Iranian Journal of Applied Animal Science

# Effects of Feeding Rumen Protected Choline and Vitamin E on Milk Yield, Milk Composition, Dry Matter Intake, Body Condition Score and Body Weight in Early Lactating Dairy Cows

Research Article

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Received on: 25 Nov 2013
Revised on: 21 Jan 2014
Accepted on: 30 Jan 2014
Online Published on: Dec 2014

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Online version is available on: www.ijas.ir



## ABSTRACT

Twenty four primiparous and multiparous Holstein cows on early lactation, beginning five weeks postpartum, were used for four weeks to investigate the effects of supplemention of rumen-protected choline (RPC) or vitamin E on milk yield, milk composition, dry matter intake, body condition score and body weight. The cows were randomly assigned to one of the following treatments: I) no supplement (control), II) 90 g/d of RPC and III) 4400 IU/d of vitamin E. In this study, dry matter intake, body weight, body condition score, milk yield and the contents and yields of all milk constituents, except the content of solid non fat (SNF) for RPC group, were not affected by choline or vitamin E supplementation (P>0.05). The RPC increased (P<0.05) the content of SNF compared with the control and vitamin E groups. The results showed that RPC or vitamin E supplementation into the diets of early lactating dairy cows did not affect dry matter intake, milk yield, milk composition, body condition score and body weight.

KEY WORDS choline, cow, feed intake, live weight, milk production, vitamin E.

## INTRODUCTION

Due to rapid increase in milk production after calving, cows undergo a period of reduced feed intake, negative energy balance and lipolysis (Goff and Horst, 1997). Glycerol, which is released from lipolysis, is used by liver for gluconeogenesis and the fatty acids which are released as non-esterified fatty acids (NEFA) are distributed with blood flow to all body tissues (Drackley *et al.* 2001). When NEFA concentrations are elevated during early lactation, mammary glands take them up efficiently and convert them to milk fat. Thus, high milk fat concentrations, or high milk ratios of fat to protein, are useful indicators for ketosis in dairy cows. Choline is a vitamin-like compound which is an essential component of phospholipids. Choline involves in the structure of lipoproteins and export lipids from liver in the form of very low density lipoproteins (VLDL). Choline may directly increase VLDL synthesis or serve to increase methionine availability for lipoprotein synthesis to indirectly alter liver triacylglyceride clearance as VLDL (Bell, 1995). Hence, choline deficiency in dairy cows may be associated with hepatic lipidosis (Hartwell *et al.* 2000). Most dietary choline is degraded by rumen microorganisms (Erdman and Sharma, 1991), thus choline must be in rumen-protected form when fed. Rumen-protected choline (RPC) is fed to dairy cows to increase the supply of choline to small intestine with the goal of increasing milk or component yields, or alleviating the development of fatty liver through enhancing export of fat from liver (Piepenbrink and Overton, 2003; Cooke *et al.* 2004); because RPC supplementation may spare methionine (Pinotti *et al.* 2002; Piepenbrink and Overton, 2003; Overton and Waldron, 2004). In a study, increasing post-ruminal supply of choline by an infusion of choline into the abomasum increased milk yield and milk fat yield (Erdman and Sharma, 1991) and in another study it was shown that feeding RPC tended to increase yields of milk fat, 3.5% fat-corrected milk (FCM) and total solids (Piepenbrink and Overton, 2003). It is suggested that early lactating cows produce more milk when they receive RPC (Erdman and Sharma, 1991; Pinotti *et al.* 2003). There may be several explanations for the effects of choline supplementation on milk yield and milk protein in dairy cows. First, choline may serve to spare methyl groups and hence S-adenosylmethionine, as the methyl group donor usually necessary for the synthesis of lecithin. Second, choline may serve as the methyl group donor for remethylization of homocysteine via its metabolite betaine. Third, betaine may also spare methionine by substituting for S-adenosylmethionine as the methyl group donor in some metabolic processes (Brüsemeister and Südekum, 2006).

