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Endogenous Components of Digesta Protein from the Terminal Ileum of Pigs Fed a Casein-Based Diet

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To gain a clearer understanding of the nature and composition of endogenous nitrogen containing substances lost from the upper mammalian digestive tract, digesta were collected from the terminal ileum of six growing pigs that had been fed a casein-based diet with titanium dioxide as an indigestible marker. Total nitrogen lost at the terminal ileum was in excess of 63 $mg \cdot g^{-1}$ digesta dry matter. Of this, nearly 73% was proteinaceous, with nearly 45% being bacterial protein, 13% from soluble free protein, and 11% from mucin. Of the nonprotein nitrogen, 11% was as ammonia and 5% as urea. Bacterial and porcine cellular DNA nitrogen were collectively 0.2% of the total nitrogen. Only 8.3% of the total nitrogen remained unidentified and was assumed to include free amino acids, RNAs, amines, and the tetrapyrroles bilirubin and biliverdin. Although mucin contributed just 10.4% of the nitrogen losses, it was the single most abundant truly endogenous component, comprising 13% of the total dry matter. Bacterial nitrogen, combined with ammonia and urea nitrogen, represented nearly 61% of the total nitrogenous losses: this suggests substantial microbial activity in the stomach and small intestine of the pig. Centrifugal separation of a bacterial fraction from the digesta produced a microbial amino acid profile that, when subtracted from the overall amino acid content, provided an amino acid profile more representative of true endogenous amino acid losses.

KEYWORDS: Pig; digesta; ileal; casein; composition

INTRODUCTION

Although the principal functions of the gastrointestinal tract are the chemical breakdown of exogenous dietary macromolecules and the absorption of the resultant smaller and simpler products, there are significant amounts of material secreted into the gut. Salivary, gastric, hepatic, pancreatic, and intestinal secretory cells all secrete endogenous proteins into the lumen of the gut (*1*) that are vital for digestion and absorption (*2*). It has been estimated that secreted materials originating from the pancreas might contribute 3−5 g of nitrogen per day (*3*), from the gall bladder 2 g of nitrogen per day (*4*), and from salivary and gastric secretions 0.3−0.6 g of nitrogen per day (*5*), together with sloughed epithelial cells and secretions from the duodenal mucosa amounting to 3−5 g of nitrogen per day (*6*). Although not strictly endogenous, bacterial protein is commonly included in estimations of endogenous materials (*7*) and may be the largest single contributor to the nitrogen of terminal ileal digesta (*8*). The mass of the endogenous proteinaceous secretions has been estimated to be equal to that of ingested protein (*9*). The protein content of the digesta is thus a dynamic equilibrium between dietary intake and the secretion of endogenous material into the lumen on one hand and the concomitant absorption from the gut of digested materials, both exogenous and endogenous in origin, on the other.

Residual amounts of unabsorbed proteinaceous materials, both exogenous and endogenous, arriving at the terminal ileum are thought to have no further nutritional value (*10*) because, although limited uptake of amino acids may occur in the large intestine, such trivial amounts are of little nutritional benefit to the host (*11*). However, the result of microbial fermentation in the hindgut is that the composition of nitrogenous materials excreted in the feces bears little relation to that in the ileal effluent: in fact, some 80% of fecal nitrogen originates from microbial activity (*12*). Because of this, the composition of gut endogenous nitrogen losses cannot be determined from fecal analysis. The nitrogenous materials leaving the ileum thus represent the net balance between secretion and reabsorption.

Classically, losses of endogenous nitrogen and amino acids in the ileal effluent have been determined by giving a protein-free diet, when any proteinaceous material in the digesta is assumed to be from endogenous sources (*13*). However, such a diet is physiologically unnatural (*13*), as it decreases the rate of both protein synthesis by the gut tissues (*14*) and of protein secretion into the gut, leading to the underestimation of endogenous nitrogen losses (*15*). For this reason, several alternative methods have been developed to measure ileal endogenous nitrogen and amino acid losses (*11*).

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Table 1. Composition of the Experimental Diet (g/kg Air Dry Weight)

ingredient	experimental diet[a]
maltodextrin	453
sucrose	161
soybean oil	154
sodium hydrogen carbonate	18
titanium dioxide	3
lactic casein	211

[a] No vitamins, minerals, or fiber were added to this diet as the same diet was used in an acute feeding study with human subjects. A preliminary casein based diet contained a vitamin−mineral premix and cellulose.

