Journal of Dairy Science Interpretive Summary

Short Communication: The effect of dry period duration and dietary energy density on the rennet gelation properties of milk in early lactation. *By Butler et al., page XXX.* The rennet gelation characteristics of milk samples collected at 2, 6, and 10 weeks postpartum were compared in cows given one of two planned dry period lengths (0 or 8 weeks) and one of two feeding levels (standard or high energy TMR). Decreasing dry period duration resulted in higher postpartum milk protein concentrations, and was associated with greater maximum curd firming rate and gel strength of milk following rennet addition. Feeding level had no effect on milk protein concentration or rennet gelation characteristics. Decreasing dry period duration may have beneficial effects on the processability of milk in the subsequent lactation.

Running Title: SHORT COMMUNICATION: DRY PERIOD DURATION AND MILK PROCESSABILITY

Short Communication: The effect of dry period duration and dietary energy density in early lactation on the rennet gelation properties of milk

S. T. Butler,[*1] M. A. de Feu,[*] B. O'Brien,[*] T. P. Guinee,[†] and J. J. Murphy[*]

* Teagasc, Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland

† Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland

[1] Corresponding author: stephen.butler@teagasc.ie

ABSTRACT

This study was carried out to examine the effects of decreasing dry period duration (DP) and altering the energy density of the diet during early lactation on the rheological characteristics of milk. Forty mature Holstein-Friesian cows were used in a completely randomized design with a 2 × 2 factorial arrangement of treatments. Cows were randomly assigned to one of two dry period treatments and one of two nutritional treatments. The dry period treatments were continuous milking (CM) or an 8-week standard dry period (SDP), and the nutritional treatments were a standard energy diet (SE) or a high energy diet (HE). Actual dry period lengths were 6.3 ± 1.7 days and 62.1 ± 1.9 days for cows for the CM and SDP treatments, respectively. Milk samples were collected at 2, 6 and 10 weeks postpartum. The concentration of fat, protein and lactose was determined in each sample. The rennet gelation properties were measured at 31 ° C using dynamic low-amplitude strain oscillatory rheometry. The following parameters were obtained from the resultant elastic shear modulus (G'): gelation time (GT), maximum curd firming rate (CFR_{max}) and gel strength (GS). Reducing dry period duration from 62 to 6 days resulted in increases in milk protein concentration (31.8 vs. 34.7 g/kg; $P < 0.001$), CFR_{max} (2.58 vs. 3.60 Pa/min; $P < 0.001$) and GS (69.4 vs. 90.5 Pa; $P = 0.003$). Raising the dietary energy density decreased percentage milk fat (43.1 vs. 37.7 g/kg; $P < 0.001$) but otherwise had no effect. GS was correlated with CFR_{max} ($r = 0.98$; $P < 0.001$), and both variables were correlated with milk protein concentration ($r = 0.71$; $P < 0.001$, and $r = 0.73$; $P < 0.001$, respectively). The results indicate that decreasing the duration of DP increased milk protein concentration and improved the rennet gelation properties of milk, but that dietary energy density had little effect.

(KEYWORDS: milk, dry period duration, dietary energy density, rennet gelation)

Abbreviation key: **G´** = elastic shear modulus; **GS** = gel strength; **GT** = Gelation time; $\mathbf{CFR_{max}}$ = Maximum curd firming rate.

Introduction

Decreasing the duration of the dry period between lactations has recently gained considerable attention in the management of dairy cows (Annen et al., 2004a, Grummer and Rastani, 2004). Omitting the dry period improves energy balance in early lactation, with a consequent reduction in body condition score loss (Rastani et al., 2005). This is achieved through the combined effects of higher dietary energy intake and decreased milk energy output (Rastani et al., 2005), though administration of bovine somatotropin prevents the decrease in milk output in multiparous cows (Annen et al., 2004b). It is well documented that severe negative energy balance and excessive body condition loss in early lactation are risk factors for fatty liver, ketosis, and compromised reproductive performance (Butler and Smith, 1989, Drackley, 1999). Thus, decreasing dry period duration may have potentially important benefits for dairy cow health and longevity. The effect of decreasing dry period duration on milk processability has not been examined, but a previous report indicated that a decrease in dry period duration increased milk protein concentration in the subsequent lactation (Rastani et al., 2005). This change in protein concentration is expected to have marked implications for rennet gelation of milk, and the manufacturing efficiency and composition of cheese (Guinee et al., 2006).

