Kafkas Univ Vet Fak Derg
24 (2): 289-293, 2018
DOI: 10.9775/kvfd.2017.18854

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://submit.vetdergikafkas.org

Research Article

Improved Lactational Performance in Dairy Cows Supplemented with Methionine or Rumen-Protected Choline During the Transition Period [1]

Ismail CETIN [1] Ibrahim Ismet TURKMEN [2] Cagdas KARA [2]
Abdulkadir ORMAN [3] Erkan SEN [4]

[1] *This study was funded by the Uludag University Scientific Research Unit Grant (Project No: OUAP (V)-2014/4)*

[1] Namik Kemal University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, TR-59030 Tekirdag - TURKEY
[2] Uludag University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, TR-16059 Bursa - TURKEY
[3] Uludag University, Faculty of Veterinary Medicine, Department of Zootechnics, TR-16059 Bursa - TURKEY
[4] Uludag University, Institute of Health Science, Department of Animal Nutrition and Nutritional Diseases, TR-16059 Bursa - TURKEY

Article Code: KVFD-2017-18854 Received: 13.10.2017 Accepted: 22.01.2018 Published Online: 22.01.2018

How to Cite This Article

Cetin I, Turkmen II, Kara C, Orman A, Sen E: Improved lactational performance in dairy cows supplemented with methionine or rumen-protected choline during the transition period. *Kafkas Univ Vet Fak Derg,* 24 (2): 289-293, 2018. DOI: 10.9775/kvfd.2017.18854

Abstract

The current study was aimed to determine the effects of dietary supplementation with rumen-protected choline and methionine on dry matter intake, milk yield, milk composition and body condition score of transition cows. In this study, 32 Holstein dairy cows which made one parturition at least was used as the animal material. The cows were randomly assigned to four groups according to lactation number, previous lactation milk yield and body condition score before close-up. Cows within each group was fed a basal control (CON) diet (n=8). Cows in treatment group methionine (MET, n=8) were supplemented with 42 g/d of methionine (Metasmart®). Cows in treatment group choline (CHOL, n=8) were supplemented with 75 g/d of rumen protected choline (RPC) source (CholiPEARL™). Cows in treatment group Mix (MIX, n=8) were supplemented with 75 g/d RPC and 42 g/d methionine. Milk yields and dry matter intakes were determined during the trial as daily. At the results of analyses that were detected; milk yield and composition the highest level in MET group (P<0.028). In conclusion of this study that was determined; the supplementation of methionine to the rations of dairy cows affected milk yield and composition positively.

Keywords: *Methionine, Rumen-protected choline, Transition periods, Dairy cows*

Geçiş Dönemindeki Süt İneklerinde Metiyonin veya Korunmuş Kolin İlavesinin Laktasyon Performansını Geliştirmesi

Öz

Bu çalışma, geçiş dönemindeki süt sığırlarında, rasyonlarına ilave edilen korunmuş kolin ve metiyoninin, kuru madde tüketimi, süt verimi, süt kompozisyonu ve vücut kondisyon skoru üzerine etkilerini değerlendirmek amacıyla yapıldı. Çalışmada, en az bir doğum yapmış 32 adet Holştayn inek kullanıldı. İnekler, laktasyon sayısı, önceki süt verimi ve geç kuru dönem öncesindeki kondisyon skorlarına göre rastgele dört gruba ayrıldı. Bütün gruplardaki inekler bazal kontrol rasyonu ile beslendi. Metiyonin grubundaki ineklere günlük 42 g metiyonin ilave edildi. Kolin grubundaki ineklere günlük 75 g korunmuş kolin ilave edildi. Miks grubundaki ineklere günlük 75 g korunmuş kolin ve 42 g metiyonin ilave edildi. Süt verimleri ve kuru madde tüketimleri günlük belirlendi. Yapılan analizler sonucunda süt verimi ve süt bileşenlerinin MET grubunda en yüksek düzeyde olduğu tespit edilmiştir (P<0.028). Sonuç olarak, yüksek verimli süt sığırlarının rasyonlarına korunmuş metiyonin ilavesinin, süt verimi ve bileşenlerini olumlu yönde etkilediği belirlenmiştir.

