



Effect of Dietary Antioxidant and Energy Density on Performance and Anti-oxidative Status of Transition Cows

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ABSTRACT : This study was conducted to evaluate the effect of dietary antioxidant and energy density on performance and anti-oxidative status in transition cows. Forty cows were randomly allocated to 4 dietary treatments in a 2×2 factorial design. High or low energy density diets (1.43 or 1.28 Mcal NE_L/kg DM, respectively) were formulated with or without antioxidant (AOX, a dry granular blend of ethoxyquin and tertiary-butylhydroquinone; 0 or 5 g/cow per d). These diets were fed to cows for 21 days pre-partum. During the post-partum period, all cows were fed the same lactation diets, and AOX treatment followed as for the pre-partum period. Feeding a high energy diet depressed the DMI, milk yield, and 4% fat-corrected milk (FCM) of cows. However, AOX inclusion in the diet improved the milk and 4% FCM yields. There was an interaction of energy density by AOX on milk protein, milk fat and total solids contents. Feeding a high energy diet pre-partum increased plasma glucose and β-hydroxybutyrate, whereas dietary AOX decreased plasma β-hydroxybutyrate value during the transition period. There were also interactions between time and treatment for plasma glutathione peroxidase activity and malondialdehyde content during the study. Cows fed high energy diets pre-partum had higher plasma glutathione peroxidase activity 3 days prior to parturition, compared with those on low energy diets. Inclusion of AOX in diets decreased plasma glutathione peroxidase activity in cows 3 and 10 days pre-partum. Addition of AOX significantly decreased malondialdehyde values at calving. Energy density induced marginal changes in fatty acid composition in the erythrocyte membrane 3 days post-partum, while AOX only significantly increased *cis*-9, *trans*-11 conjugated linoleic acid composition. The increase in fluidity of the erythrocyte membrane was only observed in the high energy treatment. It is suggested that a diet containing high energy density pre-partum may negatively affect the anti-oxidative status, DMI and subsequent performance. Addition of AOX may improve the anti-oxidative status and reduce plasma β-hydroxybutyrate, eventually resulting in improved lactation performance; the response to AOX addition was more pronounced on the high energy diet. (**Key Words :** Antioxidant, Energy Density, Transition Cow, Blood Metabolism, Anti-oxidative Status)

INTRODUCTION

Transition is a critical period for dairy cows due to the physiological and endocrine challenges to homeostasis, which can impose significant metabolic stressors on cows (Goff and Horst, 1997). Oxidative stress is caused by an imbalance between the production of free radicals and the capacity of antioxidants to neutralize them (Sies, 1991). The detection of free radicals and anti-oxidative status in plasma has become a complementary tool in the evaluation of metabolic status (Castillo et al., 2005). Several studies supported the possibility that metabolic activity may determine oxidative status. Cows with higher triglyceride

and NEFA levels showed higher reactive oxygen metabolites and lower anti-oxidative capacity (Bernabucci et al., 2005; Castillo et al., 2005). Cows with high BCS are susceptible to oxidative stress, because the excessive ingestion of macronutrients may induce rapid lipid metabolism, and increase the secretion of pro-inflammatory cytokines by adipose tissue (Dandona et al., 2004).

Pre-partum energy intake affected the post-partum lipid metabolism and performance, but the results were controversial (Drackley, 1999; Douglas et al., 2006). High energy density for close-up cows may offset the negative energy balance induced by the pregnancy requirements. In addition, it may avoid the ill effects of rapidly changing to a high concentrate diet post-partum (NRC, 2001). However, recent work has explained different results (Agenäs et al., 2003; Douglas et al., 2006). Douglas et al. (2006) reported that diets containing moderate to high energy densities

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throughout the entire dry period could be detrimental to peri-partum health (high frequency of displaced abomasum) and post-partum performance (low DMI and milk yield). Agenäs et al. (2003) found that high energy intake during the dry period could lead to significant increases in body fat, which may depress appetite and thereby cause health disorders and poor milk production.