Rennet gelation of milk is a central step in the manufacture of most cheese varieties such as Cheddar, Mozzarella and Gouda. The resultant gel is subjected to a number of operations (e.g. cutting, cooking, acidification, pressing and salting) which differ in degree with cheese variety and result in the formation of cheese (curd). The rennet gelation characteristics (curd firming rate, set-to-cut time, firmness) of the milk have marked effects on cheese composition (e.g. moisture), percentage recovery of fat from milk to cheese, and, hence, manufacturing efficiency and quality (Lelievre and

Gilles, 1982; Banks et al., 1982, 1984; Mayes and Sutherland, 1989; O'Brien et al., 1999; Guinee et al., 2005). Consequently, the rennet gelation characteristics are a valuable indicator of the suitability of milk for cheese manufacture. However, the rennet gelation of milk is also influenced by numerous factors other than protein including *inter alia* other compositional factors, pH, somatic cell count, and calcium level (Fox et al., 2000). The objective of the current study was to evaluate the effects of dry period duration, dietary energy density, and their interaction on the composition and rennet gelation of milk.

Forty multiparous Holstein-Friesian dairy cows were used in a completely randomized 2 × 2 factorial design. The 2 factors examined in the study were dry period duration and dietary energy density. For dry period duration, cows were assigned to either continuous milking (CM) or a 10-week standard dry period (SDP). Cows on the CM treatment were dried off when daily milk yield was <2 kg/day. Actual dry period lengths were 6.3 ± 1.7 days and 62.1 ± 1.9 days for cows on the no planned dry period and the 8 week dry period, respectively. Dietary energy density treatments consisted of either standard energy (SE) or high energy (HE) diets. Full details of the study design, management of the experimental animals, and effects on milk production have been previously reported (de Feu et al., 2009).

Milk samples were collected at weeks 2, 6 and 10 postpartum from all cows at the afternoon milking for composition, somatic cell count, and rheology analysis. Samples were pooled when cows within treatment at a common week postpartum were sampled on the same week; this resulted in the number of cows contributing to each composite milk sample for rennet gelation analysis ranging from 1 to 4; each dry period duration and feeding level treatment combination had 7 replicates at weeks 2, 6, and 10 postpartum. Milk samples were stored overnight at 4 °C, and analysis was carried out the day after sample collection. An aliquot of each pooled milk sample was analyzed for fat, protein and lactose concentrations by near-infrared reflectance spectroscopy (Milkoscan 605; Foss Electric, Hillerød, Denmark), and somatic cell count (SCC) was measured by laser based flow cytometry (Somacount 300; Bentley Instruments Inc., Chaska, MN).

The rennet gelation properties were measured using low amplitude strain oscillation (Advanced Rheometer ER550; TA instruments). The pH of 100 ml of milk was standardized to 6.55 at room temperature. The temperature of the milk was then brought to 31 °C by immersing the milk sample in a water bath, and the pH readjusted to 6.55 if necessary. Rennet (Chymax Plus, Pfizer Inc., Milwaukee, WI, USA), diluted to 1:20 with de-ionized water, was added to milk at a level of 0.18 mL undiluted rennet per L milk. The sample was subjected to a low amplitude shear strain of 0.025 at a frequency of 1 Hz and the elastic shear modulus, G´, was measured continuously as a function of time (Guinee et al., 1997). The following variables were calculated from the resultant G´/time profiles: gelation time (GT), defined as the time in seconds for G' to reach a value ≥ 0.2 Pa; maximum curd firming rate (CFR_{max}) defined as the maximum slope of the G'-time curve; and gel strength (GS) defined as the G' value at 50 minutes.

