Effect of feeding grape seed *Proanthocyanidin* extract on production performance, metabolic and anti-oxidative status of dairy cattle

[*Efeito da alimentação do extrato de semente de uva* Proanthocyanidin *sobre o desempenho da produção e o* status *metabólico e antioxidante de gado leiteiro*]

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ABSTRACT

Aims to investigate the effects of grape seed *proanthocyanidin* extract (GSPE) on production performance, metabolism, and anti-oxidative status of Holstein dairy cattle in early lactation. Forty-eight multiparous Holstein dairy cattle were assigned to four groups (CON, G20, G40 and G80) and supplied with 0, 20, 40, and 80mg GSPE/kg of body weight/day. G20 significantly increased milk yield compared with other groups. Milk protein and non-fat-solids were increased in G20, G40 and G80 groups compared with the control group only at the 7th day during the experiment. No significant difference was observed in milk fat and somatic cell count, nor on parameters of energy metabolism in blood, liver function and kidney function between the four groups. There was no significant difference in glutathione peroxidase, superoxide dismutase, total antioxidant capacity, and hydrogen peroxide between the groups; but the malondialdehyde content of G20 significantly increased at day 14 in comparison with CON, and tended to increase at the 28th day. In conclusion, feeding 20mg GSPE/kg of body weight/day was associated with a significant increase in milk yield without detrimental effects on liver or kidney function and with substantial energy metabolism and antioxidant parameters improvement in early lactation dairy cattle.

Keywords: dairy cattle, grape seed, *proanthocyanidin*, milk production, antioxidant properties

RESUMO

O presente trabalho visa investigar os efeitos do extrato de semente de uva Proanthocyanidin *(GSPE) sobre o desempenho da produção, o metabolismo e o* status *antioxidante de gado leiteiro Holstein em lactação precoce. Quarenta e oito vacas leiteiras multíparas Holstein foram divididas em quatro grupos (CON, G20, G40 e G80) e receberam 0, 20, 40 e 80mg de GSPE/kg de peso corporal/dia, respectivamente. O G20 aumentou significativamente o rendimento do leite em comparação com os outros grupos. A proteína e os sólidos não gordurosos do leite foram aumentados nos grupos G20, G40 e G80 somente no sétimo dia durante a experiência. Não foi observada diferença significativa na gordura do leite e na contagem de células somáticas, bem como nos parâmetros de metabolismo energético no sangue, na função hepática e na função renal entre os grupos em relação ao grupo controle. Não houve diferença significativa na glutationa peroxidase, na dimutase de superóxido, na capacidade antioxidante total e no peróxido de hidrogênio entre os grupos, mas o conteúdo malondialdeído do G20 aumentou significativamente no dia 14 em comparação com o CON, e tendia a aumentar no dia 28. Em conclusão, a alimentação de 20mg de GSPE/kg de peso corporal/dia foi associada a um aumento significativo no rendimento do leite, sem efeitos nocivos sobre a função hepática ou a renal, com o metabolismo de energia substancial e a melhoria dos parâmetros antioxidantes de gado leiteiro no início da lactação.*

Palavras-chave: gado leiteiro, sementes de uva, Proanthocyanidin*, produção de leite, propriedades antioxidantes*

INTRODUCTION

During the early lactation period, dairy cattle, similarly to every mammal, experience a state of negative energy balance where the energy from feed intake fails to meet the increasing energy requirement of maintaining body tissues and milk production (Block *et al.,* 2001*,* Koller *et al.,* 2003*,* Grummer *et al.,* 2004). This phenomenon is particularly exacerbated in high producing Holstein dairy cattle which will mobilize adipose tissue (storage triacylglycerol) increasing the blood concentration of Non-esterified Fatty Acids (NEFA) (Gessner *et al.,* 2015; Ringseis *et al.,* 2015). High NEFA blood concentrations may lead to metabolic diseases like ketosis and fatty liver disease (Drackley *et al.,* 2001*,* Ringseis *et al.,* 2015). Moreover, during this period dairy cattle are vulnerable to mastitis, metritis, subacute rumen acidosis, and abomasal displacement, due to the high exposure to variable inflammatory agents, such as lipopolysaccharides, tumor necrosis factor, reactive oxygen species, interleukin-6, etc. (Zebeli and Metzler-Zebeli, 2012*,* Akbar *et al.,* 2014). Above all, dairy cattle are susceptible to oxidative and inflammatory stress, which has a negative effect on milk yield (Sordillo and Mavangira, 2014).

