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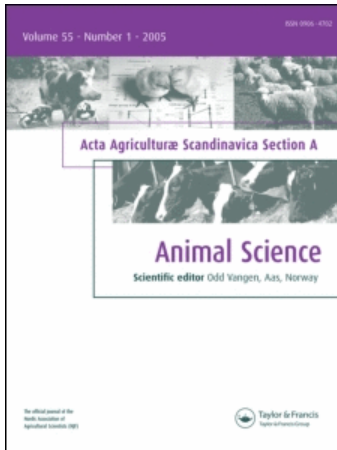
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### Nutritional strategies to combat physiological imbalance of dairy cows during early lactation: The effect of changes in dietary protein to starch ratios

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ORIGINAL ARTICLE

## Nutritional strategies to combat physiological imbalance of dairy cows during early lactation: The effect of changes in dietary protein to starch ratios

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### Abstract

Thirty Danish Holstein cows were used to determine how cows in early lactation adapt to changes in protein to starch supply in order to manipulate metabolism to combat physiological imbalance. During weeks 4 through 6 of lactation, 10 cows were fed either a high protein to starch ratio (high) diet, 10 cows were fed a low protein to starch ratio (low) diet while 10 others continued on the control diet. During weeks 7 through 9 of lactation, all cows returned to the control diet. The diets were formulated to consist of 15.0%, 18.6% and 22.2% crude protein and 13.3%, 7.5% and 1.7% of starch for the low, control and high diets, respectively. Besides milk urea nitrogen, no other production or metabolic parameters were affected by treatment. In conclusion, manipulation of dietary protein to starch is not a potential strategy to combat physiological imbalance during early lactation.

**Keywords:** Cow, physiological imbalance, metabolism, protein, starch.

### Introduction

Disease problems in dairy cows are major cow welfare and economic issues for the dairy industry and the farmers. The disease incidence is most pronounced in early lactation (Ingvarsen et al., 2003) and it has been hypothesised that physiological imbalance in individual cows is a major cause for at least certain diseases in early lactation (Ingvarsen & Friggens, 2005) and that changes in nutrient supply to individual cows may reduce the physiological imbalance and thereby the risk of cows developing diseases (Ingvarsen, 2006).

Hormonal changes, as well as changes in the nervous system and immune system, play a pivotal role with regard to the cow's ability to adapt to changes in physiological state and the partitioning of nutrients in support of lactation (Ingvarsen & Andersen, 2000). During early lactation, however, energy intake increases at a slower rate than what is required to meet the energy needed for milk production and this results in a period of negative

energy balance (NEB; Drackley, 1999). In order to provide the energy required during early lactation, dairy cows must mobilise lipid energy stored in adipose tissue to compensate for insufficient dietary energy intake which in cases of excessive mobilisation may lead to diseases (Ingvarsen, 2006).

The severity and duration of NEB is reflected in the degree of increase in circulating non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) and the degree of decrease in glucose concentrations (Drackley, 1999). All dairy cows experience some degree of NEB; the rate and extent of tissue mobilization, however, may lead to physiological imbalance in individual cows defined as cows whose physiological parameters (reflecting the function of the digestive tract, metabolic state and immune state) deviate from the normal at a given physiological state and stage of lactation, and who consequently have an increased risk of developing production diseases (clinical or subclinical) and reduced production and/or reproduction

(Ingvarstsen, 2006). Several factors, such as genetics, age, body condition score (BCS), milk production and diet may influence the metabolic status (Fronk et al., 1980; Detilleux et al., 1994; Horst et al., 1997). Few studies have examined the effect of short-term changes in dietary crude protein (CP) and starch content in early lactation in order to affect metabolic status of dairy cows (Grings et al., 1992; Cunningham et al., 1996; Chapa et al., 2001). In general, increased dietary CP levels result in increased dry matter intake (DMI) and increased milk, fat and protein yield (Cadorniga & Satter, 1993; Law et al., 2009) which may lead to increased physiological imbalance. Determining the individual metabolic and production responses of cows fed varying dietary protein to starch for a short period of time may provide further insight into the mechanisms controlling the partitioning of nutrients of dairy cows that will be beneficial for a better understanding of how to maintain an optimal metabolic status and thereby animal health and productivity during early lactation. The key questions are: how can we manipulate metabolism while maintaining DMI in order to help cows in physiological imbalance during early lactation? Will a short-term reduction in protein supply put less emphasis on milk production and contribute to bringing cows in physiological imbalance in balance? What are the short-term consequences of feeding a high protein diet on metabolism and risk of physiological imbalance during early lactation? The objective was to determine how cows in early lactation adapt to changes in protein supply with the aim to study the possibilities to manipulate metabolism, while maintaining DMI, to combat physiological imbalance and reduce the risk of diseases.

