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Influence of dietary starch contents on milk composition of Friesian cows in early lactation

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ABSTRACT

The aim of this research was to evaluate the effect of the modification of diet starch content on milk composition and on its nitrogen and mineral fractions. Ten Italian Friesian primiparous cows were randomly assigned to two groups and fed a basal total mixed ration, (BSD, basal starch diet, 24.9% starch/DM) until 42 days in milking (DIM). At 43 DIM, 5 animals (control group, CTR) continued to receive the same ration and the remaining 5 cows (experimental group, EXP) were fed a low starch diet (LSD, 21.0% starch/DM) until 65 DIM, followed by a high starch diet (HSD, 28.3% starch/DM) 66 to 85 DIM. From 86 DIM until 94 DIM, cows of the EXP group returned to the BSD. Milk samples were collected at 37, 50, 60, 70, 80, 94 DIM. Starch intake was lower for EXP at DIM 50, 60 and higher at 70 and 80 DIM ($P < 0.01$). Milk yield and fat corrected milk (FCM) did not vary between groups and times of sampling, but HSD caused a significant ($P < 0.05$) reduction of milk fat in the EXP animals. NPN and MUN contents were affected by dietary treatments at 80 DIM ($P < 0.05$), being higher in CTR and lower in EXP group in comparison to their basal values (37 DIM). The α_s1-casein percentages at 80 and 94 DIM increased in the EXP but not in the CTR group, and a marked decrease of α_s2-casein percentage for the EXP group at 94 DIM was observed ($P < 0.05$). Milk sodium content decreased at 80 and 94 DIM and the sodium to potassium ratio was reduced after the return to the basal diet in the EXP group (94 DIM), indicating that dietary starch variations can be involved in the control of epithelium integrity of mammary gland in early lactation.

Key Words: Dairy cows, Dietary starch, Milk protein fractions, Milk minerals

RIASSUNTO

INFLUENZA DEL CONTENUTO IN AMIDO DELLA DIETA SULLA COMPOSIZIONE DEL LATTE IN VACCHE PRIMIPARE DI RAZZA FRISONA ITALIANA ALL'INIZIO DELLA LATTAZIONE

La ricerca ha avuto come scopo di valutare l'effetto della modifica del contenuto di amido della razione sulla composizione del latte e sulle sue frazioni azotate e minerali. A tal fine, 10 bovine primipare di razza Frisona Italiana sono state suddivise in modo casuale in due gruppi e alimentate con una razione unifeed (BSD, 24,9% amido/SS) sino a 42 giorni di lattazione (DIM). Dal 43° DIM, 5 primipare continuavano a ricevere la stessa razione (gruppo di controllo, CTR), mentre le altre 5 primipare (gruppo sperimentale, EXP) venivano alimentate, sino al 65° DIM, con una dieta a basso contenuto di amido (LSD, 21,0% amido/SS); dal 66° all'85° DIM al gruppo sperimentale veniva somministrata una dieta ricca di amido (HSD, 28,3% amido/SS). Dall'86° al 94° DIM le vacche del gruppo sperimentale ritornavano a ricevere la dieta

BSD. I campioni di latte sono stati raccolti a 37, 50, 60, 70, 80, 94 DIM e analizzati per contenuto in grasso, proteina, lattosio, frazioni azotate, caseiniche e minerali. Come atteso, l'ingestione media giornaliera di amido è risultata più bassa nel gruppo EXP a 50 e 60 DIM (4,135 e 4,111 g/capo/d), e più alta a 70 e 80 DIM (5,760 e 5,715 g/capo/d; P < 0,01). L'ingestione di energia non è stata molto diversa fra i due, a parte una differenza significativa a 70 DIM, e, di conseguenza, non sono state registrate differenze significative nella produzione di latte e di latte corretto per il contenuto in grasso (FCM) tra le due tesi sperimentali e in funzione del momento di prelievo. La dieta HSD ha causato una significativa (P < 0,05) riduzione del contenuto in grasso del latte nel gruppo EXP (2,92 vs 3,39%). I contenuti di azoto non proteico (NPN) e ureico (MUN) del latte sono stati influenzati dalla dieta a 80 DIM (P < 0,05), risultando più elevati nel gruppo CTR e inferiori nel gruppo EXP a 80 DIM, durante la fase di somministrazione di HSD, in confronto ai valori registrati a 37 DIM. La proporzione percentuale di α_s1-caseina a 80 e 94 DIM è risultata aumentata nel gruppo EXP a 80 DIM e a 94 DIM nel gruppo EXP è stata osservata una significativa (P < 0,05) diminuzione della proporzione percentuale di α_s2-caseina. Nel gruppo EXP è stata registrata una diminuzione del contenuto di sodio nel latte a 80 e a 94 DIM, mentre nel gruppo EXP, con il ritorno alla dieta di base (94 DIM), il rapporto sodio/potassio ha mostrato una riduzione.
I dati della presente ricerca suggeriscono che l'aumento di amido nella dieta comporta modificazioni non soltanto del contenuto in grasso del latte ma anche nella ripartizione delle frazioni caseiniche e a livello di contenuto di minerali. Quest'ultimo risultato, potrebbe indicare un miglioramento della funzionalità dell'epitelio della ghiandola mammaria in presenza di una maggiore contenuto di amido nella dieta.

