Orange juice industry by-product silage can increase fat and protein in Holstein cow's milk

Luís Carlos Vinhas Ítavo[1], Noemila Débora Kozerski[1], Camila Celeste Brandão Ferreira Ítavo[1], Alexandre Menezes Dias[1], Hélène Veronique Petit[2], Chaouki Benchaar[2], Tadeu Vinhas Voltolini [3], Cloves Cabreira Jobim[4] and Geraldo Tadeu dos Santos[4]

SUPPLEMENTARY FILE

Supplementary Material and Methods

Cows, Foods, Diets and Design

We used eight 587.5±39.6 kg Holstein lactating cows, multiparous, fitted with large diameter (4 inch) rumen cannula, on 110±22 days in milking. During the experimental periods, the cows were fed a lactation herd base total mixed diet (TMD) until their assignment to treatment. Cows were housed in tie stalls, bedded with softwood shavings on rubber mats and provided free access to water.

All cows were fed the same 750:250 g/kg roughage:concentrate ratio TMD with treatment differences were achieved by replacement levels of corn silage by orange peel silage (0, 250, 500 and 750 g/kg DM) into each cow's individually weighed allocation of TMR at feeding. The TMD was fed at 07:00 h (500 g/kg of daily allocation), at 12:00 h (150 g/kg of daily allocation) and at 17:00 h (350 g/kg of daily allocation).

Fifty tons in natura orange peel (*Citrus sinensis* L. Osbeck) from the COCAMAR-CITRUS SA in Paranavaí, PR, Brazil, in vertical silos trench were ensiled, uncoated, being the first, opened after 120 days.

The whole-plant corn (Zea mays L.) was harvested at stage 2/3 grain-hard using a forage harvester, with adjustment of particle size to 0.5-1.0 cm, and ensiled in trench silos kind and open to 120 days of anaerobiosis.

The concentrate, based on soybean meal, corn meal and minerals, was formulated to meet the nutritional needs of dry cows (National Research Council, 2001). The total mixed diet (TMD) was provided with the proposal to provide isoprotein diet for groups of cows, at a forage:concentrate ratio of 750:250. The orange peel silage was used for animal feed, replacing 250, 500 and 750 g/kg of dry matter (DM) of corn silage, and was also supplied to the control treatment, only corn silage (assuming 0 g/kg replacement).

The experiment was in the form of a 4x4 Latin square design with four cows and four replacement levels of corn silage by orange peel silage and four periods (21 days each) of the sampling.

Measurements and analytical methods

To determine the nutrient intake and the ruminal parameters, the animals remained in the adaptation period of 14 days followed by seven days of samples collection (food, leftovers and milk). Diet was offered in excess to allow nearly 50 g/kg leftovers. Both the offered diet and the leftovers collected by composite sampling and were sampled on seven last days of each period.

The samples of food and leftovers were stored in plastic bags until analysed. The samples were dried in a forced ventilation oven at 55ºC for 96 h and ground to pass through a 1 mm mesh screen. The diets and the leftovers were analysed according to Association of Official Analytical Chemists (2000) methods as follows: method 930.15 to determine dry matter (DM); method 942.05 to determine organic matter (OM); method 976.05 to determine crude protein level from total nitrogen (CP); method 920.39 to determine ether extract (EE). Determination of neutral detergent fibre (NDF) was performed according to Mertens (2002), using thermostable amylase (Termamyl 120 L®), without sodium sulphite, and expressed with residual ashes.

The milk samples were stored in plastic tubes until analysed. The samples were dried in a forced ventilation oven at 55ºC for 96 h. The samples of milk of milking of morning and afternoon were analysed according to AOAC (2000) methods as follows: method 930.15 to determine dry matter (DM); method 942.05 to determine organic matter (OM); method 976.05 to determine crude protein level from total nitrogen (CP) x 6.34 factor. The Gerber method chemical test was used to determine the fat content on milk samples.

It was determined the pH and Ammoniac-N on rumen fluid immediately upon withdrawal of the liquid (Ítavo *et al.*, 2002). Analyses of volatile fatty acids in rumen fluid were done according to Wilson (1971).

Calculations

Orange peel and corn silage dried samples with 5 mm grounded were kept on nylon bags and incubated in rumen of the lactating Holstein cows, multiparous, fitted with large diameter rumen cannula. The incubation times were 3, 6, 12, 24, 48 and 72 hours, and the zero time was used with washing in water.

The DM, CP and NDF degradation for each incubation time was calculated by disappearance after incubation (g/kg). Degradation of feeds was calculated using the equation of Ørskov & McDonald (1979):

$d = a + b\,(1 - e^{-ct})$ where:

d = degradation rate at time t;

a = an intercept representing the portion of feed solubilized at time 0;

b = rumen potentially degradable fraction;

c = degradation ratio of b fraction;

t = incubation time

$a + b \leq 100$

Non-linear parameters (a, b and c) were estimated by minimal square iterative procedures by Gauss-Newton procedure. The DM, CP and NDF effective degradability (ED) was calculated using the following equation (Ørskov & McDonald, 1979):

ED = a + (b x c / c + k)

Where: k = estimated rate of outflow from the rumen, and a, b and c are same

parameter described earlier.

Effective degradability of feed was estimated for each ingredient assuming rumen solid outflow rates of 20, 50 or 80 g/kg hour^{-1}, which is represent of low, medium and high feeding amounts (ARC, 1984).

