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Research Article

Evaluation of Malondialdehyde Concentrations and Antioxidant Status in Dairy Cows Fed Close-Up Energy Density and Rumen-Protected Lysine during the Transition Period

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ABSTRACT

Overproduction of free radicals during oxidation of free fatty acids and deficiency of antioxidants caused oxidative stress and metabolic disorders. Reactive oxygen species can initiate lipid peroxidation because lipids are prone to oxidation. Carnitine is a methylated form of lysine that acts as a potent antioxidant that counteracts the occurrence of oxidative stress. However, the interaction of high energy diet and rumen protected lysine in dairy cows has not been explored well. The objective of the current study was to investigate the effect of pre-calving dietary energy levels with or without bypass lysine on malondialdehyde concentrations and antioxidant status in dairy cows during the transition period. Forty 3rd lactation Holstein cows were randomly allocated to one of the four dietary treatments (HEByls=1.53 NEL plus 40 g bypass Lysine, HECK=1.53 NEL without bypass Lys, LEbyls=1.37 NEL plus 40 g bypass Lysine and LECK=1.37 NEL without bypass Lysine) arranged in a 2 × 2 factorial design. Blood samples were collected at day -21, -14, -7, 7, 14, 21 relative to calving and malondialdehyde concentrations, glutathione peroxidase activity, superoxide dismutase activity and total antioxidant capacity were analyzed using assay kits. The results indicated that a pre-calving high-energy diet significantly increased glutathione peroxidase activity and reduced malondialdehyde concentrations in prepartum cows. Rumen protected lysine significantly decreased malondialdehyde concentrations and increased total antioxidant capacity in postpartum cows. It was concluded that a high-energy diet and rumen protected lysine substantially improved antioxidant status and reduced malondialdehyde concentrations in dairy cows during the transition period.

Keywords: Malondialdehyde; Total antioxidant capacity; Rumen protected lysine; Transition cow

INTRODUCTION

During the transition period dramatic physiological, metabolic and hormonal changes occur in dairy cows which lead to oxidative stress. The transition from late pregnancy to early lactation is associated with lipid and protein metabolic changes. The dry matter intake of the cow is significantly reduced even though the energy requirement of the cow is increased three times to meet the high energy demand for milk synthesis and secretion in early lactation. At this time the cows make many metabolic and physiological adjustments to support transition from pregnancy to lactation. For instance hepatic uptake of oxygen significantly increased for production of energy around calving to meet high energy demand during transition period. Dairy cows start to mobilize body reserve fatty to adapt and to support the increased energy demands for udder development and milk production. However, unable to adapt to high energy demand for fetal growth, calving and to the onset of lactation caused generation of lipid peroxidation which leads to metabolic stress. Mobilization and oxidation of NEFA in the liver as a result of high energy demands leads an excess production of reactive oxygen species. Excess production of reactive oxygen species is recognized as risk factors for diseases during the transition period [1].

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Under the normal physiological condition, the body usually has sufficient antioxidant reserves to neutralize overproduction of reactive oxygen species (free radicals). However, an excess production of free radicals or deficiencies of antioxidants put dairy cow under oxidative stress and increased the susceptibility of the cow to production diseases in early lactation. This augmented metabolic stress and negative energy balance caused overproduction of reactive oxygen species beyond the neutralizing capacity of antioxidant resulted in oxidative stress. Oxidative stress can damage tissue as a result of oxidation of DNA, cellular proteins, and lipids. The oxidative destruction of lipid (lipid peroxidation) is a destructive and releasing Malondialdehyde (MDA) as the end product. Lipid peroxidation has occurred as the consequences of oxidative stress and it can be used to evaluate the severity of oxidative stress [2].

The previous study showed that prepartum energy intake affected the postpartum lipid metabolism in dairy cows. Carnitine which is synthesized in the liver and kidneys through methylation of lysine have important functions in maintain cellular energy, reduce oxidative stress acts as a potent antioxidant to enhance antioxidant system and prevents DNA damage induced by the actions of free radicals and minimize cell death by apoptosis. Moreover, to alleviate the risk of metabolic disorder carnitine play an important role in β-oxidation of long fatty acid in mitochondrial to produce energy in the cell [3].

Therefore, manipulating pre-calving dietary energy density and addition of rumen protected lysine in the transition cow diet during the transition period is very vital to control negative energy balance consequently to reduce the degree of lipid peroxidation, and to enhance the antioxidant status in dairy cows. Hence, in the present study we hypothesized that closeup dietary energy density and rumen protected lysine supplementation would lower the concentration of Malondialdehyde (MDA) through impacting the degree of lipid peroxidation and improve antioxidant status in the dairy cow during the transition period. The objective of the current study was to investigate the effect of closeup dietary energy density (1.53 or 1.37 NE_L Mcal/kg DM) and supplementation of rumen protected lysine (0 or 40 g/hd/d; AscorChimiciSrl, Beijing, China) on malondialdehyde concentrations and antioxidants status in dairy cows during the transition period [4].

