Effect of Conjugated Linoleic Acid Supplement and Sesame as Source of N-6 on Performance and Milk Fatty Acid Profile of Holstein Dairy Cows During Transition Period

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Abstract:

BACKGROUND: Feeding unsaturated fatty acids sources in dairy cows is considered as a strategy to improve animal health during postpartum period and milk fatty acid profile.

OBJECTIVES: This study aimed to investigate the effects of conjugated linoleic acid supplement and combination of sesame meal and oil on performance and milk fatty acid composition of dairy cows during transition period.

METHODS: Twenty-one multiparous Holstein cows (249 days of pregnancy) were randomly assigned to 3 dietary treatments including: 1) control with a source of calcium salts of palm oil (0.95 and 1.68% of the dry matter in prepartum and postpartum diets, respectively), 2) conjugated linoleic acid supplementation (0.4 and 0.5% of the dry matter in prepartum and postpartum diets, respectively) 3) sesame meal (2.18 and 4.32% of the dry matter in prepartum and postpartum diets, respectively) and calcium salt of sesame oil (0.83 and 1.4% of the dry matter in prepartum and postpartum diets, respectively) as source of n-6.

RESULTS: Dry matter intake, body weight, body condition score, average milk yield and component including fat, protein and lactose were not affected by dietary treatments. The proportion of short and medium chain fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids were not affected by dietary treatments. Conjugated linoleic acid supplement numerically increased content of cis-9, trans-11 conjugated linoleic acid and reduced short chain fatty acids (<12 carbons) in milk fat compared to control and sesame treatments.

CONCLUSIONS: Feeding conjugated linoleic acid supplement and combination of sesame meal and oil had no effect on performance, milk composition and fatty acid profile.

Keywords: CLA, Sesame, Transition period

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Introduction

Nearly all fatty acids with 4 to 14 carbons and half of palmitic acid in milk fat originate from de-novo synthesis in mammary gland. The major carbon sources for de-novo synthesis, acetate and 3-hydroxy butyrate are produced from rumen fermentation. The highest of palmitic acid and all of the long chain fatty acids are uptaken by mammary gland from circulation. The long chain fatty acids in plasma either originate from diet or from fatty acid mobilization (Grummer, 1991). Saturated fatty acids can lead to cardiovascular diseases through affecting LDL and HDL cholesterol (Hu et al., 2001) and saturated fatty acid induced enhancement of plasma cholesterol in human diet was attributed to C12:0, C14:0 and C16:0 (Ney, 1991). On the other hand, mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acids (PUFA; linoleic and linolenic acids) showed positive effects on human health (Haug et al., 2007). Conjugated linoleic acid in a geometric and positional isomer of linoleic acid and cis-9, trans-11 is the predominant isomer of CLA in ruminant’s milk which is produced from vaccenic acid in mammary gland (Kay et al., 2004). Conjugated linoleic acid has been reported to have many beneficial effects including anticancer, prevention of atherosclerosis, anti-obesity, anti-diabetic and stimulation of immune system (Bauman et al., 2001). Linoleic and alpha-linolenic acids which are predominant PUFA in ruminant’s diet, are the precursor of cis-9, trans-11 CLA in rumen biohydrogenation process (AbuGhazaleh et al., 2003). Milk fat can be influenced by physiological and environmental factors. The physiological factors usually affect energy balance which has less potential for affecting milk fat, whereas nutrition as an important and influential environmental factor can be used as a practical strategy for manipulation of milk fat yield and composition (Bauman and Griinari, 2001). In some studies, feeding of lipid sources rich in linoleic acid decreased milk fat content (ALZahal et al., 2008; He and Armentano, 2011). Supplementation of CLA in diet reduced de-novo fatty acid synthesis and also C16:0 and C16:1 content in milk fat, whereas proportion of long chain fatty acids longer than 17 carbons was increased in response to CLA supplementation. In addition, cis-9, trans-11 CLA to trans-11 ration and proportion of PUFA in milk fat increased in response to CLA supplementation (Odens et al., 2007). Concentration of cis-9, trans-11 CLA and PUFA in milk fat were enhanced by feeding sources of n-3 and n-6 fatty acids, whereas saturated fatty acids concentration was reduced in response to n-3 and n-6 fatty acids supplementation (AbuGhazaleh, et al., 2002). These shifts in composition of milk fat fatty acids would be beneficial for human health, since medium chain and saturated fatty acids of milk fat in human diet can increase plasma cholesterol content (Ney, 1991). The results of a meta-analysis from 145 experiments considering the effects of oilseed supplementation on milk fatty acid profile demonstrated that n-3 and n-6 enriched fat sources do not influence short and medium chain fatty acid and also trans 18:1 fatty acids, however the isomers of 18:2 and 18:1 were affected individually (Glasser et al., 2008). Hence, this experiment aimed to investigate the effects of feeding CLA supplementation and combination of sesame meal and calcium salt of sesame oil on milk fat...
fatty acid profile during transition period.

