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Influence of Small Intestinal Protein on Carbohydrate Assimilation in Beef and Dairy Cattle

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Abstract

The long term goal of the proposed research, "Influence of small intestinal protein on carbohydrate assimilation and metabolism in beef and dairy cattle" was to define the limits of small intestinal starch digestion and clarify regulatory mechanisms involved in starch assimilation in cattle. It was hypothesized that dietary protein plays a critical role in the regulation of intestinal digestion; however, studies clearly identifying this role were lacking. The first two experiments quantified starch digestion (disappearance from the small intestine) in response to known increments in duodenal protein supply and found that the quantity of DM, OM and starch disappearing from the small intestine increased linearly ($P < .01$) with protein infusion. A follow-up experiment also demonstrated that casein infusion linearly increased pancreatic α -amylase concentration and secretion rate. The final experiment provided critical data on metabolic fates of glucose derived from intestinal starch digestion. These data demonstrated that increasing postruminal starch supply does increase the metabolism of glucose by visceral tissues; however, this increase is minor (20%) compared with the increase in portal production (70%). These changes can have a dramatic impact on the glucose economy of the animal and result in large increases in the amount of glucose reaching peripheral tissues.

Objectives:

- 1) quantify intestinal starch digestion in response to increased duodenal supply of protein
- 2) elucidate the role of the pancreas in this response
- 3) integrate glucose supply from starch digestion with postabsorptive glucose metabolism.

Results

Experiment 1. Starch digestion in growing steers (Kentucky)

Materials and Methods

Animals and Diets. Five Angus steers (345 ± 14 kg) surgically fitted with ruminal and double-L shaped intestinal cannulas in the duodenum (10 cm distal to the pylorus) and ileum (15 cm cranial to the ileal-cecal junction) were used in a 5 x 5 Latin square design. Steers were housed in individual pens (2.5 x 2.5 m) in a temperature-controlled facility (20 °C) with a 16 h light:8 h dark cycle. Steers were tethered during infusion and collection

periods. Steers were fed at 1.65% of BW with corn silage (9.7% CP), composing 93% of the diet (DM basis) and a protein supplement (42.5% CP) based on soybean meal and urea, composing 7% of the diet (DM basis), formulating a 12.0% CP diet. The protein supplement composition (DM basis) was: 44.41% soybean meal, 11.82% corn, 7.14% urea, 12.71% limestone, 7.57% dicalcium phosphate, 6.43% choice white grease, 6.71% TMS + Se, 2.85% Cr_2O_3 and 0.36% ADE. The diet was provided every 2h using automated feeders and steers were allowed ad libitum access to fresh water. Steers were weighed at the beginning of each period and DMI adjusted to 1.65% of BW.

Treatments. Steers were infused abomasally with 43.5 g of raw cornstarch/h (1,044 g/d, DM basis) and with the following treatments: 1) 0 g of casein/d (OC), 2) 50 g of casein/d (50C), 3) 100 g of casein/d (100C), 4) 150 g of casein/d (150C), and 5) 200 g of casein/d (200C). Casein was prepared as a stock solution containing 6.67% casein and 0.53% Na_2CO_3 . Casein stock solution and cornstarch were mixed daily with tap water to reach treatments concentration and a infusion volume of 6 liters/d. The solution was infused at a rate of 250 mL/h. Peristaltic infusion pumps fitted with Tygon pump tubing (i.d. 2.29 mm) were used. Infusion tubing was passed through the ruminal cannula and omasal orifice into the abomasum. Retention in the abomasum was aided by an 8-cm plastisol washer on the end of the infusion line. The solution was stirred continuously during the infusion (14 d). Cr_2O_3 was included in the supplement to provide a final concentration of 0.2% in the diet as a digesta flow marker.

