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Milk Production, Milk Fatty Acid Profiles and Blood Metabolites in Holstein Dairy Cows Fed Diets Based on Dried Citrus Pulp

Research Article

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ABSTRACT

The objective of the present study was to determine the effect of substitution of corn grain with dried citrus pulp (DCP) on milk composition, fatty acids profiles and blood metabolites in Holstein dairy cows. These parameters were measured in a replicated 4 × 4 latin square design experiment using eight Holstein cows. Each experimental period lasted 4 weeks. The four treatments were: control (without DCP), and supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (dry matter basis), respectively. The experimental diets was fed as a total mixed ration. The applied DCP led to a decrease in dry matter intake (DMI), milk yield, milk fat (MF) concentration, de novo fatty acid synthesis, milk protein concentration, milk protein yield, MF yield, milk lactose yield and MF:protein ratio (FPR). Inclusion of DCP in the dites showed significant differences in blood metaboites containing blood urea nitrogen (BUN), cholestrol, triglyceride (TG) and glucose ($P<0.05$). In addition, milk urea nitrogen (MUN) was affected by replaced DCP ($P<0.05$). The contents of C16:0 and C18:0 in the milk of cows fed the control diet, were higher and lower than the cows fed DCP diets, respectively ($P<0.05$). The β-hydroxybutyrate (BHB) and acetone as ketosis index in the cows fed with DCP were increased significantly ($P<0.05$). It was concluded that inclusion of DCP in dairy cow rations improved the fatty acid profile of the milk and increased blood serum glucose concentration and can be used as an energy supplement in the diet of lactating cows to support milk production.

KEY WORDS dried citrus pulp, milk composition, milk fatty acid profile, lactating Holstein cow.

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 acidosis 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 cornwas 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 Griinari, 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 theonset 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 usinga Dairy Master swing-over milking machine. Samples were taken at from 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:

$$y_{ij(k)m} = \mu + SQ_m + Period(SQ)_{im} + Cow(SQ)_{jm} + \tau_{(k)} + \varepsilon_{ij(k)m}$$

$i, j, k = 1,\ldots,4;\ m = 1, 2$

Where:
yij(k)m: observation ij(k)m.
μ: overall mean.
SQm: effect of square m.
Period(SQ)im: effect of period i within square m.
Cow(SQ)jm= the effect of Cow j within square m.
τ(k): effect of treatment k.
εij(k)m: random error with mean 0 and variance σ2.

RESULTS AND DISCUSSION

Replacing of corn grain with DCP can decreased risk of ruminal acidosis due to low production of lactate resulting to increase of energy intake in high performance dairy cows (Gao and Oba, 2018). The use of DCP significantly reduced the DMI (P<0.05) (Table 2). This finding was in contrast to Lanza *et al.* (2015), who found that the use of DCP significantly increased the DMI. However, Lopez *et al.* (2014) reported similar DMI to this study when substituting DCP for corn grain in the diets of lactating Murciano-Granadina goats. These differences between studies can be related to the chemical composition of DCP, processing method, base diet variation, feeding system, animal species and level of feeding. Also these discrepancy can be reacted to low insoluble fraction (B), greater rate of gas production in diet containing of DCP, sorrunding of starch by protein matrix was predictable (Ferrari *et al.* 2018). DCP can be used as a corn grain alternative without negative effects on the nutritive value of the diet, milk yield, and milk quality (Gao and Oba, 2018).

