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Effects of dietary supplementation of pioglitazone on metabolism, milk yield and reproductive performance in transition dairy cows

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Abstract

The objective of this study was to investigate the effect of dietary supplementation of pioglitazone (PGT), a specific ligand for PPARγ, on metabolic dynamics, milk production, and reproductive performance of transition dairy cows. Eighty multiparous Holstein cows in their second or more lactations were blocked by the calving date and parity and assigned randomly to four dietary groups (n=20 cow/treatment) including control (no PGT-/-), supplemented with PGT (6 mg PGT/kg body weight) from day -14 to +21 relative to parturition (PGT+/+) or only during prepartum (PGT+/-) or postpartum periods (PGT-/+). Postpartum body condition score and body weight loss decreased ($P < 0.05$) in all PGT supplemented groups. Milk yield was not affected by PGT supplementation ($P > 0.05$). Percentage of milk fat decreased ($P < 0.05$) in all PGT-treated groups; however, milk fat yield was lower ($P < 0.05$) in PGT (+/+) and PGT (+/-) groups compared to PGT (-/-). Peripartum (d -7 to +7) concentrations of plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) decreased in PGT (+/+) but not in the PGT (-/-) group ($P < 0.05$). During the postpartum period, PGT reduced ($P > 0.05$) plasma concentrations of NEFA in all PGT-treated groups but did not affect BHBA level. Plasma concentrations of triglycerides (TG) decreased in all PGT supplemented groups. Supplementation of PGT decreased the peripartum concentrations of plasma glucose in PGT cows. Plasma concentrations of IGF-I were higher in PGT (+/+) compared to the control group during both the peri- and postpartum periods. Plasma concentrations of growth hormone and insulin were not affected by PGT treatment ($P > 0.05$). Mean days to ovulation were lower and the proportion of cows ovulating by d 14 postpartum was higher in PGT (+/+) and PGT (+/-) compared to control. Days open were shorter in PGT (+/+), PGT (+/-) and PGT (-/+) groups compared to control.

The proportion of pregnant cows at 120 DIM was higher in PGT (+/+) and PGT (+/-) compared to the control. The results showed positive effects of dietary supplementation of PGT, especially supplementation during both the pre- and postpartum periods, on metabolic dynamics, ovarian function and reproductive performance in transition dairy cows.

***Keywords**:* Insulin resistance; pioglitazone; metabolic dynamics; milk yield; reproductive performance; dairy cows.

1. Introduction

The transition period is a crucial period in dairy cows, affecting their postpartum health, reproduction and milk performance, and is accompanied by homeorhetic adaptations in glucose and lipid metabolism [1, 2]. The increased overall energy demand in transition cows is supported by a simultaneous decrease in glucose oxidation by peripheral tissues [2], an increase in glucose output by the liver [3], and also by stimulation of body fat mobilization, that is facilitated through a decrease in the response of adipose tissue to insulin [4], known as insulin resistance.

Insulin resistance during the prepartum period is a part of the homeorhetic mechanisms that develop in peripheral tissues, and continues into early lactation to direct the nutrients toward the fetus and mammary glands [1]. Decreased sensitivity of peripheral tissues to insulin facilitates mobilization of non-esterified fatty acids (NEFA) to compensate and mitigate the period of negative energy balance (NEB); however, it may be followed by a rapid decrease in dry matter intake and a high spike of NEFA during the transition period [5, 6]. High concentrations of NEFA in transition cows contribute to energy and immune related metabolic disorders, such as displaced abomasum, ketosis, fatty liver, metritis and mastitis [7], and result in a greater risk of insulin resistance [8].

The potential for modulation of adipose tissue metabolism may suggest ways to moderate the acute spike in circulating NEFA concentrations. In this regard, several strategies have been tried to decrease insulin resistance and attenuate metabolic disorders [9-13]. A pharmacological method, based on administration of an exogenous ligand of peroxisome proliferator and activator receptors (PPARs), has been proposed as a newer approach for controlling the prepartum insulin resistance in dairy cows [14-16].

The PPARs which are widely distributed in the body, especially in the bovine adipose tissue as well as the reproductive organs [17] and mammary glands [18-20], have tremendous effects on body fat metabolism as a differentiation regulator of adipocytes [21]. Activation of these receptors in adipose tissues, influence adipocytes capacity for fatty acid storage and regulate several adipokines affecting insulin resistance [22]. PPARγ, the most prevalent PPAR in the adipose tissues, is activated by several endogenous [21, 22] and exogenous ligands such as thiazolidinediones (TZDs).

There is evidence that TZD_S injection was able to reverse the TNFα-induced insulin resistance in steers [23], and improve energy efficiency in beef cattle [24]. It was also shown that administration of TZDs during late pregnancy in dairy cows maintained higher postpartum BCS, decreased plasma concentrations of NEFA and liver TG accumulation, increased peripartum DMI and plasma glucose levels, and stimulated postpartum ovarian activity [15, 16, 25]. Accordingly, dietary supplementation of about 0.114 mg TZDs per kg BW in beef cattle also improved energy efficiency, and liver and muscle fatty acid oxidation [24]. These findings demonstrate the effectiveness of TZDs on attenuating insulin resistance and improving metabolic dynamics of transition dairy cows.