## Materials and methods

All procedures involving animals were evaluated and approved by the Danish Animal Experiments Inspectorate and complied with the Danish Laws concerning animal experimentation and care of experimental animals.

### *Animals and housing*

Thirty Danish Holstein dairy cows from the resident herd at Research Centre Foulum (Tjele, Denmark) were used as experimental animals. Data were collected from September 2007 to January 2008. Cows were housed and fed in individual tie-stalls with mats of hard rubber material and sawdust as bedding. Cows had free access to water and were milked twice daily at 0615

and 1615 h. Cows were eligible for the experiment after parturition. At the start of the study (i.e. first week of lactation), cows averaged 634 ( $\pm 45.0$ ) kg body weight (BW), produced 32.8 ( $\pm 7.1$ ) kg milk/d and had an average parity of  $2.1 \pm 0.3$  ranging from lactations 1 through 6. Lactation number was balanced over treatments.

### *Experimental design and diets*

Three diets formulated to consist of 15.0%, 18.6% and 22.2% CP (dry matter basis; DM) and 13.3%, 7.5% and 1.7% of starch (Table I), respectively, were used in this study. Diets were formulated to maintain similar forage to concentrate ratios among the three dietary treatments and, therefore, changes in CP content were achieved by supplementing soybean meal (SBM) with barley grain. The protein level in the low protein to starch diet (i.e. 15%) was chosen to assure DMI was not reduced due to low protein content in the diet (i.e.  $< 12\%$ ; Allen, 2000; Ipharraguerre, 2004). Maintaining DMI allows examination of the effect of changes in dietary protein to starch ratio without the confounding effect of DMI on metabolism and production. The 63-d experiment consisted of 3 periods. Period 1 (i.e. weeks 1–3 of lactation) was a 21-d adjustment period during which feed intake, BW, daily milk production and milk composition were measured. During Period 1, all cows were fed a total mixed ration (TMR, i.e. control diet; Table I). Throughout the study period, cows were fed to ensure 10% daily refusals and daily feed intake was measured by the differences in feed offered and refused. The TMR was mixed once daily, with approximately half of the ration fed at 0800 h and the remainder fed at 1400 h.

During weeks 4 through 6 of lactation (i.e. Period 2), one treatment group ( $n = 10$ ) was changed to a high protein to starch diet (i.e. group High), whereas another treatment group ( $n = 10$ ) was changed to a low protein to starch diet (i.e. group Low) and the control/moderate group ( $n = 10$ ; i.e. group Control) continued to be fed the standard TMR consisting of a mixture of the high and low diets in a 1:1 ratio (Table I). During weeks 7 through 9 of lactation (i.e. Period 3), all cows returned to the control diet. To clarify, data collection for week 1 of lactation started on the first Monday after calving and changes in dietary treatments took place on the last Wednesday of Periods 1 and 2.

### *Sampling and analysis*

A grass silage sample was taken prior to the start of the study and analysed for DM content, CP, crude

Table I. Ingredients and nutrient composition of experimental diets fed to Holstein dairy cows during early lactation.

Item	Diets (% of dry matter) <sup>a</sup>		
	Low	Control	High
<b>Ingredient</b>			
Grass silage	60.0	60.0	60.0
Spring barley	20.8	10.4	0.0
Sugar beet pulp, dried, unmolassed	16.0	16.0	16.0
Soybean meal, 44% CP	0.0	11.4	22.8
Urea, dried (46.7%N)	0.9	0.45	0.0
Sodium sulfate, water free	0.1	0.05	0.0
Mineral mix <sup>b</sup>	2.0	1.5	1.0
Vitamin mix <sup>c</sup>	0.2	0.2	0.2
<b>Composition<sup>d</sup></b>			
% DM	50.5	50.3	51.0
CP	15.0	18.6	22.2
Starch	13.3	7.49	1.72
Sugar	3.18	4.35	5.52
Crude fat	3.48	3.47	3.47
Neutral detergent fiber	36.2	35.4	34.5
Ash	8.64	8.76	8.87

<sup>a</sup>Experimental diets were fed as a total mixed ration consisting of either a high protein to starch ratio (i.e. High), a low protein to starch ratio (i.e. Low) or a control group (i.e. Control) consisting of a mixture of the high and low protein to starch ratio diets in a 1:1 ratio.