Experimental Periods and Sampling. Experimental periods consisted of 17 d with 14 d of infusion and sampling followed by 3 d for rest (no infusion). During the 14 d of solution infusion, the first 12 d were used for adaptation and the last 2 d for sampling of ruminal fluid, ruminal contents, duodenal and ileal digesta, feces and blood. On day 13 samples were taken from 08:00 to 18:00 and on day 14 samples were taken from 09:00 to 19:00. Duodenal digesta (250 g) and ruminal fluid (100 ml) were sampled every 2 hours, and ileal digesta (250 g) and feces (100 g) were sampled every 4 hours. Ruminal fluid was sampled only on day 13. This schedule allowed the collection of 12 duodenal digesta samples, and 6 ruminal fluid, ileal digesta and feces samples. After the last sampling on day 14, jugular blood samples were taken and frozen for subsequent glucose analysis. Duodenal and ileal digesta composite samples for each animal and period were formed by homogenizing both days samples for 1 min. Composite samples were stored (-20 °C) for later analysis. Duodenal and ileal digesta were lyophilized. Feed and fecal samples were dried in a forced-air oven at 55 °C for 48 and 96 h, respectively. Samples were ground through a 1-mm screen and analyzed for DM, OM and N, starch, Co and Cr by wet ashing and atomic absorption using an air/acetylene flame.

Ruminal fluid (100 mL) was collected and pH measured immediately. Ruminal fluid samples (5 mL) were acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for subsequent analysis of VFA and $\text{NH}_3\text{-N}$.

Statistical Methods. The data were analyzed as a 5 x 5 Latin square design using the GLM procedure of SAS (1996). The model contained period, steer, and infused casein treatment. Linear and quadratic orthogonal contrasts were performed for the effects of casein infusion treatment. Treatment effects were considered different when $P < .05$.

Table 1. Quantity of starch disappearance in steers abomasally infused with starch and protein

Item	Casein infused, g/d					P value		SEM ^a
	0	50	100	150	200	Linear	Quadratic	
Intake, g/d	1550	1584	1476	1590	1584	.49	.27	33
Duodenal flow, g/d	1302	1261	1810	1444	1539	.03	.06	83
Ileal flow, g/d	559	680	709	619	570	.90	.28	136
Apparent Starch Disappearance, g/d								
Pre-intestinal	1279	1371	701	1195	1092	.14	.08	109
Small intestinal	743	582	897	825	969	.01	.38	83
Large intestinal	392	434	501	501	423	.61	.38	113
Apparent total tract	2414	2387	2273	2521	2484	.20	.18	64

^aSEM = Standard error of the mean.

Results. A summary of the major findings is presented in Table 1. Quantity of DM and OM disappearance from the small intestine increased linearly ($P < .01$) with protein infusion. Percent DM and OM disappearance from the small intestine also increased (linear; $P < .05$) with increased protein infusion. Two hundred g/d protein infusion increased small intestinal starch disappearance by 204 g/d over the control and resulted in a linear ($P < .01$) increase in the quantity of starch disappearing from the small intestine. Level of protein infusion did not significantly affect ($P > .10$) the quantity or percent of DM, OM, or starch disappearance in the large intestine. Increasing protein flow to the duodenum appears to increase the quantity of starch disappearing from the small intestine in steers.

Experiment 2. Starch digestion in cows (Israel)

Animals. Six pleuriparous Israeli Holstein dairy cows in early lactation having ruminal and abomasal t-cannula and blood sample catheters in both vein and artery were arranged in a 3X3 Latin square design, for periods each 13 days (10 days adaptation and 3 days sampling). The animals were fed 4 equal meals at 6-h intervals, a total mixed ration (composition g/kg DM; corn silage 400g, legume hay 200g, barley grain 300g and vitamin premix 100g).

Treatments. Powdered corn starch (Sigma) in a water slurry was infused continuously abomasum via catheters passing through the rumen into the aqueous solution was also infused concomitantly with starch to provide treatments 0 (control), 400 (treatment 1) 800g (treatment 2) of casein daily. During the sampling period the feed intake and feed refusals were recorded and sampled. Rumen abomasal and fecal grab samples were collected every four hours on the second day of the collection period; on the third day arterial and venous blood was collected hourly between milkings to estimate urea concentration, and milk was sampled on the first and third days.

The markers were analyzed using ICP atomic absorption spectrophotometry. Plasma urea nitrogen was estimated colorimetrically and rumen ammonia was analyzed using an Auto Analyser and the salicylic acid procedure.

Statistical Analyses. Data were analyzed as a replicated 3 x 3 Latin square (SAS 1988). The statistical model included square, period(square), steer(square), and treatment. Results were considered significant at the $P < .05$ level.