MATERIALS AND METHODS

Experimental design

All the procedures were approved by the animal care and use committee of the institute of animal science, Chinese Academy of Agricultural Sciences, Beijing. In brief this experiment was conducted in a 2 × 2 factorial design with two level of energy (1.37 Mcal/kg DM and 1.53 Mcal/kg DM with or without bypass lysine during transition period. The dietary treatment used were high energy with bypass lysine (HEbyls)=1.53 Mcal/kg DM plus 40 g bypass lysine/day per cows, high energy without bypass lysine (HECK)=1.53 Mcal/kg/DM, low energy with bypass lysine (LEbyls)=1.37 Mcal/kg/DM plus 40 g bypass lysine/d per cow and low energy without bypass lysine (LECK)=1.37 Mcal/kg DM [5].

Experimental cows

Cows in the same parity (3rd lactation) entering their 4th lactation were chosen based on body condition scores (BCS) ≥ 3.25 and ≤ 3.5 (1 to 5 scale) from a large herd. During cow selection the expected calving date of each cow was considered to select all cows with similar expected calving dates and previous milk yield. All selected cows were balanced for their BCS, expected calving date, and previous milk yield (13679.65 ± 2370.4 kg 305 d milk yield, P=0.80) before being randomly assigned to dietary treatment groups. Sixty-cows were randomly allocated to each of the four dietary treatment groups (n=17 each) [6].

Dietary treatment ration and feeding of cows

Prior to feeding the closeup HE or LE rations, all cows were fed the same diet (NE_L=1.34 Mcal/kg DM) during the dry period until d -21 when cows were switched to either HE or LE rations until d 0 (calving date). After calving up to d 21 all cows received the same lactation ration (NE_L=1.69 Mcal/kg DM). Lysine was top dressed on the TMR once per day at a rate of 40 g Lysine/cow per day by using 50 g of ground corn as a carrier. The same amount 50 g ground corn was also top dressed to control diets. The rumen protected lysine (Ascor Chimici Srl, Beijing) supplement contained 55% lysine with 44% bioavailability therefore per 40 g of RPL product the pre and post-partum Lysine-fed cows received 9.68 g of metabolizable lysine. The values for RUP, RDP, Lys and Met were predicted using the model and are presented in Table 2.

All experimental cows were housed in a ventilated enclosed barn during the experimental period and were individually fed their respective diet. Cows had access to stand and bedding areas until 3 d before expected parturition, when they were moved to individual maternity pens bedded with straw until parturition. After parturition, cows were individually fed a common lactation diet, with or without lysine supplementation. Total mixed rations were mixed daily and provided twice per day at 0600 and 1400 h (Tables 1 and 2). All cows were fed ad libitum with their respective dietary rations [7].

Table 1: Ingredient composition of diets fed during close-up cows (-21 d to calving) and early lactation (calving to 21 d) periods.

Ingredients (%)	Close-up cow diet[1]				Fresh cow diet			
	LECk	LEByls	HECK	HEByls	LECK	LEByls	HECK	HEByls

Corn silage processed[2]	22.6	22.6	-	-	-	-	-	-
Grass hay	24.3	24.3	14.7	14.7	-	-	-	-
Oats hay	22.1	22.1	21.7	21.7	4.9	4.9	4.9	4.9
Alfalfa[3]	-	-	-	-	20.0	20.0	20.0	20.0
Corn silage	-	-	22.1	22.1	22.8	22.8	22.8	22.8
Steam flaked corn	-	-	-	-	8.9	8.9	8.9	8.9
Cornmeal	-	-	9.9	9.9	9.4	9.4	9.4	9.4
Soybean meal[4]	3.7	3.7	3.6	3.6	12.4	12.4	12.4	12.4
Cottonseed meal	3.8	3.8	2.2	2.2	-	-	-	-
DDGS[5]	1.5	1.5	3.6	3.6	-	-	-	-
Extracted soy bean[6]	-	-	-	-	2.5	2.5	2.5	2.5
Canola meal solvent	4.3	4.3	5.0	5.0	2.5	2.5	2.5	2.5
Molasses cane	-	-	-	-	1.8	1.8	1.8	1.8
Corn gluten meal	1.5	1.5	1.1	1.1	1.5	1.5	1.5	1.5
Brewers grains	9.6	9.6	10.6	10.6	6.1	6.1	6.1	6.1
Fresh cow mineral premix[7]	-	-	-	-	3.7	3.7	3.7	3.7
Close up mineral premix	5.3	5.3	4.3	4.3	-	-	-	-
Sta-chol (choline)	0.2	0.2	0.5	0.5	0.1	0.1	0.1	0.1
Bergafat100[8]	-	-	-	-	0.8	0.8	0.8	0.8
DCAD Premix DCAD[9]	0.8	0.8	0.4	0.4	-	-	-	-
$KHCO_3$	-	-	-	-	0.3	0.3	0.3	0.3
MT-BOND	-	-	-	-	0.1	0.1	0.1	0.1
Optigen-slow[10]	-	-	-	-	0.2	0.2	0.2	0.2
Dimond V-XPC Yeast product[11]	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1

Glycoline	-	-	-	-	1.6	1.6	1.6	1.6
Lactation B vitamin	-	-	-	-	0.2	0.2	0.2	0.2
Transition B vitamin	0.4	0.4	0.4	0.4	-	-	-	-