Parole chiave: *Vacche da latte, Dieta con amido, Frazioni proteiche del latte, Minerali del latte*

Introduction

Dietary starch and non structural carbohydrates (NSC) have been considered major factors affecting milk yield and quality in dairy cows (Beauchemin *et al.*, 1997; Lykos *et al.* 1997; Fitzgerald and Murphy, 1999; Khalili *et al.*, 2001), both in early and late lactation (Kennelly *et al.*, 1999; Khorasani and Kennelly, 2001). However, the effect of dietary NSC on milk production is sometimes controversial. Kennelly *et al.* (1999) and Lykos *et al.* (1997) have reported a positive effect of NSC degradation on milk yield in early lactation or for high yielding cows. However, Uchida *et al.* (2001) did not show variations in milk yield and composition substituting maize for steam-rolled maize, a process known to increase rumen degradability and total tract digestibility. In another experiment, the amount of dietary NSC did not cause a variation in milk yield and composition that was affected from the grain source (Beauchemin *et al.*, 1997). The different physiological conditions of cows in these experiments, as well as their genetic merit, do not make it possible to generalise the role of NSC content and its degradability on milk production and composition.

Data of milk production and its composition are not easy to interpret, especially in early lactation, since during the initial phases of lactation, modifications of total mixed rations (TMR, variations in NSC or NDF contents, grain and protein sources, forage to concentrate ratio, particle length) are required to meet the nutrients and dietary requirements of high producing dairy cows (NRC, 2001). These factors, together with the concomitant variations of dry matter intake (DMI) which occurs in early lactation, can interact with each other at a rumen and metabolic level, leading to somehow unpredictable flows of nutrients and microbial yields (Rode and Satter, 1988; Stefanon *et al.*, 2001).

The high dietary NSC contents required to sustain the onset of lactation are claimed to increase metabolic disorders, mastitis and laminitis (Drackley, 1999). Oxidative stress has been generally associated with these clinically relevant pathologic conditions, as well as with poor reproductive performance and milk quality (Miller *et al.*, 1992). The outcome of these pathologies is characterised by inflammatory reactions that could induce the presence of xenobiotic components affecting metabolic pathways and related enzymes needed for milk production, such as mammary gland lipases and protein kinase C (Neville and Walsh, 1995).

The complexity of nutritional, metabolic, endocrine and cellular facts involved in lactation are not thus not easy to separate, especially in a long-term study. In the present research a short-term experiment with a repeated measure model

Table 1. Composition of experimental rations used in the trial (DM basis).

	Diet[1]		
Ingredients (%)	BSD	LSD	HSD
Maize silage	29.41	22.25	26.25
Maize	22.03	19.39	27.32
Lucerne, hay	13.52	12.89	12.07
Cotton seeds	6.83	5.97	6.10
Grass hay	6.76	6.44	6.03
Soybean meal, 44%	6.06	4.62	5.41
Supplement[2]	5.35	5.10	4.77
Brewer's grain	5.20	9.80	4.64
Lucerne, dehy	4.55	4.34	4.07
Soybean, extruded	___	5.23	3.15
Sugar beet pulp	___	3.97	___
Urea	0.30	___	0.19

[1] *BSD = basal starch diet, LSD = low starch diet, HSD = high starch diet*

[2] *Supplement contains minerals, soybean, sunflower, brewer yeast, barley, wheat bran and sugar beet pulp; chemical composition (DM basis) is 33.9% crude protein, 14.4% fat, 3.9% crude fibre, 33.3% ash.*

was applied to minimise metabolic and endocrine differences related to the distance from calving and to the variation of DM intake.

