-1 secretion (Contreras et al., 2017). O'Boyle et al (2006), shown that over-conditioned cows have higher plasma TNF- α concentrations, lead the authors to hypothesize that obesity in dairy cows may lead to a state of chronic low-grade inflammation, causing insulin resistance and metabolic disorders. In the present study insulin secretion increased after vitamin D3 injection and better insulin secretion may have increased glucose uptake in insulin-dependent tissues such as skeletal muscle cells and reduced lipid mobilization from adipose tissue (less NEFA mobilization). Keep in mind that some degree of insulin resistance in insulin-dependent tissue especially muscle and adipose tissues are functional in the transition period because it saves glucose for using by the fetus and after parturition by the mammary gland for milk production. Although, insulin can alter the activity of gluco kinase and gluconeogenesis in hepatic cells the exact mechanism of insulin action in relation to lowering glucose concentration in treatment group is not clear. Vitamin D may be affected the synthesis and secretion of insulin by beta pancreatic cells in different ways. Rising the amounts of the pro-inflammatory cytokines after calving could inhibit β -cells function and vitamin D may facilitate insulin secretion by

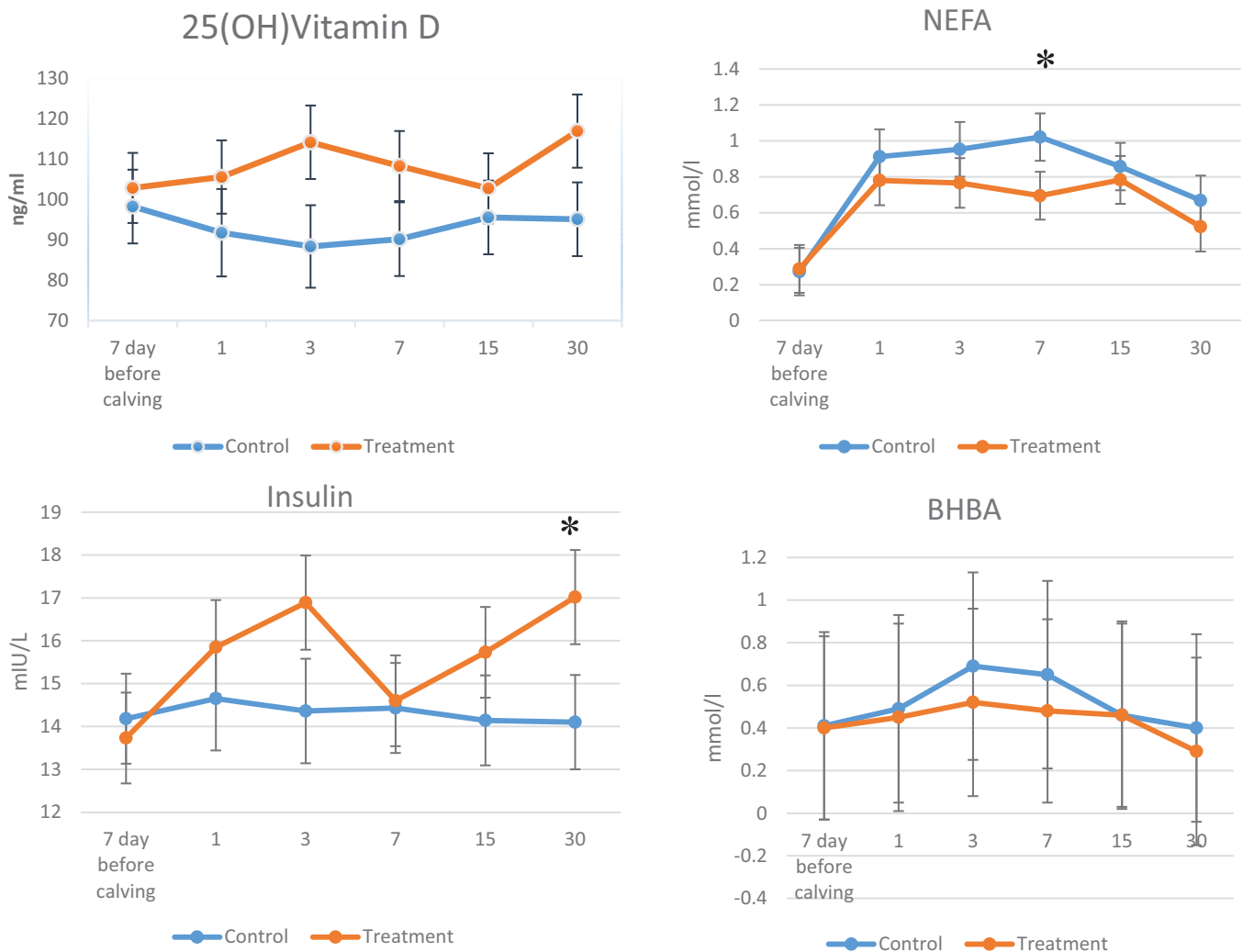


FIGURE 1 Time related changes and pairwise comparisons (LSM \pm SE) of 25(OH) vitamin D, NEFA, insulin and BHBA amounts in vitamin D injected and control groups. Refer to Table 4 for the effects of group, time and interaction of group and time for each measured variable. *: significant difference between groups ($p < 0.05$)

immune modulation mechanisms (Mathieu, 2005; Trevisi et al., 2015). In addition, a previous study has shown that vitamin D can increase insulin secretion by affecting intracellular Ca concentration in pancreatic β cells and increased insulin secretion (Borges et al., 2011). In our companion paper, the amounts of serum total calcium were not significantly