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The effects of fructose and phosphate infusions on dry matter intake of lactating cows

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ABSTRACT

The objective of this study was to examine the effects of fructose and phosphate (Pi) infusions on dry matter intake by dairy cows to further understand the mechanisms controlling feed intake related to hepatic energy status. We performed 3 experiments in which we infused fructose and Pi intravenously or abomasally to Holstein cows. The first experiment used 8 cows (4–8 d postpartum) in a duplicated 4 × 4 Latin square experiment with 1 square of multiparous and 1 square of primiparous cows. A 2 × 2 factorial arrangement of treatments was used including jugular infusions of solutions (1 L/h) containing fructose or glucose (0.6 mol/h) and Pi (NaH_2PO_4) or NaCl (0.3 mol/h). Periods were 24 h, including 2 h for infusions and 22 h for recovery. The second experiment used 4 multiparous cows (74–81 d postpartum) in a 4 × 4 Latin square design and infused fructose or glucose and either Pi or no Pi at the same rates as experiment 1. Periods were 24 h, including 1 h for infusions and 23 h for recovery. The third experiment used 4 ruminally cannulated multiparous cows (15–26 d postpartum) in a 4 × 4 Latin square design and infused fructose or glucose and either Pi or NaCl at the same rates as experiment 1 but to the abomasum. Periods were 24 h, including 1 h for infusions and 23 h for recovery. In each experiment, feed intake was recorded by a computerized data acquisition system; blood was analyzed for the concentrations of glucose, nonesterified fatty acids, and Pi; and the liver was analyzed for the concentration of Pi (experiments 2 and 3 only). Overall, fructose infusion increased DMI by fresh cows when infused intravenously and abomasally, but it did not affect DMI by mid-lactation cows. Fructose infusion also reduced hepatic Pi, and Pi infusion increased hepatic Pi when infused abomasally but not intravenously. These results suggest that fructose increases feed intake, likely by sequestering Pi and preventing ATP production. When infused intravenously to multiparous cows, Pi increased DMI and did not affect hepatic Pi content. However, when infused abomasally, Pi reduced DMI and increased hepatic Pi content. These results suggest that although Pi infusion prevents the effect of fructose loading and reduces DMI, it also increases intake through a competing mechanism. Examining long-term effect of Pi infusion on DMI could determine if competing mechanisms complicate the determination of P requirement for dairy cows. These results are consistent with the control of feed intake by hepatic energy status in dairy cows.

Key words: control of feed intake, hepatic oxidation theory, ATP, energy charge

INTRODUCTION

The control of energy intake in dairy cows is complex, including mechanisms that act independently (e.g., distention, osmotic effects) as well as many interacting factors that affect feed intake via their effects on metabolism (Ingvartsen and Andersen, 2000; Allen, 2014).

Moreover, the control of intake changes during the lactation cycle. A growing consensus exists that during the critical first few weeks of lactation, when cows are in negative energy balance because milk energy output greatly exceeds energy intake, the control of feed intake is dominated by fuel-based sensing mechanisms, specifically hepatic oxidation of fuels (Allen et al., 2009; Schäff et al., 2012; Derno et al., 2013; Martineau et al., 2016) and not by gut fill (Allen et al., 2009). Afterward, in the following months, distention by undigested feed residues in the gastrointestinal tract likely dominates the control of feed intake as milk yield and nutrient requirements are high (Allen, 1996).

Research in nonruminants revealed the effect of oxidation of fuels on feed intake by showing that inhibition of fuel oxidation stimulates feed intake and stimulation of oxidation inhibits feed intake; the research also showed that the signal to brain feeding centers is via hepatic vagal afferents (Langhans, 1996). Control of feed intake in nonruminants was demonstrated to be related to hepatic energy status, which is determined by the balance between the rates of production and utiliza-