Data were analyzed as a factorial design using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Fixed effects in the model included dry period length, feeding level, lactation week and all possible interactions, and sample was included as a random effect. Pre-planned contrasts between SE and HE at each dry period length, and between SDP and CM at each feeding level were carried out using the ESTIMATE statement. Correlation analysis (PROC CORR) was undertaken to test for correlations between rennet gelation characteristics and milk composition results.

The mean values for milk composition and rennet gelation characteristics for the different treatments are summarized in Table 1 and Figure 1. The mean milk fat content of the cows on the HE diet was 12.5% lower than that of the cows on the SE diet (4.31 vs. 3.77%; $P < 0.001$), a result that concurs with the well documented milk fat-depressing effects of high energy diets (Bauman and Griinari, 2003). An interaction ($P = 0.016$) between dry period duration and feeding level was observed for the concentration of milk protein (Figure 1), whereby the HE diet increased ($P = 0.013$) milk protein concentration for SDP cows, but had no effect for CM cows ($P > 0.3$). CFR_{max} and GS were increased in milk from CM cows compared to SDP cows, but were not affected by dietary energy density (Table 1, Figure 2). However, for both CFR_{max} and GS the interaction between dry period duration and feeding level came close to significance ($P = 0.1$ and $P = 0.06$, respectively). In general, the effects of each factor and their interaction on CFR_{max} and GS were mirrored by the effects on milk protein content. Neither dry period duration nor feeding level had significant effects on GT, but post-hoc data analysis revealed that milk from cows on the SDP treatment fed the SE diet had a shorter GT than cows fed the HE diet (537 vs. 659 s, $P< 0.05$; Figure 2).

CFR_{max} was highly correlated with GS ($r = 0.99$; $P < 0.001$), and both variables were correlated with milk protein concentration ($r = 0.73$ and $r = 0.71$, respectively; both $P < 0.001$). Weak but significant correlations were also observed between milk fat concentration and CFR_{max} ($r = 0.37$, $P = 0.001$) and GS ($r = 0.35$, $P = 0.002$). However, this observation is likely explained by the fact that milk fat concentration was correlated with milk protein concentration ($r = 0.33$, $P = 0.004$), rather than milk fat concentration having any direct positive effects on CFR_{max} or GS. Somatic cell count was not influenced by treatment; mean SCC values for the dry period and feeding level treatments were within the range previously reported by O'Brien et al. (2006). Rennet gelation properties of the milk samples were not affected by SCC, in agreement with the report of O'Brien et al. (2006).

The higher protein content in milk from cows on the continuous milking treatment is beneficial in terms of its potential to increase cheese yield. All other factors being equal, Cheddar cheese yield increases by ~0.25-0.30 kg 100 kg^{-1} of milk for every 0.1 g 100 g^{-1} increase in milk protein in the range 3.0 to 4.5g 100 g^{-1} while retaining the protein to fat ratio constant at 0.96 (Guinee et al., 1994, Guinee et al., 1996, Guinee et al., 2006). Moreover, the increase in milk protein and associated improvement in the rennet gelation characteristics of CM treatment milk has implications for cheesemaking efficiency, e.g., percentage recovery of components such as moisture, fat and protein. These effects can be particularly manifest in large modern cheese plants (e.g. processing > 1-2 M L milk *per* day). In these operations, coagulant and starter culture are added to milk on a volume basis (rather than on a protein or casein basis), the rennet gel tends to be cut on the basis of time rather than on gel firmness or gel firming rate, and other steps such as speed and duration of cut programme are fixed. With such practices, a more rapid gelation and curd firming rate minimise the risk of the curd being cut when underset. Associated defects, such as shattering of curd particles during cutting and early stages of stirring, smaller curd particles, higher losses of moisture and fat, and lower cheesemaking efficiency are also less likely to be encountered. Nevertheless, the use of appropriate manufacturing protocols (gelation temperature, gel firmness at cutting, cut programmes) enable satisfactory cheesemaking efficiencies to be achieved across the range of protein levels observed in the current study.

