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The effect of dietary pioglitazone supplementation on milk yield, insulin sensitivity and GH–IGF-I axis in Holstein dairy cows during the transition period

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Abstract

Background: High-yielding dairy cows develop insulin resistance during late gestation associated with disruption of the growth hormone (GH)–insulin-like growth factor (IGF)-I axis and cause metabolic and reproductive disorders.

Objective: This study aimed to determine the effects of dietary pioglitazone (PIO) supplementation as an insulin sensitizer agent on milk yield, plasma metabolite status and GH–IGF-I axis in transition Holstein dairy cows.

Methods: Twenty multiparous cows were randomly assigned into two experimental groups ($n = 10$ animals per group) and either fed with a basal diet (control) or the basal diet supplemented with 6 mg PIO/kg body weight (BW) from day 14 before parturition to day 21 postpartum. The BW and body condition score (BCS), non-esterified fatty acids, beta-hydroxybutyrate (BHBA), insulin, glucose, GH and IGF-I concentrations, milk production and composition were measured weekly.

Results: BW and BCS losses were lower in PIO than in control cows ($p < 0.05$). The percentage and amount of milk fat were decreased, and the amount of protein increased only in the first post-calving week in the PIO-treated cows compared to the control ($p < 0.05$). Dietary PIO supplementation increased glucose concentration at calving, but insulin concentration was increased at calving and in the first post-calving week ($p < 0.05$). Plasma concentrations of IGF-I and the ratio of IGF to GH were increased in the PIO group ($p < 0.05$). The mean revised quantitative insulin sensitivity check index with BHBA, as an insulin sensitivity index, was greater in PIO-supplemented cows ($p < 0.05$).

Conclusions: Our results showed beneficial effects of PIO supplementation on improving insulin sensitivity and the GH–IGF-I axis that may cause lower negative energy balance and better metabolic and health status in transition dairy cows.

KEYWORDS
dairy cow, IGF-I, insulin sensitivity, thiazolidinedione, transition period

1 | INTRODUCTION

During the transition period, high-yielding dairy cows experience drastic energy increments to supply milk production and fetus growth. The increased energy requirements and decline in feed intake cause different levels of negative energy balance (NEB) during the periparturient period (Butler, 1998). Due to genetic selection for higher milk production, high-yielding dairy cows are more prone to intense NEB and lower peripheral insulin concentration (Bonczek et al., 1988). At this circumference, the decreased glucose uptake by insulin-dependent peripheral tissue helps drive more glucose towards the mammary gland as an insulin-independent tissue (Butler et al., 2003). Besides, in line with regular homeostatic changes, the transition dairy cows are exposed to various levels of insulin resistance, which would stimulate the mobilization of body lipid reservoirs to mitigate the negative consequences of NEB. Meanwhile, the intensiveness of insulin resistance in high-producing dairy cows is associated with different metabolic disorders such as fatty liver.

Insulin is a crucial metabolic essential factor/hormone in coupling the growth hormone (GH)–insulin-like growth factor (IGF)-I axis. It has been shown that hypoinsulinemia and/or insulin resistance downregulates GH receptors 1A (GHR1A) expression in the liver (Butler et al., 2003). As hepatic production of IGF-I depends on the binding of GH to these receptors, low expression of GHR1A reduces hepatic and circulatory IGF-I production. The decreased circulatory IGF-I reduces the negative feedback on GH secretion at the pituitary, which in turn increase the concentration of GH (Lucy, 2004). The higher GH concentration accelerates lipolysis in adipose tissue, increasing the plasma non-esterified fatty acids (NEFA) concentration as one of the main risk factors for developing insulin resistance (Lucy, 2004; Yousefi et al., 2016). These physiological conditions are associated with more blood fat metabolite, make worse insulin resistance and increased susceptibility of dairy cows to metabolic and reproductive disorders (Drackley, 1999; Duffield, 2000).

To date, several researchers have suggested approaches to control excessive lipolysis and insulin resistance in postpartum cows using the administration of thiazolidinediones (TZD) (Smith et al., 2007, 2009) and insulin (Butler et al., 2003), as well as the administration of oral compounds such as anti-lipolytic vitamin (Pescara et al., 2010; Pires et al., 2007) and unsaturated fatty acids (Mashek et al., 2002). However, no precise physiological regulator has been identified for the efficacy of oral compounds in mitigating insulin resistance. The TZD family is the most potent peroxisome proliferator-activated receptors (PPARs) ligand that increases insulin sensitivity through molecular and metabolic pathways (Houseknecht et al., 2002). In the TZD class, pioglitazone (PIO) is a synthetic and specific drug of PPAR-γ that affects insulin sensitivity, fat metabolism, folliculogenesis and reproductive function (Yousefi et al., 2016).

