Effects of energy and amino acid supply to the small intestine on amino acid metabolism

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Effects of increasing the supply of starch and amino acids in the small intestine on absorption of nutrients and concentration of plasma metabolites were determined. A 2 × 2 × 2 factorial arrangement of treatments was used and dietary factors were as follows: 1) 25% versus 50% dietary corn, 2) formaldehyde-treated (rumen-protected) versus untreated corn, and 3) supplemental rumen-protected methionine and lysine versus no supplemental amino acids. Treatments were randomly allotted to 40 individually fed steers for the trial. Feeding formaldehyde-treated corn resulted in increased plasma glucose and insulin concentrations. Plasma insulin and glucose concentrations were also directly proportional to dietary corn level. The plasma concentrations of urea and most individual amino acids were markedly decreased when steers were fed treated corn compared with those fed untreated corn. Increased postruminal nutrient supply enhanced metabolic utilization of amino acids in these steers.

Keywords: cattle; formaldehyde; starch; amino acids; metabolism

Introduction

Feeding starch that escapes ruminal fermentation has been shown to improve the efficiency of energy utilization in growing ruminants by 11%–30%[1] and 42%,[2] increase glucose uptake in the small intestine,[3] and increase milk yield.[4] Postruminal infusion of glucose has increased milk production[5] and improved both growth rate and feed conversion.[1]

Treatment of corn with formaldehyde has been shown to increase the supply of digestible starch flowing to the small intestine and improve nitrogen (N) retention.[6,7] Further improvements in efficiency of N utilization may be possible by proper manipulation of post-ruminal amino acid supply. In some circumstances, deficiencies of amino acids available for absorption in the small intestine may be overcome by feeding rumen-protected amino acids.[8] However, amino acid metabolism is influenced not only by supply but also by regulatory mechanisms in the body. Insulin is associated with amino acid metabolism in ruminants[9,10] and amino acids are also potential stimulators for insulin secretion.[11] These regulatory relationships affecting amino acid utilization are not well understood. The present study was undertaken to determine the effects of increasing the supply of starch and amino acids to the small intestine on absorption of nutrients, and concentration of plasma metabolites and insulin in steers. Formaldehyde-treated corn was used to increase starch supply to the small intestine[12] and rumen-protected methionine (RPMet) and lysine (RPLys) were used to increase the supply of these amino acids to the small intestine.[8] RPMet and RPLys were used because Met and Lys usually have been listed as limiting amino acids, especially for cattle fed corn-based diets.

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Materials and methods

Forty crossbred Angus steers (average weight, 285 kg) were penned and fed individually twice daily for a 42-day trial. Steers were allotted to one of eight combinations of three dietary factors with five steers per treatment. Steers in each treatment had the same average weight. A completely randomized design with

Table 1 Diet compositions for steer metabolism trial

Item	Corn level %			
	25		50	
	−RPAA[a]	+RPAA	−RPAA	+RPAA
	%, DM basis			
Corn, ground[b]	25.00	24.53	50.00	49.53
Corn cobs, ground	42.00	42.00	27.97	27.97
Soy hulls	25.30	25.30	16.00	16.00
Soybean meal	5.00	5.00	3.33	3.33
Urea	.50	.50	.50	.50
Limestone	.70	.70	.96	.96
Dicalcium phosphate	.90	.90	.64	.54
Trace mineral salt[c]	.50	.50	.50	.50
Selenium (201 mg/kg)	.05	.05	.05	.05
Vitamin A (30,000 IU/g)	.01	.01	.01	.01
Vitamin D (3,000 IU/g)	.01	.01	.01	.01
Vitamin E (44 IU/g)	.03	.03	.03	.03
RPAA		.47		.47

[a] Rumen-protected amino acid blend is approximately 64% amino acids and provided .19% dietary rumen-protected methionine and .11% dietary rumen-protected lysine.
[b] Untreated or treated with formaldehyde at 1.5% (wt/wt).
[c] Contained > 93% NaCl, .35% Zn, .28% Mn, .175% Fe, .035% Cu, .007% I, and .007% Co.

