



Original Research Article

Green tea polyphenols supplementation alters immunometabolism and oxidative stress in dairy cows with hyperketonemia



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ABSTRACT

Periparturient cows often experience negative energy balance, and are therefore prone to suffering from metabolic diseases such as hyperketonemia, which causes financial losses in dairy farms. This study aimed to investigate the effect of green tea polyphenol (GTP) supplementation during the periparturient period on production performance, oxidative stress and immunometabolism in dairy cows with hyperketonemia. One hundred Holstein cows were assigned to GTP (0.2 g/kg DM; $n = 50$) or control (without GTP; $n = 50$) group based on body weight, previous milk yield, and parity on d 15 before expected parturition. Subsequently, 10 cows with hyperketonemia were selected from each group, according to blood β -hydroxybutyric acid (BHBA) concentration between 1.2 and 2.9 mmol/L from 2 to 3 d postpartum. All cows were fed a close-up diet and a lactation diet with or without GTP supply from 15 d prepartum until 30 d postpartum. Milk and blood samples were obtained from 20 cows selected with hyperketonemia on 10, 20, and 30 d postpartum. Compared with control cows, greater milk yield and lower somatic cell count were observed in GTP cows. The GTP group had lower concentrations of BHBA, free fatty acids, cholesterol, triglyceride, reactive oxygen species, malondialdehyde, and hydrogen peroxide, greater concentrations of glucose, lower activities of aspartate aminotransferase, alanine aminotransferase, and glutamyl transpeptidase, alongside greater activities of superoxide dismutase, glutathione peroxidase, and total antioxidant capacity. Additionally, GTP supplementation up-regulated concentrations of interleukin-6 and interleukin-10, but down-regulated concentrations of tumor necrosis factor- α , interleukin-1 β , interleukin-2, interleukin-8, and interferon- γ in plasma. Greater concentrations of plasma immunoglobulin G were also detected in the GTP group. Overall, the data suggested that GTP supplementation from 15 d prepartum to 30 d postpartum improved the milk yield and health status in cows with hyperketonemia during early lactation.

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1. Introduction

The transition period, from 3 wk before to 3 wk after parturition, represents the most critical period in the productive life of high-producing dairy cows (Drackley, 1999). Periparturient cows undergo a negative energy balance (NEB) due to decreased dry matter intake (DMI), and increased energy requirements for milk production (Drackley, 1999), resulting in increased oxidative stress (Sordillo and Aitken, 2009) and compromised inflammatory response (Sordillo et al., 2009). Despite the fact that body fat mobilization is an adaptive response, extremely high rates of lipid mobilization

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promotes the production of β -hydroxybutyric acid (BHBA) by the liver, which contributes to the development of hyperketonemia. Intense body fat mobilization leads to increased circulation levels of reactive oxygen metabolites and malondialdehyde (MDA) (Bernabucci et al., 2005; Celi, 2011), and up-regulated pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) (Kushibiki, 2011), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) (Trevisi et al., 2015), which is detrimental to the health of dairy cows in transition cows. It has been demonstrated that tea, a plant rich in phytochemicals including flavonoids, tannins, caffeine, polyphenols, epigallocatechin gallate (EGCG), and quercetin, possesses potent antioxidant properties (Fang et al., 2005). Plant polyphenols, end products of the plant flavonoid biosynthetic pathway, play a role in the regulation of intolerance to elevated levels of blood glucose (GLU) (Dixon et al., 2005), which is important for non-ruminants health (Yang et al., 2001; McKay and Blumberg, 2002; Rodriguez et al., 2006). In vitro and in vivo studies prove that tea and tea polyphenols have strong antioxidant activities (Elbling et al., 2010; Hu et al., 2011; Zhao et al., 2018); thus they can be used as an antioxidant and anti-aging functional food (Zhao et al., 2018). Human and rodent studies have verified that green tea polyphenols (GTP) are responsible for decreased concentrations of plasma GLU, insulin, triglyceride (TG), and free fatty acids, as well as increased GLU uptake stimulated by insulin (Wu et al., 2004; Wolfram et al., 2006; Fernando et al., 2017). Additionally, GTP altered gene expression related to lipid-metabolism in the liver of broiler chickens by increasing the phosphorylation of adenosine 5'-monophosphate (AMP)-activated protein kinase (Huang et al., 2017). Our previous study revealed that GTP had beneficial effects on redox balance in bovine mammary epithelial cells (Ma et al., 2018). For instance, GTP reduced cellular oxidative stress via the inhibition of reactive oxygen species (ROS) accumulation, which is partly explained by the activation of the nuclear factor erythroid 2 like 2 (NFE2L2)/heme oxygenase-1 (HMOX1) pathway (Ma et al., 2019). The available data implies that GTP may potentially act as an antioxidant against oxidative stress in dairy cows. However, to our best knowledge, studies that investigate the effect of GTP supplementation on oxidative stress and immune function in dairy cows with hyperketonemia are limited. Therefore, the aim of this study was to explore the effect of GTP supplementation during the periparturient period on production performance and health status in postpartum dairy cows with hyperketonemia.

2. Materials and methods

2.1. Ethics statement

The experimental protocol used in this study was approved by the Animal Ethics Committee of the Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences (approval number IMAAAHS#1215000046002373XP), which is responsible for Animal Care and Use in the Inner Mongolia Autonomous Region of China.

