Total phenolic content in milk from cows fed cactus (*Opuntia stricta*) cladodes as a partial substitute for sorghum silage in soybean oil-supplemented diets

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ABSTRACT

Cactus cladodes (CC) contain numerous polyphenols (PP) with potential health benefits. We recently showed that associating CC with soybean oil (SO) in cows' diet markedly increased the *cis*-9, *trans*-11 CLA content in milk, probably through modulation of rumen microbiota by PP. Here, we investigated whether feeding CC to cows in our previous study increases total phenolic content (TFC) in milk. Twelve cows were used in a two-period study: a) Baseline (BL): all cows received a diet composed of sorghum silage (SS) and a concentrate without SO for 14 d; b) Treatment: cows received the following SO-supplemented diets for 21 d (n=4/group): Control: diet containing only SS as forage; CC1: CC replaced half of SS; CC2: same diet as in CC1, but CC and the concentrate were fed separately from SS. Milk samples were collected on the last day of BL period and weekly during treatment period, and were analysed for TFC using the Folin-Ciocalteu method. TFC numerically increased ($P > 0.05$) by 31% and 7.7% in CC1 and CC2 as compared to control (0.273 ± 0.03, 0.225 ± 0.03, and 0.209 ± 0.03 mg GAE/g of milk, respectively) at the end of treatment period. Cactus feeding promoted a moderate increase in milk TFC, which is expected to improve the nutraceutical value of milk.

INTRODUCTION

Around 40% of terrestrial surface is covered by drylands, and more than two billion people live in these regions. Climate change is leading to an increase in global temperature and drought frequency, and the combination of those events reduces the possibility to grow traditional crops in drylands (Dubeux et al., 2022). Cacti are drought-tolerant plants that can be used as a nutritious food for humans as well as an essential fodder for ruminant production in arid and semiarid regions of the planet (Nefzaoui & Ben Salem, 2002). Its fruits and pads (also called cladodes) are a rich source of nutrients and phytochemicals for both humans and animals (Shetty et al., 2012). Furthermore, due to the high moisture content of its cladodes, cactus can supply a significant proportion of livestock water requirements. Therefore, cacti (especially from *Opuntia* and *Nopalea* genera) help to increase the sustainability of livestock production in drylands, thereby contributing to tackle food insecurity in vulnerable populations living in these regions.

However, the benefits of using cactus as fodder for livestock production may not be restricted to those reported above. A recent study from our group (Gama et al., 2021) showed that including cactus (*Opuntia stricta*) cladodes in a plant oil-supplemented diet markedly increased the contents of *cis*-9, *trans*-11 CLA and other beneficial fatty acids in milk fat, thereby improving the nutritional value of milk. As discussed in the article, this positive effect on milk fatty acid composition was probably associated with the modulation of rumen microbiota by polyphenols (Vasta et al., 2019), secondary plant metabolites with antioxidant and anti-inflammatory properties found in abundance in

Opuntia ssp. cladodes (Astello-García et al., 2015). Some authors reported increased contents of phenolic compounds in milk from cows consuming certain diets, such as mountain grassland pastures (Besle et al. 2010), which is expected to improve the nutritional value of milk given the health benefits attributed to polyphenols (Rodríguez-Daza et al., 2021). As cactus *Opuntia* cladodes are rich sources of polyphenols, in particular flavonoids and phenolic acids (Perucini-Avendaño et al., 2021), we hypothesized that total phenolic content of milk would increase in cows fed cactus cladodes. This hypothesis was tested using the milk samples collected in our previous study where milk fatty acid composition was the primary outcome (Gama et al., 2021).

OBJECTIVE

This study aimed to investigate whether the inclusion of cactus (*Opuntia stricta*) cladodes in a SO-supplemented diet increases the total phenolic content in cow's milk. We also evaluated if this response is affected by the method of cactus feeding (total mixed ration *vs.* partial mixed ration).

