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Effects of collection time on flow of chromium and dry matter and on basal ileal endogenous losses of amino acids in growing pigs

B. G. Kim,* Y. Liu,† and H. H. Stein‡§[1]

*Department of Animal Science and Technology, Konkuk University, Seoul 05029, Korea; †University of California, Davis 95616; ‡Department of Animal Sciences, University of Illinois, Urbana 61801; and §Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT: The objectives of this experiment were to examine the diurnal patterns of chromium and DM flow at the distal ileum of pigs and to determine the effect of collection time on basal ileal endogenous losses (BEL) of CP and AA. Eight barrows with an initial BW of 34.6 kg (SD = 2.1) were individually fitted with a T-cannula in the distal ileum and randomly allotted to a replicated 4 × 4 Latin square design with 4 diets and 4 periods in each square. Three diets contained either corn, soybean meal, or distillers dried grains with solubles as the sole source of CP and AA. An N-free diet was also prepared. All diets contained 0.5% chromic oxide as an indigestible marker. Equal meals were provided at 0800 and 2000 h. Ileal digesta samples were collected in 2-h intervals from 0800 to 2000 h during the last 3 d of each 7-d period. The concentration of Cr in ileal digesta samples collected in each of the six 2-h periods exhibited a quadratic effect ($P < 0.01$) that increased and then decreased in pigs fed the CP containing diets. However, the concentration of Cr in ileal digesta collected in each of the six 2-h periods from pigs fed the N-free diet increased (linear, $P < 0.01$). These differences were possibly related to differences in DM flow, because DM flow to the distal ileum had a pattern that was opposite of that observed for the concentration of Cr in the ileal digesta samples. The BEL of all indispensable AA and the sum of indispensable AA from pigs fed the N-free diet decreased (linear, $P < 0.05$) in each of the six 2-h periods, with the exception that the BEL of Arg increased and then decreased (quadratic, $P < 0.05$). The BEL of Asp, Cys, Glu, Ser, and Tyr also decreased (linear, $P < 0.05$) during each of the six 2-h periods, whereas the BEL of Pro and the sum of dispensable AA increased and then decreased (quadratic, $P < 0.05$) over the 12 h. Collection time did not affect BEL of CP. No differences were observed in the concentration of Cr, flow of DM, or basal endogenous loss of all AA if ileal digesta samples were collected over 6-, 8-, or 10-h periods, compared with collection over a 12-h period. In conclusion, diurnal variation of Cr concentration, DM flow, and the BEL of all AA were observed, but 4 to 6 h of ileal sample collection starting 4 or 6 h after feeding may provide representative samples that allow for calculation of accurate values for CP and AA digestibility.

Key words: amino acid digestibility, basal endogenous losses, chromium, diurnal variation, dry matter, pigs

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INTRODUCTION

Digestibility of CP and AA is most correctly determined at the end of the small intestine (Sauer and Ozimek, 1986; Stein et al., 2007), which makes it necessary to collect digesta at the distal ileum. Several procedures for collection of ileal digesta have been described, including T-cannulation (McBurney and Sauer, 1993; Stein et al., 1998), re-entrant cannulation (Easter and Tanksley, 1973; Braude et al., 1976), the slaughter procedure (Moughan et al., 1992), postvalve T-cannulation (**PVTC**; van Leeuwen et al., 1991; Hodgkinson et al., 2000, 2002), or the steered ileocecal valve procedure (Mroz et al., 1991; Radcliffe et al., 2005). The slaughter procedure allows for collection of digesta at only a single time point, whereas the other procedures allow for multiple collections over an extended period of time. The

[1]Corresponding author: hstein@illinois.edu
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T-cannula procedure is the most common cannulation procedure because the T-cannula is well tolerated by pigs and relatively easy to install.

Chromic oxide is widely used as an indigestible marker to determine ileal energy, DM, or nutrient digestibility in pigs (Jagger et al., 1992; Imbeah et al., 1996; Stein et al., 1999). Diurnal variation of Cr in ileal digesta has been reported when ileal digesta were collected through a replaceable T-cannula (Graham and Åman, 1986) or via a PVTC cannula (Jørgensen et al., 1997). Diurnal variation in the composition of ileal digesta has also been reported, and it has been suggested that ileal digesta samples should be collected for 8 h over 2 consecutive days (Jørgensen et al., 1997). Diurnal variation in endogenous ileal N flow has also been reported (Hodgkinson et al., 2002), but diurnal variation of basal ileal endogenous losses (**BEL**) of AA has not been reported. Therefore, the objectives of the present experiment were to determine Cr concentration in ileal digesta, the flow of DM to the distal ileum of pigs fed different types of diets, and to determine the BEL of CP and AA at the distal ileum in different collection periods post-feeding.

MATERIALS AND METHODS

The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. All pigs used in the experiment were Landrace (3/4) by Large White (1/4) cross-bred barrows (Genetiporc, Alexandria, MN).

Animals and Diets

Eight barrows (initial BW: 34.6 ± 2.1 kg) were surgically fitted with a T-cannula at the distal ileum (Stein et al., 1998) and randomly allotted to a replicated 4 × 4 Latin square design with 4 diets and 4 periods per square using a spreadsheet-based program to balance potential residual effects (Kim and Stein, 2009). Animals were housed in individual pens (1.2 × 1.5 m) in a facility with a 12-h light-dark cycle. Each pen was equipped with a feeder and a nipple drinker and had fully slatted tribar floors and smooth-sided walls.