a 2 × 2 × 2 factorial arrangement of treatments was used. Dietary factors were as follows: 1) 25% versus 50% dietary corn, 2) formaldehyde-treated versus untreated corn, and 3) supplemental rumen-protected amino acids (RPMet + RPLys) versus no supplemental amino acids (*Table 1*). To treat corn, 591 kg batches of ground corn were mixed with 18 kg of a 50% water-50% formalin (37% formaldehyde) solution (wt/wt). Dietary levels of RPMet and RPLys (.19% RPMet + .11% RPLys), which previously resulted in the greatest weight gain and plasma concentrations, were chosen for the present trial.[8] Steers were fed the 25% untreated-corn diet for 10 days and then were switched to experimental diets for a 16-day adaptation period to establish ad libitum intake. Steers were fed at 90% of ad libitum intake of the lowest consuming steer during the 16-day experimental period. Water was available continuously. Jugular blood samples were collected 3 hr post-feeding on the first day of the adaptation period and first and last day of the experimental period. Plasma was separated by centrifugation and analyzed for amino acids, urea N, insulin, and glucose concentrations. For amino acid analysis, plasma was deproteinized by the addition of 0.5 mL of 5-sulfosalicyclic acid (50% wt/vol) and centrifuged at 15,000*g* for 20 min. Aliquots of the resulting plasma supernatant were filtered through a Millipore filter (0.45 μm) and amino acid concentrations were determined by a modified high performance liquid chromatography (Model 334, Beckman Instruments, Inc., San Ramon, CA, USA) procedure[13] using o-phthaldehyde derivatization and fluorescence detection and a reverse phase column (250 × 4.6 mm i.d.) with C-18 ultrasphere 5 μm spherical-80A pore size packing. A methanol:citrate-phosphate buffer gradient was used to achieve resolution of individual amino acids. Other aliquots of plasma were analyzed for glucose,[14] urea N,[15] and insulin concentrations. A double antibody radioimmunoassay was used to quantitate plasma insulin using a commercially prepared kit (Corning Medical, Medfield, MA, USA). All samples were assayed at a volume of 200 μL in duplicate within a single assay. Sensitivity of the assay was 2.0 μU/mL. Guinea pig anti-porcine insulin was used as the first antibody, purified bovine insulin and porcine [^{125}I] insulin were used as standard and radioligand, respectively. The intra-assay coefficient of variation was 3.9%.

Feed and fecal grab samples were collected twice daily at 6 and 12 hr post-feeding for two days during the experimental period and composited by time and animal. Feed and fecal samples were dried at 55° C and analyzed for dry matter, neutral detergent fiber, acid detergent fiber,[16] crude protein,[17] and starch.[18] Nutrient digestibilities were determined by the acid insoluble ash marker ratio technique of Van Keulen and Young.[19]

Data were analyzed by analysis of variance procedures for a 2 × 2 × 2 factorial, completely randomized design experiment using Harvey's LSML76-mixed model least squares program.[20]

Results

Diet digestibilities did not differ ($P > 0.10$) except for a trend ($P < 0.07$) for 50% corn diets to have greater neutral detergent fiber and crude protein digestibilities than 25% corn diets (*Table 2*). Plasma concentrations of insulin, glucose, and urea N at the start of the adaptation period were similar among steers, indicating they were in a similar metabolic state (*Table 3*). Plasma insulin concentrations were greater ($P < 0.05$) when treated corn was fed at either 25% or 50% levels compared with when untreated corn was fed. Plasma glucose concentrations were also greater ($P < 0.05$) for steers fed formaldehyde-treated corn compared with those fed untreated corn. Plasma insulin and glucose concentrations were also directly proportional to dietary corn level. Rumen-protected amino acid (RPAA) supplementation increased ($P < 0.08$) insulin concentration slightly, but did not affect ($P > 0.05$) glucose concentration. Increasing corn level, treatment of corn with formaldehyde, and supplementation with RPAA resulted in lower ($P < 0.05$) plasma urea N.

The effects of dietary treatments on the concentrations of individual plasma amino acids are shown in

Table 2 Effects of corn level, formaldehyde treatment of corn, and supplemental rumen-protected amino acids (RPAA) on diet digestibility

	Treatment								
	25% corn level				50% corn level				
	Untreated		Treated		Untreated		Treated		
Item	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	SE
No. of steers	5	5	5	5	5	5	5	5	
Feed intake (kg/d)	6.5	6.4	6.6	6.5	6.3	6.5	6.7	6.4	
Digestibility(%)									
DM[a]	68.5	68.9	70.5	69.9	70.0	69.8	69.2	69.8	2.0
NDF[b]	55.6	55.0	56.0	55.2	58.2	59.9	59.8	58.1	1.2[e]
ADF[c]	49.2	48.9	48.0	48.9	50.1	49.7	49.2	50.0	1.7
CP[d]	65.6	66.7	67.5	68.0	68.1	68.0	69.0	70.6	1.9[e]
Starch	98.8	98.0	97.9	98.2	97.1	98.5	99.0	98.9	1.5

[a] Dry matter.
[b] Neutral detergent fiber.
[c] Acid detergent fiber.
[d] Crude protein.
[e] Corn level effect ($P < .07$).