Results. Results from Exp. 2 are presented in Table 2. Dry matter digestion was increased (60.71, 63.74, 64.54%) by infusion of starch with casein at both 400g and 800g infusion levels. Flow of NDF to the abomasum tended to be lower with casein infusion ($P < .12$) which resulted in a lower total tract NDF digestion ($P < .05$). Digestion of CP in the stomachs and intestines were unaffected by treatment; however, total tract CP digestion was greater for the casein treatments ($P < .05$). Casein infusion resulted in a greater digestion of TNC in the rumen and the total tract ($P < .05$). Blood urea of 11.40 and 13.56 mg/dl for control and 400g infusion were lower than 19.32 mg/dl for 800g infusion ($P < .05$).

Experiment 3. Volume and composition of pancreatic secretion in beef steers (Kentucky)

Animals. One Holstein, 2 crossbred, and 5 Angus steers (305 ± 5 kg) surgically fitted with pancreatic pouch-duodenal reentrant cannulas and abomasal infusion catheters were used in a replicated 4 x 4 Latin square experiment. Steers were housed in individual (2.5 x 2.5 m) pens within a temperature (23°C) and light (16 h light: 8 h dark) controlled room with water available free choice.

Before surgeries, steers were withheld from feed (48 h) and water (24 h). Pancreatic pouch-duodenal reentrant cannulas and abomasal infusion catheters were placed during an aseptic surgery under general anesthesia. Anesthesia was introduced with 8 mg/kg BW of thiopental sodium and maintained with halothane in oxygen. All animals were allowed to recover a minimum of 14 d before being placed on treatment. Rectal temperatures were checked for 7 d after surgery as an indicator of animal health.

The reentrant cannula linked a pouch of duodenum containing the primary pancreatic duct with the duodenum. The single-unit cannula used previously was replaced by open-T cannulas in both the duodenal pouch and duodenum. These cannulas were exteriorized and connected in a manner allowing all pancreatic juice to flow into the duodenum. The open-T cannulas were constructed by removing half the continuous barrel from a 1.25 cm polyvinyl chloride "T" shaped tubing adapter. Silicone tubing (20 cm; .95 cm i.d.) and 1.43 cm o.d.) was placed over the intact barrel. The exposed portion of the "T" was primed with General Electric SS4179 primer (Waterford, NY) and coated with General Electric RTV118 self leveling silicone. A 5 cm section of 1.11 cm i.d. and 1.75 o.d. silicone tubing was used to connect the cannulas externally.

Table 2. Influence of abomasal casein infusion on digestion of dry matter (DM) neutral detergent fiber (NDF), crude protein (CP) and total nonstructural carbohydrates (TNC) in cows

Item	Casein kg/d			TRT, F	Linear P<
	0	0.4	0.8		
DMI, kg	12.9	14.6	13.6	0.19	0.1
DMI+Infusion, kg	14.5	16.6	15.9	0.087	0.11
DM Abomasum flow, kg	10.1	9.6	8.4	0.58	0.81
DM, Feces, kg	5.9	5.5	5.5	0.336	0.48
DM rumen digestion	0.3	0.41	0.47	0.43	0.85
DM intestinal digestion	0.36	0.39	0.32	0.83	0.61
DM total tract digestion	0.6	0.66	0.65	0.015	0.061
NDF intake, kg	5.5	6	5.6	0.48	0.24
NDF abomasum, kg	4.3	3.5	3.1	0.12	0.67
NDF, Feces, kg	3.5	3	3.1	0.093	0.12
NDF rumen digestion	0.21	0.4	0.42	0.17	0.42
NDF intestinal digestion	0.13	0.12	0	0.51	0.6
NDF total tract digestion	0.35	0.48	0.43	0.04	0.05
CP intake, kg	1.99	2.22	2.09	0.286	0.16
CP intake + infusion, kg	1.99	2.62	2.89	0.006	0.16
CP, abomasum flow, kg	1.58	1.58	1.78	0.406	0.49
CP, feces, kg	0.94	0.94	0.9	0.681	0.66
CP rumen digestion	0.21	0.38	0.38	0.106	0.32
CP intestinal digestion	0.38	0.37	0.45	0.4	0.37
CP total tract digestion	0.54	0.64	0.69	0.0061	0.47
TNC intake, kg	4.7	5.4	4.8	0.345	0.15
TNC intake + infusion, kg	6.3	7	6.3	0.345	0.15
TNC abomasum flow, kg	3.11	2.02	1.93	0.029	0.169
TNC feces kg	0.67	0.48	0.44	0.001	0.009
TNC rumen digestion	0.54	0.69	0.67	0.04	0.11
TNC intestinal digestion	0.76	0.77	0.77	0.62	0.53
TNC total tract digestion	0.89	0.93	0.93	0.0008	0.012
Ammonia, mg/dl	19.2	21.4	22.1	0.265	0.61
Urea, mg/dl	11.4	13.6	19.3	0.012	0.33