<sup>b</sup>Mineral mix (Optimin 1; Vitfoss, Gråsten, Denmark) contained (per kg): 180 g of Ca; 10 g of P; 65 g of Mg; 80 g of Na; 30 g of S; 693 kIU of vitamin A; 146 kIU of vitamin D; 4204 mg of  $\alpha$ -tocopherol; 3080 mg of Mn; 693 mg of Cu; 3460 of Zn; 173 mg of I; 19 mg of Co; and 38.5 mg of Se.

<sup>c</sup>Vitamin mix (Rød Suplex; Vitfoss) contained (per kg): 5000 kIU of vitamin A; 200 kIU of vitamin D; 9100 mg of  $\alpha$ -tocopherol; and 10 mg of Se.

<sup>d</sup>A grass silage sample was taken prior to the start of the study and analysed for dry matter content, crude protein, crude fat, ashes, sugar, starch and neutral detergent fiber using standard methods at Aarhus University, formerly known as the Danish Institute of Agricultural Sciences (Anonymous, 1992). Composition of all other individual feed ingredients were based on values provided by the Nordic Feed Evaluation System (NorFor<sup>TM</sup>; <http://feedstuffs.norfor.info>).

fat, ashes, sugar, starch and neutral detergent fibre using standard methods at Aarhus University, formerly known as the Danish Institute of Agricultural Sciences (Anonymous, 1992). Composition of all other individual feed ingredients was based on values provided by the Nordic Feed Evaluation System (NorFor<sup>TM</sup>; <http://feedstuffs.norfor.info>). The DM content of the TMR and grass silage was determined weekly. The composition of feed refusals was assumed similar to the diet offered.

Each week, BW was measured prior to morning feeding and BCS was measured independently by a trained individual using a five-point scale (1 = thin to 5 = obese) with 0.25-unit increments as described by Ferguson et al. (1994). Plasma samples were collected by puncture of the coccygeal vein using Vacutainer tubes containing sodium heparin (BD Vacutainer Systems, Plymouth, UK). Plasma was harvested following centrifugation at  $2000 \times g$  for 20 min at 4°C and stored at -18°C until analysis. Samples were collected after the morning milking (~0900 h) twice per week (Monday and Thursday) during the experimental period (weeks 1–9 of lactation). Plasma was analysed for concentration of NEFA, BHB and glucose using methods described

previously (Mashek et al., 2001) and autoanalyser, ADVIA 1650<sup>®</sup> Chemistry System (Siemens Medical Solution, Tarrytown, NY, USA).

Milk yield was measured (Milk Meter, S.A. Christensen, Kolding, Denmark) at each milking from Monday to Friday each week and daily milk yield was calculated as the sum of the morning and afternoon milk yields. Proportional milk samples were collected at the morning milking from Monday to Friday each week (Milk Meter, S.A. Christensen, Kolding, Denmark); these samples were analysed for per cent fat, protein, lactose and somatic cell counts (SCC) using a CombiFoss 4000 (Foss Electric, Hillerød, Denmark). From this, yield of fat, protein and lactose for the morning milking was calculated on the basis of recorded morning milk yields and concentrations (g/kg of milk) of milk components were calculated. Milk BHB was analysed using the enzymatic oxidation of the metabolite. A coupled reaction was determined by fluorometry (Larsen & Nielsen, 2005). Milk urea nitrogen (MUN) was analysed using flow injection analysis. Urease was added to the dilute milk sample; after the reaction, a strong alkali solution was added and the developing ammonia was dialysed through a membrane. pH

changes in the passing aqueous phase was followed via a pH-indicator by spectrophotometer. Application notes given by the manufacturer were followed (Foss Tecator AB, Höganäs, Sweden). Milk lactate dehydrogenase (LDH) activity was analysed according to procedures described by Larsen (2005). Milk alkaline phosphatase (AP) activities were determined by kinetic, fluorometric detection, using 4-methylumbelliferone phosphate, 4-MeU-P, as substrate (Acros Organics, 41504-0010). AP was determined at pH 10.0 (DEA buffer). Excitation wavelength was 355 nm, emission was detected at 460 nm. The intra-assay and inter-assay precisions were 3.9 and 5.2 (CV%), respectively, and inter-assay precision was 19.9 and 7.7 (CV%) for low (85 U) and high (425 U) controls, respectively.