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tion of ATP (Friedman et al., 1999). Fructose loading results in the accumulation of fructose 1-phosphate in mammalian livers (Kjerulf-Jensen, 1942), temporarily sequestering phosphate (**Pi**). Perfusion of the liver of rats with fructose rapidly (<10 min) decreased hepatic ATP content by 77% with a transient decrease in Pi (Woods et al., 1970). Fructose has a similar effect of reducing available Pi and ATP content in the rat and human liver (Woods et al., 1970; Morris et al., 1978; Abdelmalek et al., 2012), and fructose has elicited feeding in rabbits (Novin et al., 1991). The fructose analog 2,5-anhydro-D-mannitol (**2,5-AM**), which is phosphorylated but not metabolized further, decreased hepatic ATP content and elicited an eating response in rats (Tordoff et al., 1988; Rawson et al., 1994; Koch et al., 1998). The reduction in ATP content was likely from trapping Pi because Pi loading prevented the reduction in hepatic ATP content and stimulation of feeding by 2,5-AM (Rawson and Friedman, 1994). Therefore, fructose loading in the lactating cow might enable us to link hepatic ATP synthesis and feeding behavior, thus elucidating the mechanisms underlying control of feed intake by hepatic oxidation of fuels. We hypothesized that fructose will decrease hepatic ATP content, thus delaying satiety and increasing meal size and DMI, while Pi loading will attenuate its effects. Accordingly, the objective of this study was to examine the effects of fructose and Pi infusions on feeding behavior and metabolic responses of dairy cows.

MATERIALS AND METHODS

Animal Housing and Care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (East Lansing). For all experiments, cows were housed in tiestalls at the Michigan State University dairy facility, fed a TMR once daily at 1100 h at 120% of expected intake (their intake in the previous day), and milked twice daily in the milking parlor approximately at 500 h and 1600 h. Feed offered and refused was recorded and sampled daily throughout the experiment for determination of nutrient content (Table 1). The amounts of feed offered and refused were weighed, and the feed offered was adjusted daily.

Design and Treatments

Experiment 1. Eight Holstein cows in the early postpartum (**PP**) period (4–8 d PP) were used in a duplicated 4 × 4 Latin square experiment balanced for carryover effects with 1 square of multiparous cows and 1 square of primiparous cows. Cows were offered the prepartum ration beginning 21 d prepartum and the lactation ration beginning at parturition and throughout the experiment (Table 1). All cows were fitted with a single jugular catheter (left or right jugular vein) 2 to 3 d before the beginning of the experiment. Catheter patency was checked daily until removal at the end of the experiment. Cows were randomly assigned to tiestalls and treatment sequences. Periods were 24 h, beginning at the conditioned meal after feeding and including 2 h for infusions and 22 h for recovery. Cows were blocked from feed for 2 h before the beginning of each infusion. A 2 × 2 factorial arrangement of treatments was used, and treatments included jugular infusions (1 L/h) of solutions containing fructose or glucose (0.6 mol/h) and phosphate (NaH_2PO_4) or NaCl (0.3 mol/h). Glucose was used as the control for the fructose treatment because the two sugars have the same molecular mass and energy content and glucose uptake by the liver of mature bovines is negligible (Stangassinger and Giesecke, 1986). Sodium chloride was used as an osmotic control for the Pi treatment. The infusion rate for Pi was estimated based on the work of Rawson et al. (1994) with rats, which used about 2:1 ratio between

Table 1. Ingredient and nutrient composition (% of DM unless otherwise noted) of the experimental diets

	Experiment		
Item	1	2	3
Ingredient			
Corn silage	29.9	19.4	29.9
Haylage	15.1	16.6	15.1
Alfalfa hay	13.6	—	13.6
Dry ground corn	19.5	15.2	19.5
Soybean meal	16.4	8.7	16.4
Soybean hulls	4.2	7.0	4.2
Cottonseeds	—	7.6	—
High-moisture corn	—	15.6	—
Wheat straw	—	5.6	—
Vitamin-mineral mix 1[1]	1.4	—	1.4
Vitamin-mineral mix 2[2]	—	4.41	—
Nutrient composition			
DM (%)	56.4	55.7	50.6
Starch	23.9	27.3	20.9
NDF	31.0	28.6	33.0
CP	17.6	16.9	17.6
P	0.39	0.36	0.39

[1]Vitamin-mineral mix 1 contained (DM basis): 11.40–13.60% NaCl, 12.80–15.30% Ca, 0.99% Mg, 0.9% P, 10.90–13.00% Na, 14.0 mg/kg Co, 250 mg/kg Cu, 9.9 mg/kg I, 745 mg/kg Fe, 994 mg/kg Mn, 7.5 mg/kg Se, 1,100 mg/kg Zn, 149,000 IU/kg vitamin A, 23,000 IU/kg vitamin D_3, and 680 IU/kg vitamin E.