Note: [1]Close-up cow diet: HEByls=high energy (1.53 NE_L Mcal/Kg DM) with 40 g/cow per day rumen-protected lysine (Ascor Chimici Srl, Beijing), HE=High Energy (1.53 NE_L Mcal/kg DM) without rumen protected lysine, LEByls=low energy (1.37 NE_L Mcal/kg DM) with 40 g/cow per day rumen protected lysine, LE=Low Energy (1.37 NE_L Mcal/kg DM) without rumen protected lysine, lactation diet=1.69 NE_L Mcal/kg DM. After parturition, the same 40 g/cow per day rumen protected lysine has supplemented for those cows that had been fed on rumen protected lysine before calving;

[2]Corn silage processed: Sieve used 4.75 mm, and kernel processing score=50-70%;
[3]Extracted soy bean: 92.6% DM and 36.4% CP;
[4]Soybean meal: 89.1% DM and 42.6% CP;
[5]DDGS=Distiller's Dried Grains with Soluble are the nutrient rich co-product of dry-milled ethanol production;
[6]Alflafa: Alfalfa hay was used to formulate total mixed ration;
[7]Close-up and fresh cow mineral premix: Ca, P, Mg, K, Na, Cl and S;
[8]Berga Fat100: By-pass fats for ruminants: extra energy without a carrier;
[9]DCAD=Dietary Cation-Anion Difference used to prevent to hypocalcemia in close-up cow;
[10]Optigen is Alltech's Non-Protein Nitrogen (NPN) source for ruminants;
[11]Diamond VXP yeast culture supplement (FD00365CHN-XP, Diamond V, Cedar Rapids, lowa, USA)

Table 2: Chemical composition of total mixed ration fed in the experiment.

Chemical components	Close-up cow diet[1]				Fresh cow diet			
	LECK	LEByls	HECK	HEByls	LECK	LEByls	HECK	HEByls
DM%	54.07	54.07	53.502	53.502	55.6	55.6	55.6	55.6
NE_L Mcal/kg DM	1.37	1.37	1.53	1.53	1.69	1.69	1.69	1.69
CP, % of DM	15.1	15.1	15.1	15.1	18.0	18.0	18.0	18.0
ADF, %	26.1	26.1	23.265	23.262	17.2	17.2	17.2	17.2
NDF, %	44.7	44.7	40.755	40.755	27.7	27.7	27.7	27.7
Ash, %	5.6	5.6	5.6	5.6	5.5	5.5	5.5	5.5
NFC % DM	25.48	25.48	31.85	31.85	41.4	41.4	41.4	41.4
Starch, %	8.79	8.79	15.46	15.46	23.3	23.3	23.3	23.3
Sugar, %	4.99	4.99	4.96	4.96	5.91	5.91	5.91	5.91
RDP % of DM	9.92	9.92	9.91	9.92	11.35	11.34	11.35	11.34
RUP % of DM	5.17	5.21	5.17	5.21	6.71	6.74	6.71	6.74
RDP supplied (g/d)	1194	1198	1191	1192	1928	1931	1928	1931

RUP supplied (g/d)	623	629	621	626	1157	1148	1157	1148
MP supplied (g/d)	1018.47	1024.19	1108.26	1110.32	2126.10	2133.16	2126.10	2133.16
Lys:Met	2.85:1	3.1:1	2.85:1	3.07:1	2.96:1	3.12:1	2.96:1	3.12:1
Lys (% of MP)	6.52	7.06	6.62	7.11	6.5	6.84	6.5	6.84
MP-Lys (g)	66.44	72.29	73.34	78.95	138.27	145.88	138.27	145.88
Met (% of MP)	2.29	2.28	2.32	2.31	2.2	2.19	2.2	2.19
MP-Met (g)	23.33	23.32	25.77	25.68	46.77	46.72	46.77	46.72
EE (% of DM	3.23	3.40	3.4	3.57	4.56	4.67	4.56	4.67
Ca (% of DM)	1.61	1.61	1.56	1.57	0.91	0.92	0.91	0.92
P (% of DM)	0.45	0.45	0.46	0.46	0.40	0.40	0.40	0.40
Mg (% of DM)	0.55	0.55	0.54	0.54	0.43	0.43	0.43	0.43
Cl (% of DM)	0.91	0.93	0.86	0.88	0.57	0.59	0.57	0.59
K (% of DM)	1.04	1.04	0.98	0.97	1.32	1.32	1.32	1.32
Na (% of DM)	0.3	0.3	0.28	0.28	0.55	0.55	0.55	0.55
S (% of DM)	0.42	0.42	0.41	0.20	0.20	0.20	0.20	0.20

Note: [1]Close-up cow diet: HEByls=high energy (1.53 NE_L Mcal/Kg DM) with 40 g/cow per day rumen protected lysine (Ascor Chimici Srl, Beijing), HE=High Energy 1.53 NE_L Mcal/kg DM) without rumen protected lysine, LEByls=low energy (1.37 NE_L Mcal/kg DM) with 40 g/cow per day rumen protected lysine, LE=Low Energy (1.37 NE_L Mcal/kg DM) without rumen protected lysine, lactation diet=1.69 NE_L Mcal/kg DM; [2]RPL=Rumen protected lysine (Ascor Chimici Srl, Beijing)