### Statistical analyses

Two cows fed the High diet were excluded from the dataset prior to statistical analysis due to experimental error (i.e. cows were only fed the experimental diet for 2 instead of 3 weeks during Period 2). Therefore, a total of 28 cows fed the Low ( $n=10$ ), Control ( $n=10$ ) and High ( $n=8$ ) diets were used for further analysis. Plasma NEFA and BHB, and milk SCC, BHB, AP and LDH were  $\log_{10}$  transformed to normalise the data prior to statistical analysis. Week of lactation (1 through 9) was adjusted to week (1 through 3) within period. To clarify, weeks 1 through 3 of lactation were considered weeks 1 through 3 of Period 1, weeks 4 through 6 of lactation were considered weeks 1 through 3 within Period 2 and weeks 6 through 9 of lactation were considered weeks 1 through 3 within Period 3. Data for this study were analysed using the MIXED procedure of SAS, version 9.2 (2008) with the repeated measure of week (1, 2 and 3). The random effect of cow nested within dietary treatment (Low, Control and High) was used as the error term in the REPEATED statement with compound symmetry (CS) as the covariance structure. The model was used to determine the effect of dietary shift (Low, Control and High) on metabolism and performance in early lactation. The class variables included cow, diet, period and week with model:

$$Y_{ijkl} = \mu + D_i + P_k + D_i \times P_k + P_k \times W_l + D_i \times P_k \times W_l + C_j(D_i) + E_{ijkl},$$

where  $Y_{ijkl}$  = dependent variable at week  $l$  ( $l=1, 2$  or  $3$ ) within period  $k$  ( $k=1, 2$  or  $3$ ), for the  $j$ th cow within the  $i$ th diet ( $i$ =Low, Control or High);  $\mu$  = overall mean;  $D_i$  = effect of diet;  $P_k$  = effect of period;  $D_i \times P_k$  = the interaction between diet  $i$  and period  $k$ ;  $P_k \times W_l$  = the interaction between period  $k$  and week

$l$ ;  $D_i \times P_k \times W_l$  = the interaction among diet  $i$ , period  $k$  and week  $l$ ;  $C_j(D_i)$  = the random effect of cow  $j$ , nested within diet  $i$ ; and  $E_{ijkl}$  = the experimental error. The absolute change in BCS and BW were calculated as the difference between the peak (i.e. within Period 1) and nadir measurements (i.e. within Period 3) throughout the study period. For changes in BW and BCS, the class variables included cow and diet with model:

$$Y_{ij} = \mu + D_i + E_{ij},$$

where  $Y_{ij}$  = dependent variable for the  $j$ th cow within diet  $i$ ;  $\mu$  = overall mean; and  $E_{ij}$  = residual error. For both models, the degrees of freedom were estimated with the Kenward–Roger specification in the model statements. Data are presented as least squares means and standard error of least squares means for significant effects was accomplished using the Tukey's option within the MIXED procedure of SAS. Statistical differences were declared as significant and highly significant at  $P < 0.05$  and  $P < 0.01$ , respectively.

## Results

### Production responses

Figure 1 shows the diet  $\times$  week  $\times$  period effect of dietary protein to starch ratios on milk yield (A), MUN concentration (B) and DMI (C) in early lactation. DMI generally increased throughout the study (period  $P < 0.001$ ). The control group increased intake from 15.1 to 19.9 kg DM throughout the first 9 weeks of lactation. During Period 2, where all diets were fed, no differences were observed in DMI due to diet. No differences in daily milk yield were observed among cows fed the Low, Control and High protein to starch ratios at any given time point. During the dietary treatment period (i.e. Period 2), MUN concentrations were lower in cows fed the Low compared with cows fed either the Control or High diets. MUN remained lower in cows fed the Low diet during the week of realimentation (i.e. Period 3; week 7 of lactation). During Period 2, differences in MUN concentration among all three treatment groups were observed during weeks 2 and 3 within Period 2.