Pioglitazone, as a TZD drug, is a synthetic and specific ligand for PPARγ that is used for treatment of type 2 diabetes mellitus in human [26]. By binding and activating PPARγ,

pioglitazone affects plasma lipids, adipose tissue, and liver to reduce insulin resistance [26, 27]. The bioavailability of PGT after oral administration was 62% in sheep [28], 83% in humans [29], and 50% in rats [30]. In dairy cows, the bioavailability and half-life of PGT following oral administration was about 60%, showing acceptable potential of PGT for dietary supplementation [31]. Although, there are several studies on dietary supplementation of TZDs in beef cattle [23, 24] and dairy cows [15, 16, 25], the use of this drug has not been approved yet, and further investigations are needed to reveal the effects of TZDs on animal metabolism and health. To our knowledge, there are no reports on the effect of dietary supplementation of TZDs on metabolism in transition dairy cows or postpartum reproduction.

The objectives of this experiment were to determine the effect of dietary supplementation of pioglitazone hydrochloride during the transition period on metabolism, milk production and reproductive performance in dairy cows.

2. Materials and methods

2.1. Experimental design

The experiment was conducted on high-producing Holstein dairy cows (42 kg/day) in a commercial dairy farm in the north of Iran from February to April 2012. Eighty multiparous cows (parity 2-6) with no overt clinical disease history were blocked by calving date and parity, and allocated in four dietary groups (n=20 cow/treatment). PGT was obtained as pioglitazone hydrochloride from Hetero Drugs (India; Batch No: PHD 0510001) and kindly provided by Darou Pakhsh Co., Tehran, Iran. Considering about 60% bioavailability for oral administration of pioglitazone in ruminants [28, 31] and also previous studies [15, 16, 25] reporting that intravenous administration of 2 or 4 mg TZD/kg BW effectively influenced metabolism and performance of transition cows, a dose of 6 mg PGT/kg BW was used in the present experiment. Experimental period was 35 d, starting on d 14 before expected

parturition to d 21 postpartum. Diets consisted of PGT (+/+): supplementation of PGT during the pre and postpartum period (d −14 to +21 relative to parturition), PGT (+/-): supplementation of PGT only during the prepartum period (d −14 to parturition), PGT (-/+): supplementation of PGT only during the postpartum period (parturition to d +21 relative to parturition) and PGT (-/-): no PGT supplementation (control), (Figure 1). Cows were fed ad libitum, a common total mixed ration (TMR) during the pre- and postpartum periods twice per day (0800 and 1600 hours), supplemented with or without PGT.

Diets were formulated (Table 1) according to the requirements during the pre and postpartum as suggested by NRC [32]. Cow assignment to treatments was balanced for calculated previous 305-d mature-equivalent milk yield and BCS. The average (±SE) of parity in PGT (+/+), PGT (+/-), PGT (-/+), and PGT (-/-) groups was 2.80±0.25, 3.10±0.30, 3.30±0.33, and 2.70±0.24, respectively.

The diets were sampled weekly, and analyzed for crude protein (CP, method 988.05; AOAC, 1990); ether extract (method 920.39; AOAC, 1990), acid detergent fiber (method 973.18; AOAC, 1990) and neutral detergent fiber [33]. At weekly intervals, body weight and BCS were measured. BCS was evaluated using a 5-scale system by three experts and the average of which was taken as the BCS for each cow [34].

2.2. Milk and blood sampling

After parturition, cows were milked 3 times per day, at 07.00, 14.00, and 23.00 hours, and milk yield of individual cows was recorded at each milking, until d 30 postpartum. Milk samples were collected weekly from all 3 consecutive milking in plastic vials, preserved with potassium dichromate, and stored at 4°C. Milk samples were analyzed for fat, protein, lactose, and total solids using MilkoScan (134 BN Foss Electric, Hillerød, Denmark). Milk composition were calculated based on the product of the milk production yield and milk

145 composition at each milking on those days, as a weighted mean, and used for statistical
146 analysis.

147 Blood samples were collected from 10 cows in each treatment, on d -14, -7, 0, 7, 14, and +21
148 (d 0 = parturition). Blood was collected via the coccygeal vein in evacuated glass tubes
149 containing EDTA (10.5 mg, Monoject; Sherwood Medical, St. Louis, MO, USA). Within 1 h
150 following sampling, plasma was harvested by centrifugation (3000 × g, 15 min at 4°C) and
151 stored at −18°C until further analysis. Plasma concentrations of cholesterol, TG and glucose
152 were measured using Pars Azmoon kits according to the manufacturer's procedures (Pars
153 Azmoon Co., Tehran, Iran). The inter- and intra-assay coefficients were 2.8 and 1.7% for
154 cholesterol, 3.1 and 2.4% for TG and 4.5 and 3.1% for glucose assay. Plasma concentrations
155 of NEFA and BHBA were measured using commercial kits (Randox Laboratories Ltd.,
156 London, UK) with a Technicon-RA 1000 Autoanalyzer (DRG Co., Marburg, Germany). The
157 inter- and intra-assay coefficients of variations for NEFA and BHBA assays were 6.3, 4.2,
158 4.6 and 3.8%, respectively.

159 Blood concentrations of insulin and progesterone (Diaplus Inc., USA), IGF-1 (Hangzhou
160 Eastbiopharm Co., Ltd., USA) and GH (Monobind Inc Lake Forest, CA, USA) were
161 measured with specific ELISA kits, following the manufacturer's instructions. The inter- and
162 intra-assay coefficients of variation were 7.2, 5.4% for insulin, 5.1, 3.2% for progesterone,
163 6.9, 5.2% for IGF-1, 7.8 and 6.5% for GH assay.