Vitamin E ($\alpha$-tocopherol) is a powerful antioxidant that lowers oxidative stress and influences the health of dairy cows (Burton and Traber, 1990) and is not degraded in the anaerobic ruminal environment (Burton and Traber, 1990; Leedle *et al.* 1993). Vitamin E is absorbed and transported from intestine to liver following the same mechanisms as lipids. In the liver, $\alpha$-tocopherol is packaged in lipoproteins and distributed from the liver via plasma to the rest of the body (Herdt and Smith, 1996). In human, vitamin E supplementation was effective in improving liver function in patients with non-alcoholic fatty liver disease (NAFLD) (Patel and Babich, 2010). In obese children with NAFLD, vitamin E has been demonstrated to significantly improve liver function (Levine, 2000) and on rats the group which used vitamin E presented better liver function among the animals with NAFLD (Idilio-Zamin *et al.* 2010).

Since cows are susceptible to fatty liver and ketosis on early lactation, most dairy farmers use choline to improve production performance, thus we decided to study whether vitamin E also affect production performance in dairy cows on early lactation. Therefore, the objectives of this study were to investigate the effects of supplementation of RPC or vitamin E, and also to compare the effects of RPC with vitamin E on milk yield, milk components, dry matter intake (DMI), body condition score (BCS) and body weight (BW) in dairy cows on early lactation.

## MATERIALS AND METHODS

### Experimental animals

In the present study, twenty four early lactating primiparous and multiparous Holstein cows beginning five weeks postpartum (BCS=2.82±0.12; mean±SD and number of lactation=2.56; mean) were used for four weeks from October 2011 to November 2011.

The cows were housed in individual tie stalls and fed basal diet for two weeks before the experiment. All experimental procedures were in accordance with the guidelines for the use and care of experimental animals which are approved by the animal ethical committee of Tehran University.

Selection of the cows was based on parity, milk yield of previous lactation (milk yield of dams for the cows in their first lactation) and BCS. The cows were randomly divided into three groups of eight cows each, and each group received one of the following treatments: I) no supplement (control), II) 90 g/d of RPC and III) 4400 IU/d of vitamin E. The RPC (ReaShure; Balchem, New Hampton, NY, USA; 25% choline) was a rumen protected source of choline chloride and the vitamin E was the product of Roche Company (Basel, Switzerland).

The cows were fed total mixed rations (TMR) *ad libitum*. The supplements were top dressed onto the TMR. The diet (Table 1) was formulated to meet nutritional requirements of dairy cows (NRC, 2001). Throughout the experiment, the amounts of feed which were offered and refused were weighed and recorded daily, and were taken once per week and frozen until the samples were processed for analyses. Weekly feed and ort samples were collected and dried at 60 ˚C for 72 hours, and then weighed to determine moisture loss.

The dried samples were ground through a 1 mm screen and the nutrient contents of the samples were analyzed weekly. Chemical analyses of the samples were based on the AOAC Official Method (AOAC, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also determined as described by Van Soest (Van soest *et al.* 1991). The NDF was assayed with a heat-stable amylase and both NDF and ADF were expressed inclusive of residual ash. Non-fibrous carbohydrate (NFC) was calculated as the difference between 100 and the sum of crude protein, NDF, total fat and ash.

### Data and sample collection

The cows were milked thrice daily at 8-h intervals and individual milk yields were recorded daily for each cow. Besides, weekly on one day, milk samples of each cow were collected at 3 consecutive milkings and then milk samples were analyzed for the concentrations of fat, protein, lactose, solids not fat (SNF) and total solids (TS) by a milk analyzer (EkoMilk Total, Bulgaria). Then the yields of fat, protein, lactose, SNF and TS of each cow were calculated from the recorded milk weights and the concentrations of fat, protein, lactose, SNF and TS in the milk samples.

