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The effects of supplementation of rumen-protected choline on some blood and milk metabolites in the transition period of dairy cattle*

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Abstract: The aim of this study was to investigate nonesterified fatty acid (NEFA), beta-hydroxy butyric acid (BHBA), and milk urea nitrogen (MUN) profiles for dairy cows fed with rumen-protected choline (RPC) during the transition period. A total of 30 dairy cows were allocated to one control and two experimental groups (10 animals per group) and were fed from 3 weeks before calving to 21 days in milk. The control was fed only the basal diet. Treatment groups 1 and 2 were fed a basal diet containing 60 and 120 g/head/day RPC, respectively. The differences between groups were insignificant for MUN levels of colostrum and milk. There were no significant differences ($P > 0.05$) between the groups in terms of serum NEFA or BHBA levels. RPC supplementation did not have a significant effect on energy or protein metabolism of dairy cows during the transition period. The main effects of the periods without group effects in the model were highly significant ($P < 0.01$) for NEFA and BHBA. There is no need to add RPC to rations formulated as isocaloric and isonitrogenic for optimal rumen fermentation in dairy cows during the transition period. To better understand the effect of RPC on dairy cows during the transition period, there is a need for further studies of animals with known metabolic profiles, and those having high milk yields or metabolic disorders.

Key words: Beta-hydroxy butyric acid, nonesterified fatty acid, rumen-protected choline, transition period, milk urea nitrogen

1. Introduction

The incidence of metabolic diseases significantly differs based on nutrition in dairy cattle in their transition period. In this period, low or insufficient feed consumption of cows increases the incidence of metabolic diseases. In this sense, the metabolic profile provides advantages for early detection of periparturient diseases that significantly affect the profitability of dairy cattle in relation to reduced breeding performance and yield. Several studies in this field have focused on certain diseases (hypocalcemia, ketosis, abomasum displacement). However, it was proven that such periparturient diseases do not occur by themselves and they are related to each other (1).

Choline can be endogenously synthesized, and it is closely related to methionine, folic acid, and vitamin B_{12}. Choline plays a key role in the syntheses of phosphatidylcholine and acetylcholine molecules, which have important roles in the body. Phosphatidylcholine is the main phospholipid in ruminants, and it has critical significance for fat absorption and transport, as well as cellular transport and lipoprotein synthesis. Choline has a lipotropic structure that speeds up the process of metabolically burning the fats in the body and prevents fat accumulation by increasing the transformation amounts and secretion of triglycerides in the liver. Thus, choline deficiency leads to insufficiency of phospholipids that have the role of carrying fats from the liver to other tissues, and fat may accumulate in the liver (2). A large part of the choline added to rations is metabolized in the rumen. A very small part of it can avoid the microbial activity in the rumen. Namely, unprotected choline easily degrades in the rumen. This is why there is a need for choline that is protected from the rumen environment. Therefore, choline is added to the diet in a rumen-protected form. Choline requirements are still uncertain for dairy cows (3). The rumen-protected form of choline has been improved to transmit choline with less degradation to the small intestine (4). Rumen-protected choline (RPC) increases the supply of choline in the small intestine with increasing milk yield and milk components or alleviated development of fatty liver syndrome (5). In the transition from the early dry period to the late dry period, i.e. while entering the

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last 3 weeks of the dry period, due to reduction in dry matter consumption, and despite this, increased nutrient and energy requirements, a transition should be made to rations with high energy and nutrient values and low neutral detergent fiber (NDF) to prepare the rumen for feeding in the lactation period (6). Metabolic profile tests are used to observe the health of the herd, help diagnose metabolic diseases and yield disorders, and determine the metabolically superior animals. In this transition period, significant changes are observed in blood and milk parameters based on mistakes made in feeding strategies that are used for dairy cattle. This study aimed to determine the effects of RPC added to the rations of Holstein cattle in the transition period in different amounts on nonesterified fatty acid (NEFA), beta-hydroxy butyric acid (BHBA), and milk urea nitrogen (MUN) values.