[2]Vitamin-mineral mix 2 contained (DM basis): 10.50–12.60% NaCl, 9.30–11.10% Ca, 0.91% Mg, 0.91% P, 8.30–9.90% Na, 14 mg/kg Co, 230 mg/kg Cu, 9.1 mg/kg I, 685 mg/kg Fe, 914 mg/kg Mn, 6.9 mg/kg Se, 1,000 mg/kg Zn, 137,000 IU/kg vitamin A, 23,000 IU/kg vitamin D_3, and 680 IU/kg vitamin E.

the fructose analog 2,5-AM and Pi. We used a short infusion period in these experiments because long-term infusion of fructose can deplete hepatic adenine nucleotide content because low concentrations of Pi and ATP accelerate the activity of 5′-nucleotidase and AMP deaminase and facilitate the degradation of the adenine nucleotides beyond the stage of AMP (Woods et al., 1970; Morris et al., 1978). In addition, long-term infusion of Pi might affect phosphorus homeostasis. Because fructose rapidly decreases hepatic ATP content, we expected rapid stimulation of feed intake by fructose so we limited the infusion period to 2 h.

Experiment 2. Four multiparous Holstein cows past peak lactation (74–81 d PP) were used in a 4 × 4 Latin square experiment balanced for carryover effects. All cows were fitted with a single jugular catheter as in experiment 1. Cows were randomly assigned to tie-stalls and treatment sequences. A 2 × 2 factorial arrangement of treatments was used, and treatments included jugular infusions of solutions (1 L/h) containing fructose or glucose (0.6 mol/h) with or without phosphate (NaH_2PO_4; 0.3 mol/h). Periods were 24 h, beginning at the conditioned meal and included 1 h for infusions and 23 h for recovery. Cows were blocked from feed for 2 h before the beginning of each infusion.

Experiment 3. Four ruminally cannulated multiparous Holstein cows (15–26 d PP were used in a 4 × 4 Latin square experiment balanced for carryover effects. Abomasal infusion devices (L. B. Gualdron-Duarte and M. S. Allen, Michigan State University, East Lansing, unpublished data) were placed at least 3 d after calving, and feed intake was monitored for 2 d before infusion to rule out any adverse effect of the devices. Cows were randomly assigned to tiestalls and treatment sequences. A 2 × 2 factorial arrangement of treatments was used and treatments were the same as in experiment1. Periods were 24 h, beginning at the conditioned meal and included a 1-h infusion period and a 23-h recovery period. Cows were blocked from feed for 2 h before the beginning of each infusion period.

Data and Sample Collection

In all the experiments infusion solutions were administered using calibrated Baxter Flo-Gard 6201 volumetric infusion pumps (Baxter Medical Products, Deerfield, IL). Feed intake was monitored by a computerized data acquisition system (Dado and Allen, 1993), and the results were validated by daily measurements of feed offered and refused. Milk yield was recorded and milk samples collected at each milking and stored with preservative at 4°C for component analysis. Samples of TMR and all diet ingredients were collected daily during all experiments and stored in plastic bags at −20°C until processed. Blood samples were collected by venipuncture of coccygeal vessels. In experiment 1, blood was taken before the beginning of infusions and at the end of infusion (2 h), whereas in experiments 2 and 3, blood was taken before the beginning of infusion and at 30 min and 60 min after the infusion began. Blood samples were collected into 3 evacuated tubes, 2 tubes containing potassium EDTA and 1 tube containing potassium oxalate with sodium fluoride as a glycolytic inhibitor. All tubes were centrifuged at 2,000 × *g* for 15 min, and plasma was harvested and stored at −20°C until analysis. In the second and third experiment liver tissue was collected before the beginning of infusions, at 30 min into the infusion, and at the end of infusion (60 min). After local anesthetization with 2% lidocaine hydrochloride, a skin incision was performed, and the biopsy instrument (14-gauge Vet-Core biopsy needles, Global Veterinary Products, Seaforth, Australia) was inserted between the 11th and 12th ribs on a line between the olecranon and the tuber coxae on the right side. Eight samples of ~20 mg each were collected, snap-frozen in liquid nitrogen, and stored at −80°C until further processing.

Sample Analysis

Feed and TMR samples were processed and analyzed for DM, NDF, CP, and starch contents. All samples were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. Samples were then ground in a Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA) and analyzed for NDF, CP, and starch. Concentration of NDF was determined according to Mertens (2002). Crude protein was determined according to Hach et al. (1987). Starch was gelatinized with sodium hydroxide and hydrolyzed using an enzymatic method (Karkalas, 1985); glucose was then measured using a glucose oxidase method (PGO Enzyme Product No. P7119; Sigma Chemical Co., St. Louis, MO).