164 **2.3. Reproductive management**

165 A PreSynch/Ovsynch program starting on d 30 postpartum was conducted [35]. Briefly, the
166 PreSynch estrous cycle synchronization was carried out by 2 injections of $PGF_{2\alpha}$ (500 μg,
167 Cloprostenol Sodium, i.m.; Estroplan, Parnell technologies PTY. LTD., Alexandria,
168 Australia) given 14 d apart. The Ovsynch program started 14 d after the second $PGF_{2\alpha}$

169 injection. In the Ovsynch program, cows were injected an intramuscular GnRH (100 µg
170 gonadorellin acetate, Gonabreed, Parnell Technologies PTY. LTD., Alexandria, Australia),
171 followed by $PGF_{2\alpha}$ injection 7 d later. An additional GnRH was injected 48 h after PGF2α
172 injection. Cows were inseminated artificially 16 h after GnRH injection.

173 Cows were inseminated and excluded from the program if they showed estrus signs following
174 the second injection of $PGF_{2\alpha}$ in presynchronization phase (d 44 postpartum) until the end of
175 the reproductive program. Estrus was detected using a combination of behavioral
176 observations (two times daily) and pedometer activity. One technician inseminated all cows
177 until the end of the experiment. The reproductive traits including postpartum ovulation,
178 conception rate, pregnancy per AI, service per conception, days open, days to first service
179 and days to first estrus were recorded during the experiment.

180 Number of days to first ovulation was determined using both ultrasonography and
181 progesterone assay (3 times per week). Ovarian follicular activity was monitored by
182 transrectal ultrasonography using a real-time linear scanning ultrasound diagnostic system (B
183 mode; Piemedical, Falco 100; 8 MHz transducer), 2 times per week, beginning on d 8
184 postpartum and continuing through d 28 postpartum. Ovulation was considered to have
185 occurred 3 d before plasma progesterone was greater than or equal to 1 ng/mL [25].

186 **2.4. Statistical analysis**

187 Data measured over time (BCS, BW, milk yield and composition, blood hormones and
188 metabolites), were analyzed by the MIXED procedure (SAS Institute Inc., Cary, NC), for
189 peripartum (d −7 to +7) and postpartum (d 0 to +21), separately. The model included the
190 fixed effects of treatment, time, the interaction between treatment and time, and the random
191 effect of cows nested within treatments. Pretreatment values for plasma variables BW, and
192 BCS measured or assessed at the beginning of the experiment, were used as covariates to

193 their corresponding measurements during the treatment period; however, covariates were
194 removed from the model if P > 0.20, and the data reanalyzed. Mathematical model and its
195 components were:

196 $$y_{ijk} = \mu + T_i + t_j + (T \times t)_{ij} + \delta(T)_{ik} + e_{ijk}$$

197 where y_{ijk} is observation on cow k at the sampling time j given treatment i; μ is the overall
198 mean; T_i is fixed effect of treatment; t_j is fixed effect of sampling time j (weeks); $(T \times t)_{ij}$ is the
199 two-way interaction of treatment i by sampling time j; $\delta(T)_{il}$ is the random effect of cow k
200 nested within treatment i, and e_{ijkl} is residual random error.

201 Time of sampling (weeks) was used in the REPEATED statement and the tukey test was used
202 for multiple comparison tests. Results were expressed as least squares means and SEM.

203 Before analysis of the reproductive data, 6 cows were removed from the data set (1, 1, 2 and
204 2 cows from PGT +/+, PGT +/-, PGT -/+ and PGT -/-, respectively). These cows were culled
205 after d 50 postpartum because of physical injury or reproductive problems. The interval
206 between calving and day of the first ovulation, days to first estrus, days to first service,
207 interval between inseminations, and days open were analyzed using survival analysis and the
208 product limit method of the Kaplan–Meier model using the LIFETEST procedure of the SAS.

209 Number of services per conception was analyzed by the GENMOD procedure using a
210 Poisson distribution. Binomially distributed data such as conception and ovulation rate were
211 analyzed by the GENMOD procedure using a binary distribution and a logit odds ratio link.
212 Statistical significance and tendencies were declared at $P < 0.05$ and $0.05 \leq P \leq 0.10$,
213 respectively.

214

215

3. Results

3.1. Plasma metabolites

The overall plasma concentrations of plasma NEFA, BHBA, cholesterol, triglyceride and glucose during the peripartum (d −7 to +7 relative to parturition) and postpartum (parturition to d 21 postpartum) periods are presented in Table 2. Plasma NEFA concentrations decreased in PGT (+/+) compared to PGT (-/+) and PGT (-/-) groups during the peripartum period ($P < 0.05$); however, during the postpartum period, plasma NEFA concentrations decreased ($P < 0.05$) in PGT (+/+), PGT (+/-) and PGT (-/+) compared to PGT (-/-). The effect of PGT supplementation on plasma BHBA concentrations was not significant during the postpartum periods ($P > 0.05$); however, PGT supplementation decreased plasma BHBA concentrations in PGT (+/+) and PGT (+/-) compared to the control group during the peripartum period ($P < 0.1$ and $P < 0.05$, respectively). Plasma concentrations of TG were decreased ($P < 0.05$) in PGT (+/+), PGT (+/-) and PGT (-/+) compared to PGT (-/-) during both the peri- and postpartum periods (Table 2). Concentration of plasma glucose was not affected by the diets during the postpartum period, while PGT supplementation increased ($P > 0.05$) plasma concentrations of glucose in PGT (+/+) and PGT (+/-) compared to PGT (-/-) during the peripartum period (Table 2).