MATERIAL AND METHODS

Twelve multiparous Holstein cows in mid-lactation (150 ± 30 days in milk) and average milk yield of 18.2 ± 4.3 kg/day were used in the study, which was conducted in two periods: a) Baseline (BL): all cows received for 14 days a total mixed ration (TMR) composed of sorghum silage (SS) and a standard concentrate (ground corn, soybean meal, and a mineral-vitamin supplement) containing no soybean oil (SO); b) Treatment: at the end of the BL period, cows were randomly assigned to one of the following SO-supplemented diets (treatments) for 21 days: 1) Control: a TMR composed of SS (70% of diet DM) and a SO-enriched concentrate; 2) CC1: a TMR composed of cactus cladodes (CC) from *Opuntia stricta* [Haw.] Haw cv. Orelha de Elefante Mexicana as a partial substitute for SS plus the SO-enriched concentrate; and 3) CC2: same diet described for treatment 2, but differing in the method used for CC feeding. In this treatment group, CC were mixed with the SO-enriched concentrate (*i.e*. as a PMR) and fed separately from SS after morning and afternoon milking. The chemical composition of BL and experimental diets is shown in Table 1. SO (refined oil) was included at 2.7 % of diet dry matter (DM) in substitution for ground corn in all experimental diets and CC were chopped in particles of ~ 2 cm using a forage machine. Composite milk samples from morning and afternoon milking were collected in 15-mL tubes on the last day of the BL period and weekly during the treatment period. The samples were frozen at -20ºC and analysed for total phenolic content (TFC) using the Folin-Ciocalteu method (Georgé et al., 2005).

The temporal variation in milk TFC was analysed using a mixed model including the fixed effects of diet, sampling day, their interaction and BL values (used as a covariate), and the random effect of cow within diet group. As the covariate was not significant (P = 0.36), unadjusted means were compared by Tukey's test. Analyses were performed using the Mixed procedure of SAS software (version 9.4).

Table 1. Proportion of ingredients and chemical composition (% dry matter, unless otherwise stated) of baseline and experimental diets.

	Diet[1]			
Item	Baseline	Control	CC1	CC2
Ingredients				
Sorghum silage (SS)	70	70	36	36
Cactus cladodes (CC)	0	0	34	34
Soybean meal	13	13.3	13	13
Ground corn	14.3	11.3	11.2	11.2
Soybean oil (SO)	0	2.7	2.7	2.7
Mineral-vitamin mix	1.4	1.4	1.4	1.4
Salt	0.5	0.5	0.5	0.5
Urea + ammonium sulphate (9:1)	0.8	0.8	1.2	1.2
Chemical composition				
Dry matter (% as-fed)	48.5	48.9	43.5	43.5
Organic matter	89.9	87.2	86.9	86.9
Crude protein	15.2	15.1	14.8	14.8
Neutral detergent fibre	44.4	44.1	32.9	32.9
Non-fibre carbohydrate	29.2	27.2	40.0	40.0
Ether extract	2.3	4.9	4.5	4.5

[1] Baseline: a total mixed ration (TMR) composed of SS and a concentrate containing no SO; Control: a TMR composed of SS and a SO-enriched concentrate; CC1: a TMR composed of CC as a partial substitute for SS plus the SO-enriched concentrate; CC2: same diet described for CC-TMR, but with CC mixed with the SO-enriched concentrate and fed as a partial mixed ration (PMR).

RESULTS AND DISCUSSION

Temporal variation in total phenolic content (TFC) in cow's milk is shown in Figure 1. As observed, TFC sharply increased from BL period (when all cows received a basal diet containing no SO) to day 7 of treatment period (when cows received the SO-supplemented diets). As the magnitude of the increase was similar across the dietary treatments, it suggests that the inclusion of SO in the experimental diets may explain this response. It is well-known that phenolic compounds are strongly affected by the refining process, and are present only in trace amounts in refined oils (Fine et al., 2016). It should be noted that, in the present study, SO was added as a substitute for ground corn in the experimental diets (Table 1), and corn grain also contains phenolic compounds, especially phenolic acids (notably ferulic and p-coumeric acids), which are mostly bound to cell wall components, as well as small amounts of flavonols, anthocyanins, and proanthocyanidins (Salinas-Moreno et al., 2017). These authors reported TFC of 2811 and 5510 μg GAE/g dry weight in yellow corn grain, most of it present as insoluble phenolics bound to cell wall components. Hence, the replacement of corn grain with SO in the diet is expected to decrease the intake of TFC, and thereby the increase in milk TFC from BL to day 7 may be attributed to a greater bioavailability of PP from other feed ingredients due to SO addition to the diet. This hypothesis, however, needs further investigation.

