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Effects of Supplementation with Increasing Levels of Energy Concentrate on the Productive Response and Ruminal Digestion of Dairy Cows Grazing Lucerne Pasture

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Abstract

The aim of the study was to determine the effect of three levels of energy concentrate intake on dry matter (DM) and energy intake, milk yield and composition, rumen environment and pasture neutral detergent fiber (NDF) digestion. Twelve Holstein multiparous cows in early lactation (69.0 ± 5 days postpartum) producing 32.8 (±4.0) kg milk were assigned to three treatments at (kg/cow day) 3.5 (T3.5), 7.0 (T7.0) and 10.5 (T10.5) kg concentrate in a 3 × 3 Latin Square design. Parameters of ruminal environment and neutral detergent fiber (NDF) digestion were obtained using 3 additional rumen cannulated cows. Concentrate was composed (as fed) by corn grain (68%), soybean meal (22%), wheat bran (8%) and a vitamin-mineral premix including monensin and thoroughly consumed. Yields (kg/cow day^{-1}) of milk, 4% fat corrected milk (4% FCM 4%) and energy corrected milk (ECM) resulted higher ($p < 0.05$) in T7.0 (29.6, 26.1 and 25.7) compared to T3.5 (27.7, 24.5 and 24.2) but similar to those obtained in T10.5 (30.6, 26.2 and 26.0). Milk protein yield increased linearly ($p < 0.01$) from 0.82 to 0.92 kg/cow day^{-1} without effects on yield of milk fat. Concentrations (g/100 g) of milk fat (3.19), protein (2.97), total solids (11.75), non-fat solids (8.60) and casein (2.40) did not differ. Milk lactose content (g/100 g) was linearly increased ($p < 0.02$) from 4.91 to 4.98 whereas milk urea decreased ($p < 0.01$) from 0.048 to 0.043. Intakes of DM and energy increased with concentrate level without effects on conversion efficiency. Changes in live weight (LW), body condition score (BCS) and concentrations of plasma non-esterified fatty acids (NEFA),

glucose, insulin, somatotrophin (GH) and insulin-like growth factor (IGF-I) were not affected. Plasma urea levels resulted lower ($p < 0.05$) in T10.5. Ruminal pH and ammonia nitrogen (N-NH_3) resulted lower ($p < 0.05$) in T10.5 compared to T3.5. Concentration of total volatile fatty acids (VFA) was higher ($p < 0.05$) in T3.5 due to the increase in acetate and butyrate while the acetate: Propionate ratio remained unchanged. Pasture NDF digestion was affected as concentrate intake increased. To increase milk protein yield and reduce concentrations of N-NH_3 in rumen and milk, feeding an energy concentrate at 41% of total DM intake resulted an effective tool.

Keywords

Dairy Cows, Concentrate, Milk Yield, Ruminal Digestion

1. Introduction

In dairy systems feeding costs quadratically decrease as the contribution of pasture in the total diet increases [1] but its energy density is insufficient to meet total energy requirements to attain the maximum milk yield in high yielding dairy cows [2]. A lower DM and energy intakes in pasture based systems explain the suboptimal milk production even when herbage allowance and pasture quality are not limiting [3]. When high quality spring pastures are the sole feeding source, maximum milk yield is expected to be around 23.2 kg/cow day^{-1} [4] and higher yields would be achieved if the BCS of cows allows energy mobilization but excessive lipid mobilization may induce metabolic and reproductive disorders [5]. Supplementation with energy concentrates may improve the productive response of cows [6] reducing the loss of body reserves [7]. Milk production in pasture based systems may be also limited by imbalances at ruminal level between availability of fermentable energy and the excess of rumen degradable protein (RDP) as suggested by [8]. Pasture RDP utilization can be improved by feeding starch-rich concentrates [9] but when high levels of cereal-based supplements are fed, ruminal pH and pasture NDF digestion can be affected [10] leading to reductions in forage intake and milk fat content. The aim of this study was to determine the effect of feeding three levels of energy concentrate on milk yield and composition, DM and energy intake, plasma metabolite and hormone concentrations and ruminal pasture NDF digestion in grazing dairy cows.

2. Materials and Methods

2.1. Cows and Treatments

The experiment was carried out at the experimental farm of INTA Rafaela (31˚12'S, 61˚30'W) during spring time of 2008. Twelve multiparous (3.2 ± 1.3 lactations) Holstein cows in early lactation (69.2 ± 5 days in milk) were used to measure milk production and composition, BW, BCS, DM intake (DMI) and