An accurate estimation of endogenous total nitrogen and amino acid flows is necessary to allow the determination of true dietary amino acid digestibility coefficients and for the factorial estimation of dietary amino acid requirements (*7*). Although the literature is replete with studies that quantify the amino acid composition of ileal digesta (reviewed in ref *1*, *11* and *16*), there are, to the authors' knowledge, no systematic studies regarding the complete composition of ileal digesta.

The aim of this study was to quantify the endogenous components of terminal ileal digesta from pigs given a diet with a purified animal protein, lactic casein, known to be highly digested and absorbed. Although small amounts of dietary peptides and amino acids may remain unabsorbed when casein is fed to an animal, these were assumed to have little bearing on the results overall.

MATERIALS AND METHODS

Animals and Diets. Digesta samples were collected from the terminal ileum of six Large White × Duroc pigs of mean (±SEM) body weight 79 ± 4.8 kg. The pigs were kept singly in steel metabolism crates, in a room maintained at 24 ± 1 °C, at the Animal Physiology Unit, Massey University, Palmerston North, New Zealand. Approval for the study was granted by the Massey University Animal Ethics Committee (protocol 05/29). The pigs arrived in the unit one week prior to surgery. During surgery, each pig was fitted with a postvalvular T cecum (PVTC) cannula, as described by van Leeuwen et al. (*17*). The study commenced some eight weeks after surgery. Following surgery, food was progressively reintroduced within one week up to a level of 0.08 metabolic body weight ($W^{0.75}$)/d, and this level of food intake was maintained for the remainder of the trial. The pigs were fed a nutritionally balanced casein-based diet, mixed with water (1:1, w/w), 3 times daily (0800, 1200, and 1600 h), in equal portions for the remainder of the trial. Water was available ad libitum. On the day of digesta collection, the pigs were fed one-third of the daily ration of the test diet at 0800 h. The composition of the test diet, which included titanium dioxide as an indigestible marker, is presented in **Table 1**. The pigs did not receive any food during digesta collection. In the evening, following the 10 h digesta collection, they received the remaining two-thirds of their daily ration and water.

Digesta were collected into polythene bags. After weighing, sodium benzoate, (10 g·kg^{-1} of digesta), as a bactericide, and phenylmethylsulfonyl fluoride (0.37 g·kg^{-1} of digesta), as a protease inhibitor, were added according to the protocol of Salgado et al. (*18*). The digesta samples were then pooled and frozen at −20 °C until chemical analysis.

Chemical Analyses. Dry matter was measured by drying to a constant mass in a forced air oven at 95 °C. Other samples of the digesta were fractionated by differential centrifugation using the method of Metges et al. (*19*). Briefly, the pooled digesta were centrifuged first at 250 RCF for 15 min at 4 °C, giving a fraction expected to contain food particles and porcine cells, then at 14500 RCF for 30 min at 4 °C, to give a precipitate expected to contain microbial cells, and a supernatant expected to contain mainly proteins, peptides, free amino acids, mucins, neutral sugars, urea, creatinine, and ammonia. A summary of the centrifugation protocol is given in **Figure 1**.

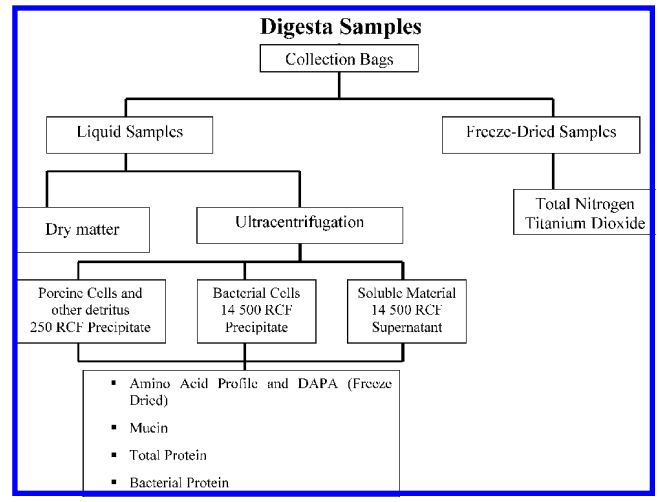

Figure 1. Schematic diagram of the processing of the digesta samples.

Total nitrogen was determined by the Leco total combustion method (AOAC 968.06 (*20*)), a variation of the Dumas method. Amino acid compositions were determined using the procedure outlined by Hodgkinson and Moughan (*15*). No corrections were made for potential losses of amino acids during hydrolysis. Methionine and cysteine were measured as methionine sulfone and cysteic acid, respectively, after the hydrolysis of samples that had been oxidized using performic acid. Diaminopimelic acid (DAPA) was quantified, following oxidation with performic acid, using an HPLC system with a UV detector. Tryptophan was not measured. Titanium dioxide was determined by the method of Short et al. (*21*), and soluble protein was determined by the Bradford method (*22*).

