Z Ernährungswiss 36: 182–190 (1997)

ORIGINAL CONTRIBUTION

K.A. Lien
W.C. Sauer
M. Fenton

Mucin output in ileal digesta of pigs fed a protein-free diet

Die Ausschüttung von Mucin im Ileuminhalt bei Schweinen nach Verfütterung einer proteinfreien Nahrung

Abstract Daily outputs of mucin in ileal digesta were estimated in three barrows fed a protein-free diet while administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously. The water soluble-ethanol precipitable fraction of ileal digesta (crude mucin; CM) was used to estimate the composition of mucin in ileal digesta. This fraction exhibited a carbohydrate composition characteristic of mucin and had a high threonine, serine and proline content (40 mol/100 mol). The proportions of soluble gastric and intestinal mucins, approximately 27 and 73 %, respectively, were estimated from the N-acetylglucosamine (GlcNAc)/N-acetylgalactosamine (GalNAc) ratio in CM. The daily outputs of soluble mucin, 2.75 and 3.41 g/day from SAI and AAI pigs (p = 0.13), respectively, were determined from the GalNAc outputs in CM, assuming the above contributions of gastric and intestinal mucins. The estimated soluble mucin outputs accounted for more than 99 % of the fucose, galactose, GalNAc and GlcNAc in CM. Total mucin outputs in ileal digesta, 5.32 and 5.65 g/day from SAI and AAI Pigs (p = 0.24), respectively, were determined from the total GalNAc output in digesta, assuming soluble and insoluble mucin had similar compositions. Based on these outputs, mucin represented approximately 30, 7 to 22, 15 and 11 % of the endogenous threonine, proline, serine and protein, respectively, in ileal digesta. Approximately 74, 76, 100 and 53 % of the fucose, galactose GalNAc and GlcNAc, respectively, in ileal digesta from pigs in this study was attributed to mucin. The results from this study demonstrate the importance of mucin as a source of some endogenous amino acids and carbohydrates.

Zusammenfassung Bei kastrierten männlichen Schweinen, die eine proteinfreie Nahrung und intravenös entweder mit Kochsalzlösung (SAI) oder ein Aminosäurengemisch erhielten, wurde die tägliche Aminosäurenausschüttung (AAI) im Ileuminhalt bestimmt. Mit der wasserlöslichen und in Ethanol ausfällbaren Fraktion des Ileuminhalts (Gesamtmucin) (CM) wurde die Zusammensetzung von Mucin im Ileuminhalt gemessen. Diese Fraktion wies eine für Mucin charakteristische Kohlegydratzusammensetzung und einen hohen Gehalt an Treonin, Serin und Prolin (40 mol/100 mol). Die Anteile an Mucinen aus dem Magen und Darm betrugen 27 bezw. 73 % und wurden nach dem Verhältnis zwischen N-Acetylgalactosamin (GiNAc):N-Acetylgalactosamin (GalNAc) im CM ermittelt. Die täglichen Ausschüttungen an löslichem Mucin betrugen bei den SAI- bzw. AAI-Schweinen (p = 0,13) und wurden aus den GalNAc-Exkretionen im CM bestimmt, wobei die obengenannten Anteile der Magen- und Darmmucine angenommen wurden. Die bestimmten löslichen Mucinausschüttungen im CM bestanden zu mehr als 90 % aus Fucose, Galactose, GaINAc und GlcNAc. Die Gesamtausschüttungen an Mucin im Ileuminhalt beliefen sich bei den SAI- bzw. AAI-

Received: 18 June 1996
Accepted: 27 November 1996

Presented in part to the Canadian Society of Animal Science at the 7th World Conference on Animal Production, Edmonton, Alberta, July 1993
(Lien, KA, Sauer WC (1993) Isolation of water-soluble crude mucus from ileal digesta of pigs. Proc. VII World Conference, ANIM Prod. Vol. 2, p 70)

Financial support provided by the Natural Sciences and Engineering Research Council.

K.A. Lien · Prof. Dr. W.C. Sauer (✉)
M. Fenton
Department of Agricultural, Food and Nutritional Science
University of Alberta
Edmonton, Alberta, Canada T6G 2P5

Schweinen (p = 0,24) auf 5,32 und 5,56 g/Tag und wurden aus der Gesamtausschüttung aus dem Ileuminhalt ermittelt, wobei davon ausgegangen wurde, daß lösliches und unlösliches Mucin die gleichen Zusammensetzungen aufwies. Basierend auf diesen Ausschüttungen, entsprach Mucin im Ileuminhalt ca. 30,7 bis 22, 15 und 11 % Threonin, Prolin, Serin und Protein. Annähernd 74, 76, 100 bezw. 53 % Fucose, Galactose, GAINAc und GlcNAc des Ileuminhalts entfielen in dieser Studie an Schweinen auf Mucin. Die Untersuchungsergebnisse der Studie unterstreichen die Bedeutung von Mucin als Lieferant von endogenen Aminosäuren und endogenen Kohlehydraten.