different between cows of trial groups, and the concentration of ionized calcium was significantly lower in the treatment group than control (Sadri et al., 2021). Thus, the effect of insulin on intracellular calcium was not probable. Another important subject is, increased uptake of glucose by muscle and adipose tissues following insulin secretion is not a beneficial effect for a fresh cow with a reduced feed intake because more glucose must be supplied for mammary gland and milk production. Thus, the effects of vitamin D on energy metabolism must be concomitantly considered with body condition score, feed intake and milk production for a better understanding of vitamin D roles in energy balance during the transition period.

Parental administration of vitamin D in our study reduced the amounts of BHBA, NEFA and glucose and enhanced better insulin

secretion and immunomodulatory effect of vitamin D reduced NEFA and BHBA in the treatment group. In dairy cows, adipose tissue had an endocrine role. It is hypothesized that cows with an over-conditioned score had inflammation that caused insulin resistance and impaired insulin production (De Koster & Opsomer, 2013). In the present study, the treatment group showed significant effects on energy characteristics and insulin resistance that was in agreement with a previous study that had shown that vitamin D has improved insulin sensitivity by its anti-inflammatory action and diminish IL-6 and TNF- α concentrations (Borges et al., 2011). If vitamin D3 injection could control inflammatory conditions then the concentrations of NEFA and BHBA are reduced and may lead to reduced insulin resistance and control of negative energy balance.

The cows in the treatment group had a higher insulin resistance index than the control group. In this regard, Viera-Neto et al. (2017) administered calcitriol after the calving period and suggested no difference in NEFA, glucose and urea concentrations between trial groups although cows in the treatment group had a slightly higher amount