Lipid membranes, such as the erythrocyte membrane, are rich in polyunsaturated fatty acid (PUFA). Free radicals formed during oxidative stress lead to peroxidation of PUFA in the lipid membranes. This phenomenon can damage cells and thus may impair the production and health of the animal (Miller and Brezeinska-Slebodizinska, 1993). The lipid mobilization imposed by characteristic metabolic demands may accelerate metabolic processes and increase the production of free radicals (Castillo et al., 2005). Use of antioxidant (AOX) may be an alternative to improve lipid metabolism and oxidative status. AOX was reported to be effective for improving oxidative balance and performance in lactating cows by improving rumen metabolism (Vázquez-Añón and Jenkins, 2007) and plasma oxidative status (Vázquez-Añón et al., 2008). The finding that pre-partum intake has a major effect on periparturient lipid metabolism raised a new question: how the pre-partum intake interacts with AOX on lipid metabolism and anti-oxidative status. We hypothesized that changes of dietary energy density pre-partum can affect the energy metabolism during the transition period, thereafter influencing the response of an antioxidant to different energy density diets. However, to our knowledge, there is little information about this topic in the literature. Therefore, the objective of this study was to determine the effects of a dietary antioxidant with pre-partum diets of varying energy density on subsequent performance and anti-oxidative status in transition cows.

MATERIALS AND METHODS

Animals, diets and experimental design

Forty Holstein multiparous cows were allocated to 10 blocks of 4 cows and fed 1 of 4 diets of high and low energy density each with or without an antioxidant in a 2×2 factorial design. Cows in each block were of similar parity (2 to 4 parity), expected calving date (21±2 d), BCS (2.75 to 3.5), and previous milk yield (7,016±306 kg/head).

The antioxidant, AOX (AGRADO® Plus, a trademark of Novus International Inc., St. Charles, MO) was formulated to consist of a dry granular blend of ethoxyquin and tertiary-butylhydroquinone. The diets were first offered 21 days before anticipated calving. Due to differences between actual and estimated calving dates, the experiment actually started from 20±4 d (mean±SD) relative to calving. Pre-partum dietary treatments were high energy (1.43 Mcal

NE_L/kg DM, HE), low energy (1.28 Mcal NE_L/kg DM, LE), antioxidant (5 g/cow per d) with high energy (HEA) and antioxidant with low energy (LEA). NE_L was calculated by the CNSAPH (2000) model. During the post-partum period, all cows were fed the same lactation diets, and the AOX treatment was the same as for the pre-partum period during the first 3 weeks after calving (Table 1). Cows were housed in a tie-stall barn and were milked using pipeline milking three times daily at 06:00, 13:30, and 20:30. The cows received feed during milking. All cows had free access to water throughout the entire experiment.

Sampling, measurement and analysis

Feed was offered in an amount to ensure approximately 10% refusals. To determine DMI, diets offered and refused were weighed for 2 consecutive days weekly. The pre-partum and post-partum dietary samples were collected weekly and composited every three weeks to analyze the chemical compositions of DM, CP, ash, ether extract (method 988.05, AOAC, 1990), ADF and NDF, (Van Soest et al., 1991). Health status (including disorders such as mastitis, displaced abomasum, and retained placenta) of cows was recorded by an experienced veterinarian. Milk production was recorded on d 5, 12 and 19 after calving, using milk sampling devices (Waikato, New Zealand), and 50 ml of milk was collected weekly for analysis of fat, protein and lactose by infrared spectrophotometer (Foss-4000, Hillerød, Denmark).

Blood samples were taken from the coccygeal vein on d 17, 10 and 3 pre-partum, on d 0 at calving and on d 3, 10 and 17 post-partum. Samples were immediately transferred into heparinized tubes. Plasma was obtained by centrifuging at 3,000×g for 10 min and was frozen at -20°C for later analysis of glucose, β-hydroxybutyrate (BHBA), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and hydrogen peroxide (H₂O₂). Plasma glucose was determined by enzymatic analysis with a commercial kit (Jiancheng Inc. Nanjing, China). Plasma concentrations of BHBA were quantified by enzyme immunoassay with a commercial kit (RT110371, ADL, USA). Values of MDA and activity of GSH-Px were measured as described by Zhang et al. (2006). One unit of GSH-Px is defined as micromoles of NADPH oxidized per minute. The reaction system included H₂O₂, glutathione, NADPH and glutathione reductase. MDA was determined by the thiobarbituric acid (TBA) method; MDA values were calculated from the extinction coefficient of MDA-thiobarbituric acid complex. Concentration of H₂O₂ was measured by reacting with molybdate, and tested with a spectrophotometric method (Chai et al., 2004).