Contrary to what was observed during the 1st week of treatment period, TFC in milk decreased for both CC2 and control diets (notably in the latter) in the last two weeks of the experimental period. In contrast, TFC in milk from cows fed the CC1 diet remained fairly stable during this period. As a result, TFC numerically increased ($P > 0.05$) by 31% and 7.7% in CC1 and CC2 as compared to control (0.273 ± 0.03, 0.225 ± 0.03, and 0.209 ± 0.03 mg GAE/g of milk, respectively) at the end of the treatment period. These results indicate that the inclusion of CC in the diet increases, to a moderate extent, the TFC in cow's milk. Besides, the magnitude of the enrichment appears to be slightly greater when CC are fed as a TMR rather than a PMR, which may be associated, at least in part, with the higher intake of CC1 diet when compared to the CC2 diet, as reported by Gama et al. (2021). Increased contents of phenolics have also been found in milk from cows fed mountain grassland pasture (Besle et al. 2010), which also contains higher levels of PP when compared to conventional forages. However, similar to what was observed in the present study, these authors also found a substantial variability in milk phenolics content among cows fed on the same diet, which should be taken into account in future studies addressing the potential of dietary strategies to enrich milk with phenolic compounds. In addition to the potential benefits on human health, enriching milk with phenolic compounds is also expected to increase its oxidative stability (Kuhnen et al., 2014).

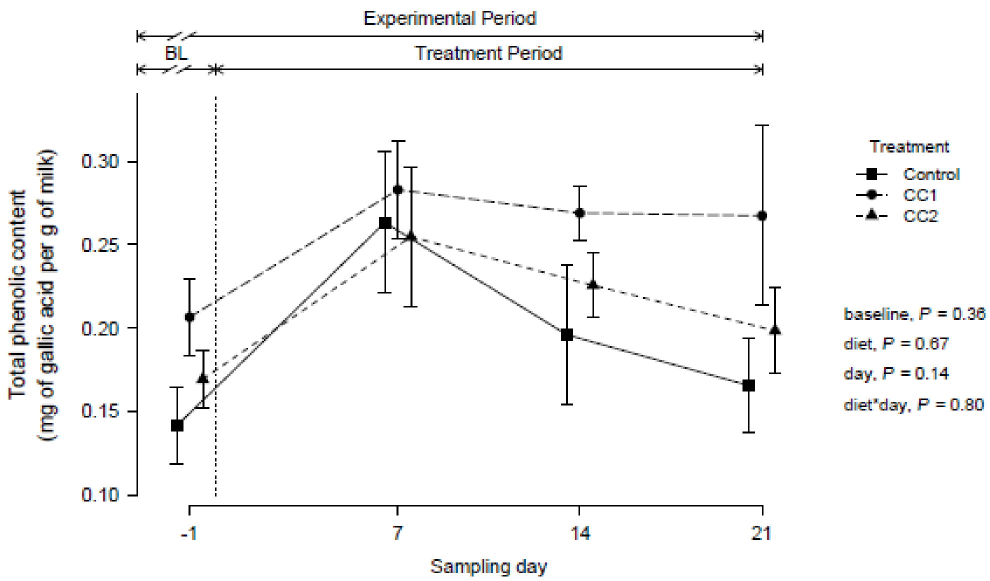

Figure 1. Temporal variation in total phenolic content in milk from cows fed cactus cladodes (CC) as a partial substitute for sorghum silage (SS) in a soybean oil (SO)-supplemented diet. BL (baseline period): all cows received the same diet containing SS as the only forage source and a standard concentrate without SO for 14 days. Treatment period: cows received the following SO-supplemented diets – 1) Control: diet containing SS as the only forage source; 2) CC1: CC replaced half of SS, and the diet was fed as a total mixed ration (TMR); and 3) CC2: CC replaced half of SS, but was mixed with the concentrate and fed as a partial mixed ration (PMR).

CONCLUSIONS

The results of this study showed that partially replacing sorghum silage with cactus (*Opuntia stricta*) cladodes in a soybean oil-supplemented diet promotes a moderate increase in total phenolic content in cow's milk, being the effect more pronounced when cladodes are fed as a total mixed ration. This is expected to improve the nutraceutical value of milk given the health benefits attributed to phenolic compounds.

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