Abomasal catheters were made by securing a 1.25 cm section of Tygon® tubing (.95 cm i.d. and 1.43 o.d.) as a cuff 1.5 cm from one end of a 45 cm section of Tygon® tubing (.64 cm i.d. and .95 cm o.d.) with cyclohexanone. After the cyclohexanone dried, a 2.5 cm vinyl flat washer with .95 cm hole was slid over the smaller tubing to the cuff. The cuff and washer were placed into the pyloric region of the abomasum with the remaining

tubing exteriorized through the adjacent body wall. Patency was maintained by filling the catheter with mineral oil between infusions.

Steers were weighed at the beginning of each period and individual intake adjusted to 1.5x the NE_m requirement. The diet contained 90% corn silage and 10% supplement on a DM basis. Diets were balanced to meet CP requirements of a 318 kg steer gaining .45 kg/d. Each day, silage and supplement were weighed out separately, thoroughly mixed, divided into 12 equal portions, and fed at 2 h increments.

Treatments. Abomasal infusions consisted of 1050 g/d (DM basis) of raw corn starch and 0, 60, 120, or 180 g/d (DM basis) sodium casein suspended in tap water to yield a total of 6000 g/d. They were prepared fresh daily and infused over a 24 h period using a peristaltic pump. Solution homogeneity was maintained by rapid continuous mixing with a stir bar and stir plate. Each period began with 3 d of adaptation by infusing 25, 50, and 75% of the final starch and treatment casein levels in 6000 g/d of total solution. Full treatment levels were infused for 8 d with samples being collected the final day. A minimum of 7 d rest was allowed between infusion treatments.

Sampling. On the sampling day, continuity of the reentrant cannula was interrupted and the duodenal reentrant section was plugged. A 24 French Foley catheter was inserted into the pancreatic pouch portion of the cannula. Approximately 200 mL of tap water was injected through the Foley catheter to flush the pancreatic pouch. The catheter was removed and a plastic "T" tubing connector inserted. A 2.5 m section of Tygon® tubing was attached to the "T" connector while the other end was placed through the center of a #8 rubber stopper. The stopper was inserted into the top of a 1000 mL sidearm Erlenmeyer flask under vacuum. The 3rd arm of the "T" was left open as a vent to prevent vacuum damage to the pouch. Fluid was continuously collected under vacuum into the Erlenmeyer flask in an ice bath for 7 h. At 30 min intervals the flasks were switched and incremental sample weights and pH measured. A composite sample was formed for each steer by retaining 10% of the 30 min samples. Remaining pancreatic fluid was returned to the duodenum using a catheter tip syringe. The first two incremental samples were returned to the duodenum without measurement or retention for composite to ensure the purity of fluids collected. Sample composites were stored at -30°C between fluid additions.

Analyses. Samples were stored (-30° C) until analysis for total protein and activities of α -amylase, trypsin, and chymotrypsin. All analyses were completed within 2 weeks after collection. Enzyme activities were expressed with 1 unit (U) being equal to the production of 1 μ mol of product/min per volume of pancreatic fluid.

Silage and supplement samples were analyzed for DM (55°C for 48 h) before beginning each period. Feed bunks were checked daily and 10% of orts retained for a composite. Composite silage and supplement samples were formed during each period. Feed samples were ground through a 1-mm screen and analyzed for N and NDF.

Statistical Analyses. Data were analyzed as a replicated 4 x 4 Latin square (SAS 1988). The statistical model included square, period(square), steer(square), and treatment. Linear and quadratic orthogonal contrasts were used to separate treatment means. Response variables included juice secretion rate, pH, protein concentration and secretion

rate, and the concentration, specific activity (U/mg protein), and secretion rate of α -amylase, trypsin, and chymotrypsin. Results were considered significant at the $P < .05$ level.