Grape seed *proanthocyanidin* extract (GSPE) is a class of polyphenol bioflavonoids, which are oligomers of epicatechin, catechin and their gallic acid esters and a class of tannins which have the same polymeric building block that could form more complex polyphenols. It has been reported that GSPE is a potent antioxidant and was found to provide better efficacy as compared to vitamin E succinate and vitamin C in vitro (Bagchi *et al.,* 1997). It was also shown that GSPE can protect human tissue injury from free radical and oxidative stress (Bagchi *et al.,* 2000).

Some antioxidant supplements can positively affect dairy cattle oxidative balance and performance; for example, the dietary antioxidant Agrado Plus, a liquid blend of ethoxyquin and tertiary-butyl-hydroquinone (Novus International, St. Louis, MO), can improve oxidative balance and lactation performance (Vázquez-Añón and Jenkins 2007*,* Vazquez-Anon *et al.,* 2008). Similarly, rumen-protected γ-aminobutyric acid acts as an antioxidant and has been associated with increased dry matter intake (DMI), and better lactation performance (Wang *et al.,* 2013). Several studies have reported the effects of feeding grape seed related products; for instance, grape seed and grape marc meal extract (GSGME), a plant extract rich in flavonoids, was associated with increased milk yield (Gessner *et al.,* 2015); likewise, grape seed supplementation was shown to increase the accumulation of ruminal acid (CLA cis-9,trans-11) and fatty acid concentration of Sarda dairy sheep (Correddu *et al.,* 2015) and could be useful to decrease the extent of oxidation of unsaturated fatty acids of sheep milk (Correddu *et al.,* 2016). Grape seed extract was also shown to modulate oxidative stress and inflammation status in humans and to improve insulin sensitivity (Kappagoda *et al.,* 2015). At the same time, it seems that grape seed extract does not have negative effects as some researchers (Davies *et al.,* 2009) found that it had no adverse effects on the intake or in respiratory rate, pulse, and temperature in horses.

To the best of our knowledge there are no reports on the effects of feeding GSPE on dairy cattle health, metabolic status and productivity. Because of the more complex composition and less *proanthocyanidin* of low purified extract, high purified extract can be better in researching the effect of *proanthocyanidin* extract on animal. So, our objective here was to study the influence of this highly purified *proanthocyanidin* on milk production, antioxidant, metabolic and health status of early lactation dairy cattle.

MATERIALS AND METHODS

All experimental procedures were assessed and approved by the Ethics Committee on Animal Experiments of Guangxi University and the care and use of animals complied with the local law and guidelines on animal experiments (Approval No. GXU2015-006).

The study took place at the Guangxi agricultural reclamation Jinguang Dairy Co., Ltd. dairy cattle farm in China. Forty-eight multiparous Holstein dairy cattle in early lactation with 2nd, 3rd, or 4th parity were selected. The dairy cattle were blocked into 12 groups based on days in milk (DIM) and milk yield (MY) according to randomized block design and randomly assigned to one of four dietary treatments: 0, 20, 40, and

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80mg GSPE/kg body weight (BW) per day per cow. And the four treatment groups were control group (CON), 20mg GSPE/kg of body weight/day (mg GSPE/kg BW/d) group (G20), 40mg GSPE/kg BW/d group (G40), and 80mg GSPE/kg BW/d group (G80).

The supplied GSPE (the proportion of *Proanthocyanidin* is 95.02%, more details are showed in Table 1) was purchased from Xi'an Fengzu Biotechnology Co., Ltd. The supplied GSPE was dissolved in drinking water and the total volume of the oral administration is 500ml. And the supplied GSPE was always administered per os at 7:00 am every day; control dairy cattle were drenched with 500ml drinking water. Dairy cattle were housed in individual tie stalls with free and ad libitum access to water and a total mixed ration (TMR).