2.2. Animals and treatments

The feeding trial was carried out on a commercial dairy farm (Hohhot, China) from Nov 15th to Dec 30th, 2018. One hundred pregnant nonlactating Holstein cows were classified into 2 groups: control (without GTP supplementation; $n = 50$) or GTP (with GTP supplementation; $n = 50$) group according to body weight (BW), previous milk yield, and parity on -15 d (-15 ± 2 d) relative to the expected calving date. Control ($n = 50$, BW = 725 ± 6.7 kg, previous milk yield = 36.34 ± 2.89 kg/d, parity = 3.85 ± 0.27 , BHBA = 0.89 ± 0.08 mmol/L, means \pm SEM) and GTP cows ($n = 50$,

BW = 729 ± 2.4 kg, previous milk yield = 36.68 ± 2.48 kg/d, parity = 3.89 ± 0.33 , BHBA = 0.93 ± 0.07 mmol/L, means \pm SEM) were fed a same basal close-up diet (6.38 MJ/kg of dry matter and 14.2% crude protein) from -15 d until parturition and the same basal lactation diet (6.85 MJ/kg of DM and 18.3% CP) from calving to 30 d postpartum with or without GTP supplementation (0.02% dry matter of total mixed ration). Green tea polyphenols (Dehe Biological Technology Co., Ltd, Jiangsu, China), composed of 91.20% total polyphenols, 41.11% EGCG, and 70.09% catechins, were mixed with a total mixed ration after being diluted with rice hull powder. The ingredient and chemical composition of these diets are shown in Table 1. Both diets were formulated to meet the cow predicted requirements according to the NRC (2001). All cows were fed 3 times daily at 06:00, 12:00 and 18:00 and had free access to water. Feed was offered to achieve 5% refusals and feed intake was recorded daily before morning feeding. Ten cows with hyperketonemia were selected from the control ($n = 10$, BW = 680 ± 18 kg, milk yield = 34.46 ± 2.49 kg/d, parity = 3.88 ± 0.25 , BHBA = 2.19 ± 0.52 mmol/L, means \pm SEM) and GTP group ($n = 10$, BW = 683 ± 22 kg, milk yield = 35.98 ± 2.53 kg/d, parity = 3.89 ± 0.29 , BHBA = 0.97 ± 0.27 mmol/L, means \pm SEM) based on concentrations of BHBA in plasma during 2 to 3 d postpartum. Hyperketonemia was defined as a plasma BHBA concentration of 1.2 to 2.9 mmol/L (Fig. 2).

2.3. Feed sample analysis

Dry matter (DM) was determined after samples were dried in an air-forced oven at 135°C for 2 h (method 930.15; AOAC, 1990). Net energy was calculated according to the NRC (2001). Nitrogen (N) was measured according to the methods of Kjeldahl, and crude protein (CP) was calculated as $6.25 \times \text{N}$. Neutral detergent fibre

Table 1
Ingredients and nutrient composition of basal close-up and lactation diets (% of DM).

Item	Close-up (15 d prepartum)	Lactation (30 d postpartum)
Ingredients		
Whole corn silage	32.36	30.16
Alfalfa	14.44	12.50
Oat hay	17.46	2.86
Cottonseed	6.09	4.07
Corn	15.09	17.71
Molasses	1.81	1.60
Soybean husk	3.21	3.61
Sodium bicarbonate	0.96	0.83
Cottonseed meal	–	4.42
Rapeseed meal	–	2.33
Distiller's dried grains with soluble	–	4.21
Wheat bran	2.33	–
Yeast	–	1.38
5% vitamin and mineral premix ¹	5.0	5.0
Soybean	–	2.49
Soybean meal	3.27	4.81
Nutrient composition ²		
DM, % as fed	45.7	49.3
Net energy, MJ/kg of DM	6.38	6.85
Crude protein	14.2	18.3
Crude fiber	19.8	16.0
Neutral detergent fiber	36.9	31.2
Acid detergent fiber	23.4	21.2
Calcium	0.59	0.69
Phosphorus	0.41	0.45

¹ Provided per kilogram of total mixed ration (on DM basis): calcium, 0.36 g; phosphorus, 0.36 g; sodium, 0.36 g; magnesium, 0.40 g; zinc, 28 mg; manganese, 17 mg; copper, 6.0 mg; cobalt, 0.24 mg; iodine, 0.80 mg; selenium, 0.21 mg; vitamin A, 4,000 IU, vitamin D, 600 IU, vitamin E, 20 mg (CVAS, Beijing, China).

² Measured values.

(NDF) and acid detergent fibre (ADF) were measured according to Van Soest et al. (1991) and Goering and Van Soest (1970), respectively.

2.4. Milk samples collection and analysis

Milk samples were collected on 10, 20, and 30 d postpartum from 20 cows selected with hyperketonemia. Cows were milked 3 times at 05:00, 11:00 and 17:00. Daily milk yield was electronically recorded at each milking. Morning, noon, and evening milk samples were collected consecutively every 10 d and mixed according to the ratio of 4:3:3. Mixed milk samples were analyzed for milk fat, protein, and lactose using a portable milk composition analyzer (LactoStar, Funke Gerber, Germany).

2.5. Blood collection

Blood from the coccygeal vein was obtained from 20 cows with hyperketonemia before morning feeding on 10, 20, and 30 d postpartum. Samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and were immediately placed on ice. Plasma was obtained by centrifugation at 3,000 r/min for 10 min (4 °C) and aliquots stored at –80 °C until further analysis.

2.6. Blood samples analysis

Activities of total antioxidant capacity (TAC; ab204519), superoxide dismutase (SOD; ab65354), glutathione peroxidase (GPX; ab102530), and concentrations of hydrogen peroxide (H₂O₂; ab102500) and MDA (ab238537) were determined using spectrophotometric diagnostic kits purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China) according to manufacturer's protocols. Absorbance was detected at 405 nm (TAC), 560 nm (SOD), 420 nm (GPX), 585 nm (H₂O₂), and 532 nm (MDA) with a microplate reader (Scientific Instrument Co. Ltd., Shanghai, China). A dichlorofluorescein staining assay (CA1410) was used to detect plasma ROS. The optical density at 450 nm was recorded using a microplate reader (Scientific Instrument Co. Ltd., Shanghai, China).