Anahtar sözcükler: *Metiyonin, Korunmuş kolin, Geçiş dönemi, Süt sığırı*

İletişim (Correspondence)
+90 282 2504734; Fax +90 282 2509960
ismailcetin@nku.edu.tr

INTRODUCTION

The periparturient period (transition period), defined as 3 weeks before calving and 3-4 weeks postpartum, dairy cattle enter into a period of negative energy and metabolizable protein balance as a result of increased metabolic demand by the mammary gland and the low dry matter intake (DMI) [1,2]. Methyl donors are needed for the synthesis of important compounds such as phosphatidylcholine and carnitine [3].

Choline and methionine are key methyl donors in mammals and their availability is important for various biological functions. Methionine is an essential amino acid and building block for protein and typically is considered one of the two most limiting amino acids for the production of milk and milk protein in lactating dairy cows [4,5]. 6% of the available choline in the body is derived from methionine and 28% of the body's methionine is used for choline synthesis [6]. The protected choline in the rations contributes to the backup of methionine [7]. Protected choline in rations can be caused to waste the methionine for synthesis of daily choline requirements, and this methionine can be used for milk production [8]. Moreover, the addition of protected choline to the rations leads to the release of more of the methionine for milk protein synthesis and affects the milk protein level positively [8-10]. Some of the studies have detected a significant effect of methionine [2,11,12] or choline [13,14] supplementation though other studies did not determine beneficial improvements on peripartal production performance with methionine [15,16] or choline [8].

The objectives of this study were to evaluate the effects of feeding methionine products or rumen-protected choline, and both (methionine + choline) on DMI, body condition score (BCS), milk yield and composition during the transitional period of high yield milk cows.

MATERIAL and METHODS

Experimental Design and Dietary Treatments

The experimental protocols were applied by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institue of Health Guide for the care and use of Laboratory Animals. The study was carried out with the permission of Uludag University Animal Experimentation Local Ethics Committee (Approval No: 2013-14/04)

In this study, a total of 32 high-yielding Holstein breed milk cows with at least one birth were used. The cows were randomly assigned to four groups according to lactation number, previous lactation milk yield and BCS before close-up. Cows within each group was fed a basal control (CON) diet (n=8). Cows in treatment group methionine (MET, n=8) were supplemented with 42 g of methionine (Metasmart^{R}). Cows in treatment group choline (CHOL, n=8) were supplemented with 75 g of rumen protected choline (RPC) source (CholiPEARL^{TM}). Cows in treatment group mix (MIX, n=8) were supplemented with 75 g RPC and 42 g methionine. The dosage of methionine and choline were supplied by the manufacturer's recommendations. All cows fed the same close-up diet from -21±2 day to calving and the same lactation diet from calving to 70 days in milk (DIM). Ingredients and chemical compositions of the diets were determined according to National Research Council [4] recommendations *(Table 1, Table 2)*.

Methionine and RPC were supplemented as a topdressed from -21±2 day to 21 DIM once daily at the a.m. feeding. The Metasmart^{R} used in this study contains 57% of 2-hydroxy-4-methylthio butanoic acid isopropyl ester. The CholiPEARl^{TM} supplement contains 24% choline chloride and is protected by spray freezing technology.

Animal Management

Experimental studies were carried out at the Omer Matli Animal Production Training and Research Center in Bursa, Turkey from November 2013 to June 2014, with an average temperature of 11.3±10.7ºC. The animals used in the research were housed in a semi-open free-standing stall and automatic feeders were used to determine the individual dry matter intake. Dry cows were fed individually the close up diet once daily (07:00 h). After calving, cows were individually fed a common lactation diet once daily (09:00) to allow for ad libitum consumption. Close-up and lactation diets were mixed daily and fed as a total mixed ration. DMI was determined daily for each animal from 21