plasma metabolite and hormone concentration. At the start of the experiment, cows were producing 32.8 (±4.0) kg milk, averaging 623.5 (±44.0) kg BW and 2.52 (±0.21) BCS. Cows were grouped by milk production, number of lactations and days postpartum and randomly assigned to one of three treatments (4 cows/treatment) in a Latin Square design with 3 experimental periods of 19 days (d) with the first 14 d for adaptation and the last 5 d for data collection. Parameters of ruminal environment and NDF digestion were obtained using 3 additional cows of the same breed fitted with rumen cannulae in a Latin Square with experimental periods of 19 d (17 d for adaptation and 2 d for measurements). All cows were fitted with transponders (ALPRO version 6.60/DeLaval, Tumba, Sweden) to individually record daily yield of milk and concentrate allocation at the milking parlor according to treatments consisting in three levels of energy concentrate at 3.5 (T3.5), 7.0 (T7.0) and 10.5 (T10.5) kg cow day^{-1}. On a wet basis, concentrate was composed by corn grain (68%), soybean meal (22%), wheat bran (8%) and a vitamin-mineral nucleus including monensin. The daily amount of concentrate was supplied by halves in individual feeders during each milking time at 4:30 a.m. and 3:30 p.m. giving enough time to the cows to consume all of it. An alfalfa (*Medicago sativa*, sp) pasture was used in a rotational grazing system adjusting herbage allowance at 30 kg DM/cow d^{-1} using a portable electric wiring. During the 3 weeks prior to the start of the trial all cows received 7.0 kg of concentrate and pasture.

2.2. Samples Collection and Analysis

Herbage mass (kg DM ha^{-1}) was weekly measured by samples cutted at 4 cm height with manual scissors in an area delimited by a metal frame of 0.125 m^2, cutting a total area of 1 m^2 in each sample. The sample composed of 8 subsamples of 0.125 m^2 was dried in an oven at 65˚C for 48 hours to determine DM content. Based on this measure, the area of the daily strips was established ensuring the target herbage allowance. During the last 5 days of each experimental period, daily and samples of concentrate and pasture were obtained. Pasture samples were taken manually in the grazing horizon simulating the selectivity of the cow by hand-plucking [11]. All samples were dried (65˚C for 48 h) and ground through a 1-mm screen (Wiley mill, Philadelphia, PA). Two representative composite samples from each experimental period were analyzed for NDF content [12], acid detergent fiber (ADF, [13], # 973.18), ether extract (EE; [14], # 920.39), acid detergent lignin (LDA; [13], # 973.18), total nitrogen (Kjeldhal method 976.05 of [14]), crude protein (CP, total nitrogen × 6.25), ash ([13] # 942.05) and *in vitro* DM digestibility (IVDMD) according to [15]. Concentrate samples were also analyzed for starch content [16].

In 2 of the 5 days corresponding to the data collection of each experimental period, milk samples were collected during each milking time using milk meters (DeLaval International AB, Tumba, Sweden) to make a single individual sample (pool) per day weighted by the respective individual milk production. In each

composite sample, concentration of milk fat, total protein, lactose, total solids (TS), non-fat solids (NFS) and urea was determined by infrared spectrophotometry (MilkoScanTM Minor, FOSS Electric, Hillerod, Denmark) according to the ISO standard method/IDF (ISO 9622 IDF 141, 2013). Milk casein content was calculated as 6.38 * (total N—non caseinic N) after semi Micro-Kjeldhal digestion and colorimetric reading in Technicon continuous flow autoanalyzer according to [17]. Fat-corrected milk (4% FCM) was adjusted according to [18] and energy-corrected milk (ECM) as proposed by [18]. The cows were weighed individually with an electronic balance at the beginning and end of each experimental period after the morning milking and preventing access to water. Along with this procedure, BCS was determined by two independent observers using a scale of 5 points (1 = excessively thin and 5 = excessively fat) with increments of 0.25 [20] taking into account the average result of both evaluators.

On the last day of each experimental period and after the 4:30 a.m. milking blood samples were taken by coccygeal vein puncture. The blood was collected in tubes containing sodium heparin (5 U/ml) and plasma was obtained by centrifugation (2000 ×g for 15 min at 4˚C) and stored at −24˚C until analized for glucose (Enzymatic blood glucose, Wiener Laboratory, Rosario, Argentina), urea (Uremia, Wiener Laboratory, Rosario, Argentina), non-esterified fatty acids (NEFA) (NEFA, Randox Laboratories Ltd., UK), insulin, growth hormone (GH) and insulin-like growth factor (IGF-I) as described in [21].

Intake of concentrate was daily and individually recorded by the difference between the quantity offered and refused throughout the trial. Pasture intake was estimated in each experimental period from the production of feces of each animal and from the IVDMD of the pasture as decribed in [22]. Intake of net energy for lactation (NE_L) was calculated based on the equations from [23] and glucogenic energy (GE) according to [24] assuming an intestinal starch digestibility of a 78% [25].

2.3. Rumen Environment and *In Situ* Pasture NDF Degradability

The rate and extent of pasture NDF degradation was estimated using the *in situ* technique [26]. At the start of the incubation period, two bags containing 5 g DM of pasture per sampling hour were introduced into the ventral sac of the rumen of the fistulated cows. The bags were extracted by duplicate for the different hours of incubation (0, 4, 8, 12, 16, 20, 24, 36 and 48 hours) and frozen (−24˚C) until the end of each period. Afterwards, they were thawed and externally washed under a cold water stream to remove contaminating and soluble material. Then all the bags were washed in a washing machine, dried in a forced air oven (60˚C for 48 hours) and weighed to determine the residual DM and NDF contents. Kinetics parameters of NDF degradation were estimated with the equation proposed by [27] using the Excel solver routine [28] as described in [22]. During the first six sampling hours, 200 mL of ruminal liquor from the ventral sac were drawn from each cannulated cow for measurements of pH, ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA). Immediately after ex-