The experiment applied was designed to assess if short-term dietary starch re-alimentation after starch restriction - within normal dietary recommendations - can influence milk compositions and technological properties, providing similar energy and protein supplies, thus avoiding relevant variations in milk output. In a previous paper (Gabai *et al.*, 2004) the relevance of these dietary variations on markers of oxidative stress, metabolic, endocrine and immune parameters have been reported. A further goal of the study was to develop an experimental model aimed at minimising the number of animals, according to the public concerns for animal welfare.

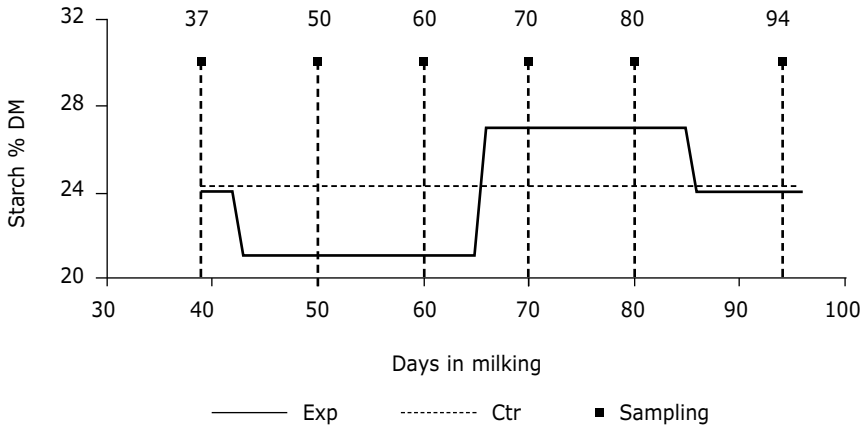
Material and methods

Animals and diet

Ten Friesian heifers were selected on the basis of genetic merit and identified at the seventh month of pregnancy according to the date of artificial insemination. At calving, animals were randomly assigned to two groups and, until 42 days in milking (DIM), they were fed a basal TMR (total mixed ration, BSD, Table 1). At 43 DIM, five animals (control group, CTR) continued to receive the same ration and the remaining five animals (experimental group, EXP) were allotted the experimental diets, which consisted of a stair-step compensated starch regimen. Experimental diets were designed to have low (LSD) and high (HSD) starch contents (Table 1), and were subsequently offered to the EXP group according the schedule of Figure 1.

Diets were formulated to be iso-energetic and to include the same forages, varying the amount of soybean, maize and sugar beet pulp. Before the beginning of the study, batch quantities of the ingredients, sufficient to cover the needs of the whole experiment, were identified and stored. Total mixed rations (TMR) were prepared daily, offered *ad libitum* and distributed twice a day, at 08:00 and 16:00. Feed residues were collected and weighed every morning, composited weekly, subsampled for DM and nutrient determinations, and used to compute nutrient intakes. Animals were milked in the milking room twice a day and milk yield recorded every two days. At DIM 37, 50, 60,

Figure 1. The graph represents starch content variations of diets administered to the control (CTR) and experimental (EXP) animals. The black boxes indicate the days of sampling.



70, 80 and 94 morning and evening milk samples were pooled and used for analysis; 4% fat corrected milk (FCM) was thus calculated.

Feed analysis

Total mixed ration samples of BSD, LSD and HSD were collected every week for chemical analysis during the whole experimental period. The number of samples were 8, 3 and 3 for BSD, LSD and HSD, respectively. TMR were dried to a constant weight in a forced-air oven at 60°C for 48 h. DM content was determined by drying samples overnight at 110°C, and ash content by ignition at 550°C for 6 h. Crude protein was analysed using a micro-Kjeldahl method and ether extract with the Soxhlet method (AOAC, 1990), NDF and ADF using a non-sequential method (Van Soest *et al.*, 1991) and starch using the enzymatic method of Herrera-Saldana *et al.* (1990). Minerals were determined using atomic absorption spectrophotometry (1100B Atomic Absorption Spectrophotometer, Perkin Elmer, Shelton, Connecticut 06484-4794, USA), with the exception of P and Cl, which were analysed colorimetrically (AOAC, 1990), using a Helios Gamma UV/Vis Spectrophotometer (Thermo Spectronic, Rochester, NY 14625, USA). The energy content of the diets was calculated using the energy concentration of the ingredients (NRC, 2001).