Table 1. *Ingredients and chemical composition of close-up diet*

Ingredients	**%DM[1]**
Wheat straw	21.75
Alfalfa hay	18.10
Corn silage	22.22
Commercial concentrate mixture[2]	37.26
Ammonium chloride	0.67
Chemical Composition	**%DM[1]**
Neutral detergent fibre	48.15
Acid detergent fibre	28.60
Crude protein	13.29
Ether extract	4.14
Ash	7.82
Non-fibre carbonhydrates[3]	26.6
Calcium	1.02
Phosphorus	0.28

[1] Dry Matter; [2] Commercial Concentrate Mixture, Matli Feed Industry, Karacabey/Turkey; [3] Non-fibre carbonhydrates, 100 – (% NDF + % CP + % EE + % Ash)

Table 2. *Ingredients and chemical composition of early lactation diet*

Ingredients	%DM[1]
Wheat straw	7.26
Alfalfa hay	21.28
Corn silage	25.98
Commercial concentrate mixture[2]	43.98
Corn gluten	0.71
Sodium bicarbonate	0.54
Magnesium oxide	0.25
Chemical composition	**%DM[1]**
Neutral detergent fibre	43.76
Acid detergent fibre	24.43
Crude protein	16.43
Ether extract	5.62
Ash	7.99
Non-fibre carbonhydrates[3]	26.2
Calcium	0.92
Phosphorus	0.63

[1] Dry Matter; [2] Commercial Concentrate Mixture, Matli Feed Industry, Karacabey/Turkey (Ingredients: Corn DDGS, corn, soybean meal,48% CP, wheat bran, rice bran, full fat soybean, limestone, sunflower meal, salt, vitamin-mineral premixes); [3] Non-fibre carbonhydrates, 100 – (% NDF + % CP + % EE + % Ash)

days before the expected parturition to the first 70 days of lactation. Cows were milked 3 times at nearly 06:00, 14:00 and 22:00 h. Milk yield was recorded daily at 70 DIM. At the end of the research, the cows returned to the farm herds at 70 DIM.

BCS were determined by the same observer for 21 days before calving (-21 day), time of calving (0 day), 21 days after calving (+21 day) and 70 days after calving (+70 day). The determination of the BCS was based on five-point scale with 0.25 unit intervals (scale 1 = thin, to 5 = obese) [17].

Feed and Milk Samples

Weekly samples were frozen at -20ºC and then composited montly for analyses. Chemical analysis (dry matter, crude protein, ether extract, ash, calcium, phosphorus) of diets were performed according to AOAC [18], neutral detergent fiber and acid detergent fiber analyses were performed according to Van Soest et al.[19].

Homogenous milk samples were collected individually from all cows for two consecutive days each week starting from the 8th day to the 70th day of lactation following the calving by means of the sampling equipment of the milking equipment. On the same day, the milk samples were analyzed with the milk analyzer (MilkoScan™ FT1 User Manual 6004 5478/Rev 1) for fat, protein, lactose, solid non-fat, total solids and milk urea nitrogen.

Statistical Analysis

BCS, DMI and milk yields were evaluated using the 'General Linear Model'. Model; The numbers of subjects (cows) were entered as random effect, duration and group as a fixed effect. One-way analysis of variance was used to compare milk components. Significance was declared at $P<0.05$. Statistical analyses of the data were performed using SPSS [20] (version 20.0, SPSS Inc, USA) program.

RESULTS

This study was conducted to determine the effect of feeding methionine products or rumen-protected choline and both (methionine + choline) on DMI, BCS, milk yield and composition during the transitional period of high yield milk cows.

DMI during the experiment are presented for all groups and periods in *Table 3*. There were no statistical differences among the experimental groups for the close-up period DMI ($P>0.05$). Significant differences were determined among the treatment groups in terms of lactation DMI ($P<0.028$). The DMI during the early lactation period averaged 21.17 kg/d for CON, 21.89 kg/d for MET, 19.90 kg/d for CHOL and 22.01 kg/d for MIX. The effects of methionine and RPC supplementation on milk yield and milk composition were shown in *Table 3*. Milk yield and milk composition were significantly affected by dietary treatments ($P<0.028$). However, dietary treatments did not any significant effect on milk urea nitrogen. Methionine supplementation significantly increased milk yield, milk fat, milk protein, milk lactose, solid non-fat and total solids at the early lactation period ($P<0.028$).