Treating genetically obese or diet-induced non-alcoholic fatty liver disease/non-alcoholic steatohepatitis mice with PPARγ ligands has decreased hepatic TAG. Activation of PPARγ triggers pathways that cause more glucose uptake and fatty acid oxidation in hepatocytes, thereby improving systemic insulin sensitivity and reducing liver steatosis (Ye and Scherer, 2013). In addition, PPARγ agonists could reduce hepatic fibrosis by restraining hepatic stellate cell (HSC) proliferation and driving activated HSC to apoptosis (Bae et al., 2010). This evidence shows the potential PPARγ activation for treating inflamed liver, a condition that is prominent in transition dairy cows and associated with impaired liver function. It has been postulated that by increasing insulin sensitivity and providing more healthy liver in dairy cows, PPARγ activation regulates the expression of the GH-IGF-I axis connecting components such as GHR 1A.

Although the beneficial effect of TZDs on the metabolism and reproduction of dairy cows has been investigated, it is not clear if the recoupling GH–IGF-I axis is a possible physiological pathway to exert their beneficial effects in postpartum dairy cows or not. Therefore, in the present study, the impact of PIO administration, as an antidiabetic drug, on milk yield and components, recoupling of GH–IGF-I axis, blood metabolites, hormones and insulin sensitivity was investigated in transition dairy cows.

2 | MATERIALS AND METHODS

2.1 | Animals and treatments

Twenty multiparous (two to four parity) Holstein dairy cows with no history of severe clinical disease were randomly allotted to two experiment groups from two weeks before to four weeks after parturition. According to NRC (2001), the cows received standard basal diets (Table 1) to meet their nutritional requirements during the pre- and postpartum periods.

Cows' allocation to the experimental groups was balanced based on their previous 305-day mature-equivalent milk yield, body condition score (BCS) and parity. They were group-housed in shaded outdoor pens. During the pre- and postpartum periods, cows were fed a total mixed ration (TMR) twice a day (0800 and 1600 hours) for ad-libitum consumption. Treatments were basal diets either supplemented with 6 mg PIO/kg BW/day or without PIO supplementation as the control group (Yousefi et al., 2015, 2019). Following the calculation of the total PIO needed for daily supplementation, it was well mixed in wheat bran and used to provide the morning TMR fed from day − 14 to 21 relative to parturition. The weekly BW of all the experimental cows was considered to adjust the dietary PIO supplementation amount. Pioglitazone was provided from Hetero Drugs (India; Batch No: PHD 0510001) as PIO hydrochloride. During the experiment, BW and BCS were recorded weekly. BW was measured using digital cattle weighing scale at 8:00, before morning feeding. Body condition score assessment was performed using three experts using a 5-point scale, and the average of the values was used to determine BCS for each cow (Wildman et al., 1982).

2.2 | Milk yield and composition

Cows were milked three times a day, at 8:00, 16:00 and 24:00. Individual cow's milk production was measured for each milking time until

TABLE 1 Ingredients and nutrient content of dairy cows' diets in the pre- and post-calving period (DM basis)

Item	Pre-calving diet	Post-calving diet
Ingredients (% of DM)		
Alfalfa hay	21.03	12.66
Corn silage	27.26	19.10
Wheat straw	9.71	1.79
Barley grain	8.23	7.08
Corn grain	17.14	15.85
Cottonseed meal	–	4.81
Soybean meal	10.40	12.52
Soybean, whole- roasted	–	8.05
Beet pulp	–	5.49
Wheat bran	3.47	1.79
Corn germ meal	–	6.95
Meat meal	–	0.39
Fat powder	–	0.40
Limestone	–	0.59
Magnesium oxide	0.3	0.16
Common salt	0.08	0.40
Sodium bicarbonate	–	0.80
Vitamin and mineral premix*	2.38	1.19
Nutrient content		
DM, %	52.48	60.13
NEL (Mcal/kg DM)	1.54	1.66
Ether extract, %	2.76	5.07
CP, %	14.80	17.79
ADF, %	22.60	17.60
NDF, %	37.00	30.5

Abbreviations: ADF, acid detergent fibre; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; NEL, net energy lactation.
*Contained (per kg): 800,000 IU vitamin A; 100,000 IU vitamin D; 5000 IU vitamin E; 10.0 g Mn; 12.0 g Zn; 2 g Cu; 0.06 g Se; 0.08 g I; and 0.01 g Co.