Table 3 Effects of corn level, formaldehyde treatment of corn, and supplemental rumen-protected amino acids (RPAA) on plasma concentrations of insulin, glucose and urea N

	Treatment								
	25% corn level				50% corn level				
	Untreated		Treated		Untreated		Treated		
Item	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	SE
				Preadaptation period[a]					
Insulin (μU/mL)	8.8	9.1	9.0	8.9	8.9	9.1	9.2	9.3	.7
Glucose (mg/dL)	52.8	53.0	52.1	51.8	51.4	52.5	51.6	52.0	1.1
Urea N (mg/dL)	13.0	13.2	13.2	12.9	12.8	13.1	12.9	13.0	.8
				Experimental period[b]					
Insulin (μU/mL)	9.2	9.9	13.1	13.8	12.2	12.7	18.5	18.9	.9[cdf]
Glucose (mg/dL)	54.1	55.0	59.8	59.2	60.5	59.8	67.3	66.9	1.2[cd]
Urea N (mg/dL)	12.7	11.8	10.8	9.4	9.9	9.1	8.6	7.4	.4[cde]

[a] Preadaptation period (steers were fed only 25% untreated diets).
[b] Mean value for two sampling times (first and last day of experimental period).
[c] Corn level effect ($P < .05$).
[d] Formaldehyde treatment effect ($P < .05$).
[e] RPAA supplementation effect ($P < .05$).
[f] RPAA supplementation effect ($P < .08$).

Table 4. The concentrations of all essential amino acids (EAA) were greater ($P < 0.05$) when 25% corn diets were fed compared to 50% corn diets. Total nonessential amino acids (NEAA) followed this same trend ($P < 0.05$). The plasma concentrations of most individual amino acids, total EAA and total NEAA declined ($P < 0.05$) when treated corn compared to untreated corn was fed at the 25% or 50% level. Supplementation with RPMet + RPLys reduced ($P < 0.05$) the plasma concentration of TEAA including Met and Lys.

Discussion

Lack of dietary treatment effects on total tract nutrient digestibilities confirms the findings of Fluharty and Loerch[7] with sheep fed formaldehyde-treated corn diets. These authors reported that treatment of corn with formaldehyde (2% by weight) resulted in a 41% decrease in ruminal starch digestion without decreasing total tract starch digestion. Thus the site of starch digestion was shifted from the rumen to the lower gastrointestinal tract. Increased plasma insulin and glucose concentrations when formaldehyde-treated corn was fed likely reflects increased intestinal absorption of glucose for treated-corn diets resulting from increased delivery of digestible starch to the small intestine as observed by Oke.[6] The effect of rumen-protected amino acid supplementation on insulin concentrations was not as great as that of formaldehyde treatment. Little information is available about the effect of amino acids on insulin production in ruminants. Plasma insulin was increased when Davis[21] infused arginine, leucine, and phenylalanine, and Chew

Table 4 Effects of corn level, formaldehyde treatment of corn, and supplemental rumen-protected amino acids (RPAA) on plasma amino acid concentrations