Table 1. Product test report of GSPE

No.	Item	Result	No.	Item	Result
1	Product name	Grape Seed Extract	11	Loss on drying	1.63%
2	Proanthocyanidin	95.02%	12	Pesticides	Negative
3	Plant part	Procyanidins	13	As	≤1.0ppm
4	Batch number	SF140624-1	14	Hg	≤0.5ppm
5	Manufacture date	10th Nov, 2015	15	Pb	≤2.0ppm
6	Testing date	13th Nov, 2015	16	Total microbiology plate count	<1000cfu/g
7	Effective date	9th Nov, 2017	17	Yeast & mold	<100cfu/g
8	Appearance	Brownish powder	18	Fungi	Negative
9	Odor & taste	Characteristic	19	E.Coli	Negative
10	Mesh size	100% pass 80 mesh	20	Salmonella	Negative

Milk yield was recorded every ten-days [six times in total: 0 (MY-0), 10, 20, 30, 40 and 50th day of the experiment]. Milk samples were collected after blood samples were taken at about 9:00 am at the 0, 7, 14, 21, 28, 35 and 42nd day of the experiment. The first three streams of milk were discarded, and then milk samples were collected and immediately closed and placed in an isothermal cooler at 4°C. We used 20ml of milk sample to detect milk fat (MF), non-fat-solids (SNF) and milk protein (MP) using a milk composition analyzer (*Milkyway, Hangzhou San Bo Technology Co., Ltd, China*). The remaining 30 ml milk samples were used for the determination of somatic cell count (SCC) via the Soma Scope Smart flow cytometer (Delta Instruments, *PerkinElmer scientific instruments Co., Ltd*, China).

Blood samples (10ml) were collected at about 7:00~8:00 am from the jugular vein in 10ml tubes with heparin sodium as an anticoagulant at day 0, 7, 14, 21, 28, 35 and 42 of the experiment. Both milk and blood samples collection schedule were hold constant for the entire 6 weeks of this study. Blood samples were centrifuged immediately at 3,000xg for 10min at 4°C for plasma separation. The following metabolic parameters in plasma were measured: glucose (GLU), total protein (TP), albumin (ALB), globulin (GLOB), blood urea nitrogen (BUN), uric acid (UA), cholesterol (CHOL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT). A HITACHI 7020E Type Automatic Biochemical Analyzer (*Hitachi, Co., Ltd, Japan*) was used and related kits (*Sichuan Maker Biotechnology Co., Ltd., China)* (Zhao P *et al.* 2016). And the plasma levels of total ketone bodies (TKB) was measured by the Modified Salicylaldehyde Colorimetric Method (Procos J, 1961); plasma levels of NEFA, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2), total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were determined by kits *(Nanjing Jiancheng Bioengineering Institute, China)* (Liu *et al.,* 2013).

Data were analyzed with JMP 10 software and the use of mixed effects multivariable regression modeling. Outcome variables were tested for normality and non-normally and distributed data were log-transformed before further analysis.

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RESULTS

The DIM, MY, parity, BW and BCS were not different between the four groups at the beginning of the experiment (Table 2).

Table 3 presents ingredients and nutrition compositions of the feedstuffs, which were fed to all the cattle in the study.

The effects of feeding different dose GSPE on milk and plasma variables in dairy cattle are showed in Table 4. During the whole experiment only MY of G20 is significantly different from CON (P< 0.05), G20 was 0.12 log kg/d higher than CON, before log-transforming, Least Sq Mean of CON is 27.11±0.83kg/d, and G20 is 30.45±0.90kg/d, so G20 was about 3.34kg/d higher than CON. And G20 and G40 have statistical trend between CON (P< 0.1), have no significant difference or statistical trend in other indexes.

Table 2. Mean and SE for the main parameters in the experiment groups[1]

| No. | Group | Mean ± SE | | | | |
		DIM	MY	Parity	BW	BCS
1	CON	33.92±16.47	30.98±6.47	2.67±0.89	571.08±53.51	2.00±0.30
2	G20	37.50±18.36	29.57±5.27	2.67±0.78	584.75±58.02	1.92±0.36
3	G40	35.67±16.98	29.82±7.22	2.17±0.39	582.83±60.93	1.79±0.40
4	G80	33.83±19.36	29.81±8.00	2.33±0.65	61.60	1.92±0.36

[1] CON, control group; G20, G40, and G80 are 20, 40, and 80mg GSPE/kg of body weight/day groups, respectively. All P-values were greater than 0.05.