In conclusion, continuous milking significantly enhanced the rennet gelation characteristics of milk (i.e., maximum curd firming rate, gel strength), an effect attributable mainly to the higher milk protein content. In contrast, increasing dietary energy density did not affect the rennet gelation characteristics. The results indicate that shortening the duration of the dry period could have beneficial effects the processability of milk.

ACKNOWLEDGMENTS

The authors thank Jonathon Kenneally for collection of milk samples and Norann Galvin for her skillful laboratory assistance. National Development Plan funding is gratefully acknowledged.

REFERENCES

Annen, E. L., R. J. Collier, M. A. McGuire, and J. L. Vicini. 2004a. Effects of dry period length on milk yield and mammary epithelial cells. J. Dairy Sci. 87(E. Suppl.):E66-E76.

Annen, E. L., R. J. Collier, M. A. McGuire, J. L. Vicini, J. M. Ballam, and M. J. Lormore. 2004b. Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. J. Dairy Sci. 87(11):3746-3761.

Banks, J. M., W. Banks, D. D. Muir, and A. G. Wilson. 1981. Cheese Yield: Composition does matter. Dairy Ind. Int. 46:15-22.

Banks, J. M., D.D. Muir, and A.Y. Tamime. 1984. A comparison of the quality of Cheddar cheese produced from seasonal and standardized milk. J. Society Dairy Technol. 37:88-92.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutr. 23:203-227.

Butler, W. R. and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.

de Feu, M. A., A. C. O. Evans, P. Lonergan, and S. T. Butler, 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status and postpartum ovarian function in Holstein-Friesian dairy cows. J. Dairy Sci., In Press.

Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci. 82:2259-2273.

Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, 2000. *Fundamentals of Cheese Science*. Aspen Publishers, Inc., Gaithersburg, MD.

Grummer, R. R. and R. R. Rastani. 2004. Why reevaluate dry period length? J. Dairy Sci. 87(E. Suppl.):E77-E85.

Guinee, T. P., D. J. O'Callaghan, E. O. Mulholland, and D. Harrington (1996). Milk protein standardization by ultrafiltration for Cheddar cheese manufacture. J. Dairy Res. 63:281-293.

Guinee, T. P., P. D. Pudja, and E. O. Mulholland (1994). Effect of milk protein standardization, by ultrafiltration, on the manufacture, composition and maturation of Cheddar cheese. J. Dairy Res. 61:117-131.

Guinee, T. P., C. B. Gorry, D. J. O'Callaghan, B. T. O'Kennedy, N. O'Brien, and M. A. Fenelon. 1997. The effects of composition and some processing treatments on the rennet coagulation properties of milk. Int. J. Dairy Technol. 50:99-106.

Guinee, T.P., J. Kelly, and D.J. O'Callaghan. 2005. Cheesemaking efficiency. End of Project Report 2003, MFRC No. 46. Teagasc, Oakpark Carlow, Ireland.

Guinee, T.P., B. T. O'Kennedy, and P. M. Kelly. 2006. Effect of milk protein standardization using different methods on the composition and yields of Cheddar cheese. J. Dairy Sci. 89:468-482.

Lelievre J., and J. Gilles. 1982. The relationship between the grade (product value) and composition of young commercial Cheddar cheese. N.Z. J. Dairy Sci. Technol. 17:69-75.

