Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://tandfonline.com/loi/taar20

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**To cite this article:** A. Pirestani & M. Aghakhani (2017): The effects of rumen-protected choline and l-carnitine supplementation in the transition period on reproduction, production, and some metabolic diseases of dairy cattle, Journal of Applied Animal Research, DOI: 10.1080/09712119.2017.1332632

**To link to this article:** http://dx.doi.org/10.1080/09712119.2017.1332632

JOURNAL OF APPLIED ANIMAL RESEARCH, 2017
https://doi.org/10.1080/09712119.2017.1332632

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# The effects of rumen-protected choline and L-carnitine supplementation in the transition period on reproduction, production, and some metabolic diseases of dairy cattle

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**ABSTRACT**

This experiment was designed to examine the effects of L-carnitine and choline on production parameters, reproduction indices, as well as ketosis and fatty liver indicators in pre- and post-partum Holstein cows. A number of 120 dairy cows were randomly allocated to four treatment groups: protected choline (60 gm/day per cow), L-carnitine (50 gm/day per cow), choline + L-carnitine (60 + 50 gm/day per cow), and control group (without supplement). Treatments were given from one week before calving to four weeks after calving. Milk and blood samples were collected at the time of calving till four successive weeks. Results showed that combined choline and L-carnitine supplementation increased milk fat % (3.93 ± 0.11), but decreased solids-non-fat % (8.04 ± 0.10) and lactose % (3.92 ± 0.05). Reproduction indices – including service/conception rate (1.24 ± 0.40), calving to first visible oestrus (53.36 ± 8.20), calving to first service (59.07 ± 4.20 days), and days open (63.36 ± 8.10 days) – were improved for combined choline and L-carnitine group. A significantly lower level of aspartate aminotransferase (50.16 ± 5.96) and a higher level of beta hydroxy butyrate acid (0.31 ± 0.10) were observed in blood serum when combined choline and L-carnitine was supplemented. In conclusion, concomitant administration of choline and L-carnitine improved reproduction indices as well as the liver health index in Holstein cows.

**ARTICLE HISTORY**
Received 3 January 2016
Accepted 15 May 2017

**KEYWORDS**
Choline; L-carnitine; (re)production; transition period; dairy cattle

## 1. Introduction

The period before the onset of lactation is very critical for the accumulation of lipids in the liver and decrease in feed intake (Hartwell et al. 2000). The post-partum period of dairy cattle is characterized by negative energy balance during the recovery status from the parturition event and subsequently for milk production as well as restarting reproduction function (Piepenbrink and Overton 2003).

Choline is a compound that metabolically interacts very closely with methionine and vitamin $B_{12}$ metabolism (Janovick Guretzky et al. 2006). Ruminally protected choline improves the growth performance of finishing cattle without negative effect on carcass characteristics. However, there are contradictory results on the interaction between dietary fat and supplemental choline (Overton and Waldron 2004; Janovick Guretzky et al. 2006). The mechanism by which choline improves growth performance may be due to alterations in lipid metabolism and/or transport. In dairy cattle, choline supplementation has improved both lactation performance and fertility (Hartwell et al. 2000; Janovick Guretzky et al. 2006). Application of protected choline could effectively increase milk fat and protein yield at the early lactation period (Zom et al. 2011), although the total milk, fat, and protein yield was not affected by the treatment during the whole period. On the other hand, recent data indicated that supplementing protected choline during the prepartum period, from −50 to −21 days before expected calving, did not associate with production traits in Holstein dairy cows (Zhou et al. 2016a).

L-carnitine is vitally important and endogenously synthesized from lysine and methionine in the liver and kidneys. L-carnitine plays an important role in the production of energy via mitochondrial β-oxidation in cells (Greenwood et al. 2001). Carnitine effectively involves in some metabolic processes, such as oxidation of long-chain fatty acids, regulation of ketosis, support of the immune system, enhancement of the antioxidant system, and improvement of reproduction (Citil et al. 2009; Pirestani et al. 2009). On the whole, carnitine supplementation can improve glucose status by decreasing liver lipid accumulation and stimulating hepatic glucose output, and diminishing the risk of developing metabolic disorders during early lactation (Carlson et al. 2007a). Therefore, considering the combined application of both choline and L-carnitine can be important compared to their individual applications in energy synthesis and subsequently production, reproduction, and health parameters of dairy cows.

