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Effect of supplementation with cracked wheat or high moisture corn on milk fatty acid composition of grazing dairy cows

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ABSTRACT

In Chile, high moisture corn (*Zea mays* L. [HMC]) has been included mainly in diets for grazing dairy cows on pastures with high crude protein, and is an alternative that improves nutrients supply and rumen synchrony between energy and protein provided by the diet. In addition, supplementation with HMC results in an increase of fatty acids (FA) in milk, desirable for human health. The aim of this study was to evaluate the effect of herbage allowance (HA) and type of supplement on milk FA composition in grazing dairy cows during spring. Thirty-two multiparous Holstein-Friesian dairy cows (533 ± 71 kg BW; 53 ± 4 d in milk [DIM] and milk yield 23.8 ± 4.2 kg d^{-1}) were randomly assigned to four dietary treatments resulting from the combination of two HA levels (low 20 *vs*. moderate 30 kg DM cow^{-1} d^{-1}) and two types of supplements HMC and cracked wheat [*Triticum aestivum* L., CW]) offered at 3.5 kg DM cow^{-1} d^{-1}. Total FA intake was greater for moderate HA (547.8 g d^{-1}) than low HA (527.2 g d^{-1}) ($P < 0.05$); but did not affect milk yield, milk constituents and milk FA composition ($P > 0.05$). Supplementation with HMC increased milk production by 2.3 kg d^{-1} compared with CW. Cows supplemented with HMC showed a greater intake of total FA (564 g d^{-1}) and had greater concentrations of long-chain monounsaturated FA in milk (27.13 g 100 g^{-1}) compared to cows supplemented with CW (511.0 g d^{-1} total FA intake and 25.39 g 100 g^{-1} in milk, respectively) ($P < 0.05$). It is concluded that grazing dairy cows supplemented with HMC during spring produced milk with higher concentrations of desirable FA, independently of the HA level.

Key words: Conjugated linoleic acid, herbage allowance, high moisture corn, *Triticum aestivum*, cracked wheat, *Zea mays*.

INTRODUCTION

Dairy production in Southern Chile is based on the utilization of non-irrigated swards dominated by perennial ryegrass (*Lolium perenne* L.) (Keim et al., 2014). During spring, relatively high herbage growth rates (50-80 kg DM ha^{-1} d^{-1}) of these types of swards allow for adequate pre-grazing herbage mass (i.e. 2400 kg DM ha^{-1}). This spring herbage growth is characterized by high concentrations of moisture and crude protein (200-300 g kg^{-1} DM), with low concentrations of non-structural carbohydrates (Pulido et al., 2009), which can limit herbage DM intake (DMI) and, thereby, milk yield. In this scenario, herbage allowance (HA) and grazing management appear as alternatives to manage and improve herbage quality and increase herbage DM and energy intake (Peyraud and Delagarde, 2013). However, grazing management alone may not be enough to meet the nutritional energy requirements of

high production dairy cows, particularly in early lactation, and supplementation is required to supply the energy requirements (Bargo et al., 2006). Therefore, cereal grains are often fed during spring to increase total DMI and optimize ruminal fermentation by providing readily available energy for ruminant microbial growth and volatile fatty acid production (Peyraud and Delagarde, 2013).

Cracked wheat (*Triticum aestivum* L.; CW), a supplement commonly used for dairy cows, can be useful as an energetic supplement if its contribution is not excessive in the ration (Cabrita et al., 2009). On the other hand, high moisture corn (*Zea mays* L.; HMC) can be a comparative source of metabolic energy in relation to traditional cereals, with its high content of starch and fat as well as small sizes of starch granules (Ruiz-Albarran et al., 2016). In addition, the impact of using grain cereals with a varied carbohydrate composition, physical structure and rumen digestibility on milk composition and milk fatty acid (FA) composition in cows can be different.

Feeding strategies can alter milk FA composition in dairy cows fed total mixed rations or grazed herbage (Doreau et al., 2011; Elgersma, 2015, Barca et al., 2017). Generally, grazing dairy cows have greater concentrations of polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (*cis*-9, *trans* 11; CLA) in the milk than cows fed total mixed rations (Elgersma, 2015). This is the product of the high concentrations of linoleic (C18:2n-6) and α-linolenic (C18:3n-3) acids present in the herbage, which are considered the main substrates for the synthesis of FA in milk (Dewhurst et al., 2006; Glasser et al., 2013). PUFA are considered beneficial for human health (Elgersma, 2015). Hence, milk from grazing cows is healthier than milk from cows fed with diets based on conserved forages or concentrates (Shingfield et al., 2013).