Results. A summary of the major findings is presented in Table 3. The quantity of pancreatic juice secreted was not altered (avg. 155 mL/h; $P > .10$) by the infusion of casein with starch. Treatments also had no affect ($P > .10$) on the pH (avg. 8.13) or protein concentration (avg. 10.23 mg/ml) of the juice secreted from the pancreas. No differences were detected ($P > .10$) in the rate of protein secretion (avg. 1536 mg/h).

Casein infusion linearly increased the α -amylase concentration (U/mL; $P < .02$) and specific activity (U/mg of protein; $P < .03$) of pancreatic α -amylase. These changes corresponded to a linear increase ($P < .01$) in the secretion rate of α -amylase activity (U/h). Infusion of casein with starch did not change ($P > .10$) the trypsin or chymotrypsin concentrations (U/L), specific activities (U/mg protein), or secretion rates (U/h).

Table 3. Influence of post-ruminal protein infusion on secretion, pH, protein, α -amylase, trypsin, and chymotrypsin

Observation	Treatment					P-value ($P < .05$)	
	0	60	120	180	SEM ^a	Linear	Quadratic
Secretion, mL/h	155	167	143	155	11	.66	.98
pH of secretion	8.09	8.14	8.16	8.13	.04	.51	.37
Protein							
mg/mL	10.35	9.65	10.03	10.87	.64	.51	.25
mg/h	1,482	1,604	1,414	1,645	117	.58	.65
α -Amylase							
Units/mL ^b	182	210	201	271	21	.02	.34
Units/mg protein	17.5	21.3	19.8	24.6	1.9	.03	.79
Units/h	26,847	36,385	26,763	41,894	2290	.01	.24
Trypsin							
Units/L	1,537	1,252	1,309	1,418	116	.57	.11
Units/mg protein	.143	.130	.131	.133	.010	.50	.44
Units/h	216	210	182	217	19	.77	.30
Chymotrypsin							
Units/L	354	296	337	407	42	.30	.15
Units/mg protein	.033	.030	.034	.036	.003	.39	.57
Units/h	50	47	49	63	7	.26	.25

^aSEM = standard error of mean, n=8.

^bOne unit = 1 μ mol product formed per min.

Experiment 4. US Glucose metabolism in beef steers (Kentucky)

Procedures

Animals: Eight Angus steers (235 ± 6 kg, average weight) were used in a crossover

design. Steers were fitted with permanent ruminal cannulas and abomasal infusion catheters for treatment infusion. Steers were also surgically fitted with chronic indwelling catheters in the hepatic portal vein, hepatic vein, two mesenteric veins, and a mesenteric artery. The right carotid artery was also elevated to provide access to arterial blood in the event a mesenteric arterial catheter became nonpatent. Before surgeries, steers were withheld from feed (48 h) and water (24 h). Catheters were placed and the carotid artery was elevated during an aseptic surgery under general anesthesia. Anesthesia was introduced with a 1.76 mg/kg ketamine and .088 mg/kg xylazine IV dose and maintained with halothane in O₂. All animals were allowed to recover a minimum of 14 d before being placed on treatment. Rectal temperatures were checked for 7 d after surgery as an indicator of animal health. The University of Kentucky Institutional Animal Care and Use Committee approved all procedures. Blood catheters were filled with 1,000 U/mL heparinized-saline solution between sampling periods to maintain patency. Patency of the abomasal infusion catheter was maintained by filling with mineral oil between infusions. Steers were tethered in individual (2.5 x 2.5 m) pens for the first 11 days of infusion and then moved to 2.2 m x .9 m metabolism crates for the final 3 days of each period. The left jugular vein and elevated carotid artery were fitted with temporary catheters 12-16 h prior to sampling. Both the pens and metabolism crates were within temperature (23°C) and light (16 h light: 8 h dark) controlled rooms and water was available free choice.

Diets and infusion treatments. Steers were weighed at the beginning of each period and individual DMI adjusted to 1.5x the NE_m requirement. A pelleted diet (Table 1) containing orchard grass (89%), corn gluten meal (5%), and Soypass® (5%) was formulated to meet the degradable intake protein requirement of steers receiving ruminal starch infusion and the metabolizable protein requirement of a steer 250 kg gaining 0.84 kg/d. The diet was weighed out daily and fed in 12 equal portions at 2 h increments.