traction and previous filtration of the ruminal liquor with cheese-like cloth, pH was measured with a portable digital pH-meter (ORION model 250 A). A sample (100 mL) was acidified with 1 mL of 1 N H_2SO_4 and stored at −20˚C until the NH_3-N and VFA determinations. The NH_3-N concentration was determined by titration with steam entrainment prior to alkalization of the samples with sodium hydroxide according to [29]. For VFA determination, the samples were purified with orthophosphoric acid (25%) on sulfuric acid 0.5 M at 0.5 mL for each 2 mL of sample and then centrifuged per 10 min with 5000 g as described in [30]. Samples were injected by auto-sampler Robokrom ® GC on a Konik GC 5000 B equipped with a flame ionization detector. VFA (injected using a 10:1 split ratio and splitless time 9) were separated on a Nukol capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; Perkin Elmer - Elite FFAP; Part. N9316354). The FID injector temperature was held at 250˚C, and the detector temperature at 250˚C. The initial oven temperature was 80˚C (held for 1 min), which was then increased to 156˚C at a rate of 9˚C/min (held for 0 min). Hydrogen was used as a carrier gas and column flow was held at 2.4 mL/min. It was used for the calibration curves the standard volatile acid mix Supelco (Cat. No. 46975-U).

2.4. Statistical Analysis

Milk production and composition (mean values from the last five days of each experimental period), changes in BW and BCS, DM intake, metabolite and hormone concentration and kinetic parameters of ruminal NDF digestion were analyzed according to a Latin Square design (3 treatments and 3 experimental periods) using the MIXED procedure of SAS [31]. The model used was:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + A_{(k)l} + E_{ijkl}$$

where Y_{ijkl} = dependent variable; μ = overall mean; T_i = treatment effects; P_j = effects of the experimental period; S_k = effects of the sequence; $A_{(k)l}$ = random effects of animal within sequence and E_{ijkl} = residual error. The rumen parameters (pH, NH_3-N and VFA) were analyzed in a 3 × 3 Latin Square design using the following model:

$$Y_{ijklm} = \mu + T_i + P_j + S_k + A_{(k)l} + H_m + E_{ijkl}$$

where Y_{ijklm} = dependent variable; μ = overall mean; T_i = treatment effects; P_j = effects of the experimental period; S_k = effects of the sequence; $A_{(k)l}$ = random effects of animal within sequence, H_m = effects of hour of sampling; $T \times H_{im}$ = interaction effects of treatment and hour and E_{ijklm} = residual error. Mean comparisons were carried out using the Tukey-Kramer test, and differences were considered significant with $p < 0.05$. Linear and/or quadratic effects of concentrate levels were also tested by orthogonal contrasts.

3. Results

3.1. Chemical Composition of Feedstuffs

Chemical composition of feedstuffs are shown in **Table 1**. Herbage mass in the

Table 1. Chemical composition of pasture and concentrate[1].

Parameters	Pasture[2]	Concentrate
DM (%)	20.3 ± 1.3	90.7 ± 1.4
	g/100 g DM	
OM	89.8 ± 0.9	93.8 ± 0.8
IVDMD	69.6 ± 2.2	84.1 ± 1.2
CP	23.9 ± 1.3	18.0 ± 1.3
NDF	41.5 ± 3.6	17.0 ± 1.9
ADF	25.7 ± 3.6	6.1 ± 1.1
ADL	5.5 ± 0.4	0.9 ± 0.4
EE	2.5 ± 0.3	4.5 ± 0.7
Starch	Nd	45.9 ± 3.4

[1]Values are expressed through the mean ± standard deviation. [2]Perennial pastures of alfalfa (*Medicago sativa*). DM = dry matter; OM = organic matter; IVDMD = *in vitro* DM digestibility; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; EE = ether extract; nd = not determined.

pregrazing strips averaged 2033 (±131) kg DM ha^{-1} with an herbage allowance of 30.6 (±1.2) kg DM cow^{-1} day^{-1} during the trial.

Pasture contents of CP, NDF, ADF, ADL and ash were consistent to the average values for spring alfalfa pastures while contents of DM and EE were located in the lower limit of the ranges informed in [32] for this type of pastures (20% to 27% and 2.0% to 6.0% for DM and EE, respectively).

3.2. Milk Production and Composition

Yields of milk, 4% FCM and ECM in T7.0 were significantly higher ($p < 0.05$) compared to T3.5 and similar to that obtained in T10.5 while protein production increased ($p < 0.01$) with the level of supplementation (50 g/day) without significant differences in milk fat output (**Table 2**).

Milk concentrations of fat, protein, ST, SNG and casein were similar between treatments. Lactose content resulted higher in T10.5 compared to T3.5 while milk urea content was lower in T10.5. These results suggest a greater glucose availability to the mammary gland and a better protein-energy balance in the rumen respectively. This last statement is compatible with the lower values of NH_3-N in the rumen observed in T10.5 treatment (see later). The contrasts also revealed linear increases for milk lactose content, yields of milk, 4% FCM, MCE and protein with linear decrements for milk urea content when concentrate intake increased. No quadratic effects were detected for any of the analyzed variables.