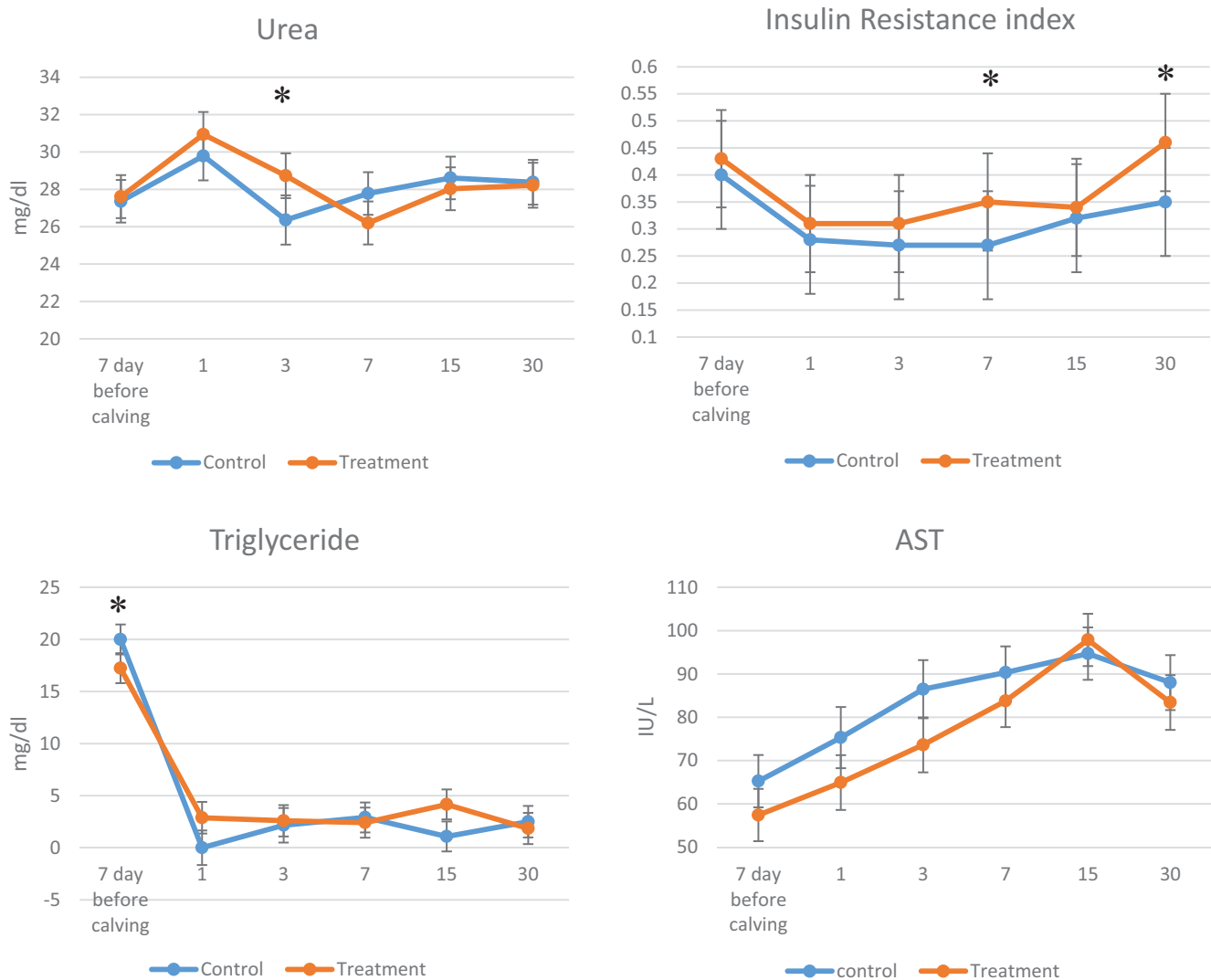


FIGURE 2 Time related changes and pairwise comparisons (LSM \pm SE) of urea, triglyceride, AST and insulin resistance index amounts in vitamin D injected and control groups. Refer to Table 4 for the effects of group, time and interaction of group and time for each measured variable. *: significant difference between groups ($p < 0.05$)

of BHBA. As previously mentioned, the amounts of insulin and glucose were significantly higher and lower in the treatment group than in control, respectively. Regulation of glucose haemostasis by insulin is based on two major components, glucose intake by tissue and suppression of hepatic glucose output (gluconeogenesis). The cause of the lower amount of glucose in the treatment group was attributed to better secretion of insulin by pancreatic beta cells which resulted in lower insulin resistance and better glucose utilization by body cells.