Bacterial protein in the 14500 RCF precipitate was determined using a bacterial protein extraction lysis buffer (PELB) kit combined with a Noninterfering Protein Assay Kit, both obtained from G-Biosciences, St Louis, MO. Following the manufacturer's instructions, total protein estimation in the 14500 RCF precipitate, mainly comprising bacterial matter, was achieved by first using the bacterial PELB kit to lyse the bacterial cells, before using the Noninterfering Protein Assay Kit. The extracellular protein concentration was then determined for the same quantity of the 14500 RCF precipitate without the initial lysis step. The difference between the resultant concentrations was considered to be the bacterial protein concentration.

Mucins were quantified by the determination of the amino sugars *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc), carbohydrate markers peculiar to glycoproteins. The method used was similar to that described by Lien et al. (*23*). Gas−liquid chromatography was undertaken using a Shimadzu 2010 chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD), with a DB-17 fused silica capillary column (J & W Scientific, Folsom, CA; 0.25 mm internal diameter × 30 m) with helium (1.5 mL/min) as the carrier. A Shimadzu GC solutions V2-30su6 data system (Shimadzu Scientific Instruments Inc., Columbia, MD) was used for peak area integration.

Mucin output was estimated using regression equations devised by Lien et al. (*23*). The regression equations for native mucin, assuming no digestion, were as follows where GalNAc is equal to GalNAc output in g·day^{-1}.

For native mucin:

$$\%\ \mathrm{GalNAc} = 32.30 - 22.74x + 8.83x^2 - 1.37x^3$$

where x = GlcNAc/GalNAc ratio.

Thus,

$$\mathrm{mucin\ \ output} = \mathrm{GalNAc}/\%\ \mathrm{GalNAc} \times 0.01$$

Ammonia and urea were determined using an adaptation of the method devised by Chaney and Marbach (*24*). DNA was determined using a QIAamp DNA Stool Mini Kit obtained from QIAGEN Inc. Valencia, CA. The kit was used following the manufacturer's instructions for the isolation of DNA from stools for pathogen detection. The

Table 2. Nitrogen content of Terminal Ileal Digesta for Pigs Given a Casein-Based Diet

	g·kg^{-1} DMI[a]		mg·g^{-1} DDM[b]		
	mean	SEM[c]	mean	SEM	percentage
total nitrogen	3.9	0.45	63.2	1.48	100
protein nitrogen	2.8	0.30	46.4	1.83	73.0
nonprotein nitrogen	1.1	0.04	16.8	0.98	18.2
soluble protein nitrogen	1.7	0.21	27.8	1.25	60.0[d]
insoluble protein nitrogen	1.1	0.18	18.6	2.03	39.9[d]

[a] DMI = dry matter intake. [b] DDM = digesta dry matter. [c] SEM = standard error of the mean. [d] Percentage values of total protein nitrogen.

absorbance of the extracted samples was determined using a NanoDrop ND-1000 UV−vis Spectrophotometer (NanoDrop Technologies Wilmington, DE 19810). The concentration of DNA was determined from its absorbance at A_{260} nm and its purity, with respect to contaminants that absorb UV, such as protein, from the ratio of the readings at A_{260} nm and A_{280} nm.

RESULTS AND DISCUSSION

In this study, the experimental diet contained the protein, casein, which is almost completely digested and absorbed (*25*, *26*); indeed because of its high digestibility, casein is a preferred protein source to determine basal ileal endogenous losses (*26*, *27*). It is expected that almost all of the soluble protein found in the ileal effluent would have originated from endogenous or bacterial sources. The study allowed the direct determination of endogenous proteinaceous components in the digesta of protein fed animals.

A description of the nitrogenous components of the digesta is given in **Table 2**. Total nitrogen made up 6.3% of digesta dry matter (DDM). Of the total nitrogen in the digesta, 73% was protein nitrogen and 18% nonprotein nitrogen. Some 60% of the protein nitrogen was soluble and present in the 14500 RCF supernatant, with the remaining 40% being insoluble and found in the two centrifugation precipitates.

The differential centrifugation method used here separated the digesta into three fractions. Microscopic examination of the 250 RCF precipitate and the 14500 RCF supernatant confirmed that although there were bacterial cells in these fractions, their numbers were proportionally insignificant compared to the precipitate from the 14500 RCF centrifugation stage.