Table II shows the differences in production responses among Holstein cows fed the Low, Control and High dietary protein to starch ratio in early lactation. Changes in BW and BCS were similar among treatment groups throughout the study period. In addition, no differences in content of fat, protein, lactose, SCC and AP were observed among cows fed varying dietary protein to starch ratios during early lactation. A diet  $\times$  period effect

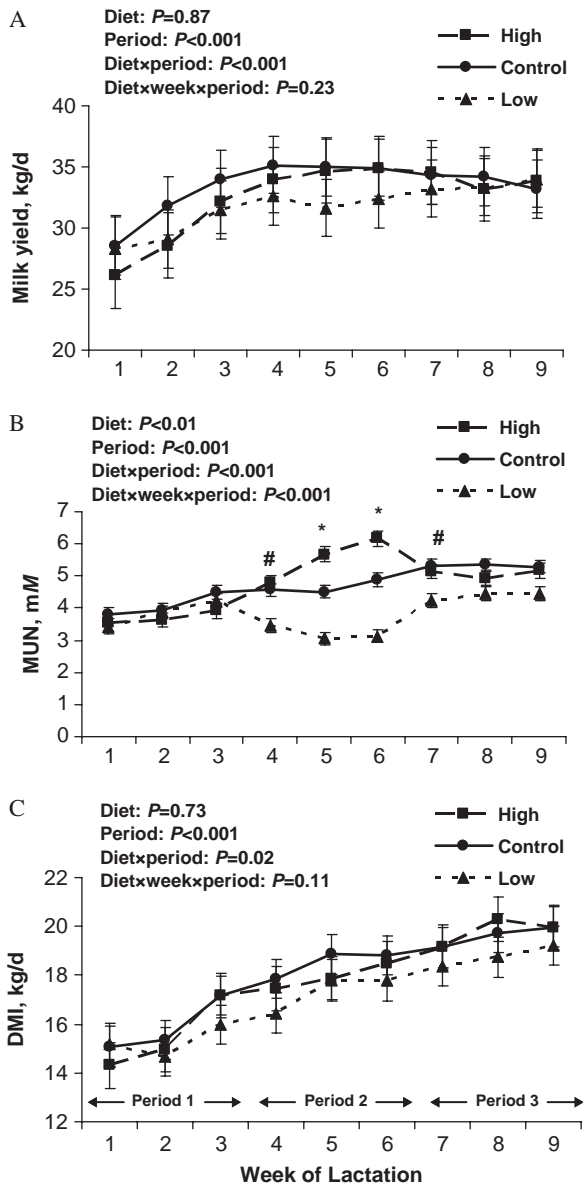


Figure 1. Differences in daily milk yield (A), concentration of milk urea nitrogen (MUN; B) and dry matter intake (DMI; C) among Holstein cows fed a diet with a low protein to starch ratio (Low; 1:2;  $\blacktriangle$ ;  $n=10$ ; 15.0% crude protein [CP] and 13.3% starch, dry matter basis), a moderate protein to starch ratio (Control; 1:1;  $\bullet$ ;  $n=10$ ; 18.6% CP and 7.49% starch), and a high protein to starch ratio (High;  $\blacksquare$ ;  $n=8$ ; 22.2% CP and 1.72% starch) during weeks 4 through 6 of lactation (i.e. Period 2).

\*Differences ( $P<0.05$ ) among dietary treatment groups (i.e. Low, Control, and High) at a given time point.

#Differences ( $P<0.05$ ) between Low and Control/High cows at a given time point.

was observed for milk LDH activity, where, regardless of diet, LDH activity increased throughout the study period. A diet  $\times$  week  $\times$  period effect was observed for milk BHB, where milk BHB concentration decreased in all treatment groups as lactation progressed; however, no differences were observed among dietary treatments within a given period.

### Metabolic responses

Figure 2 shows the effect (diet  $\times$  week  $\times$  period) of dietary protein to starch ratio on plasma NEFA (A), BHB (B) and glucose concentration (C) in early lactation. There was no effect of diet or the diet  $\times$  week  $\times$  period interaction on plasma metabolite concentration throughout the study period. The effect of period, week  $\times$  period (data not shown) and the diet  $\times$  period interaction were significant. Regardless of dietary treatment, NEFA concentration decreased as days in milk (DIM) increased, glucose concentration was lowest during Period 1 when compared to Periods 2 and 3, and BHB concentration was higher during Periods 1 and 2 than Period 3. No differences were observed among dietary treatments at any given time point.