Mayes, J. J., and B. J. Sutherland. 1989. Further notes on coagulum firmness and yield in Cheddar cheese manufacture. Aust. J. Dairy Technol. 44: 47-48

O'Brien, B., R. Mehra, J.F. Connolly, and D. Harrington. 1999. Seasonal variation in the composition of Irish manufacturing and retail milks. 1. Chemical composition and renneting properties. Irish J. Agric. Food Res. 38:53-64.

O'Brien, B., T. P. Guinee, A. Kelly, and P. Joyce, 2006. Processability of late-lactation milk from a spring-calved dairy herd. Aust. J. Dairy Technol. 61:3-7.

Rastani, R. R., R. R. Grummer, S. J. Bertics, A. Gumen, M. C. Wiltbank, D. G. Mashek, and M. C. Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. J. Dairy Sci. 88:1004-1014.

Table 1. Mean milk composition and rennet gelation results during weeks 2, 6 and 10 postpartum[1].

	Dry period (DP)		Feeding level (FL)		P-values			
	SDP	CM	SE	HE	SEM	DP	FL	DP × FL
Protein (%)	3.18	3.47	3.29	3.37	0.046	<0.001	0.22	0.015
Fat (%)	3.93	4.15	4.31	3.77	0.099	0.11	<0.001	0.5
Lactose (%)	4.76	4.70	4.72	4.74	0.039	0.3	0.7	0.7
SCC[2]	218	360	309	269	51	0.06	0.6	0.2
SCS[3]	4.32	5.59	5.10	4.81	0.22	<0.001	0.4	0.5
GT (s)	598	613	575	636	29.3	0.7	0.14	0.15
CFR_{max} (Pa/min)	2.58	3.60	3.06	3.18	0.003	<0.001	0.9	0.098
GS (Pa)	69.4	90.5	81.0	78.9	4.85	0.18	0.8	0.060

[1] SDP = standard dry period; CM = continuous milking; SE = standard energy diet; HE = high energy diet; DP = dry period; FL = Feeding level.
[2] SCC = somatic cell count; values reported are cells/ml ÷ 1000
[3] SCS = somatic cell score; calculated as the natural log of SCC values.

Figure 1. Effects of dry period duration and feeding level on milk composition during weeks 2, 6 and 10 postpartum on milk fat, protein and lactose. Panel A: Milk fat concentrations were not affected by decreasing dry period duration (P > 0.1), but were decreased by increasing dietary energy density (P < 0.001). The effect of lactation week was also significant (P = 0.015). Panel B: Milk protein concentration was increased by decreasing dry period duration (P < 0.001), but dietary energy density did not have a significant effect (P > 0.2). A significant interaction between dry period duration and dietary energy density was observed (P = 0.016), and lactation week was also a significant effect (P = 0.006). Panel C: Milk lactose concentrations were not affected by dry period duration, dietary energy density, lactation week or any interaction term (all P > 0.3). SDP = standard dry period; CM = continuous milking; SE = standard energy diet; HE = high energy diet.

Figure 2. Effects of dry period duration and feeding level on the rennet gelation characteristics of milk. Milk samples were collected at weeks 2, 6, and 10 postpartum. The fixed effect ‘lactation week’ was not significant for any of the three rheological variables, and therefore overall means are presented. Panel A: Mean gelation time was not affected by either dry period duration or dietary energy density (P > 0.1). Panel B: Maximum curd firming rate was increased (P < 0.001) by decreasing dry period duration, but dietary energy density did not have a significant effect (P > 0.8). The interaction between dry period length and dietary energy density tended to be significant (P = 0.10). Panel C: Gel strength was increased (P < 0.01) by decreasing dry period duration, but dietary energy density did not have a significant effect (P > 0.7). The interaction between dry period length and dietary energy density tended to be significant (P = 0.06). SDP = standard dry period; CM = continuous milking; SE = standard energy diet; HE = high energy diet.