Table 3. Ingredients and nutrition compositions of the feedstuffs[1]

No.	Item	Diet	No.	Item	Diet
	Ingredients, % of DM		11	Calcium hydrophosphate	1.02
1	Corn in northeast china	24.16	12	Sodium bicarbonate	0.41
2	Corn silage	4.35	13	Animal salt	0.48
3	Wheat bran	3.17	14	Vitamin mineral premix[2]	0.7
4	Bean pulp	9.05	15	Beer brewing wastes	10.88
5	Rape seed dregs	5.43		Nutrient compositions[3]	
6	Alfalfa silage	16.83	16	DM (% of feedstuffs)	54.93
7	Soybean Hulls	10.76	17	CP (% of DM)	20.32
8	Elephant grass	3.55	18	NDF (% of DM)	32.02
9	Chinese leymus	8.7	19	ADF (% of DM)	12.77
10	Calcium carbonate	0.51	20	NE_L[4] (Mcal/kg DM)	1.73

[1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; NE_L, net energy lactation.
[2] Content per kilogram of premix: 450 to 550K IU of vitamin A, 140 to 160K IU of vitamin D_3, 2900 to 3100IU of vitamin E, 3.9 to 4.1g of Fe, 1.2 to 1.4g of Cu, 2.9 to 3.1g of Mn, 5.9 to 6.1g of Zn, 75 to 85mg of I, 45 to 55mg of Se, 75 to 85mg of Co.
[3] Calculated from the analyzed value of the feedstuffs ingredients.
[4] NE_l, Net Energy Lactation; calculated based on feedstuffs according to the *Chinese National Station of Animal Production and Health (2000)*.

The time point effect of feeding GSPE on milk and plasma variables in dairy cattle are showed in Table 5, which shows that some variables related to energy metabolism, liver function and kidney function, and anti-oxidative status are significantly different(P< 0.05), and even extremely significantly different (P< 0.001) at the time point. However, combined with Table 4,

there is no significant difference or statistical trend besides MY and SNF, so we just need to focus on MY and SNF. As time goes by, the total MY of four groups cattle is extremely significant decrease. The total SNF of four groups cattle is extremely significant increase from T1 to T2, and then extremely significant decrease from T2 to T3, until T5.

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Table 4. Effects of feeding different dose GSPE on milk and plasma variables in dairy cattle[1)]

Item[2)]	CON	G20	G40	G80
MY, log kg/d	3.29±0.12[B]	3.41±0.12[A]	3.33±0.11	3.3±0.12
MF, log %	1.30±0.12	1.20±0.13	1.07±0.13	1.28±0.13
MP, log %	1.12±0.033	1.14±0.03	1.15±0.03[A]	1.10±0.03[B]
SNF, %	8.18±0.31[#]	8.41±0.31[A#]	8.42±0.29[A#]	8.02±0.31[B]
SCC, log k cells/mL	4.74±0.42	4.40±0.46	4.78±0.46	5.86±0.46
TKB, log mg/dL	1.14±0.05	1.17±0.06	1.13±0.05	1.18±0.05
NEFA, log umol/L	5.50±0.08	5.46±0.09	5.54±0.09	5.47±0.09
GLU, log mmol/L	1.19±0.03	1.23±0.03	1.18±0.03	1.19±0.03
ALT, log U/L	3.41±0.04	3.41±0.05	3.34±0.05	3.41±0.05
AST, log U/L	4.72±0.04	4.68±0.04	4.72±0.04	4.69±0.04
GGT, log U/L	3.45±0.07	3.41±0.07	3.57±0.07	3.37±0.07
ALB, g/L	32.62±0.52	33.27±0.58	31.70±0.51	32.23±0.54
GLOB, log g/L	4.16±0.03	4.11±0.04	4.18±0.03	4.10±0.03
TP, g/L	97.23±1.80	94.32±2.00	97.57±1.77	93.21±1.85
BUN, mmol/L	7.40±0.25	7.56±0.27[a]	6.64±0.27[b]	6.91±0.27
CHOL, mmol/L	4.51±0.22	4.51±0.24	4.48±0.24	4.45±0.24
UA, log mmol/L	3.76±0.07	3.86±0.07	3.64±0.07	3.73±0.07
GSH-PX, log U/mL	4.66±0.02	4.66±0.02	4.72±0.02	4.67±0.02
H$_2$O$_2$, log mmol/L	2.79±0.03	2.78±0.04	2.74±0.04	2.78±0.04
MDA, log nmol/mL	1.22±0.04	1.33±0.05	1.22±0.05	1.23±0.05
SOD, U/mL	161.06±7.26	164.58±7.96	154.98±7.96	160.09±7.96
TAC, log U/mL	1.54±0.04	1.55±0.04	1.49±0.04	1.52±0.04