Feed and blood samples collections and analysis

Samples of feed offered were collected at 0700 h 4 times per week. Samples were frozen at -20°C until further analysis. Samples of TMR from each treatment were analyzed for DM content by oven drying at 60°C to a constant weight. The dried samples were ground through a 1 mm screen using a Cyclotec 1093 Mill (Tecator 1093, Tecator AB, Hoganas, Sweden) before analysis. Samples were further dried at 105°C for 2 h to determine the absolute DM and chemical analyses were expressed on the basis of the final absolute DM. The CP (N × 6.25) content of feed samples was determined using the macro-Kjeldahl nitrogen test (AOAC, 2000; method 976.05) with a Kjeltec digester 20 and a Kjeltec System 1026 distilling unit (Tecator AB). The contents of NDF and ADF were determined using the Van Soest procedure using heat stable amylase (type XI-A of Bacillus subtilis; Sigma-Aldrich Corporation, St. Louis, MO). The ash content was determined by incineration at 550°C overnight and the OM content calculated (AOAC, 2000; method 942.05). The ether extract content was determined using a Soxtec system HT6 apparatus (Tecator AB) according to AOAC (2000; method 920.39) [8].

Approximately 15 mL of duplicate blood samples from individual cows were collected at 0700 h daily on d -21, -14, -7, 0, 7, 14, and 21 relative to calving *via* a coccygeal vein.

Approximately 15 mL of duplicate blood samples from individual cows were collected at 0700 h daily on d -21, -14, -7, 0, 7, 14 and 21 relatives to calving *via* a coccygeal vein. One aliquot of blood sample was collected in serum separator tubes (Serum Clot Activator, Greiner Bio-one GmbH, A 4550 Kremsmunster, Austria) and the samples allowed to clot for a minimum of 25 min at 20°C and stored in the refrigerator overnight. The samples were then centrifuged at 3,000 × g for 15 min at 4°C prior to separation of the serum. The separated blood serum samples were kept under -20°C until further analysis [9].

Analysis of antioxidant status and MDA concentrations in blood serum

Total Antioxidant Capacity (T-AOC), Superoxide Dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity and Malondialdehyde (MDA) concentrations were analyzed using the commercial the assay kits of according to the manufacturer's instructions [10].

Superoxide dismutase activity in the blood serum was determined spectrophotometrically at 450 nm using the assay kit (96T). Superoxide Dismutase (SOD) enzyme involved in oxidation-antioxidant balance by removing of superoxide anion radicals (O^{2-}) to protect cells from damage, its activity is expressed in unit/ml. SOD is the first intracellular defense against reactive oxygen species and its activity is expressed in unit/ml. One unit of SOD is defined as the amount of enzyme exhibit 50% of nitro blue tetrazolium at 37°C in 20 min and 20 µL serums.

Glutathione Peroxidase (GSH-Px) activity was analyzed by colorimetric method using the assay kit. GSH-Px catalyzes peroxide (H_2O_2) and reduced Glutathione (GSH) to produce H_2O and oxidized glutathione (GSSG). GSH-Px activity is represented by its enzymatic reaction rate. The enzyme activity was calculated by measuring GSH consumption in this enzymatic reaction. In this experiment the GSH-Px activity represented by catalyzed GSH reaction rate. Two substrates undertake redox reaction without enzyme (non-enzymatic reaction, hence GSH consumption caused by the non-enzymatic reaction was deducted to calculate enzyme reaction. GSH reacts with dithio-dinitro benzoic acid to produce 5thio-dinitro benzoic acid anions with relatively stable yellow color. The GSH content was measured by spectrophotometer at 412 nm absorbance. One unit of GSH-PX is defined as the amount of enzyme depleting 1 µmol of GSH per 5 minutes at 37°C in 0.1 mL of serum.

Total Antioxidant Capacity (T-AOC) in the blood serum was measured using a commercial colorimetric assay kit according to the manufacturer's instructions. The value of optical density all test tubes were measured at 520 nm by using the spectrophotometer. One unit of total antioxidant capacity is defined as the increment in the absorbance by 0.01 due to the reduction of Fe^{3+} at 37°C per minute in 0.1 mL of serum.

Malondialdehyde (MDA) concentrations were analyzed with 2-thiobarbituric acid, monitoring the change of absorbance at 532 nm with the spectrophotometer flowed manufacturer's instructions. The intra and inter assay coefficient of variation were 3.1 and 4.3%, 1.7 and 3.5%, 3.2 and 6.8%, 3.5 and 4.1% for GSH-Px, SOD, T-AOC and MDA respectively [11].