2. Materials and methods

2.1. Animal material

This study was approved by the Local Animal Experiments Ethics Board of Ondokuz Mayıs University (dated 10.03.2014 and numbered B.30.2.ODM.0.20.09.00-050.04-15).

The research was carried out at the Gökkale Agriculture and Stockbreeding establishment, operated with a capacity of about 2500 cattle near the Çakallar village of the district of Devrekani in Kastamonu, Turkey. Groups were formed by the method of random sampling from animals that were determined to be in the last 3 weeks of pregnancy by examining their artificial insemination records and animals whose lactation numbers and milk yields in their previous lactation periods were known. All the animals were taken into the study 3 weeks before the expected birth date in such a way as to cover the transition period and subjected to treatments until 3 weeks after birth. The study was carried out between 15 March and 20 May 2014 and included a total of 30 Holstein cattle so that each of the control and two experimental groups contained 10 animals.

2.2. Feed material

The control group was fed only with a basal diet, while treatment groups 1 and 2 were fed with a basal diet containing 60 and 120 g/head/day RPC, respectively. The RPC was provided by YEM-VIT Vitamin-Added Feed Additives Inc. in Kemalpaşa, İzmir. The nutrient contents of the feed materials that were used in the study are presented in Table 1, while the characteristics of the rations that were used in the prepartum and postpartum periods are given in Table 2.

2.3. Chemical analyses of the feeds

The feeds were used in the analyses by grinding them in a mill with a mesh diameter of 1 mm. The dry matter (DM) contents of the feeds were determined by drying them in an oven at 105 °C for 8 h, while their crude ash contents were determined by burning in an ash oven at 550 °C for 4 h. The nitrogen (N) contents were determined with the Kjeldahl method. Crude protein was determined with the N × 6.25 formula. Crude fat analysis was conducted with an automated Soxhlet system of the Büchi brand (Büchi B-811) (7). Metabolic energy levels were calculated according to the Turkish Standards Institution (8). The NDF and acid detergent fiber (ADF) contents, which form the cell wall compounds of feeds, were analyzed by the method reported by Van Soest et al. (9) using an ANKOM 200 Fiber Analyzer device (ANKOM Technology Corp., Fairport, NY, USA).

2.4. Collection and analyses of blood and milk samples

The blood samples were collected from an abdominal vein of the animals in the beginning of the prepartum period, at parturition or the 1st days after calving, 1 and 3 weeks after parturition while the milk samples were collected at parturition, and 1 week and 3 weeks after birth. Daily milk yield records were identified by utilizing the computer-controlled system of the enterprise. Milk obtained from morning and evening milking of animals was rated per yield, and that day's milk mixture was prepared. In order to prevent microbial deterioration, 2-bromo-2-nitropropane-1.3-diol was added to the milk. Then milk was preserved

Table 1. Nutrient content (%) and metabolic energy values of feedstuffs (kcal/kg).

Ingredient	*DM*	*CP*	*Ash*	*CF*	*CS*	*ADF*	*NDF*	*ME*
Alfalfa	90.8	15	9.68	3.10	27.6	30.2	44.9	2063
Straw	96.6	2.8	6.87	1.64	39.8	47.1	76.6	1416
Mix feed	91.2	22.9	8.33	4.20	5.30	8.6	23.4	2739
Barley	88.7	11	2.34	1.78	4.75	6.1	18.5	2835
Cottonseed	93.8	23.3	5.53	20.67	19.40	23.9	46.7	3093
SoyPass	88	40	6.65	1.57	4.20	8.1	24.6	2520
Corn silage	29.8	7.54	5.58	2.53	21.8	26.3	45.6	2514

Table 2. Characteristics of ration used in prenatal and postnatal periods, kg/day.