There were no statistical differences for body condition scores among treatments. But body condition variation was significantly affected by the addition of methionine and RPC. Cows lost 0.97, 0.72, 0.81 and 0.53 body score unit between close-up period and week 10 of lactation for CON, MET, CHOL and MIX, respectively *(Table 4)*. Body condition variation was the lowest in MIX.

DISCUSSION

The effects of rumen-protected methionine supplementation on DMI in previous studies have been conflicted by some researchers [2,11,12,16]. For example, Zhou et al.[2] observed an increase in prepartal and postpartal DMI with Smartamine M. Although Ordway et al.[16] determined an increase in postpartal DMI with Metasmart supplementation, there is no differences in groups fed by Smartamine M. On the other hand, Socha et al.[15] detected that there was no effect of feeding Smartamine M or Smartamine ML during the prepartum period on prepartum DMI during the last week of gestation. In the present study, differences in mean prepartum DMI were not determined; but postpartum DMI for MET and MIX treatments were significantly higher

Table 3. *Effect of supplementing Holstein cows during the peripartal period with methionine (Metasmart®), rumen-protected choline (CholiPEARL™) or both on DMI, milk yield and milk composition*

Parameters	CON X±Sx	MET X±Sx	CHOL X±Sx	MIX X±Sx
Close-up DMI (kg/d)	14.70±2.66	14.79±3.24	13.99±2.82	13.92±3.55
Lactation DMI (kg/d)	21.17±4.4^{b}	21.89±4.12^{a}	19.90±4.27^{c}	22.01±4.13^{a}
Milk yield (kg/d)	40.85±5.5^{c}	42.88±4.72^{a}	40.05±5.93^{c}	42.00±5.09^{b}
Milk fat (%)	3.80±1.07^{b}	4.02±1.20^{a}	3.49±1.13^{c}	3.73±1.05^{b}
Milk protein (%)	3.06±0.37^{b}	3.17±0.35^{a}	2.93±0.43^{c}	3.04±0.32^{b}
Milk lactose (%)	4.61±0.16^{b}	4.74±0.20^{a}	4.63±0.22^{b}	4.63±0.17^{b}
Solids non fat(%)	8.51±0.49^{b}	8.76±0.48^{a}	8.38±0.49^{c}	8.51±0.40^{b}
Total solids (%)	12.42±1.3^{b}	12.93±1.46^{a}	11.93±1,42^{c}	12.35±1.30^{b}
Milk urea nitrogen (mg/dL)	14.45±3.73	14.30±3.54	14.27±2.94	14.26±3.29

Different superscripts indicate stastical differences ^{a,c} P<0.028

Table 4. *Effect of supplementing Holstein cows during the peripartal period with methionine (Metasmart®), rumen-protected choline (CholiPEARL™) or both on body condition score*

Weeks	CON X±Sx	MET X±Sx	CHOL X±Sx	MIX X±Sx
-3. week	3.53±0.16	3.66±0.38	3.47±0.28	3.53±0.36
Calving	3.56±0.18	3.59±0.35	3.47±0.28	3.59±0.38
3. week	2.87±0.30	3.19±0.37	2.87±0.46	3.16±0.42
10. week	2.64±0.32	2.93±0.28	2.64±0.43	3.00±0.38
Variation*	0.97±0.21^{a}	0.72±0.31^{ab}	0.81±0.35^{ab}	0.53±0.16^{b}

** -3. week body condition score and lactation 10. week body condition score difference; Different superscripts indicate stastical differences ^{a,b} P<0.046*

than for the CON treatment. In accordance with previous reports, rumen-protected choline supplementation did not affect DMI [8,21,22]. As a result of many scientific studies have shown that supplemented with methionine and rumen-protected choline to transition rations in dairy cattle may be caused different DMI. It has been suggested that the contradictory results both in our and other studies may be due to the differences in the level of methionine and choline supplementation, length of feeding, stage of lactation, or combination of these.