Concentrations of interferon-γ (IFN-γ; ab193681), IL-1β (ab100704), interleukin-2 (IL-2; ab193682), IL-6 (ab205080), interleukin-8 (IL-8; ab113352), interleukin-10 (IL-10; ab108870), immunoglobulin G (IgG) (ab205078), and TNF-α (ab193683) were determined via ELISA kits purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China) according to manufacturer's protocols. Absorbance was detected at 450 nm (IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IgG, and TNF-α) using a microplate reader (Scientific Instrument Co. Ltd., Shanghai, China). Beta-hydroxybutyric acid, non-esterified fatty acids (NEFA), GLU, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyl transpeptidase (GGT), albumin (ALB), cholesterol, and triglyceride (TG) were measured using an automatic biochemical analyzer (H794-SUNMATIK-6008, Beijing, China).

2.7. Statistical analysis

All data were analyzed using the mixed procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC, 2014) according to the following model with repeated measures:

$$Y_{ijkl} = \mu + B_i + M_j + T_k + MT_{jk} + C_l(B_i) + e_{ijkl}$$

where Y_{ijkl} = dependent, continuous variable, μ = overall mean, B_i = random effect of block, M_j = fixed effect of treatment (j = CON or GTP), T_k = fixed effect of time, MT_{jk} = interaction between treatment and time, $C_l(B_i)$ = random effect of cow within block, and e_{ijkl} = residual error. Single-factor variance analysis was carried out for the control and treatment group, and Duncan's method was used for multiple comparisons. All data were presented as the mean ± SEM. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Feed intake, milk yield and milk composition

Effects of green tea polyphenols (GTP) supplementation on milk components were shown in Table 2 and Fig. 1. The Day × GTP interaction was significant for milk yield ($P < 0.01$; Fig. 1B), milk protein yield ($P = 0.02$; Fig. 1C), milk fat yield ($P = 0.05$; Fig. 1D), and somatic cell count (SCC, $P = 0.01$; Fig. 1E). Green tea polyphenols supplementation increased milk yield ($P < 0.01$; Fig. 1B) and decreased somatic cell count (SCC, $P < 0.01$; Fig. 1E) compared to the control. Dry matter intake ($P = 0.03$; Fig. 1A), milk protein yield ($P < 0.01$; Fig. 1C), and milk fat yield ($P < 0.01$; Fig. 1D) increased over time during early lactation, regardless of treatment.

3.2. Plasma parameters associated with liver function and energy metabolism

The Day × GTP interaction was not significant for plasma parameters associated with liver function and energy metabolism ($P > 0.05$), except for BHBA ($P < 0.01$; Fig. 2A) and GLU ($P = 0.0006$; Fig. 2C). GTP cows had lower concentrations of plasma BHBA (Fig. 2A), NEFA (Fig. 2B), GGT (Fig. 3C), ALB (Fig. 3D), cholesterol (Fig. 3E) and TG (Fig. 3F) than controls (all $P < 0.01$). Greater concentrations of GLU ($P < 0.01$; Fig. 2C) in plasma were observed in GTP cows compared with the controls. Additionally, GTP supplementation up-regulated activities of AST ($P < 0.01$; Fig. 3A) and ALT ($P < 0.01$; Fig. 3B) in plasma.

3.3. Plasma parameters associated with oxidative stress

A Day × GTP interaction was observed for GPX ($P = 0.01$; Fig. 4C). Compared to control group, activities of TAC (Fig. 4A), SOD (Fig. 4B), and GPX (Fig. 4C) in plasma were greater in the GTP group (all $P < 0.01$). Meanwhile, GTP supplementation decreased

Table 2
Effects of green tea polyphenols (GTP) supplementation on milk components in multiparous Holstein cows with hyperketonemia during early lactation (% of DM, $n = 10$).

Item	10 d postpartum				20 d postpartum				30 d postpartum				P-value		
	Control	SEM	GTP	SEM	Control	SEM	GTP	SEM	Control	SEM	GTP	SEM	GTP	Day	GTP × Day
Fat	4.06	0.06	4.10	0.08	4.08	0.08	4.02	0.06	4.09	0.05	4.05	0.05	0.22	0.47	0.68
Protein	3.25	0.11	3.23	0.08	3.25	0.07	3.25	0.05	3.29	0.04	3.27	0.04	0.44	0.15	0.86
Fat-to-protein ratio	1.25	0.03	1.27	0.01	1.26	0.04	1.24	0.02	1.24	0.01	1.24	0.01	0.14	0.55	0.36
Lactose	5.17	0.12	5.15	0.2	5.19	0.16	5.10	0.21	5.02	0.36	5.06	0.36	0.68	0.23	0.69