Individual milk samples were analyzed for fat, true protein, and lactose concentration by mid-infrared spectroscopy (AOAC, 1990; method 972.160) by the NorthStar Michigan Laboratory (Grand Ledge, MI). Yields of milk components were calculated using milk yield and component concentrations for each milking.

Plasma samples were analyzed in duplicate. Plasma concentration of nonesterified fatty acids (**NEFA**) was determined using a commercial kit [NEFA-HR (2) kit, Wako Chemicals USA Inc., Richmond, VA]. Plasma glucose concentration was analyzed using the glucose oxidase method (PGO Enzyme P7119, Sigma-Aldrich,

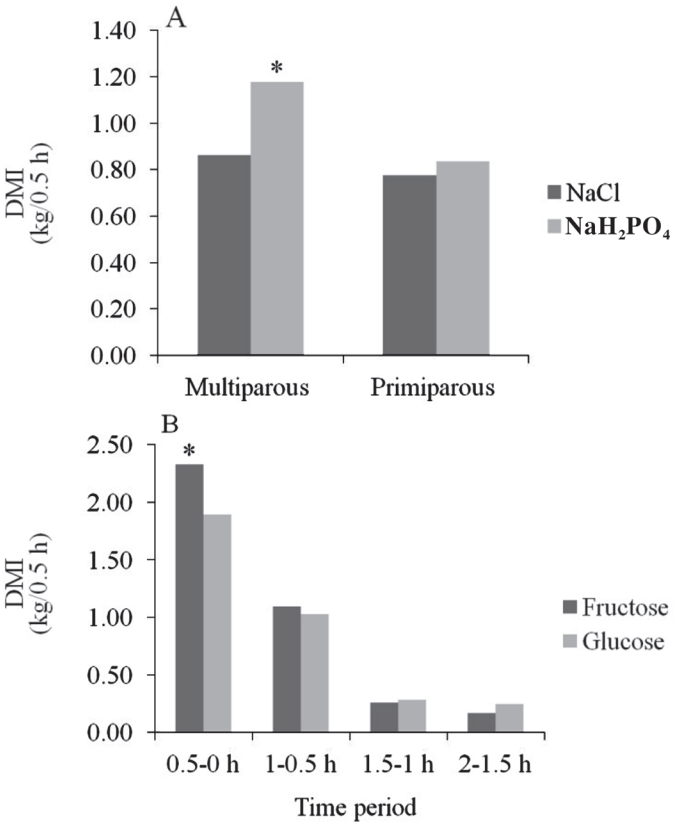

Figure 1. Statistical interactions for DMI (kg/0.5 h) during the 2-h jugular infusion for experiment 1. (A) Interaction between square (parity) and phosphate infusion; (B) interaction between time and fructose infusion; $^{*}P \leq 0.05$.

St. Louis, MO). Plasma and liver Pi concentrations were analyzed using a commercial kit (MAK030, Sigma-Aldrich).

Statistical Analysis

In experiment 1, DMI data for the 2-h infusion period were analyzed by repeated measures (four 0.5-h periods within the 2-h infusion period) using the PROC MIXED of SAS (version 9.4, 2013; SAS Institute, Cary, NC). The fixed effects were time, fructose infusion, Pi infusion, square (parity; only in the first experiment), period, and their interactions, and cow was included as a random effect. All other data for experiment 1 and data for experiments 2 and 3 were analyzed using the fit model procedure of JMP (version 12.1, SAS Institute). For all experiments (other than DMI on experiment 1) the fixed effects were fructose infusion, Pi infusion, square (parity; only in the first experiment), period, and their interactions, and cow was included as a random effect. For the analysis of liver and plasma parameters, initial values before infusion treatment were used as a covariate. Main effects were declared significant at $P \leq 0.05$, and tendencies were declared at $P \leq 0.10$. Interactions were declared significant at $P \leq 0.10$.