To convert synthesis and production of fatty volatile acids, were used the factors 0.864, 1.0 and 0.618 for acetate, propionate and butyrate, respectively. Relations between effective production and VFA concentration in the rumen was described by the following regression equations cited by Ítavo *et al.* (2000c), being Y(acetate) = 0.410 + 0.04 X; Y (propionate) = 0.134 + 0 0344 X, and Y (butyrate) = -0.020 + 0.028 X; Where X is the concentration in μmoles/litre of each acid and Y is the effective production, obtaining the result in moles per day (mol/day).

Statistical Analysis

Data of milk production, milk composition and nutrients intake were analysed as a 4 x 4 Latin square with diet, period and cow as factors using the general linear model procedures of Statistical Analysis System - SAS (2000). The ruminal parameters data experimental design was randomized blocks, split plots (plots = four levels, five times subplot = sampling of rumen fluid), with four replicates per treatment. Replacement level, Time of sampling of rumen fluid and period as factors using the general linear model procedures of SAS (2000).

Data were evaluated by analysis of variance and regression. Means at 0.05 significance level by Tukey test were compared, and the regression equations followed by the matrix model: $\underline{\boldsymbol{Y}} = \boldsymbol{X}\underline{\boldsymbol{\beta}} + \underline{\boldsymbol{\varepsilon}}$

Where:

$\underline{\boldsymbol{Y}}$ = Vector of observed values;

$\boldsymbol{X}$ = matrix corresponding to the values of independent variables;

$\underline{\boldsymbol{\beta}}$ = Vector of unknown parameters;

$\underline{\boldsymbol{\varepsilon}}$ = vector of random errors (N x 1); where $\underline{\boldsymbol{\varepsilon}} \sim N(\phi, I\sigma^2)$;

Table S1

Chemical composition of mixed total diet (750 g/kg DM silage: 250 DM g/kg concentrate) in function of the replacement level of orange peel silage in replacement of corn silage

Nutrient	Food (g/kg DM)		Concentrate[#]	Replacement levels (g/kg DM)			
	Corn Silage	Orange Peel Silage		0	250	500	750
DM (g/kg)	310.0	120.0	915.0	461.2	425.6	390.0	354.4
OM (g/kg of DM)	935.9	954.5	919.0	931.7	935.2	938.6	942.1
CP (g/kg of DM)	83.1	90.9	260.0	127.3	128.8	130.2	131.7
EE (g/kg of DM)	11.2	20.4	31.1	16.2	17.9	19.6	21.3
aNDF (g/kg of DM)	451.3	301.6	108.8	365.7	337.6	309.5	281.5
ADF (g/kg of DM)	200.9	216.8	54.6	164.3	167.3	170.3	173.3
NFC (g/kg of DM)	454.4	587.1	519.1	470.6	495.5	520.3	545.2

# Corn ground (612 g/kg), Soybean meal (338 g/kg), Urea (20 g/kg), Mineral supplement (30 g/kg),
DM = Dry matter; OM = Organic matter; CP = Crude protein (total-N x 6.25); EE = Ether extract;
NDF = Neutral detergent fibre, ADF = Acid detergent fibre; NFC = no-fibrous carbohydrates

Table S2

Values and regression equations adjusted for volatile fat acids (μM/mL), in function of collection time (t), for replacement levels (RL) of DM corn silage by orange peel silage.

	Replacement levels (g/kg DM)				SEM	P-*value*	
	0	250	500	750		Linear	Quadratic
				Acetic acid (μM/mL)[1]			
Before	73.9	59.5	59.3	43.7	0.38	0.001	0.155
1 hour	85.4	89.4	74.2	67.3	0.18	0.001	0.001
3 hours	82.8	96.1	78.8	66.2	0.23	0.001	0.001
5 hours	82.7	78.9	68.0	60.0	0.24	0.001	0.001
7 hours	72.8	75.3	59.8	54.4	0.22	0.001	0.001
				Propionic acid (μM/mL)[2]			
Before	16.4	15.8	14.9	14.8	0.31	0.001	0.001
1 hour	20.8	21.5	16.6	16.1	0.25	0.001	0.001
3 hours	20.7	21.9	17.2	15.3	0.32	0.001	0.001
5 hours	20.4	17.8	14.5	14.1	0.31	0.001	0.001
7 hours	18.18	16.84	13.5	13.9	0.32	0.001	0.001
				Butyric acid (μM/mL)[3]			
Before	8.9	8.1	7.3	6.5	0.33	0.001	0.154
1 hour	11.9	12.6	8.9	7.7	0.27	0.001	0.001
3 hours	12.3	13.5	9.9	7.5	0.33	0.001	0.001
5 hours	12.2	10.7	8.1	7.1	0.34	0.001	0.123
7 hours	10.0	9.5	6.8	6.8	0.31	0.001	0.105
				VFA total (μM/mL)[4]			
Before	75.6	74.4	78.7	78.7	0.18	0.045	0.001
1 hour	78.1	78.7	80.6	81.2	0.16	0.001	0.102
3 hours	74.4	74.4	78.1	78.7	0.16	0.001	0.104
5 hours	83.7	86.9	79.4	83.7	0.11	0.001	0.001
7 hours	83.7	63.1	80.0	73.1	0.11	0.001	0.041

SEM = Standard error of means; RL = Replacement levels of DM corn silage by orange peel silage (g/kg DM)

[1]$Y= 75,7775 - 0,303673*RL + 9,37295*t - 1,36007*t^2$ ($R^2 = 0,84$)

[2]$Y= 18,6433 - 0,0663669*RL + 1,51290*t - 0,237360*t^2$ ($R^2 = 0,90$);

[3]$Y= 10,3538 - 0,0577799*RL + 1,47959*t - 0,215252*t^2$ ($R^2 = 0,91$)

[4]Y=78,28 (NS)