### Discussion

Early lactation is the most metabolically challenging period in the life cycle of dairy cows. During this time, total body metabolism is altered in order to support the energy demands for milk synthesis. The partitioning of nutrients for lactation involves numerous endocrine and metabolic changes that lead to a state of NEB in support of lactation (Bauman & Currie, 1980; Van Kneegsel et al., 2007a, 2007b). Some dairy cows may experience a severe or extended period of NEB during early lactation that may lead to a state of physiological imbalance (Ingvarsten, 2006). Developing new strategies that can manipulate metabolism to contribute to bringing cows in physiological imbalance in balance may prevent the risk of diseases during early lactation (Ingvarsten, 2006). Feeding varying dietary protein to starch ratios for 3 weeks during early lactation had no effect on various metabolites and production parameters measured for this study and did not alter the degree of physiological imbalance. Therefore, short-term changes in dietary CP and starch are not promising nutritional strategies to combat physiological imbalance for cows in early lactation. The only short-term consequence to varying dietary protein and starch ratios for cows in early lactation was MUN concentration. MUN concentration reflected the substantial changes in dietary CP content formulated for the Low, Control and High dietary treatments; where MUN was highest in cows fed the High and lowest in cows fed the Low protein to starch ratio. Positive relationships between increases in dietary CP and MUN have been previously reported (Broderick, 2003; Charbonneau et al., 2007), and MUN has been used as a model to predict dietary CP intake and nitrogen excretion (Jonker et al., 1998).

Table II. The effect of dietary protein to starch ratio<sup>a</sup> ( $\mu \pm \text{SE}$ ) on body weight (BW), body condition score (BCS) and production responses of Holstein dairy cows in early lactation.

Variable	Diet			P-value			
	Low	Control	High	Diet	Period	Diet $\times$ period	Diet $\times$ period $\times$ week <sup>b</sup>
Change BW <sup>c</sup> , kg	65.5 $\pm$ 12	74.4 $\pm$ 12	72.0 $\pm$ 13	0.86	N/A	N/A	N/A
Change BCS	0.62 $\pm$ 0.09	0.49 $\pm$ 0.09	0.69 $\pm$ 0.11	0.39	N/A	N/A	N/A
Milk components <sup>d</sup>							
Fat, g/kg of milk	44.9 $\pm$ 1.3	42.3 $\pm$ 1.3	42.1 $\pm$ 1.4	0.25	<0.001	0.17	0.95
Protein, g/kg of milk	32.5 $\pm$ 0.78	33.6 $\pm$ 0.77	33.8 $\pm$ 0.86	0.49	<0.001	0.15	0.98
Lactose, g/kg of milk	49.3 $\pm$ 0.45	49.5 $\pm$ 0.45	50.2 $\pm$ 0.51	0.37	<0.001	0.25	0.73
SCC, log <sub>10</sub> (cells/mL)	4.91 $\pm$ 0.10	4.72 $\pm$ 0.10	4.81 $\pm$ 0.12	0.44	<0.01	0.19	0.74
BHB, log <sub>10</sub> (mM)	3.88 $\pm$ 0.10	3.67 $\pm$ 0.10	3.74 $\pm$ 0.11	0.35	<0.001	0.11	0.03
LDH, log <sub>10</sub> ( $\mu$ mol/min/L)	0.82 $\pm$ 0.11	0.57 $\pm$ 0.11	0.59 $\pm$ 0.13	0.21	<0.001	0.01	0.52
AP, log <sub>10</sub> (IU/L)	4.36 $\pm$ 0.15	4.47 $\pm$ 0.15	4.42 $\pm$ 0.17	0.88	<0.001	0.06	0.24

Note: MUN, milk urea nitrogen; SCC, somatic cell counts; BHB, beta-hydroxybutyrate; LDH, lactate dehydrogenase; and AP, alkaline phosphatase.

<sup>a</sup>Experimental diets were fed as a total mixed ration consisting of either a high protein to starch ratio (i.e. High;  $n = 8$ ; 22.2% crude protein [CP] and 1.72% starch, on a dry matter basis), a low protein to starch ratio (i.e. Low;  $n = 10$ ; 15.0% CP and 13.3% starch) or a control group (i.e. Control;  $n = 10$ ; 18.6% CP and 7.49% starch) consisting of a mixture of the high and low protein to starch ratio diets in a 1:1 ratio.

<sup>b</sup>Weeks 1–3 of lactation were equivalent to weeks 1–3 of Period 1, weeks 4–6 of lactation were equivalent to weeks 1–3 within Period 2, and weeks 6–9 of lactation were equivalent to weeks 1–3 within Period 3.