Four experimental diets were prepared (Tables 1 and 2). Three diets contained either ground corn (97.0%), soybean meal (**SBM**; 34.2%), or distillers dried grains with solubles (**DDGS**; 62.4%) as the sole source of AA. An N-free diet was used to estimate BEL of CP and AA. All diets contained 0.5% chromic oxide as an indigestible marker. Vitamins and minerals were included in all diets to meet or exceed nutrient requirement estimates (NRC, 1998).

Feeding and Sample Collection

Feed was provided at daily levels of 3 times the estimated maintenance requirement for energy (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998). Equal meals were provided at 0800 and 2000 h. Feed allowance was adjusted at the beginning of each period when the BW of each pig was recorded. Animals had free access to water through a nipple drinker at all times. Each period lasted 7 d. The initial 4 d were considered an adaptation period to the diet, and ileal digesta samples were collected in six 2-h periods from 0800 to 2000 h on d 5, 6, and 7 (i.e., samples were collected from 0800 to 1000 h; 1000 to 1200 h; 1200 to 1400 h; 1400 to 1600 h; 1600 to1800 h; and 1800 to 2000 h). A 225-mL plastic bag was attached to the cannula barrel using a cable tie and digesta flowing into the bag were collected. Bags were removed whenever they were filled with digesta, or at least once every 30 min, and immediately stored at -20°C. Results of unpublished work from our laboratory indicate that the combination of using small collection bags, frequent change of bags, and immediate storage at -20°C prevents bacterial degradation of AA. Samples were kept separate for each animal and collection time, but mixed across the 3 collection days within animal and collection times. This resulted in 6 samples of digesta collected from each pig in each period with each sample representing a 2-h collection period. Collected samples were stored at -20°C to prevent bacterial degradation of AA in the digesta.

Chemical Analyses

At the conclusion of the experiment, frozen ileal digesta samples were allowed to thaw at room temperature and then mixed and weighed. A subsample was collected, weighed, lyophilized, and weighed again. Lyophilized digesta samples were finely ground prior to chemical analysis. All diets and lyophilized digesta samples were analyzed for Cr (Fenton and Fenton, 1979) and DM (Method 930.15; AOAC, 2007) and CP was analyzed using the combustion procedure (Method 990.03; AOAC, 2007). An Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) was used to analyze for total N with Asp being the calibration standard. The concentration of CP was calculated as N × 6.25. Amino acids were analyzed on a Hitachi Amino Acid Analyzer (Model L8800; Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard (Method 982.30 E [a, b, c]; AOAC, 2007). Samples were hydrolyzed with 6*N* HCl for 24 h at 110°C before being analyzed. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis. Tryptophan was determined after NaOH hydrolysis

Table 1. Ingredient composition of experimental diets, as-fed basis

	Diet			
Ingredients, %	Corn	Soybean meal	Distillers dried grains with solubles	N-free
Ground corn	97.00	–	–	–
Soybean meal, 48% CP	–	34.15	–	–
Distillers dried grains with solubles	–	–	62.40	–
Corn starch	–	40.00	15.00	68.20
Sucrose	–	20.00	20.00	20.00
Soybean oil	–	3.00	–	4.00
Solka floc[1]	–	–	–	4.00
Ground limestone	0.70	0.65	1.40	0.80
Dicalcium phosphate	1.10	1.00	–	1.30
Potassium carbonate	–	–	–	0.40
Magnesium oxide	–	–	–	0.10
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50	0.50

[1]Fiber Sales and Development Corp., Urbana, OH.

[2]Supplied per kg of complete diet: vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

for 22 h at 110°C. All diets were also analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer; Ankom Technology, Macedon, NY). Samples of corn, SBM, and DDGS were also analyzed for DM, CP, AA, ADF, and NDF (Table 3). Chromium was analyzed at South Dakota Agricultural Laboratories (Brookings, SD), and samples were analyzed for AA at University of MO, Agricultural Experiment Station Analytical Services (Columbus, MO); all other analyses were completed in the Monogastric Nutrition Laboratory at the University of Illinois (Urbana, IL).

Calculation and Statistical Analysis

The output of Cr and DM in ileal digesta was defined as the ileal Cr flow and the ileal DM flow, respectively, and these values, along with values for BEL of AA were calculated for 4-, 6-, 8-, 10-, and 12-h collection periods using the following equation:

$$\text{Concentration, \%} = \Sigma \left[\begin{matrix} \text{concentration (\%)} \\ \times \text{ sample (g)} \end{matrix} \right] / \Sigma \text{ sample (g)}$$

where sample (g) represents the weight of the ileal digesta sample after freeze-drying.

The BEL of AA was calculated as described previously (Stein et al., 2007). The ileal DM flows were calculated according to the following equation:

$$\text{Ileal DM flow, g/kg DMI} = Cr_{diet} / Cr_{ileal},$$

where Cr_{diet} = Cr concentration in diet (g/kg DM), and Cr_{ileal} = Cr concentration in ileal digesta (g/kg DM).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included collection time as the fixed effect, and period and animal as random effects. Orthogonal polynomial contrasts were used to test for linear and quadratic effects of collection time. The PDIFF option was used to compare the values for the 12-h collection period with values from all the collection times and lengths. The animal was considered the experimental unit. An α level of 0.05 was used to determine statistical significances.

