**INTRODUCTION**

Feeding of human-edible foods, such as cereal grains, to dairy cows is not economic because of cost. Therefore, strategies to reduce grain in dairy cow nutrition are required to reduce cost without reducing milk production (Ertl et al. 2015). Absence of cheap feed sources has led to the use of agricultural wastes in animal diets. Because of the ability of ruminant to digest fiberous (presence of anaerobic microorganisms in their gastro-intestinal tract) use of available feedstuffs wastes can be considered as feeds (Palangi et al. 2013). Citrus wastes are major ingredients in animal diet formulation in many parts of the world, as an energy supplement (Bampidis and Robinson, 2006; Sharif et al. 2018a). DCP is rich in sugar and pectin, but low in starch (Hindrichsen et al. 2004). It can be used as energy resource to meet of performance requirements of ruminants (Bampidis and Robinson, 2006).

Non-forage fiber (NFF) sources produced increased levels of acetic acid concentration in the rumen helps to hold milk production and MF concentration when roughage is scare or when the energy requirement is high.
Applying of non-fiber carbohydrate in diet formulation to increase production of high performance dairy cows is suggested (Gao and Oba, 2016). Since starch as a non-fiber carbohydrate (NFC) source caused increasing of acidoisis risk in the rumen, low feed intake and low MF, applying of high sugar by-products has received enough attention (Gao and Oba, 2016). A level of 40 per cent dietary roughage has been considered practical. However, a lower-level is suggested, and higher levels may reduce dry matter intake (DMI), milk composition and diet digestibility. Some studies recommended NFF to meet fiber requirements for lactating dairy cows. Dried citrus pulp as a NFC feedstuff used as energy source for ruminants that fermented fastly (Gouvea et al. 2016; Ferrari et al. 2018).

Dried citrus pulp as a replacement for corn grain or sorghum silage in the diet did not alter DMI, milk production or milk protein concentration (Assis et al. 2004; Alnaimy et al. 2017). Dried citrus pulp is usually used in substitute of high fermentable starchy sources (Hall and Eastridge, 2014). When corn was completely replaced by DCP in the diets of dairy cows yielding about 20 kg/d of milk (Assis et al. 2004), caused increasing of milk yield (Santos et al. 2001). Moreover, DCP contains flavonoids, which are antioxidant carriers (Williams et al. 2004; Bampidis and Robinson, 2006). Thus, for cows receiving diets containing a high level of polyunsaturated fatty acids (PUFA), DCP may be useful to enhance the concentration of antioxidants in milk and to improve milk quality.

The MF concentration and milk fatty acids (FA) profiles are of interest due to their relationship with human health. Altering them in dairy cow through dietary manipulation has caused large changes (Liu et al. 2016). Milk FA profile is often affected by a ruminal biohydrogenation process and Δ9-desaturase enzyme activity (Bauman and Grinnari, 2003). Large alterations of MF profiles can be achieved by changing the type of roughage in the diets (Belibasakis and Tsirogiannis, 1996).

No significant differences in blood serum metabolites in cows receiving DCP were reported, except for high cholesterol concentrations in the serum. Belibasakis and Tsirogiannis (1996) and Alnaimy et al. (2017) reported that total triglyceride, glucose and blood urea nitrogen (BUN) concentration in the blood serum were decreased when the calves were fed with DCP.

Limited literature is available about applying DCP in ruminant nutrition. Therefore, the current study was conducted to survey effect of used DCP as an energy source on milk yield, milk composition, milk FA profile and blood metabolites in lactating Holstein cows.

**MATERIALS AND METHODS**

**Animal feeding**

The trial was conducted at Ashjaei dairy farm, Astara, Ardabil, Iran. The performance study consisted of eight lactating Holstein cows, which were blocked according to the average milk yield of the 21 days before the onset of trial (30.95±1 kg/day), days in milk (55±15 days), with an average body weight (BW) of 550 ± 50 kg. Each block was randomly allocated in a replicated 4 × 4 latin square design (LSD) trial with 28-day periods according to the parity and lactation number (2.5±0.5). Dites were: control (no DCP component), and groups with 50%, 75% and 100% DCP/corn grain ratio (DM basis), respectively, formulated according to NRC (2001). The diets were offered as total mixed ration twice daily (Table 1). Cows on experiment were allocated to treatments based on a LSD for a continuous lactation trial over 4 weeks. A 14-day adaptation period was followed by data collection during days 15-28 of each period.

**Sampling, measurements, and analyses**

Milk recording was conducted automatically twice daily at 06:00 and 18:00 using a Dairy Master swing-over milking machine. Samples were taken at each milking and preserved for analyses of milk composition, milk urea nitrogen (MUN) and milk FA profile. Variables relating to milk characteristics such as protein, lactose, fat, MUN and FA profiles were determined using a foss conveyor, electric 4000. Blood samples were taken from each animal 2 h after feeding on the last day of each period.