21 days postpartum. In addition, weekly milk samples were collected from all three consecutive daily milking and stored in plastic tubes containing potassium dichromate at 4°C. Milk samples were tested using MilkoScan (134 BN Foss Electric, Hillerød, Denmark) for fat, protein, lactose and total solids.

2.3 | Plasma metabolites and hormone assays

Blood samples were collected at 13:00 h from the coccygeal vein of all cows on days −14, −7, 0, 7, 14 and 21 relative to calving using evacuated glass tubes containing EDTA (10.5 mg, Monoject; Sherwood Medical, St. Louis, MO, USA). The collected blood samples were kept at 4°C, within 1 h after sampling, centrifuged at 3000×g for 10 min, and the harvested plasma was stored at −20°C until further evaluation. Plasma concentration of glucose (ParsAzmoon Co., Tehran, Iran), NEFA and beta-hydroxybutyrate (Randox Laboratories Ltd., London, UK) were assessed using commercial kits according to the manufacturer's procedures. The NEFA and beta-hydroxybutyrate (BHBA) inter-assay coefficients were 6.1% and 4.4%, whereas their intra-assay coefficients were 4.3% and 3.9%, respectively. Blood concentrations of insulin (Diaplus Inc., USA), GH (Monobind, Inc., Lake Forest, CA, USA) and IGF-1 (Hangzhou Eastbiopharm Co., Ltd., USA) were measured using their corresponding ELISA kits as described in the manufacturer's instructions. Inter-assay and intra-assay coefficients of variation were 7.5% and 5.6% for insulin, 5.1% and 3.2% for GH, and 6.8% and 5.4% for IGF-1 assays.

2.4 | Estimation of insulin sensitivity index

In this experiment, the revised quantitative insulin sensitivity check index (RQUICKI) including BHBA was used to determine insulin sensitivity according to the following formula (Balogh et al., 2008):

RQUICKI-BHBA = 1/ [log (glucose (mg/dL)) + log (insulin (μU/mL)) + log (NEFA (mmol/L)) +log (BHBA ((mmol/L))].

2.5 | Data analysis

The data generated during the time (hormones, blood metabolites, BW and BCS) were analyzed using SAS version 9.2 software and MIXED procedure in a complete randomized block design based on parity with time as the repeated measure. However, the generalized linear model procedure was used to analyze BW and BCS losses. Pre-treatment measurements were used as covariates for the respective response variables. Significant differences and tendencies were declared at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

3 | RESULTS

The effects of PIO on BW and BCS are presented in Table 2. The mean post-parturition BW in PIO-treated cows tended to increase compared to the control group ($p = 0.06$). A significant interaction of treatment × time on BW was detected, where there were no differences between experimental groups at calving and in the first post-calving week, but the control cows had lower BW in the second and third post-parturition weeks (Figure 1). Also, the control cows lost more BW compared to the PIO cows during the postpartum period ($p < 0.05$). Accordingly, BCS loss in PIO-treated cows was lower compared to the control cows ($p < 0.05$).

The effects of PIO supplementation on milk yield and composition are shown in Table 3. Results showed that milk production was not affected by the treatments. However, the percentage ($p < 0.05$) and amount of milk fat ($p \leq 0.08$) in the PIO-treated cows were decreased as compared to the control cows. The significant interaction of treatment × time on the percentage and production of milk protein

TABLE 2 The effect of Pioglitazone feeding on mean body weight and BCS of Holstein dairy cows ($n = 10$ cows per treatment)

	Treatments*			*p* Value		
Item	Control	PIO	SEM	Treatment	Time	Treatment × time
BW (kg)	643.81	666.47	8.66	0.06	<0.01	0.04
BW loss (kg)†	33.01[a]	24.28[b]	1.91	<0.01	–	–
BCS††	3.34	3.58	0.61	0.79	0.39	0.26
BCS loss†††	0.98[b]	0.73[a]	0.05	<0.01	–	–