RESULTS AND DISCUSSION

The corn, SBM, and DDGS used in this experiment contained CP and AA close to expected concentrations (NRC, 2012) with the exception that both SBM and DDGS contained slightly more Lys than previously reported (Table 3). This observation indicates that the sources of SBM and DDGS that were used in this experiment were not heat damaged because heat damage of feed ingredients will reduce the concentration of Lys as well as the ratio of Lys to CP (Fontaine et al., 2007; González-Vega et al., 2011). Pigs consumed their diets without apparent problems throughout the experiment.

For ileal digesta collection, a T-cannula that was surgically installed 10 to 20 cm anterior to the ileocecal valve was used in the present study. The values obtained from this technique have been shown to result in minimal trial-to-trial variations (Knabe et al., 1989; Sauer and de Lange, 1992). However, the diurnal variation existing in the chemical composition of digesta

Table 2. Analyzed nutrient composition of experimental diets, as-fed basis

	Diet			
Item	Corn	Soybean meal	DDGS[1]	N-free
DM, %	88.21	92.61	92.70	92.25
CP, %	7.08	16.46	18.40	0.27
ADF	2.86	1.44	7.30	1.28
NDF	7.37	2.01	17.85	1.19
Cr, %	0.32	0.33	0.30	0.35
Indispensable AA, %				
Arg	0.35	1.23	0.88	0.01
His	0.25	0.50	0.54	0.02
Ile	0.24	0.81	0.70	0.01
Leu	0.77	1.34	2.14	0.03
Lys	0.55	1.35	0.76	0.10
Met	0.13	0.24	0.37	0.00
Phe	0.37	0.90	0.98	0.03
Thr	0.22	0.62	0.65	0.01
Trp	0.04	0.25	0.16	< 0.04
Val	0.34	0.88	0.97	0.02
Total indispensable AA	3.26	8.12	8.15	0.23
Dispensable AA, %				
Ala	0.48	0.76	1.35	0.02
Asp	0.43	1.91	1.18	0.02
Cys	0.15	0.26	0.35	0.00
Glu	1.17	2.99	2.72	0.06
Gly	0.29	0.73	0.79	0.01
Pro	0.57	0.86	1.51	0.03
Ser	0.27	0.67	0.72	0.01
Tyr	0.25	0.55	0.67	0.02
Total dispensable AA	3.61	8.73	9.29	0.17

[1]DDGS = distillers dried grains with solubles.

Table 3. Analyzed nutrient composition of corn, soybean meal, and distillers dried grains with solubles, as-fed basis

	Ingredient		
Item	Corn	Soybean meal	DDGS[1]
DM, %	85.56	87.04	87.43
CP, %	7.32	47.83	27.79
ADF	2.25	5.08	13.52
NDF	6.82	6.72	30.03
Indispensable AA, %			
Arg	0.34	3.56	1.32
His	0.23	1.26	0.85
Ile	0.24	2.31	1.04
Leu	0.77	3.78	3.18
Lys	0.26	3.09	1.04
Met	0.13	0.72	0.57
Phe	0.34	2.41	1.59
Thr	0.22	1.82	0.98
Trp	0.05	0.66	0.2
Val	0.35	2.46	1.45
Total indispensable AA	2.93	22.07	12.22
Dispensable AA, %			
Ala	0.48	2.18	1.97
Asp	0.43	5.48	1.72
Cys	0.16	0.74	0.57
Glu	1.13	8.41	3.77
Gly	0.29	2.09	1.16
Pro	0.56	2.49	2.19
Ser	0.26	2.04	1.12
Tyr	0.21	1.70	1.06
Total dispensable AA	3.52	25.13	13.56

[1]DDGS = distillers dried grains with solubles.

may affect the determination of nutrient digestibility of diets or ingredients fed to pigs (Graham and Åman, 1986; Jørgensen et al., 1997; Hodgkinson et al., 2002). Therefore, it is important that the proportion of the digesta that are collected through the ileal cannula during the digesta collection periods is representative of the total digesta passing the distal ileum. In the present experiment, the concentrations of Cr in ileal digesta samples collected in each of the six 2-h periods increased and then decreased (quadratic, $P < 0.01$) if pigs were fed diets based on corn, SBM, or DDGS (Table 4). The maximum concentration of Cr was observed 4 to 8 h after the meal and this observation is in close agreement with observations reported by Livingstone et al. (1980) and Graham and Åman (1986). This observation also indicates that diurnal variation in the chemical composition of ileal digesta collected from pigs fitted with a T-cannula does exist, which is consistent with previous reports (Graham and Åman, 1986; Köhler et al., 1990). Diurnal variation in the concentration of Cr in ileal digesta collected from PVTC-cannulated pigs also has been reported (van Leeuwen et al., 1997; Hodgkinson et al., 2002). This variation is possibly related to the flow of digesta, which is assumed to pass the ileum 3 to 5 h after feeding (Zebrowska, 1973; Braude et al., 1976; Darcy et al., 1980). However, the concentration of Cr in ileal digesta samples collected in each of the six 2-h periods increased (linear, $P < 0.05$) if pigs were fed the N-free diet. The reason for the difference in the changing patterns of Cr in the digesta collected from pigs fed the N-free diet compared with pigs fed diets containing corn, SBM, or DDGS is most likely that nutrients in all ingredients in the N-free diet except Solka floc are easily digestible and likely had been absorbed before the end of the small intestine, which resulted in a progressively increasing concentration of Cr in the digesta in the distal ileum. In contrast, the nutrients in corn, SBM, and DDGS are less digestible and greater proportions of DM, therefore, likely reached the distal end of the small intestine without being digested, and passed the distal ileum into the large intestine. The amount of digesta passing into the large intestine, therefore, influenced the concentration of Cr in the digesta, which resulted in the quadratic pattern for the concentration of Cr in the digesta.