3.3. Dry Matter and Energy Intake

Concentrate was thoroughly consumed by cows and no refusals were observed in any treatment. The estimated pasture DM intake resulted higher in T3.5, without differences between T7.0 and T10.5 (**Table 3**). Pasture substitution rate

Table 2. Milk production and composition in grazing dairy cows supplemented with an energy concentrate at 3.5 (T3.5), 7.0 (T7.0) or 10.5 (T10.5) kg·d^{-1}.

Variable	Treatment[1]			SEM	*p*<		
	T3.5	T7.0	T10.5		Treat[2]	Lineal[3]	Quadratic[3]
Milk, kg cow^{-1} d^{-1}	27.7^{b}	29.6^{a}	30.6^{a}	0.91	0.01	0.01	0.36
4% FCM. kg cow^{-1} d^{-1}	24.5^{b}	26.1^{a}	26.2^{a}	0.62	0.01	0.01	0.11
ECM, kg cow^{-1} d^{-1}	24.2^{b}	25.7^{a}	26.0^{a}	0.62	0.01	0.01	0.18
Milk fat							
kg cow^{-1} d^{-1}	0.90	0.95	0.93	0.03	0.23	0.30	0.15
g/100g	3.26	3.25	3.05	0.11	0,14	0,08	0,33
Milk protein							
kg cow^{-1} d^{-1}	0.82^{c}	0.87^{b}	0.92^{a}	0.03	0.01	0.01	0.86
g/100g	2.97	2.95	2.99	0.05	0.56	0.63	0.35
Lactose, g/100g	4.91^{b}	4.93^{ab}	4.98^{a}	0.02	0.04	0.02	0.55
ST, g/100g	11.79	11.78	11.67	0.15	0.65	0.40	0.66
SNG, g/100g	8.57	8.57	8.66	0.06	0.27	0.15	0.43
Urea, g/100g	0.048^{a}	0.047^{a}	0.043^{b}	0.001	0.01	0.01	0.17
Casein, g/100g	2.40	2.38	2.42	0.02	0.21	0.42	0.12

[1]Values are expressed as least square means (LS Means) and standard error of LS Means (SEM). [2]Effect of treatment (Treat). [3]Contrast. [a,b,c]Within rows LS Means with different letters differs (Tukey-Kramer test, $p < 0.05$). 4% FCM = 4% fat corrected milk; ECM = energy corrected milk; TS = total solids; NFS = non-fat solids.

Table 3. Dry matter and energy intakes in grazing dairy cows supplemented with an energy concentrate at 3.5 (T3.5), 7.0 (T7.0) and 10.5 (T10.5) kg·d^{-1}.

Variable	Treatment[1]			SEM	*p*<[2]	
	T3.5	T7.0	T10.5		Treat.	Period
Pasture intake						
DM, kg·d^{-1}	17.42^{a}	14.59^{b}	13.85^{b}	0.54	0.01	0.04
NE$_L$, Mcal·d^{-1}	24.92^{a}	20.87^{b}	19.80^{b}	0.77	0.01	0.04
Total intake						
DM, kg·d^{-1}	20.60^{b}	20.94^{b}	23.37^{a}	0.54	0.01	0.04
NE$_L$[3], Mcal·d^{-1}	31.43^{c}	33.88^{b}	39.33^{a}	0.77	0.01	0.04
GE[4], Mcal EN$_L$ d^{-1}	7.96^{c}	8.98^{b}	9.67^{a}	0.18	0.01	0.04
Conversion efficiency						
Milk kg·MS^{-1}	1.36	1.43	1.32	0.06	0.16	0.01
Milk Mcal EN$_L^{-1}$	0.89^{a}	0.88^{a}	0.78^{b}	0.04	0.01	0.01
ECM kg·MS^{-1}	1.18	1.24	1.12	0.04	0.09	0.01
ECM Mcal EN$_L^{-1}$	0.78^{a}	0.76^{a}	0.67^{b}	0.03	0.01	0.01

[1]Values are expressed as least square means (LSMeans) and standard error of LSMeans (SEM). [2]Effects of treatment (Treat) and period. [3]Calculated using [23]. NE$_L$ values for the pasture and concentrate: 1.43 and 2.05 Mcal kg DM^{-1} respectively. [4]Energy provided by glucogenic precursors available from the rumen and small intestine [31]. [a,b,c]Within rows LSMeans with different superscripts differ (Tukey-Kramer test, $p < 0.05$). NE$_L$ = net energy for lactation; GE = glucogenic energy; ECM = energy corrected milk.

(kg DM pasture/kg MS concentrate) was 0.88 between T3.5 and T7.0 and 0.23 between T7.0 and T10.5.

The total DM intake was higher in T10.5 without differences between T3.5 and T7.0 while total NE_L intake increased with the level of concentrate. Milk conversion efficiency per kg of DM intake was similar between treatments while expressed as an energy consumed basis resulted lower in T10.5 without differences between T3.5 and T7.0.

3.4. Changes in Body Weight and Body Condition Score

No treatment effect was detected for any of the variables associated with the variation of body reserves although the loss of BW in group T3.5 was 80% higher than observed in T10.5 (Table 4).