**Table 1** Ingredients and chemical composition of the diet

| Ingredients | Amounts (g/kg of DM) | Chemical composition | Values |
|---|---|---|---|
| Alfalfa hay (medium chopped) | 204.7 | DM (g/kg)[3] | 590 |
| Corn silage | 175.8 | CP (g/kg)[3] | 171.7 |
| Beet pulp | 41.3 | Ash (g/kg)[3] | 57.6 |
| Ground barley grain | 198.8 | Total fat (g/kg)[3] | 43.8 |
| Ground corn grain | 58.7 | NDF (g/kg)[3] | 304.8 |
| Ground wheat grain | 28.5 | ADF (g/kg)[3] | 183.8 |
| Solvent extracted soybean meal | 79.9 | NFC (g/kg)[3,5] | 382.1 |
| Wheat bran | 7.1 | Ca (g/kg)[3] | 8.1 |
| High lint whole cottonseed | 29.5 | P (g/kg)[3] | 5.0 |
| Canola meal | 100.2 | NEL (Mcal/kg)[4] | 1.66 |
| Corn gluten meal | 11.5 | RUP (g/kg of CP)[4] | 314.5 |
| Minerals and vitamins supplement[1] | 6.3 | RDP (g/kg of CP)[4] | 685.5 |
| Fat supplement (energy booster)[2] | 15.9 | Met (g/kg of MP)[4] | 22.1 |
| Salt | 2.5 | Lys (g/kg of MP)[4] | 76.9 |
| Calcium carbonate | 3.2 | Vitamin E (IU/kg)[4] | 18.9 |
| Sodium bicarbonate | 10.2 | - | - |
| Di calcium phosphate | 3.7 | - | - |
| Magnesium oxide | 1.9 | - | - |
| Mycosorb | 0.6 | - | - |
| Biotin premix | 0.7 | - | - |
| Zeolit | 19.0 | - | - |

[1] Contained 190 g Ca/kg; 90 g P/kg; 30 g Mg/kg; 4 g Fe/kg; 0.5 g Cu/kg; 5 g Mn/kg; 4 g Zn/kg; 0.1 g Co/kg; 0.1 g I/kg; 0.03 g Se/kg; 0.4 g antioxidant/kg; $5 \times 10^5$ IU vitamin A/kg; $10^5$ IU vitamin D/kg and $3 \times 10^3$ IU vitamin E/kg.
[2] Rumen protected fat - energizer RP10.
[3] The nutrients which were determined by laboratory. Analysis conducted with four TMR samples.
[4] The nutrients which were calculated using the standards (NRC, 2001).
[5] NFC= 1000 - (CP+ash+total fat+NDF).
DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non fibrous carbohydrate; NEL: net energy for lactation; RUP: rumen undegradable protein and RDP: rumen degradable protein.

The 4% FCM (fat corrected milk) for each cow was calculated from daily milk yield and the percentage of milk fat using the formula as given by Gains (Gaines, 1928):
FCM= [(0.4×kg milk) + (0.15×kg milk×fat %)].

Also, the yield of energy corrected milk (ECM) was calculated by the following formula (Defrain *et al.* 2006):

ECM= [(0.327×kg milk) + (12.95×kg fat) + (7.2×kg protein)].

Feed efficiency is defined as kilograms of milk produced per kilogram of dry matter consumed, and ECM efficiency is expressed as the ratio of ECM to DMI.

### Body weight and body condition score

In the first and last day of the experiment, the cows were weighed after milking at the same time of the day to record the initial and final BWs. The cows were also scored for body condition from 1 (thin) to 5 (fat) in the first and the last day of the experiment.

### Statistical analyses

Statistical analyses were performed with SAS (SAS, 2002), using Mixed procedure for DMI, milk production and milk components and GLM procedure for milk urea nitrogen (MUN), BCS and BW.

Standardized residuals were also inspected graphically to assess fit to a normal distribution. Raw data were transformed to their natural logarithm to achieve a normal distribution for analysis.

All transformed data were back-transformed for reporting least squares means. Statistical model included the effects of treatments and parity. Significant differences in the mean values among the treatments were analyzed by Tukey's tests. Significant levels were declared at $P < 0.05$.

## RESULTS AND DISCUSSION

### Milk yield and milk composition

In this research, feeding RPC and vitamin E did not increase milk yield, FCM and ECM significantly (P>0·05; Table 2).

In comparison, Zahra *et al.* (2006) reported that Holstein cows which received 56 g/day of RPC 3 weeks preparum through 4 weeks postpartum, produced on average, 1.2 kg/day more milk yield compared with unsupplemented group.