Key words Pigs – mucin – endogenous amino acids – endogenous carbohydrates

Schlüsselwörter Schweine – Mucin – endogene Aminosäuren – endogene Kohlenhydrate

Abbreviations *CM* = crude mucin *GalNAc* = N-acetylgalactosamine *GlcNAc* = N-acetylglucosamine *AAI* = amino acid infused · *SAI* = saline infused · *N* = nitrogen *MW* = molecular weight

Introduction

There is a scarcity of information on the recovery of endogenous substances from different sources at the distal ileum of pigs. In this respect, the recovery of mucin is of particular interest since little digestion of mucin occurs prior to the large intestine (10). With proteolysis, mucus gels are reduced first to their component mucins, and further to mucin subunits by digestion of exposed protein regions (2, 19, 24). Although exhaustive proteolysis results in a loss of up to 30 % of mucus protein, this represents only approximately 5 % of the total mucus molecule (20, 33). The remaining protein, comprised predominantly of threonine, serine and proline (70 mol/100 mol of amino acids), is protected from further proteolysis by a coat of oligosaccharides (10).

The prevalence of mucin in ileal digesta has been inferred from the high threonine content of endogenous protein (e.g. 5) and from the low apparent digestibility of this amino acid in many feedstuffs (32). Mucin is also considered a primary source of endogenous carbohydrates in ileal digesta (7, 17, 22). Thus, in addition to providing important information concerning the recovery of endogenous protein and carbohydrates, an estimate of the recovery of mucin could provide insight into the effects of dietary treatments in the digestive tract since mucin provides a protective lining for the gastrointestinal mucosa (2, 19, 24). Allen (2) has suggested that proteolysis, augmented by physical abrasion, is the primary factor governing the presence of mucin in the intestinal lumen.

The purpose of this study was to estimate the daily output of mucin in ileal digesta of pigs fed a protein-free diet and to estimate the contribution of mucin to total endogenous protein and carbohydrates. Digesta samples were collected during a previous study in which pigs were fed a protein-free diet while being administered either saline or a complete amino acid mixture intravenously, therefore, data regarding the effect of the protein status of the pig on the recovery of mucin in ileal digesta are also presented.

Materials and methods

This study was performed using samples collected in a previous experiment (5). Sufficient samples for analyses were available for three barrows, therefore, three observations were obtained for each treatment. Briefly, barrows, with an average initial body weight of 55 kg, were fitted with a simple T-cannula at the distal ileum and a catheter in the external jugular vein. The animals were fed twice daily, at 08:00 and 20:00 hours, 700 g of a protein-free diet while administered either saline or a complete mixture of amino acids intravenously. The experimental diet consisted of 79.7 % cornstarch, 10 % sucrose, 3 % canola oil, 3 % Alphafloc and a vitamin-mineral premix. Digesta were collected continuously for 24 h on the eighth day of the infusion period and immediately frozen prior to freeze-drying and grinding.

Chemical and statistical analysis

Crude mucin (CM) was isolated according to modifications of procedures described by Allen (2) and Miller and Hoskins (21). Approximately 3 g of freeze-dried digesta was weighed into a 50 mL polystyrene test tube and 25mL of 0.15M NaCl containing 0.02M sodium azide, maintained at 4 °C, were added. Samples were homogenized for 1 min using a Polytron Homogenizer (Kinematica, Kriens, Switzerland) and immediately centrifuged for 30 min at 12 000 x g at 4 °C. The aqueous layer was decanted into a second 50 mL polystyrene test tube and once again centrifuged at 12 000 x g for 30 min to ensure the complete removal of insoluble material. Fifteen mL of the aqueous fraction were pipetted into a pre-weighed 50 mL test tube, cooled in an ice-bath, and ice cold ethanol added to a final concentration of 60 % (v/v). The samples were allowed to precipitate overnight at -20 °C.

The following day samples were centrifuged at 1 400 x g for 10 min and the precipitate recovered by decanting the supernatant. The pellet was resolubilized in 15 mL

of 0.15M NaCl and cooled in an ice-bath. Pre-cooled ethanol was added to a final concentration of 60 % (v/v) and the samples were again left overnight to precipitate. The CM precipitate was recovered by centrifugation. Successive rinsing was continued until a clear supernatant was obtained. The final precipitate was resolubilized in 10 mL of water and freeze-dried.

Amino acid analysis, following procedures adapted from Jones and Gilligan (11), were repeated in ileal digesta samples for this study. Samples were prepared as previously described (16) and analyses were performed using a Varian 5000 high performance liquid chromatography system (Varian Instruments Inc., Walnut Creek, CA). Approximately 30 mg of CM or 100 mg of ileal digesta were hydrolyzed for 24 h in 6 mL of 6M HCl prior to amino acid analysis. Proline was estimated as its trimethylsilyl derivative by gas chromatography-mass spectrometry using leucine in the samples as a standard.