On the third day post-partum, erythrocytes were collected for extraction of the erythrocyte membranes after centrifuging and decanting the plasma. Erythrocyte

Table 1. Ingredients and composition of the experimental diets

Items	Pre-partum ¹				Post-partum ²
	HE	HEA	LE	LEA	
Ingredients (% DM basis)					
Corn silage	20	20	18.5	18.5	19.5
Grass hay	35	35	52	52	14
Ground corn grain	27	27	10	10	29
Wheat bran	4.5	4.5	10	10	7.2
Soybean meal 42.5% CP	3.2	3.2	0	0	9
Sesame meal	2.5	2.5	2	2	0
Cottonseed meal	4.5	4.5	4.2	4.2	0
Others ³	0	0	0	0	19.3
Limestone	0.7	0.7	0.7	0.7	0.5
Premix ⁴	0.7	0.7	0.7	0.7	0.5
Salt	0.45	0.45	0.45	0.45	0.25
Saleratus	0.45	0.45	0.45	0.45	0
Dicalcium phosphate	1	1	1	1	0.75
Antioxidant ⁵	0	0.05	0	0.05	0 or 0.05
Composition					
DM (%)	58.9	59.3	59.1	59.3	52.0
CP (% DM)	13.7	13.9	11.8	11.8	14.8
Ether extract (% DM)	2.51	2.54	2.42	2.54	3.0
NDF (% DM)	43.1	43.8	54.6	55.1	39.0
ADF (% DM)	26.3	26.5	33.0	33.4	21.8
Ash (% DM)	6.81	6.84	6.36	6.43	7.3
NE _L (Mcal/kg DM) ⁶	1.43	1.43	1.28	1.28	1.48

¹ Pre-partum: HE = High energy; HEA = High energy with antioxidant; LE = Low energy; LEA = Low energy with antioxidant.

² All cows fed the same diets after calving except the antioxidant was still added in HEA and LEA groups.

³ 4.6% DDGS, 11.0% alfalfa meal and 3.7% sugar beet pulp pellet.

⁴ Formulated to provide (per kg of premix) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3,000 mg of Fe, 40 mg of Co, 3,000 mg of Mn, and 3,000 mg of Cu.

⁵ Dietary antioxidant was added to HEA and LEA groups from week 3 pre-partum until week 3 post-partum in the form of AGRADO Plus dry granular.

⁶ Calculated based on individual feedstuffs in CNSAPH (2000).

membranes were prepared by osmotic lysis as described by Dodge et al. (1963). The fatty acids in the erythrocyte membranes were methylated by *in situ* transesterification with 0.5 N methanolic NaOH followed by 14% boron trifluoride in methanol. Samples were injected by autosampler into a Hewlett-Packard 6890A gas chromatograph equipped with a flame-ionization detector (Hewlett-Packard, Sunnyvale, CA). Results for each fatty acid were expressed as a percentage of the sum of all identified fatty acids. Membrane fluidity was determined using an FP-550 polarization spectrofluorimeter (Jasco, Japan) as previously described (Lindi et al., 1993).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS[®] (SAS Institute, 2000), with the cow as the error term, to test for main effects and interactions. When measurements were taken over time, repeated measurement data were analyzed using the mixed procedure of SAS (SAS

Institute, 2000) with cow within treatment as the subject and the error term to test for main effects and interaction, and the residual error was used to test for week and week by treatment interaction. Mean comparisons across treatments were evaluated when the interaction terms of the model were significant ($p \leq 0.05$) using LSMEANS and PDIFF separation of all the treatments. The statistic model was as below:

$$Y_{ijk} = \mu + \text{block} + E_i + A_j + T_k + EA_{ij} + ET_{ik} + AT_{jk} + EAT_{ijk} + C_{ijk}$$

Where, Y_{ijk} = dependent variable, μ = average, E_i = energy density effect, A_j = AOX effect, T_k = time effect, EA_{ij} = interaction of energy and AOX, ET_{ik} = interaction of energy and time, AT_{jk} = interaction of AOX and time, EAT_{ijk} = interaction of energy and AOX and time, C_{ijk} = error.