**Material and Methods**

The experiment was conducted at the Natural Resources & Agricultural Research Farm of Tehran University, Karaj, Iran. Twenty-one multiparous pregnant Holstein cows were divided into 3 treatment groups based on parity, body weight and milk yield of the last lactation. The cows were placed in individual stalls and had free access to water. Treatment administration was initiated 21 days before anticipated calving date and continued until 21 DIM. Experimental diets consisted of: 1) calcium salt of saturated fatty acid (40% palmitic acid; control), 2) CLA supplement (Lutrel Pure, BASF, Ludwigshafen, Germany, supplied by Golbar Navid Bahar Company, 9% cis-9 trans-11 CLA and 9% trans-10 cis-12 CLA), 3). Combination of sesame meal and calcium salt of sesame oil as source of n-6 fatty acid (Kimiya Alvand Science-based Company, Ghom, Iran). The rations were formulated according to NRC (2001) requirements for dairy cows during pre and postpartum period and were isocaloric and isonitrogenous. The cows were fed total mixed ration (TMR) in 2 morning and afternoon meals and feed refusals were collected daily before the morning meal during the whole experiment. The ingredients, chemical composition and fatty acid profile of dietary treatments are presented in Table 1 and 2, respectively. Body Weight (BW) and Body Condition Score (BCS) of the cows were determined 21 days before parturition and 5 and 21 days postpartum. The BCS of the cows was assigned according to 1 to 5 score scale (score 1 and 5 respectively for very thin and very fat cows). After parturition, the cows were milked 3 times daily and milk samples were collected weekly for measuring milk components including fat, protein and lactose. At the last week of the experiment another milk sample was collected which was the proportional mixture of 3 milking times, and frozen at -20 °C for determination of milk fatty acid profile. The preparation of the sample for milk fatty acid analysis was according to national standard of Iran with the number of 8818 (milk fat-preparation of methyl-ester of fatty acid-Azmon method). The fatty acid profile was determined by Gas-chromatograph machine (Y1 6100GC, Youngling, South Korea) with the column length of 60 m and diameter of 0.25 mm (BPX70, SGE Analytic Science, Australia). Hydrogen gas (with the purity of 99.999 % and flow rate of 2 ml/min) was used as carrier gas. The initial temperature was 50 °C and was constant for 5 min and then increased with the rate of 40 °C per min until 200 °C and after 6 min started to increase with the rate of 40 °C per min to reach 240 °C and remained constant for 10 min to allow all gases to be exited from the column. Identification of the peaks was done by comparing each individual peak with the peak of pure fatty acid standard and the results were expressed proportionately. Data were analyzed by MIXED procedure of SAS software for repeated measurements (DMI, milk yield and components) and GLM procedure of SAS was used for analyzing single measurements (milk fat fatty acid profile). The experimental model for repeated data is as following:

\[ Y_{ijk} = \mu + Ti + Pj + TP_{ij} + e_{ijk} \]

Where \( Y_{ijk} \) is the dependent variable, \( \mu \) is the mean value, \( Ti \) is the treatment effect, \( Pj \) is the period effect, \( TP_{ij} \) is the interaction of treatment and period and \( e_{ijk} \) is the experimental error.
Results

**Performance:** The results related to DMI, milk yield and composition, BW and BCS are presented in Table 3. Dry matter intake was not affected by dietary treatments during pre and postpartum period, whereas period effect was statistically significant during both pre and postpartum period ($P<0.05$). Dry matter intake was reduced in all treatments during prepartum period as calving time came on and after calving, DMI was increased gradually by progression of lactation. Moreover, DMI was affected by interaction of period and treatment during postpartum period ($P<0.05$). Average daily milk yield and components of milk including fat, protein and lactose were not affect-
ed by dietary treatments. Milk yield and lactose content were affected by period during postpartum period (P<0.05) and milk protein content and yield were numerically affected (P<0.06). Body weight and BCS were not affected by dietary treatments.