3.5. Plasma Concentration of Metabolites and Hormones

Despite the increased NE_L intake (Table 3), circulating levels of glucose, insulin and IGF-I were not affected by the treatments (Table 5).

The absence of treatment effect on changes in BW and BCS is compatible with the similar plasma NEFA and insulin concentation. Uremia was significantly lower in T10.5, a result consistent with the lower values of milk urea content registered in that treatment.

Table 4. Changes in body weight and body condition score in grazing dairy cows supplemented with 3.5 (T3.5), 7.0 (T7.0) and 10.5 (T10.5) kg cow^{-1} d^{-1} of concentrate.

Variable	Treatment[1]			SEM	$p<$[2]
	T3.5	T7.0	T10.5		
Initial BW, kg	603.5	601.2	604.8	14.6	0.86
Final BW, kg	588.0	591.2	596.2	13.9	0.19
BW change, kg	−15.5	−10.0	−8.6	5.3	0.63
Initial BCS, 1 a 5	2.48	2.50	2.46	0.07	0.64
Final BCS, 1 a 5	2.46	2.43	2.44	0.07	0.79
Change	−0.01	−0.07	−0.03	0.05	0.68

[1]Values are expressed as least square means (LSMeans) and standard error of LSMeans (SEM). [2]Effects of treatment (Treat); [a,b]Within rows LSMeans with different superscripts differ (Tukey-Kramer test, $p < 0.05$).

Table 5. Plasma metabolite and hormone concentrations in grazing dairy cows supplemented with 3.5 (T3.5), 7.0 (T7.0) and 10.5 (T10.5) kg/cow d^{-1} of energy concentrate.

Parameter	Treatment[1]			EEM	$P<$[2]	
	T3.5	T7.0	T10.5		Treat	Period
Glucose, mmol·l^{-1}	1.99	2.14	2.21	0.13	0.23	0.01
Urea, mmol·l^{-1}	8.10[a]	8.38[a]	7.38[b]	0.29	0.04	0.01
AGNE, µEq·l^{-1}	361.56	296.22	333.89	24.13	0.19	0.57
GH, ng·ml^{-1}	5.74	6.38	6.11	2.26	0.98	0.28
Insulin, ng·ml^{-1}	0.70	0.59	0.88	0.17	0.26	0.10
IGF-I, ng·ml^{-1}	139.03	125.00	130.24	10.11	0.62	0.10

[1]Values are expressed as least square means (LSMeans) and standard error of LSMeans (SEM). [2]Effects of treatment (Treat) and period. [a,b]Within rows LSMeans with different superscripts differ (Tukey-Kramer test, $p < 0.05$). NEFA = non esterified fatty acids; GH = somatotrophin; IGF-I = somatomedin C.

3.6. Ruminal Environment and Pasture NDF Digestion

The treatment × hour interaction was not significant for any variable of ruminal environment. Total VFA concentration was significantly higher in T3.5 without differences between T.7 and T10.5. This result could be linked to the observed increase in the acetate and butyrate concentrations (Table 6). The molar proportions of VFA were not affected by treatments. The pH values decreased with the increase in concentrate intake. The concentration of NH_3-N in T10.5 resulted significantly lower respect to T3.5 but similar to that observed in T7.0 without differences between T3.5 and T7.0. This result was consistent with the lowest values of plasma and milk urea concentrations observed in T10.5.

Increasing levels of concentrate intake tended ($p < 0.12$) to decrease the fractional rate of forage CW degradation. The effective degradability of forage CW decreased between 8.3 to 9.7 percentage points with the increase in concentrate intake, a result consistent with the decrease in ruminal pH values observed as concentrate intake increased. The rest of the parameters associated with the kinetics of ruminal CW digestion were not affected (Table 7).

Table 6. Parameters of rumen environment in grazing dairy cows supplemented with 3.5 (T3.5), 7.0 (7.0) and 10.5 (T10.5) kg/cow d^{-1} of an energy concentrate.

Parameter	Treatment[1]			SEM	$p<$[2]		
	T3.5	T7.0	T10.5		Treat	Hour	Treat * Hour
Total VFA, mmol·L^{-1}	176.1[a]	129.4[b]	133.9[b]	6.62	0.01	0.01	0.22
Ac, mmol·L^{-1}	105.9[a]	77.0[b]	83.7[b]	4.12	0.01	0.01	0.29
Ac, mol 100 mol^{-1}	60.0	59.3	62.6	0.89	0.07	0.74	0.93
Pr, mmol·L^{-1}	41.6	31.9	29.1	4.54	0.24	0.01	0.16
Pr, mol 100 mol^{-1}	23.7	24.7	21.6	0.92	0.11	0.78	0.98
Butyrate, (mmol·L^{-1})	22.0[a]	14.1[b]	15.5[b]	0.76	0.01	0.01	0.16
Butyrate, mol 100 mol^{-1}	11.9	10.9	11.5	0.12	0.11	0.01	0.13
Ac:Pr ratio	2.59	2.55	2.95	0.35	0.70	0.08	0.21
pH	6.07[a]	6.03[ab]	5.81[b]	0.07	0.02	0.04	0.66
NH_3-N, mg·dl^{-1}	49.11[a]	44.63[ab]	36.17[b]	5.49	0.01	0.09	0.94

[1]Values are expressed as least square means (LSMeans) and standard error of LSMeans (SEM). [2]Effects of treatment (Treat), Hour and Treat * Hour interaction. [a,b,c]Within rows LSMeans with different superscripts differ (Tukey-Kramer test, $p < 0.05$). VFA = volatile fatty acids.