Carbohydrates were analyzed as their alditol acetates according to procedures adapted from Blakeney et al. (3) and Kraus et al. (14). Approximately 50 mg of CM or ileal digesta were treated with 12M sulfuric acid (1.5 mL) for 1 h at room temperature. The solution was diluted to 3M with 4.5 mL of water and the samples hydrolyzed for 1 h at 110 °C. Following hydrolysis, 200 µL of internal standard were added (N-methylglucamine and *myo*-inositol, for amino sugars and neutral sugars, respectively, at 10 mg/mL of distilled water). Aliquots (1 mL) of the acid hydrolysates were cooled in an ice-bath and made basic with the addition of 0.7 mL concentrated ammonium hydroxide. To 100 µL of this, 1 mL sodium borohydride (30 mg/mL in anhydrous dimethylsulphoxide) was added and reduction allowed to proceed for 90 min at 40 °C. Excess sodium borohydride was decomposed with the addition of 200 µL concentrated glacial acetic acid. Following this, 0.2 mL 1-methylimidazole and then 2 mL acetic anhydride were added. The solution was mixed and acetylation occured at room temperature for 10 to 15 min. Thereafter, 5 mL of water was added to decompose excess acetic anhydride and the mixture cooled to room temperature. Alditol acetates were extracted into 4 mL of dichloromethane by vigorous shaking and the upper aqueous layer removed. The dichloromethane layer was rinsed twice with 4 mL of water and evaporated to dryness under a stream of nitrogen. Standard sugars and derivitization reagents were obtained from Sigma (Sigma Chemical, St. Louis, MO).

Prior to analysis by gas-liquid chromatography (Varian 3400), the alditol acetates were redissolved in 1 mL of dichloromethane and approximately 0.5 µL of derivitized sample was injected onto a DB-17 fused silica capillary column (0.25 mm i.d. x 30 m; J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a rate of 1.5 mL/min. Injector temperature was programmed from 60° to 270 °C at 150 °C/min and maintained for 20 min. Oven temperature was raised at 30 °C/min from 50° to 190 °C, maintained for 3 min, then increased 5 °C/min to 270 °C and maintained for 5 min. Detector temperature was set at 270 °C. Peak area integration for amino acid and carbohydrate analyses were made using a Shimadzu Ezchrom Data System (Shimadzu Scientific Instruments Inc., Columbia, MD).

To determine treatment effects, data were subjected to statistical analysis using the SAS (31) General Linear Model Procedure. The data were analyzed using a cross-over design, with group (animals that moved together from one period to the next), animals within each group, period and treatment as sources of variation (27).

Calculations

Regression equations were derived from the N-acetylglucosamine (GlcNAc) to N-acetylgalactosamine (GalNAc) ratios in purified gastric (33) and intestinal (18, 20) mucins to calculate the contribution of gastric mucin and the GalNAc content. Two formulas were derived, assuming complete native (no proteolytic digestion) or subunit (proteolytic digested) mucin, to estimate the range of mucin output. The relationship between the GlcNAc/GalNAc ratio and contribution of gastric mucin is described by the following regression equations:

$$\text{Native: } \%\text{gastric} = -80.23 + 183.26x - 71.19x^2 + 11.05x^3 \quad (1a)$$
$$\text{Subunit: } \%\text{gastric} = -82.07 + 188.36x - 74.50x^2 + 11.69x^3 \quad (1b)$$

where x = the GlcNAc/GalNAc ratio. The GalNAc content of mucin mixtures is described by the following regression equations:

$$\text{Native: } \%\text{GalNAc} = 32.30 - 22.74x + 8.83x^2 - 1.37x^3 \quad (2a)$$
$$\text{Subunit: } \%\text{GalNAc} = 34.87 - 25.36x + 10.03x^2 - 1.57x^3 \quad (2b)$$

where x = the GlcNAc/GalNAc ratio. The daily output of mucin was calculated from the estimated GalNAc content and daily outputs of GalNAc in CM or ileal digesta by the following equation:

$$\text{mucin output} = \text{GalNAc}/\%\text{GalNAc} \quad (3)$$

where GalNAc = GalNAc output in g/day. (3)

Results and discussion

The isolation of mucin from ileal digesta is compounded by the presence of undigested dietary materials (4, 17) and the incomplete solubility of mucin (4, 17, 19). It has been convenient, therefore, to estimate mucin outputs using marker carbohydrates, particularly the amino sugars (4, 7, 17, 36). As discussed previously (17), GalNAc is particularly well suited for this purpose because of its limited occurence in dietary and endogenous sources. The usefulness of GlcNAc as a marker for mucin is dimin-

ished by its contributions from other sources, particularly proteoglycans. N-acetylglucosamine and uronic acids are the primary constituents of proteoglycans (30) and, although not determined in this study, a considerable quantity of uronic acids are present in ileal effluent from humans fed polysaccharide-free diets (7).

The reliability of estimates for mucin outputs, using marker carbohydrates, depends on the accuracy with which the composition of mucin in ileal digesta can be determined. In a previous study (17) we derived a composition for mucin in ileal effluent from ileostomates fed increasing amounts of soy fiber from the carbohydrate composition of CM after regression to zero soy fiber intake. Regression to zero soy fiber intake was necessary to remove soluble carbohydrates derived from the diet. The approach taken in this study is to take advantage of the large differences in the carbohydrate compositions of gastric and intestinal mucins, particularly with respect to the GlcNAc/GalNAc ratios, to determine the relative proportions of these mucins in ileal digesta. Gastric mucin contains approximately 30 % GlcNAc and 13 % GalNAc (33, 35), whereas the contributions of these sugars in intestinal mucin is approximately 20 and 40 % (18, 20), respectively. Once the relative proportions of gastric and intestinal mucins are known, mucin outputs can be estimated from the known compositions of these mucins and the GalNAc output. In this respect, the carbohydrate composition of CM is of value since it is unlikely that nonmucin glycoproteins will contribute to the amino sugar content in this fraction of digesta (4).