Abbreviations: BCS, body condition score; BW, body weight; PIO, pioglitazone; SEM, standard error of the mean.
*Animals either fed by basal diet (control) or fed by the basal diet supplemented with 6 mg PIO/kg BW from day 14 before parturition to day 21 postpartum. BW and BW losses were measured during the postpartum period, and BCS was measured from day 14 calving to day 21 after calving.
[a,b]Within the row, values with different superscripts are significantly different ($p < 0.05$).
†Calculated as BW at day 21 post parturition − BW at parturition.
††Based on a 5-point scale.
†††Calculated as BCS at day 21 post-parturition − BCS at day 14 pre-parturition.

TABLE 3 The effect of pioglitazone feeding on milk production and composition of Holstein dairy cows during postpartum period ($n = 10$ cows per treatment)

	Treatments*			*p* Value		
Item	Control	PIO	SEM	Treatment	Time	Treatment × time
Milk yield, kg/d	40.02	38.98	2.06	0.72	<0.01	0.63
4% FCM**, kg/d	42.80	39.06	2.03	0.21	<0.01	0.91
Fat, %	4.48[a]	4.05[b]	0.11	0.02	<0.01	0.36
Fat, kg/d	1.77	1.56	0.05	0.08	0.02	0.09
Protein, %	3.40[b]	3.82[a]	0.10	<0.01	<0.01	0.01
Protein, kg/d	1.35	1.47	0.08	0.30	<0.01	0.01
Lactose, %	4.68	4.61	0.07	0.50	<0.01	0.60
Total solids, %	12.76	12.45	0.15	0.16	<0.01	0.23
Total solids, kg/d	5.08	4.83	0.25	0.48	<0.02	0.66

Abbreviations: PIO, pioglitazone; SEM. standard error of the mean.
[a,b]Within the same row, values with different superscripts are significantly different ($p < 0.05$).
*Animals either fed by basal diet (control) or fed by the basal diet supplemented with 6 mg PIO/ kg body weight (PIO) from day 14 before parturition to day 21 postpartum.
**4% FCM = fat-corrected milk, calculated as: [0.4 × milk production (kg d−1)] + [15 × fat yield (kg d−1)].

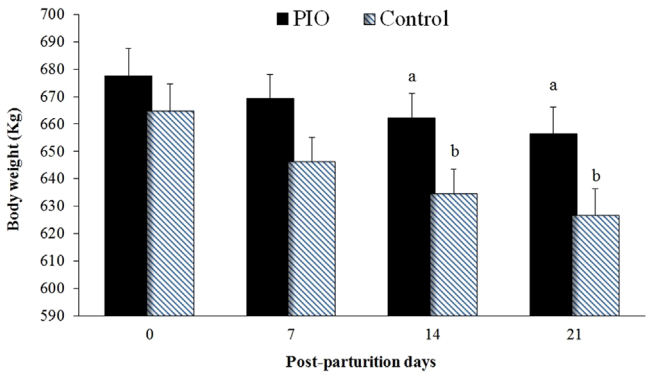

FIGURE 1 The effect of pioglitazone on body weight in Holstein dairy cows during the postpartum period. Note that within each time point, means with different superscripts (a,b) are significantly different ($p < 0.05$).

(Figure 2) revealed that PIO supplementation increased milk protein during the first week of lactation in PIO cows than that of the control cows ($p < 0.05$). However, PIO supplementation did not significantly affect the total milk protein, lactose and solids.

Table 4 represents the effect of PIO supplementation on plasma metabolites and hormones. Dietary inclusion of PIO decreased plasma concentrations of NEFA and BHBA in PIO-treated compared to the control cows ($p < 0.05$). The interaction of treatment × time on plasma NEFA showed that PIO reduced NEFA concentrations during the first and second postpartum weeks ($p < 0.05$; Figure 3). PIO supplementation had no significant effect on plasma glucose concentrations; however, there was a significant interaction of treatment × time, where PIO-treated cows had a higher concentration of glucose at calving ($p < 0.05$; Figure 4). Plasma insulin concentrations were not different in PIO and control cows; however, the significant interaction between

TABLE 4 The effect of pioglitazone feeding on blood metabolites, hormones, and insulin sensitivity index in Holstein dairy cows during transition period ($n = 10$ cows per treatment)