Although some studies have shown the effect of grazing management and supplementation on milk FA of grazing dairy cows, there is still a lack of information on how FA can be modified by HA and supplementation with different carbohydrate sources (Bargo et al., 2006; Elgersma, 2015). We hypothesize, that milk yield and milk FA of grazing dairy cows fed at two HA are influenced by type of supplement as a consequence of the increase of energy and linoleic acid (C18:2n-6) intake. The aim of this study was to determine the effect of HA and type of supplement on milk FA in grazing dairy cows in early lactation during spring.

MATERIALS AND METHODS

Experimental site and pasture

The experiment was conducted at Vista Alegre Experimental Research Station, University Austral of Chile, Valdivia (39°47'46" S, 73°13'13" W), Chile, from 7 October to 22 December 2010. The sward was a 13-yr-old permanent ryegrass (*Lolium perenne* L.), which had been subjected to rotational grazing. The soil type has been classified as a medial, mesic, typic Hapludalf (Soil Survey Staff, 1992).

Cows and dietary treatments

Thirty-two multiparous Holstein-Friesian dairy cows from the university's dairy herd (milk yield 29 ± 5.7 kg^{-1} cow d^{-1}; 60 ± 3.3 d in milk [DIM]; 530 ± 63 kg body weight [BW]) were grouped according to milk yield, DIM and BW. The four dietary treatments resulted from the combination of two herbage allowances (HA): low (20 kg DM cow^{-1} d^{-1}) and moderate (30 kg DM cow^{-1} d^{-1}), and two types of supplements: high moisture corn (HMC) and cracked wheat (CW) offered at 3.5 kg DM cow^{-1} d^{-1}, distributed twice daily during milking sessions in the parlor (06:00 and 15:00 h). A mineral mix (Anasal High Production, ANASAC, Santiago, Chile) (140 g Ca kg^{-1}, 100 g P kg^{-1}, 60 g Mg kg^{-1}, 40 g Na kg^{-1}, 2 g S kg^{-1}, 5000 mg Zn kg^{-1}, 1500 mg Cu kg^{-1}, 20 mg Co kg^{-1}, 200 mg I kg^{-1}) was fed with the concentrate at a rate of 0.25 kg cow^{-1} d^{-1} to avoid mineral deficiency. The amount of supplement offered was calculated using the metabolizable energy (ME) requirements for producing about 30 kg milk d^{-1} (NRC, 2001). Fresh water was freely available in the pasture and in the holding area of the milking parlor.

Cows were strip-grazed on a 13.5 ha sward dominated by perennial ryegrass (45% *Lolium perenne* L., 13.3% *Holcus lanatus* L., 12% *Bromus catharticus* Vahl var. *elatus* [É. Desv.] Planchuelo, 9.3% *Agrostis capillaris* L., 6% *Trifolium repens* L., 4.7% broad-leaf and 9.7% dead material) with each treatment herd at the same paddock, but separated by an electric fence according to the correspondent HA. Herbage allowance was distributed twice daily after each milking. The rotation interval was approximately 16 d, with four rotations over the duration of this study.

Herbage measurements

Daily HA was calculated on an herbage mass basis and grazing area per cow adjustment. Herbage mass was estimated from 100 rising plate meter (Ashgrove Plate Meter, Ashgove Pastoral Products, Palmerston North, New Zealand, using the following equation: $Y = 100x + 400$, where Y is the herbage mass (kg DM cow^{-1} d^{-1}), 100 is the slope, x is the height 0.5 cm of the pasture and 400 is the intercept (Canseco et al., 2009). Plate meter measurements were made by walking along the paddocks in a "W" pattern. This procedure was repeated post grazing, to calculate herbage mass disappearance and estimate individual herd herbage intake.

Experimental procedures and sampling

Samples of supplements and herbage were collected for chemical analysis three times a week, pooled by week, and dried for 48 h at 60 °C. Concentrate samples for FA profile analysis were collected and stored at -20 °C before being freeze-dried. Both, concentrate and herbage were ground through a 1 mm screen (Wiley Mill, Philadelphia, Pennsylvania, USA) and analyzed for DM, CP, ether extract (EE), acid detergent fiber and ash (AOAC, 1996), starch (MAFF, 1996), and neutral detergent fiber (NDF) (Van Soest et al., 1991). Metabolizable energy of herbage, supplement was estimated as per the equation of Garrido and Mann (1981): ME (Mcal kg^{-1} DM) = 0.279 + 0.0325 OMD % where OMD is *in vitro* (organic matter digestibility/DM × 100, determined using the methodology of Tilley and Terry (1963), as suggested by Goering and Van Soest (1970).