For milk fatty acid composition and fluidity of the

Table 2. Effect of dietary antioxidant and energy density pre-partum on milk yield and milk composition in dairy cows post-partum

Items	Treatment ¹				SEM	p value ²						
	HE	HEA	LE	LEA		E	AOX	Week	E×AOX	E×Week	AOX×Week	E×AOX×Week
DMI pre-partum	11.0	10.9	11.8	12.4	0.17	<0.01	0.19	0.06	0.35	0.47	0.28	0.57
DMI post-partum	14.3 ^b	15.5 ^{ab}	15.9 ^{ab}	16.2 ^a	0.61	0.04	0.08	0.02	0.03	0.24	0.35	0.48
Yield (kg/d)												
Milk	19.1	21.8	22.3	23.7	0.72	0.05	0.10	<0.01	0.62	0.83	0.12	0.59
4% fat-corrected milk	17.8	22.0	22.2	22.6	0.75	0.06	0.08	0.13	0.16	0.96	0.48	0.59
Milk protein	0.56	0.70	0.72	0.71	0.03	0.08	0.16	0.54	0.12	0.58	0.43	0.19
Milk fat	0.70	0.90	0.91	0.89	0.04	0.10	0.26	0.39	0.36	0.98	0.47	0.56
Milk composition (%)												
Protein	3.10 ^b	3.32 ^a	3.36 ^a	3.09 ^b	0.06	0.82	0.73	<0.01	<0.01	0.95	0.44	0.12
Fat	3.90	4.30	4.30	3.86	0.14	0.93	0.93	0.04	0.07	0.45	0.32	0.46
Lactose	4.70	4.61	4.65	4.65	0.05	0.96	0.61	<0.01	0.56	0.90	0.05	0.18
Total solids	12.25 ^{ab}	12.76 ^a	12.75 ^a	12.06 ^b	0.16	0.71	0.73	<0.01	0.03	0.36	0.84	0.55
Non-fat solids	8.35	8.46	8.46	8.20	0.08	0.50	0.55	<0.01	0.11	0.55	0.16	0.98

^{a,b} Means within same row with different superscripts differ ($p < 0.05$).

¹ HE = High energy; HEA = High energy with antioxidant; LE = Low energy; LEA = Low energy with antioxidant.

² E = Energy density effect, AOX = Antioxidant effect, E×AOX = Interaction of energy density and antioxidant.

erythrocyte membrane, single measurement data were analyzed using the PROC MIXED model as below:

$$Y_{ij} = \mu + \text{block} + E_i + A_j + EA_{ij} + C_{ij}$$

Where, Y_{ij} = dependent variable, μ = average, E_i = energy density effect, A_j = antioxidant effect, EA_{ij} = interaction of energy and antioxidant, C_{ij} = error.

Significant differences were declared at p-values less than 0.05 and trends at p-values less than or equal to 0.10 and higher than 0.05.

RESULTS

Feed intake and lactation performance

Compared with those fed low energy density diets pre-partum, cows fed high energy density diets consumed lower dry matter pre-partum ($p < 0.01$) and post-partum ($p < 0.05$). Addition of AOX tended to increase the DMI post-partum ($p = 0.08$) (Table 2). There was an interaction between energy density and AOX for DMI post-partum ($p < 0.05$). Cows fed high energy diets pre-partum showed lower milk yield ($p = 0.05$) and 4% FCM ($p = 0.06$) during the experimental periods. The addition of AOX tended to increase milk yield ($p = 0.10$) and 4% FCM ($p = 0.08$). No effect was detected for energy density or AOX supplementations on milk protein, fat, lactose, total solids and non-fat solids, but there was an interaction of energy density by AOX on milk protein, milk fat and total solids concentrations (Table 2). Although LEA decreased milk protein, fat and total solids concentration compared with LE, milk yield of protein, fat and total solids was not different

between the two treatments.

Plasma parameters

Some parameters related to energy metabolism and oxidative status are shown in Figure 1 and 2. Concentration of glucose peaked around calving and declined after calving. Feeding high energy density pre-partum increased plasma glucose ($p = 0.05$) and BHBA ($p = 0.06$), and AOX significantly decreased plasma BHBA ($p < 0.05$) for the entire period. For specific time points, response of feeding AOX on glucose was not observed, but AOX decreased plasma BHBA on d 10 and 17 post-partum ($p < 0.05$). Although neither energy density nor AOX treatment showed significant effects on plasma GSH-Px and MDA throughout the experiment, high energy diet increased GSH-Px activity 3 days prior to parturition ($p < 0.05$). Addition of AOX lowered the GSH-Px activity at 3 and 10 days pre-partum. Cows fed diets without AOX showed higher MDA values at calving, whereas cows supplemented with AOX showed a sharp drop in MDA values at calving ($p < 0.05$). Cows fed the high energy diet pre-partum tended to have higher H_2O_2 in plasma; AOX decreased the concentration of plasma H_2O_2 , especially when cows were fed HE diets pre-partum.