Table 7. Parameters of pasture NDF degradation in grazing dairy cows supplemented with 3.5 (T3.5), 7.0 (7.0) and 10.5 (T10.5) kg/cow d^{-1} of ane energy concentrate.

Variable	Tratamiento[1]			EEM	$p<$[2]
	T3.5	T7.0	T10.5		
Cell Wall (CW)					
PDFCW, %	54.08	54.55	50.00	4.06	0.69
FRDCW, % $hour^{-1}$	12.17	8.29	7.53	0.95	0.12
Lag time, hours	0.13	1.78	1.05	0.92	0.55
Effective degradability,[3] kp = 5% $hour^{-1}$	38.09[a]	29.82[b]	28.38[c]	0.32	0.01

[1]Values are expressed as least square means (LSMeans) and standard error of LSMeans (SEM). [2]Treatment (Treat) and period effects. [3]Rate of passage [22]. [a,b,c]Within rows LSMeans with different superscripts differ (Tukey-Kramer test, $p < 0.05$). PDFCW = Potentially digestible fraction of cell wall; FRDCW = fractional rate of cell wall digestión.

4. Discussion

4.1. Pasture Quality

Herbage allowance (kg DM/cow day^{-1}) and pasture biomass (kg DM/Ha) are two non-nutritional factors that affect voluntary intake of grazing animals [33]. To maximize pasture intake, an herbage allowance of at least 31 kg DM/cow day^{-1} is needed [34] [35] and with alfalfa pastures under rotational grazing, maximum pasture DM intake (19.5 kg DM/cow day^{-1}) was observed at an herbage allowance of 30 kg DM/cow day^{-1} [36]. When high quality forage biomass is around 2000 kg DM ha^{-1} and then close to the average value recorded in the present trial, maximum herbage intake would be achieved when the grazing time is not restricted [37]. Pasture CP content (**Table 1**) was within the range (150 - 250 g CP kg DM^{-1}) proposed by [37] in order to obtain a high DM digestibility. In our trial, pasture DM content (**Table 1**) was above the critical range of 15% - 18% that would negatively affect herbage intake [38]. Pasture NDF content (**Table 1**) was well below the values considered critical (500 - 550 g NDF kg DM^{-1}) for high forage intake and adequate production of milk [39]. It could be concluded that forage quantity and quality did not limit pasture DM intake.

4.2. Milk Yield and Composition

Milk production increased with level of concentrate but above 7 kg/cow day^{-1} additional changes were not detected (**Table 2**). In controlled feeding conditions when pasture silage was the only forage source and total energy intake was kept constant, the maximum response in milk production was observed when glucogenic energy intake was about 8-10 Mcal EN_L in cows producing 27 - 30 kg milk day^{-1} [24]. These results are consistent with those obtained in the present work and contribute to explain the lack of milk response when cows consumed T7.0 and 10.5 kg of concentrate since glucogenic energy intake (8.98 and 9.67 Mcal **Table 3**) was in the limit zone (plateau) of the response in milk production.

Feeding energy concentrates to grazing dairy cows increased milk protein content [6] [40] a result not observed in this study (**Table 2**). The increase would be explained by a higher proportion of ruminal propionate and enhanced rumen microbial protein synthesis [40] [41] with a concomitant increase in the amino acid available at the small intestine. The absorbed amino acids together with preformed or neo synthesized glucose from propionate in the liver represent the main substrate for milk protein synthesis. Increases in milk protein concentration (+0.04%) for each extra Mcal of GE consumed in a range from 4 to 12 Mcal/day were reported in [24]. According to the estimated GE intake achieved in the present trial (**Table 3**) an increase in milk protein content would have been expected. The lack of a significant increase in milk protein content was probably masked by a dilution effect when milk production was enhanced. Milk protein yield increased linearly with concentrate intake (**Table 2**) a result also reported when dairy cows grazed alfalfa-ryegrass based pastures and were supplemented at 0, 5 and 10 kg DM/day with a corn-based concentrate [42].

Milk fat content was not affected by concentrate intake (**Table 2**) a result not observed by [6] who reported a linear decrease in milk fat when levels of concentrate increased up to 10 kg DM/day in grazing dairy cows. The main factor affecting milk fat percentage is the fiber content of the diet [43]. To prevent reductions in milk fat content, dairy cow diets should contain a minimum of 25% NDF with 16% of the NDF provided by forage [23]. In the present trial, even in cows that consumed the highest dose of concentrate, the NDF content in total diet represented 31.5% with 24.6% NDF arising from forage. This amount of fiber was apparently sufficient enough to avoid a decrease in milk fat content or fat yield (**Table 2**). A significant reduction in milk fat concentration and yield in grazing cows was reported when energy concentrate exceeded 50% DM of the diet [44]. In the present work, concentrate intake represented only 40.7% of total DM in T10.5 which would contribute to explain the absence of effects on milk fat.