**Milk fatty acid profile:** Milk fatty acid profile affected by dietary treatments is presented in Table 4. The reduction in the proportion of the short chain fatty acids (<12 carbons) was numerically greater in milk fat of cows receiving CLA (4.96%) compared to control group (8.21%). Also, the milk fat of CLA treatment tended (P=0.11) to have a greater content of cis-9, trans-11 CLA compared to control treatment. The proportion of PUFA was higher in milk fat from cows fed sesame meal and oil comparing to cows

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**Table 2.** Fatty acid composition of experimental diets (Control: diet containing calcium salt of palm oil; CLA: diet containing CLA supplement and Sesame: diet containing sesame meal and calcium salt of sesame oil).

<table>
<thead>
<tr>
<th>Fatty acids, % of total fatty acids</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.95</td>
<td>0.94</td>
</tr>
<tr>
<td>C16:0</td>
<td>25.50</td>
<td>24.09</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.42</td>
<td>3.24</td>
</tr>
<tr>
<td>C18:1 trans</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>C18:1 cis</td>
<td>26.59</td>
<td>25.02</td>
</tr>
<tr>
<td>C18:2</td>
<td>32.58</td>
<td>34.28</td>
</tr>
<tr>
<td>C18:3</td>
<td>9.04</td>
<td>9.80</td>
</tr>
</tbody>
</table>

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**Table 3.** Least squares means for DMI (dry matter intake), BW (body weight), BCS (body condition score), milk yield and milk composition among dietary treatments. (Diets include: Control: calcium salt of saturated fatty acids; CLA: CLA supplement; Sesame: Combination of sesame meal and calcium salt of sesame oil). * Standard error of means.

<table>
<thead>
<tr>
<th>Item</th>
<th>*Diet</th>
<th>CLA</th>
<th>Sesame</th>
<th>*SEM</th>
<th>P-value</th>
<th>DMI (Kg)</th>
<th>Time</th>
<th>Diet*Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepartum DMI (Kg)</td>
<td>14.49</td>
<td>13.08</td>
<td>14.39</td>
<td>0.88</td>
<td>0.47</td>
<td>0.001</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Postpartum DMI (Kg)</td>
<td>19.23</td>
<td>18.33</td>
<td>18.80</td>
<td>0.77</td>
<td>0.73</td>
<td>0.0001</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Milk yield (Kg/d)</td>
<td>34.19</td>
<td>35.53</td>
<td>35.15</td>
<td>1.98</td>
<td>0.89</td>
<td>0.002</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>3.73</td>
<td>3.64</td>
<td>3.47</td>
<td>0.22</td>
<td>0.70</td>
<td>0.22</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Milk protein (%)</td>
<td>2.89</td>
<td>2.86</td>
<td>2.74</td>
<td>0.09</td>
<td>0.45</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Milk lactose (%)</td>
<td>4.67</td>
<td>4.63</td>
<td>4.41</td>
<td>0.11</td>
<td>0.26</td>
<td>0.42</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Milk fat (kg/d)</td>
<td>1.35</td>
<td>1.24</td>
<td>1.25</td>
<td>0.1</td>
<td>0.72</td>
<td>0.64</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Milk protein (kg/d)</td>
<td>1.07</td>
<td>0.99</td>
<td>1.01</td>
<td>0.08</td>
<td>0.79</td>
<td>0.06</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Milk lactose (Kg/d)</td>
<td>1.72</td>
<td>1.57</td>
<td>1.67</td>
<td>0.12</td>
<td>0.7</td>
<td>0.01</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>BW at 249 d of pregnancy</td>
<td>165.71</td>
<td>659.17</td>
<td>664.57</td>
<td>30.46</td>
<td>0.97</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BW 5 d post calving</td>
<td>589.29</td>
<td>580.83</td>
<td>614.43</td>
<td>25.92</td>
<td>0.64</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BW at 21 DIM, kg</td>
<td>563.36</td>
<td>561.83</td>
<td>574.28</td>
<td>22.04</td>
<td>0.91</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BCS at 249 d of pregnancy</td>
<td>3.50</td>
<td>3.54</td>
<td>3.54</td>
<td>0.09</td>
<td>0.93</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BCS 5 d post calving</td>
<td>3.21</td>
<td>3.21</td>
<td>3.29</td>
<td>0.09</td>
<td>0.77</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BCS at 21 days in milk</td>
<td>3.12</td>
<td>3.16</td>
<td>3.12</td>
<td>0.05</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
fed CLA supplemented and control diets \((P=0.15)\). The saturated to unsaturated fatty acid ratio and proportion of MUFA in milk fat was not affected by dietary treatments \((P>0.05)\).