3.2. Plasma hormones

The overall plasma concentrations of plasma insulin, GH and IGF-1 during the peri- and postpartum periods are presented in Table 3. Plasma insulin and GH concentrations were not affected ($P > 0.05$) by treatment during the peri- or postpartum periods (Table 3). Plasma concentration of IGF-1 increased ($P < 0.05$) in PGT (+/+) compared to PGT (-/-) during the peri- and postpartum periods. However, during the postpartum period, plasma concentration of IGF-1 tended ($P < 0.1$) to be higher in PGT (+/+) than PGT (-/+) group (Table 3).

3.3. Productive traits

The overall effects of PGT supplementation on BW, BCS and milk yield and milk composition are shown in Table 4. There was no significant effect of PGT supplementation on BW and BCS; however, PGT supplementation decreased ($P < 0.05$) BW loss in PGT (+/+) and PGT (+/-) compared to PGT (-/-), and BCS loss in PGT (+/+), PGT (+/-) and PGT (-/+) compared to PGT (-/-) ($P < 0.05$, table 4).

Supplementation of PGT did not affect milk yield and 4% fat-corrected milk yield during the first 30 d postpartum (Table 4). The percentage of milk fat was decreased by PGT supplementation in PGT (+/+), PGT (+/-) and PGT (-/+) compared to PGT (-/-); however, daily milk fat production (kg) decreased in PGT (+/+) and PGT (+/-) and tended to be decreased ($P < 0.10$) in PGT (-/+) compared to PGT (-/-). Supplementation of PGT did not affect other milk constituents (Table 4).

3.4. Reproductive traits

The effects of PGT supplementation on reproductive traits are shown in Table 5. The proportion of cows ovulating by 14 d postpartum was higher in PGT (+/+) and PGT (+/-) than the PGT (-/-) group ($P < 0.05$). The proportion of cows ovulating by 21 d postpartum tended to be higher in PGT (-/+) compared to PGT (-/-) cows. Mean days to first ovulation was lower in PGT (+/+) and PGT (-/+) groups compared to PGT (-/-). The mean days open was decreased in cows fed with PGT diets compared to control cows. The number of days to first estrus was not affected by PGT supplementation; however, days to first service was decreased in PGT (+/+) and PGT (-/+) compared to PGT (-/-) cows ($P < 0.05$; table 5). The interval between the first and second inseminations was shorter in PGT (+/+) and PGT (+/-) than that in PGT (-/-) cows ($P < 0.05$). The number of service per conception was lower in PGT (+/+) and PGT (+/-) compared to PGT (-/-) cows ($P < 0.05$). Conception rate at first

service tended to be higher in PGT (+/-) than in the PGT (-/-) group ($P < 0.10$). The proportion of pregnant cows by 120 DIM was higher in PGT (+/+), PGT (+/-) and PGT (-/+) compared to PGT (-/-) cows ($P < 0.05$).

4. Discussion

Insulin resistance in peripheral tissues during the prepartum period and early lactation in dairy cows directs nutrients toward the uterus and mammary glands in support of the fetus and lactation [1]. However, in pre- and postpartum cows, insulin resistance is probably followed by an increase in circulating concentrations of NEFA, and the decrease in dry matter intake leads to several postpartal metabolic disorders and poor reproductive performance [5, 6].

Prepartal administration of TZD was beneficial in controlling the circulating NEFA level, improving DMI and attenuating the insulin resistance [15, 16, 25]. We expected that dietary supplementation of PGT, as a specific ligand of PPAR-γ, would improve energy and fat metabolism and consequently improve reproductive performance in transition dairy cows. Although, there are several studies on the use of these TZDs in dairy and beef cattle [15, 16, 24, 25, 31], it has not been approved and further investigations are required; however, the present study is the first study to investigate the effect of dietary TZDs on the metabolism and reproductive performance in transition dairy cows.

Plasma NEFA concentrations decreased in cows receiving PGT during the pre- and postpartum periods; however, in the peripartal period, NEFA was decreased when cows received PGT during both pre- and postpartum periods. In agreement with these results, Smith et al. [15] showed that administration of TZD decreased plasma NEFA concentrations during the prepartal period and tended to decrease peripartal NEFA concentration. Ghoreishi [36] showed that dietary supplementation of 4.0 mg PGT/kg BW during the transition period

288 decreased plasma peripartal concentrations of NEFA. It was also reported that administration
289 of 2.0 mg TZD/kg BW to steers was effective in alleviating insulin resistance, induced by
290 administered TNF-α, and reducing the increased plasma NEFA after d 2 of treatment [23].

291 These results are explained by the possible effect of TZDs on increasing DMI during the
292 peripartal and postpartal periods [15, 25, 36], and/or by direct effect of TZDs on re-
293 esterification of fatty acids and induction of phosphoenol pyruvate carboxykinase in adipose
294 tissue, that increases glyceroneogenesis and promotes a futile cycle [37]. Probably, the
295 glyceroneogenesis-dependent fatty acid-lowering effect of TZDs could be an essential aspect
296 of the antidiabetic action of these compounds. Generally, lower levels of plasma NEFA
297 showed that PGT may decrease fat mobilization or increase free fatty acid re-esterification or
298 stimulate hepatic capacity for oxidation of NEFA.