Although the mechanism of insulin resistance differs in humans, dogs and cats when compared to ruminants (Dandona et al., 2004) there are similarities between human and ruminant insulin resistance (De Koster & Opsomer, 2013). The development of insulin resistance may be specific to a certain metabolic pathway in a certain tissue. Dairy cattle tolerated some degree of NEB after parturition concomitant with a reduction in glucose and increasing BHBA and NEFA amounts. These situations are caused by glucose reduction followed by lipolysis and insulin resistance. The severity of these conditions depending on

the BCS of the cow before calving (Leblanc, 2010). On the other hand, AST activity was significantly lower in the treatment group than in the control group. This suggests liver status in the treatment group was in better physiologic and metabolic condition. Cows receiving the vitamin D3 injection showed mild but potentially significant reductions in lipolysis and transfer and accumulation of lipid in the liver. Lipid mobilization and accumulation in the liver caused damage to the hepatocyte and rising in AST activity in the serum. Clinically, dairy cows suffering from ketosis and/or fatty liver exhibit reduced feed intake, reduced milk yield, loss of body weight and central nervous system involvement. Cases of fatty liver syndrome do not respond well to treatment and mortality rates of up to 50% can occur (Adewuyil et al., 2005).

Urea is another variable that was significantly higher in the treatment group. Kawashima et al. (2016) clarified that cows with more insulin resistance had lower BCS during the transition period and urea value at the prepartum period. It is postulated that malnutrition in insulin-resistant cows caused lower BCS and urea values than in