Treatments were ruminal or abomasal infusion with 800 g/d of partially hydrolyzed starch (SH; 16%) for 14 d. The first 6 d were used for adaptation by infusing 25, 50, and 75% of the final SH quantity. Equal quantities of tap water were infused into the site opposite the SH.

Collection. On the last day of infusion, 500 µCi of 6-³H glucose was pulse dosed into the hepatic vein at time 0. After the pulse dose of 6-³H glucose was performed, a primed (40 µCi) continuous infusion (.8 µCi/min) of [U-¹⁴C]-glucose was infused through a sterile .45-mm filter. A primed (15 mL) continuous infusion (.8 mL/min) of P-amino hippurate (PAH; 10% wt/vol, pH 7.4) was begun into a mesenteric vein through a sterile .45-µm filter at 240 min.

Arterial samples (7 mL) were taken period at the following times after the 6-³H glucose pulse dose: 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, and 240 min. At 300, 360, 420, 480, 540, and 600 min simultaneous arterial, hepatic, and portal blood samples (15 mL) were taken over a 5 min period. Samples were collected into heparinized syringes, transferred to centrifuge tubes containing 2 mg NaF per mL of blood, mixed by inversion, and placed on ice until centrifugation (15,000 x g, 15 min at 4°C) and plasma harvested. At the final 6 sampling times an additional 2 mL of arterial, hepatic, and portal blood were collected into 5 mL heparinized glass syringes, capped, and immediately analyzed for hemoglobin and O₂

saturation using a Hemoximeter (Radiometer America, Westlake, OH). The remaining sample was used to measure packed cell volume by drawing whole blood into microhemotocrit capillary tubes and centrifuging (15,000 x g, 5 min).

Analysis. All samples were analyzed for glucose specific activity. Plasma (2 mL) was deproteinized for determination of specific activity by adding 2 mL of .3 N Ba(OH)₂ followed by 2 mL .3N ZnSO₄. The sample was mixed and allowed to stand for 5 min after each addition. After centrifuging (15 min at 15,000 x g), the supernatant was poured into a cation (AG 50W; BioRad Laboratories, Hercules, CA) exchange column and allowed to drip completely through an anion (BIO-REX 5; BioRad Laboratories, Hercules, CA) exchange column. The columns were rinsed by allowing 5 mL of H₂O to completely pass through both columns. The total eluent was collected, lyophilized, and reconstituted in 1 mL of H₂O. An aliquot (700 µL) was combined with 15 mL of scintillation cocktail and counted on a dual channel liquid scintillation counter. The remaining sample was analyzed for glucose concentration using an enzymatic procedure.

Hourly arterial, hepatic, and portal plasma samples were also analyzed for glucose, lactate, PAH, ammonia nitrogen, β-hydroxybutyrate, α-amino nitrogen (AAN), and VFA. Glucose and lactate plasma concentrations were determined with a YSI Model 27 (Yellow Springs Instruments, Yellow Springs, OH). Whole plasma was analyzed for PAH and NH₃-N using glutamate dehydrogenase. The PAH standards were prepared from the infused solutions. Plasma was deproteinized with an equal volume of .6 N HClO₄, centrifuged (15,000 x g 10 min), and the supernatant analyzed for AAN and β-hydroxybutyrate. Threonine was used for the AAN standards. Plasma PAH, β-hydroxybutyrate, AAN, and NH₃-N methods were adapted for use on a COBAS FARA II centrifugal analyzer (COBAS FARA II, Roche Diagnostic Systems, Montclair, NJ). Two mL of each hourly sample was used to form a single composite for determination of VFA content by gas chromatography.

Calculations. Plasma flow and net flux of nutrients across the portal drained viscera (PDV), hepatic, and total splanchnic (TS) vascular beds were calculated as described previously (Krehbiel et al., 1992). Blood flow was used to determine oxygen and hemoglobin fluxes rather than plasma flow since the analysis were performed on whole blood. A positive net flux indicates a release or net absorption of the nutrient into the plasma pool, whereas a negative net flux implies net uptake or utilization by gastrointestinal tissues. 6-[³H] glucose disappearance curves were fitted to a 3-pool open system model. [U-¹⁴C] glucose continuous infusion calculations were performed according to (Bergman et al., 1973).

