Published March 3, 2017

Effects of ileal digesta collection time on standardized ileal digestibility of amino acids in corn, soybean meal, and distiller's dried grains with solubles fed to growing pigs

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ABSTRACT: The objective of this experiment was to determine the minimum collection time needed to obtain representative samples of ileal digesta from pigs fed different types of diets. 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 per square. Three diets contained corn, soybean meal (SBM), or distiller's dried grains with solubles (DDGS) as the sole source of CP. 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. When pigs were fed the corn diet, the standardized ileal digestibility (SID) of most indispensable AA and dispensable AA increased and then decreased (quadratic, $P < 0.05$) during each of the six 2-h periods. The SID of most AA were less ($P < 0.05$) if the ileal digesta samples were collected from 1800 to 2000 h or from 1600 to 2000 h compared with the SID values from the ileal samples collected over the entire 12-h period. When pigs were fed the SBM diet, the SID of Ile, Leu, Thr, Trp, Val, Ala, and Cys increased and then decreased (quadratic, $P < 0.05$) but the SID of all other AA linearly decreased ($P < 0.05$) during the six 2-h periods. The SID of almost all AA were greater ($P < 0.05$) if the ileal samples were collected from 0800 to 1000 h, from 0800 to 1200 h, from 1000 to 1400 h, or from 0800 to 1400 h but less ($P < 0.05$) if collected from 1600 to 1800 h, from 1800 to 2000 h, from 1600 to 2000 h, or from 1400 to 2000 h compared with the SID values from the ileal samples collected over the entire 12-h period. When pigs were fed the DDGS-based diet, the SID of Ile, Leu, Lys, Phe, Trp, Val, and Tyr increased and then decreased (quadratic, $P < 0.05$) during the six 2-h periods. The SID of most AA were less ($P < 0.05$) if the ileal samples were collected from 1800 to 2000 h compared with the SID values from the ileal samples collected over the entire 12-h period. In conclusion, diurnal variations in SID of AA in pigs indicate that 6 h of ileal sample collection starting 4 or 6 h after feeding may provide representative samples of ileal digesta from pigs fed different types of diets.

Key words: amino acids, collection times, diurnal variation, pigs, standardized ileal digestibility

 J. Anim. Sci. 2017.95:789–798
doi:10.2527/jas2016.1082

INTRODUCTION

Collection of ileal digesta in experiments to determine the standardized ileal digestibility (**SID**) of AA usually takes place over a period of time following a meal, and a subsample is then collected for analysis. However, it is not known how long the time of digesta collection needs to be, and digesta collection in 24-h periods has been described (de Lange et al., 1989; Marty et al., 1994; Hodgkinson et al., 2000; Urbaityte et al., 2009), whereas others have used 12-h collection periods (Rudolph et al., 1983; Fastinger and Mahan, 2003; Bohlke et al., 2005; Opapeju et al., 2006; Lan et al., 2008). Collection of digesta for 10 h (Stein et al., 2005) or only 8 h (Woodworth et al., 2001; Baker and Stein, 2009; Almeida et al., 2011; Kim et al., 2012) has also been used. In most cases, digesta collections are initiated immediately after feeding the morning meal, but more sophisticated collection schedules with digesta collection at 6 time points throughout the day (Mariscal-

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Received September 30, 2016.
Accepted January 2, 2017.

Landín and Reis de Souza, 2006) or in alternating 8-h periods (Caine et al., 1997) have also been described.

The reason for the reported differences in collection protocols among experiments is most likely that diurnal differences in the flow of DM and Cr have been described (Graham and Åman, 1986; Jørgensen et al., 1997; Hodgkinson et al., 2002; Kim et al., 2016), and it is, therefore, not known how long the digesta collection period needs to be to obtain a representative sample. From a human welfare and an economic point of view, it is, however, desirable that the collection period is as short as possible, but it is not known how a shorter collection time may impact calculated values for AA digestibility in pigs. Therefore, in this experiment, we tested the hypothesis that differences in the diurnal flow of AA to the distal ileum results in variable values for SID of AA. The objective was to determine the minimum collection time needed to obtain representative samples of ileal digesta from pigs fed different types of diets.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Care and Use Committee at the University of Illinois. Eight growing barrows (34.6 ± 2.1 kg initial BW) equipped with a T-cannula in the distal ileum were randomly allotted to a repeated 4 × 4 Latin square design with 4 diets and four 7-d periods in each square. Three of the diets were based on corn (97.0%), soybean meal (**SBM**) and cornstarch, or distiller's dried grains with solubles (**DDGS**) and cornstarch, and an N-free diet that was used to determine basal ileal endogenous losses of CP and AA was also included in the experiment. All diets also contained 0.50% chromic oxide. Further details about ingredient compositions and diet formulations have been described (Kim et al., 2016). Pigs were fed their respective diets at 3 times the estimated requirement for maintenance ME, and daily feed allotments were provided in equal meals at 0800 and 2000 h, and feed provisions were adjusted at the beginning of each period when individual pig BW were recorded. Ileal digesta was collected in 6 consecutive 2-h periods from 0800 to 2000 h on d 5, 6, and 7 of each collection period, and digesta from the 3 collection days was mixed within animal and collection period. Subsamples of digesta from each collection period were then lyophilized, and digesta and diet samples were analyzed for DM, CP, and AA as described by Kim et al. (2016).