Ingredient	*Prenatal period, –3 to 0**	*Postnatal period, 0* to +3*
Alfalfa	2.00	5.00
Straw	2.50	-
Mix feed	4.00	10.85
Barley	1.00	1.00
Cotton seed	0.50	1.05
SoyPass	0.10	0.30
Diamond XP**	0.050	0.055
Sodium bicarbonate	0.04	0.10
Bypass fat (7100 kcal/kg)	0.10	0.50
Corn silage	10.00	21.00

**At parturition or the 1st days after calving; **manufacturer: Diamond, Cedar Rapids, IA, USA.

at –20 °C until analysis. The blood samples that were collected from the animals before morning feeding were taken into tubes without anticoagulants and centrifuged for 10 min at 3000 rpm at 4 °C. The clear blood serum at the top was then collected and stored at –20 °C until the time of analysis. The NEFA quantities were determined using a commercial kit (Sigma Aldrich, Catalog Number MAK044) in a Thermo Scientific Multiskan GO microplate spectrophotometer device, and the BHBA quantities were determined by using beta ketone test strips in a beta ketone monitoring device (Model TD-4235).

2.5. Statistics

Lactation numbers were analyzed and compared by using a link = normal link function with the method of generalized linear models (Genmod). For the parameters of MUN, NEFA, and BHBA, comparisons were made among weeks and groups with the ANOVA method, which was repeated weekly. The weekly changes were analyzed based on third-degree polynomial functions. Descriptive statistics were calculated and are presented in tables.

3. Results

The mean numbers of lactation in the control and treatment groups 1 and 2 were 2.30 ± 0.423, 2.10 ± 0.277, and 2.18 ± 0.325, respectively (P = 0.9217). When the numbers of lactations were examined, it was seen that the studied cattle were close in the three groups in terms of their ages.

3.1. Milk production and milk metabolite values

The average milk yields per week according to groups are given in Table 3. While there was no statistical difference among groups in terms of weekly milk yields, milk yield of experimental group 1 was found numerically higher than the others. The postpartum MUN levels of the animals in the groups in colostrum and milk are presented in Table 4. The RPC that was used in different amounts did not have a statistically significant effect on the MUN levels in the colostrum or the milk. However, the MUN levels of the animals in experimental group 2, which was given at a daily amount of 120 g RPC per animal, were numerically higher in all periods.

3.2. Blood metabolite values

Based on the groups, the BHBA and NEFA levels of the animals are given in Table 4. There was no significant difference among the groups based on serum BHBA and NEFA levels. However, the BHBA levels in the prepartum period were numerically higher in comparison to those in the postpartum period. There was a decrease in serum BHBA values in the 3rd week of lactation. The NEFA concentration expectedly increased up to birth and reached its peak during birth. There was a decrease in serum NEFA levels in the control group and the experimental groups. This decrease became more noticeable in the 3rd week of lactation. Additionally, at the end of the study (3rd week of lactation), the serum NEFA levels of the animals in the experimental groups in which RPC was consumed reached lower levels in comparison to those in the control group. Mean values of NEFA, BHBA, and MUN in the dairy cows by periods are presented in Table 5. The change in NEFA levels was found to be statistically significant. The highest NEFA value was reached during parturition. This provides the energy required by the cattle while going into parturition by oxidation of fats. The blood BHBA levels were minimized in the 3rd week of the postpartum period. This may be explained by the animal leaving a state of both hormonal and parturition stress and entering a period where it could consume sufficient quantities of dry matter.

4. Discussion

The mean numbers of lactations in the dairy cattle in the transition period in the control and experimental groups 1 and 2 were respectively 2.30 ± 0.423, 2.10 ± 0.277, and 2.18 ± 0.325. There was no significant difference among the groups (P > 0.05). Therefore, the potential effect of number of lactation on the results of the study was eliminated.

Weekly milk yields in the control and experimental groups 1 and 2 in the 3rd week after parturition were determined as 38.37 ± 2.433, 39.90 ± 2.395, and 36.34 ± 3.509 kg, respectively. Milk yields of experimental group 1 were numerically high in terms of weekly milk yields. There was no significant difference (P > 0.05) in fat-free dry matter or fat and milk protein content among the control, experimental group 1, and experimental group

Table 3. Means of milk production and milk composition by groups (mean ± SEM).