[1)] CON, control group; G20, G40, and G80 are 20, 40, and 80mg GSPE/kg of body weight/day groups.
[2)] MY, milk yield; MF, milk fat; MP, milk protein; SNF, non-fat-solids; SCC, somatic cell count; TKB, total ketone body; NEFA, non-esterified fatty acid; GLU, glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALB, albumin; TP, total protein; GLOB, globin; BUN, blood urea nitrogen; CHOL, cholesterol; UA, uric acid; GSH-Px, glutathione peroxidase; H$_2$O$_2$, hydrogen peroxide; MDA, malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity.
[ABab] Having different uppercase indicate that the data are extremely significant difference (P< 0.01), having different lowercase indicate that the data are extremely significant difference (P< 0.05).
[#] Mens there is a statistical trend.

Table 5. The time point effect of feeding GSPE on milk and plasma variables in dairy cattle

Item[1]	T[2]0	T1	T2	T3	T4	T5	T6	SE	*P*-value
MY, log kg/d	3.40	3.40	3.40	3.26	3.17	3.11		0.125	<0.0001
MF, log %	0.86	1.30	1.24	1.84	1.92	1.37	0.86	0.213	<0.0001
MP, log %	1.10	1.10	1.31	1.07	1.08	1.07	1.12	0.038	<0.0001
SNF, %	8.01	8.03	9.88	7.76	7.79	7.69	8.13	0.357	<0.0001
SCC, log k cells/mL	3.99	4.94	4.31	5.19	4.60	5.30	4.84	0.556	0.199
TKB, log mg/dL	1.06	1.18	1.12	1.11	1.16	0.72	1.06	0.109	0.109
NEFA, log umol/L	5.75	5.11	5.45	5.46	5.48	5.58	5.66	0.220	0.520
GLU, log mmol/L	1.27	1.21	1.21	1.17	1.12	1.16	1.22	0.075	0.0855
ALT, log U/L	3.18	3.47	3.43	3.45	3.38	3.49	3.50	0.065	0.0003
AST, log U/L	4.66	4.91	4.76	4.72	4.62	4.59	4.76	0.053	<0.0001
GGT, log U/L	3.39	3.52	3.51	3.44	3.40	3.44	3.46	0.082	0.489
ALB, g/L	32.00	33.39	33.56	33.15	31.71	31.61	32.90	0.717	0.027
GLOB, log g/L	4.16	4.17	4.20	4.16	4.10	4.13	4.17	0.039	0.111
TP, g/L	97.46	98.99	100.96	98.01	92.61	94.44	98.15	2.412	0.016
BUN, mmol/L	6.17	8.42	9.50	7.08	7.25	6.33	7.08	0.353	<0.0001
CHOL, mmol/L	3.82	4.67	4.64	4.70	4.34	4.50	4.93	0.277	0.002
UA, log mmol/L	3.51	3.73	3.98	3.85	3.82	3.73	3.73	0.106	0.016
GSH-PX, log U/mL	5.16	4.15	4.21	4.66	4.55	4.50	5.43	0.059	<0.0001
H$_2$O$_2$, log mmol/L	2.78	2.62	2.93	2.77	2.95	2.80	2.68	0.083	0.051
MDA, log nmol/mL	1.10	1.10	1.31	1.07	1.08	1.07	1.12	0.088	<0.0001
SOD, U/mL	185.71	124.05	174.90	146.19	149.78	174.60	172.19	11.550	<0.0001
TAC, log U/mL	1.16	1.95	1.41	1.62	1.25	1.46	1.97	0.97	<0.0001

[1] The same as Table 4.
[2] For the MY, the T0, T1, T2, T3, T4 and T5 indicate the 0, 10, 20, 30, 40 and 50th day respectively, but for other indexes, the T0, T1, T2, T3, T4, T5 and T6 indicate the 0, 7, 14, 21, 28, 35 and 42th day respectively.