	Treatment								
	25% corn level				50% corn level				
	Untreated		Treated		Untreated		Treated		
Item	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	−RPAA	+RPAA	SE
	μmol/dL								
Plasma EAA[a]									
Val	15.0	15.1	14.4	13.6	13.2	12.0	10.9	11.8	1.0[bc]
Thr	9.1	8.6	8.0	7.2	7.0	6.9	6.3	5.8	.9[bc]
Met	4.9	4.4	4.8	4.0	4.1	3.8	3.0	2.5	.4[bcd]
Ile	11.6	11.3	11.0	10.6	10.9	10.2	8.6	8.8	.7[bc]
Leu	15.2	14.8	14.2	13.0	13.2	12.5	11.2	10.6	.4[bcd]
Phe	9.5	9.1	8.2	7.5	7.6	6.5	4.0	3.9	.2[bcd]
His	10.1	9.6	9.1	8.3	8.6	8.2	7.0	5.9	.5[bcd]
Lys	15.2	14.8	14.2	12.9	13.1	11.6	10.4	9.3	.8[bcd]
Arg	12.0	11.6	10.3	9.9	10.2	9.4	7.2	6.5	.4[bcd]
TEAA	102.6	99.3	94.2	87.0	87.8	81.1	68.5	65.1	1.6[bcd]
Plasma NEAA									
Glu	20.2	20.6	19.0	18.4	17.5	17.0	16.2	15.5	.9[bc]
Asp	11.7	11.1	10.2	10.5	10.0	9.8	9.0	8.4	.4[bc]
Cit	8.2	8.6	7.5	6.8	7.8	6.2	5.9	6.2	.3[bcd]
Gly	16.0	15.2	13.6	13.0	12.7	13.1	12.8	12.4	.2[bcd]
Tyr	15.8	14.0	12.2	12.5	12.0	11.2	10.9	10.4	.5[bcd]
Ala	19.9	21.8	23.6	23.2	22.0	22.8	24.0	23.5	1.0[bcd]
Orn	9.6	9.1	8.4	7.5	7.0	7.2	6.2	6.8	.3[bcd]
Trp	8.1	7.3	6.9	6.5	6.0	6.3	5.9	5.6	.4[bc]
TNEAA	109.5	107.7	101.4	98.4	96.0	93.6	90.9	88.7	2.8[bc]

[a] EAA, essential amino acids; TEAA, total essential amino acids; NEAA, nonessential amino acids; TNEAA, total nonessential amino acids.
[b] Corn level effect ($P < .05$).
[c] Formaldehyde treatment effect ($P < .05$).
[d] RPAA supplementation effect ($P < .05$).

et al.[22] intravenously infused arginine. The patterns of plasma concentration of glucose, urea N, and insulin in the present trial were consistent with the results reported for sheep fed diets containing two levels of formaldehyde-treated corn.[6]

The higher plasma urea N concentrations for steers fed 25% corn compared to those fed 50% corn may indicate increased absorption of ruminal ammonia. However, the higher plasma amino acid concentrations may also indicate elevated plasma urea N resulted from inefficient utilization of absorbed amino acids for tissue protein synthesis. Plasma urea N concentration has been reported to be a function not only of digestible N intake and absorption of ammonia from the gastrointestinal tract, but also of the array of amino acids absorbed in relation to the physiologic function they are required to support.[23]

Lower concentrations of plasma amino acids as a result of feeding formaldehyde-treated corn and higher corn levels may reflect enhanced protein utilization via insulin as a result of increased starch absorption in the small intestine. The magnitude of the decline in plasma amino acid concentrations observed for treated corn was greater ($P < 0.05$) when 50% corn diets were fed than when 25% corn diets were fed. The lower plasma level of EAA observed when 50% treated corn + RPAA diet was fed suggests that this diet provided a better balance between energy supply and the supply of essential amino acids for these steers than did the other diets. Supplementation with RPMet + RPLys reduced ($P < 0.05$) the plasma concentration of TEAA. Somewhat surprisingly, plasma Met and Lys were also reduced when these rumen-protected amino acids were fed, suggesting that these amino acids were indeed limiting in these diets. Broderick et al.[24] reasoned that when extra protein is digested in the small intestine, EAA will not accumulate in plasma unless supplied in excess of requirements.

A marked rise in plasma glucose concentration was observed in steers fed treated corn at the 50% level. Glucose has been shown to have a number of actions that may lead to altered amino acid absorption and metabolism. When the 25% untreated corn diet was fed, essential amino acids may have accumulated in plasma because of insufficient energy to promote their utilization. Potter et al.[25] showed that exogenous administration of glucose causes a decline in plasma amino acid levels in sheep. Eskeland et al.[26] also showed that the energy-induced plasma amino acid depressions were accompanied by an increase in N retention indicating tissue uptake and utilization for protein synthesis of the amino acids removed from the plasma pool. The glucose-induced plasma amino acid depression in ruminants has been suggested to be mediated by insulin.[9] It is apparent that supplementation with rumen-protected amino acids stimulated a decline in plasma EAA less than corn treatment (*Table 4*), indicating the possibility of other limiting amino acids being supplied as a result of formaldehyde treatment of corn. Sparing of amino acids from gluconeogenesis may have occurred when formaldehyde-treated corn was fed.

It is concluded that under the dietary conditions imposed in the present study, the primary response to formaldehyde-treated corn + RPAA was the correction of an amino acid deficit. The net effect was a decrease in plasma amino acid concentration and this effect appeared to result from a change in carbohydrate and endocrine status that enhanced the utilization of amino acids for protein synthesis and spared their use for gluconeogenesis.

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