The objectives of the present study were to evaluate the co-supplementation effect of dietary choline chloride and L-carnitine supplements in Holstein dairy cattle. We evaluated the reproduction indices (calving to first visible oestrus, calving to first service, days open, and number of service per conception),

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Supplemental data for this article can be accessed doi:10.1080/09712119.2017.1332632

milk composition (fat, protein, lactose, solids non-fat [SNF], milk yield, and somatic cell count [SCC]), as well as liver health and ketosis indices (aspartate aminotransferase [AST] and beta hydroxy butyrate acid [BHBA]).

## 2. Materials and methods

### 2.1. Animals and management

One hundred and twenty Holstein cows were selected with parity number, live weight, and milk production in their previous lactation period ranging from 2 to 4, from 450 to 500 kg, and 33 to 35 kg, respectively. In the meantime, the temperature and humidity ranged from 3 to 12 and from 49.3% to 56%, respectively. The cows were housed in straw yard free stalls and offered fresh feed three times a day (total mix ratio at 7 am, 3 pm, and 11 pm) with free access to water. During this study, two diets were used: pre- and post-partum, which corresponded to dry periods and early lactation periods based on the NRC 2001 (Table 1). It should be noteworthy that dry matter intake (DMI) was not recorded in this study. However, the nutrient composition of pre- and post-partum diet has been included as Supplement Tables 1 and 2. Anionic salts were used in the diets during pre-parturition for the prevention of hypocalcaemia for all cows. Calves were separated from their mothers and kept individually in a special compartment. After parturition, the cows were checked daily for placenta extraction and uterine involution. The cows were milked three times daily at 6 am, 2 am, and 10 pm during four consecutive weeks after parturition.

### 2.2. Experimental design

The cows were allocated to the following four groups randomly, so that the mean parity number and mean milk yield were almost similar in all groups: (A) protected choline (25%, Soda Food Supplement, Sana Dam Pars Company, Iran); (B) L-carnitine (20%, Lohmann Animal Health, Germany); (C) protected choline + L-carnitine; and (D) control group or no supplement. Protected choline (60 gm/day per cow) (Janovick Guretzky et al. 2006) and L-carnitine (50 gm/day per cow) (Pirestani et al. 2009) were used as top-dress from one week (7.0 ± 2.1 days) before the expected calving date to four weeks after calving. Two hours after feeding, blood samples (10 ml) were collected at the time of calving followed by weekly sampling through four successive weeks. Blood serum was separated from the clot by centrifugation at 3000 rpm for 15 minutes followed by AST analysis using an autoanalyser system (RA1000 Model) and BHBA analysis using Kits Biochemical (RANDOX®), as liver health and ketosis indicators, respectively.

Milk samples were analysed for fat, protein, lactose, and SNF using the Milkoscan system (Foss-4000 Co.). SCC was measured by the autoanalyser system (Foss-5000 Co.) in a commercial laboratory. In this study, fat percentage was estimated based on 4% fat corrected milk (FCM) (Piepenbrink and Overton 2003). Reproductive indices including days from calving to first visible heat, days from calving to first service, days open or days from calving to successful pregnancy, and number of services per conception were evaluated.

**Table 1.** Composition of pre- and post-partum diet.

| Materials (kg) | Concentrate | Corn silage | Alfa Alfa | Sugar beet pulp | Water | Total |
|---|---|---|---|---|---|---|
| Prepartum | 23.2 | 53.6 | 17.9 | – | 5.3 | 100 |
| Post-partum | 38.5 | 34 | 12.2 | 4.4 | 10.9 | 100 |

### 2.3. Statistical analysis

The combination of choline (0, 60) and L-carnitine (0, 50) was considered as a two-way factorial effect. Records for reproductive indices were analysed using the general linear model procedure considering the combined effect as the only known variation source. Weekly data from each cow for the afore-mentioned productive traits were regarded as repeated measurements and analysed using repeated option in the linear model. Mean comparisons were performed by the Tukey *post hoc* method and considering $P$-value < .05 as the significant level. All data analyses were conducted using the SAS package.