Table 4. Concentrations of Cr and the flow of DM (g/kg DMI) in ileal digesta samples according to collection times from pigs fed a corn-based diet, a soybean meal-based diet, a distillers dried grains with solubles-based (DDGS) diet, or an N-free diet[1,2], DM basis

	Diet							
	Corn		Soybean meal		DDGS		N-free	
Item	Cr, %	DM flow	Cr, %	DM flow	Cr, %	DM flow	Cr, %	DM flow
2-h period								
0800 to 1000 h	1.39*	274*	1.82**	194**	0.89**	406**	2.70*	157*
1000 to 1200 h	1.43*	264	2.42	149	0.92*	383	2.63*	159*
1200 to 1400 h	2.14**	176**	2.43	147	1.04	342	3.09	133
1400 to 1600 h	1.89	210	2.47*	147	1.08*	331	3.17	119
1600 to 1800 h	1.62	233	1.94**	188**	1.10**	322*	3.36	112
1800 to 2000 h	1.35**	298**	1.86**	194**	0.88**	406**	3.32	115
SEM	0.13	21	0.13	9	0.04	18	0.33	22
P-value								
Linear	0.908	0.723	0.169	0.097	0.147	0.209	0.001	0.006
Quadratic	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.569	0.545
4-h period								
0800 to 1200 h	1.42*	268	2.28	161	0.91*	388	2.74*	147
1000 to 1400 h	1.78	222	2.47	146*	0.98	363	2.89	143
1200 to 1600 h	2.04**	193*	2.46	147	1.06	337	3.09	129
1400 to 1800 h	1.79	219	2.30	160	1.09*	327*	3.26	115
1600 to 2000 h	1.53	257	1.92**	189**	1.00	361	3.33	114
SEM	0.10	16	0.13	8	0.03	11	0.31	17
6-h period								
0800 to 1400 h	1.69	234	2.38	153	0.96	369	2.89	141
1000 to 1600 h	1.85	214	2.48*	145	1.01	353	2.96	137
1200 to 1800 h	1.92	204	2.35	155	1.07	333	3.17	124
1400 to 2000 h	1.70	235	2.19	168	1.03	349	3.26	116
SEM	0.08	11	0.12	7	0.03	9	0.32	17
8-h period								
0800 to 1600 h	1.77	224	2.42	150	0.99	360	2.96	136
1000 to 1800 h	1.81	217	2.40	152	1.03	347	3.04	132
1200 to 2000 h	1.83	218	2.27	161	1.04	346	3.19	123
SEM	0.06	8	0.12	7	0.02	8	0.31	17
10-h period								
0800 to 1800 h	1.75	225	2.35	156	1.01	353	3.03	132
1000 to 2000 h	1.75	226	2.33	157	1.01	354	3.07	130
SEM	0.05	6	0.12	7	0.02	7	0.31	17
12-h period								
0800 to 2000 h	1.71	233	2.29	160	1.00	359	3.06	130
SEM	0.04	6	0.11	7	0.02	7	0.30	16

*Means different from the value in the 12-h period ($P < 0.05$); **Means different from the value in the 12-h period ($P < 0.01$).

[1]Each least squares mean represents 8 observations.

[2]Data from collection for 4, 6, 8, 10, or 12 h were calculated based on collections in the six 2-h periods.

When pigs were fed the corn-based diet, the concentration of Cr in ileal digesta samples collected in 2-h periods (0800 to 1000 h, 1000 to 1200 h, or 1800 to 2000 h) and in the 4-h period from 0800 to 1200 h was less ($P < 0.05$) than the concentration of Cr in ileal digesta samples collected over the entire 12-h period. However, ileal digesta samples collected in the 2-h period from 1200 to 1400 h or in the 4-h period from 1200 to 1600 h had greater ($P < 0.05$) concentrations of Cr than samples collected during the entire 12-h period. When pigs were fed the SBM-based diet, ileal digesta samples collected in 2-h periods (0800 to 1000 h, 1600 to 1800 h, or 1800 to 2000 h) or in the 4-h period from 1600 to 2000 h had less ($P < 0.05$) concentration of Cr than samples collected over the 12-h period, whereas ileal digesta samples collected in the 2-h period from 1400 to 1600 h or the 6-h period from 1000 to 1600 h had a greater ($P < 0.05$) concentration of Cr than for the entire 12-h period. When pigs were fed the DDGS-based diet, ileal digesta samples collected in 2-h periods

Table 5. Basal endogenous losses (g/kg DMI) of indispensable AA calculated based on different times of ileal digesta collection from pigs fed the N-free diet[1,2]