Milk analysis

Fat, protein and lactose contents of the individual milk samples collected at 37, 50, 60, 70, 80 and 94 DIM were analysed by means of infrared readings (Biggs, 1978) using a Milk-o-Scan 134 A/B apparatus (Foss-Electric, DK-3400 Hillerød, Denmark). SCC in quarter milk samples were counted on a Bentley Somacount 150 (Bentley, USA) and the natural logarithm (ln) of the values obtained were used for the statistical analysis (IDF, 1997).

Individual milk samples collected at 37, 60, 80 and 94 DIM were also analysed for nitrogen fractions, mineral elements and rennet-coagulation properties. Total nitrogen (TN), non-casein nitrogen (NCN), non-protein nitrogen and proteose-peptone nitrogen were determined by Kjeldahl according to Aschaffenburg & Drewry (1959), using a Digester DK6 and a Steam distilling unit UDK126A (Velp Scientifica, 20040 Usmate, Italy). From these nitrogen fractions, the following variables were calculated: casein N (TN - NCN) and casein number (casein N x 100 / TN). Milk urea nitrogen (MUN) was measured using a Bun Analyzer 2 (Beckman Coulter, Inc., 92621 Brea, California, USA) and the Bun reagent kit P/N 667510 (Beckman Coulter, Inc., Galway, Ireland). Casein was fractionated using reverse-phase high performance liquid chromatography (Spectra

Physics, D-64289 Darmstadt-Kranichstein, Germany) according to Visser et al (1986), with detection at 220 nm.

Calcium, sodium and potassium were determined by 1100B Atomic Absorption Spectrophotometer (Perkin Elmer, Shelton, Connecticut 06484-4794, USA) (Anon., 1982) ashing milk samples by muffle furnace at 530°C and re-dissolving with HCl. Phosphorus was determined by colorimetry, according to Allen (1940), using a Helios Gamma UV/Vis Spectrophotometer (Thermo Spectronic, Rochester, NY 14625, USA) and chloride (Cl-) by titration with $AgNO_3$ using the volumetric method of Charpentier-Volhard (Savini, 1946). The same milk samples were also used to measure potentiometric acidity (pH) and titratable acidity, with 0.25 N NaOH, according to the Soxhlet-Henkel method (Anon., 1963). Milk rennet coagulation properties were determined at 35°C (McMahon and Brown, 1982) by Lattodinamografo apparatus (Foss-Italia, Padova, Italy).

Statistical analysis

Data were analysed using the repeated measure analysis of the general linear model procedure of SPSS (1997) with trend analysis. Factor terms included in the model were treatment (T, CTR vs EXP), animal within group (Subject), sampling times (S), and the interaction of treatment and sampling times (TxS), which provided 46 degrees of freedom for the error term. Data of N fractionation, pH, technological parameters and

Table 2. Dry matter content and chemical compositions of experimental diets.

		Diet[1]		
		BSD SD n = 8	LSD SD n = 3	HSD SD n = 3
DM	%	53.72 + 0.86	52.73 + 0.61	55.99 + 0.12
DM basis:				
Ash	%	7.3 + 0.05	6.8 + 0.02	7.0 + 0.02
CP	"	16.6 + 0.14	17.1 + 0.14	16.6 + 0.20
EE	"	4.3 + 0.17	5.5 + 0.11	4.6 + 0.09
NDF	"	38.0 + 0.43	38.4 + 0.47	35.3 + 0.19
ADF	"	23.0 + 0.27	23.3 + 0.29	21.1 + 0.11
Starch	"	24.9 + 0.34	21.0 + 0.25	28.3 + 0.10
NSC	"	34.9 + 0.37	32.6 + 0.27	37.6 + 0.02
NSC/NDF		0.92 + 0.02	0.85 + 0.02	1.07 + 0.01
Cl	%	0.33 + 0.01	0.31 + 0.01	0.29 + 0.01
P	„	0.51 + 0.01	0.51 + 0.01	0.48 + 0.01
Ca	„	0.86 + 0.03	0.84 + 0.02	0.78 + 0.02
Mg	„	0.33 + 0.01	0.32 + 0.00	0.31 + 0.00
Na	„	0.35 + 0.02	0.34 + 0.01	0.30 + 0.01
K	„	1.27 + 0.01	1.23 + 0.01	1.21 + 0.00
DCAB[2]	mEq/kg	383 + 5.52	383 + 0.02	360 + 3.30
NEL[3]	Mcal/kg	1.52 + 0.02	1.54 + 0.00	1.57 + 0.00

[1] *BSD = basal starch diet, LSD = low starch diet, HSD = high starch diet*

[2] *DCAB = dietary cation anion balance*

[3] *NE_L = Net energy lactation, estimated on the basis of TMR composition*

mineral content of milk were analysed with the same statistical model, which provided 24 degrees of freedom for the error term. Simple contrasts of TxS effects between the first level of sampling time (37 DIM) vs the other DIMs were calculated and reported in the tables.