They were immediately transferred into centrifuge tubes containing 0.1 mL of 10% ethylenediaminetetraacetic acid (EDTA) solution. They were then centrifuged at 3000 rpm for 10 min. Determination of blood serum metabolites (glucose, BUN, cholesterol and triglyceride levels) were conducted by laboratory kits (Belibasakis and Tsirogiannis, 1996).

**Statistical analysis**

Statistical analyses of data were conducted by the generalized linear model (GLM) procedure of SAS (SAS, 2014). Difference between means was compared by using a Tukey test (P<0.05). The test data were analyzed using of a 4 × 4 replicated Latin square design as the following model:

\[
y_{ij(k)m} = \mu + SQ + \text{Period}(SQ) + \text{Cow}(SQ) + \tau(k) + \epsilon_{ij(k)m}
\]

where, i, j, k= 1,…,4; m= 1, 2
The replacement of DCP showed a significant effect on MUN (Table 2) and de novo fatty acid synthesis (Table 3). However, the MUN content in experimental groups was within the normal range (Alnaimy et al. 2017). But in the case of parameters BHB and acetate, were increased significantly (P<0.05) in animals fed DCP. By reducing ruminal proteolytic activity, due to high CP associated with ADF and NDF, a significant portion of the crude protein passes through the rumen degradation, there by being available for digestion in the abomasum (Lashkari et al. 2014). The presence of DCP in the diet, as a replacement of corn grain, caused a reduce in the concentrations of palmitic acid (C16:0) in the milk, (P<0.05) (Table 3). This finding was in agreement with that reported by Kostas et al. (1995), while the concentration of stearic acid (C18:0) was significantly increased. Fatty acid composition of milk triglycerides was affected by DCP (Table 3). The long-chain fatty acids were unaffected by treatment except of stearic acid that was increased (P<0.05). This finding was in contrast with some studies (Broderick and Clayton, 1997; Rocha Filho et al. 1999). Overall DCP can be included effectively in concentrate rations fed to ruminants as an energy and a fiber source. Also DCP can be considered as an antioxidant due to containing of phenolic compounds in diets for lactating Holstein cows.

The FA profiles of MF were not statistically changed by treatments. Several factors such as low ruminal protozoa population and reduced rumen digestion of cellulose, acetate insufficiency, butyrate deficiency, cyanocobalamin insufficiency and decreased insulin secretion have all been expressed as possible causes for diminished fat concentration in milk when cows were fed high cereal grains diets or diets rich in fat containing of high fat (Erdman, 1999; Ivan et al. 2013). Major changes were reported for short and medium chain FA in the milk (Erdman, 1999; Ivan et al. 2013).

Dietary citrus pulp caused an increase in C16:0 FA in milk which was in consistent with the results reported by Fegeros et al. (1995) who fed DCP to ewes. The FA is dependent on the high content of C16:0 FA in DCP to be secreted in the milk (Santos et al. 2014). It, it is suggested that possible changes in ruminal butyrate synthesis may have increased the milk C16:0 content. However, the impacts of DCP on synthesis of butyrate in the rumen varies between ruminant species. For instance, in small ruminants fed DCP, a reduce of butyrate concentration in the rumen was reported (Piquer et al. 2009; Gilaverte et al. 2011). An enhancement in butyrate concentration in the rumen was reported in dairy cows fed DCP-based diets (Broderick and Clayton, 1997; Rocha Filho et al. 1999). It is possible that the impact of DCP on milk C16:0 concentration is related to other components in the diet.
Most PUFA are biohydrogenated by ruminal microorganisms and DCP can alter ruminal biohydrogenation processes, thus interrupting their completion. This produces vaccenic acid (11E-Octadec-11-enoic acid) probably causing numerically higher MUFA and lower palmitic acid concentrations, as was achieved in the MF of cows that received DCP (Kalscheur et al. 1997; Bateman and Jenkins, 1998). The inclusion of DCP in the diets, as a replacement for corn grain, resulted in a lower C16:0 concentration (Table 3). The significant effects on milk lactose concentration and milk yield of cows receiving DCP could indicate interactions between different feed ingredients (Doyle et al. 2005).

Dried citrus pulp sugar content produce less ruminal volatile fatty acids (VFA) per unit of mass compared to starch (Hall and Herejk, 2001), and resulted in an increase in carbohydrates escaping fermentation in the rumen (Sutoh et al. 1996; Ribeiro et al. 2005). This reduction in ruminal fermentation causes low fermentable metabolisable energy, which is required for microbial protein synthesis in the rumen. Diets including DCP resulted in higher C18:0 concentrations in MF (Table 3). High concentration of PUFA in cows fed DCP caused partly bio-hydrogenated into C18:0, and transfered into milk (Bauman and Griinari, 2003). This resulted in high concentrations of C18:0 in the milk.
The MF concentration of C18:0 is usually correlated with the reducing of MF synthesis (Bauman and Grinari, 2003). De novo fat acid synthesis was decreased when the cows received DCP (P<0.05). Low de novo fatty acid synthesis supported decreased MF concentration in cows receiving DCP (Tables 2 and 3). These findings are in agreement with the findings of Woolpert et al. (2016). There were no significant differences in cows receiving DCP and corn grain (Table 3).

