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

Andrea Summer, Primo Mariani, Michela Bellotti, Alfonso Zeconci, Sandy Sgorlon & Bruno Stefanon

To cite this article: Andrea Summer, Primo Mariani, Michela Bellotti, Alfonso Zeconci, Sandy Sgorlon & Bruno Stefanon (2005) Influence of dietary starch contents on milk composition of Friesian cows in early lactation, Italian Journal of Animal Science, 4:1, 35-47, DOI: 10.4081/ijas.2005.35

To link to this article: http://dx.doi.org/10.4081/ijas.2005.35
Influence of dietary starch contents on milk composition of Friesian cows in early lactation

Andrea Summer¹, Primo Mariani¹, Michela Bellotti², Alfonso Zecconi², Sandy Sgorlon³, Bruno Stefanon³

¹ Dipartimento di Produzioni animali, Biotecnologie veterinarie, Qualità e Sicurezza degli Alimenti. Università di Parma, Italy
² Dipartimento di Patologia animale, Igiene e Sanità Pubblica. Università di Milano, Italy
³ Dipartimento di Scienze della Produzione Animale. Università di Udine, Italy

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 αs1-casein percentages at 80 and 94 DIM increased in the EXP but not in the CTR group, and a marked decrease of αs2-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 due razioni 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 ricca di amido (HSD, 28,3% amido/SS). Dall’86° al 94° DIM le vacche del gruppo sperimentale ritornavano a ricevere la dieta richa di amido. Il contenuto di amido (LSD, 21,0% amido/SS) dal 66° al 85° DIM e la razione unifeed sino al 65° DIM, con una dieta a basso contenuto di amido (LSD, 21,0% amido/SS); dal 66° all’85° DIM il 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, caseiche 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 $\alpha_1$-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 $\alpha_2$-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 caseiche e a livello di contenuto di minerali. Quest’ultimo risultato, potrebbe indicare un miglioramento della funzionalità dell’epitelio della ghiandola mammaria in presenza di un 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
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,

---

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

| 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       | 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.
SUMMER et al.

38 ITAL.J.ANIM.SCI. VOL. 4, 35-47, 2005

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).

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.

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
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.**

| Diet¹ | BSD SD | LSD SD | HSD SD |
|-------|--------|--------|--------|
| 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 |
| DCAB² | mEq/kg | 383 ± 5.52 | 383 ± 0.02 | 360 ± 3.30 |
| NEL³ | 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 NEL = Net energy lactation, estimated on the basis of TMR composition