RESULTS AND DISCUSSION

Phosphate infusion increased DMI by 37% for multiparous cows during the 2-h infusion period but did not affect DMI by primiparous cows (Figure 1A). The effect of Pi infusion on DMI of multiparous cows was sustained after treatment ceased, increasing DMI by 47% compared with NaCl infusion (6.5 and 4.4 kg, respectively) over the 4 h since infusion began (data not shown). The Pi treatment increased daily milk yield for multiparous cows in experiment 1 by 5% ($P = 0.03$) and tended to increase daily lactose yield by 5% ($P = 0.07$) but tended to reduce milk yield for primiparous cows by 5% ($P = 0.08$). In both squares, Pi tended to increase MUN by 5% (Table 2; Figure 2). Moreover, Pi tended to increased fat percentage by 18% for the fructose treatment only (interaction, $P = 0.07$), and the fructose treatment increased milk protein percentage by 3% (Table 2). Fructose infusion increased DMI by 23% in the first 0.5 h of infusion in experiment 1, but the effect was transient with no effect of fructose on DMI detected afterward (Figure 1B).

Table 2. Daily milk and milk component yields for experiment 1[1]

	Fructose		Glucose		Significance, *P*-value				
Variable	Cl	Pi	Cl	Pi	F/G	Cl/Pi	F × Pi	F × square	Pi × square
Milk yield (kg/d)	32.3	32.1	31.7	32.2	0.76	0.66	0.48	0.31	0.01
Fat (%)	4.22	4.96	4.80	4.35	0.96	0.60	0.04	0.92	0.21
Fat (kg/d)	1.40	1.53	1.51	1.38	0.81	0.99	0.07	0.24	0.89
Protein (%)	3.34	3.35	3.27	3.23	0.01	0.57	0.56	0.97	0.62
Protein (kg/d)	1.20	1.23	0.90	0.85	0.34	0.79	0.99	0.51	0.15
Lactose (%)	4.80	4.91	4.90	4.91	0.19	0.11	0.26	0.59	0.88
Lactose (kg/d)	1.55	1.59	1.57	1.59	0.68	0.44	0.79	0.60	0.07
MUN (mg/dL)	11.6	12.2	11.8	12.6	0.37	0.06	0.72	0.58	0.45

[1]Treatments were 2-h infusions of fructose or glucose (F/G; 0.6 mol/h) and NaCl or NaH_2PO_4 (Cl/Pi; 0.3 mol/h). Square = parity.

Table 3. Plasma phosphate (Pi), nonesterified fatty acids (NEFA), and glucose concentrations at the end of the infusion period of experiment 1[1]

	Fructose		Glucose		Significance, *P*-value				
Variable	Cl	Pi	Cl	Pi	F/G	Cl/Pi	F × Pi	F × square	Pi × square
Pi (mg/dL)	29.1	66.0	33.8	59.6	0.79	<0.0001	0.11	0.09	0.08
NEFA (μEq/L)	326	316	176	270	0.02	0.30	0.19	0.72	0.72
Glucose (mg/dL)	38.2	39.2	74.8	69.3	<0.0001	0.40	0.22	0.17	0.36

[1]Treatments were 2-h infusions of fructose or glucose (F/G; 0.6 mol/h) and NaCl or NaH_2PO_4 (Cl/Pi; 0.3 mol/h). Square = parity.

Glucose and Pi infusions increased their respective concentrations in plasma compared to their control treatments as expected; glucose infusion increased plasma glucose concentration 86% compared with fructose infusion and Pi infusion increased plasma Pi concentration 100% compared with NaCl infusion (Table 3). Both glucose and fructose infusions reduced NEFA during the infusion period (data not shown), and although no time by treatment interaction was detected, glucose infusion decreased plasma NEFA concentration 31% compared with fructose infusion (Table 3).

Although the fructose treatment stimulated DMI compared with glucose treatment as expected, the effect was detected only during the first 0.5 h of infusion. The majority (50–70%) of blood fructose in humans and rats is cleared and metabolized by the liver, but fructose is also metabolized by other tissues including intestine, testis, kidney, skeletal muscle, adipose, and brain (Mayes, 1993; Douard and Ferraris, 2008). It is possible that, during the first 0.5 h, most of the fructose was cleared by the liver, sequestering Pi and decreasing ATP production, but as liver fructose concentration increased, the rate of fructose clearance from the blood was reduced, enabling greater clearance by other tissues. The greater reduction in plasma NEFA concentration for the glucose infusion compared with fructose infusion might have masked the stimulation of DMI by fructose over the first 0.5 h because a greater reduction in plasma NEFA concentration following feeding caused a reduction in hepatic acetyl CoA content and was positively related to DMI among cows in the PP period, likely by reducing hepatic oxidation (Piantoni et al., 2015).

