Influence of rumen-protected choline on blood red-ox potential and biochemical biomarkers of dairy cows during the transition period*)

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Summary

The aim of this study was to determine the serum oxidative status, antioxidant capacity, and oxidative stress index, as well as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine profiles, of dairy cows fed rumen-protected choline during the transition period. A total of thirty dairy cows allotted to one control and two experimental groups (10 animals per group) were fattened from 3 weeks prior to the expected calving date until 3 weeks postpartum. The control group was fed a basal diet, and treatment groups 1 and 2, were fed a basal diet containing 60 g/head/day and 120 g/head/day or rumen-protected choline, respectively.

Serum total antioxidant status (TAS) levels for the control group and treatment groups 1 and 2 at the time of parturition were 0.56 ± 0.029^{ab} , 0.45 ± 0.028^b , and 0.59 ± 0.036^a , respectively. The differences between the groups were statistically significant ($P < 0.05$). Rumen-protected choline supplementation did not have a significant effect on TAS levels in the third week before parturition and in the first and third weeks of lactation. There were no significant differences ($P > 0.05$) between the groups in terms of total oxidative status (TOS), oxidative stress index (OSI) or creatinine levels. Serum ALT levels were significantly different ($P < 0.05$) between the groups at parturition and in the first week of lactation, but not significant ($P > 0.05$) for the other periods. In addition, the serum AST level was significantly different ($P < 0.05$) between the groups in the first and third weeks of lactation, but not significantly different ($P > 0.05$) for the other periods. After removing the effect of groups, time-dependent changes were significant ($P < 0.001$) for all parameters. The highest values for TAS, TOS and OSI were obtained in the third week before parturition. The values for these parameters decreased during parturition and after the postnatal period. The lowest values for serum ALT and creatinine occurred at parturition.

Rumen-protected choline supplementation did not have a significant effect on serum TAS and TOS levels during the transition period. There is a need for further studies of animals with known metabolic profiles, malnourished animals, and those with high milk yields or metabolic disorders.

Keywords: antioxidant, choline, cow, oxidant, transition period

Cows in the transition period are exposed to physiological, metabolic and nutritional changes. Both the rapid growth of the fetus and the postnatal onset of lactation substantially increase the nutritional requirements of cows. Energy, vitamin and mineral intake by dairy cows during the transition period are generally much lower than their requirements. This condition leads to a physiologically negative energy balance

status of the animal (17, 19). Energy metabolism in the periparturient period is associated with elevated rates of production of reactive oxygen species (ROS) (11). The proper balance between oxidants and antioxidants plays an essential role in the maintenance of the health and performance of dairy cows. The total antioxidant capacity can act as a natural defense system in transitioning dairy cows and can neutralize and prevent damage caused by free radicals, which can reduce oxidative stress (20). A decrease in the antioxidant levels in serum and tissues can cause oxidative stress and free radical damage. Oxidative stress can be

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monitored with several markers, and a number of methods have been developed to assess total antioxidant capacity by measuring each antioxidant component separately (9).

Choline, an essential component of cell membranes, has a role in the synthesis of phosphatidylcholine, which is necessary for very-low-density lipoprotein synthesis and secretion from the liver. Choline is also a methyl donor for the synthesis of carnitine from methionine and lysine (16). Choline deficiency decreased carnitine concentration in the liver. Choline supplementation resulted in a significantly increased carnitine concentration in the liver and significantly lower urinary carnitine excretion. Choline indirectly enhanced fatty acid oxidation and carnitine concentration in the liver, which may reduce the accumulation of lipids in the liver (2). Choline, used separately as an antioxidant (8), provides substantial protection to cells (24). Only a small amount of dietary choline escapes degradation in the rumen (6). Dietary choline in cattle is rapidly and extensively degraded in the rumen (up to 99%). Therefore, there is need to protect choline in the rumen environment.

The objective of the present investigation was to determine the effects of rumen-protected choline on the oxidative status, antioxidant capacity and oxidative stress index, as well as on AST, ALT and creatinine profiles, of dairy cows during the transition period.

Material and methods

The use of all experimental protocols was approved by The Ondokuz Mayıs University Local Ethics Committee on Animal Experiments.

Animals and experimental design. The animal feeding experiment was conducted at the Gökkale Agriculture and Livestock Company Limited facility in Devrekani, Kastamonu, Turkey. A total of thirty Friesian-Holstein dairy cows were allotted to one control and two experimental groups (10 animals per group) and then fattened from 3 weeks prior to the expected calving date until 3 weeks postpartum. The research was conducted from 15 March to 20 May 2014. The control group was fed the basal diet, and treatment groups 1 and 2 were fed a basal diet containing 60 g/head/day and 120 g/head/day of rumen-protected choline, respectively. The rumen-protected choline was provided by YEM-VIT Vitamin Feed Additives Inc. in Kemalpaşa, İzmir.