The amino acid composition of the digesta is presented in **Table 3**, and the distribution of amino acids in the three centrifugation fractions is shown graphically in **Figure 2**. The six most abundant amino acids detected in the digesta, in decreasing order of abundance (mg·g^{-1} DDM), were glutamic acid, aspartic acid, proline, threonine, serine, and glycine. These six amino acids are present in the highest proportions in the protein core of glycoproteins from the gastrointestinal tract. Of these six, threonine, serine, and proline are most abundant in the glycosylated region of mucin polymers, a region that holds almost 90% of these three amino acids (*28*). The other three amino acids, glutamic acid, glycine, and aspartic acid, are predominant in the nonglycosylated regions of mucin. The high proportions (nearly 52% of the total by mass of amino acids) of these six mucin-associated amino acids, is consistent with a high rate of secretion of mucins into the gastrointestinal tract and a relatively poor digestion and absorption of their amino acids from the small intestine. These mucin- associated amino acids predominate in the 14500 RCF supernatant (**Figure 2**), from which it may be inferred that a large proportion of soluble mucin is present in this fraction.

Table 3. Amino Acid Composition of Terminal Ileal Digesta[a]

	mol/100 mol	SEM[b]	mg·g^{-1} DDM[c]	SEM[b]
Indispensable Amino Acids				
arginine	2.5	0.19	11.4	1.09
cysteine	2.0	0.28	5.9	0.69
histidine	3.0	0.21	11.8	0.98
isoleucine	3.8	0.30	12.7	0.96
leucine	5.8	0.42	19.7	1.58
lysine	3.5	0.24	13.3	1.29
methionine	1.1	0.06	4.4	0.37
phenylalanine	2.2	0.23	11.4	1.16
threonine[d]	8.8	1.00	25.8	2.13
valine	6.7	0.50	20.0	1.47
Dispensable Amino Acids				
alanine	7.7	0.60	17.9	1.67
aspartic acid	9.5	0.68	33.3	2.91
glutamic acid	15.1	1.06	59.4	6.67
glycine	9.1	0.86	16.9	0.97
proline[d]	8.9	0.64	26.4	2.21
serine[d]	7.8	0.64	20.7	1.36
tyrosine	2.1	0.13	10.0	0.87

[a] Tryptophan was not determined. [b] SEM = standard error of the mean. [c] DDM = digesta dry matter. [d] Predominant amino acids in mucin.

Diaminopimelic acid (DAPA) is a component of peptidoglycans found in bacterial cell walls. Although traces of DAPA may be found in protozoa, it is almost unique to bacteria and consequently has been used as a marker of bacterial protein (*29*). The accuracy of this approach, however, has been called into question by a number of researchers because the concentration of DAPA varies with bacterial size and species (*30*). Although alternative markers have been proposed (*31*), the degree of error associated with bacterial nitrogen estimations using DAPA is not known (*8*). While DAPA was judged by Robinson et al. (*32*) to underestimate the microbial nitrogen pool, Csapo et al. (*31*) suggested that it overestimated it by some 10% in comparison with D-aspartic acid and D-glutamic acid, used as alternative bacterial protein markers (*31*). Although there may be limitations in the use of DAPA, it was adopted in this study as a useful comparative method in line with the work of other researchers.

The concentrations of DAPA and DAPA nitrogen were estimated to be 0.62 and 0.09 mg·g^{-1} digesta dry matter, respectively, values that are in accord with Rowan et al. (*33*). Using the ratio of 2.88 mg of DAPA per gram of bacterial dry matter (*34*), it was calculated that the mass of bacterial dry matter was 216 mg·g^{-1}, a value also in accord with Rowan et al. (*33*), and represents 21% of the total dry matter of the digesta.

As bacterial amino acids are not of direct dietary origin, it is pertinent to determine the amino acid composition of endogenous ileal digesta adjusted for the bacterial component. The amino acid profile of the 14500 RCF precipitate, the bacterial pellet (**Table 4**), can be considered, with some certainty, to be derived solely from bacterial protein. The flows of amino acids related to this fraction of the digesta were thus subtracted from the total endogenous flow to give an estimate (**Table 4**) of the true endogenous ileal amino acid flows. However, it should be noted that there will be quantities of soluble bacterial protein present in the 14500 RCF supernatant that have originated from lysed bacterial cells: these are not accounted for in this correction.