## 3. Results

### 3.1. Milk production and composition

According to Figure 1(A), milk yield varied from 33.8 kg/day (choline) to 37.0 kg/day (Control). This difference was not statistically significant ($P$-value = .1499). For fat% trait, higher performance was recorded in all groups compared to control animals ($P < .05$). As depicted in Figure 1(B), application of either choline or L-carnitine increased the fat%. Moreover, the combination usage of choline and L-carnitine simultaneously caused the highest fat%. Assessing protein % indicated that dairy cows which received choline or L-carnitine were associated with the highest protein %. Interestingly, when both choline and L-carnitine were administrated, protein% decreased significantly compared to each individual treatment (Figure 1). Surveying SNF% and lactose % in Figure 1(D–E) indicates that the supplementation of choline or L-carnitine into the basal diet did not significantly compared to the control group. However, both SNF% and lactose % dramatically dropped through combined choline and L-carnitine effect ($P < .05$). Regarding the SCC parameter, no significant differences were detected among the assessed groups ($P < .05$).

### 3.2. Blood biochemical parameters

According to Figure 2, the BHBA level in the choline group was similar to that of the control group ($P$-value = .78), while the use of L-carnitine increased the BHBA level compared to choline and control groups ($P = .0388$ and $P = .066$, respectively). The combined effect of choline and L-carnitine led to a drastic increase in the BHBA level compared to the control group ($P$-value = .0027). The control group had higher levels of AST compared to other groups (27.70). Except for the comparison between choline + L-carnitine and L-carnitine ($P = .39$), significant difference existed among other groups. Use of L-carnitine resulted in effectively reduction of AST compared to choline group ($P$-value = .0373). However, using combination of

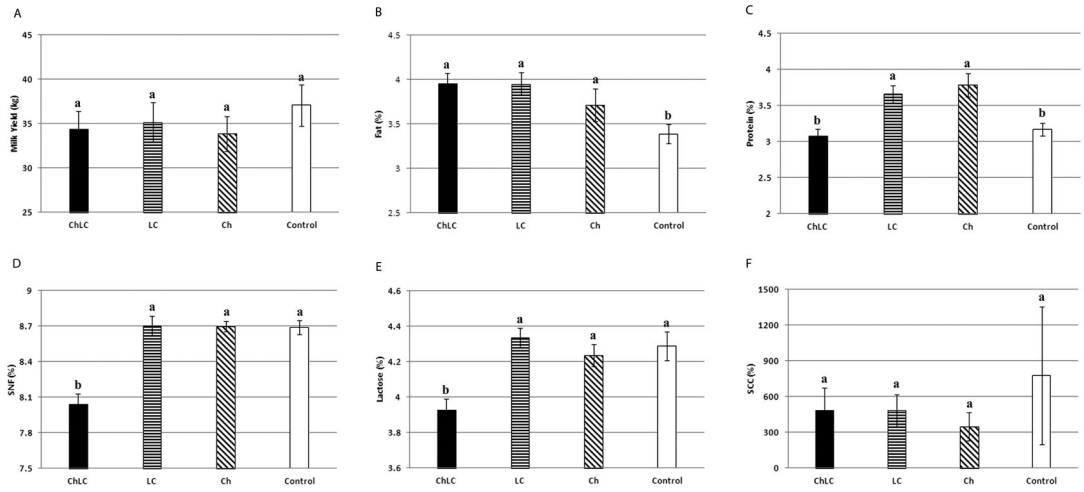


**Figure 1.** Mean comparison for production and milk composition in different supplementation groups. Least square means and standard errors for production traits using choline (Ch), L-carnitine (LC), choline + L-carnitine (ChLC), and control groups. Least square means with common letters are not significantly different (*P*-value < .05). All cows in each group were multiparous.

choline and L-carnitine resulted to the highest decrease in AST ($P < .0001$).

### 3.3. Reproductive indices

According to Table 2, choline + L-carnitine treatment significantly ($P < .05$) decreased days open, service/conception rate, calving to first service, and calving to first visible oestrus compared to the control as well as individual choline or L-carnitine.

## 4. Discussion

### 4.1. Milk production and composition

Some of the difficulties faced during the transitional period from calving until milk production are the restriction of DMI and adjustment of energy intake to minimize a negative energy balance (especially in high-producing cows). This study investigated the ability of choline and L-carnitine to assist prevention of negative energy balance, reproductive problems, and metabolic disorders.