Table 1 Recovery and composition of crude mucin from ileal digesta of pigs fed a protein-free diet while simultaneously administered either saline (SAI) or a complete amino acid mixture (AAI) intravenously

Items	SAI	AAI	SEM[a]	P[b]
Crude mucin, g/day	5.2	5.6	0.13	0.47
Composition, %				
Protein[c]	34.3	35.1	2.73	0.79
Fucose	3.5	4.8	0.36	0.21
Galactose	8.2	10.3	0.31	0.12
GalNAc[d]	10.7	12.5	0.28	0.14
GlcNAc[d]	8.7	9.7	0.11	0.11
Glucose	1.6	1.2	0.40	0.09
Mannose	1.2	1.0	0.04	0.11
GluNAc/GalNAc	0.82	0.78	0.109	0.83
Protein/Carbohydrate	1.01	0.90	0.085	0.56

[a] Standard error of the mean.
[b] Probability of difference between SAI and AAI pigs (n = 3).
[c] Sum of individual amino acids.
[d] N-Acetylgalactosamine and N-Acetylglucosamine, respectively.

Table 2 Carbohydrate and amino acid compositions of crude mucin from saline (SAI) and amino acid (AAI) infused pigs

Treatment	SAI	AAI	SEM[a]	P[b]
Carbohydrates, mol/100 mol				
Fucose	13.7	15.6	0.56	0.21
Galactose	29.4	30.4	0.25	0.19
GalNAc[c]	31.5	30.5	0.16	0.13
GlcNAc[c]	25.4	23.5	0.97	0.34
Indispensable amino acids, mol/100 mol				
Arginine	2.5	2.4	0.03	0.19
Histidine	1.3	1.3	0.07	0.47
Isoleucine	3.0	2.7	0.07	0.22
Leucine	5.8	5.2	0.30	0.38
Lysine	2.5	2.3	0.14	0.50
Methionine	0.7	0.6	0.07	0.70
Phenylalanine	2.4	2.5	0.16	0.43
Threonine	15.0	16.4	0.33	0.17
Valine	5.9	6.0	0.06	0.35
Dispensable amino acids, mol/100 mol				
Alanine	9.2	9.9	0.63	0.59
Aspartate	7.8	7.0	0.34	0.33
Glutamate	8.7	8.2	0.29	0.42
Glycine	8.9	8.7	0.65	0.79
Proline	12.5	12.4	0.69	0.83
Serine	11.5	12.4	0.74	0.56
Tyrosine	2.6	2.1	0.13	0.23

[a] Standard error of the mean.
[b] Probability of difference between saline and amino acid infused pigs (n = 3).
[c] N-Acetylgalactosamine and N-Acetylglucosamine, respectively.

The daily output and composition of CM in ileal digesta of pigs fed a protein-free diet are presented in Table 1. Daily outputs of CM in ileal digesta were not different (p = 0.47) between amino acid (AAI) and saline infused (SAI) pigs. Carbohydrate plus protein accounted for 70 % of the CM in both treatments. The remaining portion may be comprised of a variety of components including sialic acid which represents approximately 18 % and 2 % of intestinal and gastric mucin, respectively (2, 19, 24). Moisture and ash, in similarily prepared samples from this laboratory, ranged from 11 to 16 % and 11 to 21 %, respectively. The high ash content is most likely due to the presence of salt, since no effort was made to remove it from the final product.

The CM preparations contained predominantly (> 90 %) those sugars characteristic of mucus glycoproteins (mucin carbohydrates), namely fucose, galactose, GalNAc and GlcNAc (Table 1). Mannose and glucose, common contaminants of mucus preparations (18, 34), were also present. These results are consistent with those of a previous study (22) in which mucin carbohydrates represented 84 % of the total carbohydrate in the soluble fraction of ileal digesta from rats fed a fiber-free diet. The high

protein to carbohydrate ratios, 1.0 and 0.9 in SAI and AAI pigs, respectively, compared to 0.2 to 0.4 in purified mucus (18, 33), suggest the presence of coprecipitating contaminant proteins.

The compositions of the protein and carbohydrate fractions of CM are presented in Table 2. Threonine, serine and proline comprised 40 mol/100 mol of the amino acids in both CM preparations. The contributions of these amino acids were lower than in purified mucin, 50 to 70 mol/100 mol (18, 20, 33). However, similar contributions were reported in other studies (8, 26, 34). Although the low content of threonine, serine and proline confirms the presence of some nonmucin protein, their high contributions, relative to the other amino acids, is suggestive of a high content of mucin in the water soluble-ethanol precipitable fraction of ileal digesta from pigs fed a protein-free diet.

There were no differences ($p > 0.10$) in the composition of mucin carbohydrates in CM between AAI and SAI pigs (Table 2). The contents of galactose and GalNAc were highest (approximately 30 % each) followed by GlcNAc (24 %) and fucose (14 %). The carbohydrate composition was intermediate to that reported for purified pig gastric (33) and small intestinal mucins (18, 20), but more closely resembled that of intestinal mucin, especially with respect to the GlcNAc/GalNAc ratios (Table 1). The GlcNAc to GalNAc ratio for pig gastric mucin is 2.4 (33) compared to 0.6 for mucin from the small intestine (18). The GlcNAc/GalNAc ratios in CM from SAI and AAI pigs were 0.82 and 0.78, respectively, suggesting that mucin in ileal digesta of pigs fed a protein-free diet originates primarily in the small intestine. The higher contribution of threonine, relative to serine (Table 2), is also consistent with a higher proportion of intestinal mucin (2, 19, 24).