However, in agreement with the results of our study, it was reported that RPC did not affect the daily yields of 3.5% FCM, fat, protein and DMI significantly for Holstein and Jersey cows which were top dressed once daily with 60 g of RPC from 21 d prepartum through 21 d postpartum (Janovick Guretzky *et al.* 2006).

**Table 2** The milk yield, milk composition, dry matter intake, body condition score and body weight in the experimental cows fed diets with supplemental rumen-protected choline (RPC) and vitamin E

| | Treatments | | | |
|---|---|---|---|---|
| Parameters[1] | I) Control | II) RPC | III) Vitamin E | P-value |
| **BW (kg)** | | | | |
| Initial | 565.54 | 557.26 | 559.61 | - |
| Final | 552.14 | 547.95 | 553.47 | - |
| Change | -13.40±6.91 | -9.31±7.02 | -6.14±4.36 | 0.71 |
| **BCS** | | | | |
| Initial | 3.12 | 3.00 | 2.98 | - |
| Final | 2.98 | 2.90 | 2.92 | - |
| Change | -0.14±0.11 | -0.10±0.10 | -0.06±0.06 | 0.84 |
| **DMI (kg/d)** | 21.43±0.68 | 22.68±0.60 | 23.26±0.70 | 0.75 |
| **Yield (kg/d)** | | | | |
| Milk | 34.70±0.98 | 35.01±0.73 | 36.75±0.90 | 0.65 |
| 4% FCM | 31.15±0.83 | 31.23±0.59 | 32.46±1.11 | 0.75 |
| ECM | 34.51±0.91 | 34.74±0.64 | 35.91±1.20 | 0.78 |
| Fat | 1.15±0.03 | 1.16±0.02 | 1.18±0.06 | 0.87 |
| Protein | 1.12±0.03 | 1.13±0.02 | 1.17±0.03 | 0.71 |
| Lactose | 1.65±0.05 | 1.68±0.04 | 1.80±0.05 | -0.47 |
| SNF | 3.01±0.08 | 3.11±0.06 | 3.22±0.08 | 0.57 |
| TS | 4.16±0.11 | 4.26±0.08 | 4.40±0.12 | 0.65 |
| **Milk composition (%)** | | | | |
| Fat | 3.34±0.07 | 3.30±0.06 | 3.20±0.11 | 0.49 |
| Protein | 3.24±0.03 | 3.22±0.03 | 3.18±0.04 | 0.48 |
| Lactose | 4.75±0.02 | 4.79±0.02 | 4.86±0.03 | 0.15 |
| SNF | 8.67±0.04[b] | 8.87±0.05[a] | 8.74±0.03[ab] | 0.04 |
| TS | 12.01±0.09 | 12.17±0.08 | 11.94±0.11 | 0.29 |
| **MUN (mg/dL)** | 13.05±1.06 | 14.41±0.58 | 14.55±0.48 | 0.40 |
| **Feed efficiency** $_{(Milk/DMI)}$ | 1.56±0.03 | 1.57±0.03 | 1.61±0.04 | 0.83 |
| **ECM efficiency** $_{(ECM/DMI)}$ | 1.55±0.03 | 1.55±0.03 | 1.56±0.04 | 0.98 |

Values are expressed as mean ± SE (standard error).
FCM: fat corrected milk; ECM: energy corrected milk; SNF: solid non fat; TS: total solid; DMI: dry matter intake; MUN: milk urea nitrogen; BW: body weight and BCS: body condition score.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

In this study, the lack of any significant effects of dietary vitamin E supplementation on DMI, milk yield, milk protein, milk fat and lactose agree with others (Givens *et al.* 2004).

But in a study, the addition of 12000 IU of vitamin E for 21 days to fat diets increased milk fat content by 18% (Pottier *et al.* 2006) and in another study, it was reported that milk fat percentage was 6% higher when cows were fed TMR containing 10000 IU of α-tocopherol (Kay *et al.* 2005).

In this experiment, the content of SNF was affected by RPC supplementation (P<0.05) but other milk constituents were not affected by either RPC or vitamin E supplementation (P>0.05; Table 2).

The RPC increased (P<0.05) the content of SNF compared with the control and vitamin E. The lactose concentrations were not affected through the use of the supplements (P=0.16), which is in agreement with Xu *et al.* (2006) who reported that feeding RPC did not affect milk lactose in Chinese Holstein dairy cows which were supplemented with 0, 30, 60 or 90 g/d RPC from 15 d before expected calving to 15 d postpartum.