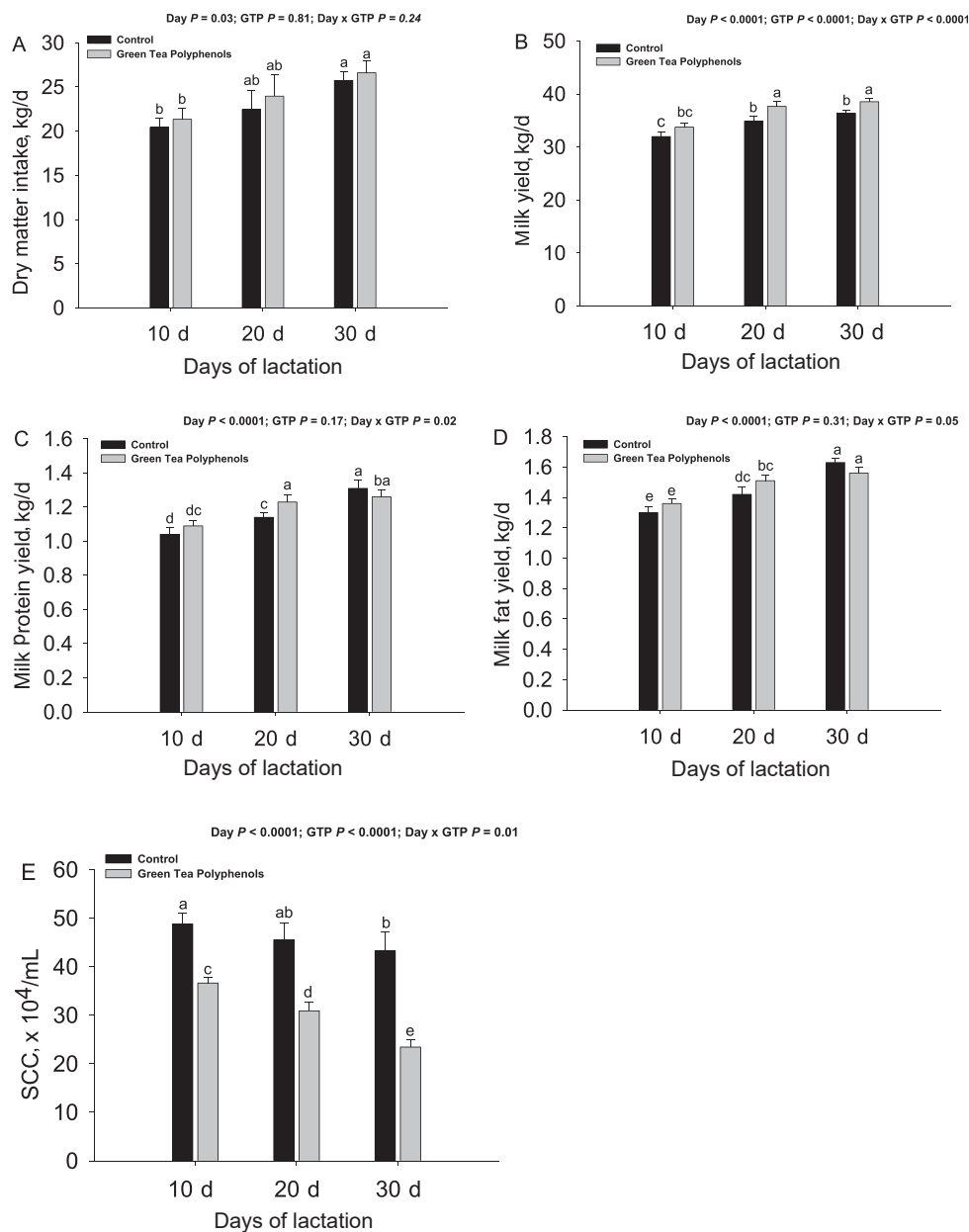


Fig. 1. Effects of green tea polyphenols (GTP) supplementation on dry matter intake (DMI, A), milk yield (B), milk protein yield (C), milk fat yield (D) and somatic cell count (SCC, E) in multiparous Holstein cows with hyperketonemia during early lactation. *n* = 10. Data are presented as the mean ± SEM. ^{a-e} means with different letters differ significantly (*P* < 0.05).

concentrations of ROS (Fig. 4D), MDA (Fig. 4E) and H₂O₂ (Fig. 4F) in plasma (all *P* < 0.01).

3.4. Plasma parameters associated with inflammation

The Day × GTP interaction was significant for IgG (*P* = 0.0005; Fig. 5A), IL-1β (*P* = 0.0003; Fig. 5C), IL-2 (*P* = 0.0003; Fig. 5D), IL-8 (*P* = 0.0002; Fig. 5F), and IL-10 (*P* < 0.01; Fig. 5G). Higher concentrations of IgG (Fig. 5A), IL-6 (Fig. 5E), and IL-10 (Fig. 5G) were observed in the plasma of cows supplemented with GTP (all *P* < 0.01). Moreover, GTP cows had lower concentrations of TNF-α (Fig. 5B), IL-1β (Fig. 5C), IL-2 (Fig. 5D), IL-8 (Fig. 5F), and IFN-γ (Fig. 5H) in plasma than the controls (*P* < 0.05).

4. Discussion

4.1. Hyperketonemia and energy metabolism

Hyperketonemia, a common metabolic disorder, usually occurs in the postpartum period. It is associated with NEB resulting from decreased DMI and increased energy requirements that are needed for postpartum milk production (Drackley, 1999; Dann and Drackley, 2005; Xu et al., 2008). Hyperketonemia is characterized by high concentrations of BHBA in the blood, urine, and milk, and is identified by a blood BHBA concentration of 1.2 to 2.9 mmol/L (McArt et al., 2012; Itle et al., 2015). Additionally, low circulating GLU (<3.75 mmol/L) is another characteristic of