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	TIAA[3]
2-h period											
0800 to 1000 h	0.75**	0.30**	0.62**	1.07**	0.93**	0.16**	0.62**	1.02**	0.20**	0.85**	6.51**
1000 to 1200 h	0.97	0.23	0.40	0.67	0.64	0.11	0.40	0.60	0.15	0.53	4.69
1200 to 1400 h	1.46**	0.22	0.31	0.49	0.50	0.08	0.29	0.44	0.12	0.39	4.29
1400 to 1600 h	1.20	0.20	0.32	0.52	0.54	0.09	0.31	0.48	0.12	0.41	4.20
1600 to 1800 h	0.93	0.20	0.34	0.55	0.54	0.10	0.33	0.50	0.11	0.44	4.05
1800 to 2000 h	0.78*	0.20	0.36	0.59	0.62	0.09	0.36	0.57	0.11	0.48	4.16
SEM	0.20	0.04	0.08	0.14	0.12	0.02	0.08	0.14	0.02	0.11	0.87
P-value											
Linear	0.849	0.025	0.008	0.007	0.025	0.008	0.010	0.023	0.002	0.010	0.018
Quadratic	< 0.001	0.154	0.006	0.006	0.007	0.017	0.00 6	0.010	0.077	0.006	0.090
4-h period											
0800 to 1200 h	0.89	0.23	0.42	0.71	0.67	0.11	0.42	0.65	0.15	0.56	4.82
1000 to 1400 h	1.29	0.22	0.34	0.55	0.55	0.09	0.33	0.50	0.13	0.44	4.44
1200 to 1600 h	1.32*	0.21	0.31	0.50	0.52	0.09	0.30	0.46	0.12	0.40	4.23
1400 to 1800 h	1.08	0.20	0.33	0.53	0.54	0.09	0.32	0.49	0.11	0.43	4.12
1600 to 2000 h	0.88	0.20	0.35	0.57	0.58	0.09	0.34	0.53	0.11	0.46	4.12
SEM	0.19	0.02	0.05	0.07	0.08	0.02	0.04	0.06	0.01	0.06	0.53
6-h period											
0800 to 1400 h	1.18	0.22	0.36	0.59	0.58	0.10	0.35	0.54	0.14	0.47	4.54
1000 to 1600 h	1.25	0.22	0.33	0.54	0.55	0.09	0.32	0.49	0.13	0.43	4.35
1200 to 1800 h	1.19	0.20	0.32	0.51	0.52	0.09	0.30	0.47	0.12	0.41	4.14
1400 to 2000 h	1.01	0.20	0.34	0.55	0.56	0.09	0.33	0.51	0.11	0.44	4.14
SEM	0.19	0.02	0.04	0.07	0.07	0.01	0.04	0.05	0.01	0.06	0.51
8-h period											
0800 to 1600 h	1.19	0.22	0.35	0.57	0.56	0.10	0.33	0.52	0.13	0.45	4.40
1000 to 1800 h	1.17	0.21	0.33	0.54	0.54	0.09	0.32	0.49	0.12	0.43	4.25
1200 to 2000 h	1.13	0.20	0.33	0.53	0.54	0.09	0.31	0.49	0.12	0.42	4.15
SEM	0.19	0.02	0.04	0.06	0.07	0.01	0.04	0.05	0.01	0.05	0.50
10-h period											
0800 to 1800 h	1.12	0.21	0.34	0.56	0.55	0.09	0.33	0.51	0.12	0.45	4.29
1000 to 2000 h	1.11	0.21	0.34	0.55	0.55	0.09	0.32	0.50	0.12	0.44	4.24
SEM	0.17	0.02	0.04	0.07	0.07	0.01	0.04	0.05	0.01	0.05	0.48
12-h period											
0800 to 2000 h	1.07	0.21	0.35	0.56	0.56	0.09	0.33	0.52	0.12	0.45	4.27
SEM	0.16	0.02	0.04	0.07	0.07	0.01	0.04	0.05	0.01	0.05	0.96

*Means different from the value in the 12-h period ($P < 0.05$); **Means different from the value in the 12-h period ($P < 0.01$).

[1]Each least squares mean represents 8 observations.

[2]Data from collections for 4, 6, 8, 10, and 12 h were calculated based on collections in the six 2-h periods.

[3]TIAA = total indispensable AA.

(0800 to 1000 h, 1000 to 1200 h, or 1800 to 2000 h) or in the 4-h period from 0800 to 1200 h had less ($P < 0.05$) Cr concentration, but ileal digesta samples collected in 2-h periods (1400 to 1600 h or 1600 to 1800 h) or in the 4-h period from 1400 to 1800 h had a greater ($P < 0.05$) concentration of Cr compared with that collected over the 12-h period. When pigs were fed the N-free diet, ileal digesta samples collected in 2-h periods (0800 to 1000 h or 1000 to 1200 h) or in the 4-h period from 0800 to 1200 h contained less ($P < 0.05$) Cr than for the 12-h period. No differences in concentration of Cr were observed if ileal digesta samples were collected in 6-h or 8-h periods, compared with the 12-h period. These differences were mainly due to differences in DM flow because the DM flow had an opposite pattern compared with the concentration of Cr in the ileal digesta samples (Table 4). These results indicate that 6 h of ileal digesta sample collection starting 4 h after feeding may provide a representative sample for the entire 12-h period.

The average concentration of Cr in ileal digesta and the average flow of DM to the distal ileum over the 12-h collection period were 1.71% and 233 g/kg DMI from pigs fed the corn-based diet, 2.29% and 160 g/kg DMI for pigs fed the SBM-based diet, 1.00% and 359 g/kg

Table 6. Basal endogenous losses (g/kg DMI) of dispensable AA and CP calculated based on different collection periods for ileal digesta from pigs fed the N-free diet[1,2]