The daily outputs of carbohydrates and the estimated daily outputs of mucin in CM and in ileal digesta are presented in Table 3. In contrast to CM, in which mucin carbohydrates represent greater than 90 % of the total carbohydrates, fucose, galactose, GalNAc and GlcNAc represented less than 10 % of carbohydrates in ileal digesta. However, glucose and xylose, representing 90 % of total carbohydrates in ileal digesta, were likely derived from the diet since these are relatively minor components of endogenous carbohydrates (7, 13, 22). Traces of arabinose, rhamnose and ribose were also detected in digesta. Although not significant, the daily outputs of mucin carbohydrates in CM were higher in AAI, versus

Table 3 Carbohydrate and mucin outputs in crude mucin and in ileal digesta, and the contribution of carbohydrates from mucin to carbohydrates in crude mucin and in ileal digesta of saline (SAI) and amino acid (AAI) infused pigs

	Crude mucin				Ileal digesta			
	SAI	AAI	SEM[a]	P[b]	SAI	AAI	SEM	P
Carbohydrates, g/day								
Fucose	0.17	0.27	0.022	0.18	0.57	0.58	0.008	0.28
Galactose	0.44	0.60	0.020	0.11	1.50	1.37	0.038	0.25
GalNAc[c]	0.55	0.70	0.021	0.14	1.07	1.16	0.003	0.05
GlcNAc[c]	0.45	0.54	0.009	0.11	1.89	1.55	0.021	0.05
Glucose	0.08	0.07	0.005	0.22	41.24	37.74	1.005	0.26
Mannose	0.06	0.05	0.002	0.10	0.96	0.67	0.005	0.02
Xylose	ND	ND			7.44	7.46	0.384	0.92
Mucin, g/day[d]								
Gastric	0.80	0.85	0.014	0.34	1.51	1.38	0.128	0.39
Intestinal	1.95	2.57	0.096	0.14	3.81	4.27	0.082	0.17
Total	2.75	3.41	0.082	0.13	5.32	5.65	0.047	0.24
Contribution, %[e]								
Fucose	123.4	99.6	8.261	0.23	73.3	74.3	0.915	0.45
Galactose	131.7	117.7	7.465	0.37	71.3	82.0	5.900	0.50
GalNAc	99.4	99.8	0.119	0.21	99.8	100.0	0.220	0.54
GlcNAc	99.5	99.8	0.067	0.19	47.2	58.2	0.334	0.03

[a] Standard error of the mean.
[b] Probability of difference between saline and amino acid infused pigs (n = 3).
[c] N-Acetylgalactosamine and N-Acetylglucosamine, respectively.
[d] Mucin outputs in crude mucin and in ileal digesta estimated from the GlcNAc/GalNAc ratio in crude mucin and the outputs of GalNAc in crude mucin and ileal digesta, respectively (see calculations in text).
[e] Output of carbohydrates from mucin, for the calculation of the contributions of carbohydrates from mucin to carbohydrates in crude mucin and ileal digesta, calculated from the estimated mucin output and the carbohydrate composition of purified mucin presented previously (18, 20, 33).

Table 4 Ileal output of amino acids in crude mucin in saline (SAI) and amino acid (AAI) infused pigs

	SAI	AAI	SEM[a]	P[b]	%Cont[c]
Indispensable amino acids, mmol/day					
Arginine	0.36	0.38	0.024	0.65	0.81
Histidine	0.17	0.22	0.012	0.18	2.02
Isoleucine	0.42	0.45	0.017	0.58	1.21
Leucine	0.82	0.87	0.055	0.95	2.02
Lysine	0.35	0.40	0.012	0.31	2.02
Methionine	0.09	0.10	0.010	0.70	0.40
Phenylalanine	0.31	0.42	0.032	0.23	4.44
Threonine	2.12	2.74	0.011	0.02	25.00
Valine	0.82	0.99	0.033	0.18	6.85
Dispensable amino acids, mmol/day					
Alanine	1.36	1.68	0.012	0.04	12.90
Aspartate	1.11	1.18	0.067	0.97	2.82
Glutamate	1.23	1.37	0.029	0.23	5.65
Glycine	1.28	1.43	0.036	0.30	6.05
Proline	1.76	2.05	0.142	0.48	11.69
Serine	1.68	2.09	0.022	0.05	16.53
Tyrosine	0.36	0.35	0.023	0.45	-0.40

[a]Standard error of the mean.
[b]Probability of difference between saline and amino acid infused pigs (n = 3).
[c]Contribution of individual amino acids to the total increase in amino acids in crude mucin between SAI and AAI pigs.