The lysine derived from the bacterial pellet, present in the 14500 RCF precipitate (see **Table 4**), represented nearly 25% of the total amount of lysine present in the ileal digesta. There is a marked difference between the lysine concentrations of microbial protein and of porcine intestinal tissue. The concentra-

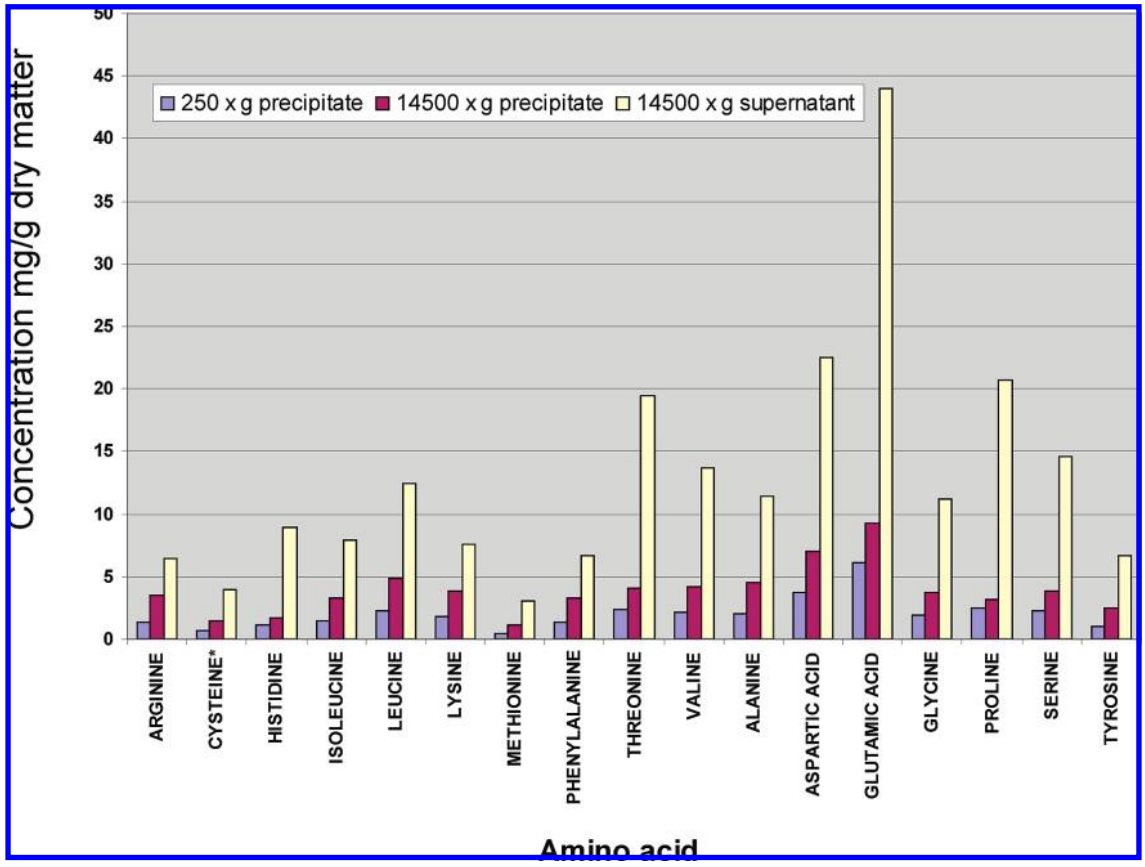

Figure 2. Amino acid concentrations for the different centrifugation fractions of ileal digesta.

Table 4. Amino Acid Composition of Total and Endogenous (Corrected for Bacterial Amino Acids) Ileal Digesta Flows[a]

	total amino acids	bacterial amino acids[c]	endogenous amino acids[d]
	mg·g^{-1} DDM[b]	mg·g^{-1} DDM	mg·g^{-1} DDM
Indispensable Amino Acids			
arginine	11.4	3.1	8.2
cysteine	5.9	1.4	4.6
histidine	11.8	1.7	10.7
isoleucine	12.7	3.0	9.8
leucine	19.7	4.4	15.3
lysine	13.3	3.3	10.0
methionine	4.4	1.1	3.3
phenylalanine	11.4	2.9	8.5
threonine[e]	25.8	4.0	21.8
valine	20.0	4.0	16.0
Dispensable Amino Acids			
alanine	17.9	4.0	13.9
aspartic acid	33.3	7.0	26.3
glutamic acid	59.4	10.9	48.5
glycine	16.9	3.6	13.3
proline[e]	26.4	3.5	22.9
serine[e]	20.7	3.6	17.0
tyrosine	10.0	2.1	7.9