299 The lower plasma concentrations of BHBA during the peripartal period in cows treated with
300 PGT was in agreement with the results of Smith et al. [15], who reported a decrease in
301 plasma BHBA concentration by administering 2.0 mg TZD/ kg BW. However, Smith et al.
302 [25] reported an increase in peripartal BHBA concentration by TZD administration, and
303 Ghoreishi [36] found no significant effect of dietary supplementation of 4.0 mg PGT on
304 plasma BHBA concentration. The lower plasma concentrations of BHBA may be a result of
305 lower plasma NEFA availability in PGT-treated cows (Table 2) and increased hepatic
306 capacity to oxidize palmitic and stearic acids [38] which lowers plasma BHBA. However,
307 because dietary or pharmacologic manipulation of hepatic PPARs in dairy cows would
308 increase β-oxidation of fatty acids and likely increase plasma BHBA [39], it is probable that
309 lower BHBA levels in this experiment are caused by an indirect effect of PGT on the liver, by
310 lowering the plasma NEFA, or by increasing DMI.

311 The effectiveness of PGT on fat metabolism was also approved where plasma concentration

of TG decreased in PGT-treated cows during both the peri- and postpartal periods. Ghorieshi et al [36] reported no effect of dietary supplementation of 4.0 mg PGT per kg BW on plasma TG in transition cows; however, consistent with the results of the present study, calves from cows treated with PGT had lower plasma TG concentration. According to results of Palmer et al [40], PGT may decrease expression of MTTP (microsomal triglyceride transfer protein) and consequently decreases incorporation of TG in VLDL and plasma concentration of TG. In addition, limited mobilization of body fat as a result of PGT treatment may reduce precursors required for TG synthesis in the liver. Generally, the approved beneficial effects of TZDs on enhancing insulin sensitivity in cow's peripheral tissues [15, 16, 23] is the most likely pathway by which PGT treatment decreased body fat mobilization in dairy cows, as previously documented for the human adipocytes [41].

In the present study, peripartum plasma concentrations of glucose increased in cows which received PGT for 14 d prepartum (PGT +/+ and PGT +/-). This finding supported the results of previous studies in which perpartal administration of 2.0 or 4.0 mg TZDs/ kg BW increased plasma concentrations of glucose during the peripartal period [16, 25]. However, the effect of TZDs on plasma glucose was not significant in other studies [15, 36]. It has been demonstrated that administration of TZDs increased DMI in transition dairy cows [15, 36] and laboratory animals [42, 43]. Therefore, the higher concentration of glucose during the peripartal period may be a direct effect of higher DMI in PGT- treated cows. Moreover, prepartal administration of TZDs decreased accumulation of TG in the liver of dairy cows [25] and humans [44, 45] and that would increase gluconeogenic capacity [46, 47] and plasma concentration of glucose.

It has been suggested that TZDs may increase plasma concentration of insulin in dairy cows [15]; however, in agreement with results of Schoenberg and Overton [16], no significant change in plasma concentration of insulin was observed in the current study. A previous

study in animal models [21] showed that TZDs administration may improve pancreatic β-cell function and insulin production; however, Kushibiki et al. [23] reported a decrease in plasma concentrations of insulin in TZD-treated steers following improvement in glucose utilization. The differences between animals, dosage and/or type of administration might have resulted in inconsistent responses to this compound. On the other hand, we expected an increase in plasma insulin in those cows that had a higher plasma concentration of glucose. However, no increase in insulin levels may be due to an inadequate increase in plasma concentration of glucose or a failure of the liver to secrete insulin at a level appropriate for plasma glucose concentrations.

Plasma concentration of IGF-1 was significantly increased in PGT (+/+) and non-significantly in PGT (+/-) and PGT (-/+) compared to PGT (-/-), but plasma concentrations of GH were not affected by PGT treatment. In peripartal dairy cows, concentrations of plasma IGF-1 are low but GH level is high [48]. It has been suggested that decreases in liver growth hormone receptor 1A (GHR 1A) before calving causes the uncoupling of the somatotropic axis in postpartal cows [48]. The subsequent recoupling of the somatotropic axis has been linked to postpartal nutrition and energy balance and is probably dependent on GHR 1A [49]. In response to insulin, GHR 1A and IGF-I expression increased in the liver of postpartal dairy cows [49], humans and other species [50]. In the present study, NEB indexes (NEFA and BHBA) decrease and plasma concentration of glucose increased in PGT-treated cows. These effects were also demonstrated by increased DMI and a decrease in the NEB during the postpartum in dairy cows that were subjected to TZDs [15, 25]. As an insulin sensitizing agent, PGT may also improve the responsiveness of the liver and other peripheral tissues to insulin. Therefore, there is a possibility that PGT increased the sensitivity of liver to insulin and caused greater expression of GHR 1A receptor and IGF-1 synthesis in the liver.

In this experiment, milk yield was not affected by PGT, but milk fat percentage and yield

were decreased by d 30 postpartum. Smith et al. [25] showed that prepartal administration of 4.0 mg TZDs/kg BW tended to decrease milk fat percentage by d 63 postpartum. Considering the fact that a part of plasma NEFA and blood fat metabolites incorporated directly into milk fat [48], the decrease in milk fat is explained by lower plasma NEFA and other fat metabolites in PGT-treated cows. Moreover, administration of endogenous or exogenous ligands of PPAR-γ to dairy sheep and goats regulated genes involved in triacylglycerol synthesis and secretion in mammary gland epithelial cells and milk fat content [18, 19]. TZDs also altered lipogenic gene networks in the bovine mammary epithelial cells [20]. Therefore, it has been postulated at least a part of the change in milk fat may be due to the effect of PGT on milk fat synthesis in mammary glands.