Results

The same ingredients were used for the experimental diets, with the exception of sugar beet pulp inclusion in the LSD ration, and urea addition in the BSD and HSD rations (Table 1). Sugar beet pulps were required to reduce starch content, compensating for the decrease in maize meal and maize silage in the LSD diet. Moreover, to keep constant the estimated net energy for lactation (NE_L) concentration in the LSD diet, full fat extruded soybean was included. In the HSD diet, maize content was increased and full fat extruded soybean included to maintain the dietary CP content of this diet similar to the BSD. For all three diets, maize was almost the only source of starch, apart from a marginal contribution (about 3% of total starch) of barley, contained in the supplement.

Starch contents (Table 2) were 24.9, 21.0 and 28.3% DM for the BSD, LSD and HSD diets respectively, and NSC 34.9, 32.6 and 37.6% DM, respectively, reflecting starch variations between diets. The increase in starch content in HSD was compensated by the reduction of NDF, which caused a modification of the NSC to NDF ratio from 0.85 of LSD to 1.07 of HSD, compared to 0.92 for BSD. Some differences were observed for the CP and lipid contents of the diets, higher in the LSD diet. Mineral contents and dietary cation anion balance (DCAB) were always lower in the HSD than in BSD and LSD diets.

Nutrient intakes were not different between treatments (CTR vs EXP), while significant interactions of treatment and time of sampling (TxS) were observed for DMI, starch, NDF and NSC intakes, but not for CP intake (Table 3). Starch and NDF intakes dramatically varied between CTR and EXP groups at DIM 50, 60, 70 and 80 ($P < 0.01$), but were comparable at the beginning and at the end of the trial (94 vs 37 DIM, $P>0.05$). DMI increased in the CTR group with DIM, and was slightly reduced for the EXP group during the administration of LSD (significant interactions 50 vs 37 and 60 vs 37 DIM). After the subsequent increase in dietary starch concentration in the EXP group (HSD, DIM 70 and 80), animals recovered the gap of DMI relative to the CTR group. The contrast analysis of 94 vs 37 DIM controls was never significant, indicating that cows in the experimental group returned to the initial nutrient supplies. Estimated NEL intake was significantly higher during the HSD diet administration ($P < 0.05$).

Treatment (T) and time of sampling (S) did not significantly affect milk (Table 4) and FCM production between the CTR and EXP groups. A significant ($P < 0.05$) increase in milk SCC was observed in EXP group during the period of LSD (50 and 60 DIM). Milk protein content and yield did not vary between treatments and time of sampling. A significant reduction of fat content, but not in fat yield, in the EXP animals (contrast 80 vs 37 DIM, $P < 0.05$) was observed and contrast analysis for lactose content indicated a significant treatment and time of sampling (TxS) interaction at DIM 50, 70 and 94 ($P < 0.01$).

The NPN and MUN contents (Table 5) were affected by dietary treatments at 80 DIM ($P < 0.05$), being higher in CTR and lower in EXP group in comparison to their basal values (37 DIM). Technological parameters, such as pH, titratable acidity and rennet coagulation properties of milk were unaffected by dietary treatments (data not shown). A reduction of k-casein ($P < 0.01$) and an increase in $\beta+\gamma$ casein percentages in milk with DIM were observed ($P < 0.01$). The α_s1-casein percentages at 80 and 94 DIM increased in the EXP but not in the CTR group, and a marked decrease in α_s2-casein percentage at 94 DIM ($P < 0.05$) was observed for the EXP group.

Calcium and phosphorus, but not potassium, contents in milk (Table 6) were unaffected by dietary treatments, and a significant reduction of sodium content and sodium to potassium ratio in milk of EXP animals was observed at 94 DIM ($P < 0.05$).

Table 3. Mean DM and nutrients intakes concentrations of cows fed control (CTR) and experimental (EXP) diets.