<sup>c</sup>The change in BCS and BW were calculated as the absolute difference between the peak and nadir measurements throughout the study period (i.e. weeks 1–6 of lactation).

<sup>d</sup>Composite milk samples were collected at each morning (i.e. am) milking from Monday to Friday for all analyses.

The primary objective of this study was to manipulate milk production and metabolites while maintaining DMI during early lactation. Our results show that DMI was not altered by varying dietary protein to starch ratios and is consistent with the results of others that observed no changes in DMI unless dietary CP is  $\leq 12.0\%$  (Allen, 2000; Ipharraguerre, 2004; Law et al., 2009). Furthermore, the addition of urea to the Low diet potentially prevented the reductions in microbial growth in the rumen due to deficiency of rumen degradable protein while increasing intestinal digestibility of organic matter (Cameron et al., 1991; Ipharraguerre et al., 2005).

Several studies have investigated the effect of long-term changes in dietary CP on metabolism and production of dairy cows during early lactation. In support of the present results, Cunningham et al. (1996) observed no effect when replacing SBM with high moisture corn in order to increase dietary CP from 14.4 to 16.4 or 18.4% CP (DM basis) while reducing the nonstructural carbohydrate content from 41.3 to 36.6 and 31.0 (DM basis), respectively, on DMI, daily milk yield, milk fat and protein contents, BW and BCS. It is important to note that this study fed cows in early lactation (32  $\pm$  10 DIM) for a longer duration (i.e. 12 weeks) coupled with a more narrow range in per cent dietary CP when compared with the present study. Similar results with regard to blood

metabolites have been previously reported (Chapa et al., 2001), where no changes in circulating concentration of NEFA, BHB and glucose were observed at 50 DIM when cows were fed either 16.6 or 22.8% CP (DM basis; corn and SBM) during early lactation. In contrast, Law et al. (2009) fed Holstein cows either 114, 144, or 173 g of CP/kg of DM from 1 to 150 DIM by supplementing with SBM and rape meal. Results showed that decreasing dietary CP concentration decreased DMI in cows fed 114 than either 144 or 173 g/kg of DM. In addition, milk yield, milk fat and protein yield decreased with decreasing concentration of dietary CP. The results of Law et al. (2009) contrast those of Cunningham et al. (1996) and the present study. However, Law et al. (2009) fed low levels of CP that have been shown to decrease DMI (i.e. <12%; Allen, 2000; Ipharraguerre, 2004).

The majority of studies evaluating the effect of varying dietary protein and starch on metabolic and production responses of lactating dairy cows have been conducted by supplementing with corn grain rather than barley as the primary starch source (Chapa, et al., 2001; Ipharraguerre et al., 2005). Differences in the amount and source of starch on metabolism and production was not the objective of the present study, however, it is important to note that the site of digestion of starch may differ between diets supplemented with barley versus