When pasture silage was the only forage source, an increase in the availability of glucose and glucogenic precursors maximized lactose synthesis [24] [45] [46]. In our study, lactose content increased linearly with the level of concentrate intake (**Table 2**).

Yields of 4% FCM and ECM increased with concentrate intake without additional differences between T7.0 and T10.5 (**Table 2**). In grazing dairy cows, a curvilinear relationship was reported for yield of 4% FCM when intake of a cereal based concentrate increased from 0 to 10.4 kg DM/cow day^{-1} but it tended to plateau before reaching the highest levels of concentrate intake [44].

This pattern of response would be explained by two mechanisms, an increase in substitution rate of pasture by concentrate and a decrease in ruminal pH affecting forage fiber digestion as the level of concentrate increases [47]. In the present work pasture substitution rate did not increase with the level of supplementation averaging 0.56 or 0.23 between T7.0 and T10.5. However, increasing levels of concentrate caused a decrease in ruminal pH and affected the effective degradability of forage FDN (**Table 7**). This result may explain in part the absence of changes in yield of 4% FCM and ECM when the level of supplementation exceeded 30% of total DM intake.

Milk urea and ruminal NH_3-N concentrations decreased as concentrate intake was enhanced (**Table 2** and **Table 6**) according to results from [9] [43] suggesting an improved nitrogen utilization in supplemented cows.

4.3. DM and Energy Intake

Pasture intake decreased (−3.6 kg·day^{-1}) and total DM intake increased (+2.8 kg·day^{-1}) when cows consumed 10.5 compared to 3.5 kg/cow day^{-1} concentrate (**Table 3**) results that resulted consistent with those reviewed by [6] in grazing dairy cows. The inclusion of supplements generally depresses pasture DM intake in grazing dairy cows although the relationship between the amount of supplement fed and the substitution rate is somehow inconsistent [6]. Substitution rate

may be linked to a lower ruminal pH that may affect the rate of forage NDF digestion and hence pasture intake [48]. In our study, supplementation with increasing levels of concentrate reduced the effective degradability of forage FDN (Table 7), which would help explain the higher substitution rate observed between T7.0 and T10.5.

As pasture NE_L intake decreased after supplementation (−4.05 and −5.12 Mcal/cow d^{-1} for T7.0 and T10.5, respectively) the increase in total NE_L intake (Table 3) was explained by energy arising from the concentrate (6.51 and 13.02 Mcal d^{-1} for T7.0 and T10.5, respectively). The conversion efficiency of consumed DM was similar between treatments while that of NE_L resulted lower in T10.5 compared to T3.5 and T7.0 (Table 3). Since in T3.5 the cows apparently mobilized greater endogenous energy to sustain milk production (Table 4) the real differences in conversion efficiency could partly be masked. The lack of response in efficiency was mainly explained by the high substitution rates observed, although the differences between treatments in the mobilization of body reserves could have also contributed to explain this result.

4.4. Changes in BW, BCS and Plasma Metabolite and Hormone Concentration

The lack of changes in parameters associated to body energy stores after concentrate feeding (Table 4) were aldo reported in other studies [9] [49]. It is important to state that changes in BW is an imprecise parameter because real records may be masked in part by filling effects explained by DM intake, digestive tract content and other factors like changes in uterine involution. Circulating plasma NEFA are more useful indicators to reflect changes in lipomobilization in short-term periods but this parameter was also not affected by treatments (Table 5) keeping with the absence of differences in body parameters. Plasma glucose and regulatory hormones such as insulin, GH and IGF-I also act as dynamic or short-term indicators of energy balance [50] [51]. When cows are in negative energy balance, a decrease in plasma glucose, insulin and IGF-I concentrations are expected with a concomitant increase in NEFA [52] [53]. In this trial, plasma glucose and insulin concentrations were similar between treatments (Table 5). Liver production of IGF-I is positively correlated with energy intake and circulating GH levels [54] but even though the higher energy intake plasma concentration of IGF-I was not affected (Table 5) a result compatible with the similar circulating levels of GH.

Milk urea is derived mainly from blood urea which in turn arise from ruminal $N\text{-}NH_3$ excess and amino acids catabolism in the liver [55]. The lower plasma concentration of urea in T10.5 (Table 5) kept with the observed lower ruminal concentration of $N\text{-}NH_3$ (Table 6) and urea in milk (Table 2).

4.5. Ruminal Environment and Digestion

The lower ruminal pH associated with the increase in concentrate intake (Table

6) was an effect also reported in the review of [6] when energy concentrate intake ranged from 1.1 to 10 kg DM/cow day^{-1}. Intake of fermentable non-structural carbohydrates decrease chewing and rumination, reduce ruminal motility, change VFA production and reduce the buffer capacity leading to a decreased ruminal pH. In line with previous studies [9] [10] [42] the lower ruminal N-NH_3 concentration (**Table 6**) was probably reflecting a higher capture of N-NH_3 because total CP intake was similar between treatments (4.80 ± 0.13 kg·day^{-1}, p = 0.11). As unexpected, the highest total VFA concentration was observed with the lower concentrate intake in the T3.5 (**Table 6**) a result probably explained by the high quality of the pastures (**Table 1**) that lead to a high VFA production [56].