In the present study, the postpartal BCS and BW loss was less as a result of PGT- treatment. These findings supported those of Smith et al. [25] that showed higher BCS in cows receiving 2.0 or 4.0 mg/kg TZDs for 25 d prepartum. However, Smith et al. [15] and Schoenberg and Overton [16] did not find any differences in BCS or BW in cows treated prepartum with TZDs. The lower concentrations of NEFA and BHBA indicated a lower negative energy balance during the peri- and postpartal periods in PGT-treated cows. Moreover, lesser amounts of energy used for milk fat synthesis and possibly more energy intake (as DMI) are possible reasons for less BW and BCS loss during postpartum in PGT-supplemented cows.

The proportion of cows ovulated by d 14 postpatum increased by PGT supplementation (PGT +/+ and PGT -/+), while the average number of days to ovulation was decreased. Smith et al. [25] also showed that prepartal administration of 4.0 mg TZDs/kg BW for 25 d decreased the interval from calving to first ovulation. It has been shown that the NEB adversely influenced the interval from calving to ovulation [51]; however, reproductive efficiency improved as BCS loss decreased [52] in dairy cows. In this study, dietary supplementation of PGT decreased peri- and postpartal NEFA, BHBA and postpartum loss of BCS and BW, and

caused a decrease in the NEB. In agreement with the results of this study, there are studies showing that administration of insulin or insulin sensitizing agents during the prepartum period stimulate ovulation and improve the reproductive performance [49, 53, 54]. In addition, studies in ruminants and rats [17, 55, 56] showed that PPARγ was widely expressed in reproductive tissues and ovarian follicles and expression of these receptors increased as ovulation was approached. This implies a possible direct effect of PGT on specific receptors leading to a higher proportion of cows ovulating postpartum. However, PGT increased postpartal concentration of IGF-1 which is considered as a crucial factor for early postpartum ovulation. In this regard, Kawashima et al [57] demonstrated that ovulation and final development of the preovulatory follicle is strictly dependent on increasing plasma levels of IGF-1 and insulin during the first postpartal follicular wave, while low concentration of IGF-1 delays ovulation [58]. Accordingly, PGT may provide a more appropriate metabolic milieu as well as having a direct effect on the ovulatory process.

The average number of days open was decreased and proportion of pregnant cows during 120 DIM increased in PGT-supplemented cows. Decreased days open is described by shorter intervals between inseminations and more conception per insemination. Interestingly, PGT supplementation during both the pre- and postpartal periods (PGT +/+) resulted in 14 d and 19 d decreases in days open compared to PGT (+/-) and PGT (-/+), respectively. This indicated a synergic interaction between pre- and postpartal supplementation of PGT, and a relative advantage of supplementing PGT during both the pre- and postpartal periods.

Butler et al. [49] reported that prepartal administration of insulin resulted in earlier postpartal ovarian activity and improved the reproductive performance. Consistent with the results of the present study, it has been demonstrated that conception rate was higher in cows that showed fewer days to first service [59]. The ovarian follicles in the cows supplemented with PGT during the transition period might experience more appropriate metabolic and hormonal

conditions, resulting in more competent oocytes. Insulin resistance decreases the quality of oocytes, leading to a decline in reproductive performance and early embryo development [60]. Consistent with the results of the present study, supplementation of chromium, an insulin sensitizing element, reduced plasma concentrations of NEFA and negative energy balance in heat-stressed animals [61], resulting in improvement of the reproductive performance in transition dairy cows. Therefore, improved hormonal and metabolic conditions as a result of PGT supplementation may be involved in the improvement of the reproductive performance in dairy cows.

4.1. Conclusions

Dietary supplementation of PGT during the transition period decreased peri- and postpartal plasma NEFA, BHBA, and TG concentrations and also decreased milk fat content, BCS and BW loss during the postpartal periods in Holstein dairy cows. The improved metabolic health in PGT-supplemented cows effectively enhanced the postpartum ovulation resumption activity and reproductive performance. However, better productive and reproductive performances were observed when PGT was supplemented during both the pre- and postpartum periods. According to the results of the present study and the previous studies on the effect of TZDs on transition dairy cows, oral or intravenous administration of these drugs seems to have beneficial effects on metabolic dynamics and reproduction; however, further research should be conducted to confirm the mechanisms involved in the effects of PGT and PPAR-γ on metabolic dynamics, ovulation and reproductive performance in dairy cows.

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602 **Figure caption**

603 **Figure 1.** An outline of the experimental design indicating initiation of dietary treatment and
604 duration of pioglitazone (PGT) supplementation. Dairy cows (n=20 cow/treatments) were fed
605 four experimental diets from d -14 to +21, relative to parturition. PGT was supplemented at 6
606 mg /kg BW to TMR.

607 Table 1. Ingredients and chemical composition (DM basis) of the experimental diets

Ingredients	Prepartum diet	Postpartum diet
Alfalfa hay	26.15	22.00
Corn silage	31.64	20.33
Cottonseed, whole	2.74	7.42
Soybean, whole- roasted	---	2.47
Meat meal	---	1.28
Beet pulp	---	4.08
Barley grain	12.27	10.48
Corn grain	9.29	13.83
Soybean meal	9.07	12.1
Wheat bran	3.15	1.5
Corn germ meal	---	1.05
Common salt	---	0.43
Sodium bicarbonate	---	0.90
Dicalcium phosphate	---	0.3
Calcium carbonate	0.89	0.8
Magnesium oxide	---	0.18
Bentonite	---	0.27
Vitamin and mineral premix[1]	0.90	0.67
Ammonium chloride	3.10	---
Magnesium sulfate	0.80	---
Composition		
DM, %	50	55
NEL (Mcal/kg DM)	1.53	1.66
EE, %	3.2	4.6
CP , %	14.1	17.1
ADF, %	24.1	22.13
NDF, %	36.2	32.2