Week	Control	Treatment group 1	Treatment group 2	P
Milk (kg/day)				
1	27.15 ± 1.306	31.86 ± 2.116	31.70 ± 2.593	0.2084
2	34.59 ± 1.711	36.91 ± 2.252	35.36 ± 2.918	0.7778
3	38.37 ± 2.433	39.90 ± 2.395	36.34 ± 3.509	0.6747
P	0.3733			
Fat-free dry matter (%)				
0*	18.87 ± 0.274	19.15 ± 0.224	19.13 ± 0.395	0.7805
1	8.82 ± 0.099	8.42 ± 0.156	8.66 ± 0.124	0.1093
3	8.64 ± 0.091	8.23 ± 0.205	8.60 ± 0.120	0.1219
P	0.3286			
Fat (%)				
0*	6.75 ± 0.107	6.93 ± 0.225	6.81 ± 0.272	0.8532
1	3.41 ± 0.093	3.38 ± 0.073	3.39 ± 0.119	0.9791
3	3.46 ± 0.098	3.60 ± 0.080	3.38 ± 0.086	0.2428
P	0.9097			
Protein (%)				
0*	14.74 ± 0.179	14.36 ± 0.253	14.23 ± 0.783	0.7676
1	3.21 ± 0.082	3.30 ± 0.108	3.41 ± 0.081	0.2982
3	3.19 ± 0.037	3.24 ± 0.107	3.30 ± 0.082	0.6279
P	0.7663			

*At parturition or the 1st days after calving.

2. Cooke et al. (10) found that RPC addition to rations of dairy cows positively influenced milk yields in the transition period, which is in agreement with results for 60 g of RPC in the present study. Increasing the intestinal supply of RPC, experimental group 1 had improved milk production in the 3rd week of the postpartum period, approximately 4% higher than that of the control. Erdman and Sharma (11) reported that RPC supplementation to rations of dairy cows increased milk yield, but milk fat and milk protein levels did not change, which were similar in terms of milk fat and protein. Pineda and Cardoso (12) determined that effects of RPC on milk yield and milk fat were not statistically significant, which is in line with the results of this study, and they found statistically significant differences in terms of milk protein, which is different from the results of this study. However, some researchers did not observe any significant effects on milk fat yield, milk protein, total solids, and milk urea nitrogen concentrations in RPC-supplemented cows compared to controls (13–15), which is in line with the results of the present study. On the other hand, supplementation of RPC has not been positively associated with milk concentrations of fat, protein, or other milk components (13).

The protein level in a ration is an important factor for reproductive performance. Increased urea nitrogen in milk and blood creates problems especially in the animals' conception. In this study, the mean MUN levels in the colostrum of the animals in the control and experimental groups 1 and 2 were respectively 15.46 ± 0.504, 14.63 ± 1.267, and 16.73 ± 0.663 ml/dL, while the levels in the milk of the animals were respectively 14.52 ± 0.653, 15.71 ± 0.967, and 16.38 ± 0.695 ml/dL. The difference among the MUN levels in the colostrum and milk of the groups throughout the study was not significant ($P > 0.05$). Minuti et al. (16) found MUN values in the 1st and 3rd weeks respectively as 14.7 and 12.2 ml/dL. Their results were lower than those found in this study. Pineda and Cardoso (12) found the effects of calcium salts and RPC on the levels of MUN in animals in the middle and advanced lactation periods in the control and experiment groups as 15.4 and 16.1 ml/dL, respectively. These results were similar to those in this study. Hartwell et al. (5) did not find a significant effect of RPC added to the rations of dairy cattle in their transition period on MUN levels, which supported the results in this study.

BHBA analysis may be utilized in the postpartum period in monitoring the health status of animals (17,18).

Table 4. Serum MUN, BHBA, and NEFA levels in dairy cows during transition period (mean ± SEM).