Concentration of VFA in ruminal fluid depends on the balance between production and absorption through ruminal wall and the rate of absorption is directly related to VFA production and inversely with ruminal pH thus preventing its accumulation in the rumen [57]. It was also reported that the fractional absorption rates of propionic and butyric VFA increased as the pH decreased from 7.2 to 4.5 [57]. In the pH range observed in the present experiment (5.57 - 6.33) it is possible that the absorption rates of propionic and butyric acids were high, while the acetic acid absorption rate was not affected by the pH level partly explaining the higher total VFA concentration recorded in T3.5. The lack of effect on ruminal VFA proportions or in the acetate: propionate ratio (**Table 6**) was consistent with the similar milk fat content (**Table 2**).

The observed trend to lower forage cell wall degradation ($p < 0.12$) and the lower degradability of forage FDN (**Table 6**) were previously observed in [9] [58]. Compared to unsupplemented cows, intake of 8.6 kg DM day^{-1} of a corn-based concentrate reduced pasture DM and NDF degradation [9]. Increasing levels of a barley based concentrate from 4.5 to 8.1 kg DM/cow day^{-1} affected pasture NDF digestibility [58]. It is generally accepted that NDF digestion is affected when the ruminal pH falls below 6.0 [59]. The average ruminal pH registered in T10.5 (5.81, **Table 6**), would contribute to explain the reduction in the effective NDF degradability of the pasture observed in that treatment. In alfalfa based pastures, feeding increased levels of energy concentrate caused a decrease in ruminal pH negatively affecting digestion pasture FDN without effects on milk fat content.

4.6. Integration of Experimental Results on Concentrate Feeding Levels

Results obtained from similar trials carried out at INTA [22] [60] [61] were analyzed by multiple regression with the incorporation of qualitative variables [62] to remove effects associated to different groups of animals, years and statistical designs. The parallelism and coincidence test were also implemented. Concentrate composition was similar for the four trials involved. Intake of concentrate ranged between 2.7 and 11.6 kg DM/cow day^{-1} and herbage allowance from 27.0 to 35.9 kg DM cow^{-1} day^{-1}. Parameters of pasture quality ranged from 20.3% to

30.8% for DM, 18.3% to 25.1% for CP; 34.8% to 50.2% for NDF; 19.9% to 25.8% ADF and 69.6% to 75.2% in vitro DM digestibility. Average milk production at the start of the trials was 34.1 kg/cow day^{-1}. The four regression lines for milk production were parallel and coincident and the following marginal response equation was obtained: Milk (kg/cow day^{-1}) = 25.95 + 0.774 * kg concentrate DM ($p < 0.042$, $R^2 = 0.37$). The equation suggest that unsupplemented cows may produce up to 25.95 kg milk when only pasture is fed and above this yield milk production increases linearly at a rate of 0.774 kg milk per kg DM of additional concentrate as the amount of concentrate intake increases from 2.7 to 11.6 kg MS/cow day^{-1}. For the rest of the variables of production and composition of milk analyzed, the lines were parallel but not coincident giving rise to 4 different equations. The immediate or marginal response is one that is expressed in the short term after supplementation resulting from total nutrients absorbed and the way they are partitioned between milk production and weight gain. It has been described as curvilinear with increasing amounts of concentrate [42]. Thus, in some studies the marginal response decreased above intakes of 3 to 4 kg DM day^{-1} of concentrate for cows of moderate (less than 25 kg·day^{-1}) milk production potential [62]. The greater response to concentrate supplementation in cows of high genetic merit can be attributed to a greater partition of nutrients for milk production with respect to cows with lower production potential [63]. The linear increase in milk production observed in the present work was similar to that reported by [6] in high yielding dairy cows producing from 28.3 to 45.8 kg milk day^{-1} supplemented with increasing levels of concentrate in a range of 1.8 to 10 kg DM day^{-1} . The average production level of the cows used in the trials included in the present analysis was within the range reported in [6]. However, the marginal response obtained in the present work (0.774) was lower than reported in [6]. Substitution rate is higher when herbage mass is not limiting pasture intake and in one of the main factors affecting the productive response to supplementation [64]. Responses (kg milk/kg concentrate) of 0.4 to 0.6 and 0.92 were reported in [64] for papers published before and after 1990 respectively and the increase in the marginal was explained by the decrease in susbstitution rate from 0.6 to 0.4 kg DM pasture/ kg DM concentrate. The substitution rate observed in two trials of the present analysis was near to 0.6 and then contributed to explain the global lower marginal response obtained with respect to trials reviewed in [65].

5. Conclusion

Increasing intake levels of energy concentrate improved yields of milk, 4% FCM and ECM without additional responses when concentrate represented more than 30% of total dry matter intake of grazing cows producing about 30 kg milk day^{-1} without affecting chemical composition of the milk. The increase in total DM and energy intake explained in part the lack of positive effects on conversion efficiency although the increased energy absorbed was not apparently channeled

to body reserves or lower lipid mobilization results that were consistent with the lack of effect on changes in insulin and growth hormone concentrations that may induce alterations in the partition of the absorbed energy. Results obtained suggest that in order to avoid negative effects on pasture fiber digestion starch based concentrate intake does not exceed 30% of total dry matter intake of cows.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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