SAI, pigs resulting in estimated intestinal and total mucin outputs which were 32 % (2.6 versus 2.0 g/day) and 24 % (3.4 versus 2.8 g/day, respectively) higher, respectively, with amino acid infusion. Daily carbohydrate outputs in digesta were usually lower for AAI pigs, although this was only significant for mannose and GlcNAc. N-Acetylgalactosamine output in digesta were higher ($p = 0.05$) with amino acid infusion, however, the estimated daily outputs of mucin were not different, 5.7 g/day versus 5.3 g/day ($p = 0.24$), between SAI and AAI pigs, respectively. In both CM and digesta, estimates for the output of intestinal mucin were more than twice that of gastric mucin. The higher estimates for mucin output in ileal digesta, compared to CM, reflects the contribution of insoluble mucin. The solubility of mucus gels is increased as they are proteolytically degraded to their component mucin subunits (2, 19). In addition, the solubility of mucin may be influenced by pH and the presence of bile (19).

The contributions of fucose, galactose, GalNAc and GlcNAc from mucin to total fucose, galactose, GalNAc and GlcNAc, respectively, in CM and ileal digesta (Table 3) were calculated from the estimated mucin outputs and the composition of purified mucins presented previously (18, 20, 33). The estimated contributions of mucin carbohydrates to total carbohydrates in CM exceeded 99 %, suggesting that essentially all of the carbohydrates in the water soluble-ethanol precipitable fraction of digesta (CM) from the pigs in this study could be derived from mucin. Clamp and Gough (4) have suggested that non-mucin glycoproteins, which could contribute to the amino sugar content of CM, are not important constituents of the soluble fraction of human ileostomy effluent. These results demonstrate that, not only does the GlcNAc/GalNAc ratio in CM provide a reasonable estimate of the relative contributions of soluble gastric and intestinal mucins, it can also be used, in conjunction with previously described mucin compositions, to derive an estimate of soluble mucin output.

Total mucin output was estimated from the GlcNAc/GalNAc ratio in CM and the GalNAc content of ileal digesta, assuming all GalNAc in digesta was associated with mucin. The estimated mucin output accounted for more than 70 % of the fucose and galactose, but only 50 % of the GlcNAc (Table 3). The low contribution of GlcNAc may indicate the presence of proteoglycans. Uronic acids and GlcNAc are the primary constituents of proteoglycans (30) and, although not determined in this study, a considerable quantity of uronic acids are present in ileal effluent from humans fed polysaccharide-free diets (7). The results presented in Table 3 are consistent with several previous studies which point to mucin as a primary source of endogenous carbohydrates in ileal digesta. A high content of mucin sugars was observed in secretions from canine Heidenhain pouches (13). Fucose and galactose represent 65 % of the endogenous neutral carbohydrates in ileal effluent from humans fed polysaccharide-free diets (7). More recently, Monsma et al. (22) reported that fucose, galactose and the amino sugars represented 77 % of the total endogenous carbohydrates in ileal digesta from colectomized rats.

Although no significant differences were observed in mucin outputs between SAI and AAI pigs, the proportionately higher amount of soluble mucin (in CM), compared to total mucin (in digesta), in AAI pigs presents an interesting tendency that is worth noting. These data, presented in Table 3, imply an increase in the solubility of mucin following amino acid infusion, possibly resulting from an enhanced proteolytic activity. Studies *in vitro* (e.g. 20, 33) and *in vivo* (e.g. 23, 25) demonstrate that mucus is proteolytically degraded to mucin subunits, increasing the solubility of mucus gels (2, 19). Solubilization of mucus is directly correlated with an increase in proteolytic activity (15, 23). Kowalewski et al. (13) reported a 44 % increase in the amount of mucin carbohydrates, in conjunction with a threefold increase in the amount of pepsin, in canine Heidenhain pouches following the consumption of food. Daily amino acid outputs in CM (Table 4) provide some support for this. Similar to carbohydrates in CM, the daily output of amino acids increased by an average of 15.5 % with amino acid infusion. Significant increases were observed for threonine, serine and alanine. More importantly, threonine, serine and proline contributed more than 50 mol/100 mol

Table 5 The contribution of amino acids from mucin to amino acids in ileal digesta from saline (SAI) and amino acid (AAI) infused pigs[a]

	Subunit[b]				Native[c]			
	SAI	AAI	SEM[d]	P[e]	SAI	AAI	SEM	P
Protein, %[f]	5.6	7.8	0.49	0.19	7.8	11.0	0.69	0.19
Indispensable amino acids, %								
Arginine	2.4	3.5	0.20	0.18	6.9	9.7	0.50	0.17
Histidine	3.9	5.0	0.14	0.11	9.0	11.6	0.36	0.12
Isoleucine	3.9	4.7	0.08	0.07	7.3	8.9	0.16	0.08
Leucine	2.0	2.4	0.05	0.11	5.8	6.9	0.22	0.16
Lysine	1.8	2.1	0.07	0.17	5.6	6.7	0.26	0.18
Phenylalanine	2.4	2.5	0.12	0.57	5.3	5.5	0.25	0.57
Threonine	28.0	33.2	1.31	0.22	29.0	34.4	1.43	0.22
Valine	6.5	7.1	0.12	0.17	10.4	11.3	0.17	0.16
Dispensable amino acids, %								
Alanine	1.6	1.9	0.03	0.09	3.7	4.6	0.14	0.13
Aspartate	1.1	1.4	0.05	0.13	4.2	5.5	0.22	0.14
Glutamate	1.9	2.4	0.03	0.06	4.2	5.2	0.07	0.06
Glycine	1.9	2.8	0.17	0.17	3.0	4.4	0.26	0.17
Proline	7.2	22.4	0.09	0.01	7.7	23.8	0.10	0.01
Serine	13.3	16.3	0.23	0.07	13.2	16.3	0.30	0.09
Tyrosine	3.1	3.8	0.01	0.01	6.8	8.2	0.01	0.01