[a] Tryptophan was not assayed. [b] DDM = digesta dry matter. [c] 14500 RCF precipitate. [d] Total amino acid flow corrected for bacterial amino acid flow. [e] Predominant amino acids in mucin.

tion of lysine in the 14500 RCF precipitate, which represents the microbial fraction of the digesta, was 3.6 g/16 g of bacterial nitrogen, a figure that is in accord with that of Metges et al. (*35*) and very different from the accepted lysine concentration of porcine intestinal tissue (7.8 g/16 g nitrogen (*36*). The concentration of lysine in the other fractions, the 250 RCF precipitate and the supernatant from the high speed centrifugation, which together are considered to be nonmicrobial, was found to be 8.3 g/16 g nitrogen, a value that is more in line with the value for cellular protein determined by Munro and Fleck (*36*).

Table 5. Concentrations of DNA and DAPA in Terminal Ileal Digesta

	DAPA mg/g DDM[a]	DNA mg/g DDM		
centrifugation fraction	total[b]	total[b]	bacterial cellular	porcine cellular[e]
250 RCF precipitate	0.01	0.18	0.02	0.16
14500 RCF precipitate	0.26	0.45	0.45[c]	
14500 RCF supernatant	0.36	0.71	0.68[d]	0.03
totals	0.62	1.34	1.15	0.19

[a] DDM = digesta dry matter. [b] These totals represent the DAPA and DNA detected in the ileal digesta. [c] It was assumed that all of the DNA detected in the 14500 precipitate was bacterial in origin. A DNA/DAPA ratio of 1.77 was calculated (when expressed as mg·g^{-1} DDM), and used to estimate the bacterial DNA in the other two centrifugation fractions. [d] The concentration of bacterial DNA in this fraction was assumed to originate from lysed bacterial cells and was determined from the DNA/DAPA ratio of 1.77, (see [b] above). [e] Cellular DNA was determined as being the difference between the total DNA detected and estimated bacterial DNA.

The concentrations of DNA (DNA) and DAPA in the different centrifugation fractions are presented in **Table 5**. The mean value for digesta DNA was 1.34 mg·g^{-1} of dry matter, a value that compares well with 1.1 mg·g^{-1} of digesta dry matter reported by Rowan et al. (*33*). It was assumed that the DNA concentration of 0.45 mg·g^{-1} of digesta dry matter (DDM) detected in the 14500 RCF precipitate was solely of bacterial origin. Using this concentration of DNA together with the estimate of DAPA in the same centrifugation fraction (0.26 mg·g^{-1} of DDM), a DNA/DAPA ratio of 1.73 was determined, a figure also in agreement with that obtained by Rowan et al. (*33*). The latter ratio was then used to determine the amounts of bacterial DNA in the other centrifugation fractions. DNA in

Table 6. Distribution of Protein of Microbial or Nonmicrobial Origin within the Different Centrifugation Fractions of Ileal Digesta

	g/kg DMI[a]	SEM	mg/g DDM	SEM	percentage of total protein[d]
total protein	20.5	2.25	329.7	11.69	100
total microbial protein[b]	11.1	0.83	180.4	17.3	54.1
total nonmicrobial protein	9.4	0.86	149.3	15.3	45.9
250 RCF Precipitate					
protein	2.1	0.25	43.4	3.08	10.2
microbial protein	1.0	0.18	24.3	2.87	4.9
porcine cellular protein	1.1	0.19	19.0	2.07	5.4
14500 RCF Precipitate					
protein[c]	6.7	0.73	91.0	10.91	32.7
microbial protein	3.5	0.31	62.7	7.65	17.1
nonmicrobial protein	3.2	0.37	28.2	3.52	15.6
14500 RCF Supernatant					
protein	11.7	1.28	195.4	10.92	57.1
microbial protein	6.6	0.79	93.3	7.51	32.2
nonmicrobial protein	5.1	0.58	102.0	9.63	24.9

[a] Mean ± standard error of the mean. [b] Microbial protein was determined using the value of 26.03 mg DAPA/g of bacterial nitrogen. [c] Protein in this fraction was estimated using the bacterial protein extraction lysis buffer, (PELB), kit combined with a noninterfering protein assay kit, obtained from G-Biosciences. [d] Percentages of total protein calculated from protein values expressed in $g \cdot kg^{-1}$ DMI.

the 14500 RCF supernatant was assumed to have originated from lysed bacterial and porcine cells (inspection using light microscopy revealed that the number of intact cells in this fraction was negligible). Porcine cellular DNA material was determined as the difference between total DNA detected and the estimated quantity of bacterial DNA. The higher proportion of porcine cellular DNA in the 250 RCF centrifugation fraction supports the effectiveness of the separation, by centrifugation, of the porcine cellular material and bacterial material from the soluble components of the digesta. Using the QIAamp DNA Stool Mini Kit, the DNA extraction gave a particularly pure sample, such that protein interference was minimal.