Item	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	TDAA[3]	CP
2-h period										
0800 to 1000 h	0.99	1.26**	0.30**	1.66**	2.94	4.4**	0.80*	0.50*	12.8*	27.4
1000 to 1200 h	1.02	0.90	0.19	1.20	2.50	10.2	0.55	0.30	16.8	25.7
1200 to 1400 h	1.15*	0.80	0.14	1.03	3.18*	14.9	0.50	0.22	21.9**	29.5
1400 to 1600 h	1.02	0.82	0.14	1.01	2.57	12.0	0.51	0.24	18.3	26.0
1600 to 1800 h	0.88	0.79	0.15	1.04	2.12	9.0	0.47	0.25	14.7	22.3
1800 to 2000 h	0.81	0.84	0.17	1.05	2.05	6.8**	0.50	0.28	12.5*	21.4
SEM	0.17	0.17	0.04	0.22	0.54	1.90	0.12	0.07	2.80	4.50
P-value										
Linear	0.081	0.051	0.009	0.012	0.066	0.616	0.049	0.012	0.448	0.099
Quadratic	0.080	0.082	0.009	0.041	0.454	< 0.001	0.137	0.007	< 0.001	0.365
4-h period										
0800 to 1200 h	0.99	0.93	0.21	1.24	2.39	8.8	0.57	0.32	15.4	24.7
1000 to 1400 h	1.11	0.85	0.16	1.10	2.94	13.4*	0.53	0.25	20.3*	28.3
1200 to 1600 h	1.09	0.81	0.14	1.02	2.87	13.4*	0.50	0.23	20.1*	27.8
1400 to 1800 h	0.96	0.80	0.15	1.02	2.36	10.5	0.49	0.25	16.6	24.3
1600 to 2000 h	0.86	0.81	0.16	1.05	2.11	8.2	0.49	0.26	13.9	22.1
SEM	0.16	0.10	0.02	0.14	0.38	2.0	0.05	0.03	2.7	3.4
6-h period										
0800 to 1400 h	1.07	0.86	0.17	1.13	2.79	11.9	0.53	0.27	18.8	27.2
1000 to 1600 h	1.08	0.84	0.15	1.07	2.81	12.9	0.52	0.25	19.6	27.5
1200 to 1800 h	1.01	0.80	0.14	1.02	2.62	11.9	0.49	0.24	18.3	25.9
1400 to 2000 h	0.92	0.81	0.15	1.03	2.29	9.7	0.49	0.25	15.6	23.6
SEM	0.16	0.09	0.02	0.13	0.38	1.9	0.05	0.03	2.7	3.4
8-h period										
0800 to 1600 h	1.05	0.85	0.16	1.09	2.72	12.0	0.52	0.26	18.6	26.8
1000 to 1800 h	1.02	0.82	0.15	1.05	2.63	11.8	0.51	0.25	18.2	26.1
1200 to 2000 h	0.98	0.80	0.15	1.02	2.53	11.1	0.49	0.24	17.3	25.1
SEM	0.16	0.09	0.02	0.13	0.36	1.9	0.05	0.03	2.6	3.2
10-h period										
0800 to 1800 h	1.00	0.83	0.16	1.07	2.56	11.2	0.51	0.26	17.6	25.6
1000 to 2000 h	0.99	0.82	0.15	1.05	2.54	11.1	0.50	0.25	17.4	25.4
SEM	0.15	0.09	0.02	0.13	0.34	1.8	0.05	0.03	2.4	3.1
12-h period										
0800 to 2000 h	0.98	0.83	0.16	1.07	2.49	10.6	0.51	0.26	16.9	25.0
SEM	0.15	0.09	0.01	0.13	0.33	1.7	0.05	0.03	2.3	3.0

*Means different from the value in the 12-h period ($P < 0.05$); **Means different from the value in the 12-h period ($P < 0.01$).

[1]Each least squares mean represents 8 observations.

[2]Data from collection for 4, 6, 8, 10, and 12 h were calculated based on collections in the six 2-h periods.

[3]TDAA = total dispensable AA.

DMI for pigs fed the DDGS-based diet, and 3.06% and 130 g/kg DMI for pigs fed the N-free diet. This observation indicates that the chemical composition of ileal digesta depends on the type of diet that is fed and that the greater the concentration of fiber in the diet is, the more DM flows to the distal ileum and the less is the concentration of Cr in the digesta. This observation is in agreement with previous data (Low et al., 1978; Livingstone et al., 1980) and reflects the fact that fiber is not digested in the small intestine. It has also been demonstrated that concentrations of fiber and fat in the diet may affect the digestibility of CP and DM (Leterme et al., 1996; Jørgensen et al., 1997; Hodgkinson et al., 2000; Kil et al., 2010). The current data demonstrating that the flow of DM increased as the concentration of fiber in the diet increased support the observation that dietary fiber may influence nutrient digestibility.

The BEL of all indispensable AA and the sum of indispensable AA from pigs fed the N-free diet decreased (linear, $P < 0.05$) over the six 2-h periods with the exception that the BEL of Arg increased and then decreased (quadratic, $P < 0.05$; Table 5). The BEL of all indispensable AA except of Arg was greater ($P < 0.05$) during the 2-h period from 0800 to 1000 h compared with that collected over the entire 12-h period. No differences were observed in the BEL of indispensable AA if ileal digesta samples were collected in 4-, 6-, or 8-h periods, compared with the 12-h period.