Statistical analysis

The data for Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px), Total Antioxidant Capacity (T-AOC) and Malondialdehyde (MDA) were analyzed as a completely randomized design with repeated measures using PROC MIXED of SAS (version 9.2, SAS Institute Inc., Cary, NC). The MIXED statistical model used for analysis was as follow:

$y_{ijkl}=\mu+L_i+E_j+LE_{ij}+A_{ijk}+T_l+TL_{jl}+TE_{kl}+TLE_{jkl}+\varepsilon_{ijkl}$, where y_{ijkl} was the dependent, continuous variable, µ was the overall mean; L_i was the fixed effect of lysine (I=with or without supplementary lysine); E_j was the fixed effect of energy (j=1.37 Mcal/kg DM or 1.53 Mcal/kg DM); A_{ijk} was the random effect of the k^{th} cow in the ij^{th} combination of lysine and energy; T_l was the fixed effect of time (day) of the experiment; the two and three-way interactions of the time, lysine and energy, all considered fixed and ε_{ijkl} was the residual error [12].

Serum SOD, GSH-Px, T-AOC and MDA were analyzed at the various time points that were not equally spaced, hence the covariance structure for the repeated measurements was modeled using the spatial power option. The Kenward-Roger option was used for the computing the denominator degrees of freedom for testing hypotheses. Least squares means were compared using LSD and statistical differences were declared significant at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$.

RESULTS

Main effects of rumen protected lysine and energy are illustrated in Figures 1-4 and Table 4, The Interaction effects of pre-partum energy density and rumen protected lysine on antioxidant status and malondialdehyde concentrations are shown in Table 3 [13].

Table 3: Interaction effects of pre-partum energy density and rumen protected lysine on antioxidant status during the transition period.

Variables[1]	Energy × RPL				SEM[6]	P-values		
	LECK[2]	LERPL[3]	HECK[4]	HERPL[5]		Energy × RPL[7]	Time[8]	Time × Energy × RPL[9]
Prepartum								
SOD, U/mL	10.1	8.7	9.18[b]	10.8[a]	0.34	0.01	0.01	0.01
GSH-Px, U/mL	151	163.2	157.1	185.5	7.26	0.28	0.01	0.08
T-AOC, U/mL	2.87	3.8	3.37	4.24	0.45	0.95	0.17	0.8

MDA, nmol/ml	2.9	2.1	2.3	1.98	0.16	0.11	0.01	0.25
Postpartum								
SOD, U/mL	10.96	10.64	10.27	11	0.67	0.44	0.01	0.27
GSH-Px, U/mL	177.8	173.76	160.01	173.9	4.9	0.07	0.01	0.78
T-AOC, U/mL	2.76	3.56	2.8	3.98	0.31	0.54	0.01	0.31
MDA, nmol/ml	2.95	2.67	3.32	2.48	0.16	0.09	0.02	0.66

Note: [a,b]Means from interactions in the same row with different superscripts are significantly different (P<0.05) using the least significant difference method; [1]Variables: SOD=Superoxide Dismutase, GSH-Px=Glutathione Peroxidase, T-AOC=Total Antioxidant Capacity and MDA=Malondialdehyde;

[2]LECK: Pre-calving low energy diet without rumen protected lysine (LE=1.37 NEL Mcal/kg DM) without RPL; [3]LERPL: Pre-calving low energy diet with rumen protected lysine (1.37 NEL Mcal/kg DM) with 40 g RPL/day per cows); [4]HECK: Pre-calving high energy diet without rumen protected lysine (1.53 NEL Mcal/kg DM) without RPL); [5]HERPL: Pre-calving high energy diet with rumen protected lysine (1.53 NEL Mcal/kg DM) with 40 g RPL/day per cows); [6]SEM: Standard Error of Mean; [7]Energy × RPL: Energy interaction with rumen-protected lysine; [8]Time: d -21, -14, -7, 0, 7, 14 and 21 relative to calving; [9]Time × Energy × RPL: Interaction of time, energy and rumen protected lysine

Table 4: Effect of close-up dietary energy levels and rumen-protected lysine on antioxidant status and MDA levels during the transition period.

Variables[1]	RPL[2]		Energy[3]		SEM[5]	P-value[4]				
	0	40	1.37	1.53		RPL	Energy	Time[6]	Time × RPL[7]	Time × Energy[8]
Prepartum										
SOD, U/mL	9.6	10	9.4	10	0.24	0.73	0.09	0.01	0.33	0.58
GSH-Px, U/mL	154.07[b]	174.4[a]	157.2	171.3	5.13	0.008	0.06	0.01	0.3	0.02
T-AOC, U/mL	3.12	4.02	3.34	3.81	0.32	0.06	0.31	0.17	0.81	0.81
MDA, nmol/ml	2.60[a]	2.03[b]	2.49[a]	1.13[b]	0.12	0.03	0.01	0.01	0.66	0.53
Postpartum										
SOD, U/mL	10.6	10.8	10.8	10.6	0.47	0.77	0.78	0.01	0.93	0.63
GSH-PX, U/mL	168.91	173.8	175.7	166.9	3.47	0.32	0.08	0.01	0.02	0.95
T-AOC, U/mL	2.78[b]	3.77[a]	3.16	3.39	0.22	0.003	0.46	0.01	0.59	0.8