**Discussion**

**Performance:** There was no difference in DMI between dietary treatments during pre and postpartum period. Supplementation of CLA in dairy cows’ diet during transition period (Rezai et al., 2016; Bernal-Santos et al., 2003), feeding rolled soybean seed to dairy cows during various prepartum periods (Gardinal et al., 2016) and also inclusion of various proportion of sesame meal during early lactation of dairy cows (Shirzadegan & Jafari, 2014) did not affect DMI significantly all of which are consistent with current research. Furthermore, using a mixture of plant oil with different proportions up to 4.07 % of dietary dry matter did not
negatively affect DMI (He et al., 2011).

Abomasal infusion of incremental doses of trans-10, cis-12 CLA in dairy cows did not affect milk yield and protein percentage and yield, whereas milk fat percentage and yield were reduced in response to trans-10, cis-12 CLA infusion (Peterson et al., 2002). Moreover, feeding CLA to dairy cows during transition period comparing to palm oil increased milk yield and reduced milk fat content (Rezai et al., 2016). The lack of milk fat depression in response to CLA supplementation during the first weeks of lactation in current study is in agreement with some other studies (Schlegel et al., 2012; Bernal-Santos et al., 2003; Kay et al., 2006). Feeding sesame in dairy cows during early lactation reduced milk yield and protein content and increased milk fat content, however, milk lactose was not affected (Shirzadegan & Jafari, 2014) which is not consistent with current study. Increasing the level of soybean oil supplementation as a source of linoleic acid caused a linear reduction in milk fat content and increased milk yield (Al Zahal et al., 2008). In addition, increasing the level of sunflower oil, source of linoleic acid in dairy cows’ diet reduced milk fat content (He et al., 2011). N-6 fatty acid enriched fat supplement can reduce milk fat content through production of trans-10, cis-12 CLA during biohydrogenation (Shingfield et al., 2010). In current study, milk fat yield and percentage were lower in cows fed CLA supplement and sesame meal and oil comparing to control group, however the difference was not statistically significant \( (P > 0.05) \). Lack of milk fat depression in response to CLA supplementation during the first weeks of lactation has been attributed to low CLA absorption by the mammary gland. Elevated concentration of plasma NEFA as a consequence of adipose tissue mobilization during the transition period may restrict absorption of CLA by the epithelial cell of mammary gland (Schlegel et al., 2015). On the other hand, CLA supplementation in dairy cows from 2 weeks prepartum until 20 weeks postpartum did not influence milk fat content during the first weeks of lactation (Bernal-Santos et al., 2003). They stated that the lack of milk fat reduction cannot be attributed to reduced uptake of trans-10, cis-12 CLA by the mammary gland as it was continuously transferred to milk fat during the experiment. They stated that the lack of milk fat reduction cannot be attributed to reduced uptake of trans-10, cis-12 CLA by the mammary gland as it was continuously transferred to milk fat during the experiment. Enhanced mobilization of fat reserves during early lactation increases the proportion of absorbed NEFA by the mammary gland for triglyceride synthesis, however, it is not clear why enhanced proportion of NEFA for milk fat synthesis completely eliminates the inhibitory effect of CLA on milk fat synthesis. It has been reported that due to attenuation of cellular signaling systems, trans-10, cis-12 CLA does not have the ability to induce the coordinated reduction in expression of lipogenic enzyme (Bernal-Santos et al., 2003). In the current study, body weight and body condition score were not affected by dietary treatments. This is in agreement with previous research (Souza et al., 2016; Prado et al., 2016). Milk fat synthesis utilizes 50% of energy required for milk fat synthesis and is the major factor for increasing energy requirement during early lactation which leads to mobilization of body reserves (von Soosten et al., 2011). As milk yield and milk fat content and yield were not affected by
dietary treatments in current study, lack of difference in BW and BCS between dietary treatment would be expectable.