608 1. Contained (per kg): 16,000,000 IU vitamin A; 3,200,000 IU vitamin D; 48,000 IU vitamin
609 E; 24.0 g Mn; 24.0 g Zn; 24.0 g Fe; 12.8 g Cu; 1.44 g I; 0.32 g Se; and 0.32 g Co.
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Table 2. Least squares means for the effect of dietary supplementation of pioglitazone (6 mg PGT/kg BW) on plasma metabolites during transition periods in Holstein cows (n=10/treatment[1])

Item	Diets[1]				SEM
	PGT(+/+)	PGT(+/-)	PGT(-/+)	PGT(-/-)	
NEFA (mmol/L)					
Peripartum[2]	0.65[b]	0.72[ab]	0.82[a]	0.79[a]	0.05
Postpartum[3]	0.52[b]	0.59[b]	0.60[b]	0.72[a]	0.05
BHBA (mmol/L)					
Peripartum	0.56[b]	0.57[ab]	0.61[ab]	0.65[a]	0.04
Postpartum	0.56	0.55	0.73	0.63	0.07
Cholesterol (mg/dL)					
Peripartum	96.13	90.30	88.90	98.90	4.40
Postpartum	133.52	131.22	134.25	122.58	8.90
Triglyceride (mg/dL)					
Peripartum	19.99[c]	23.61[b]	20.68[bc]	28.72[a]	1.30
Postpartum	18.08[b]	19.97[b]	19.37[b]	24.07[a]	1.76
Glucose (mg/dL)					
Peripartum	58.98[a]	55.38[ab]	47.86[bc]	46.83[c]	2.60
Postpartum	49.93	52.10	45.76	53.66	3.66

[a–c] Values with different superscripts within a row indicate a significant difference, $P < 0.05$.

[1] Diets consisted of PGT (+/+): supplementation of PGT in pre- and postpartum periods (d -14 to +21 relative to parturition), PGT (+/-): supplementation of PGT only during the prepartum period (d -14 to parturition), PGT (-/+): supplementation of PGT only during the postpartum period (d 0 to +21 relative to parturition) and PGT (-/-): no PGT supplementation.

[2] Represents data collected weekly from 7 d before parturition through 7 d postpartum.

[3] Represents data collected weekly from parturition through 21 d postpartum.

640 Table 3. Least squares means for the effect of dietary supplementation of pioglitazone (6 mg
641 PGT/kg BW) on some plasma hormones during the transition period in Holstein cows
642 (n=10/treatment)
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	Treatments[1]				
Item	PGT(+/+)	PGT(+/-)	PGT(-/+)	PGT(-/-)	SEM
Insulin (µIU/mL)					
Peripartum[2]	8.68	9.21	8.30	8.11	0.69
Postpartum[3]	8.21	9.13	8.18	9.00	0.59
GH (µg/L)					
Peripartum	3.68	3.98	4.22	4.02	0.31
Postpartum	2.83	3.19	3.10	3.46	0.40
IGF-1 (µg/L)					
Peripartum	48.11[a]	40.06[ab]	38.92[ab]	34.03[b]	4.27
Postpartum	39.91[a]	35.03[ab]	32.67[ab]	30.55[b]	3.31

644 [a–c] Values with different superscripts within a row indicate a significant difference, $P < 0.05$.
645 [1] Diets consisted of PGT (+/+): supplementation of PGT in pre- and postpartum periods (d -
646 14 to +21 relative to parturition), PGT (+/-): supplementation of PGT only during the
647 prepartum period (d -14 to parturition), PGT (-/+): supplementation of PGT only during the
648 postpartum period (d 0 to +21 relative to parturition) and PGT (-/-): no PGT supplementation.
649 [2] Represents data collected weekly from 7 d before parturition through 7 d postpartum.
650 [3] Represents data collected weekly from parturition through 21 d postpartum.

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662 Table 4. Least squares means for the effect of dietary supplementation of pioglitazone (6 mg
663 PGT/kg BW) on production traits during the postpartum period in Holstein cows
664 (n=20/treatment)

	Treatments[1]				
Item	PGT (+/+)	PGT (+/-)	PGT(-/+)	PGT (-/-)	SEM
Body weight (kg)	639.15	639.51	637.64	635.87	1.26
Body weight loss (kg)	30.86^{c}	37.44^{b}	39.16^{ab}	41.60^{a}	0.09
BCS[2]	3.34	3.33	3.37	3.25	0.05
BCS loss	0.54^{b}	0.60^{b}	0.63^{b}	0.77^{a}	0.04
Milk yield, kg/d	40.92	42.80	42.21	42.97	1.50
4% FCM[3], kg/d	40.06	40.71	41.35	43.53	1.33
Fat, %	3.91^{b}	3.90^{b}	3.89^{b}	4.15^{a}	0.11
Fat, kg/d	1.57^{b}	1.60^{b}	1.63^{ab}	1.77^{a}	0.05
True protein, %	3.16	3.14	3.19	3.27	0.08
True protein, kg/d	1.27	1.29	1.31	1.39	0.07
Lactose, %	4.78	4.67	4.71	4.77	0.05
Lactose, kg/d	1.96	1.97	2.00	2.06	0.08
Total solids, %	12.12	12.29	12.34	12.40	0.12
Total solids, kg/d	4.95	5.07	5.17	5.29	0.18