MDA, nmol/ml	3.12a	2.58[b]	2.81	2.9	0.11	0.001	0.58	0.02	0.59	0.8

Note: [a,b]Means from main effect or interactions in the same row with different superscripts are significantly different, P<0.05, using the least significant difference method; [1]Variables: SOD=Superoxide Dismutase, GSH-Px=Glutathione peroxidase, T-AOC=Total Antioxidant Capacity and MDA=Malondialdehyde; [2]RPL effect=Rumen-Protected Lysine (Ascor Chimici Srl, Beijing, China) was top dressed into total mixed rations at a rate of 40 g/cows per day from d -21 to d 21 after calving; [3]Energy effect: Low Energy (LE)=1.37 NEL Mcal/kg DM); High Energy, (HE=1.53 NEL Mcal/kg DM) fed from d -21 to d 0 (calving date); [4]P-value: Statistical differences were declared significant at P ≤ 0.05 and tendencies were determined at P ≤ 0.10. P-Values are from a repeated measures analysis of variance; [5]SEM: Standard Error of Mean; [6]Time: d -21, -14, -7, 0, 7, 14 and 21 relative to calving; [7]RPL × Time: Interaction Rumen Rotected Lysine and Time; [8]Energy × Time: Interaction of Energy and Time

Effect on blood serum Superoxide Dismutase (SOD) activity

Neither pre-calving energy density nor rumen protected lysine or their interaction affected the serum SOD activity in postpartum cow (Table 4). However, cows fed a close-up high energy diet and supplemented with RPL had numerically higher serum SOD activity. Pre-calving high energy diet tended to increase the serum SOD activity in prepartum cow (P=0.09). Supplementation of RPL did not affect SOD activity in prepartum cows. However the interaction of high energy diet and rumen protected lysine increased SOD activity by 17.6% in prepartum cows (P=0.001) compared to control group (Table 3). No significant effect of pre-calving energy diet and RPL supplementation in SOD activity was observed in postpartum cows (Figure 1) [14].

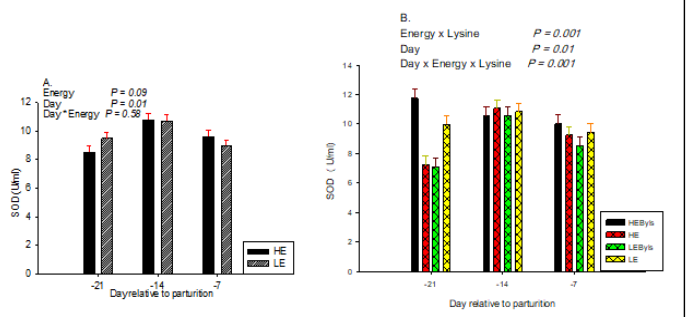

Figure 1: Effect of pre-calving energy density and rumen protected lysine on Superoxide Dismutase (SOD) activity in dairy cows during the transition period. HE: High Energy; LE: Low Energy; HEByls: High Energy with Bypass lysine; LEByls: Low Energy with Bypass lysine.

Effect on blood serum Glutathione Peroxidase (GSH-Px) activity

Supplementation of rumen protected lysine impacted serum GSH-Px activity in prepartum cows (P=0.008) but not in postpartum cows.

Cows supplemented with RPL increased mean serum GSH-Px activity by 13.2% (P<0.01) more than non-lysine supplemented group in prepartum cows. Pre-calving dietary energy density tended to increase serum GSH-Px activity in prepartum (P=0.06) and postpartum cows (P=0.08). Prepartum cows fed a pre-calving high energy diet tended to have a higher serum GSH-Px activity compared with cows fed a low energy diet during the close-up period (Figure 2) [15].

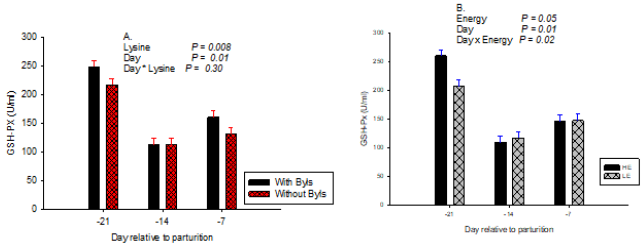

Figure 2: Effect of pre-calving energy density and rumen-protected lysine on glutathione peroxidase (GSH-Px) activity in dairy cows during the transition period. HE: High Energy; LE: Low Energy; Byls: Bypass lysine.

However, after calving, cows fed the pre-calving high energy diet tended to have lower serum GSH-Px activity than cows fed the low energy diet. Time and pre-calving energy interactions significantly increased serum GSH-PX activity in prepartum cows (P=0.02). However, the effect of pre-calving high energy diet on serum GSH-PX activity was more pronounced in the first 3 weeks before calving. Cows supplemented with RPL had higher serum GSH-Px activity (213.4 U/ml *vs.* 146.8 I U/ml) at the first 3 weeks after calving. No interaction between pre-calving energy diet and RPL supplementation was observed in GSH-PX activity in pre and postpartum cows. However, the interaction of pre-calving low energy diet and RPL supplementation tended to increase serum GSH-Px activity in postpartum cows (P=0.07) [16].