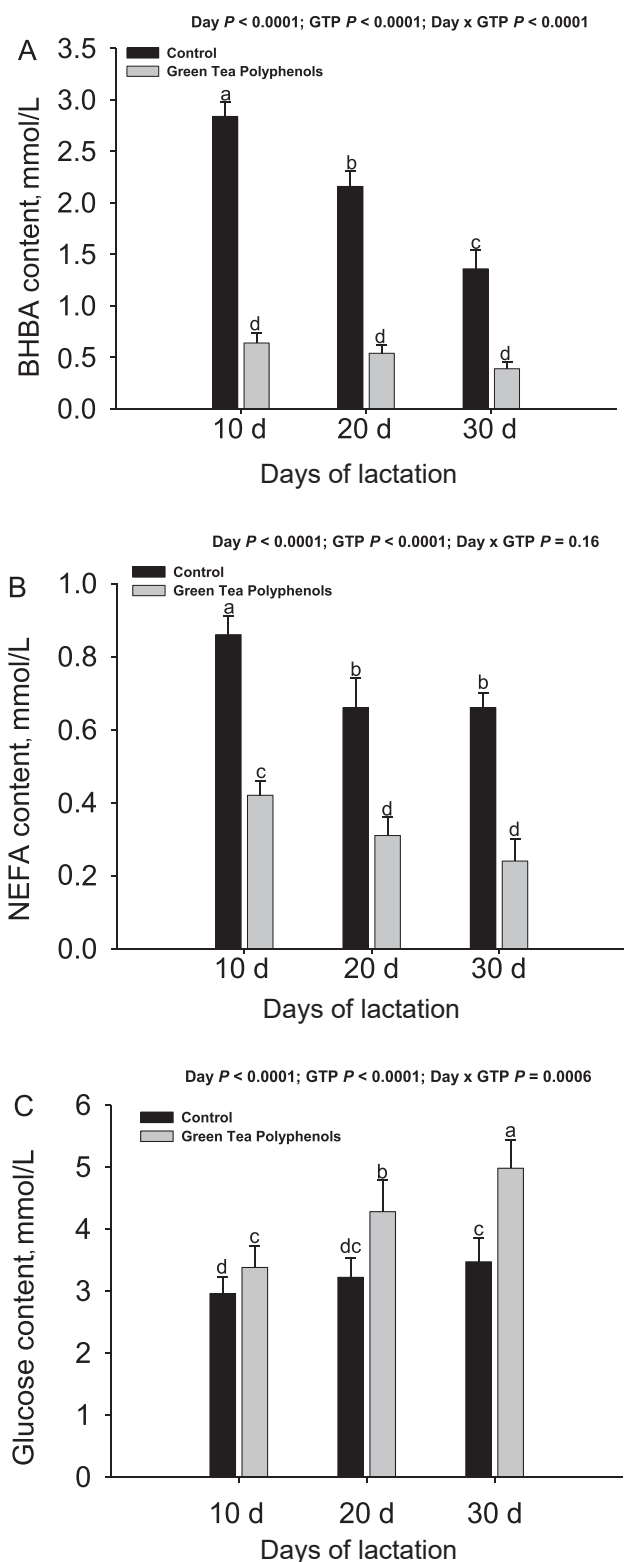


Fig. 2. Effects of green tea polyphenols (GTP) supplementation on plasma bio-markers related to energy metabolism in multiparous Holstein cows with hyperketonemia during early lactation. A to C: concentrations of β -hydroxybutyric (BHBA, A), non-esterified fatty acids (NEFA, B) and glucose (GLU, C). $n = 10$. Data are presented as the mean \pm SEM. ^{a-d} means with different superscripts differ significantly ($P < 0.05$).

hyperketonemia. Blood GLU level decreases dramatically before the occurrence of hyperketonemia, which results in the mobilization of body fat reserves along with elevated concentrations of NEFA in blood (Xu et al., 2010). Non-esterified fatty acids are mainly derived from lipolysis, reflecting the extent of body fat mobilization. In the current study, lower concentrations of BHBA and NEFA, coupled with greater concentrations of GLU in plasma in GTP cows, suggest that GTP supplementation during the periparturient period helps alleviate NEB during early lactation (Cao et al., 2007; Winkler et al., 2015). Other studies of monogastric animals have revealed that dietary GTP supplementation could alleviate oxidative stress and insulin resistance (Hininger-Favier et al., 2009). Although the exact mechanism of how GTP regulates NEB is unclear, it is possible that this might be related to the antioxidant property of GTP (Ma et al., 2018).

4.2. Oxidative stress

It is well-known that ROS are essential for mitohormesis; however, excessive ROS generation results in cellular oxidative stress (Jain et al., 2019). The protective role of GTP against the ROS-mediated cellular damage is widely recognized, at least in non-ruminants (Schroeder et al., 2003; Raza and John, 2005). Malondialdehyde is one product of lipid peroxidation, and elevated plasma MDA concentrations indicate lipid peroxidation (Kohen and Nyska, 2002). In vivo studies found that appropriate doses of GTP reduced the increase of the MDA level caused by cerebral ischemia (Lee et al., 2004). Our previous in vitro studies using bovine mammary epithelial cells revealed that GTP enhanced activities of antioxidant enzymes and reduced ROS and MDA concentrations through activating the NFE2L2/HMOX1 pathway (Ma et al., 2018, 2019). This suggests that GTP contributes to eradicating ROS, which subsequently promotes cell growth and metabolism (Ma et al., 2019). In the current study, lower concentrations of ROS, MDA, and H_2O_2 in cows supplemented with GTP, indicate that GTP might potentially help attenuate oxidative stress in vivo, which is consistent with our previous in vitro results (Ma et al., 2018, 2019).