**Milk fatty acid profile:** The proportion of short and medium chain fatty acids was not affected by dietary treatments in current study. Inclusion of CLA supplement during transition period of dairy cows reduced the proportion of short and medium chain fatty acids and palmitic acid and increased trans 18:1 fatty acid, whereas cis-9, trans-11 CLA concentration was not affected by CLA supplementation (Rezaei et al., 2016). In another study, CLA supplementation in dairy cows’ diet increased the proportion of trans-10, cis-12 CLA in milk fat, however the concentration of other fatty acids was not affected by CLA supplementation comparing to control group (Chandler et al., 2017). Feeding extruded soybean seed as a source of acid linoleic and calcium salt of long chain fatty acids did not show any significant effect on C4:0, C6:0 and C16:1, whereas decreased proportion of C8:0, C10:0, C12:0, C14:0, C14:1, C15:0 and C17:0 and increased C18:0, C18:1, C18:2 and C18:3 in milk fat (Schaufl et al., 1992). Feeding rolled soybean seed for various periods during prepartum increased cis-9, cis-12 C18:2, cis-9, trans-11 and total PUFA in milk fat, whereas the proportion of short and long chain fatty acids was not affected (Gardinal et al., 2016). Inclusion of processed cottonseed reduced the proportion of fatty acids with 14 to 16 carbons and increased cis-9, trans-11 CLA concentration in milk fat (Chen et al., 2007). Also, dietary supplementation of 2% sunflower seed oil (Dai et al., 2011) or corn oil and palm kernel oil (Girón et al., 2016) enhanced the cis-9, trans-11 CLA content in milk fat. Generally, diets containing long chain PUFA reduce de-novo synthesis of medium chain fatty acids by the mammary gland (Kennelly, 1996). In current study, total short chain fatty acids (lower than 11 carbons) in milk fat in CLA and sesame groups was lower than control group, however, the difference was not statistically significant. Total PUFA proportion was higher in cows receiving sesame compared to CLA and control groups, however the difference was not statistically significant (P=0.14). Increased PUFA content in milk fat of cows receiving sesame can be related to direct transfer of calcium salt of linoleic acid scaping from ruminal biohydrogenation to mammary gland. Cis-9, trans-11 CLA isomer concentration was numerically higher in milk fat of cows receiving CLA supplement comparing to sesame and control groups (P=0.11), which demonstrates transference of this isomer from ruminal protected CLA to mammary gland. The length of experimental period, amount of dietary fat supplement, lactation phase, energy balance and genetic potential for milk production are the influential factors on milk fatty acid composition. Any factors that can cause negative energy balance in dairy cows, can overshadow the effect of fat supplement of milk fatty acid composition due to mobilization of long chain fatty acids from body reserves and their transference to milk fat (Schaufl et al., 1992).

Inclusion of CLA supplement and mixture of sesame meal and oil in transition cows diet compared to saturated fat powder (calcium salt of palmitic acid) did not affect performance, milk component and milk fat fatty acid profile. It seems that due to negative energy balance condition, the effects of dietary fatty acids are mitigated by the NEFA mobilized from body reserves during negative energy balance.
Acknowledgments

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Conflicts of interest

The author declared no conflict of interest.

References


He, M., Perfield, K.L., Green, H.B., Armentano, L.E. (2011) Effect of dietary fat blend enriched in oleic or linoleic acid and mon-


اثر تغذیه مکمل اسید لینولئیک مزدوج و کنجد (به عنوان منبع امگا-6) بر عملکرد و ترکیب اسیدهای جبر شیر گاوی‌های شیری هولشتاین طی دوره انتقال

ندا شیخ‌ابوالفضل رالی، مهدی گنج‌خانلو، و مهدی دهقان‌نوبادگی
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(دریافت مقاله: 22 فروردین 1397، پذیرش نهایی: 23 تیر ماه 1397)

چکیده
زمینه مطالعه: تغذیه منابع اسیدهای جبر غیراشباع در گاوی‌های شیری به عنوان راهکاری جهت بهبود وضعیت سلامت دام بعد از زایش و اگل دیوی جبر دریافت شده است.

هدف مطالعه: این آزمایش با هدف بررسی اثر تغذیه مکمل اسید لینولئیک مزدوج و مخلوط کنجاله و روغن کنجد طی دوره انتقال بر عملکرد و ترکیب اسیدهای جبر شیر گاوی‌های شیری هولشتاین انجام شد.

روش‌کار: تعداد 21 راس گاو چند پاکتایی به تعداد 349 روز در قالب طرح کاملاً تصادفی به گروه‌های حاوی نمک‌های کلسیمی روغن پالم (1/4 درصد ماده خشک در پیش و پس از زایش)، 2 مکمل اسید لینولئیک مزدوج (1/4 درصد ماده خشک در پیش و پس از زایش) و کنجد (3/2 درصد ماده خشک در پیش و پس از زایش) اختصاص داده شدند.

نتایج: تغذیه مکمل اسید لینولئیک مزدوج و کنجد، کنجد دوره انتقال، و اسید لینولئیک مزدوج در تولید شیر و کیفیت شیر بین تیمارهای مختلف متفاوت می‌باشد.

واژه‌های کلیدی: تغذیه، کنجد، دوره انتقال، CLA