Results of fixed effect test in fit least squares are showed in Table 6, which shows which effects had a significant impact on the variables, for example, MP was statistically significantly associated with MY-0 (Estimate= 0.0254, P<

0.0001), and MP was statistically significantly associated with BCS at enrollment (BCS= 1: 1.12±0.03; BCS= 1.5: 1.12±0.01; BCS= 2: 1.16±0.01; BCS= 2.5: 1.16±0.01, P= 0.0310).

Table 6. Some results of fixed effect test in fit least squares

Role variables[1]	Effects	Estimate/SE	*P*-value
MY	MY-0	0.0254	< 0.0001
MF	MY-0	0.0258	0.0088
MP	MY-0	-0.0026	< 0.0009
	BCS=1	1.12±0.03	
MP	BCS=1.5	1.12±0.01	0.0310
	BCS=2	1.16±0.01	
	BCS=2.5	1.16±0.01	
	parity=2	1.12±0.03	
MP	parity=3	1.16±0.03	0.0336
	parity=4	1.13±0.03	
SNF	BCS=1	8.18±0.31	0.0119
	BCS=1.5	8.26±0.11	
	BCS=2	8.6±0.09	
	BCS=2.5	8.45±0.13	
SNF	MY-0	-0.0273	0.0002
TKB	DIM	-0.0059	0.0003
	parity=2	1.14±0.05	0.0463
	parity=3	1.01±0.07	
	parity=4	0.98±0.07	
NEFA	MY-0	0.0144	0.0268
GGT	BW	0.0020	0.0042
ALB	parity=2	32.62±0.52	0.0013
	parity=3	30.00±0.71	
	parity=4	31.43±0.72	
GLOB	parity=2	4.16±0.03	0.0137
	parity=3	4.27±0.04	
	parity=4	4.25±0.05	
TP	parity=2	97.23±1.80	0.0425
	parity=3	102.21±2.45	
	parity=4	102.22±2.49	
CHOL	MY-0	0.0399	0.0235

[1] MY, milk yield; MF, milk fat; MP, milk protein; SNF, non-fat-solids; TKB, total ketone body; NEFA, non-esterified fatty acid; GGT, gamma glutamyl transferase; ALB, albumin; GLOB, globin; TP, total protein; CHOL, cholesterol.

Least square means for different treatment and different time point are presented in Figure 1.

For variables related to production performance and udder health, analysis results show that a statistically significant treatment group by time point interaction was noticed (*P*0.0025) in MY. Especially, MY of G20 is significantly higher than CON at day 40th (P= 0.0329), the MY of CON is 3.15±0.05 log kg/d, G20 is 3.39±0.05 log kg/d, before log-transforming, Least Sq Mean of CON is 23.87±1.25kg/d, and G20 is 30.23±1.37kg/d, so G20 was 6.36kg/d higher than CON. As Figure 1 (A) shows that MY of

G20 has different trends compared to other groups, MY of G20 always higher than CON, G40 and G80 from day 20th to day 50th. A statistically significant treatment group by time point interaction was also noticed in MP (P= 0.0023), least square means plot describing this interaction are presented in Figure 1 (B). A statistically significant treatment group by time point interaction was also noticed in SNF (*P*=0.0029), the least square means plot describing this interaction are presented in Fig.1 (C). MP and SNF have similar trend, and both had significant difference at day 7th, which showed that G20 was significantly higher than

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CON. However, both MP and SNF of G20 had not significant difference between other groups. And no significant difference was observed among the four groups in somatic cell count.