Biochemical analysis. Blood samples were taken in the third week before parturition, at the time of parturition, and in the first and third weeks of lactation. The samples were taken from the coccygeal vein into 10 ml non-heparinized tubes and stood at room temperature for up to 30 min to facilitate clotting. Serum was removed by centrifugation at 1150 g for 10 minutes in a refrigerated centrifuge at 4°C. Serum samples were stored at -20°C until analysis.

Serum ALT, AST and creatinine levels in the cows were measured with commercial autoanalyzer test kits (Audit Diagnostics, Ireland) in a biochemistry autoanalyzer (Autolab, AMS srl, Autoanalyzer, Netherlands).

Serum total antioxidant status (TAS) was measured with Rel Assay commercial kits by the colorimetric method. This method of measurement is based on decolorization in proportion to the total concentration of the antioxidant molecules of the coloring radical as a result of reduction of 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) cationic radicals by the antioxidant molecules. The results were expressed as mmol Trolox equivalent/L (10).

Serum total oxidative status (TOS) was measured with Rel Assay commercial kits by the colorimetric method. In this method, the oxidant molecules within a sample are based on the oxidation of the ferrous ion-o-dianisidine complex to the ferric ion. The results were expressed as $\mu\text{mol H}_2\text{O}_2$ Equivalent/L (9).

The TOS-to-TAS ratio was also calculated as the oxidative stress index (OSI) value (Erel, 2005). The results were expressed as Arbitrary Units (AU) (9). $\text{OSI} = (\text{TOS}/\text{TAS}) \times 100$.

Statistical analysis. The data were analyzed using the one-way ANOVA procedure of SPSS software (IBM SPSS Version 20 software package, Ondokuz Mayıs University, Samsun, Turkey). Data were checked for normality with the Kolmogorov-Smirnov Test and for homogeneity of variance with the Levene Test. Differences were considered significant at $P < 0.05$. All variables were expressed as the mean and standard error (SE).

Results and discussion

During the transition period, dairy cows undergo a variety of physiological and metabolic changes. These occur in the blood plasma due to metabolic changes. Antioxidants play an important role in controlling oxidative reactions; this activity is related to the reactivity of the antioxidants with free radicals (25). Mean values of TAS, TOS, OSI, ALT, AST and creatinine activities in dairy cows during the third week before parturition, during parturition, and during the first and third weeks of lactation by groups within the periods are given in Table 1. Mean values of TAS, TOS, OSI, ALT, AST and creatinine activities in dairy cows by periods are shown in Table 2. In the present study, serum TAS levels for the control group and treatment groups 1 and 2 at parturition time were $0.56 \pm 0.029^{\text{ab}}$, $0.45 \pm 0.028^{\text{b}}$, and $0.59 \pm 0.036^{\text{a}}$, respectively. The differences between groups were significantly different ($P < 0.05$) for the TAS levels in serum at parturition. The TAS levels of cows administered 120 g/head/day of rumen-protected choline were significantly higher at parturition, and thus would be expected to have improved antioxidant status and reduced oxidative stress. Rumen-protected choline supplementation did not have a significant effect on TAS levels in the third week before parturition and in the first and third weeks of lactation. The TAS concentration was only significantly higher at three weeks before calving, thus indicating a lower antioxidant status and higher oxidative stress at weeks one and three after calving. The TAS levels indicated a reduction in overall antioxidant potential at parturition time and in the early lactation

Tab. 1. Mean values of TAS, TOS, OSI, ALT, AST and creatinine activities in dairy cows during the third week before parturition, during parturition, and during the first and third weeks of lactation by groups within the periods (n = 10)