[a] Output of amino acids from mucin, for the calculation of the contributions of amino acids and protein from mucin to amino acids and protein, respectively, in crude mucin and ileal digesta, calculated from the estimated mucin output and the amino acid composition of purified mucin presented previously (18, 20, 33).
[b] Calculated assuming mucin is completely in its subunit form (complete proteolytic degradation of the naked region).
[c] Calculated assuming mucin is completely in its native form (no proteolytic digestion).
[d] Standard error of the mean.
[e] Probability of difference between saline and amino acid infused pigs (n = 3).
[f] Sum of amino acids.

of the total increase in the amino acid output, indicating that much of the increase in amino acid output could be attributed to an increase in the mucin content of CM.

Several previous studies provide evidence that a large quantity of dietary starch, in conjunction with amino acid infusion, could lead to an enhanced proteolytic activity in the intestinal lumen. Mucosal mass and nutrient absorption were greater in parenterally fed rats with intralumenal infusion of 30 % glucose compared to 10 % glucose or saline (29). Mucosal enzyme activities, reduced by starvation, are rapidly restored following the consumption of diets containing free amino acids (28), however, in the absence of lumenal amino acids, the mucosa utilizes plasma amino acids for the synthesis of at least some of these enzymes (6). Thus providing amino acids parenterally might not only enhance mucosal enzyme activity, but might also prevent the decline in pancreatic protease activity observed in pigs fed protein-free diets (9). In this respect, it is of interest to note that the ileal outputs of GlcNAc and mannose decline with amino acid infusion (Table 3) because their prevalence in proteoglycans (30) and membrane glycoproteins (12), respectively, could be interpreted to indicate an improvement in mucosal integrity. This aspect of the effect of amino acid infusion in pigs fed protein-free diets requires further investigation, particularly since the feeding of protein-free diets is a standard method for determining endogenous protein and amino acid outputs.

The contribution of amino acids from mucin to total amino acids in ileal digesta (Table 5) was calculated from the estimated mucin outputs (Table 3) and the composition of purified mucin presented previously (20, 21, 34). Data are presented assuming either complete proteolytic digestion of the naked region (Subunit) or no digestion of the naked region (Native) to provide the range of mucin amino acid contributions. It is necessary to consider this range because the extent of proteolytic digestion of mucin in ileal digesta is unknown. Endogenous amino acid outputs determined in this study were similar to those presented previously (5) and are not included here. Although mucin represented a relatively small proportion (5 to 11 %) of the total endogenous amino acids in ileal digesta, the contributions of threonine (28 to 35 %), serine (13 to 16 %) and proline (7 to 24 %) were higher. Contributions of these amino acids were similar whether it was assumed that mucin was either completely in its

native or subunit forms because these amino acids are located predominantly in the glycosylated region, protected from proteolysis (20, 33). Protein in the naked region (approximately 35 % of the total protein) has a composition similar to that of an average protein (2, 19, 24), thus the contributions of the remaining amino acids were approximately twice as high when it was assumed that mucin was completely in its native, rather than its subunit, form.

The data presented in this study provides information concerning the output of mucin in ileal digesta of pigs fed a protein-free diet and its contribution to endogenous protein and carbohydrates. An estimation of the mucin content of ileal digesta has important consequences for nutritional studies since the separation of endogenous from undigested dietary protein is a major impedement in the assessment of the effects of dietary treatments on the digestion of protein and the true digestibility of dietary ingredients. In this respect, this study has presented important information on the contribution of at least one source of endogenous protein, to total endogenous protein recovered at the distal ileum. In addition, this study has also presented information concerning the effect of feeding protein-free diets.