Statistics. Data were analyzed as a crossover design using the GLM procedure of SAS with steer, period, and treatment included in the model. Only 7 observations were used for nutrient flux and [U-¹⁴C] glucose measurements of the ruminal treatment due to catheter function failing in one steer. Only 7 measurements were reported for the 6-³H glucose measurements of the abomasal treatment due to arterial sampling problems at the early time points. Results were considered significant if probability of type 1 error was equal to or less than .05.

Results

A summary of the major findings from Exp. 4 are presented in Tables 4,5 and 6. Shifting carbohydrate supply to the small intestine increased portal ($P=.02$) and hepatic ($P=.06$) plasma flow, whereas hematocrit and blood hemoglobin were unaffected. Arterial, portal and hepatic blood glucose specific activities were all higher ($P=.01$) when starch was infused ruminally. Portal flux of glucose was greater ($P=.01$) when starch was infused abomasally. Net portal flux of lactate, β -hydroxybutyrate, α -amino-N and oxygen were unaffected by treatment whereas, ammonia-N net portal flux was greater ($P = .07$) with abomasal starch infusion.

Changing the site of starch infusion seemed to have a profound impact on hepatic metabolism. Hepatic flux of glucose was unchanged; however, hepatic uptake of lactate, α -amino-N and oxygen were lower ($P<.06$) and hepatic uptake of ammonia was greater ($P=.04$) with abomasal starch infusion. Abomasal starch infusion also decreased ($P=.01$) net hepatic β -hydroxybutyrate output. Combined these changes in PDV and hepatic metabolism resulted in a two-fold increase in net TS glucose output ($P=.01$), decreased lactate uptake ($P=.07$) and decreased β -hydroxybutyrate output ($P=.04$) for steers receiving the abomasal infusion. Total splanchnic α -amino-N, ammonia-N and oxygen were unaffected.

Table 4. Arterial, portal, and hepatic plasma flow and hematocrit, hemoglobin, [$U-^{14}$] glucose specific activities in steers infused abomasally with casein and starch

Item	Treatment			
	Ruminal	Abomasal	SEM	P value
Plasma Flow, L/h				
Arterial	109.0	113.7	7.9	.67
Portal	405.9	454.5	7.4	.01
Hepatic	514.8	568.2	12.9	.03
Hematocrit, %				
Arterial	27.0	26.6	.3	.40
Portal	26.8	26.7	.3	.90
Hepatic	27.0	26.6	.1	.47
Hemoglobin, g/dl				
Arterial	9.9	9.7	.1	.36
Portal	10.0	9.9	.2	.49
Hepatic	10.1	10.0	.2	.45
[$U-^{14}$] glucose, dpm/mmol glucose				
Arterial	725254	467602	37513	.01
Portal	722627	441995	37023	.01
Hepatic	681757	428137	36050	.01

Portal glucose production ($P = .01$), portal glucose utilization ($P = .03$) and glucose irreversible loss ($P = .01$) were greater when starch was supplied to the abomasum. Hepatic glucose production, and hepatic and peripheral glucose utilization and production did not change due to treatment. Peripheral glucose utilization was also greater ($P=.08$) for steers receiving abomasal starch infusion.

Only glucose irreversible loss was affected by treatment for the [6-³H] glucose kinetics. Post-ruminal starch infusion increased glucose irreversible loss while pool size, volume, space, total entry rate and recycling were unaffected.

Discussion

The aims of the proposal were to study mechanisms of nutritional adaptation in ruminants. Specifically, the response to intestinal protein (casein) and carbohydrate. The initial experiments (Exp. 1, US and Exp. 2, Israel) demonstrated that casein does affect the disappearance of nutrients and specifically carbohydrate in the small intestine (Exp. 1 US) a concept that had been suggested in the literature but never clearly demonstrated.

This increased intestinal disappearance of carbohydrate demonstrated in Exp. 2 could be the result of increased pancreatic α -amylase secretion as was demonstrated in Exp. 3 (US). This adaptive response to increased intestinal protein appears somewhat unique to ruminants. Nonruminants typically adapt to increased protein intake by increasing the secretion of pancreatic proteases, not α -amylase. Why ruminants increase α -amylase secretion and proteases remain unchanged remains unclear. However, this response does suggest that digestion may be enhanced through nutritional manipulation in situations where α -amylase may be limiting.