Parameters	Groups	Periods			
		Third week before parturition	Parturition	First week of lactation	Third week of lactation
TAS, mmol Trolox equivalent/L	Control	0.70 ± 0.021	0.56 ± 0.029 ^{ab}	0.40 ± 0.023	0.35 ± 0.033
	Treatment Group 1	0.68 ± 0.029	0.45 ± 0.028 ^b	0.42 ± 0.049	0.45 ± 0.067
	Treatment Group 2	0.71 ± 0.022	0.59 ± 0.036 ^a	0.49 ± 0.043	0.38 ± 0.056
	P	NS	*	NS	NS
TOS, μmol H ₂ O ₂ equivalent/L	Control	30.79 ± 3.217	8.67 ± 0.574	16.80 ± 1.573	10.32 ± 1.439
	Treatment Group 1	26.02 ± 3.974	7.72 ± 0.158	14.35 ± 0.846	12.96 ± 1.060
	Treatment Group 2	34.64 ± 5.086	9.69 ± 1.223	15.12 ± 1.045	14.47 ± 1.294
	P value	NS	NS	NS	NS
OSI, Au	Control	0.44 ± 0.043	0.16 ± 0.011	0.42 ± 0.045	0.32 ± 0.052
	Treatment Group 1	0.38 ± 0.053	0.18 ± 0.012	0.39 ± 0.065	0.35 ± 0.079
	Treatment Group 2	0.48 ± 0.061	0.16 ± 0.013	0.33 ± 0.029	0.45 ± 0.066
	P value	NS	NS	NS	NS
ALT, IU/L	Control	10.22 ± 1.412	5.67 ± 0.471 ^{ab}	3.67 ± 0.527 ^b	13.11 ± 1.438
	Treatment Group 1	8.75 ± 1.031	4.38 ± 0.532 ^b	14.25 ± 2.094 ^a	14.50 ± 1.669
	Treatment Group 2	11.11 ± 1.882	6.33 ± 0.601 ^a	7.11 ± 0.920 ^b	15.44 ± 1.119
	P value	NS	*	*	NS
AST, IU/L	Control	63.89 ± 3.743	68.22 ± 6.778	41.78 ± 4.146 ^a	28.67 ± 4.500 ^b
	Treatment Group 1	66.00 ± 2.686	54.13 ± 6.096	42.13 ± 5.740 ^a	69.00 ± 6.030 ^a
	Treatment Group 2	56.89 ± 4.188	54.00 ± 4.031	22.22 ± 2.943 ^b	45.67 ± 5.094 ^b
	P value	NS	NS	*	*
Creatinine, mg/dL	Control	2.11 ± 0.0906	1.40 ± 0.219	1.32 ± 0.138	1.60 ± 0.204
	Treatment Group 1	1.97 ± 0.0697	1.18 ± 0.076	1.41 ± 0.245	1.93 ± 0.237
	Treatment Group 2	2.03 ± 0.0792	1.29 ± 0.120	1.18 ± 0.040	1.32 ± 0.105
	P value	NS	NS	NS	NS

Explanations: NS: non-significant; means within the same column with a different letter are significantly different (P < 0.05)

Tab. 2. Mean values of TAS, TOS, OSI, ALT, AST, and creatinine activities in dairy cows by periods (n = 30)

Parameters	Periods				
	Third week before parturition	Parturition	First week of lactation	Third week of lactation	P
TAS, mmol Trolox Eq/L	0.70 ± 0.013 ^a	0.54 ± 0.021 ^b	0.44 ± 0.023 ^c	0.39 ± 0.030 ^c	*
TOS, μmol H ₂ O ₂ Eq/L	30.65 ± 2.420 ^a	8.73 ± 0.480 ^c	15.46 ± 0.706 ^b	12.57 ± 0.796 ^{bc}	*
OSI, Au	0.44 ± 0.030 ^a	0.16 ± 0.007 ^b	0.38 ± 0.027 ^a	0.37 ± 0.038 ^a	*
ALT, IU/L	10.08 ± 0.861 ^b	5.50 ± 0.339 ^c	8.12 ± 1.120 ^{bc}	14.35 ± 0.804 ^a	*
AST, IU/L	62.12 ± 2.174 ^a	58.96 ± 3.450 ^{ab}	35.12 ± 3.037 ^b	46.96 ± 4.345 ^b	*
Creatinine, mg/dL	2.05 ± 0.046 ^a	1.30 ± 0.088 ^c	1.30 ± 0.089 ^c	1.59 ± 0.113 ^b	*

Explanations: means within the same row with a different letter are significantly different (P < 0.05)

period. Castillo et al. (3) reported that serum TAS levels were not significantly different between the first weeks of the early lactation period and the late lactation period. They further reported that serum TAS levels fluctuated markedly during the first weeks of the early lactation period, a finding which is supported by our study. Jian Gong and Min Xiao (13) determined that serum TAS in early-lactation cows was significantly higher than in dry and peak-lactation cows. This finding is not consistent with the results of the present study.

In addition, Aydılek et al. (1) reported that the estrous cycle affected TAS values in dairy cows, which were higher than in our study.

The TOS and OSI indices are important parameters for monitoring the health of dairy cows during the transition period. The OSI index is based on the ratio between reactive oxygen substances and serum antioxidant capacity. An increase in the ratio indicates risk of oxidative stress because of the increase in ROS production or defensive antioxidant consumption (5).