Cows were milked twice a day at 06:00 and 15:00 h. Milk yield was measured at milking times throughout the 11 wk of the experiment using the Waikato MKV Milk Meter (Waikato Milking Systems, Hamilton, New Zealand). Representative subsamples of milk were collected during two consecutive days of each week, at both milkings, and analyzed for fat, protein and urea by infrared spectrophotometer (Foss 4300 Milko-scan; Foss Electric, Hillerod, Denmark). On days 21, 42 and 63, 100 mL milk each cow was sampled at the morning and afternoon milking for analysis of FA profile. These samples were mixed according to the milk yield of each cow to get one representative sample per day, which was then frozen at -20 °C until analysis. Cows were weighed once a week after morning milking, and body condition score was recorded by two experienced observers using the five-point scale.

Individual herbage DM intake was estimated, indirectly, once during this study from animal performance results (Baker, 2004) as follows:

$$\text{Herbage DM intake (kg DM d}^{-1}) = \frac{(ME_m + ME_{my} + ME_{lwc} + ME_g) - (\text{supplement ME})}{\text{Herbage ME}}$$

where, ME_m, ME_{my}, ME_{lwc} and ME_g are the ME requirements for maintenance, milk production, live weight change and gestation, respectively (AFRC, 1993), supplement ME supplied by the supplement, and herbage ME is the estimated ME concentration of hand-plucked herbage samples. Individual intake of HMC and CW was calculated daily by the difference between the amount offered and refused.

Dietary intake (g d^{-1}) of specific fatty acids (FA) was estimated by summing the products of herbage and concentrate intake (g d^{-1}) and their respective FA concentrations (g DM d^{-1}). Total lipids of concentrate, herbage and milk were extracted according to Bligh and Dyer (1959) and trans-esterified to fatty acid methyl esters (FAME) (Hartman and Lago, 1973). The FAME were prepared for analysis using a GC 2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector (1:100 split ratio), a flame ionization detector (FID), a Supelco 100-m RT-2560 fused silica capillary column (0.25 mm id X 0.2 μm film thickness) (Sigma-Aldrich, Supelco, Bellefonte, Pennsylvania, USA), stationary phase and using helium as a carrier gas at a flow of 30 mL min^{-1}, air flow at 400 mL min^{-1} and hydrogen at 30 mL min^{-1}. Injection conditions were achieved by setting an initial temperature of 140 °C for 3 min, then increasing at a rate of 3 °C min^{-1} until reaching 200 °C, at which point temperature was held for 2 min, then increased by 3 °C min^{-1} up to 250 °C. This temperature was maintained for 3 min. Temperatures used in the injector and the detector were 230 and 250 °C, respectively. The sample injection volume was 1.0 μL. FA were identified by comparison with retention times of pure standards of FAME with the corresponding times of the separate components of the samples. The quantification was made by area normalization (g 100 g^{-1}). FA profile was generated using external standards (FAME mix C4-C24) and another mixture of CLA (*cis*-9 *trans*-11, *trans*-9 *cis*-11, *cis*-10 *trans*-12, *trans*-10 *cis*-12) (Sigma-Aldrich, Supelco). The desaturase index was calculated based on FA pairs representing Δ-9 desaturase products and substrates: *cis*-9 14:1/14:0; *cis*-9 16:1/16:0; *cis*-9 18:1/18:0 (Kelsey et al., 2003).

Statistical analysis

The experimental design was completely randomized with a 2 × 2 factorial arrangement of treatments to study milk yield, milk composition, milk fatty acids, DM intake, live weight, and body condition score (BCS). Treatments were analyzed by ANOVA using a Proc Mixed model of SAS (2004). The statistical model was: $Y_{ijkl} = \mu + H_i + S_j + HS_{ij} + e_{ijk}$, where Y_{ijkl} is dependent variable, μ is intercept, H_i is fixed effect of the ith herbage allowance, S_j is effect of the jth type of supplement, HS_{ij} is effect of the interaction between herbage allowance and type of supplement, and e_{ijk} is residual random effect ~ N (0,σ^2). Differences between treatments were declared at $P < 0.05$, and tendencies from $P > 0.05$ to $P < 0.10$.