Fatty acid composition and fluidity of the erythrocyte membrane

Feeding a high energy diet pre-partum decreased the relative concentrations of $C_{12:0}$, $C_{16:0}$ and $C_{16:1}$ in the erythrocyte membrane ($p = 0.03$, 0.09 , and 0.03 , respectively), whereas it significantly increased *cis*-9, *trans*-11 conjugated linoleic acid (CLA) and *trans*-10, *cis*-12,

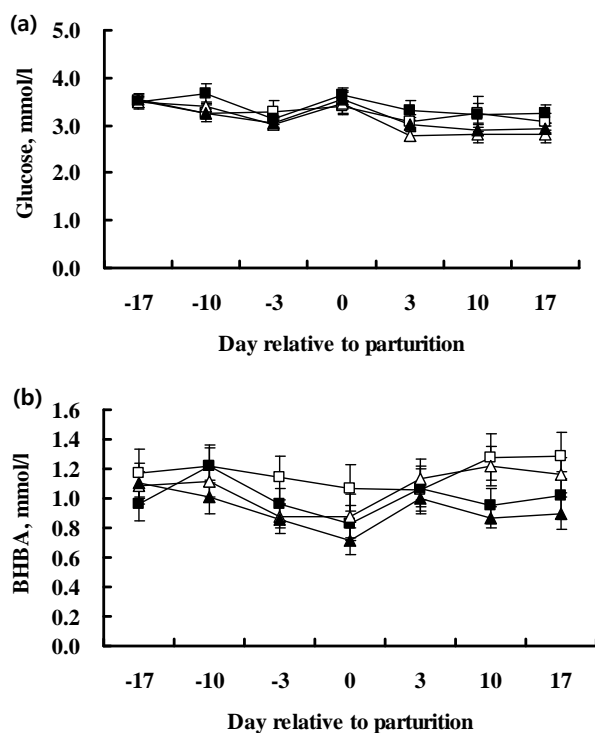


Figure 1. Glucose (a) and β -hydroxybutyrate (b) metabolites in plasma. The pooled SEM of glucose and BHB were 0.07 and 0.05 respectively. \square means HE, \blacksquare means HEA, \triangle means LE, \blacktriangle means LEA. HE = high energy, HEA = high energy with antioxidant, LE = low energy, LEA = low energy with antioxidant.

CLA concentrations in the erythrocyte membrane ($p < 0.01$). The proportion of $C_{18:0}$ tended to increase due to feeding a high energy diet pre-partum ($p = 0.09$). Addition of AOX significantly increased *cis*-9, *trans*-11 CLA and tended to increase relative concentration of $C_{18:2}$. No significant interaction of AOX by energy density was observed for any of the fatty acids. Cows fed high energy density pre-partum showed higher total fatty acid concentrations in the erythrocyte membrane regardless of AOX (Table 4). When the fatty acid profile was expressed as concentration, cows fed on both HE diets had higher values, because of the higher concentration of fatty acid in the membrane (Table 4). Feeding a high energy diet pre-partum improved the fluidity of the erythrocyte membrane (1.51 vs. 1.41, $p < 0.05$) at 3 days post-partum, but no effect of AOX was observed. Fluorescence polarization and viscosity showed the opposite result due to their negative relationship with fluidity (Table 4).