Superoxide dismutase converts superoxide to H_2O_2 , which plays an essential role in the first defense line, and GPX, which converts H_2O_2 to H_2O , belongs to the secondary defense mechanisms; thus, they regulate the recovery of cellular oxidative damage (Masella et al., 2005). Overall, increased activities of antioxidant enzymes including TAC, SOD and GPX in GTP and decreased concentrations of ROS and MDA imply that GTP supplementation might alleviate oxidative stress and lipid peroxidation in cows with hyperketonemia.

4.3. Liver function

Liver is a major site for lipid, GLU, and protein metabolism. Transcriptomic and proteomic analyses demonstrated that NEB led to marked alterations of many biochemical pathways in the liver of dairy cows (Loor et al., 2007; McCarthy et al., 2010; McCabe et al., 2012). Postpartum hyperketonemia alters the biomarkers associated with liver function; for example, concentrations of AST were higher in cows with hyperketonemia than that in healthy cows. In the current study, lower concentrations of AST, ALT and GGT in GTP cows indicate that GTP supplementation can improve liver function in cows with hyperketonemia. One particularly interesting finding

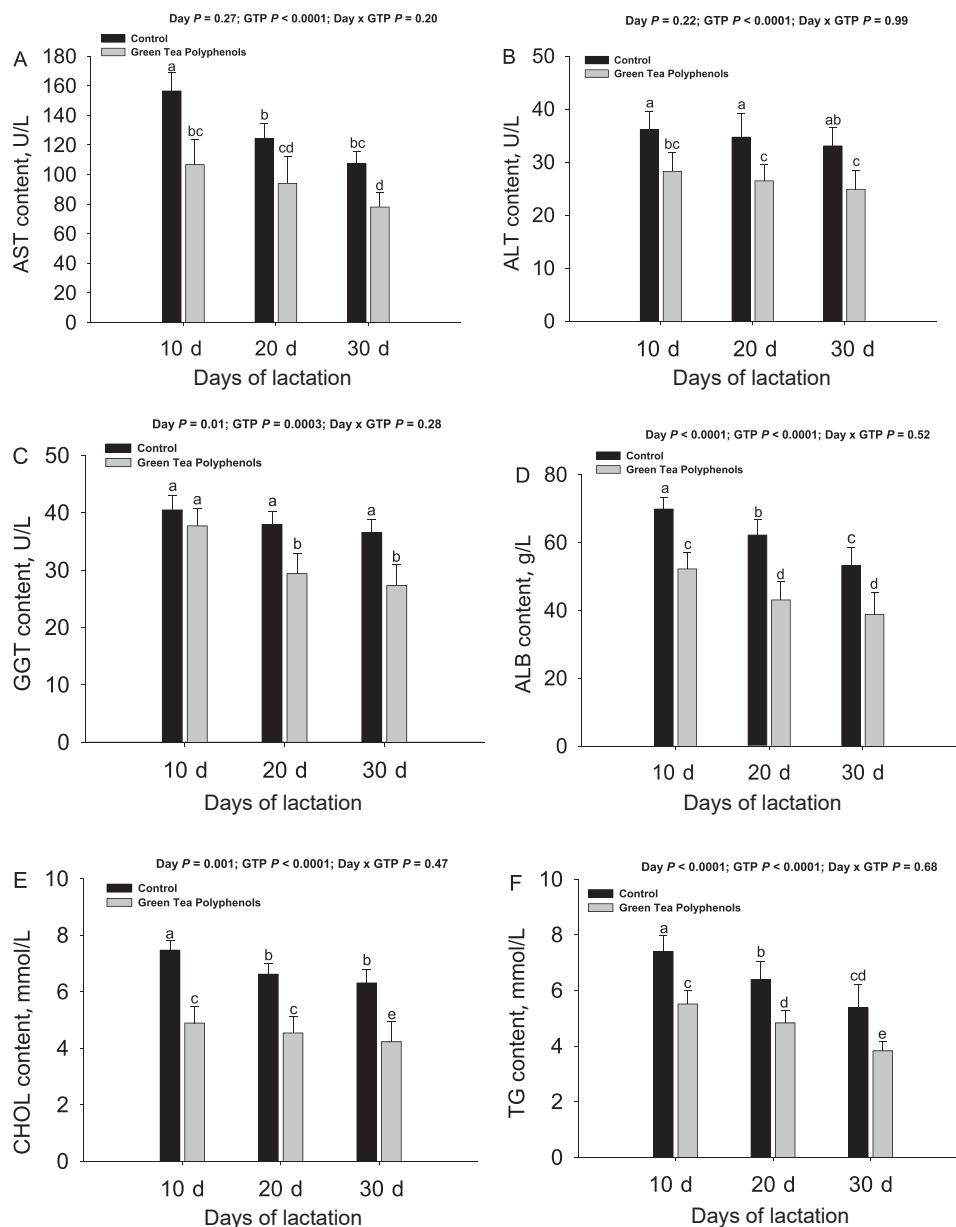


Fig. 3. Effects of green tea polyphenols (GTP) supplementation on plasma bio-markers associated with liver function in multiparous Holstein cows with hyperketonemia during early lactation. (A) to (F): concentrations/activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyl transpeptidase (GGT), albumin (ALB), cholesterol, and triglyceride (TG). $n = 10$. Data are presented as the mean \pm SEM. ^{a-d} means with different superscripts differ significantly ($P < 0.05$).

was how the supplementation of GTP could reduce concentrations of ALB, cholesterol and TG in periparturient cows, which suggests an altered liver hormone sensitivity to lipase activity and cholesterol absorption in response to GTP supply (Niu et al., 2013; Winkler et al., 2015). However, improved liver function would lead to increased levels of ALB, TG and cholesterol, as the secretion of these lipids from liver into plasma is enhanced. Therefore, we speculate that the lack of elevated concentrations of ALB, cholesterol and TG in cows fed GTP might be due to the fact that liver function has not recovered to healthy levels. Further studies are warranted to elucidate the exact mechanism on how GTP regulates liver metabolism.