---

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 AgNO₃ using the volumetric method of Charpentier-Volhard (Savini, 1946). The same milk samples were also used to measure potentimetric acidity (pH) and titratable acidity, with
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\textsubscript{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 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 \(\alpha_1\)-casein percentages at 80 and 94 DIM increased in the EXP but not in the CTR group, and a marked decrease in \(\alpha_2\)-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).
| 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  | 20.17  | 20.00  | 19.73  | 20.13  | 0.625 | *** | *** | 0.0411 |
|        | EXP   | 19.89 | 19.47  | 19.73  | 20.36  | 20.19  | 20.29  |
| NE_L Mcal/d | CTR | 30.19 | 30.34  | 30.62  | 30.38  | 30.19  | 29.45  | 1.440 | *** | ** | 0.0920 |
|        | EXP   | 30.25 | 30.50  | 30.87  | 31.25  | 31.05  | 29.68  |
| Starch kg/d | CTR | 4.966 | 5.170  | 4.952  | 4.977  | 4.880  | 5.035  | 0.0386 | *** | *** | 0.0028 |
|        | EXP   | 4.976 | 4.135  | 4.111  | 5.760  | 5.715  | 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  | 7.790  | 7.534  | 7.397  | 7.618  | 0.0883 | *** | *** | 0.0059 |
|        | EXP   | 7.577 | 7.380  | 7.637  | 7.173  | 7.127  | 7.679  |
| NSC %  | CTR   | 6.946 | 7.169  | 6.941  | 7.017  | 6.877  | 7.052  | 0.0767 | *** | *** | 0.0051 |
|        | EXP   | 6.958 | 6.408  | 6.414  | 7.645  | 7.587  | 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.
| 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 * | 4.99 * | 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.
| Item                  | Treat. | DIM      | Main effects and interactions (P <) |
|----------------------|--------|----------|-------------------------------------|
|                      |        | 37       | 60       | 80       | 94       | 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    |          |       |      |     |      |
| αs2-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        |        |      |     |      |
| αs1-Casein           | "      | CTR      | 38.70    | 37.54    | 38.70    | 38.28   | b       | 0.951 | *  | * | 0.683 |
|                      | EXP    | 37.59    | 37.93    | 39.04    | 39.17    | a        |        |      |     |      |
| β+γ- 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. |          |          |          |          | T       | MSE1   | S       | TxS     | MSE2   |
|----------|--------|----------|----------|----------|----------|---------|--------|---------|---------|--------|
|          |        | DIM      |          |          |          |         |        |         |         |        |
|          |        | 37       | 60       | 80       | 94       |         |        |         |         |        |
|          |        | BSD      | LSD      | HSD      | BSD      |         |        |         |         |        |
| Ca kg/100g |       | CTR      | 117.4    | 117.3    | 115.5    | 115.9   | 15.054 | 23.269  |         |        |
|          |       | EXP      | 118.8    | 119.6    | 114.5    | 113.9   |         |         |         |        |
| P kg/100g |       | CTR      | 91.9     | 93.9     | 91.1     | 91.7    | 48.764 | 28.754  |         |        |
|          |       | EXP      | 100.5    | 101.7    | 98.9     | 92.1    |         |         |         |        |
| Na kg/100g |       | CTR      | 45.6     | 40.0     | 44.3     | 42.6    | **      | **      | **      | 7.499  |
|          |       | EXP      | 39.6     | 40.0     | 36.6     | 32.7    |         |         |         |        |
| K kg/100g |       | 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 kg/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, αs1-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.

Research supported by the Ministry of University and Scientific and Technological Research, Italy (MURST, 1999-2001).

REFERENCES

ALLEN, R. J. L., 1940. The estimation of phosphorus. Biochem. J. 34:858-865.
ANON., 1963. Säuregradbestimmung nach Soxhlet-Henkel (SH). Milchwissenschaft 18: 520.
ANON., 1982. Analytical methods for atomic absorption spectrophotometry. Bodenseewerk Perkin-Elmer & Co GmbH, Überlingen, Germany.
ASCHAFFENBURG, R., DREWRY, J., 1959. New procedure for the routine determination of the various non-casein proteins of milk. Proc. 15th International Dairy Congress, London, UK, 3: 1631-1637.
AOAC, 1990. Official methods of analysis. 15th ed. AOAC, Arlington, USA.
AULDIET, M. J., THOMSON, N. A., MACKLE, T. R., HILL, J. P., PROSSER, C. G., 2000. Effects of pasture allowance on the yield and composition of milk from cows of different b-lactoglobulin phenotypes. J. Dairy Sci. 83: 2069-2074.
BAUMGARD, L. H., CORI, B. A., Dwyer, D. A., SAEGP, A., BAUMAN, D. E., 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol., Regulatory Integrative Comp. Physiol. 279: R179-R184.
BEAUCHMIN, K. A., RODE, L. M., YANG, W. Z., 1997. Effects of nonstructural carbohydrates and source of cereal grain in high concentrate diets of dairy cows. J. Dairy Sci. 80: 1640-1650.
BERTRAND, J. A., PARDEE, F. E., JENKIN, T. C., 1998. Effect of ruminally protected amino acids on milk yield and composition of Jersey cows fed whole cottonseed. J. Dairy Sci. 81: 2215-2220.
BIGG, D. A., 1978. Instrumental infrared estimation of fat, protein and lactose in milk: collaborative study. J. Assoc. Off. Anal. Chem. 61: 1015-1034.
DEPETRES, E. J., CANT, J. P., 1992. Nutritional factors influencing the nitrogen composition of bovine milk: a review. J. Dairy Sci. 75: 2043-2070.
DRAKELEY, J. K., 1999. Biology of the dairy cows during the transition period: the final frontier? J. Dairy Sci. 82: 2959-2973.
FITZGERALD, J. J., MURPHY, J. J., 1999. A comparison of low starch maize silage and grass silage and the effect of concentrate supplementation of the forages or inclusion of maize grain with the maize silage on milk production of dairy cows. Livest. Prod. Sci. 57:95-111.
GARAI, G., TESTONI, S., PICCININI, R., AND STRADAFOI, G. (2004). OXIDATIVE STRESS IN PRIMIPAROUS COWS IN RELATION TO DIETARY STARCH AND THE PROGRESS OF LACTATION. ANIM. SCI., 79:99-108.
HERRERA-SALDANA, R., GOMEZ-ALARCON, G., TORACI, M., HUBER, J. T., 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. J. Dairy Sci. 73: 142-148.
INTERNATIONAL DAIRY FEDERATION, 1997. Recommendations for presentation of mastitis-related data. FIL-IDF Bulletin 321: 6-25.
KENNELLY, J. J., ROBINSON, B., KHORASANI, G. R., 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation Holstein cows. J. Dairy Sci. 82: 2486-2496.
KHALLIL, H., SAHRAIEN, A., HISSI, K., HUHTANEN, P., 2001. Effects of type and treatment of grain and protein source on dairy cow performance. Anim. Sci. 72: 573-584.
KHORASANI, G. R., KENNELLY, J. J., 2001. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in late-lactation Holstein cows. J. Dairy Sci. 84: 1707-1716.
LYKOS, T., VARIA, G. A., CAMPER, D., 1997. Varying degradation rates of total nonstructural carbohydrates: effects on ruminal fermentation, blood metabolites, and milk production and composition in high producing Holstein cows. J. Dairy Sci. 80: 3341-3355.
McMARCH, D. J., BROWN, R. J., 1982. Evaluation of Formagraph for comparing rennet solutions. J. Dairy Sci. 65: 1639-1642.
DIETARY STARCH AND MILK COMPOSITION