For variables related to energy metabolism, a trend toward an increase in treatment group by

time point interaction was noticed (P= 0.0692) in NEFA, but there was no significant difference compared with CON, the least square means plot describing this interaction is presented in Figure 1 (D). No significant difference or statistical trend in treatment group by time point interaction was observed in TKB and GLU.
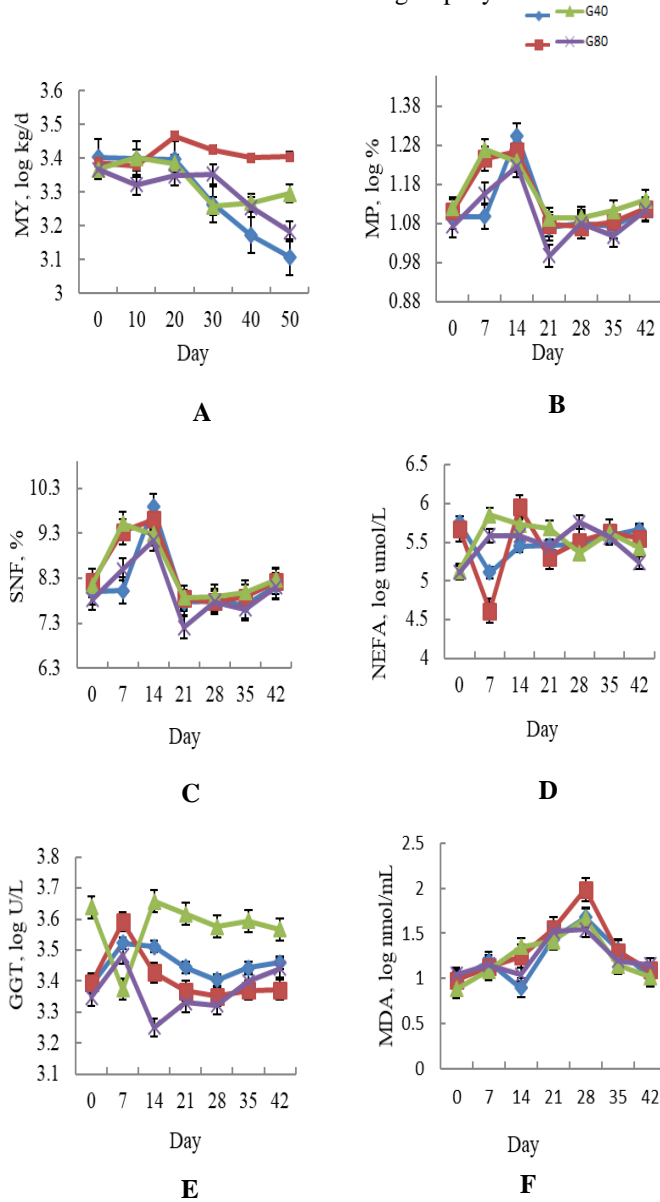

Figure 1. Least square means of clinical indicators of four groups. (A)MY, (B)MP, (C) SNF, (D) NEFA, (E) GGT and (F) MDA typical lesions of the milk yield, milk protein, non-fat-solids, Non-esterified Fatty Acids, gamma glutamyl transferase and malondialdehyde respectively least square means for different treatment and different time point.

For variables related to liver function and kidney function, a statistically significant treatment group by time point interaction was noticed (P= 0.0040) in GGT, the least square means plot describing this interaction is presented in Figure 1 (E). Analysis results showed that G40 was extremely significantly different from G20 at day 7th (P< 0.0001), and day 7th (P< 0.0001) and day14[th] (P= 0.0094), respectively. However, there was no significant difference observed compared with CON. A trend toward an increase in treatment group by time point interaction was also noticed (P= 0.0709) in BUN. No significant difference or statistical trend in treatment group by time point interaction was observed in other liver function and kidney function indexes.

For variables related to anti-oxidative status. A statistically significant treatment group by time point interaction was noticed in MDA (P= 0.0463), least square means plot describing this interaction are presented in Figure 1 (F). At day 14th, MDA of G20 was significantly higher than CON, and G40 was extremely significantly higher than G80, but no significant difference was observed at any other time points and between any other groups. And no significant difference or statistical trend in treatment group by time point interaction was observed in other anti-oxidative indexes.