In the present study, the serum TOS levels for the control group and treatment groups 1 and 2 at the time of parturition were 8.67 ± 0.574 , 7.72 ± 0.158 , and 9.69 ± 1.223 , respectively. The serum OSI values for the control group and treatment groups 1 and 2 at the time of parturition were 0.16 ± 0.011 , 0.18 ± 0.012 , and 0.16 ± 0.013 , respectively. However, there was no statistically significant difference between the control and treatment groups for TOS and OSI status. The TOS level at parturition was significantly lower than in the third week before parturition and in the first and third weeks of lactation. In Table 2, the main effects of the periods without group effects in the model were highly significant ($P < 0.001$) for all parameters. The highest values for TOS and OSI were obtained in the third week before parturition. These values decreased during parturition.

The results of the present study therefore evidence significant changes in the indicators of the oxidative stress and antioxidant status of dairy cows during the transition period. Our results suggest more oxidative stress and lower antioxidant defense capacity just after parturition or during early lactation than during advanced pregnancy, which may contribute to the incidence of many metabolic diseases (27). Therefore, regular monitoring of the antioxidant profile and supplementation with antioxidants could help to reduce the incidence of these diseases in transition cows. The levels of serum TOS in the colostrum period of cows were between 4.98 and 6.52 (22). These findings are consistent with results for parturition in the present study on dairy cattle.

Serum creatinine gives an insight into the renal health of cows during the transition period. The serum creatinine concentration, which is an indicator of renal function, is affected by the dietary intake of proteins. In this study, serum creatinine levels were not significantly different ($P > 0.05$) between the groups. However, the level decreased during the calving period and in the first and third weeks of lactation when compared to the third week before the calving period. In the present study, serum creatinine levels were not affected by the physiological period and rumen-protected choline administration. Castillo et al. (3) reported that creatinine levels at 1 and 2 weeks after calving were 1.35 ± 0.07 and 1.14 ± 0.03 mg/dL, respectively, which were similar to those in the present study. In Table 2, serum creatinine levels of the periods without group effects were significantly lower ($P < 0.05$) in the third week before calving and in the calving period than in the first and third weeks of lactation.

ALT activity shows difference in cows during the transition period. In this study, there was a significant ($P < 0.05$) difference in the serum ALT level between the groups at parturition and the first week of lactation, but no significant difference ($P > 0.05$) between three weeks before parturition and the third week of

lactation. ALT activity in cows during the transition period reached its highest level during the third week of lactation, although there was no significant ($P > 0.05$) difference between the groups. Specifically, ALT activity in the third week of lactation was 260% more than at parturition (Tab. 2). Stojević et al. (29) reported a mean of 8.91 U/L of ALT between days 10 and 45 days of lactation. For the same period in the present study, serum ALT levels were within the range of 8.12 and 14.35 U/L. Serum ALT levels in cows differ during certain periods. The serum ALT levels in the current study were similar to those found by some researchers (12, 21) but different from those found by others (18, 26). The authors of the present study believe that this difference may be due to the animal's production capability, clinical healthy status, and the composition of rations in the dry and lactation periods.

Serum AST levels were significantly different ($P < 0.05$) between the groups at the first and third weeks of lactation, but not significantly different ($P > 0.05$) for the other periods. Aspartate aminotransferase (AST) can be used to assess liver function. Its activity is mostly related to fatty liver syndrome in dairy cows (4). Furthermore, it is also associated with the lack of appetite and the appearance of ketosis in dairy cows during early lactation (28). Gonzalez et al. (14) stated that hepatic lesions can occur when the AST value is above 100 U/L. The present study showed a decrease in the serum AST values in the first week of lactation in cows administered rumen-protected choline. Serum AST levels in treatment groups 1 and 2 were higher than in the control group in the third week of lactation. The negative energy balance that can occur in early lactation in high-producing dairy cows may cause cellular damage in the liver as a result of lipid mobilization. Choline that is protected from rumen degradation in this period may reduce the accumulation of liver lipid, enabling very-low-density lipoprotein synthesis and secretion from the liver (15). Djoković et al. (7) stated that the serum AST level of cows in early lactation was higher ($P < 0.05$) than that of cows in late pregnancy, which was different from the findings of the present study. Separately, Ögün et al. (23) reported that AST levels increased during parturition and in the postpartum period. These levels were much higher than the ones in the current study. This may be due to cellular damage in the liver caused by the negative energy balance in cows during parturition and in the early postpartum period.

Overall, the present study demonstrated that administering rumen-protected choline at 120 g/head/day enhanced antioxidant capacity at parturition in dairy cows. Rumen-protected choline supplementation did not have a significant effect on serum TAS and TOS levels in the third week before parturition and in the first and third weeks of lactation. To better understand the effectiveness of rumen-protected choline, there

is a need for further studies of animals with known metabolic profiles, malnourished animals, and those producing large volumes of milk or with metabolic disorders.

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