RESULTS

Sward and supplement characteristics

During the experiment, the offered daily HA per cow were 20 and 30 kg DM cow^{-1} d^{-1} for the low and moderate treatments, respectively. The herbage offered had similar pre-grazing herbage mass (2294 ± 352 kg DM ha^{-1}), while post-grazing was different (1319 ± 124 and 1204 ± 193 kg DM ha^{-1}, $P < 0.05$) for low and moderate HA, respectively. Table 1 shows the chemical composition and FA profiles of herbage and supplements. Nutritional value of the herbage was similar in both HA. HMC had lower concentrations of DM, CP, NDF and ADF than CW ($P < 0.05$). In addition, HMC had a greater content of starch and EE than CW, but a similar concentration of ME (3.2 Mcal EM kg^{-1} DM). The major FA in the herbage was C18:3n-3, followed by C16:0 and C18:2n-6. The FA profile between C16:0 to C18:3n-3 was different for the both types of supplements.

Animal performance

The effect of HA and type of supplement on milk yield and composition is shown in Table 2. The results show that increasing HA had no effect on milk yield, 4% fat-corrected milk (FCM) yield and the constituents of the milk ($P > 0.05$). Supplementation with HMC increased milk yield by 2.3 kg^{-1} d^{-1}, compared with CW ($P < 0.05$). Milk fat content tended ($P = 0.07$) to be greater for cows supplemented with CW than those with HMC. There was an interaction between HA and type of supplement for milk protein ($P < 0.05$). Milk urea N ($P < 0.01$) was greater for cows grazed with moderate *vs*. low HA. Daily live weight gain was lower ($P < 0.05$) for cows with moderate HA (Table 2), but had no effect on BCS change. The type of supplement had an effect on BCS change, and CW had greater BCS change than HMC ($P < 0.05$).

Table 1. Chemical composition and fatty acid (FA) composition of herbage and supplements.

	Herbage allowance			Type of supplement		
	Moderate	Low	SD	HMC	CW	SD
DM, g kg^{-1}	188	188	2.79	692	840	0.05
CP, g kg^{-1} DM	208	207	1.96	77	124	0.41
NDF, g kg^{-1} DM	392	402	2.11	106	144	1.70
ADF, g kg^{-1} DM	241	252	1.33	19	34	0.24
Total ash, g kg^{-1} DM	86	81	0.75	15	14	0.11
ME, Mcal kg^{-1} DM	2.7	2.7	0.04	3.3	3.2	0.04
Starch, g kg^{-1} DM	-	-	-	704	631	1.23
EE, g kg^{-1} DM	30	30	0.91	33	13	0.16
C16:0, g 100 g^{-1} FA	12.73	12.65	1.410	9.79	23.19	0.492
cis-9 C16:1, g 100 g^{-1} FA	1.57	1.65	0.192	0.09	0.26	0.011
C18:0, g 100 g^{-1} FA	1.04	1.17	0.224	1.92	2.37	0.204
cis-9 C18:1, g 100 g^{-1} FA	1.34	1.64	0.303	22.92	18.10	0.812
C18:2n-6, g 100 g^{-1} FA	9.19	9.93	1.371	56.87	48.00	2.743
C18:3n-3, g 100 g^{-1} FA	68.49	66.52	2.074	1.29	2.67	0.052
Other FA, g 100 g^{-1} FA	5.55	5.66	0.411	5.41	5.40	0.022

SD: Standard deviation, HMC: high moisture corn, CW: cracked wheat, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ME: metabolizable energy, EE: ether extract.
-Values not determined.

Table 2. Milk production, milk composition, body weight and body condition score of dairy cows fed with two herbage allowances and two types of supplements.

	Herbage allowance		Type of supplement			*P* value		
	Moderate	Low	HMC	CW	SEM	HA	TS	HA×TS
Milk production, kg d^{-1}	26.7	26.4	27.7	25.4	0.852	0.809	0.050	0.108
4% FCM production, kg d^{-1}	24.1	24.1	24.9	23.1	0.724	0.083	0.999	0.230
Fat, kg d^{-1}	34.1	35.0	34.0	36.4	0.096	0.657	0.073	0.351
Protein, kg d^{-1}	33.1	33.4	32.1	34.4	0.034	0.523	0.001	0.017
Urea, mmol L^{-1}	7.5a	6.3b	6.8	7.0	0.319	0.011	0.595	0.818
BW, kg^{-1}	532	530	514	547	15.70	0.934	0.154	0.265
BW gain, kg d^{-1}	0.76b	1.09a	1.00	0.85	0.108	0.049	0.395	0.785
BCS	2.8	2.6	2.6	2.8	0.098	0.380	0.380	0.526
BCS change, points	0.00	0.03	-0.11a	0.08b	0.065	0.775	0.046	0.535

HMC: High moisture corn, CW: cracked wheat, HA×TS: interaction between herbage allowance and type of supplement, SEM: standard error of the mean, FCM: fat-corrected milk, BW: body weight, BCS: body condition score.
Different letters within a row indicate significant differences according to one way ANOVA ($P < 0.05$).