DISCUSSION

Feed intake and performance

Although the energy density was low in LE and LEA treatments, the cows in the two treatments consumed 1.2 times NE_L requirement due to higher DMI during the pre-

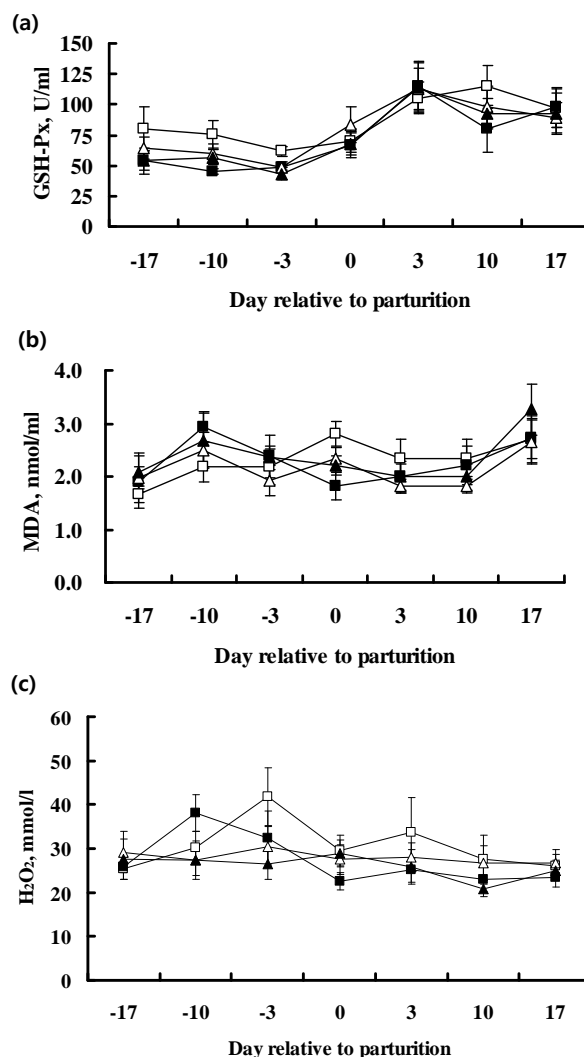


Figure 2. GSH-Px (a), MDA (b) and H_2O_2 (c) metabolism in plasma. The pooled SEM of GSH-Px, MDA and H_2O_2 were 5.08, 0.12 and 3.1 respectively. \square means HE, \blacksquare means HEA, \triangle means LE, \blacktriangle means LEA. HE = high energy, HEA = high energy with antioxidant, LE = low energy, LEA = low energy with antioxidant.

partum period. Cows fed high energy diets pre-partum had lower DMI throughout the study, and consequently decreased milk yield in the first 3 weeks after calving, compared with those on low energy diets. The high non-structural carbohydrate in HE and NDF in LE contributed to the difference in energy density. Although the non-structural carbohydrate in HE may not be enough to induce disorders in transition cows, the carry-over effect on post-partum performance cannot be neglected, since it at least showed less productivity than LE. In addition, the LE diets containing more grass hay were bulky and the cows should have higher DMI to satisfy the energy requirement. Our finding was similar to that of Tesfa et al. (1999), where cows fed high energy density diets pre-partum showed low feed intake and milk production. However, others found

Table 3. Effect of dietary antioxidant and energy density pre-partum on fatty acid composition in the erythrocyte membrane (% total fatty acids measured)

Fatty acids (%)	Treatment ¹				SEM	p value ²		
	HE	HEA	LE	LEA		E	AOX	E×AOX
C _{12:0}	0.50	0.52	0.64	0.61	0.05	0.03	0.97	0.59
C _{16:0}	16.60	15.79	17.91	16.94	0.69	0.09	0.21	0.91
C _{16:1}	2.27	2.18	2.66	2.55	0.16	0.03	0.54	0.94
C _{18:0}	10.46	10.61	9.78	9.83	0.45	0.09	0.83	0.92
9cC _{18:1}	12.84	12.92	12.35	12.38	0.56	0.37	0.92	0.96
11cC _{18:1}	2.15	2.14	1.99	2.06	0.17	0.48	0.87	0.80
C _{18:2} (n = 6)	15.08	16.32	14.84	15.66	0.59	0.47	0.10	0.73
C _{18:3}	1.37	1.36	1.39	1.37	0.14	0.91	0.93	0.98
9c,11t CLA	0.033	0.043	0.020	0.029	0.003	<0.01	<0.01	0.85
10t,12c CLA	0.036	0.029	0.013	0.021	0.004	<0.01	0.88	0.09
C _{20:4} (n = 6)	29.03	29.17	28.96	29.17	1.09	0.97	0.88	0.97
C _{20:5} (n = 3)	1.10	1.09	0.99	1.03	0.07	0.22	0.86	0.77
C _{22:4} (n = 6)	0.38	0.42	0.39	0.37	0.04	0.60	0.79	0.54
C _{22:6} (n = 6)	8.08	7.41	8.06	7.98	0.84	0.75	0.66	0.73
SFA ³	27.62	26.93	28.33	27.38	0.82	0.49	0.33	0.88
MUFA ⁴	17.26	17.24	17.00	16.99	0.60	0.68	0.98	0.99
PUFA ⁵	55.12	55.73	54.67	55.64	0.87	0.76	0.38	0.84