Milk yield was lower in choline than control group without significant difference ($P = .15$). This might be attributable to feeding protected choline with high rumen undegradable protein in prepartum period. This effect may represent changes in methyl metabolism in liver (Hartwell et al. 2000). After control group, L-carnitine group had favourable effect on milk production. A study on the effects of L-carnitine on milk production in lactating female pigs concluded that adding L-carnitine to their diets during lactation increases milk production; this is consistent with our results in Holstein cows. This can be attributed to the positive effects of L-carnitine on reducing the negative balance of energy and protein production. Reduction of the negative balance of energy and protein production leads to weight gain in piglets, and research has indicated that milk production is higher for overweight piglets (Ramanau et al. 2005). On the other hand, this reduction could be due to this fact that we are presenting the least square means, while comparing raw means for each group indicated increase in milk yield in choline and/or L-carnitine groups compared to the control animals (data not shown). The reported means in other studies were also based on arithmetic means rather than least square means which is based on model adjustment.

The control group had significantly decreased milk fat; this was likely due to increased insulin secretion and/or metabolism of non-esterified fatty acids by choline or L-carnitine, which can lead to the activation of a growth hormone-sensitive lipase. The dietary fat resulted in production and storage of triglycerides, cholesterol, and other fatty acids in adipose tissue (Hartwell et al. 2000). Another study showed that 48.7 and 73.2 g/day of choline added to the diet caused an increase in fat content, total fat, and FCM in the milk (Piepenbrink and Overton 2003); this agrees with our results. In another study, the high amount of unprotected choline (325–282 g/day) added to the diets of dairy cows during the late period of lactation reduced rumen pH and propionate and DMI and increased the acetate/propionate ratio and amount of ammonia in the rumen. In fact, high breakdown of choline in the rumen is the primary reason for the desired results of adding unprotected choline in the diet (Janovick Guretzky et al. 2006). Because L-carnitine transports non-esterified fatty acids into the mitochondria, it affects fat metabolism (Carlson et al. 2007a).

**Table 2.** Mean comparison for reproductive indices in different supplementation groups.

| | Reproductive indices | | | |
|---|---|---|---|---|
| Treatment[1] | Service/ conception rate | Days open (d) | Calving to first service (d) | Calving to first visible oestrus (d) |
| Control | $2.50 \pm 0.6^{a}$ | $90.07 \pm 17.8^{a}$ | $60.78 \pm 8.0^{bc}$ | $53.64 \pm 14.5^{b}$ |
| Choline | $1.79 \pm 0.6^{b}$ | $82.21 \pm 14.0^{ab}$ | $66.50 \pm 5.9^{a}$ | $62.21 \pm 11.0^{a}$ |
| L-carnitine | $1.50 \pm 0.5^{bc}$ | $74.71 \pm 13.8^{b}$ | $64.71 \pm 6.2^{ab}$ | $63.29 \pm 9.3^{a}$ |
| Choline + L-carnitine | $1.24 \pm 0.4^{c}$ | $63.36 \pm 8.1^{c}$ | $59.07 \pm 4.2^{c}$ | $53.36 \pm 8.2^{b}$ |

[a,b,c]Means ± standard deviation within each column with common superscripts are not significantly different (*P*-value < .05).

[1]Control (no supplement), choline (60 gm/daily/cow), L-carnitine (50 gm/daily/cow), and choline + L-carnitine (60 + 50 gm/daily/cow).