4.4. Inflammation

Enhanced pro-inflammatory cytokines (TNF- α , IL-1 β , IL-2, IL-6, IL-8, and IFN- γ) lead to the development of inflammation and impair liver function in cows during the transition period. Additionally, it has been demonstrated that NEB contributes to the induction of hepatic inflammation (Looor et al., 2007; McCarthy et al., 2010). Tumor necrosis factor- α , IL-1 β , IL-2, IL-6, IL-8, and IFN- γ are major pro-inflammatory cytokines that play crucial roles in cell survival and apoptosis of normal and malignant cells (Brenner et al., 2015). Interleukin 6 exerts a regulatory effect on anti-inflammatory response (Pedersen et al., 2007). Interleukin 10,

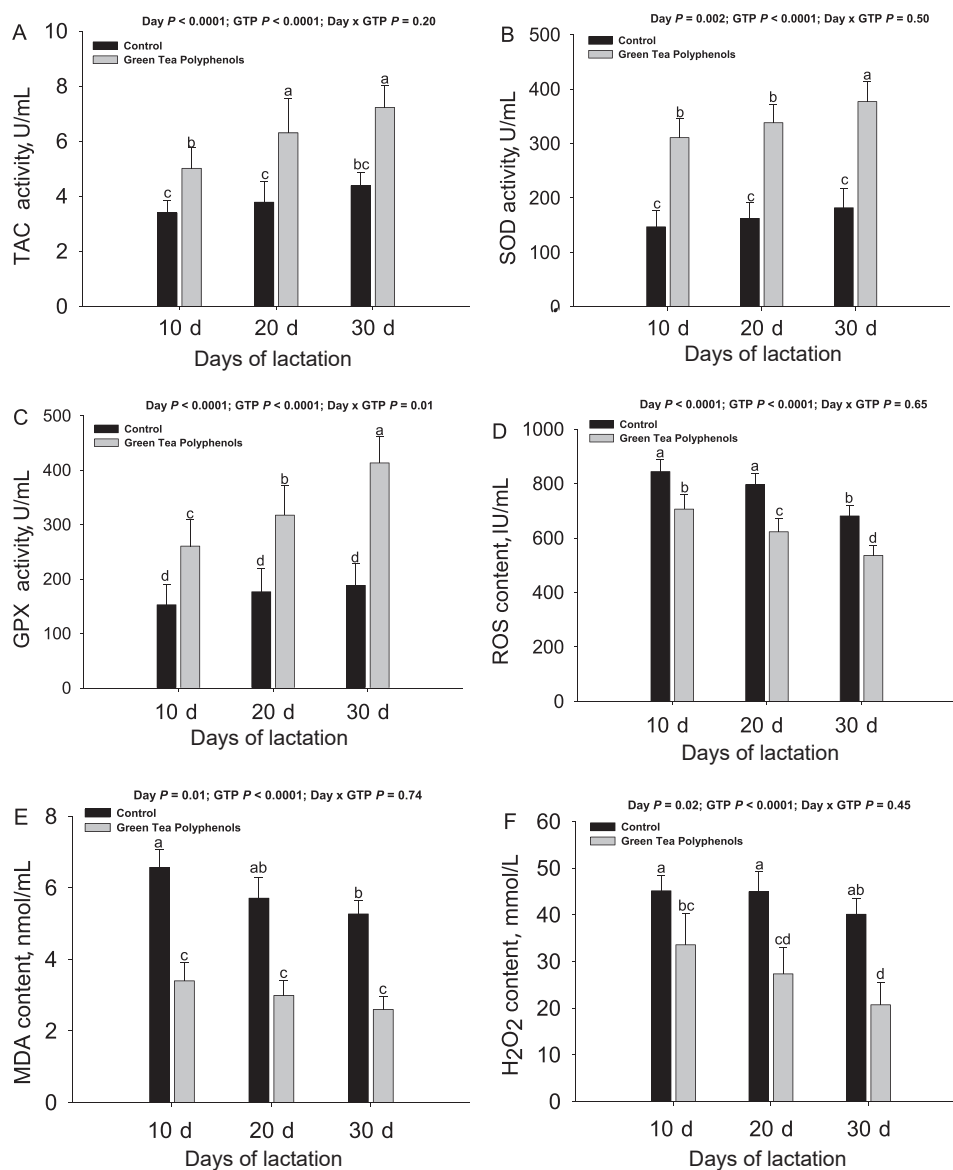


Fig. 4. Effects of green tea polyphenols (GTP) supplementation on plasma bio-markers associated with oxidative stress in multiparous Holstein cows with hyperketonemia during early lactation. (A) to (C): total antioxidant capacity (TAC), activities of superoxide dismutase (SOD), and glutathione peroxidase (GPX). (D) to (F): concentrations of reactive oxygen species (ROS), malondialdehyde (MDA) and hydrogen peroxide (H₂O₂). Data are presented as the mean ± SEM. ^{a-d} means with different superscripts differ significantly ($P < 0.05$).

secreted by monocytes, macrophages, T cells, and dendritic cells in response to systemic inflammation, is an anti-inflammatory cytokine and plays a critical role in modulating the inflammatory response to protect host tissue against damage induced by overt inflammation (Kwon and Kaufmann, 2010). In the current study, GTP supplementation increased concentrations of anti-inflammatory cytokines and decreased concentrations of pro-inflammatory cytokines in plasma, suggesting that GTP supplementation alleviated inflammatory responses in dairy cows with hyperketonemia.