Effect on serum Total Antioxidant Capacity (T-AOC) concentrations

Neither energy nor time, nor any interaction affected serum Total Antioxidant Capacity (T-AOC) concentrations both in pre and postpartum cows (Table 4). However, rumen protected lysine treatment increased serum T-AOC concentrations by 36.7% (P=0.003) in postpartum cows and tended to improve T-AOC concentrations in prepartum cows as compared to non-lysine supplemented group (P=0.06). The pre-calving energy × RPL interaction significantly increased Total Antioxidant Capacity (T-AOC) in pre and postpartum cows (Figure 3) [17].

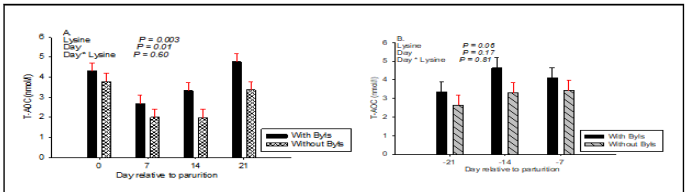

Figure 3: Effect of pre-calving energy density and rumen protected lysine on Total Antioxidant Capacity (T-AOC) in dairy cows during the transition period. HE: High Energy; LE: Low Energy; Byls: Bypass lysine.

Effect on serum Malondialdehyde (MDA) concentrations

Pre-calving energy diet, time and their interaction had a significant effect on MDA concentrations in prepartum cows (Tables 3 and 4). Cows fed a low energy diet in the close-up to calving period had the highest MDA levels (2.86 U/ml) at week 2 before calving and the high energy diet reduced the concentration of MDA by 54.6% (P=0.03) in prepartum but not in postpartum cows. Feeding a pre-calving HE diet did not lower the concentration of MDA in postpartum cows and supplementation of rumen protected lysine decreased MDA concentrations by 17.3% in prepartum cows (P<0.001). The interaction of close-up energy diet and rumen-protected lysine did not impact MDA levels both in pre and postpartum cows. Mean MDA concentrations tended to be lowest in cows fed HE and BLys than LE no Lys cows during the postpartum period (Figure 4) [18].

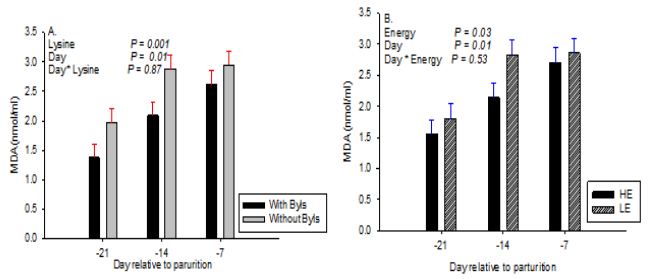

Figure 4: Effect of pre-calving energy density and rumen protected lysine on Malondialdehyde (MDA) levels in dairy cows during the transition period. HE: High Energy; LE: Low Energy; Byls: Bypass lysine.

DISCUSSION

Reactive oxygen species and antioxidant status

During the transition period, the dietary energy required to meet physiological and metabolic demands is significantly increased. At the same time, the cell requires more oxygen for cellular oxidation in order to generate the energy required for milk synthesis and secretion. Through normal cellular respiration, tissues consume more oxygen to meet the dramatically increased energy demand at the onset of lactation. These increased metabolic activities lead to an increased accumulation of reactive oxygen species and a consequent depletion of antioxidant defenses around calving. In the present study, we found that feeding a pre-calving high energy diet alone resulted in lowering the serum SOD activity in postpartum cows compared with cows fed a low energy diet. This result may be because cows fed a high energy diet were exposed to a greater negative energy balance and had increased production of reactive oxygen species which reduced the serum SOD activity. However, supplementation of RPL to a pre-calving high energy diet increased serum SOD activity during early lactation. The increased serum SOD activity during this time is probably due to the increased synthesis of reactive oxygen species. Superoxide Dismutase (SOD) is the major antioxidant defense in protecting cell damage from excess production and accumulation of reactive oxygen species around calving by converting superoxide radicals to a less toxic hydrogen peroxide which is subsequently degraded by catalase to $2H_2O$ and O_2. In this trial, feeding a pre-calving HE diet together with RPL ameliorated the deleterious effect of negative energy balance by reducing the production of free radicals and increased serum SOD activity. Rumen protected lysine is a precursor for carnitine which enhances the export of long fatty acid from the cytosol (cytoplasmic matrix) to the mitochondria for β-oxidation. Carnitine also acts as a potent antioxidant in the cell. An alternative hypothesis is potentially caused by an up regulation of SOD to neutralize superoxide radicals and enhance the adaption of animals to oxidative stress. In the present study, we found higher SOD activity in the first week after calving; conversely, Sharma, et al. found low SOD activity in early lactation cows compared to the SOD activity in late gestation [19].