Table 5. Portal and hepatic flux of metabolites in steers infused with ruminal or abomasal starch infusion

Item	Treatment		SEM	P value
Portal flux, mmol/h				
Glucose	-30	64	5	.01
Lactate	41	39	3	.56
β -hydroxybutyrate	58	56	5	.72
α -amino N	131	96	18	.21
Ammonia N	70	91	7	.07
Oxygen	-945	-999	25	.16
Hepatic flux, mmol/h				
Glucose	124	137	9	.34
Lactate	-75	-39	8	.02
β -hydroxybutyrate	68	41	5	.01
α -amino N	-58	-33	16	.01
Ammonia N	-76	-100	7	.04
Oxygen	-1066	-943	38	.06
Total splanchnic flux, mmol/h				
Glucose	95	201	11	.01
Lactate	-33	0	11	.07
β -hydroxybutyrate	126	97	8	.04
α -amino N	73	63	14	.63
Ammonia N	-6	-8	1	.28
Oxygen	-2010	-1942	46	.31

^aND = nondetectable

Table 6. Portal, hepatic, and peripheral glucose production and utilization, and glucose irreversible loss in steers infused with ruminal or abomasal starch infusion

Item	Treatment		SEM	P value
[U- ¹⁴ C] glucose, mmol/h				
Portal glucose production	-2	129	9	.01
Portal glucose utilization	28	65	9	.03
Hepatic glucose production	145	123	11	.19
Hepatic glucose utilization	21	-14	18	.20
Peripheral glucose utilization	72	140	24	.08
Peripheral glucose production	-23	-61	18	.16
Glucose irreversible loss	121	192	14	.01
[6- ³ H] glucose				
Pool size, mmol	187	215	13.7	.18
Distribution volume, L	40.4	44.8	3.3	.36
Space, %	17.1	18.31	1.2	.47
Irreversible loss, mmol/h	186	271	4.8	.01
Total entry rate, mmo/h	1073	1282	157	.36
Recycling, mmol/h	886	1011	161	.58

The metabolic consequences of shifting the site of starch digestion from the rumen to the small intestine were studied in Exp. 4 (US). Infusing starch into the small intestine dramatically increased the supply of glucose to peripheral tissues. The treatment supplied 185 mmol/h of glucose to the small intestine, of this 70% reached the portal circulation (portal glucose production 129 mmol/h). The increase in portal glucose utilization (65-28 mmol/h) represents an additional 20% of the infused carbohydrate. These changes occur without a change in hepatic glucose production. Combined these changes result in a substantial (59%) increase in whole body glucose metabolism (glucose irreversible loss) and a 112% increase in peripheral glucose supply (total splanchnic flux) when carbohydrate was infused into the abomasum compared with the rumen.

These data demonstrate that increasing postruminal starch supply does increase the metabolism of glucose by visceral tissues; however, this increase is minor (20%) compared with the increase in portal production (70%). These changes can have a dramatic impact on the glucose economy of the animal and result in large increases in the amount of glucose reaching peripheral tissues.

Evaluation of Research Achievements

The specific objectives of the project were met. We were able to demonstrate that intestinal protein supply affects carbohydrate disappearance. This relationship will be useful as we refine nutrient requirements and dietary formulations to predict what nutrients are supplied to the small intestine. There may be a critical amount of certain nutrients necessary for optimal digestion. This was demonstrated in that we were also able to show that intestinal protein supply affects pancreatic enzyme secretion. In addition to the ability to manipulate intestinal digestion it was demonstrated that shifting the site of starch digestion has a profound effect on visceral and whole-body glucose metabolism. This information will be useful as we attempt to formulate diets that provides the optimal

balance of nutrients to the entire gastrointestinal tract and will enable us to more accurately predict animal performance based on diet formulation.

Description of Cooperation

Research protocols were circulated among investigators in the grant. Regular communication among investigators included telefax and e-mail messages, with consultation on preparation of animals for experiments, and progress during sample collection. Experimental results were circulated among investigators and both groups contributed information to the yearly reports. Scientists met at the American Society of Animal Science meetings prior to planning the final year of experiments.

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