Feed intake and fatty acid intake

Total DM, herbage and FA intake are shown in Table 3. HA had no effect on herbage intake (15.0 kg DM cow^{-1}) or total DMI (18.3 kg DM cow^{-1}) ($P > 0.05$). Total FA intake was greater for moderate HA (547.8 g d^{-1} $P < 0.05$) than low HA. Cows grazed with the moderate HA had a greater intake of C18:3n-3 ($P < 0.05$), and a lower intake of C18:0 (stearic) and *cis*-9 C18:1 (oleic) ($P < 0.05$) than cows grazed with low HA. Cows fed HMC showed a greater intake in total FA (564 g d^{-1}, $P < 0.001$), and greater intakes of C18:0, *cis*-9 C18:1 and C18:2n-6 than those supplemented with CW.

Table 3. Total dry matter, herbage and fatty acid (FA) intake of daily cows fed with two herbages allowances and two types of supplement.

	Herbage allowance		Type of supplement			*P* value		
	Moderate	Low	HMC	CW	SEM	HA	TS	HA×TS
Herbage intake, kg DM d^{-1}	15.2	14.8	14.5	15.2	1.409	0.429	0.527	0.480
Total intake, kg DM d^{-1}	18.4	18.3	18.2	18.8	0.390	0.353	0.527	0.480
Total intake of FA, g d^{-1}	547.8a	527.2b	564.0a	511.0b	8.908	0.048	0.007	0.730
C16:0	70.5	68.1	68.7	70.0	1.516	0.980	0.290	0.759
cis-9 C16:1	7.48	7.57	7.41	7.64	0.193	0.922	0.178	0.785
C18:0	6.51b	6.92a	7.19a	6.24b	0.13	0.050	0.001	0.826
cis-9 C18:1	23.5b	24.6a	32.8a	15.2b	0.179	0.001	0.001	0.866
C18:2n-6	86.5	88.3	108.1a	66.68b	1.146	0.480	0.001	0.800
C18:3n-6	322.8	301.7	308.0	316.5	8.085	0.022	0.221	0.706
Other FA	30.8	30.0	31.6	29.7	0.671	0.899	0.341	0.769

HMC: High moisture corn; CW: cracked wheat; HA×TS: interaction between herbage allowance and type of supplement; SEM: standard error of the mean.
Different letters within a row indicate significant differences according to one way ANOVA ($P < 0.05$).

Milk fatty acids

Table 4 presents the FA profile of the milk. The content of short, medium and long-chain FA, as well as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA and CLA in the milk were not affected by HA ($P > 0.05$). The cows supplemented with HMC had less concentrations of C10:0, C12:0, C14:0 and C16:0, and greater concentrations of C18:0 and *cis*-9 C18:1 compared to those supplemented with CW. Supplementation with HMC reduced the concentrations of short-chain, medium-chain and saturated FA ($P < 0.05$), while supplementation with CW increased the long-chain FA and MUFA concentrations in the milk. The type of concentrate had no effect on PUFA or CLA content of the milk. The desaturase index and the relationship between n-6:n-3 were not affected by HA or type of supplement ($P > 0.05$).

Table 4. Milk fatty acid (FA) composition of dairy cows fed with two herbages allowances and two types of supplement (TS).