Several techniques have been used to estimate BEL of AA, including the N-free diet, the peptide alimentation technique, and the regression technique (Carlson and Bayley, 1970; Taverner et al., 1981; Moughan et al., 1992). The N-free diet method used in the present experiment is the most commonly used procedure for estimating endogenous protein. The BEL of Asp, Cys, Glu, Ser, and Tyr decreased (linear, $P < 0.05$) over the six 2-h period, whereas the BEL of Pro and total dispensable AA increased and then decreased (quadratic, $P < 0.05$; Table 6). The BEL of most dispensable AA was greater ($P < 0.05$) if ileal digesta samples were collected during the 2-h period from 0800 to 1000 h compared with samples collected over the 12-h period. However, the BEL of Pro was less ($P < 0.05$) if samples were collected from 0800 to 1000 h or from 1800 to 2000 h, but greater ($P < 0.05$) if samples were collected in 4-h periods (1000 to 1400 h or 1200 to 1600 h), compared with the BEL of Pro for the entire 12-h period. The BEL of the sum of dispensable AA was less ($P < 0.05$) if ileal digesta samples were collected in 2-h periods (0800 to 1000 h or 1800 to 2000 h), but greater ($P < 0.05$) if ileal digesta samples were collected from 1200 to 1400 h or in 4-h periods (1000 to 1400 h or 1200 to 1600 h) compared with values for the 12-h period. No differences were observed in the BEL of all dispensable AA except Pro if ileal digesta samples were collected in 4-, 6-, or 8-h periods, compared with the 12-h period. The observation that the flow of Pro had a different time pattern compared with the flow of other AA may be a result of the fact that mucin protein is rich in Pro. Mucin protein is largely resistant to hydrolysis in the intestinal tract, and therefore, is not reabsorbed (Moughan and Schuttert, 1991).

The BEL results indicate that a constant amount of endogenous AA is moving through the lower small intestine 6 h after feeding; therefore, 4- to 6-h ileal collection starting 6 h after feeding may provide a representative sample. These results are in close agreement with previous data (Hodgkinson et al., 2002).

The BEL of CP that was calculated from samples over 2 h were not different from losses calculated for the entire 12-h period, regardless of which 2-h period was used. Likewise, no differences were observed in the BEL of CP if ileal digesta samples were collected in 4-, 6-, or 8-h periods, compared with the 12-h period. This observation is consistent with data from Hodgkinson et al. (2002), who used the peptide alimentation technique and PVTC-cannulated pigs to estimate the BEL of N in ileal digesta, and also concluded that there was a relatively constant endogenous N flow over time postfeeding.

In the present experiment, ileal digesta were collected only during the diurnal period, i.e., during the 12-h daytime, whereas we did not attempt to collect ileal digesta during the nocturnal period (i.e., during the nighttime). It is, therefore, not possible to determine if conclusions for the diurnal period obtained in the present experiment are also representative for the nocturnal period. However, it has been reported that data obtained during a 12-h diurnal period are not different from data obtained over a 16-h period that includes some of the nocturnal period (Graham and Åman, 1986). Likewise, it was demonstrated that if pigs are fed meals at 0800 and 1700 h, the flow to the distal ileum of AA is constant over 24 h (Hodgkinson et al., 2002). Nevertheless, it is possible that if pigs are left undisturbed during the nocturnal period, and if no digesta are collected during this period, values for BEL of AA will be different, because the behavior and the physical movement of pigs associated with digesta collection may impact peristalsis in the small intestine and, therefore, also DM flow to the distal ileum. However, to our knowledge, there are no data published to determine if physical activity of pigs influences BEL of AA.

In conclusion, diurnal variation in Cr and DM flow exists as the concentration of Cr increased and then decreased if diets contained corn, SBM, or DDGS, but DM flow decreased and then increased if ileal digesta samples were collected in 2-h periods. However, DM flow and, therefore, also concentration of Cr in ileal digesta are influenced by the type of diet being fed with the amount of fiber in the diet resulting in increased ileal flow of DM and reduced concentration of Cr in the digesta. During the diurnal period, values for BEL of CP and all AA that are calculated by analyzing ileal digesta samples collected for 4 to 6 h starting 4 or 6 h after feeding are not different from values calculated from digesta samples collected for 12 h starting immediately after feeding.

LITERATURE CITED

AOAC. 2007. Official methods of analysis. 18th ed. W. Hortwitz and G. W. Latimer Jr., editors, Assoc. Off. Anal. Chem. Int., Gaithersburg, MD.

Braude, R., R. J. Fulford, and A. G. Low. 1976. Studies on digestion and absorption in the intestines of growing pigs. Measurements of the flow of digesta and pH. Br. J. Nutr. 36:497–510.

Carlson, K. H., and H. S. Bayley. 1970. Nitrogen and amino acids in the feces of young pigs receiving a protein-free diet and diets containing graded levels of soybean meal or casein. J. Nutr. 100:1353–1362.

Darcy, B., J. P. Laplace, and P. A. Villiers. 1980. Digestion in the pig small intestine. 2. Comparative kinetics of the passage of digesta according to the mode of fistulation, ileocaecal or postvalvular ileocolic, in various feeding conditions. Ann. Zootech. 29:147–177.

Easter, R. A., and T. D. Tanksley, Jr. 1973. A technique for re-entrant ileocecal cannulation of swine. J. Anim. Sci. 36:1099–1103.

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631–634.

Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J. Agric. Food Chem. 55:10737–10743.

González-Vega, J. C., B. G. Kim, J. K. Htoo, A. Lemme, and H. H. Stein. 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci. 89:3617–3625.

Graham, H., and P. Åman. 1986. Circadian variation in composition of duodenal and ileal digesta from pigs fitted with T-cannulas. Anim. Prod. 43:133–140.

Hodgkinson, S. M., P. J. Moughan, P. C. H. Morel, and G. W. Reynolds. 2002. The diurnal pattern of ileal dry matter and endogenous ileal nitrogen flows in the growing pig. J. Sci. Food Agric. 82:1860–1866.