An average DAPA/bacterial nitrogen ratio of 26.42 $mg \cdot g^{-1}$ bacterial nitrogen was determined, a value very close to that of 26.4 reported by Wünsche et al. (*37*). Utilizing this value, the proportions of the protein from microbial and nonmicrobial sources can be calculated; they are presented in **Table 6**. It is evident that a substantial proportion of protein of microbial origin is present in the digesta, nearly 54%, with over half of this being present in the 14500 RCF supernatant.

Different types of mucin are secreted by different regions of the gastrointestinal tract, and the composition of such mucins is known to vary between different animal species and the various sites of secretion (*28*). However, mucins in digesta taken from the terminal ileum would be expected to originate mostly from the stomach and the small intestine. The amino sugars *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GluNAc) are useful mucin markers, and once the ratio of the two is known, the relative proportions of gastric and intestinal mucin can be estimated. This is possible because the GluNAc/GalNAc ratio differs considerably between the two types of mucin, with gastric mucin containing approximately 30% GlcNAc and 13% GalNAc, whereas intestinal mucin contains approximately 20% and 40% (*23*, *28*, *38*). Regression equations developed by Lien et al. (*23*) can be used to estimate the proportions of gastric and intestinal mucin. Of several methods that may be used to detect mucin (*39*), the assay used here provides the most accurate information as to the type of mucin present in digesta.

The concentration of both amino sugars and the calculated mucin output are presented in **Table 7**. Although the concentrations of both GalNAc and GluNAc are in accord with those reported by Piel et al. (*40*), the total mucin output determined in this study is much higher. This may be because of differences between the control diet used by Piel et al. (*40*) and the casein diet used in this study or, more likely, as noted by Piel et al. (*40*), due to differences in the composition of mucins in weaned piglets and older pigs.

In their studies of gastrointestinal mucus, Mantle and Allen (*41*) reported GalNAc/GluNAc ratios of 2.8 and 0.6 for purified pig gastric and intestinal mucin respectively. In this study, a

Table 7. Mean Concentrations of the Amino Sugars *N*-Acetylgalactosamine and *N*-Acetylglucosamine, and the Concentration of Mucin in Terminal Ileal Digesta[a]

		mean	SEM
N-acetylgalactosamine	diet	ND[b]	
	ileal digesta $g \cdot 100\ g^{-1}$ DDM[d]	2.0	0.28[c]
	ileal digesta $g \cdot kg^{-1}$ DMI[e]	5.35	0.32
N-acetylglucosamine	diet	ND	
	ileal digesta $g \cdot 100\ g^{-1}$ DDM	2.6	0.22
	ileal digesta $g \cdot kg^{-1}$ DMI	7.0	0.49
GluNAc/ GalNAc ratio[f]		1.3	0.06
mucin output	$g \cdot kg^{-1}$ DMI	36.9	2.22
	$mg \cdot g^{-1}$ DDM	131.0	10.69
mucin nitrogen	$g \cdot 100\ g^{-1}$ digesta total nitrogen	9.4	0.68

[a] The estimated proportions of gastric and intestinal mucins in the digesta were 64% and 36%, respectively. [b] ND = not detected. [c] SEM = standard error of the mean. [d] DDM = digesta dry matter. [e] DMI = dry matter intake. [f] When expressed as $g \cdot 100\ g^{-1}$ DDM.

Table 8. Determined Concentrations of Urea, Ammonia, and Creatinine in Terminal Ileal Digesta[a]

	$g \cdot kg^{-1}$ DMI	SEM	$mg \cdot g^{-1}$ DDM	SEM
urea	1.9	0.34	7.1	0.84
ammonia	1.8	0.29	8.4	1.43
creatinine	0.12	0.03	3.1	0.73

[a] Values are means ± standard error of the mean.