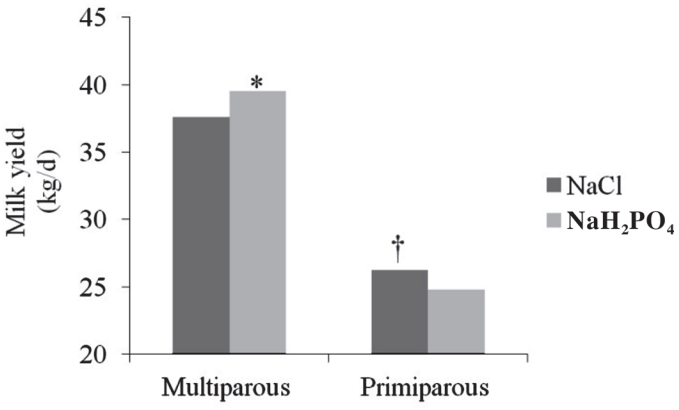

Figure 2. Statistical interaction between phosphate infusion and square (parity) for daily milk yield (kg/d) in the first experiment; *$P = 0.03$, †$P = 0.08$.

In contrast, the stimulation of DMI by Pi infusion for multiparous cows was unexpected and may have occurred because it increased daily milk yield for these cows clearing fuels from the blood. Because phosphorus requirement is related to the production level of the cow (NRC, 2001) and the multiparous cows produced 51% more milk than the primiparous cows (38.6 and 25.5 kg/d, respectively), the multiparous cows may have had a higher requirement for Pi and thus benefited from the Pi infusion. The lack of effect of Pi infusion on DMI of primiparous cows might be because it tended to reduce milk yield compared to control.

The unexpected effect of Pi on intake in experiment 1 raised the following questions: Was this a positive effect of Pi or a negative effect of NaCl, and was Pi affecting intake through mechanisms related to hepatic activity? To answer these questions, we conducted experiment 2, in which the control treatment for Pi was no infusion (not NaCl) and liver samples were taken to evaluate effects of treatment on hepatic Pi content. Generally, fructose had no effect on DMI throughout experiment 2, but Pi increased DMI by 28% at 0.5 h and tended to increase DMI by 24% at 1 h and 37% at 2 h after the beginning of infusion (Table 4). After 8 h, Pi increased intake by 43% only with the fructose treatment (interaction, $P = 0.06$).

Treatments did not affect yields of milk or milk components in experiment 2 (Table 5). However, Pi treatment interacted with fructose treatment to affect milk fat concentration; Pi reduced milk fat concentration by 17% when infused with fructose, but it did not affect milk fat concentration when infused with glucose. Consistent with our expectations, glucose treatment increased plasma glucose concentration by 32% at 30 min and 43% at 60 min after the start of infusion compared with fructose treatment (Table 6). Similarly, Pi treatment increased plasma Pi concentration 173% at

Table 4. Dry matter intake (kg) during the infusion and postinfusion period of experiment 2[1]

Time since infusion start (h)	Fructose		Glucose		Significance, *P*-value		
	XPi	Pi	XPi	Pi	F/G	XPi/Pi	F × Pi
0.5	1.80	2.73	2.16	2.35	0.96	0.05	0.15
1	2.33	3.40	2.90	3.10	0.69	0.10	0.24
2	3.46	5.80	4.40	5.02	0.92	0.10	0.31
4	6.16	8.30	8.02	7.32	0.73	0.58	0.29
8	11.4	16.3	14.2	14.1	0.79	0.06	0.06
24	24.6	26.8	25.5	25.6	0.86	0.17	0.18

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and with NaH_2PO_4 (Pi, 0.3 mol/h) or without (XPi).

60 min after the start of infusion but did not increase hepatic Pi concentration.

Failure of fructose to increase DMI of cows in experiment 2 that were just past peak lactation is consistent with a signal from gut distention dominating control of feed intake during this period with diminished signals from hepatic oxidation (Allen, 1996; Allen et al., 2009). Increased DMI by Pi treatment in this experiment indicates that intravenous infusion of Pi increases intake rather than the NaCl infusion reducing intake, answering the question raised from experiment 1. Moreover, intravenously infused Pi failed to increase hepatic Pi content and increased DMI in mid-lactation cows, suggesting it increases intake through mechanisms unrelated to hepatic oxidation and energy charge. Furthermore, the increase in DMI was not related to greater clearance of fuels from the blood by the mammary gland because yields of milk and milk components were not affected by treatment. Because intravenous infusion of Pi failed to increase hepatic Pi content, a different approach was required to understand the effect of hepatic Pi on intake and treatments were infused directly to the abomasum in experiment 3.