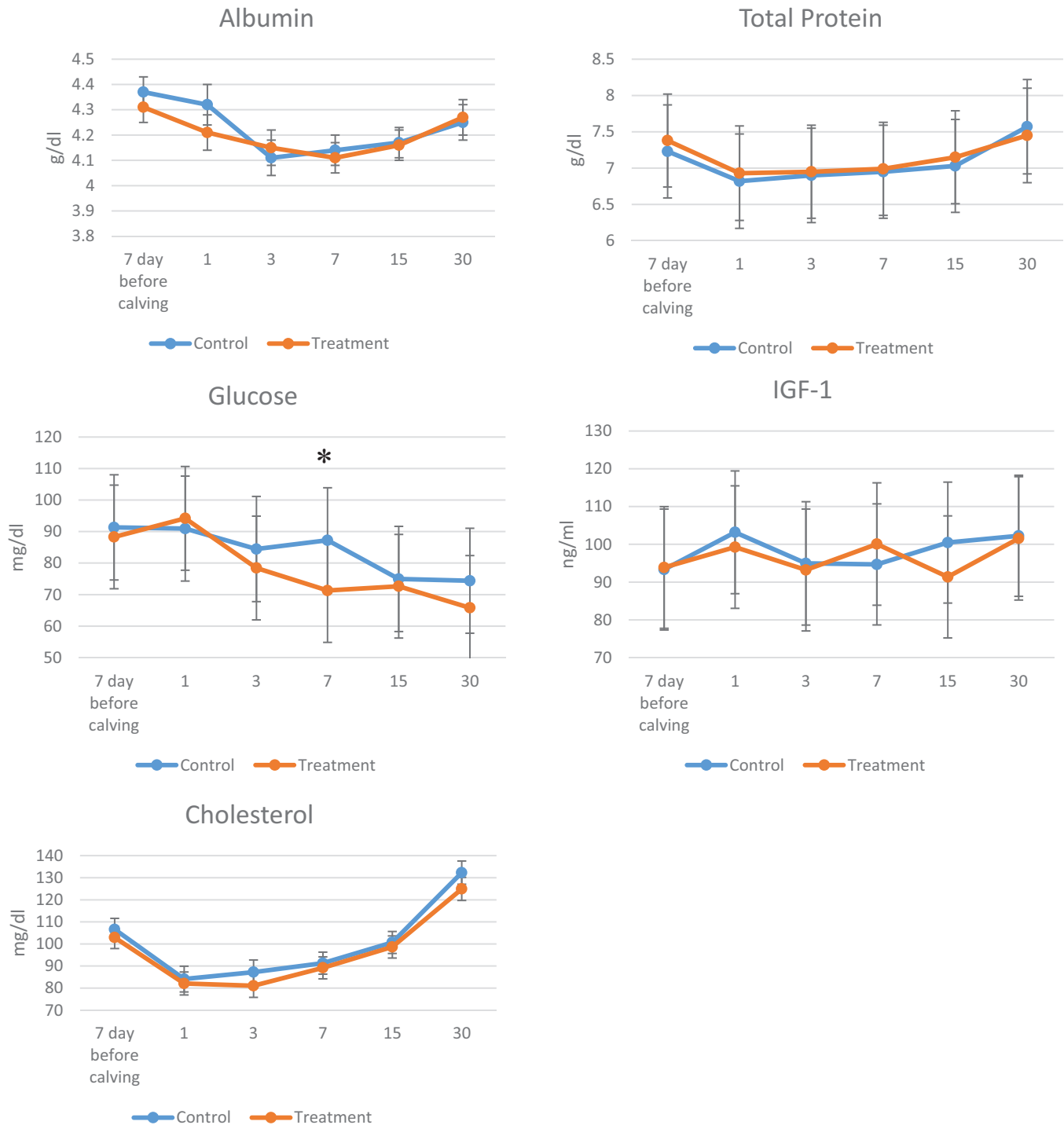


FIGURE 3 Time related changes and pairwise comparisons for the (LSM \pm SE) of albumin, total protein, BHBA, NEFA and cholesterol amounts in vitamin D injected and control groups. Refer to Table 4 for the effects of group, time and interaction of group and time for each measured variable. *: significant difference between groups ($p < 0.05$)

non-insulin resistant cows. In our study, the cows did not suffer from malnutrition and the results have not supported these descriptions.

In the present study, the amounts of albumin and total protein were not significantly different between trial groups. It was in agreement with a previous study that reported vitamin D administration did not have any effects on serum albumin and total protein amount (Rivera et al., 2005).

IGF-1 assessment in early post-partum during the negative energy balance could be used to predict nutritional and reproductive status in dairy cows and is associated with circulating concentrations of glucose, insulin and are negatively associated with plasma concentrations of NEFA. Also, IGF-1, cholesterol and triglyceride concentrations are dependent on dry matter intake. A possible explanation for unchanging amounts of IGF-1, cholesterol and triglyceride in the treatment

group was possible that vitamin D injection did not affect dry matter intake after parturition (Martinez et al., 2018). Trummer et al. (2017) shown that there was no significant effect of vitamin D supplementation on IGF-1 concentrations in humans. The study in humans suggested that the effects of vitamin D on lipid metabolism are potentially unfavourable (Schwetz et al., 2018).

Inconsistent with our result, it is suggested that a decrease of 25(OH)D in dairy cattle after parturition in comparing with late prepartum was established, although the biological significance of this change in healthy cattle is unclear (Holcombe et al., 2018). In our study, the effects of vitamin D3 injection are seeing in cows where the serum concentration of 25(OH)D are really at the high end of the reference value for cows undergoing parturition. It is likely that since these animals were in the upper limit of serum 25(OH)D amount, we did not see large effects following injection because the concentration of 25(OH)D remains in the middle value of the reference range after parturition. It seems that a better model would be cows with serum concentrations in the lower limit of serum 25(OH)D amount to clarify the vitamin D effects on insulin resistance and other energy characteristics in dairy cows. Since, in the cows in the lower mid-range of 25(OH)D, the concentration decreasing to a low level and/or lower than the reference value that may be resulted in many abnormalities or disorders in calcium homeostasis, immune responses and metabolism.

5 | CONCLUSION

The close-up administration of vitamin D3 could improve transition period management by controlling insulin resistance. Furthermore, energy characteristics have been refined following the mentioned treatment. The magnitude of changes and direction of changes of BHBA, NEFA, glucose and insulin is important in RQUICKI index calculation. Based on solely this index insulin resistance was lower in the treatment group but the effects of insulin must be interpreted with changes of other parameters such as glucose uptake by insulin-dependent tissues. Since many economical lose in dairy farming are caused by production-related diseases, decreasing insulin resistance may help with the prevention of negative energy balance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL 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 (3/43622). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

AUTHOR CONTRIBUTION

Morteza Hasanabadi: Conceptualization, Investigation, Writing – Original Draft Preparation. **Mehrdad Mohri:** Conceptualization, Funding Acquisition, Methodology, Investigation, Project Administration, Data Curation, Resources, Writing – Review & Editing. **Hesam. A. Seifi:** Conceptualization, Formal Analysis, Methodology, Project Administration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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