	Treatments*			*p* value		
Item	Control	PIO	SEM	Treatment	Time	Treatment × time
NEFA (mmol/L)	0.65[a]	0.49[b]	0.04	<0.01	<0.01	0.04
BHBA (mmol/L)	0.70[a]	0.48[b]	0.04	<0.01	<0.01	0.15
Glucose (mg/dL)	57.95	59.67	1.82	0.52	<0.01	0.04
Insulin (μIU/mL)	9.76	10.83	0.49	0.13	<0.01	<0.01
GH (μg/L)	3.37	3.23	0.28	0.74	<0.01	0.99
IGF-1 (μg/L)	35.12[b]	49.95[a]	3.73	0.02	<0.01	0.97
IGF/GH ratio	10.42[a]	15.46[b]	4.59	0.04	<0.01	0.86
IGF/ Insulin ratio	3.65	4.61	0.55	0.20	0.37	0.25
RQUICKI-BHBA index**	0.41[b]	0.46[a]	0.01	0.03	<0.01	<0.01

Abbreviations: BHBA, beta-hydroxybutyrate; GH, growth hormone; IGF-1, insulin like growth factor-1; NEFA, non-esterified fatty acids; PIO, pioglitazone; SEM, standard error of the mean.

[a,b]Within the same row, values with different superscripts are significantly different ($p < 0.05$).

*Animals either fed by basal diet (control) or fed by the basal diet supplemented with 6 mg PIO/ kg body weight (PIO) from day 14 before parturition to day 21 postpartum.

**RQUICKI-BHBA index = revised quantitative insulin sensitivity check index including BHB, calculated as: 1/ [log (glucose (mg/dL)) + log (insulin (μU/mL)) + log (NEFA (mmol/L)) +log (BHB ((mmol/L))].

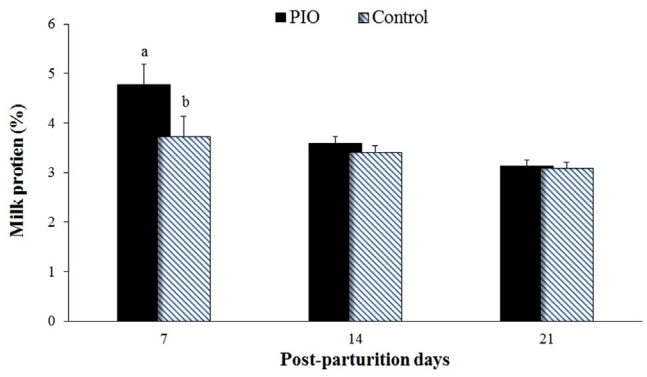

FIGURE 2 Effect of pioglitazone on milk protein percentage in Holstein dairy cows during the postpartum period. Note that within each time point, means with different superscripts (a,b) are significantly different ($p < 0.05$).

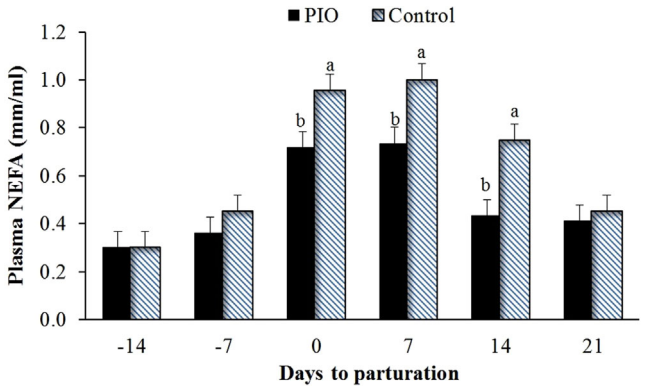

FIGURE 3 Effect of pioglitazone on plasma NEFA concentration in Holstein dairy cows during the transition period. Note that within each time point, means with different superscripts (a,b) are significantly different ($p < 0.05$).

treatment × time on plasma insulin revealed that PIO feeding increased the plasma insulin at calving and the first post-calving week ($p < 0.05$; Figure 5).