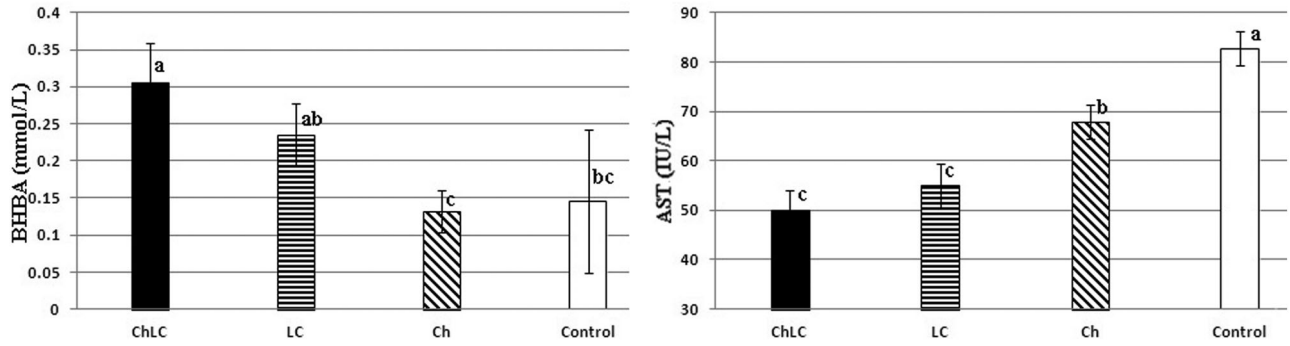


**Figure 2.** Mean comparison for BHBA and AST in different supplementation groups. Least square means and standard errors for blood BHBA and AST using choline (Ch), L-carnitine (LC), choline + L-carnitine (ChLC), and control groups. Least square means with common letters are not significantly different (*P*-value < .05). All cows in each group were multiparous.

During the experiment period, the choline and L-carnitine increased milk protein, while choline treatment had the best effect on milk protein production. Other studies have indicated that supplementing cows' diets with protected choline increased milk yield (43.3 kg/day), fat % (4.29%), fat yield (1.78 kg/day), protein % (3.09%), and protein yield (1.28 kg/day) (Piepenbrink and Overton 2003). However, choline plays an important role in the production of milk proteins by sparing methionine, a necessary component in milk proteins (Janovick Guretzky et al. 2006). Among three L-carnitine dosages (6, 50, and 100 g/day), 100 g/day was sufficient to increase milk protein (Carlson et al. 2006b). In our study, the simultaneous implementation of choline and L-carnitine unfavourably decreased milk protein production. This could be due to differences among breeding values of dairy cows in terms of protein %, as we did not consider equal milk protein % in animal randomly allocation to the above-mentioned groups. A similar result was detected for milk lactose and SNF % in the choline + L-carnitine group compared to separate choline and L-carnitine administration. Milk lactose is less likely to be influenced by diet and it is an osmotic pressure factor in the mammary gland (Toghdory et al. 2009). SNF and milk lactose are directly related and decrease in milk SNF could be originated from milk lactose % (Piepenbrink and Overton 2003).

The supplementation of choline and L-carnitine did not reduce SCC significantly. However, more uniformity or lower variation in SCC was obtained from supplementing either choline or L-carnitine or their combination. Even though a decrease in SCC is the ideal index for milk producers, extreme records for this trait could substantially influence the milk price and lower marketable yield.

### 4.2. Blood biochemical parameters

According to our results, the choline + L-carnitine group showed a considerable increase in BHBA. The increase in the BHBA level which subsequently increases the chance of ketosis in lactating cows could be due to the failure of carbohydrate and fat metabolism (Carlson et al. 2006a). Studies have indicated that the use of rumen-protected choline at 0, 45, 60, and 75 g/day did not affect the concentration of liver triglycerides, but caused a linear increase in the concentration of liver glycogen. Using protected choline at the prepartum period decreased the creatinine level in the plasma after calving without having any effect on blood cholesterol biomarkers (Zhou et al. 2016b). This effect may be due to the positive impact of choline on metabolism and the reduction of ketone bodies such as BHBA in the liver (Zahra et al. 2006). Choline shortage causes fatty liver since it restricts the liver triglycerides transfer and subsequently reduces very low density lipoproteins. Thus, the use of diets supplemented with rumen-protected choline can prevent the incidence of fatty liver in dairy cows during calving and early lactation (Davidson et al. 2008). The effect of betaine on the metabolism of blood in dairy goats showed that bile acids cause cell apoptosis in the liver (Eklund et al. 2005). The oxidation of choline and conversion of choline to betaine result in the destruction of bile acid and improvement in lipid metabolism in the liver by lipophilic activity (Fernandez et al. 2009). Unlike these reports, our current results indicated that BHBA increased through choline + L-carnitine and L-carnitine supplementation. However, this raising could be attributed to the time of sampling after feeding. Plasma metabolites can be affected by the absorption of dietary lipids during 6–8 h after feeding. This leads to the lipoprotein lipase activity which could increase BHBA mobilization in response to L-carnitine supplementation (Greenwood et al. 2001). Moreover, BHBA increase following L-carnitine administration could be due to an imperfect bypassing pathway of unprotected L-carnitine from the rumen (Bryant et al. 1999). However, clear mechanisms for these possibilities are yet to be investigated.