4.5. Production performance

Milk yield is an important indicator of dairy cow production performance. Milk yield is influenced by many factors, such as

management, health status, genetic background, and stress factors including oxidative stress. Cows undergoing greater NEB, coupled with increased oxidative stress and inflammation postpartum, are likely to suffer from hyperketonemia. Up-regulated oxidative stress and inflammation biomarkers in the blood suggest a more pronounced oxidative stress status and inflammatory response in postpartum dairy cows, which is consistent with previous findings (Winkler et al., 2015). Cows in a state of metabolic disease will lead to the decline of the cow's health and production performance (Drackley et al., 2001). In the present study, despite greater milk yield, cows with GTP supplementation did not have more DMI, suggesting that an increased milk yield might be at least, in part, attributed to a decreased oxidative stress status and down-regulated inflammatory response, in reaction to GTP supply.

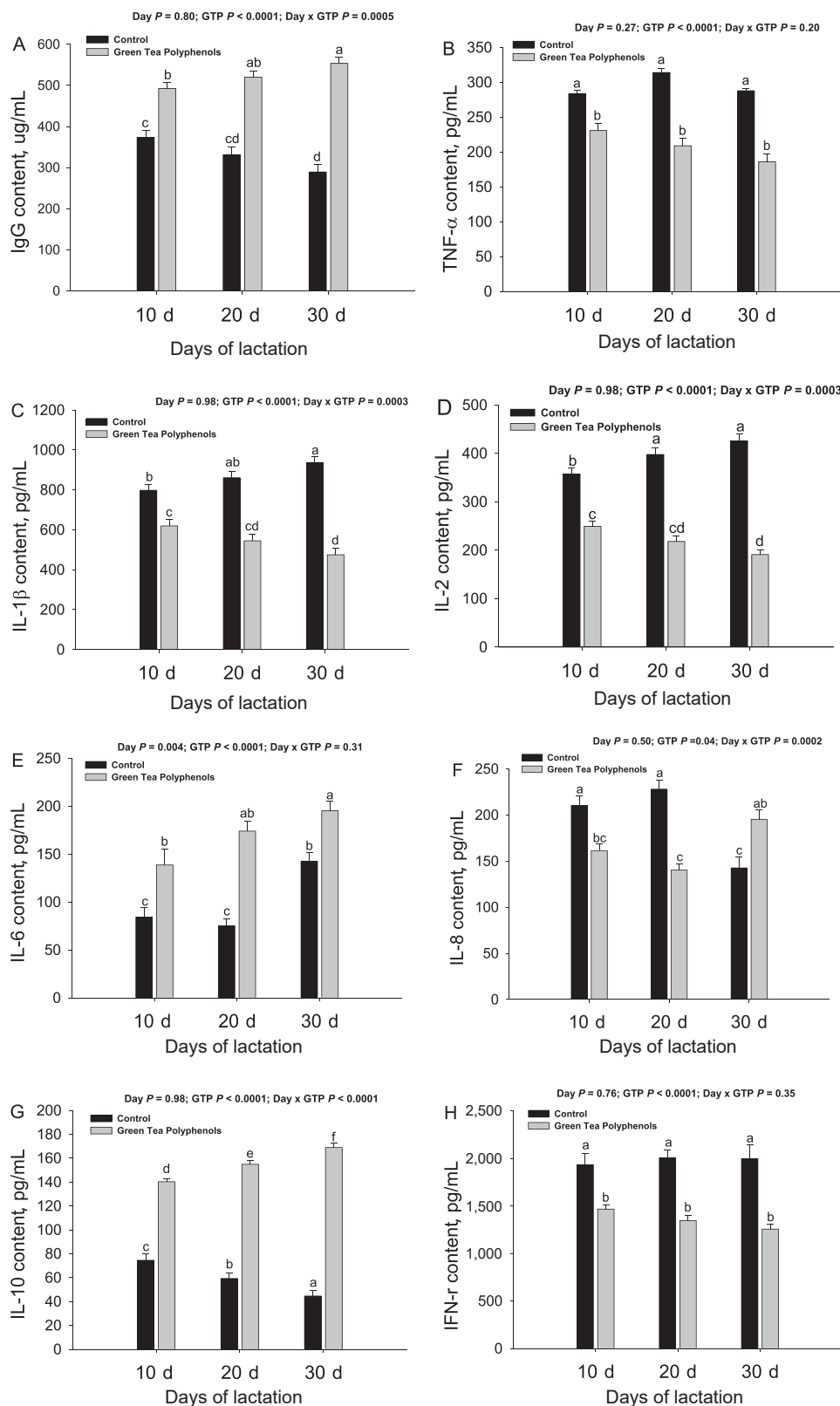


Fig. 5. Effects of green tea polyphenols (GTP) supplementation on plasma bio-markers associated with inflammation in multiparous Holstein cows with hyperketonemia during early lactation. A to H: concentrations of immunoglobulin G (IgG), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), and interferon- γ (IFN- γ). $n = 10$. Data are presented as the mean \pm SEM. ^{a-f} means with different superscripts differ significantly ($P < 0.05$).

5. Conclusions

Supplementation of GTP during the transition period reduced concentrations of oxidative stress bio-markers including ROS, H₂O₂ and MDA, and pro-inflammatory cytokines, such as TNF- α , IL-1 β , IL-2, IL-8, and IFN- γ in plasma. This might partly explain the enhanced milk yield and improved health status in dairy cows during early lactation. Overall, GTP supply during the periparturient period contributes to improving the milk yield and well-being of postpartum dairy cows with hyperketonemia.

Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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