665 [a,b] Values with different superscripts within a row indicate a significant difference, $P < 0.05$.
666 [1] Diets consisted of PGT (+/+): supplementation of PGT in pre- and postpartum periods (d -
667 14 to +21 relative to parturition), PGT (+/-): supplementation of PGT only during the
668 prepartum period (d -14 to parturition), PGT (-/+): supplementation of PGT only during the
669 postpartum period (d 0 to +21 relative to parturition) and PGT (-/-): no PGT supplementation.
670 [2] Based on a 5-point scale.
671 [3] 4% FCM = fat-corrected milk, calculated as [0.4 × milk production (kg d^{-1})] + [15 × fat
672 yield (kg d^{-1})].
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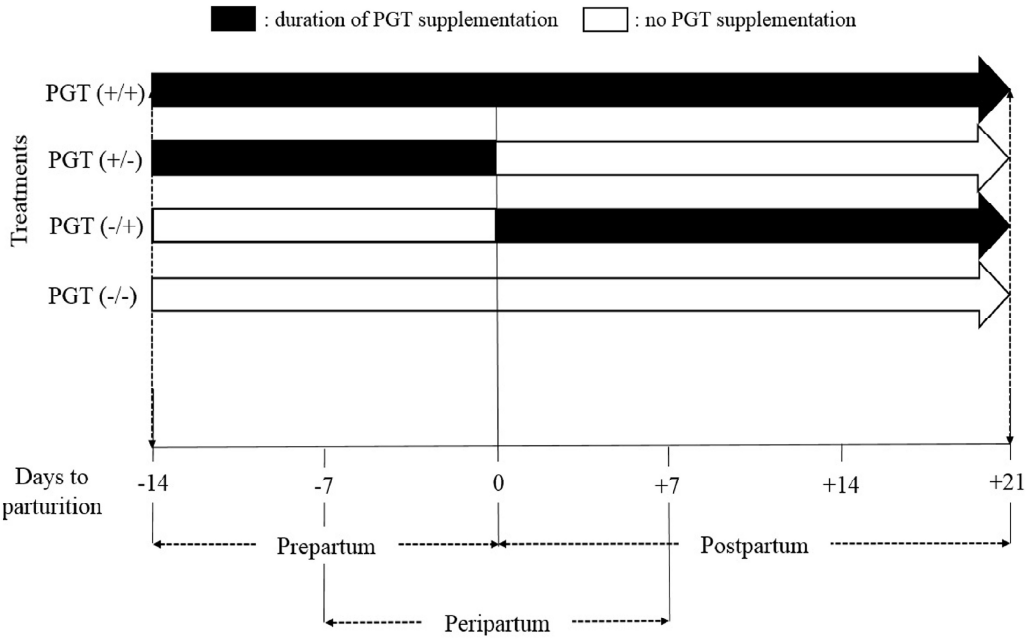
Table 5. Least squares means for the effect of dietary supplementation of pioglitazone (6 mg PGT/kg BW) on reproductive traits in Holstein cows (n=20/treatment)

	Treatments [1]				
Item	PGT(+/+)	PGT(+/-)	PGT(-/+)	PGT(-/-)	SEM
First ovulation ≤ 14 d, %(n/group)	45%(9/20)^{a}	15%(3/20)^{b}	30%(6/20)^{ab}	15%(3/20)^{b}	0.11
First ovulation ≤ 21 d, % (n/group)	65%(13/20)	60%(12/20)	70%(14/20)	45%(9/20)	0.13
First ovulation (d)	18.37 ^{b}	21.00 ^{ab}	19.60 ^{b}	23.05 ^{a}	1.29
Median days to first estrus	40	41	40	45	2.3
Median days to first service	44^{b}	50^{ab}	46^{b}	53^{a}	2.8
First to second insemination interval (d)	34^{b}	30^{b}	45^{ab}	55^{a}	5
Median days open	133 ^{b}	147^{b}	152^{b}	169 ^{a}	7
Number of services per conception	2.56^{b}	2.28^{b}	3.00^{ab}	3.80^{a}	0.55
Pregnant to first insemination, %(n/group)	10.5%(2/19)	26%(5/19)	16.5%(3/18)	5.5%(1/18)	0.10
Pregnant by 120 DIM, % (n/group)	53%(10/19)^{a}	42% (8/19)^{a}	39% (7/18)^{a}	11% (2/18)^{b}	0.13

$^{a, b}$ Values with different superscripts within a row indicate a significant difference, $P < 0.05$.

[1] Treatments included PGT (+/+): supplementation of PGT in pre- and postpartum periods (d -14 to +21 relative to parturition), PGT (+/-): supplementation of PGT only during the prepartum period (d -14 to parturition), PGT (-/+): supplementation of PGT only during the postpartum period (d 0 to +21 relative to parturition) and PGT (-/-): no PGT supplementation.


: duration of PGT supplementation
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Treatments
PGT (+/+)
PGT (+/-)
PGT (-/+)
PGT (-/-)
Days to parturition
-14
-7
0
+7
+14
+21
Prepartum
Postpartum
Peripartum

Highlights

- Dietary supplementation of PGT during the transition period decreased peri- and postpartal plasma NEFA, BHBA, and TG concentrations and also decreased milk fat content, BCS and BW loss during the postpartal periods in dairy cows.
- PGT had positive effects on postpartum resumption of ovarian activity and increased the number of ovulated cows during the postpartum period.
- The average number of days open was decreased and proportion of pregnant cows during 120 DIM increased in PGT-supplemented cows.
- Better productive and reproductive performances were observed when PGT was supplemented during both the pre- and postpartum periods