Table 9. Summary of the Sources of Nitrogen in Terminal Ileal Digesta of Pigs Given a Casein-Based Diet

source of nitrogen	mg/g DDM[a]	SEM	percentage of total protein	percentage of nonbacterial protein	percentage of total nitrogen
			Protein		
bacterial	28.3[b,c]	3.80	60.9		44.7
porcine cellular[d]	3.1	0.69	6.7	17.2	4.9
soluble free protein[e]	8.5	0.80	18.2	46.7	13.4
mucin	6.6	0.48	14.1	36.1	10.4
			Nonprotein		
DNA	0.12	0.02			0.2
urea	3.3	0.40			5.2
ammonia	6.9	0.98			11.0
creatinine	1.2	0.20			1.8
nonspecific[f]	5.2	0.71			8.3
total	63.2	2.31			100.0

[a] Values are the means ± standard errors. [b] Equivalent to 26.4 mg DAPA/g bacterial nitrogen. [c] Bacterial nitrogen was principally determined from the 14500 RCF with corrections for DAPA found in other centrifugation fractions. [d] Porcine cellular nitrogen was calculated from the 250 RCF centrifugation precipitate with corrections for the DAPA found in that fraction. This fraction represents intact porcine cells and does not include cellular debris. [e] Soluble free protein was determined from the 14500 RCF supernatant using the Bradford reagent and may include albumin, immunoglobulins, digestive enzymes, and small peptides such as digestion resistant bioactive peptides and soluble cell debris. [f] This fraction contains nonidentified nitrogenous material that may include nonprotein compounds such as free amino acids, RNAs, amines, and the tetrapyrroles, bilirubin and biliverdin.

value of 1.3 for total mucin was determined using the regression equations developed by Lien et al. (*23*). The proportions of gastric and intestinal mucin present in the digesta were estimated to be 64% and 36%, respectively.

It is known that the presence of dietary proteins in the abomasum of the calf increases the secretion of pepsin and chymosin into the stomach (*42*) and that these enzymes can hydrolyze gastric mucus to release mucins into the abomasal digesta, which in turn flow into the duodenum. The high proportion of gastric mucin found in the ileal digesta in the present study supports the thesis that dietary casein stimulates the secretion of gastric proteases and acid, which in turn erode the gastric mucus layer and release gastric mucins into the chyme, which are not fully digested before arriving at the terminal ileum.

When the threonine/serine ratios of the different centrifugation fractions were determined (see **Table 3**), the ratio for the supernatant was nearly 30% higher than that of the 250 RCF precipitate and nearly 34% higher than that in the 14500 RCF precipitate. Once again, this indicates that a large proportion of the mucin in the digesta is soluble and is in the supernatant, with a much smaller amount of insoluble mucin in the low-speed centrifugation fraction. At 13% of the digesta dry matter, mucin is the single most abundant truly endogenous component secreted into the gastrointestinal tract.

Concentrations of ammonia, urea, and creatinine are shown in **Table 8**. When expressed in terms of nitrogen, these three components accounted for nearly 65% of the nonprotein nitrogen in the digesta. Although accounting for only 1.6% of the digesta dry matter, urea and ammonia contribute a disproportionate amount of nitrogen.

Ammonia, urea, and the metabolic activity of intestinal bacteria are all linked to the nitrogen cycling systems of the gut in animals with simple stomachs, including humans. The release of ammonia into the intestinal lumen may result from the catabolism and oxidation of amino acids by both enterocytes and bacteria, as well as the enzymatic breakdown of urea by microbial flora. The enteric metabolism of amino acids has important implications for the apparent digestibilities of proteins and amino acids, becoming, as Stoll et al. suggest, a source of nutritional inefficiency (*43*). In this study, the combined contributions of bacteria, urea, and ammonia account for nearly 55% of the total nitrogen in the digesta at the terminal ileum and may reflect nutritional inefficiency (*43*). It is interesting to note that the nitrogenous composition of the digesta of pigs fed a casein-based diet are in accord with the results of Chacko and Cummings, who suggested that 80−85% of nitrogen lost from the small bowel was from proteins and peptides (*44*).

The estimate of bacterial protein nitrogen, nearly 45% of total digesta nitrogen (see **Table 9**), is suggestive of large populations of bacteria in the small intestine, contrary to the earlier view that the upper digestive tract is virtually devoid of microbial activity (*45*). Although the number of bacteria in the small intestine may have been influenced by the presence of the PVTC cannula, its effect on the proportion of microbial protein relative to total protein is considered to be much less than that found with other collection techniques such as ileo-rectal anastomosis (*46*).

Present in the 14500 RCF supernatant was a protein fraction that was determined using the Bradford reagent. This fraction was estimated to contain nearly 9 mg of soluble free protein per gram of dry matter (see **Table 9**) and at 13% represents a significant proportion of total nitrogen present in the digesta dry matter. This fraction would be expected to contain immunoglobulins, digestive enzymes, and small protease-resistant peptides.

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