The β-hydroxybutyrate (BHB) and acetone concentration in milk as ketosis indicators were increased significantly (P<0.05) in cows receiving DCP. This was supported by low fat/protein (FPR), an indicator of negative energy balance (Enjalbert et al. 2001).

The inclusion of DCP in the diet, resulted in a decrease in BUN, whereas blood serum cholesterol, TG and glucose levels were increased (P<0.05; Table 4). The high glucose concentration in the blood of cows fed DCP was in contrast to the findings of Santos et al. (2014), whereas blood cholesterol concentration was in agreement with their report. Increasing of blood cholesterol concentration in cows fed with diet containing of DCP was in agreement with that reported by Alnaimy et al. (2017), but Sharif et al. (2018b) reported no significant differences between blood glucose concentration in lambs received DCP with lambs did not fed DCP. However they reported no significant differences in blood concentration of triglycerides in cows fed on DCP. The blood glucose and total cholesterol concentrations in present study were increased in cows fed DCP that was in contrast with Jingzhi et al. (2017) that reported no significant differences for mentioned parameters in blood serum of rabbit fed DCP. The BUN concentrations in cows fed DCP was lower than that those did not received DCP. Thid finding was in contrast with Sharif et al. (2018b) who reported no significant differences between BUN concentration in lambs received DCP with lambs did not fed DCP.

### Table 3: Fatty acid profile of milk from Holstein dairy cows fed diets supplemented dry citrus pulp

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DCP0</th>
<th>DCP50</th>
<th>DCP75</th>
<th>DCP100</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denovo (g/100g milk)</td>
<td>1.08</td>
<td>0.85</td>
<td>0.89</td>
<td>1.00</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>NEFA (μEq/L)</td>
<td>359.66</td>
<td>421.71</td>
<td>426.75</td>
<td>368.25</td>
<td>27.81</td>
<td>0.24</td>
</tr>
<tr>
<td>BHB</td>
<td>0.01</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Acetone (mmol/L)</td>
<td>0.087</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>64.00</td>
<td>69.12</td>
<td>68.94</td>
<td>71.73</td>
<td>4.74</td>
<td>0.71</td>
</tr>
<tr>
<td>UFA (%)</td>
<td>18.67</td>
<td>22.18</td>
<td>22.61</td>
<td>20.04</td>
<td>1.37</td>
<td>0.18</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>16.87</td>
<td>20.49</td>
<td>20.70</td>
<td>18.13</td>
<td>1.27</td>
<td>0.14</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>1.94</td>
<td>2.01</td>
<td>2.34</td>
<td>2.377</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Fatty acid (FA) composition (g/100 g of FA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>30.78</td>
<td>28.18</td>
<td>27.57</td>
<td>28.83</td>
<td>0.59</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:0</td>
<td>12.97</td>
<td>14.89</td>
<td>14.99</td>
<td>13.18</td>
<td>0.52</td>
<td>0.02</td>
</tr>
<tr>
<td>C18:1</td>
<td>14.96</td>
<td>19.08</td>
<td>19.14</td>
<td>16.20</td>
<td>1.19</td>
<td>0.05</td>
</tr>
</tbody>
</table>
| DCP0: no dried citrus pulp (DCP) supplementation and DCP50, DCP75 and DCP100: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.

### Table 4: Effect of dry citrus pulp on blood metabolites in dairy cow (mg/dL)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DCP0</th>
<th>DCP50</th>
<th>DCP75</th>
<th>DCP100</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>16.45</td>
<td>15.64</td>
<td>15.91</td>
<td>16.05</td>
<td>0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>52.82</td>
<td>54.68</td>
<td>54.74</td>
<td>54.91</td>
<td>0.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>202.11</td>
<td>202.43</td>
<td>205.00</td>
<td>202.74</td>
<td>0.22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TG</td>
<td>15.45</td>
<td>17.79</td>
<td>16.80</td>
<td>16.13</td>
<td>0.06</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
| DCP0: no dried citrus pulp (DCP) supplementation and DCP50, DCP75 and DCP100: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.


### Conclusion

Replacing of corn grain with DCP resulted in a reduction in milk composition. Inclusion of DCP significantly changed blood serum metabolites (BUN, cholesterol, TG concentrations). The use of DCP as a substitute for corn grain in the diet of Holstein dairy cows can be considered due to the lower cost and the overall feed efficiency.

### Acknowledgement

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