Item		Treat.	DIM						Main effects and interactions (P <)				
			37	50	60	70	80	94	T	MSE1	S	TxS	MSE2
			BSD	LSD	LSD	HSD	HSD	BSD					
DMI	kg/d	CTR	19.85	20.23 ^{A}	20.17 ^{A}	20.00	19.73	20.13		0.625	***	***	0.0411
		EXP	19.89	19.47 ^{B}	19.73 ^{B}	20.36	20.19	20.29					
NE_L	Mcal/d	CTR	30.19	30.34	30.62	30.38 ^{b}	30.19	29.45		1.440	***	**	0.0920
		EXP	30.25	30.50	30.87	31.25 ^{a}	31.05	29.68					
Starch	kg/d	CTR	4.966	5.170 ^{A}	4.952 ^{A}	4.977 ^{B}	4.880 ^{B}	5.035		0.0386	***	***	0.0028
		EXP	4.976	4.135 ^{B}	4.111 ^{B}	5.760 ^{A}	5.715 ^{A}	5.075					
CP	"	CTR	3.263	3.343	3.328	3.339	3.319	3.340		0.0174	***		0.0012
		EXP	3.269	3.368	3.369	3.382	3.364	3.366					
NDF	"	CTR	7.563	7.610 ^{A}	7.790 ^{A}	7.534 ^{A}	7.397 ^{A}	7.618		0.0883	***	***	0.0059
		EXP	7.577	7.380 ^{B}	7.637 ^{B}	7.173 ^{B}	7.127 ^{B}	7.679					
NSC	"	CTR	6.946	7.169 ^{A}	6.941 ^{A}	7.017 ^{B}	6.877 ^{B}	7.052		0.0767	***	***	0.0051
		EXP	6.958	6.408 ^{B}	6.414 ^{B}	7.645 ^{A}	7.587 ^{A}	7.108					

Means with different superscripts are significantly different for P<0.01 (^{A,B}) or P<0.05 (^{a,b}), after simple contrast analysis.
BSD = basal starch diet; LSD = low starch diet; HSD = high starch diet; T= Probability of F for Treatment effect; MSE1 = Between group mean square error; S = Probability of F for Sampling effect; TxS = Probability of F for Treatment x Sampling effect; MSE2 = Within subject mean square error.
NE_L = Net Energy Lactation; CP = Crude Protein; NSC = Non Structural Carbohydrates

Table 4. Mean milk yield and composition of cows fed control (CTR) and experimental (EXP) diets.

Item		Treat.	DIM						Main effects and interactions (P <)				
			37	50	60	70	80	94	T	MSE1	S	TxS	MSE2
			BSD	LSD	LSD	HSD	HSD	BSD					
Milk	kg/d	CTR	26.02	28.54	27.92	28.92	26.86	26.28		18.175			5.059
		EXP	24.98	25.42	24.40	27.84	26.06	26.62					
Milk	SCC ln	CTR	4.49	4.21 ^{b}	3.51 ^{b}	2.86	3.23	3.44		4.185	*	*	1.167
		EXP	3.55	5.47 ^{a}	4.99 ^{a}	4.27	3.00	3.63					
4% FCM	kg/d	CTR	21.86	27.34	28.10	26.09	24.55	24.71		20.820			9.919
		EXP	22.03	21.65	23.35	24.69	21.73	23.70					
Fat	"	CTR	0.76	1.06	1.13	0.97	0.92	0.95		0.0401			0.0326
		EXP	0.80	0.77	0.91	0.90	0.75	0.87					
Protein	"	CTR	0.80	0.85	0.85	0.88	0.88	0.84		0.0023			0.0050
		EXP	0.82	0.85	0.79	0.92	0.87	0.90					
Lactose	"	CTR	1.37	1.43	1.41	1.45	1.41	1.36		0.0419			0.0167
		EXP	1.28	1.35	1.19	1.45	1.34	1.40					
Fat	%	CTR	2.92	3.63	3.97	3.44	3.39 a	3.60		0.124			0.374
		EXP	3.19	3.03	3.72	3.28	2.92 b	3.29					
Protein	"	CTR	3.08	2.96	3.03	3.04	3,26	3.19		0.0531			0.0176
		EXP	3.27	3.38	3.25	3.30	3.33	3.36					
Lactose	"	CTR	5.27	5.02 ^{B}	5.06	5.01 ^{B}	5.25	5.18 ^{a}		0.0040	***	***	0.0122
		EXP	5.13	5.28 ^{A}	4.88	5.23 ^{A}	5.16	5.28 ^{b}					

Means with different superscripts are significantly different for P<0.01 (^{A,B}) or P<0.05 (^{a,b}), after simple contrast analysis.
BSD = basal starch diet; LSD = low starch diet; HSD = high starch diet; T= Probability of F for Treatment effect; MSE1 = Between group mean square error; S = Probability of F for Sampling effect; TxS = Probability of F for Treatment x Sampling effect; MSE2 = Within subject mean square error.