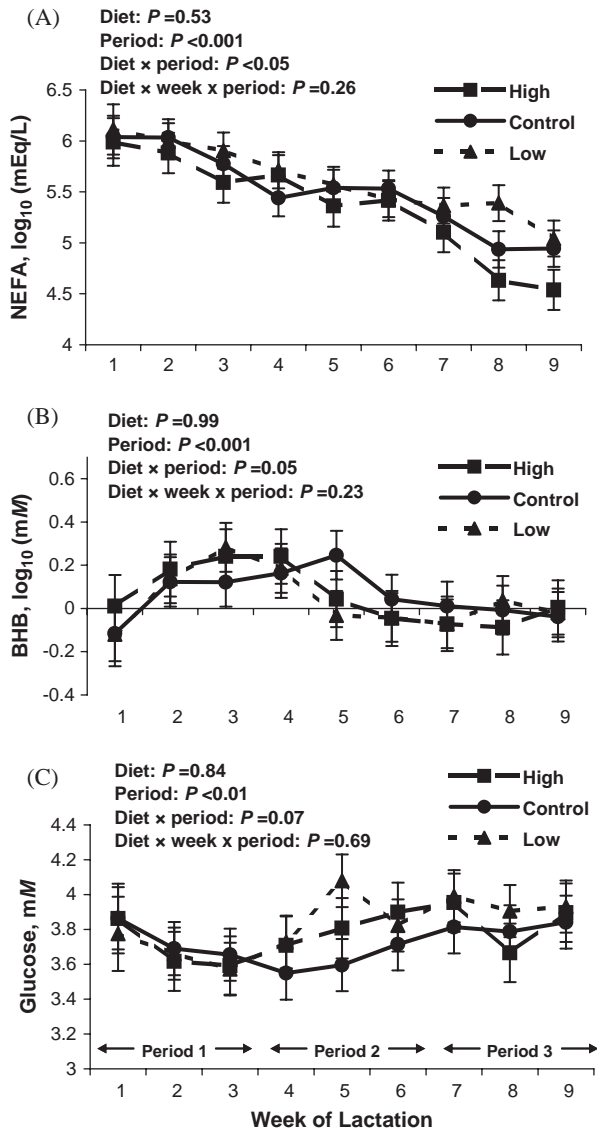


Figure 2. Differences in plasma non-esterified fatty acid (NEFA; A), beta-hydroxybutyrate (BHB; B) and glucose (C) concentration among Holstein cows fed a diet with a low protein to starch ratio (Low; 1:2; ▲;  $n=10$ ; 15.0% crude protein [CP] and 13.3% starch, dry matter basis), a moderate protein to starch ratio (Control; 1:1; ●;  $n=10$ ; 18.6% CP and 7.49% starch) and a high protein to starch ratio (High; ■;  $n=8$ ; 22.2% CP and 1.72% starch) during weeks 4 through 6 of lactation (i.e. Period 2). No differences ( $P<0.05$ ) were observed among dietary treatment groups (i.e. Low, Control and High) at a given time point.

corn (Khorasani et al., 2001) that may have contributed to the lack of effects observed. For example, rumen fermentation of barley starch is greater than that of corn (Nocek & Tamminga, 1991) and, therefore, less barley starch is digested and absorbed in the small intestine. The researchers speculate that more propionate, a gluconeogenic precursor, may have been produced and absorbed from the rumen for cows fed the Low diet. In addition, animals fed barley-based diets have been

shown to have higher microbial N synthesis that may lead to increased microbial protein synthesis than animals fed corn-based diets (Boss & Bowman, 1996; Suber & Bowman, 1998). Therefore, cows fed the Low diet may have compensated for the low protein intake via increased microbial protein synthesis observed when feeding barley-based diets thereby increasing supply of gluconeogenic amino acids for maintenance and uptake into the mammary gland. This compensatory effect may have been further exacerbated by the supplementation of urea to the Low diet as described previously.

For cows fed the High diet, no differences were observed with regard to DMI, metabolism or production when compared with cows fed the Control or Low protein to starch diet. One explanation may be an increase in supply of gluconeogenic amino acids that is commonly observed when cows are fed high protein diets during lactation (Cunningham et al., 1996; Ipharraguerre et al., 2005). Increasing dietary CP increases intake of all amino acids including essential, non-essential and total amino acids and enhances the passage of amino acids to the duodenum while simultaneously decreasing the digestion of starch in the rumen and intestines (Cunningham et al., 1996; Ipharraguerre et al., 2005). Therefore, a sparing effect may partly explain the lack of differences observed for cows fed the High diet via an increased supply of gluconeogenic amino acids. However, no differences in milk protein content were observed indicating that uptake of gluconeogenic amino acids to the mammary gland was similar among treatment groups (Lemosquet et al., 2004). Perhaps milk production prior to the start of the experimental period may partly explain the lack of effects observed between cows fed the Control and High protein to starch diet. For all treatment groups in the present study, average daily milk yield throughout the study period was greater than 30 kg/d and studies have indicated that cows producing over 30 kg/d is insufficient at detecting increases in milk yield in response to increased dietary CP (e.g. 16–19%; Santos et al., 1998; Ipharraguerre & Clark, 2005). DMI was not altered for cows fed varying dietary protein to starch ratios during early lactation, and likewise milk yield was not manipulated during the treatment period which in part may explain the lack of effects observed with regards to changes in metabolites in plasma (i.e. NEFA, BHBA and glucose).

## Conclusions

Despite the substantial differences in dietary protein to starch ratios, short-term changes in dietary

protein to starch by supplementing SBM for barley grain did not alter DMI, BW and BCS, indicators of metabolic imbalance (i.e. NEFA, BHB, and glucose) and production data (i.e. milk and component yield, except MUN) in early lactation. In conclusion, short-term changes in dietary protein to starch ratios did not alter milk yield and the metabolic status of the cows in early lactation. Consequently, manipulation of dietary protein to starch ratios is not a promising nutritional strategy to combat physiological imbalance and reduce the risk of production diseases during early lactation.

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