In the present study, the use of citrus pulp led to a reduction in daily milk production and FCM (P<0.05). However, Lanza (1984) reported that the replacement of whole or part of maize or barley with dry pulp from oranges or lemons does not have a negative effect on milk production or MF content, which is not consistent with the results of this study. In dairy cows, citrus pulp leads to an increase in acetic acid, therefore, to an adverse effect on the acetate to propionateratio (Drude *et al.* 1971). The decrease in milk yield as dietary DCP increased is probably related to these change in the balance of this ratio. As presented in Table 2, MF ranged from 3.15 to 3.43%, the highest MF was obtained from the control diet, with the lowest in 50% replacement of citrus pulp. The fat/protein ratio (FPR) in the control treatment was reduced by the use of citrus pulp. The soluble carbohydrates in citrus pulp are fermented more rapidly and therefore, produce more propionate, thus reducing the acetate:propionate ratio, resulting low MF in citrus pulp replacements. The results obtained in this study for milk components are consistent with the other studies (Broderick and Radloff, 2004; Benchaar *et al.* 2006; Lechartier and Peyraud, 2010).

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 withthat reported by Kostas *et al.* (1995), while the concentration of stearicacid (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.

Table 1 The feed ingredients and chemical composition of experimental diets

Ingredient*	DCP_0	DCP_{50}	DCP_{75}	DCP_{100}
Alfalfa hey	18.04	18.04	18.04	18.04
Corn silage	15.71	15.71	15.71	15.71
Barley	10.27	10.27	10.27	10.27
Corn	8.94	4.47	2.24	0.00
Corn gluten feed	7.66	7.66	7.66	7.66
Dry citrus pulp	0.00	4.47	6.7	8.94
Wheat bran	6.67	6.67	6.67	6.67
Fish meal	3.99	3.99	3.99	3.99
Soybean meal	19.44	19.44	19.44	19.44
Beet pulp	5.97	5.97	5.97	5.97
Salt	0.4	0.4	0.4	0.4
Calcium carbonate	0.57	0.57	0.57	0.57
Vegetable oil	1.77	1.77	1.77	1.77
Vitamin/min premix	0.57	0.57	0.57	0.57
Total	100	100	100	100

* Neutral detergent fiber (NDF): 33.9 (% DM); ADF: acid detergent fiber (ADF): 19.8 (% DM); non-fiber carbohydrate (NFC): 32.1(% DM); Undiscounted total digestible nutrients (TDN): 71 (% DM); Metabolizable energy (ME): 2.62 (Mcal/kg DM); Net energy for lactation (NE_L): 1.67 (Mcal/kg DM); Crude protein (CP):18 (% DM); Ca: 0.8 (% DM); P: 0.6 (% DM); Ether extract (EE): 4.1 (% DM); DCAD: 177 (mEQ/kg).

DCP_0: no dried citrus pulp (DCP) supplementation and DCP_{50}, DCP_{75} and DCP_{100}: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.

Table 2 The dry matter intake, milk yield and milk composition of Holstein dairy cows fed diets supplemented dry citrus pulp

Item	DCP_0	DCP_{50}	DCP_{75}	DCP_{100}	SEM	P-value
Dry matter intake (kg/day)	21.48^{a}	21.00^{ab}	20.60^{ab}	20.56^{c}	0.18	< 0.05
Milk						
Yield (kg/day)	31.85^{a}	29.95^{b}	30.92^{ab}	29.09^{c}	0.21	< 0.05
4% FCM*	29.13^{a}	27.17^{ab}	27.19^{b}	26.89^{c}	0.28	< 0.05
Fat (%)	3.43^{a}	3.15^{b}	3.20^{ab}	3.32^{ab}	0.05	0.05
Protein (%)	2.87	2.60	2.84	2.92	0.05	0.26
Lactose (%)	4.74^{a}	4.36^{b}	4.40^{b}	4.52^{b}	0.05	< 0.05
Fat (kg/d)	1.09^{a}	0.98^{b}	0.98^{ab}	0.99^{c}	0.01	< 0.05
Protein (kg/d)	0.91^{a}	0.80^{b}	0.88^{ab}	0.87^{ab}	0.01	0.05
Lactose (kg/d)	1.50^{a}	1.35^{b}	1.36^{c}	1.35^{d}	0.02	< 0.05
FPR (fat/protein)	1.26^{a}	1.24^{b}	1.14^{b}	1.13^{b}	0.02	0.05
Milk urea nitrogen (mg/dL)	13.51^{a}	13.34^{ab}	13.08^{b}	13.07^{ab}	0.07	0.05
Feed efficiency	1.48^{b}	1.43^{ab}	1.50^{a}	1.42^{ab}	0.01	0.05

* Fat corrected milk (FCM)= 0.4 × milk yield (kg) + 15 × fat yield (kg).