Methionine or its analogue supplements have an important role in milk yield production [2,12,23]. In our study, methionine supplementation resulted in an increased milk yield and milk components whereas milk urea nitrogen was not significantly affected. Some other studies there were no differences defined by rumen-protected methionine [15] or methionine analog supplement [16]. The methionine has been identified as one of the two most limiting amino acid for lactating dairy cows and this is a greater DMI which increase the daily protein intake [4]. Thereby, milk yield was being optimum as expected due to the optimum Lys:Met ratio. Many studies have shown that supplementing with rumen-protected methionine has improved milk protein synthesis [2,12,16]. It was not a surprise increase in milk protein due to increased milk yield in MET group. Moreover, the significant increase in milk composition were due to the increase in milk yield. High percentages of total solids in the MET group was probably associated with over milk protein and milk fat percentage in those cows. Hartwel et al.[24] reported that milk yield and composition were not affected by supplementing with choline chloride. Also, Guretzky et al.[8] observed that supplementing choline from -21 days to 42 days or longer did not affect the milk yield and composition. The result of our study was in agreement with Hartwel et al.[23] and Guretzky et al.[8]. Although significant effects on milk yield were reported in studies when choline was supplemented during the peripartal period [13,25,26], in the present study, the milk yield is lower in the CON and CHOL groups than in the other treatment groups, suggesting that lactation DMI is less in these groups. Zahra et al.[25] determined greater milk yield with supplemental choline in dairy cows with a BCS of 4 beginning the close-up period that was driven primarily by greater DMI. So, our results from the present study do not seem to support the suggestion by Zahra et al.[25]. Nevertheless, there were no statistical differences in BCS among treatments. In the previous studies, methionine or choline supplementation did not effect of BCS were reported [2,16]. However, in the present study, the differences in body condition variations were significant which would suggest that fat mobilization was the highest in CON group.

In conclusion, current study results suggested that supplementation of methionine to the rations of dairy cows affected milk yield and composition positively. Given the change in body condition variations, the control group was observed more loss of condition than the supplemented with methionine, rumen protected-choline or both. More studies must be conducted to evaluate effects of supplemented with methionine, rumen-protected choline or both during the transition period on dairy cow performance.

REFERENCES

1. Drackley JK: Biology of dairy cows during the transition period: the final frontier. *J Dairy Sci,* 82, 2259-2273, 1999. DOI: 10.3168/jds.S0022-0302(99)75474-3

2. Zhou Z, Vailati-Riboni M, Trevisi E, Drackley JK, Luchini DN, Loor JJ: Better postpartal performance in dairy cows supplemented with rumen protected methionine compared with choline during the peripartal period. *J Dairy Sci,* 99, 8716-8732, 2016. DOI: 10.3168/jds.2015-10525

3. Pinotti L, Baldi A, Dell'Orto V: Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. *Nutr Res Rev,* 15, 315-332, 2002. DOI: 10.1079/NRR200247

4. NRC: National Research Council. Nutrient Requirements of Dairy Cattle. 7th revised edn., National Academy Press, Washington, DC, 2001.

5. Civelek T, Birdane F, Kabu M, Cıngı CC, Acar A: Effects of methionine and lysine on metabolic profile in dairy cattle during periparturients period. *Kafkas Univ Vet Fak Derg,* 19 (3): 423-432, 2013. DOI: 10.9775/kvfd.2012.7968

6. Emmanuel B, Kennelly JJ: Kinetics of methionine and choline and their incorporation into plasma lipids and milk components in lactating goats. *J Dairy Sci,* 67, 1912-1918, 1984. DOI: 10.3168/jds.S0022-0302(84)81524-6

7. Hutjens MF: Feed additives. *Vet Clin North Am: Food Anim Pract,* 7 (2): 525-540, 1991.

8. Janovick Guretzky NA, 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,* 89, 188-200, 2006. DOI: 10.3168/jds.S0022-0302(06)72083-5

9. Pinotti L, Baldi A, Savoini G, Dell'Orto V: Effects of rumen protected choline on lipid metabolism in periparturient high yielding dairy cows. *Livest Prod Sci,* 70, 176 (Abstr.): 2001.