Our data showed that blood AST was favourably decreased using choline implementation. In addition, the L-carnitine supplementation could significantly reduce blood AST compared to the choline supplementation. Fatty liver is a metabolic disease that occurs when the fatty acid uptake into the liver is higher than the rate of fat oxidation and removal from the liver. Studies show that choline prevents the accumulation of fat in the liver by creating a positive pressure and improving the metabolism of esterified fatty acids for energy production (Evans et al. 2006). However, L-carnitine is a donor of acetyl groups and, therefore, increases the intracellular levels of carnitine, which serves as a major transporter of fatty acid across the mitochondrial membranes. L-Carnitine is converted into acetyl carnitine using acetyl coenzyme A for transport into the

membrane lipids, or it can be bonded to long-chain fatty acids and transferred into the mitochondrial matrix (Malaguarnera et al. 2008). The changeover of the acylic group activates the intra-mitochondrial salvage pathway, reactivates coenzyme A, which finally reduces intracellular peroxidation and malonyl aldehyde levels, and improves the liver function (Ramanau et al. 2005).

Even though the BHBA trend among studied groups was antagonistic to the AST trend, there was a positive association between these two variables in animals within each group. We estimated the phenotypic correlation between BHBA and AST for each group individually and found that this index varied from 2.9% to 42.5% for choline + L-carnitine and L-carnitine groups, respectively. The whole data analysis indicated that there is a positive correlation ($r = 21.2\%$) between BHBA and AST in all animals with their repetitions (data not shown). This correlation is weaker than those reported by Seifi et al. (2007) and Taghipour et al. (2010). Overall, no health disorders, including ketosis, dystocia, lameness, and mastitis, were clinically observed in the animals during this study.

### 4.3. Reproductive indices

In terms of reproductive indices, the choline + L-carnitine group showed a significant reduction in the days open, service/conception rate, calving to first service, and calving to first visible oestrus compared to the other groups. Data indicated that combined choline + L-carnitine supplementation is more effective and favourable than individual or no supplementation for the reproduction indices. Research showed that during particular reproductive periods, choline helps cows to balance negative energy, resulting in increased follicular development and fertility. In addition, choline deficiency caused decreased hormones production (FSH and LH) because of the necessity of choline in cell membrane structures (Evans et al. 2006). Otherwise, a non-significant reduction in the number of days open, calving to first service, and calving interval can be justified by the consumption of L-carnitine in a 40-day period. L-Carnitine supplementation reduces ketone bodies, and cholesterol and TG, and it prevents delayed post-partum oestrus and fatty liver and ketosis in livestock and reduces fertility indices including days open and calving interval (Carlson et al. 2007b). Increasing L-carnitine content in the pig diet resulted in higher numbers and weights of newborn piglets than that in control groups, as well as fewer days open (Ramanau et al. 2004). L-Carnitine plays an important role in intrauterine membrane growth because of its effects on the metabolism of insulin, and it is likely that its growth hormone-like action affects intrauterine embryonic nutrition, stimulation, and oxidation of glucose. This article reflects the need for L-carnitine supplementation for pigs during pregnancy (Ramanau et al. 2004). However, further experiment is suggested using a defined DMI to provide a more accurate estimation on the effects of L-carnitine and protected choline chloride.

## 5. Conclusion

Our study revealed that concomitant choline and L-carnitine supplementation in dairy cows' diet could significantly improve the reproduction indices which nowadays are of main concern in the management of Holstein dairy cows. In addition, in terms of main production indices, milk yield was not affected by choline and L-carnitine supplementation, while fat % and SCC uniformity were increased compared to that in the control group. Moreover, a significant decrease in blood AST as the liver health index was achieved through a combined choline and L-carnitine effect. However, further experiments are needed to assess different aspects of the combined choline and L-carnitine effect important parameters of the dairy cow industry.

## Acknowledgements

The authors would like to thank Drs. Shahin Eghbalsaied, Nasrollah Hesam, and Farid Saeedeih for their kind assistance in conducting the project and analysing the data, and for their valuable comments on the manuscript.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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