Week	Control	Treatment group 1	Treatment group 2	P
MUN levels (ml/dL)				
0*	15.46 ± 0.504	14.63 ± 1.267	16.73 ± 0.663	0.2332
1	13.80 ± 0.710	15.48 ± 0.842	15.59 ± 0.717	0.1950
3	14.52 ± 0.653	15.71 ± 0.967	16.38 ± 0.695	0.2462
P	0.3045			
BHBA levels (mmol/L)				
–3	0.92 ± 0.104	0.82 ± 0.051	0.75 ± 0.093	0.3674
0*	0.75 ± 0.131	0.73 ± 0.062	0.73 ± 0.079	0.9830
1	0.91 ± 0.139	0.76 ± 0.065	0.82 ± 0.103	0.6176
3	0.62 ± 0.081	0.60 ± 0.047	0.75 ± 0.111	0.4326
P	0.0109			
NEFA levels (mmol/L)				
–3	0.78 ± 0.210	0.53 ± 0.077	0.45 ± 0.071	0.2290
0*	0.83 ± 0.185	0.70 ± 0.084	0.71 ± 0.141	0.7929
1	0.56 ± 0.108	0.46 ± 0.099	0.54 ± 0.130	0.8143
3	0.48 ± 0.093	0.34 ± 0.126	0.34 ± 0.112	0.5930
P	<0.0001			

*At parturition or the 1st days after calving.

Table 5. Mean values of NEFA, BHBA, and MUN values in dairy cows by periods[¥] (mean ± SEM).

Period	NEFA, mmol/L	BHBA, mmol/L	MUN, ml/dL
–3 weeks	0.59 ± 0.440 ab	0.83 ± 0.050 a	-
0[¥¥]	0.75 ± 0.443 a	0.74 ± 0.053 ab	15.64 ± 0.507
1 week	0.52 ± 0.345 b	0.83 ± 0.060 a	14.98 ± 0.448
3 weeks	0.39 ± 0.337 c	0.66 ± 0.049 b	15.56 ± 0.457
P	*	*	NS

[¥] n = 30; a, b, c: Values within periods in columns followed by different letters are significantly different at the 5% level; [¥¥] at parturition or the 1st days after calving.

In this study, the BHBA levels of the animals in the prepartum period (3rd week before parturition) in the control and experimental groups 1 and 2 were respectively 0.92 ± 0.104, 0.82 ± 0.051, and 0.75 ± 0.093 mmol/L, while these values in the 3rd week of the postpartum period were respectively 0.62 ± 0.081, 0.60 ± 0.047, and 0.75 ± 0.111 mmol/L. For the prepartum (–3 weeks) and postpartum (+3 weeks) periods, the difference among the groups in terms of BHBA levels was found to be statistically insignificant (P > 0.05). On the other hand, the BHBA levels in the control and experimental group 1 were found to be higher in comparison to those in the 3rd week of the postpartum period. When the time-related changes based on the groups were analyzed, the intragroup time × group interactions were found to be insignificant (P > 0.05). The main effects of the periods without group effects in the model were highly significant (P < 0.01) for BHBA. Uyarlar (19) conducted a study on dairy cattle in the transition period and found the effect of RPC on blood BHBA concentration (0.17–0.42 mmol/L) to be

insignificant, which was similar to the result of this study. However, the BHBA values obtained in that study were lower than those in this study. Higher serum BHBA levels before parturition, at parturition, and in the 1st week of the postpartum period are caused by the need for energy that arises as a result of glucose and gluconeogenesis deficiency. In the 3rd week of the postpartum period, there were decreases in the BHBA levels in all the groups. The finding that RPC did not have an effect on the BHBA levels of the cattle in the transition period was similar to the conclusion reached by Uyarlar (19).