Table 5. Mean values of nitrogen fractions of milk of cows fed control (CTR) and experimental (EXP) diets.

Item		Treat.	DIM 37	DIM 60	DIM 80	DIM 94	Main effects and interactions (P <) T	MSE1	S	TxS	MSE2
			BSD	LSD	HSD	BSD					
Total N (TN)	mg/100g	CTR	460.0	475.3	472.0	462.2		832.243			937.89
		EXP	490.8	510.1	492.3	493.8					
Non protein N	"	CTR	27.0	27.7	27.6 a	24.5		23.542	**	*	3.95
		EXP	26.3	27.8	22.4 b	22.1					
Proteose-peptone N	"	CTR	4.7	7.5	8.5	11.2		6.260			9.49
		EXP	8.8	8.8	7.7	10.0					
MUN	"	CTR	9.8	11.4	11.7 a	10.5		2.626		*	1.07
		EXP	11.4	11.9	10.5 b	11.2					
Casein number (CN/TN)	%	CTR	75.4	76.3	75.6	75.9		3.676			1.42
		EXP	75.0	75.0	75.8	75.3					
k-Casein	"	CTR	11.57	11.48	10.74	10.97		0.534	**		0.637
		EXP	11.91	11.96	10.46	11.23					
α_s2-Casein	"	CTR	11.18	12.16	11.13	11.30 a		0.302	***	*	0.690
		EXP	12.72	12.73	11.74	10.18 b					
α_s1-Casein	"	CTR	38.70	37.54	38.70 b	38.28 b		0.951	*	*	0.683
		EXP	37.59	37.93	39.04 a	39.17 a					
$\beta+\gamma$- Casein	"	CTR	38.55	38.82	39.44	39.45					
		EXP	37.78	37.38	38.77	39.42		0.958	*		1.154

Means with different superscripts are significantly different for P<0.01 (^{A,B}) or P<0.05 (^{a,b}), after simple contrast analysis.
BSD = basal starch diet; LSD = low starch diet; HSD = high starch diet; T= Probability of F for Treatment effect; MSE1 = Between group mean square error; S = Probability of F for Sampling effect; TxS = Probability of F for Treatment x Sampling effect; MSE2 = Within subject mean square error.

Table 6. Mean value of mineral elements of milk of cows fed control (CTR) and experimental (EXP) diets.

Item		Treat.	DIM				Main effects and interactions (P <)				
			37	60	80	94	T	MSE1	S	TxS	MSE2
			BSD	LSD	HSD	BSD					
Ca	mg/100g	CTR	117.4	117.3	115.5	115.9		15.054			23.269
		EXP	118.8	119.6	114.5	113.9					
P	„	CTR	91.9	93.9	91.1	91.7		48.764			28.754
		EXP	100.5	101.7	98.9	92.1					
Na	„	CTR	45.6	40.0	44.3 [a]	42.6 [a]	**	5.918	**	**	7.499
		EXP	39.6	40.0	36.6 [b]	32.7 [b]					
K	„	CTR	158.2	160.3	154.7	157.6	*	20.522			20.330
		EXP	148.0	151.2	149.2	155.5					
Na : K		CTR	0.28	0.25	0.28	0.26 [a]		0.0001	**	*	0.0004
		EXP	0.26	0.26	0.25	0.22 [b]					
Cl	mg/100g	CTR	73.8	70.7	68.4	72.6		20.639			20.110
		EXP	76.8	77.6	74.1	72.8					

Means with different superscripts are significantly different for P<0.01 ([A,B]) or P<0.05 ([a,b]), after simple contrast analysis.
BSD = basal starch diet; LSD = low starch diet; HSD = high starch diet; T= Probability of F for Treatment effect; MSE1 = Between group mean square error; S = Probability of F for Sampling effect; TxS = Probability of F for Treatment x Sampling effect; MSE2 = Within subject mean square error.