¹ HE = High energy; HEA = High energy with antioxidant; LE = Low energy; LEA = Low energy with antioxidant.

² E = Energy effect, AOX = Antioxidant effect, E×AOX = Interaction of energy density and antioxidant.

³ Saturated fatty acid. ⁴ Monounsaturated fatty acid. ⁵ Polyunsaturated fatty acid.

that diets high in energy density pre-partum improved transition success (McNamara et al., 2003). McNamara et al. (2003) assumed that a higher concentrate ration stimulated an increase of rumen epithelium area and VFA absorption. Additionally, there may be an advantage for cows to adapt to high-energy rations during the dry period to avoid the ill effects of rapidly changing to a high energy diet with different feed ingredients (Holcomb et al., 2001). In fact, even a small amount of concentrate may be adequate to maintain the rumen epithelia area (Olsson et al., 1997). Feeding a transition diet with an energy content of 1.71 Mcal/kg pre-partum was associated with higher tissue mobilization and plasma BHBA compared with abruptly changing to a lactation diet post-partum (Guo et al., 2007). Although energy density in our diet was lower than that of Tesfa et al. (1999) and Guo et al. (2007), even the LE diet

could satisfy the pre-partum requirement of dairy cows as indicated by high plasma glucose concentration and positive energy balance pre-partum (not shown). Post-partum cows were found to have a potential to compensate for low energy intake pre-partum, resulting in higher DMI and milk production (Agenäs et al., 2003). In an experiment carried out by Doepel et al. (2002), cows were found to be in negative energy balance just a few days before calving, which was associated with a sharp reduction in DMI at calving. Although no significant effect was observed on DMI with AOX addition, cows fed the HEA diet had numerically higher DMI post-partum, compared with the HE diet (15.5 kg/d vs. 14.3 kg/d). Response to AOX on milk production was similar to an experiment in lactating cows (Vázquez-Añón et al., 2008). Addition of AOX increased DM, OM and fiber digestibility, contributing to

Table 4. Effects of antioxidant with varying energy density on fatty acid concentrations in the erythrocyte membrane and fluidity of the erythrocyte membrane (% total fatty acids measured)

Items	Treatment ¹				SEM	p value ²		
	HE	HEA	LE	LEA		E	AOX	E×AOX
Fatty acid (%)	54.9	55.9	52.0	52.5	0.83	0.01	0.24	0.73
Fluorescence polarization	0.33	0.33	0.34	0.34	0.002	0.01	0.20	0.82
Viscosity	5.33	5.20	5.72	5.50	0.13	0.01	0.19	0.76
Fluidity	1.49	1.53	1.38	1.44	0.04	0.02	0.21	0.85

¹ HE = High energy; HEA = High energy with antioxidant; LE = Low energy; LEA = Low energy with antioxidant.

² E = Energy density effect, AOX = Antioxidant effect, E×AOX = Interaction of energy density and antioxidant.

the improvement in milk production (Han et al., 2002; Vázquez-Añón and Jenkins, 2007). Interaction of AOX and energy density on milk contents was minor with limited biological meaning, because, compared with LE, the decrease of milk protein in LEA might be a dilution effect due to higher milk yield.