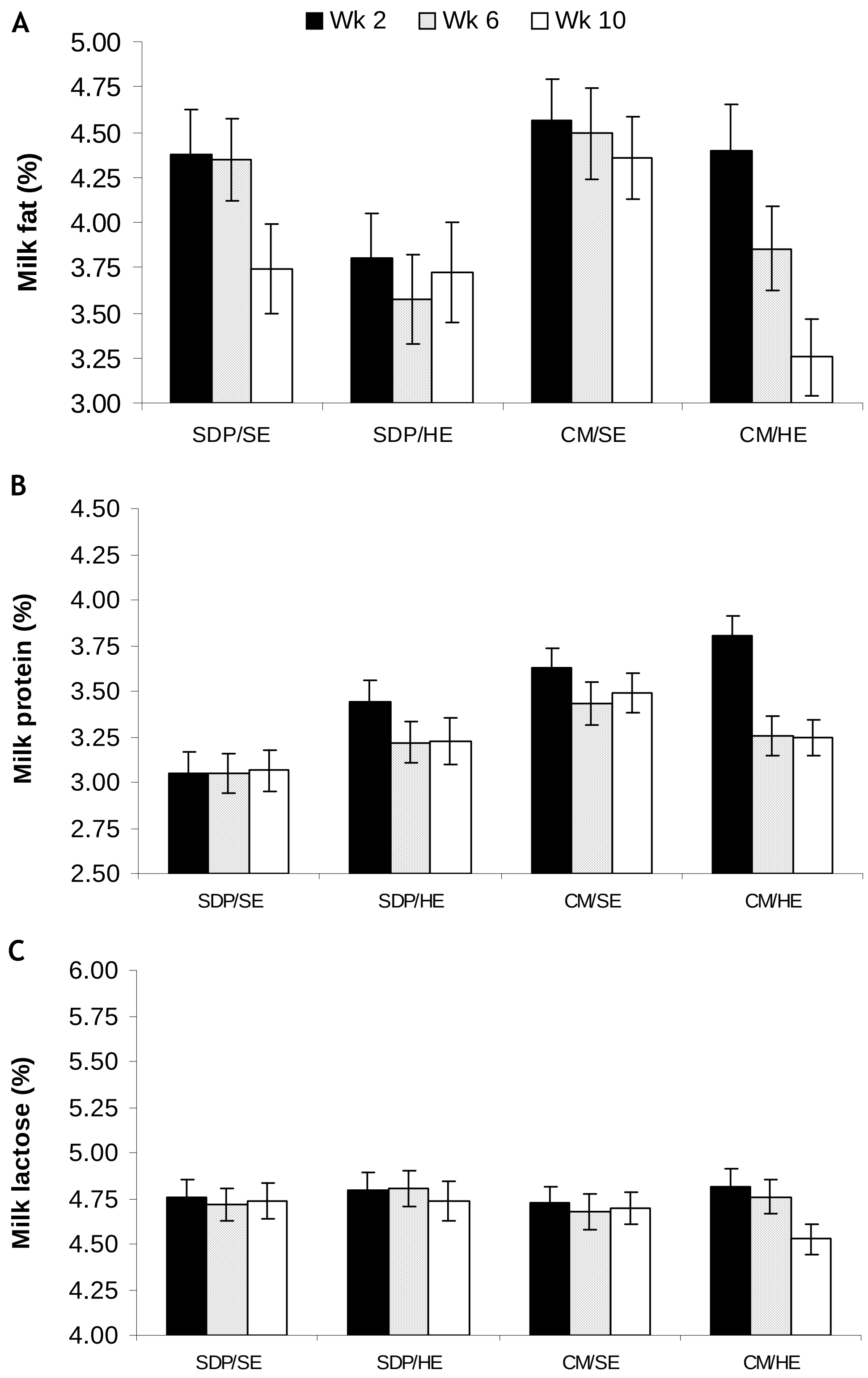

Figure 1 - Butler