In experiment 3, interactions of main effects of treatment were detected at 2 h ($P = 0.03$), 4 h ($P = 0.003$), and 8 h ($P = 0.008$) after the start of the 1-h infusion. The fructose treatment increased DMI by 4 h after the start of infusion and Pi decreased DMI by ~33% only for the fructose treatment at 2, 4, and 8 h after the start of infusion (Table 7). Treatments did not affect yields of milk or milk components, but Pi increased MUN by 10% ($P = 0.01$, Table 8). Glucose compared with fructose treatment tended to increase plasma glucose concentration by 5% ($P = 0.06$) at 30 min, but the effect diminished ($P = 0.15$) by 60 min after the start of infusion (Table 9). The Pi treatment increased plasma Pi concentration at 60 min after the start of infusion by 11% ($P = 0.04$) compared to the NaCl control treatment. The Pi treatment increased hepatic Pi content at 30 min ($P = 0.002$), but the increase was greater when infused with glucose than with fructose ($P = 0.07$), and treatment had no effect on hepatic Pi content at 60 min. This finding suggests that fructose probably sequestered the Pi taken up by the liver and possibly saturated because plasma Pi was only increased 60 min after Pi infusion begun.

Our primary hypotheses were that fructose increases intake by sequestering Pi and that Pi infusion will reduce the effect of fructose. The results of experiment 3 fit these hypotheses, are consistent with hepatic ATP concentration affecting DMI, and present another level by which hepatic energy charge controls and af-

Table 5. Daily milk and milk component yields for experiment 2[1]

Variable	Fructose		Glucose		Significance, *P*-value		
	XPi	Pi	XPi	Pi	F/G	XPi/Pi	F × Pi
Milk yield (kg/d)	50.4	53.1	52.8	50.7	0.99	0.88	0.27
Fat (%)	3.58	2.98	3.23	3.34	0.95	0.13	0.05
Fat (kg/d)	1.81	1.58	1.71	1.68	0.95	0.23	0.34
Protein (%)	2.61	2.68	2.63	2.81	0.40	0.14	0.53
Protein (kg/d)	1.32	1.43	1.39	1.41	0.51	0.15	0.35
Lactose (%)	5.00	5.03	5.06	5.00	0.65	0.65	0.27
Lactose (kg/d)	2.52	2.67	2.67	2.54	0.91	0.92	0.22
MUN (mg/dL)	15.6	15.1	14.4	14.8	0.31	0.92	0.52

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and with NaH_2PO_4 (Pi, 0.3 mol/h) or without (XPi).

Table 6. Plasma and liver content during the infusion period of experiment 2[1]

Variable	Time into infusion (min)	Fructose		Glucose		Significance, *P*-value		
		XPi	Pi	XPi	Pi	F/G	XPi/ Pi	F × Pi
Plasma Pi (mg/dL)	30	11.3	21.7	12.9	14.9	0.40	0.15	0.22
	60	4.75	23.7	13.8	27.0	0.13	0.01	0.46
Plasma nonesterified fatty acids (μEq/L)	30	298	230	207	213	0.28	0.47	0.40
	60	189	230	167	133	0.30	0.94	0.50
Plasma glucose (mg/dL)	30	62.6	60.7	82.3	80.0	0.007	0.67	0.95
	60	57.1	55.6	77.9	83.7	0.005	0.70	0.52
Liver Pi (μmol/g)	30	15.0	14.8	13.8	15.1	0.88	0.85	0.81
	60	10.4	12.8	11.9	10.6	0.94	0.88	0.68

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and with NaH_2PO_4 (Pi, 0.3 mol/h) or without (XPi).