Hodgkinson, S. M., P. J. Moughan, G. W. Reynolds, and K. A. C. James. 2000. The effect of dietary peptide concentration on endogenous ileal amino acid loss in the growing pig. Br. J. Nutr. 83:421–430.

Imbeah, M., K. Angkanaporn, V. Ravindran, and W. L. Bryden. 1996. Investigations on the guadination of lysine in proteins. J. Sci. Food Agric. 72:213–218.

Jagger, S., J. Wiseman, D. J. A. Cole, and J. Craigon. 1992. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. Br. J. Nutr. 68:729–739.

Jørgensen, H., J. E. Lindberg, and C. Andersson. 1997. Diurnal variation in the composition of ileal digesta and the ileal digestibilities of nutrients in growing pigs. J. Sci. Food Agric. 74:244–250.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959–2967.

Kim, B. G., and H. H. Stein. 2009. A spreadsheet program for making a balanced Latin square design. Rev. Colomb. Cienc. Pecu. 22:591–596.

Knabe, D. A., D. C. LaRue, E. J. Gregg, G. M. Martinez, and T. D. Tanksley, Jr. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441–458.

Köhler, T., J. Huisman, L. A. Den Hartog, and R. Mosenthin. 1990. Comparison of different digesta collection methods to determine the apparent digestibilities of the nutrients at the terminal ileum in pigs. J. Sci. Food Agric. 53:465–475.

Leterme, P., A. Théwis, P. van Leeuwen, T. Monmart, and J. Huisman. 1996. Chemical composition of pea fibre isolates and their effect on the endogenous amino acid flow at the ileum of the pig. J. Sci. Food Agric. 72:127–134.

Livingstone, R. M., B. A. Baird, T. Atkinson, and R. M. J. Crofts. 1980. Circadian variation in the apparent digestibility of diets measured at the terminal ileum in pigs. J. Agri. Sci. 94:399–405.

Low, A. G., I. G. Partridge, and I. E. Sambrook. 1978. Studies on digestion and absorption in the intestines of growing pigs. 2. Measurements of the flow of dry matter, ash and water. Br. J. Nutr. 39:515–526.

McBurney, M. I., and W. C. Sauer. 1993. Fiber and large bowel energy absorption: Validation of the integrated ileostomy-fermentation model using pigs. J. Nutr. 123:721–727.

Moughan, P. J., and G. Schuttert. 1991. Composition of nitrogen containing fractions in digesta from the distal ileum of pigs fed a protein-free diet. J. Nutr. 121:1570–1574.

Moughan, P. J., G. Schuttert, and M. Leenaars. 1992. Endogenous amino acid flow in the stomach and small intestine of the young growing pig. J. Sci. Food Agric. 60:437–442.

Mroz, Z., A. W. Jongbloed, P. A. Kemme, H. Everts, A. M. van Vuuren, and R. Hoste. 1991. Preliminary evaluation of a new cannulation technique (steered ileo-caecal valve) for quantitative collection of digesta from the small intestine of pigs. In: M. W. A. Verstegen, J. Huisman, and L. A. den Hartog, editors, Digestive physiology in pigs. EAAP Publ. No. 54. Pudoc Wageningen, Netherlands. p. 334–339.

NRC. 1998. Nutrient requirements of swine. 10th ed. Natl. Acad. Press, Washington, DC.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Radcliffe, J. S., J. P. Rice, R. S. Pleasant, and G. A. Apgar. 2005. Technical Note: Improved technique for fitting pigs with steered ileocecal valve cannulas. J. Anim. Sci. 83:1563–1567.

Sauer, W. C., and K. de Lange. 1992. Novel methods for determining protein and amino acid digestibilities in feedstuffs. In: S. Nissen, editor, Modern methods in protein nutrition and metabolism. Academic Press, San Diego, CA. p. 87–120.

Sauer, W. C., and L. Ozimek. 1986. Digestibility of amino acids in swine: Results and their practical applications. A review. Livest. Prod. Sci. 15:367–388.

Stein, H. H., S. Aref, and R. A. Easter. 1999. Comparative protein and amino acid digestibilities in growing pigs and sows. J. Anim. Sci. 77:1169–1179.

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172–180.

Stein, H. H., C. F. Shipley, and R. A. Easter. 1998. Technical Note: A technique for inserting a T-cannula into the distal ileum of pregnant sows. J. Anim. Sci. 76:1433–1436.

Taverner, M. R., I. D. Hume, and D. J. Farrell. 1981. Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal grains. Br. J. Nutr. 46:149–158.

van Leeuwen, P., G. J. M. van Kempen, A. J. M. Jansman, and M. W. A. Verstegen. 1997. Feeding frequency and the length of digesta collection periods in ileal digestibility experiments. In: J.-P. Laplace, C. Février, and A. Barbeau, editors, Proc. VII Int. Symp. Digest. Physiol. Pigs, Publ. No. 88, Eur. Assoc. Anim. Prod., INRA, St. Malo. p. 540–543.

van Leeuwen, P., D. J. van Kleef, G. J. M. van Kempen, J. Huisman, and M. W. A. Verstegen. 1991. The post valve T-caecum cannulation technique in pigs applicated to determine the digestibility of amino acid in maize, groundnut and sunflower meal. J. Anim. Physiol. Anim. Nutr. 65:183–193.

Zebrowska, T. 1973. Influence of dietary protein source on the rate of digestion in the small intestine of pigs. Part 1. Amount and composition of digesta. Rocz. Nauk Roln. B 95:115–132.