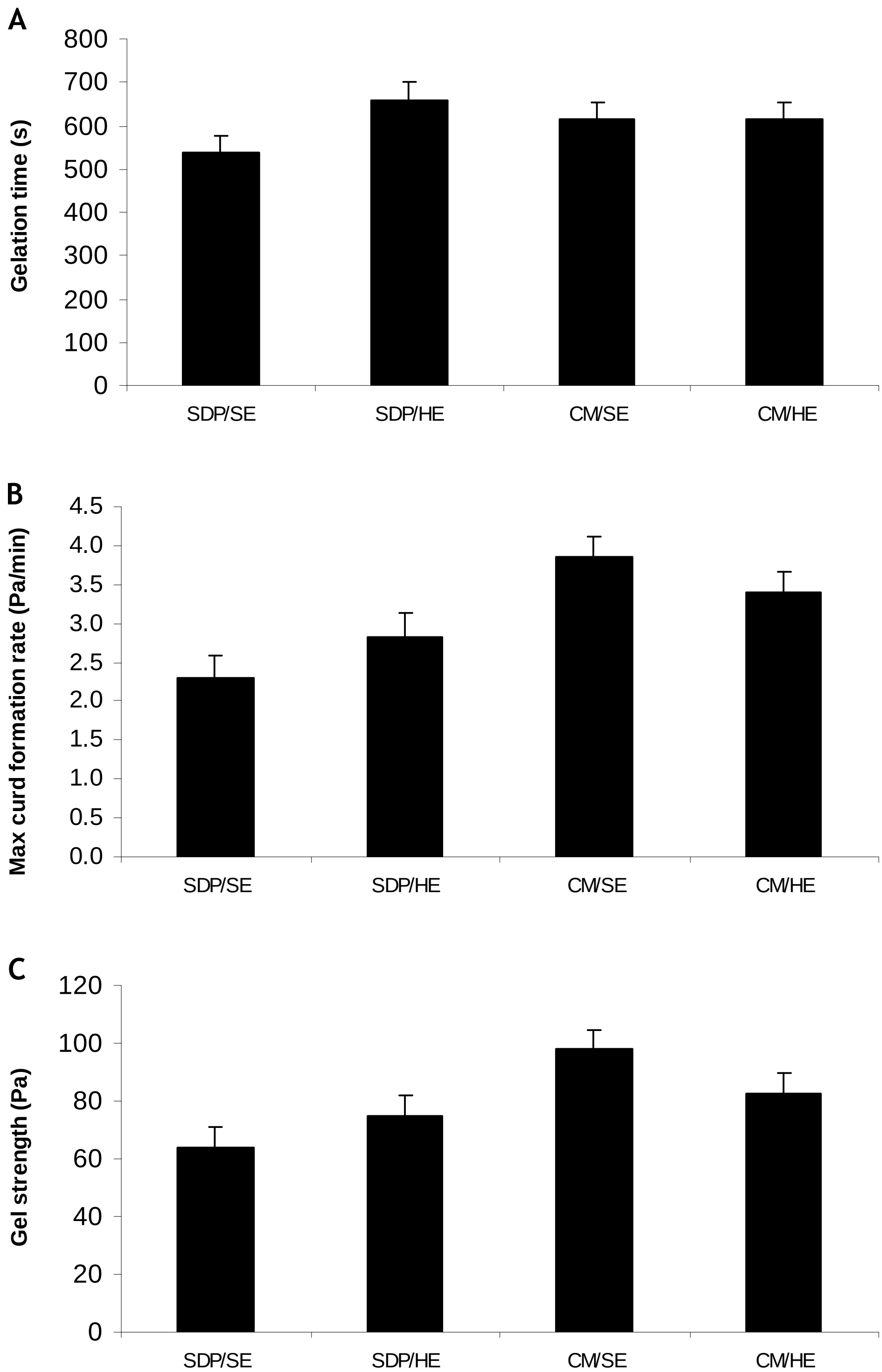

Figure 2 - Butler