Plasma concentration of GH and the ratio of IGF-I to insulin were not influenced by PIO supplementation; meanwhile, the concentration of IGF-I and the ratio of IGF-I to GH were increased in the PIO cows compared to the control cows ($p < 0.05$). Moreover, PIO supplementation improved the RQUICKI-BHBA index compared to the control cows ($p < 0.05$). There was a significant interaction of treatment × time on the RQUICKI-BHBA index, where it was higher in PIO cows than in the control cows at day −7 and day +14 relative to parturition ($p < 0.05$; Figure 6).

4 | DISCUSSION

The health of dairy cows is endangered by metabolic changes around calving (Celi and Gabai, 2015; Ospina et al., 2010). The present study showed that PIO supplementation improved dairy cows' metabolic parameters and insulin sensitivity index during the transition period.

Plasma NEFA concentration is a well-known lipolysis and NEB biomarker that not only is increased by insulin resistance but also a high level of NEFA adversely affects insulin sensitivity during late gestation and early lactation in dairy cows (Tordjman et al., 2003). Previous studies have indicated that TZD administration reduced the plasma NEFA concentration during the postpartum period

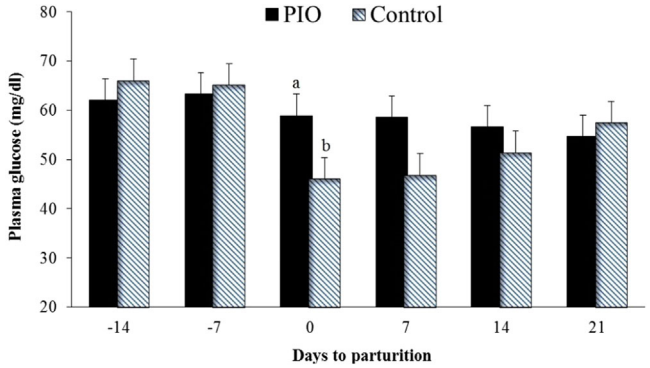

FIGURE 4 Effect of Pioglitazone on plasma glucose concentration in Holstein dairy cows during the transition period. Note that within each time point, means with different superscripts (a,b) are significantly different ($p < 0.05$).

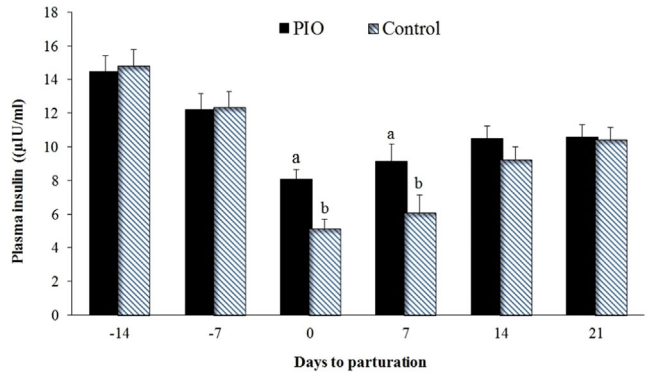

FIGURE 5 Effect of Pioglitazone on plasma insulin concentration in Holstein dairy cows during the transition period. Note that within each time point, means with different superscripts (a,b) are significantly different ($p < 0.05$).

(Schoenberg and Overton, 2011; Smith et al., 2007, 2009). Ghoreishi (2012) also showed that supplementation of 4 mg PIO/kg BW could reduce the concentration of NEFA. It has been suggested that TZD supplementation decreases insulin resistance associated with lower plasma NEFA in dairy steers (Kushibiki et al., 2001). By activating PPRγ, PIO has a significant increase in improvement in hepatic and peripheral insulin sensitivity and modulation of fat metabolism. In dairy cows, however, increasing dry matter intake during the postpartum period (Ghoreishi, 2012; Smith et al., 2007, 2009), mitigated NEB, as well as re-esterification of fatty acids that reduces fat mobilization, and stimulation of liver capacity for free fatty acids oxidation (Tordjman et al., 2003; Yousefi et al., 2016) were also suggested for lower NEFA by treatment of TZDs.

In addition to NEFA, BHBA is another metabolite associated with energy balance and liver functioning in lactating cows (Ospina et al., 2010). In this study, the decrease in BHBA concentration with PIO intake was consistent with the study of Yousefi et al. (2016), which showed that feeding 6 mg PIO/kg BW decreased plasma BHBA concentration in dairy cows. Smith et al. (2007) also stated that administering 2 or 4 mg TZD/kg BW decreased plasma BHBA concentration. Nevertheless, others did not find plasma BHBA changes (Gheise et al., 2018; Ghoreishi, 2012) or even observed higher plasma BHBA (Smith et al., 2009) after the administration of TZDs in dairy cows. Decreased plasma NEFA availability and increased hepatic capacity for free fatty acids oxidation explain the reduced BHBA in PIO-supplemented cows (Allen et al., 2005; Ide et al., 2000; Yousefi et al., 2016).