The NEFA levels of the animals in the control group and experimental groups 1 and 2 were respectively 0.78 ± 0.210, 0.53 ± 0.077, and 0.45 ± 0.071 mmol/L in the prepartum period (–3 weeks) and 0.48 ± 0.093, 0.34 ± 0.126, and 0.34 ± 0.112 mmol/L in the postpartum period (+3 weeks). The differences among the groups in the prepartum (–3 weeks) and postpartum (+3 weeks) periods in terms of NEFA levels were insignificant ($P > 0.05$). However, these levels decreased in the postpartum period. Increase in NEFA concentrations in the postpartum period and at parturition is an indicator of fat mobilization. The finding that RPC supplementation did not have an effect on the plasma NEFA levels of the dairy cattle in the periparturient period was similar to the result of Pineda and Cardoso (12). When the time-related changes based on the groups were examined, the intragroup time × group interactions were found to be insignificant ($P > 0.05$). The main effects of the periods without group effects in the model were substantially significant ($P < 0.01$) for NEFA. Whitaker (20) found the upper threshold for plasma NEFA levels as 0.4 mmol/L at the end of parturition and 0.7 mmol/L in the early lactation period. Whitaker also defined the upper threshold for BHBA as 1.0 mmol/L for dairy cattle and 0.6 mmol/L for cattle in their last period of pregnancy. The NEFA and BHBA values before parturition and at parturition in this study were higher than those determined by Whitaker (20). However, this may be explained not by a state of negative energy balance, but by high body condition scores (>3.10) and heavier live weights. The finding that RPC addition did not have an effect on plasma NEFA concentrations was in agreement with the results of Hartwell et al. (5). On the other hand, some studies reported that plasma NEFA concentrations decreased as a response to RPC addition (10).

Plasma BHBA and NEFA concentrations are frequently used to demonstrate the energy status in dairy cattle and detect subclinical ketosis. Subclinical ketosis diagnosis may be made if the plasma BHBA level is <0.9 mmol/L (21). The threshold level for NEFA concentration that indicates a negative energy balance was reported as 0.6 mmol/L (21). It was observed that limiting concentrated feed and adding choline to the ration in the prepartum period led to a decrease in serum BHBA and NEFA levels and prevented fatty liver syndrome (22). The findings of another study (10) that choline supplementation in rations did not change NEFA and BHBA levels in comparison to control groups were in agreement with those obtained in this study. Evans et al. (23) reported that choline addition to the rations of dairy cattle in the transition period may help reduce NEFA levels in the blood. In this study, there were numerical reductions in the serum NEFA levels in the groups for which RPC was added to the rations. Młynek et al. (24) reported that BHBA concentration was higher in high-yielding dairy cows than in low-yielding dairy cows, which indicates an excess of the fatty acid released from adipose tissue for the intensity of milk secretion. The results of the present study were similar to those of low-yielding dairy cows.

Consequently, it was determined that the RPC that was supplemented in the rations of dairy cattle in the transition period at different levels did not have a significant effect on their energy and protein metabolism. The main effects of the periods without group effects in the model were highly significant ($P < 0.01$) for NEFA and BHBA. There is no need to add RPC to rations formulated as isocaloric and isonitrogenic for optimal rumen fermentation in dairy cows during the transition period. To better understand the effect of RPC on dairy cows during the transition period, there is a need for further studies of animals with known metabolic profiles, and those having high milk yields or metabolic disorders.

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References

1. Drackley JK. Biology of dairy cows during the transition period: the final frontier. J Dairy Sci 1999; 82: 2259-2273.
2. Reece WO. *Dukes*' Physiology of Domestic Animals. 13th ed. New York, NY, USA: John Wiley & Sons; 2015.
3. NRC. Nutrient Requirements for Dairy Cattle. 7th Revised Edition. Washington, DC, USA: National Academy Press; 2001.
4. Pawar SP, Kewalramani N, Thakur SS, Kaur J. Effect of dietary rumen protected choline supplementation on milk choline content in crossbred cows. Indian J Anim Nutr 2015; 32: 30-35.
5. Hartwell JR, Cecava MJ, Donkin SS. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. J Dairy Sci 2000; 83: 2907-2917.