	Herbage allowance		Type of supplement			*P* value		
	Moderate	Low	HMC	CW	SEM	HA	TS	HA×TS
g 100 g^{-1} FA								
C4:0	1.53	1.45	1.52	1.46	0.052	0.334	0.419	0.422
C6:0	1.73	1.66	1.69	1.70	0.043	0.313	0.850	0.773
C8:0	1.26	1.19	1.19	1.25	0.025	0.115	0.154	0.885
C10:0	3.09	2.98	2.89b	3.19a	0.072	0.320	0.001	0.318
C11:0	0.34	0.33	0.33	0.34	0.011	0.646	0.491	0.186
C12:0	3.73	3.63	3.49b	3.87a	0.100	0.465	0.010	0.252
Total short-chain	11.71	11.28	11.14b	11.84a	0.254	0.232	0.050	0.538
C13:0	0.14	0.15	0.14	0.15	0.000	0.081	0.530	0.586
C14:0	11.65	11.7	11.39b	11.96a	0.172	0.858	0.023	0.187
C14:1	1.00	0.99	0.99	1.00	0.032	0.762	0.779	0.320
C15:0	1.23	1.28	1.23	1.27	0.023	0.182	0.332	0.210
cis-10 C15:1	0.25	0.25	0.28	0.27	0.012	0.898	0.518	0.492
C16:0	27.53	28.3	26.93b	28.89a	0.505	0.283	0.001	0.089
cis-9 C16:1	1.73	1.71	1.70	1.74	0.051	0.791	0.640	0.875
C17:0	0.72	0.66	0.61	0.78	0.080	0.627	0.172	0.322
cis-10 C17:1	0.20	0.21	0.20	0.21	0.000	0.142	0.165	0.819
Total medium-chain	44.45	45.25	43.32b	46.05a	0.691	0.422	0.001	0.107
C18:0	11.14	11.10	11.87a	10.37b	0.275	0.935	0.001	0.049
trans-9 C18:1	3.74	3.40	3.57	3.57	0.173	0.178	0.998	0.468
cis-9 C18:1	19.29	19.57	20.3a	18.57b	0.340	0.563	0.001	0.118
trans-6 C18:2	0.42	0.48	0.37	0.33	0.021	0.679	0.298	0.606
cis-9 *cis*-12 C18:2	1.11	1.19	1.12	1.17	0.023	0.066	0.265	0.211
C18:3n-3	0.78	0.81	0.79	0.81	0.031	0.455	0.568	0.456
cis-9 *trans*-11 CLA	1.24	1.11	1.14	1.21	0.064	0.182	0.403	0.936
trans-9 *cis*-11CLA	0.024	0.021	0.021	0.024	0.000	0.714	0.643	0.139
cis-10 *trans*-12 CLA	0.025	0.021	0.021	0.025	0.000	0.659	0.659	0.203
trans-10 *cis*-12 CLA	0.033	0.037	0.029	0.021	0.000	0.700	0.305	0.494
∑ > C20:0	0.66	0.62	0.67	0.61	0.053	0.357	0.608	0.088
Total long-chain	38.46	38.36	39.92a	36.92b	0.674	0.787	0.001	0.777
Others	5.33	5.11	5.41	5.14	0.162	0.631	0.260	0.653
∑SFA	64.41	64.76	63.63b	65.55a	0.623	0.691	0.032	0.263
∑MUFA	26.3	26.22	27.13a	25.39b	0.421	0.897	0.001	0.149
∑PUFA	5.11	4.89	4.93	5.07	0.121	0.213	0.457	0.943
n6/n3	1.84	1.69	1.84	1.82	0.141	0.605	0.860	0.995
C14:1/C14:0	0.086	0.084	0.086	0.083	0.003	0.867	0.615	0.615
C16:1/C16:0	0.063	0.061	0.063	0.062	0.001	0.455	0.214	0.214
cis-9 C18:1/C18:0	1.78	1.80	1.75	1.80	0.040	0.834	0.209	0.216

HMC: High moisture corn; CW: Cracked wheat, HA×TS: interaction between herbage allowance and type of supplement; SEM: standard error of the mean; CLA: conjugated linoleic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Different letters within a row indicate significant differences according to one way ANOVA ($P < 0.05$).

DISCUSSION

There has been considerable interest in the milk FA profile of dairy cows on pastoral systems, particularly in PUFA and CLA levels. One of the characteristics of grazed herbage is that it contains high concentrations of n-3 FA, particularly C18:3n-3 acids within the lipids. These FA generally lead to an increased level of PUFA in milk (Elgersma, 2015; Barca et al., 2017). The nutritive value of the pre-grazing herbage of the present study can be considered high and representative of permanent swards used in Chile for feeding grazing dairy cows (Pulido et al., 2009; Keim et al., 2014). In the present study, grazing two HA had no effect on FA composition in the herbage. Consequently, dairy cows were always harvesting leafy material of similar FA concentration above grazing height for the high HA and moderate HA treatments. In addition, the concentrations of major herbage FA (C18:3n-3, C16:0 and C18:2n-6) were like those reported by Dewhurst et al. (2006) and Dierking et al. (2010) on the FA composition of temperate grasses during spring.