DCP_0: no dried citrus pulp (DCP) supplementation and DCP_{50}, DCP_{75} and DCP_{100}: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.

SEM: standard error of the means.

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 palmtic acid concentrations, as was achieved in theMF 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 metablisable 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.

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

Treatment	DCP$_0$	DCP$_{50}$	DCP$_{75}$	DCP$_{100}$	SEM	P-value
Denovo (g/100g milk)	1.08^{a}	0.85^{b}	0.89^{b}	1.00^{ab}	0.04	0.01
NEFA (μ/Eq/L)	359.66	421.71	426.75	368.25	27.81	0.24
BHB (mmol/L)	0.01^{b}	0.04^{a}	0.04^{a}	0.04^{a}	0.03	< 0.05
Acetone (mmol/L)	0.0875^{b}	0.14^{a}	0.13^{a}	0.12^{a}	0.01	0.05
SFA (%)	64.00	69.12	68.94	71.73	4.74	0.71
UFA (%)	18.67	22.18	22.61	20.04	1.37	0.18
MUFA (%)	16.87	20.49	20.70	18.13	1.27	0.14
PUFA (%)						
Fatty acid (FA) copmposition (g/100 g of FA)	1.94	2.01	2.34	2.377	0.13	0.07
C16:0	30.78^{a}	28.18^{b}	27.57^{b}	28.83^{ab}	0.59	0.01
C18:0	12.97^{b}	14.89^{a}	14.99^{a}	13.18^{ab}	0.52	0.02
C18:1	14.96	19.08	19.14	16.20	1.19	0.05

DCP$_0$: no dried citrus pulp (DCP) supplementation and DCP$_{50}$, DCP$_{75}$ and DCP$_{100}$: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.

De novo: synthesis of fatty acids by mammary gland; NEFA: non-esterified fatty acids; BHB: β-hydroxybutyrate; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated and PUFA: polyunsaturated fatty acids.

SEM: standard error of the means.

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

Treatment	DCP$_0$	DCP$_{50}$	DCP$_{75}$	DCP$_{100}$	SEM	P-value
BUN	16.45^{a}	15.64^{c}	15.91^{b}	16.05^{b}	0.04	< 0.05
Glucose	52.82^{b}	54.68^{a}	54.74^{a}	54.91^{a}	0.20	< 0.05
Cholesterol	202.11^{b}	202.43^{b}	205.00^{a}	202.74^{b}	0.22	< 0.05
TG	15.45^{d}	17.79^{a}	16.80^{b}	16.13^{c}	0.06	< 0.05

DCP$_0$: no dried citrus pulp (DCP) supplementation and DCP$_{50}$, DCP$_{75}$ and DCP$_{100}$: supplemented groups with 50%, 75% and 100% DCP:corn grain ratio (DM basis), respectively.

BUN: blood urea nitrogen and TG: triglyceride.

SEM: standard error of the means.

The MF concentration of C18:0 is usually correlated with the reducing of MF synthesis (Bauman and Griinari, 2003). De novo fatty 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 finding are in agreement with the findungs of Woolpert *et al.* (2016). There were no significant differences in cows reciving 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 cholestrol, 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 cholestrol concentration was in agreement with their report. Increasing of blood cholestrol 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 reproted no signifcant differences in blood concentration of triglycerides in cows fed on DCP. The blood glucose and total cholestrol 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 parametres 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.

CONCLUSION

Replacing of corn grain with DCP resulted in a reduction in milk composition. Inclusion of DCP significantly changed blood serum metablites (BUN, cholestrol, 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.

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