6. Winkleman LA, Elsasser TH, Reynolds CK. Limit-feeding a high-energy diet to meet energy requirements in the dry period alters plasma metabolite concentrations but does not affect intake or milk production in early lactation. J Dairy Sci 2008; 91: 1067-1079.

7. AOAC. Official Methods of Analysis. 18th ed. Arlington, VA, USA: Association of Official Analytical Chemists; 2006.

8. Turkish Standards Institution. Hayvan Yemleri-Metabolik Enerji Tayini (Kimyasal Metot). TSE No: 9610. Ankara, Turkey: TSE; 1991 (in Turkish).

9. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 74: 3583-3597.

10. Cooke RF, Silva Del Rio N, Caraviello DZ, Bertics SJ, Ramos MH, Grummer RR. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. J Dairy Sci 2007; 90: 2413-2418.

11. Erdman RA, Sharma BK. Effect of dietary rumen-protected choline in lactating dairy cows. J Dairy Sci 1991; 74: 1641-1647.

12. Pineda A, Cardoso FC. Effects of rumen-protected choline with calcium salts of long chain fatty acids on milk yield and milk composition of middle and late lactation Holstein cows. Livest Sci 2015; 175: 37-46.

13. Guretzky NAJ, Carlson DB, Garrett JE, Drackley JK. Lipid metabolite profiles and milk production for Holstein and jersey cows fed rumen protected choline during the periparturient period. J Dairy Sci 2006; 89: 188-200.

14. Pawar SP, Kewalramani N, Thakur SS, Kaur J. Effect of dietary rumen protected choline supplementation on milk choline content in crossbred cows. Indian J Anim Nutr 2015; 32: 30-35.

15. Zom RLG, Baal JV, Goselink RMA, Bakker JA, Veth MJ, Vuuren AM. Effect of rumen protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. J Dairy Sci 2011; 94: 4016-4027.

16. Minuti A, Palladino A, Khan MJ, Alqarni S, Agrawal A, Piccioli-Capelli F, Hidalgo F, Cardoso FC, Trevisi E, Loor JJ. Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the peripartal period in dairy cows. J Dairy Sci 2015; 98: 8940-8951.

17. Cetinkaya N, Ozcan H, Gucus AI, Uluturk S, Tosun I. The use of blood metabolite profiles for monitoring health and nutritional status of dairy cattle. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 1997; 37: 37-46 (in Turkish with an abstract in English).

18. Salman M, Bölükbaş B. Metabolic profiling and analytical testing in dairy cow during transition period. In: 1st International Animal Nutrition Congress; Antalya, Turkey; 2016. pp. 298-302.

19. Uyarlar C. Geçiş dönemindeki süt ineklerine rasyona ilave olarak verilen niasin, kolin ve biotinin bazı kan ve süt parametreleri üzerine etkisi. PhD, Afyon Kocatepe University, Afyonkarahisar, Turkey, 2010 (in Turkish).

20. Whitaker DA. Interpretation of metabolic profiles in dairy cows. Cattle Pract 1997; 5: 57-60.

21. Hiss S, Weinkauf C, Hachenberg S, Sauerwein H. Short communication: Relationship between metabolic status and the milk concentrations of haptoglobin and lactoferrin in dairy cows during early lactation. J Dairy Sci 2009; 92: 4439-4443.

22. Bertics SJ, Grummer RR. Effects of fat and methionine hydroxy analog on prevention or alleviation of fatty liver induced by feed restriction. J Dairy Sci 1999; 82: 2731-2736.

23. Evans E, Mair DT, Gauthier R, Fontaine J. Effects of a protected vitamin and choline supplement in the transition period on dairy cow metabolic parameters and health. Professional Animal Scientist 2006; 22: 164-169.

24. Młynek K, Głowińska B, Salomończyk E, Tkaczuk J, Styś W. The effect of daily milk production on the milk composition and energy management indicators in Holstein–Friesian and Simmental cows. Turk J Vet Anim Sci 2018; 42: 223-229.