References

1. AOAC (1988) Official methods of analyses (14th Ed). Association of Official Analytical Chemists. Washington DC
2. Allen A (1981) Structure and function of gastrointestinal mucus. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 617–639
3. Blakeney AB, Harris PJ, Henry RJ, Stone BA (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. Carbohydrate Res 113:291–299
4. Clamp JR, Gough M (1991) The glycoconjugate content of human faeces and ileostomy effluents. Clinica Chimica Acta 203:243–248
5. de Lange CFM, Sauer WC, Souffrant WB (1989) The effect of protein status of the pig on the recovery and amino acid composition of endogenous protein in digesta collected from the distal ileum. J Anim Sci 67:755–762
6. Dudley MA, Nichols BL, Rosenberger J, Perkinson JS, Reeds PJ (1992) Feeding status affects *in vivo* prosucrase-isomaltase processing in rat jejunum. J Nutr 122:528–534
7. Englyst HN, Cummings JH (1986) Digestion of the carbohydrates of banana (*Musa paradisiaca sapientum*) in the human small intestine. Am J Clin Nutr 44:42–50
8. Forstner J, Roomi N, Fahim R, Gall G, Perdue M, Forstner G (1984) Acute and chronic models for hypersecretion of intestinal mucin. In: Mucus and Mucosa (Ciba Found. Symp. 109). Pitman, London, pp 61–71
9. Hee J, Sauer WC, Mosenthin R (1988) The measurement of pancreatic secretions in the pig with the pouch technique. J Anim Physiol Anim Nutr 60:241–252
10. Hoskins LC (1984) Mucin degradation by enteric bacteria: ecological aspects and implications for bacterial attachment to gut mucosa. In: Boedeker EC (ed) Attachment of organisms to the gut mucosa. Volume II. CRC Press, Boca Raton, FL, pp 51–67
11. Jones BN, Gilligan JP (1983) O-phthaldialdehyde precolumn derivitization and reverse-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J Chromatogr 266:471–492
12. Kim YS, Morita A, Miura S, Siddiqui B (1984) Structure of glycoconjugates of intestinal mucosal membranes: role in bacterial adherence. In: Boedeker EC (ed) Attachment of organisms to the gut mucosa. Volume II. CRC Press, Boca Raton, FL, pp 99–109
13. Kowalewski K, Pachkowski T, Secord DC (1976) Mucinous secretion from canine Heidenhain pouch after stimulation with food, pentagastrin and histamine. Eur Surg Res 8:536–544
14. Kraus RJ, Shinnick FL, Marlett JA (1990) Simultaneous determination of neutral and amino sugars in biological materials. J Chromatogr 513:71–81
15. Laszewicz W, Slomiany A, Murty VLN, Liau YL, Slomiany BL (1985) Effect of ethanol on the peptic degradation of gastric mucus glycoproteins. Digestion 31:47–53
16. Li S, Sauer WC, Hardin RT (1994) Effect of dietary fiber level on amino acid digestibility in young pigs. Can J Anim Sci 74:327–334
17. Lien KA, McBurney MI, Beyde BI, Thomson ABR, Sauer WC (1996) Ileal recovery of nutrients and mucin in humans fed total enteral formulas supplemented with soy fiber. Am J Clin Nutr 63:584–595
18. Mantle M, Allen A (1981) Isolation and characterization of the native glycoprotein from pig small-intestinal mucus. Biochem J 195:267–275
19. Mantle M, Allen A (1989) Gastrointestinal mucus. In: Davison JS (ed) Gastrointestinal secretion. Butterworth and Co Ltd, London, pp 202–229
20. Mantle M, Mantle D, Allen A (1981) Polymeric structure of pig small intestinal mucus glycoprotein. Biochem J 195:277–285
21. Miller RS, Hoskins LC (1981) Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a most "probable number" method. Gastroenterology 81:759–765
22. Monsma DJ, Vollendorf NW, Marlett JA (1992) Determination of fermentable carbohydrates from the upper gastrointestinal tract by using colectomized rats. Appl Environ Microbiol 58:3330–3336
23. Munster DJ, Bagshaw PF, Wilson JG (1987) Peptic erosion of gastric mucus in the rat. Comp Biochem Physiol 87A:509–513
24. Neutra MR, Forstner JF (1987) Gastrointestinal mucus: synthesis, secretion and function. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 975–1009
25. Ofosu F, Forstner JF, Forstner GG (1978) Mucin degradation in the intestine. Biochim Biophys Acta 543: 476–483
26. Ohara S, Kakei M, Ishihara K, Katsuyama T, Hotta K (1984) Effects of fasting on mucous glycoprotein in rat stomach. Comp Biochem Physiol 79: 325–329
27. Peterson RG (1985) Design and analysis of experiments. Marcel Dekker Inc, New York
28. Poullain MG, Cezard JP, Marche C, Roger L, Mendy F, Broyart JP (1989) Dietary whey proteins and their peptides or amino acids: effects on the jejunal mucosa of starved rats. Am J Clin Nutr 49:71–79
29. Richter GC, Levine GM, Shiau Y (1983) Effects of luminal glucose versus nonutritive infusates on jejunal mass and absorption in the rat. Gastroenterology 85:1105–1112
30. Roden L, Horowitz MI (1978) Structure and biosynthesis of connective tissue proteoglycans. In: Horowitz MI,

Pigman W (eds) The glycoconjugates. Volume II. Academic Press, New York, NY, pp 3–71
31. SAS Institute Inc (1989) SAS/STAT User's Guide. SAS Institute, Cary, NC
32. Sauer WC, Ozimek L (1986) Digestibility of amino acids in swine: results and their practical applications. A review. Livest Prod Sci 15:367–388
33. Scawen M, Allen A (1977) The action of proteolytic enzymes on the glycoprotein from pig gastric mucus. Biochem J 163:363–368
34. Snary D, Allen A (1971) Studies on gastric mucoproteins. The isolation and characterization of the mucoprotein of the water-soluble mucus from pig cardiac gastric mucosa. Biochem J 123:845–853
35. Stanley RA, Lee SP, Roberton AM (1983) Heterogeneity in gastrointestinal mucins. Biochim Biophys Acta 760:262–271
36. Stephen AM, Haddad AC, Phillips SF (1983) Passage of carbohydrate into the colon: direct measurements in humans. Gastroenterology 85:589–595