The results of our study disagree with those obtained by others (Piepenbrink and Overton, 2003; Janovick Guretzky *et al.* 2006) who showed that the cows fed RPC had increased milk fat, total solids contents and yields. It was also reported that supplemental RPC had no significant effect on the contents of protein, lactose and SNF, but their yields increased significantly (Zahra *et al.* 2006; Zom *et al.* 2011).

In the present study, the concentrations of MUN were not affected by feeding either RPC or vitamin E (P=0.40). The lack of change in MUN due to feeding RPC agrees with the study of Hartwell *et al.* (2000), who reported no change in MUN when RPC was fed either 0, 6, or 12 g/d from 28 d prepartum until 120 d postpartum. It is well known that MUN mirrors changes in blood urea level and blood urea level reflects liver function. Increased triglyceride infiltration in hepatocytes can decrease liver ability to perform ureagenesis, thus it lowers blood urea concentration.

In this study, feeding RPC or vitamin E had little or no effect on milk composition, which is affected by many factors such as breed, forage: concentration ratio, etc.

There have been conflicting results with respect to the effects of RPC supplementation.

The differences in response to rumen-protected choline might be related to the quality of the products, or the protection method which used against rumen degradation as differences in the degree of rumen-protection for different products (Kung *et al.* 2003). Also it could be due to differences in the nutritive value of the diets being fed and the production of choline by rumen bacteria.

**Dry matter intake**

Dietary RPC or vitamin E did not affect DMI (P=0.75; Table 2). It shows that these supplements could not increase the experimental cow's appetite. In agreement, several studies have reported that RPC supplementation had no effect on DMI in dairy cows (Piepenbrink and Overton, 2003; Janovick Guretzky *et al.* 2006; Zahra *et al.* 2006; Davidson *et al.* 2008).

In a study, Erdman and Sharma indicated that supplementing early lactating cows with 0, 15, 30, or 45 g/d of RPC from wk 5 to 21 postpartum did not affect DMI (Erdman and Sharma, 1991). Similarly, Piepenbrink and Overton (2003) fed cows 0, 45, 60, or 75 g/d of RPC from 21 d before expected calving to 63 d postpartum and found no effect of RPC supplementation on DMI. Likewise, Pinotti *et al.* (2003) found no influence of RPC supplementation on DMI when transition cows were fed 20 g/d of RPC from 14 d prepartum until 30 d postpartum.

**Energy corrected milk**

Greater ECM requires greater glucose and amino acids. Glucose is essential to provide NADPH for *de-novo* milk fat synthesis and ATP for protein synthesis from amino acids. In our study the supplements did not affect ECM which is in contrast with Davidson *et al.* (2008) who stated that ECM yield was higher in cows fed choline from 21 to 91 days in milk.

But Zom *et al.* (2011) reported that choline supplementation had no effect on ECM from 3 wk before until 6 wk after calving. In our study the lack of significant effects of the supplements was due to non-significant effects of the supplements on milk yield, milk fat and milk protein, because according to ECM calculation formula, these factors influence ECM.

**Body weight and body condition score**

The BW and BCS changes were not affected by RPC and vitamin E supplementation (P>0.05) but the treated groups lost less BWs than the control group, indicating that the cows fed on diets supplemented with choline or vitamin E were mobilized less body tissue in early lactation period. The results of our study for choline supplementation are in accordance with Zom *et al.* (2011) who reported that choline supplementation had no effect on BW and BCS from 3 wk before until 6 wk after calving. The lack of any significant treatment effects on BW and BCS corresponds to the lack of a significant treatment effect on energy balance.

## CONCLUSION

The results of the current study indicated that RPC and vitamin E supplementation into the diets of early lactating dairy cows did not affect dry matter intake, milk yield, milk composition, BCS and BW significantly, thus further studies are required for better understanding and also to explore the mechanism of the action of RPC and vitamin E in dairy cows on early lactation.

## ACKNOWLEDGEMENT

The authors sincerely appreciate the animal science department of Tehran University.

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