Table 7. Dry matter intake (kg) during the infusion and postinfusion period of experiment 3[1]

Time into infusion (h)	Fructose		Glucose		Significance, *P*-value		
	Cl	Pi	Cl	Pi	F/G	Cl/Pi	F × Pi
0.5	1.93	1.35	1.67	1.48	0.67	0.05	0.26
1	1.96	1.54	1.78	1.69	0.92	0.18	0.35
2	3.20[a]	2.16[b]	2.65[ab]	3.08[a]	0.49	0.28	0.03
4	5.14[a]	3.50[b]	4.00[b]	3.59[b]	0.05	0.03	0.003
8	7.93[a]	5.19[c]	6.19[bc]	7.40[ab]	0.66	0.18	0.008
24	16.5	14.5	15.1	15.8	0.99	0.50	0.20

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and NaCl or NaH_2PO_4 (Cl/Pi; 0.3 mol/h).

Table 8. Daily milk and milk component yields for experiment 3[1]

Variable	Fructose		Glucose		Significance, *P*-value		
	Cl	Pi	Cl	Pi	F/G	Cl/Pi	F × Pi
Milk yield (kg/d)	34.7	33.5	33.4	34.2	0.73	0.81	0.32
Fat (%)	3.79	3.96	3.85	3.95	0.88	0.50	0.85
Fat (kg/d)	1.32	1.33	1.27	1.36	0.87	0.47	0.49
Protein (%)	2.48	2.42	2.43	2.44	0.47	0.26	0.11
Protein (kg/d)	0.86	0.81	0.81	0.83	0.54	0.63	0.22
Lactose (%)	4.80	4.78	4.80	4.81	0.62	0.70	0.62
Lactose (kg/d)	1.67	1.61	1.60	1.64	0.76	0.79	0.34
MUN (mg/dL)	12.3	13.7	13.0	14.2	0.14	0.01	0.72

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and NaCl or NaH_2PO_4 (Cl/Pi; 0.3 mol/h).

Table 9. Plasma and liver content during the infusion period of experiment 3[1]

Variable	Time into infusion (min)	Fructose		Glucose		Significance, *P*-value		
		Cl	Pi	Cl	Pi	F/G	Cl/Pi	F × Pi
Plasma Pi (mg/dL)	30	22.2	24.5	23.7	23.5	0.89	0.51	0.47
	60	22.1	24.7	21.4	23.6	0.35	0.04	0.82
Plasma nonesterified fatty acids (μEq/L)	30	595	580	677	563	0.67	0.46	0.52
	60	484	460	658	583	0.32	0.74	0.86
Plasma glucose (mg/dL)	30	47.9	46.2	49.9	49.1	0.06	0.26	0.69
	60	46.1	45.6	50.3	46.3	0.15	0.17	0.32
Liver Pi (μmol/g)	30	8.19[b]	10.4[b]	10.1[b]	15.5[a]	0.03	0.002	0.07
	60	7.10	7.60	6.68	9.85	0.69	0.40	0.54

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments were 1-h infusions of fructose or glucose (F/G; 0.6 mol/h) and NaCl or NaH_2PO_4 (Cl/Pi; 0.3 mol/h).

fects feed intake in dairy cows. That Pi reduces DMI when infused abomasally and increases DMI when infused intravenously suggests that Pi affects DMI by 2 independent mechanisms; abomasal infusion probably affects DMI through a liver-related mechanism, and intravenous infusion likely affects DMI by a liver-independent mechanism. Higher producing cows may have a higher metabolic requirement for phosphorus than what is currently provided in rations, and the fact that increasing the phosphorous content in diets has no effect on DMI (Peterson et al., 2005; Grünberg et al., 2011; Puggaard et al., 2011) might be because of the hypophagic effect of Pi through a mechanism related to hepatic oxidation. This hypothesis needs to be further verified in longer-term experiments to understand whether competing mechanisms exist for effects of Pi on DMI, which would make it difficult to determine the true P requirements of dairy cows.

Fructose infusion increased intake when infused to fresh cows, abomasally or intravenously. Because fructose is rapidly fermented in the rumen, mainly to lactate and butyrate (Golder et al., 2012), feeding fructose is not expected to increase DMI. However, sorbitol can be metabolized to fructose in the liver of nonruminants and ruminants (Blakley, 1951; Shaw, 1974; Mayes, 1993). Part of the sorbitol consumed by ruminants may escape ruminal fermentation; for example, in sheep as much as 5 to 7% of the sorbitol escaped ruminal metabolism (Geay et al., 1992). Accordingly, sorbitol feeding may potentially increase DMI in fresh cows.

The results of this research demonstrate the complex mechanisms that control feed intake in dairy cows, provide evidence for an effect of ATP production on DMI, and suggest that Pi affects intake by 2 competing mechanisms.

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