Cows supplemented with RPL had a higher serum GSH-Px activity (213.4 U/ml *vs.* 199.3 U/ml) in the first 3 weeks following calving. Pintea, et al. reported decreased GSH-Px activity one week after parturition followed by increased GSH-Px activity in the second week with activity normalizing after 6 weeks. Similarly, Bühler, et al. Reported that the GSH-Px and SOD activities reached a peak around calving and the present serum GSH-Px activity is comparable with these findings with a higher GSH-Px activity (212.86 U/ml) during early lactation. Feeding a pre-calving high energy diet tended to increase serum GSH-Px activity as compared to cows fed the low energy diet. Similarly, Buhler, et al. reported that supplementation of higher dietary energy level induced higher Glutathione peroxidase activity and higher expression levels for some genes and anti-oxidative parameters that correlated with energy and metabolic parameters. Similarly, Pilarczyk, et al. Reported higher GSH-Px activity and MDA levels in early lactation compared to dry period and peak lactation. Gong and Xiao, reported similar GSH-Px activity levels (229.56 U/ml) in early lactation. The increased in serum GSH-PX activity in early lactation in the present study indicated a protective response to increased oxidative stress during early lactation. Colakoglu, et al. reported that GSH-Px activity is decreased from d -21 to d 0 and then gradually increased until 21 days after calving. In a similar fashion, serum GSH-Px activity decreased from d -21 to d 0 (calving) in the present study, although it did not decrease uniformly among all dietary treatments. We found the highest serum GSH-Px activity at d -21 before calving in cows fed a high energy diet with RPL as did Colakoglu, et al., who reported higher GSH-Px activity before calving. Serum antioxidant concentrations are not only dependent upon dietary feed intake but also upon the systematic capacity of the liver. Antioxidants are used both for neutralizing ROS and transferred to colostrums which may reduce the serum concentrations of some antioxidants during periparturition. In general, quantification of individual antioxidants does not provide a good image of the antioxidant capacity of the animal as different antioxidants can

act simultaneously to counteract the negative impact of oxidative stress, but this also means that a deficiency in a single antioxidant does not necessarily mean the overall antioxidant activity is decreased [20].

Malondialdehyde (MDA) concentrations

Because of lipids are prone to oxidation high levels of reactive oxygen species can initiate lipid peroxidation generation. Malondialdehyde (MDA or propanedial) is the end product of lipid peroxidation and its concentration can be used as a metabolic marker of oxidative stress. In our results, we found a higher MDA concentration and a lower total antioxidant capacity during early lactation (<3 wk). Cows fed a pre-calving high energy diet without rumen protected lysine supplementation had higher MDA concentrations in postpartum cows but in cows fed HE with RPL the MDA levels were lower yet the T-AOC was higher. Overall the total antioxidant status in cows that did not receive RPL regardless of energy density of the diet was reduced in early lactation. This may be due to overproduction of reactive oxygen species in which the antioxidant defense could not overwhelm, which resulted in per-oxidation of polyunsaturated fatty acids which resulted in MDA production. Our results indicate that an accumulation of MDA in early lactation but not gestating cows indicates an imbalance between oxidants and antioxidant activity. Tissue energy and oxygen demands are higher at the onset of lactation than prepartum, which explains the higher MDA levels observed in early lactation. Our data suggests that the concentration of MDA increased steadily prior to calving and peaked in the first week after calving, and this result has been observed previously. Konvicna, et al. found higher mean serum MDA levels in the first week compared to week 3, 6 and 9 after calving. Bernabucci, et al. also reported significantly higher MDA levels in early lactation as compared to in late pregnancy cows [21].

CONCLUSION

Feeding high energy density rations to dairy cattle during close-up period significantly lowered blood serum MDA concentration, increased serum GSH-PX activity and tended to improve serum SOD activity in prepartum cows. Pre and postpartum rumen protected lysine supplementation during the transition period (3 weeks before to 3 weeks after calving) reduced the concentrations of MDA in pre and postpartum cows, increased T-AOC, and tended to increase serum GSH-PX activity in postpartum cows. Interactions between pre-calving high energy diet and rumen-protected lysine increased serum SOD activity in prepartum cows, tended to increase serum GSH-PX activity and reduced serum MDA concentrations in postpartum cows. Feeding pre-calving high energy alone during the close-up period (3weeks before calving until calving) numerically increased serum MDA levels, decreased serum SOD and GSH-PX activities in postpartum cows. Collectively, the present results demonstrated that a high energy pre-calving diet, supplementation of rumen protected lysine and their interaction substantially improve the antioxidant status, and lowered serum MDA concentrations in dairy cows during the transition period.

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AUTHOR CONTRIBUTIONS

Conceptualization, G.D.D., D.P.B. and L.M.; methodology, D.P.B., G.D.D. and L.M; software, D.P.B.; validation, D.P.B., G.D.D. and F.W.; formal analysis, G.D.D., L.M., and F.W., D.P.B., G.D.D., and L.M. resources, D.P.B. and L.M.; data curation, G.D.D. and D.P.B.; writing-original draft preparation, G.D.D.; writing review and editing, D.P.B., T.C. and L.M; visualization, G.D.D., D.P.B and L.M.; supervision, D.P.B.; project administration, D.P.B.; funding acquisition, D.P.B.

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AVAILABILITY OF DATA AND MATERIAL

The datasets generated during and/or analyzed during study are available from the corresponding author on reasonable request.

ETHICAL APPROVAL

This study was approved by the Animal Care and Use Committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (No. IAS20180115, Beijing). Use of animals in the present study was in strict accordance with the Directions for Caring for Experimental Animals from the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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