DISCUSSION

We show here that feeding 20mg GSPE/kg BW/d had positive effects on the milk production of Holstein dairy cattle. This in agreement with results reported by other researchers showing that GSGME supplementation was associated with increased MY (Gessner *et al.,* 2015). Other study also shown that tannins was associated with increased MY, which possibly result from promoting intestinal absorption of essential amino acids (Bhatta *et al.,* 2000). During this experiment, dairy cattle supplemented with GSPE produced more milk protein in comparison with the control group. It has been reported that 5.0kg/d of dried grape marc slightly increased milk protein of dairy cattle in late lactation (Moate *et al.,* 2014), and GSGME also increased milk protein of dairy cattle in early lactation (Gessner *et al.,* 2015). An interesting result was that SNF showed a similar pattern with MP. The associations between GSPE supplementation and increased MY, MP and SNF may be due to

reducing the degradation of protein in the rumen (Patra and Saxena 2011), and to increase the protein flux of small intestine (Moate *et al.,* 2014). However, this is just a speculation; these results could also be a result of increased dry matter intake which we unfortunately did not measure.

Somatic cell count was not significantly different between different treatment groups. Previous reports showed that grape seed phenolic extract had antimicrobial activity against the gram-positive *B. linens* (Chedea *et al.,* 2011) and inhibited the growth of *E. coli* (Chedea *et al.,* 2011, Hajati *et al.,* 2014). However, we were not able to show any effects of feeding GSPE on SCC. Similarly, researcher showed that supplementation with 300g of grape seed/day had no significant effects on SCC (Nudda *et al.,* 2015).

Feeding GSPE had no significant effects on TKB, NEFA and GLU. The concentration of NEFA tended to be higher (P= 0.0798) at 14d in G20. Other researchers showed that GSGME was associated with an increase in the concentration of beta-hydroxybutyric acid at some point (Gessner *et al.,* 2015).

We found no significant influence of GSPE supplementation on ALT, AST, ALB, GLOB, TP, BUN and UA. However, except for the 7d, GGT activity of G40 higher than other groups, while G20 lower than CON, and G80 lower than CON all the time. Above all, the increasing GGT activity of G40 may not be due to GSPE supplementation. Feeding 20mg GSPE/kg BW/d GSPE had no effects on liver and kidney function parameters of ALT, AST, ALB, GLOB, TP, BUN and UA; confirming that the appropriate dose of *proanthocyanidin* is safe. This in agreement with the study of other researchers, which showed that *proanthocyanidin*-rich extract from grape seed lacked toxicity (Yamakoshi J *et al.,* 2002), and another study reported that horses were fed grape seed extract and that this induced no harmful effects on their health (Davies *et al.,* 2009).

Previous reports showed that GSPE can protect rat leukocytes from DNA damage (Morin *et al.,* 2008), and offer protection against free radicals and free radical induced lipid peroxidation, and that this bioactivity is higher than vitamins C, E and b carotene (Bagchi *et al.,* 2000). However,

our results showed that there was no significant effect of GSPE supplementation on GSH-Px, SOD, TAC and H_2O_2. Similarly, GSGME did not affect the endoplasmic reticulum in the liver and the systemic anti-oxidative system of dairy cattle in early lactation (Gessner *et al.,* 2015). The MDA content of G20 significantly increased at 14d in comparison with CON and tended to be increased at 28d, in addition, the high-dose administration of GSGME also cannot reduce the MDA content, indicating that those dairy cattle maybe suffered from some degree of oxidative stress (Armstrong and Browne 1994).

CONCLUSIONS

The supplementation of 20mg GSPE/kg BW/d had significant effects on MY, MP and SNF, which significantly increased MY in long term and increased MP and SNF at 7d, without significant negative effects for the liver or kidney function of early lactation dairy cattle. In this study, the mean weight of cattle was about 570kg, the GSPE is about $ 0.00002464 per mg, if supply 20mg GSPE/kg BW/day, it will cost about $ 0.2809 per day per cow. During experiment period 42 days, MY of G20 was 3.34kg/d higher than CON, and G20 was 6.36kg/d higher than CON at day 40th, and the price of milk was $0.7700 per kg, which means that G20 could earned about $2.5718 per day per cow more than CON, and it was about $4.8972 per cow at day 40th in this study. Above all, this study indicated that using GSPE supplement was financially beneficiary.

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