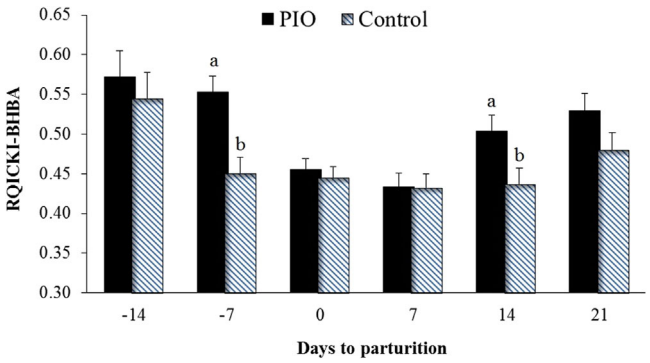

FIGURE 6 Effect of Pioglitazone on RQICKI-BHBA index in Holstein dairy cows during the transition period. Note that Within each time point, means with different superscripts (a,b) are significantly different ($p < 0.5$).

In the present study, PIO supplementation caused higher plasma concentrations of insulin during calving and the first post-calving week in PIO-treated cows. In addition, an increase in plasma glucose at calving in PIO-treated cows was noted. It has been shown that the administration of TZDs is associated with an increase in dry matter intake and plasma glucose (Ghoreishi, 2012; Larsen et al., 2003; Smith et al., 2007; Wolden-Hanson et al., 2002). Moreover, some studies have suggested that administration of 2 or 4 mg TZD/kg BW increased the concentration of glucose during the periparturient period as a result of the increased liver glycogen to triglyceride ratio and hepatic gluconeogenesis (Schoenberg and Overton, 2011; Smith et al., 2009). In animal models (Houseknecht et al., 2002), TZDs administration improved pancreatic β-Cell function and insulin production, which may explain the higher insulin concentrations in PIO-treated cows.

The PIO supplementation increased the plasma IGF-I concentration and the IGF-I to GH ratio but did not affect the GH concentration. Consistent results were reported by Yousefi et al. (2016), but Gheise et al. (2018) stated that PIO supplementation did not affect IGF-I plasma concentrations, likely due to the use of PIO in a shorter feeding period (21 days during the post-parturition period vs. 36 days during pre- and post-parturition periods). The high-producing dairy cows experience low plasma IGF-I and high GH concentrations during the transition period (Lucy, 2004), showing uncoupling between GH and IGF-I production. The subsequent recoupling of this axis depends on the reduction in NEB, improving GHR 1A receptor expression (Butler et al., 2003). In the present study, NEB indices (NEFA and BHBA) were significantly decreased in PIO-treated cows. These effects were also supported by the previous findings showing higher dry matter intake and lower NEB during postpartum in TZD-treated cows (Smith et al., 2007, 2009). It could be postulated that the higher IGF-I concentration per GH unit in the PIO-supplemented cows is due to the possible

improvement in the response of the liver and other peripheral tissues to insulin. Probably, the effect of PIO on increasing the liver's insulin sensitivity and a higher expression of the GHR 1A receptor resulted in more efficient IGF-I production. These results were supported by a better insulin sensitivity index in the PIO-treated cows. It is worth noting that insulin resistance in peripartum dairy cows is associated with low insulin and glucose concentrations and is somehow different from humans (Lucy, 2004). Hence, the increased insulin and glucose observed in PIO-tread cows at parturition and the first post-parturition week may convey an optimum metabolic condition; however, it may be explained by insulin resistance in humans. Although we did not conduct insulin or glucose clamp, as a direct evaluation and a precise method, it may help to address these contradictions.