Plasma parameters

Similar to previous research (Doepel et al., 2002), plasma glucose concentrations peaked at calving and gradually declined to a lower value compared to pre-partum in our study. Perhaps cows stored more glycogen for hepatins because of consumption of a glucogenic diet pre-partum in the HE and HEA groups, which resulted in higher plasma glucose during the first 3 weeks post-partum. In addition, lower milk production on a high energy diet would have resulted in lower demand for glucose for milk lactose synthesis. BHBA is a common and sensitive indicator of negative energy balance and adipose mobilization (Duffield, 2000). Lower BHBA concentrations with dietary AOX might indicate a positive response to AOX supplementation on energy balance. There was a possible relationship between oxidative and metabolic status in transition cows; cows with higher BHBA and NEFA showed higher reactive oxygen metabolites and thiobarbituric acid-reactive substances (Bernabucci et al., 2005). A diet with high energy pre-partum increased GSH-Px activity on d 3 pre-partum ($p < 0.05$), while AOX decreased the GSH-Px activity on d 3 and 10 pre-partum. Plasma GSH-Px activity is related to plasma lipid peroxidation (Halliwell and Chirico, 1993) and may be used as an indirect indicator of oxidative stress (Tüzün et al., 2002). Increasing levels of GSH-Px is reflective of indirect compensatory response of cells to oxidative damage, as it was observed when cows were in heat stress (Bernabucci et al., 2002). Cows fed diets without AOX showed higher MDA value at calving, whereas this value showed a sharp drop in the cows fed diets with AOX added (Figure 2). Similar to our study, the results obtained in transition cows also showed highest MDA at calving, but the addition of vitamin E was effective in cows recovering from parturition-related oxidative stress (Bouwstra et al., 2008). The above oxidative parameters associated with changes of H_2O_2 indicated that high energy may make cows more prone to oxidative stress, while AOX may partially ameliorate it. The effect of AOX on oxidative stress may relate to reducing the free radicals and sparing the endogenous antioxidant defense system (Vázquez-Añón et al., 2008). Supported by the potential correlation between energy balance and oxidative stress (Bernabucci et al., 2005; Castillo et al., 2005), we also found that cows supplemented with dietary AOX were under lower oxidative stress and had better energy metabolism indicated by lower plasma BHBA.

Fatty acid composition and fluidity of the erythrocyte membrane

Marginal changes in the fatty acid composition of the erythrocyte membrane were found. Although $C_{16:0}$ was reported to be favorable and $C_{18:0}$ unfavorable with respect to the rheological properties of erythrocytes (Persson et al., 1996), the difference can be ignored because of 6% higher fatty acid content in cows on a high energy diet pre-partum. The erythrocyte membrane was rich in PUFA that exceeded 50% in this study. These fatty acids cannot be synthesized *de novo* by the erythrocyte membrane, thus fatty acids in the diet are the main source incorporated into the erythrocyte membrane. There are many advantages of using the erythrocyte membrane to evaluate the metabolism of fatty acids, such as more stable fatty acid pattern and low fluctuation. Many studies showed changes in fatty acids of erythrocytes after several weeks of feeding treatment (Skeaff et al., 2006). Although changes of several kinds of fatty acid composition were observed, energy treatment pre-partum showed little effect on SFA, MUFA and PUFA compositions.

Compared with energy density treatments, AOX showed less effect on fatty acid composition in the erythrocyte membrane. Only $C_{18:2}$ and *cis-9, trans-11* CLA were changed due to AOX addition. Kuiper et al. (1971) observed that $C_{18:2}$ concentrations in the erythrocyte membrane were lower when the cows were in heat stress. Dietary AOX has been reported to protect lipids from oxidative damage, and reduce the loss of PUFA (Andrews et al., 2006). Unexpectedly, no significant effect of AOX was observed on concentration of PUFA in the erythrocyte membrane. Interestingly, although the high energy diet resulted in a negative influence on performance, fluidity of the erythrocyte membrane was improved by a high energy diet, but not by AOX, which was unexpected. Membrane fluidity may be modulated by compositional changes of fatty acids in membrane phospholipids, irrespective of lipid peroxidation (Keddad et al., 1996). Decreased response to AOX supplementation might be due to the fact that the diet used in our study was not oxidized, and the dietary PUFA may change little even without the addition of an antioxidant, compared with other studies involving oxidized diets. Further research is needed to elicit the relationship between dietary manipulation and membrane characteristics.

CONCLUSIONS

The pre-partum diet containing high energy density negatively affected the DMI and subsequent milk production and anti-oxidative status. AOX decreased plasma BHBA and improved the anti-oxidative status and milk production of early lactating cows. The addition of AOX and high energy diets can affect the fatty acid profile

in the erythrocyte membrane, but fluidity of the erythrocyte membrane was only affected by a high energy diet.

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