MERTENS, D. R., 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80: 1463-1481.

MILLER, J. K., BREEZINSKA-SLEBOZINSKA, R. J., MADSEN, F. C., 1992. Oxidative stress, antioxidants and animal function. J. Dairy Sci. 76: 2812-2823.

NATIONAL RESEARCH COUNCIL, 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press, Washington, DC, USA

NEVILLE, M. C., WALSH, C. T., 1995. Effects of xenobiotics on milk secretion and composition. Am. J. Clin. Nutr. 61(Suppl.): 687S-694S.

NOCEK, J. E., RUSSEL, J. B., 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71: 2070-2107.

PYORALA, S., 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 34: 565-578.

RODE, L. M., SATTER, L. D., 1988. Effect of amount and length of alfalfa hay in diets containing barley or corn on site of digestion and rumen microbial protein synthesis in dairy cows. Can. J. Anim. Sci. 68: 445-451.

SAVINI, E., 1946. Analisi del latte e dei latticini. Ed. Hoepli, Milano, Italy.

SMITH, K.L., 2002. A discussion of normal and abnormal milk based on somatic cell count and clinical mastitis. Bulletin FIL-IDF 372: 43-45.

SORENSEN, A., MUIR, D. D., KNIGHT, C. H., 2001. Thrice-daily milking throughout lactation maintains epithelial integrity and thereby improves milk protein quality. J. Dairy Res. 68: 15-25.

STASTICAL PACKAGE FOR SOCIAL SCIENCE, 1997. SPSS Advanced Statistics, 7.5. SPSS Inc., Chicago, IL, USA.

STEFANON, B., VOLPE, V., MOSCARDINI, S., GRUBER, L., 2001. Using Artificial Neural network to model the urinary excretion of total and purine derivative nitrogen fractions in cows. J. Nutr. 131: 3307-3315.

UCHIDA, K., BALLARD, C. S., MANDEBVU, P., SNIFFEN, C. J., CARTER, M. P., 2001. Effect of variation in proportion of cornmeal and steam-rolled corn in diets for dairy cows on behaviour, digestion, and yield and composition of milk. J. Dairy Sci. 84: 453-461.

VAN SOEST, P. J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY, USA.

VAN SOEST, P. J., ROBERTSON, J. B., LEWIS, B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

VISSE, S., SLANGEN, K. J., ROLLEMA, H. S., 1986. High-performance liquid chromatography of bovine caseins with the application of various stationary phases. Milchwissenschaft. 41: 559-562.