The RQUICKI-BHBA index is a significant influencing factor for the relationship between baseline and dynamic glucose, NEFA, BHBA and insulin levels in ketotic cows (Djoković et al., 2017). The revised quantitative insulin sensitivity check index (RQUICKI) and its modified formula (RQUICKI-BHBA) are the best capable indexes for estimating insulin sensitivity changes (Balogh et al., 2008). However, adding BHBA to the RQUICKI index would help to evaluate insulin sensitivity quickly and efficiently. Our findings showed that the insulin sensitivity index increased in PIO-fed cows, which could be due to a decrease in NEFA and BHBA concentration. Gheise et al. (2018) and Schoenberg et al. (2011) showed that PIO and TZD treatments did not affect RQUICKI index. The RQUICKI has a poor distinguishing capacity when applied to diagnose reduced insulin sensitivity in cows, particularly when they are affected by metabolic diseases (Kerestes et al., 2009). The use of the RQUICKI-BHBA index in the present study rather than RQUICKI may explain the inconsistent finding compared to the results reported by Gheise et al. (2018) and Schoenberg et al. (2011).

In the current study, dietary PIO supplementation reduced BCS and BW loss. Consistent with our findings, it has been reported that cows fed PIO experienced lesser BCS loss during the transition period (Gheise et al., 2018; Smith et al., 2009; Yousefi et al., 2016). However, in contrast to our findings, some studies (Schoenberg and Overton, 2011; Smith et al., 2007) did not find a significant effect of TZD on reducing BCS and BW losses. The discrepancy between the literature likely depends on the TZD level, route of administration or duration of administration. Lower concentrations of lipolysis indices (NEFA and BHBA) indicate that PIO supplementation potentially improved insulin sensitivity, lipid metabolism and, thus, energy balance. In addition, less energy used to synthesize milk fat and possibly more energy income (as dry matter) may explain lower BCS and BW loss in the PIO-treated cows.

The results showed that PIO supplementation did not affect milk production but decreased fat milk percentage. In line with this result, Yousefi et al. (2016) showed that PIO supplementation reduced the milk fat percentage in dairy cows. It has also been shown that administration of 4 mg TZD/kg BW before calving tended to reduce milk fat percentage in dairy cows (Smith et al., 2009). As a component influencing blood fat metabolites and body fat mobilization, it could be assumed that the reduced milk fat in PIO-treated cows is a consequence of lowering blood NEFA, BHBA and other fat metabolites involved in the milk fat synthesis (Lucy, 2004). On the other hand, TZD administration has been shown to alter lipogenic gene networks in bovine mammary epithelial cells (Kadegowda et al., 2009) and, therefore, could change *de novo* fat synthesis in mammary glands. In the present study, although the amount of milk protein did not change, the percentage of milk protein was improved during the first week after calving by supplementing PIO. Because milk fat content was significantly decreased in PIO-treated cows, it may increase the proportion of the other milk components, such as protein. However, further investigations are required to find plausible mechanisms beyond the increment of milk protein.

5 | CONCLUSION

Insulin resistance in dairy cows is associated with several metabolic and reproductive disorders. During the preparation period, the increase in NEB in high-yielding dairy cows causes disruption GH–IGF-I axis. Subsequent recoupling of the axis depends on improvement in insulin sensitivity and lowering NEB. In this study, dietary supplementation of PIO improved the insulin sensitivity index and IGF-I to GH ratio. Our findings also showed that PIO supplementation influenced energy output and lipolysis by reducing milk fat and plasma concentrations of NEFA and BHBA. These observations, along with lower BW and BCS losses, emphasize the mitigating effects of PIO on NEB in dairy cows. The mitigated NEB indices and the increased insulin sensitivity in PIO-fed cows may improve the condition of GH–IGF-I recoupling associated with lower metabolic disorders in dairy cows.

AUTHOR CONTRIBUTIONS

Ali Reza Yousefi and Reza Masoumi: *Conceptualization*, *Methodology*, *Supervision*, *Project administration*, *Formal analysis*, *Visualization*, *Writing—Original Draft*, *and Writing—Review and Editing*. Behnam Rostami and Hamid Amanlou: *Visualization*, *Investigation*, *Writing—Original Draft*, *and Writing—Review and Editing*. Saeed Mirzaie: *Contribution to the experimental procedure and preparing the manuscript*.

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CONFLICT OF INTEREST

No potential conflict of interest is reported by the authors.

DATA AVAILABILITY STATEMENT

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

ETHICAL APPROVAL

The experiment was conducted with approval from the Department of Animal Science, Faculty of Agriculture, University of Zanjan Ethics Committee, Zanjan, Iran.

PEER REVIEW

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