Supplementation in grazing dairy cows may influence the FA composition of milk fat, but there is considerable variation depending on the fat content and composition of the basal diet (Barca et al., 2017). In addition, supplementation with concentrated energy sources (wheat or maize) has little effect on milk yield and composition when moderate amounts of concentrate (less than 6 kg^{-1} d^{-1}) are offered in this grazing system (Bargo et al., 2006). In this study, the energetic supplements had a similar ME, but HMC had greater starch content than CW, and these differences could alter the rumen biohydrogenation of fatty acid, as described by Elgersma (2015). The HMC and CW were good sources of *cis*-9 C18:1 and C18:2n-6, although HMC had a higher EE and FA content than CW. The most representative FA in HMC were C18:2n-6 (56.87 g 100 g^{-1} FA) and *cis*-9 C18:1 (22.92 g 100 g^{-1} FA). These values are greater than those reported for HMC by Bradford and Allen (2004). They also surpass the values found for rolled maize and ground maize by Cabrita et al. (2009) and Mohammed et al. (2010), and are greater than the values for corn and wheat found by Schroeder et al. (2004).

Increasing HA did not improve milk yield and composition, contrary to results from McEvoy et al. (2008), and Peyraud and Delagarde (2013), who indicated a positive relationship between HA, grass DMI, and milk yield. However, Kennedy et al. (2008) did not find any effect of increased HA on milk yield in dairy cows given different HA (13, 16 and 19 kg DM cow^{-1} d^{-1}, measured above 4 cm height). The lack of response in milk yield could be due to intake herbage similar for both HA, and by the similarity in energy intake and nutritive value of sward. Consequently, dairy cows were always harvesting leafy material of similar FA concentration above grazing height for the low and moderate HA treatments.

Milk yield was 2.3 kg cow^{-1} d^{-1} higher when dairy cows were fed with HMC instead of CW. Wu et al. (2001) found similar results, but with a higher supplementation of HMC (9 kg cow^{-1} d^{-1}). This fact may be explained by differences in DM concentrations between HMC (692 g^{-1} kg^{-1} DM) and CW (840 g^{-1} kg^{-1} DM) these differences could also have a significant impact on starch digestibility both in the rumen and in the total tract, and, therefore, on the energy supply for milk yield. Previous studies have shown a 20% increase in ruminal starch digestibility for HMC diets compared to dry corn diets, and energy values 16% higher than those of dry corn (Wu et al., 2001; Boivin et al., 2013). In addition, improvement in milk yield might be the result of an improvement in ruminal fermentation, due to the moderate supplementation level and high quality of herbage offered. In our study, the level of supplementation used was 20% of the total DMI; therefore, the herbage was the main source of feed, and had a greater effect on digestion than the supplements given. An interaction between HA and type of supplement was observed for milk protein. The treatment that received a low HA and CW had a greater content of milk protein (34 g kg^{-1}), which might be explained by a faster degradation rate of carbohydrates in CW compared to HMC. In addition, the increase in glucogenic precursors from the supplementation with CW could reduce the utilization of some amino acids for gluconeogenesis and, therefore, increase the supply and uptake of glucogenic amino acids to the mammary gland, thus increasing milk protein (Cabrita et al., 2007).

High urea concentration in milk indicates high ruminal ammonia concentration, with excess ammonia passing from the rumen into the blood stream, and being converted into urea in the liver (Pacheco and Waghorn, 2008). The high urea concentration (above the reference limit < 7.0 mmol L^{-1}) in milk from cows grazed on the high HA (Wittwer, 2012) indicates an asynchrony between energy and protein in the rumen (Pacheco and Waghorn, 2008), and a concentration of protein in the diet above the cows' nutrient requirements.

Increasing HA did not increase herbage DMI or total DMI. In contrast, Peyraud and Delagarde (2013) reported an average increase in herbage DMI of 0.20, 0.15 and 0.11 kg DM offered of HA by grazing dairy cows, when HA (measured at ground level) increases from 20 to 30, 30 to 40 and 40 to 50 kg DM, respectively. The difference in herbage DM intake between this study and the present results can be attributed to type of sward, pre-grazing herbage mass availability, cow characteristics and milk yield.

Cows offered moderate HA had a higher total FA intake and C18:3n-3 and lower intake levels of C18:0 and *cis*-9 C18:1 compared to low HA. This effect was unexpected because there were no differences in estimated DMI and herbage FA composition for both moderate and low HA. It can be associated with the fact that the dairy cows grazing moderate HA tended to graze high strata of the sward canopy, with a greater proportion of leaves compared to low HA. Previous studies have reported that leaf blades have an unsaturated FA content greater than the stems (Rugoho et al., 2016).

The supplementation with HMC and CW may contribute with intake precursors of PUFA and CLA to dairy cows. Although intake of supplement and total DMI were similar, a higher FA total was observed with HMC. This can be associated with the fact that HMC is a supplement with a greater concentration of fat and *cis*-9 C18:1 and C18:2n-6, long-chain FA compared to CW. These long-chain FA could increase the flow of C18 FA entering the duodenum (Chilliard et al., 2007) and enhance synthesis of long-chain FA in milk (Bradford and Allen, 2004).