10. Sales J, Homolka P, Koukolova V: Effect of dietary rumen-protected choline on milk production of dairy cows: A meta-analysis. *J Dairy Sci,* 93, 3746-3754, 2010. DOI: 10.3168/jds.2010-3106

11. Chen ZH, Broderick GA, Luchini ND, Sloan BK, Devillard E: Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. *J Dairy Sci,* 94, 1978-1988, 2011. DOI: 10.3168/jds.2010-3578

12. Osorio JS, Ji P, Drackley JK, Luchini D, Loor JJ: Suplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J Dairy Sci,* 96, 6248-6263, 2013. DOI: 10.3168/jds.2012-5790

13. Pinotti L, Baldi A, Politis I, Rebucci R, Sangalli L, Dell'Orto V: Rumen-protected choline administration to transition cows: Effects on milk production and vitamin E status. *J Vet Med A Physiol Pathol Clin Med,* 50, 18-21, 2003. DOI: 10.1046/j.1439-0442.2003.00502.x

14. Zom RL, van Baal J, Goselink RM, Bakker JA, de Veth MJ,van Vuuren AM: Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. *J Dairy Sci,* 94, 4016-4027, 2011. DOI: 10.3168/jds.2011-4233

15. Socha MT, Putnam DE, Garthwaite BD, Whitehouse NL, Kierstead NA, Schwab CG, Ducharme GA, Robert JC: Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. *J Dairy Sci,* 88, 1113-1126, 2005. DOI: 10.3168/jds.S0022-0302(05)72778-8

16. Ordway RS, Boucher SE, Whitehouse NL, Schwab CG, Sloan BK: Effects of providing two forms of suplemental methionine to periparturient Holstein dairy cows on feed intake and lactional performance. *J Dairy Sci,* 92, 5154-5166, 2009. DOI: 10.3168/jds.2009-2259

17. Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt HF, Lesch TN: A dairy cow body condition scoring system and its relationship to selected production characteristics. *J Dairy Sci,* 65, 495-501, 1982. DOI: 10.3168/jds.S0022-0302(82)82223-6

18. AOAC: Official Methods of Analysis. Association of Official Analytic Chemists. 15th edition. Arlington, Chapter 4, 1-56, 1990.

19. Van Soest PJ, Robertson JB, Lewis BA: Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci,* 74 (10): 3583-3597, 1991. DOI: 10.3168/jds.S0022-0302(91)78551-2

20. SPSS: Statistical Package for the Social Science. Base System User's Guide, Version 20.0. SPSS, Armonk, NY, IBM Corp, USA, 2011.

21. Piepenbrink MS, Overton TR: Liver metabolism and production of cows fed ıncreasing amounts of rumen-protected choline during the periparturient period. *J Dairy Sci,* 86, 1722-1733, 2003. DOI: 10.3168/jds.S0022-0302(03)73758-8

22. Xu G, Ye J, Liu J, Yu Y: Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *Asian-Aust J Anim Sci,* 19 (3): 390-395, 2006. DOI: 10.5713/ajas.2006.390

23. Piepenbrink MS, Marr AL, Waldron MR, Butler WR, Overton TR, Vazquez-Anon M, Holt MD: Feeding 2-Hydroxy-4-(Methylthio)-Butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. *J Dairy Sci,* 87, 1071-1084, 2004. DOI: 10.3168/jds.S0022-0302(04)73253-1

24. Hartwell JR, Cecava MJ, Donkin SS: Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum triacylglyceride, plasma metabolites and milk production in transition dairy cows. *J Dairy Sci,* 83, 2907-2917, 2000. DOI: 10.3168/jds.S0022-0302(00)75191-5

25. Zahra LC, Duffield TF, Leslie KE, Overton TR, Putnam D, LeBlanc SJ: Effects of rumen-protected choline and monensin on milk production and metabolism of periparturient dairy cows. *J Dairy Sci,* 89, 4808-4818, 2006. DOI: 10.3168/jds.S0022-0302(06)72530-9

26. Elek P, Newbold JR, Gaal T, Wagner L,Husveth F: Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. *Animal,* 2, 1595-1601, 2008. DOI: 10.1017/S1751731108002917