Discussion

The modification of the NSC to NDF ratio from 0.85 (LSD) to 1.07 (HSD) in the EXP group was consistent with the experiment that was designed to evaluate animal response in relation to short-term variations of starch or NSC. The different trend of DMI between groups during the trial was likely to be related to the lower NSC to NDF ratio of the LSD, below the 0.90 threshold level (Nocek and Russel, 1988), compared to BSD. However, it must be considered that the observed differences of DMI between the CTR and EXP groups was numerically moderate (0.76 and 0.44 kg/d at DIM 50 and 60, respectively), and did not cause significant variations of estimated NEL intake between groups until 70 DIM. The HSD had the lowest content of NDF, a nutrient that is negatively related to DMI (Mertens, 1997), even though the effect is not always confirmed (Kennelly *et al.*, 1999; Khorasani and Kennelly, 2001). The higher DMI intake and energy content of the HSD caused an increase in NEL intake for the EXP group at 70 DIM.

This result of milk yield was expected, since diets were designed to avoid differences in milk output, a condition required to explain variations - if any - of milk composition and technological characteristics.

The higher milk SCC in EXP group during the period of LSD (50 and 60 DIM) was unrelated to an increased frequency of intramammary infections (bacterial count, data not shown). An average level of SCC of 237.000 cells/ml (5.47 ln cells /ml) was measured only at 50 DIM, a value far above of 100.000 cells/ml considered a threshold for mammary gland infection (Smith, 2002; Pyorala, 2003), but proteose-peptone content (Table 5) was within normal ranges, and it is likely that this increase of SCC was related to a triggering of the different biochemical mechanisms controlling cell trafficking, as suggested in a previous paper (Gabai *et al.*, 2004).

The variation of structural and non structural carbohydrate intakes probably affected milk fat content at 80 DIM for EXP group, which can be related to a change of volatile fatty acid contents in the rumen (Van Soest, 1994) and to a lower rumen pH reported for higher starch diets (Khorasani and Kennelly, 2001). Another possible explanation is the formation of conjugated fatty acids, trans-10 cis-12 linoleic acid in particular, associated to high dietary starch, which has been demonstrated as the main causing factor for milk fat depression (Baumgard *et al.*, 2000). The variation of milk lactose content is not easy to explain and the different NDF to NSC ratios between diets could be implied. The higher starch and lower NDF intakes after the administration of HSD could have increased rumen propionate production (Khorasani and Kennelly, 2001) and glucose availability for lactose synthesis.

Protein content and casein number did not vary during the trial but a decrease in milk NPN and MUN contents at 80 DIM was observed, a result consistent with data reported by Likos *et al.* (1997) for diets higher in rumen degradable NSC. It is likely that the higher starch intake in EXP group after feeding HSD had produced an increase in N uptake by rumen microbes with a consequent decrease in plasma urea (DePeters and Cant, 1992). In the present experiment, diets were formulated to avoid differences in protein quality and to cover nitrogen requirements of cows. Moreover, CP intake did not change between groups and sampling times. According to Bertrand *et al.* (1998) modifications of milk total N and casein N can be expected when the availability of essential amino acids, i.e. lysine, limit milk protein synthesis. However, α_s1-caseins increased for the EXP group in the last part of the trial, probably as a consequence of the higher energy intake, which could have spared AA from gluconeogenesis to milk protein synthesis. According to Auldist *et al.* (2000), the amount of casein fractions in milk are affected by both β-lactoglobulin phenotype and energy allowance, the latter effect being more pronounced.

Another consequence of starch re-alimentation can be an enhancement of mammary gland functions. At 80 and 94 DIM, a decrease in markers of metabolic and oxidative stress occurred for EXP group (Gabai *et al.*, 2004), and the concomitant reduction of sodium and of sodium to potassium ratio, although this latter is evident at 94 DIM, would indicate a better mammary gland epithelium integrity (Sorensen *et al.*, 2001).

Conclusions

The results herein presented indicate that dietary modifications of carbohydrates within the recommended range, providing that energy and protein requirements are satisfied, caused minor variations of milk compositions and technological characteristics, and that these occurred mostly during the period of starch re-alimentation (HSD).

Most of the results presented in this paper are consistent with previous published data, indicating that the repeated measure model applied here can be effectively used as a powerful tool for lactation studies in dairy cows, thus reducing the number of experimental animals. Data reported in the companion papers will give an insight to the role that nutrient availabilities could have on oxidative stress and its effects on cow welfare and health conditions.

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