Recent studies have reported the influence of grazing on the milk FA composition of dairy cows (Shingfield et al., 2013; Elgersma, 2015; Ruhogo et al., 2016). Within the levels evaluated in the present study, the greater FA intake for the moderate HA did not alter milk FA profile, which could be associated with the similar DMI and FA composition of herbage offered in both HA. Palladino et al. (2009) indicates that changes in HA (i.e. 16 *vs*. 20 kg DM cow^{-1} d^{-1}) did not greatly affect the composition of milk FA in grazing dairy cows, despite variation between FA intake. In contrast, Bargo et al. (2006) found that increasing HA from 25 to 40 kg DM cow^{-1} d^{-1} increased short, medium and long-chain FA, and reduced SFA in milk.

The FA C12:0, C14:0 and C16:0 are nutritionally undesirable because they increase cholesterol content of the low-density lipoproteins, and are associated with an increased risk of coronary heart disease (Shingfield et al., 2013). On pastoral dairy production systems, high levels of supplementation with cereal grains have shown to increase the concentrations of these FA in milk considerably (Bargo et al., 2006). In the present study, the level of supplementation of HMC decreased concentrations of C12:0, C14:0 and C16:0 in milk compared with CW. These results are supported by previous studies where grazing dairy cows were given moderate amounts of amylase concentrates and produced milk with lower concentrations of short and medium-chain FA (C4:0 to C16:0) and a high proportion of MUFA (Bradford and Allen, 2004; Chilliard et al., 2007; Cabrita et al., 2009). A reduction in concentrations of C12:0, C14:0 and C16:0 with supplementation of HMC can also be explained by the inhibition of the novo synthesis in the udder, because the acetyl CoA activity is inhibited by the greater presence of long-chain FA contained in the HMC compared to CW. In addition, these lower FA concentrations can be explained by the mammary glands' use of acetate and β-hydroxybutyrate derived from ruminal fermentation of carbohydrates for the synthesis of C4:0 to C12:0 FA, almost all myristic acid (C14:0) and about half of C16:0 in milk fat (Bauman et al., 2011; Shingfield et al., 2013). The rest of C16:0 and all of the long-chain FA derive from mammary uptake of circulating and plasmatic albumin bound to non-esterified fatty acid (Bauman et al., 2011). This could be supported by the fact that cows in our study were in a negative energy balance, where the increase of concentrations of β-hydroxybutyrate may have been the result of the mobilization of body reserves, leading to reductions in the concentration of C16:0 in milk as reported by Chilliard et al. (2007). Supplementation with HMC was the most influential factor in increasing the concentration of C18:0 and *cis*-9 C18:1 in milk. The increase in these FA in the milk was associated with concentrations of MUFA contained in the diet and, consequently, their biohydrogenation, total or partial, to C18:0 in the rumen, as well as the activity of Δ-9 desaturase in the mammary gland that contributes to 60% to 80% of the entire amount of *cis*-9 C18:1 secreted in milk (Chilliard et al., 2007; Shingfield et al., 2013). These facts suggest that cows supplemented with HMC had a more complete ruminal biohydrogenation of dietary lipids, attributed to extensive degradability of fermentable carbohydrates in the rumen, compared to CW. Therefore, extensive fermentability of HMC, compared with CW, would have increased the amount of dietary fat available for lipase action, which explains the higher concentrations of C18:0 and *cis*-9 C18:1 in milk, as mentioned by Mohammed et al. (2010).

The lower concentration of SFA and greater concentration of MUFA in milk fat in cows receiving HMC is consistent with several studies that have evaluated milk FA composition in grazing dairy cows given corn-based supplements (Bradford and Allen, 2004; Kay et al., 2005; Bargo et al., 2006). The difference between the MUFA and SFA content in milk is the result of the FA composition of the HMC and the herbage, the herbage in the current study contained approximately 76% unsaturated FA, and there was a higher concentration of unsaturated FA in the HMC. Additional factors, such as the rumen environment and associative effects of the different dietary components may have affected rumen biohydrogenation and, thereby, FA content of milk.

CONCLUSIONS

On spring grazing dairy cows supplemented with high moisture corn as energy source could lead to substantial increase in milk yield and higher concentration of long-chain and monounsaturated fatty acids in milk. This study showed that under spring grazing conditions, herbage allowance had lower effect than the type of supplement.

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