Calculation and Statistical Analysis

To calculate the Cr, DM, and AA concentrations for 4-, 6-, 8-, 10-, and 12-h collection periods, the concentrations of Cr, DM, and AA were calculated as described by Kim et al. (2016). Values for SID of AA were calculated as previously described (Stein et al., 2007). Data for SID of AA were analyzed using MIXED procedures 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, quadratic, and cubic effects of collection time for 2-h periods. The PDIFF option was used to compare digestibility values from the 12-h collection period with digestibility values from other collection periods. The pig was considered the experimental unit. An α level of 0.05 was used to determine statistical significances among means.

RESULTS AND DISCUSSION

Diurnal variation in the composition of ileal digesta has been reported (Graham and Åman, 1986; Jørgensen et al., 1997; Hodgkinson et al., 2002), and it has been suggested that ileal samples should be collected for 8 h over 2 consecutive days. Other collection times have also been used, with the most labor-intensive procedures involving 24-h collections (de Lange et al., 1989; Hodgkinson et al., 2000; Urbaityte et al., 2009), and specific collection times are often laboratory specific with one laboratory preferring one schedule and another laboratory usually using a different schedule. There are, however, very few data available to demonstrate that one collection schedule is preferable over another, and it is also not known if the same schedule provides the best results for all ingredients. As a consequence, to increase accuracy of the SID values that are calculated from the digesta collected, there is a need to investigate the times of digesta collection that provides the most representative values for digestibility of AA. However, because differences among ingredients and diets in terms of digesta passage rate and possibly protein digestibility rate may exist, it is possible that the optimum collection schedules may be different among different ingredients. In the present work, an attempt to identify the optimum collection times for a cereal grain, an oilseed meal, and a high-fiber ingredient was made to avoid making conclusions based on one type of diet that may not be representative for other types of diets.

The SID values of AA in corn (Tables 1 and 2) that were calculated for the entire 12-h period were in close agreement with previous published values (Sauvant et al., 2004; Stein et al., 2005; Yin et al., 2008; NRC, 2012). The SID of Ile, Leu, Met, Phe, Thr, Trp, Val, and total indispensable AA increased and then decreased (quadratic, $P < 0.05$) and the SID of Lys decreased (linear, $P < 0.05$) whereas the SID of Arg did not change over the six 2-h collection periods. The SID of Ala, Asp, Cys, Glu, and Tyr increased and then decreased (quadratic, $P <$

Table 1. Standardized ileal digestibility (%) of indispensable AA calculated based on collection of ileal digesta during different time periods from pigs fed a corn-based diet[1,2], as-fed basis

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	TIAA[3]
2-h periods											
0800 to 1000 h	91.0	87.3	77.1	85.9	90.2**	82.5	84.6	65.0	61.8	76.9	83.7
1000 to 1200 h	88.5	86.5	77.0	85.4	87.7	84.2	84.4	64.0	66.0	75.7	82.7
1200 to 1400 h	92.4	90.4*	84.0*	90.3**	86.4	88.1*	89.4*	75.6	76.7	82.3*	87.2
1400 to 1600 h	94.4	86.9	76.9	86.4	84.4	83.9	84.9	66.5	67.8	75.5	83.3
1600 to 1800 h	93.0	85.9	73.2	83.6	83.7	80.6*	82.3	60.2	60.8	72.3	80.7
1800 to 2000 h	88.5	83.0**	67.2**	78.9**	82.9**	76.4**	77.4**	52.4**	49.9**	66.4**	76.3**
Linear	0.91	0.07	0.01	<0.01	<0.01	0.02	0.01	0.06	0.07	<0.01	0.02
Quadratic	0.19	0.06	0.01	<0.01	0.56	0.01	0.01	0.03	0.01	0.03	0.04
4-h periods											
0800 to 1200 h	88.9	86.1	75.4	84.6	87.6	82.8	83.3	61.3	61.5	74.4	81.9
1000 to 1400 h	91.5	88.9	81.3	88.4	87.2	86.7	87.4	71.1	72.3	79.8	85.6
1200 to 1600 h	94.2	89.1	81.4	88.9	86.0	86.7	87.8	72.4	73.2	79.9	86.0
1400 to 1800 h	94.3	87.1	76.5	85.9	84.9	83.2	84.6	65.7	66.7	75.4	83.1
1600 to 2000 h	91.7	85.2	71.9**	82.5**	83.9	79.6**	81.1**	58.8*	58.4*	71.1**	79.8*
6-h periods											
0800 to 1400 h	91.5	88.4	79.6	87.4	87.3	85.4	86.3	68.3	68.9	78.4	84.7
1000 to 1600 h	93.6	88.8	80.9	88.3	86.9	86.5	87.3	71.4	72.8	79.4	85.7
1200 to 1800 h	93.5	88.5	79.6	87.8	85.7	85.3	86.6	69.9	70.7	78.3	84.9
1400 to 2000 h	93.7	87.1	76.3	85.5	85.5	83.0	84.3	65.6	66.0	75.3	82.9
8-h periods											
0800 to 1600 h	93.3	88.4	79.8	87.7	86.9	85.6	86.5	69.4	70.5	78.5	85.0
1000 to 1800 h	93.1	88.3	79.6	87.6	86.4	85.4	86.5	69.6	70.8	78.3	84.8
1200 to 2000 h	93.0	88.3	79.2	87.3	86.1	85.0	86.2	69.5	69.8	77.9	84.6
10-h periods											
0800 to 1800 h	92.8	88.0	78.8	87.1	86.4	84.8	85.9	68.1	69.0	77.6	84.3
1000 to 2000 h	92.6	88.1	79.0	87.1	86.5	85.0	86.0	69.0	69.7	77.7	84.5
12-h period											
0800 to 2000 h	92.3	87.8	78.2	86.6	86.4	84.4	85.4	67.4	68.0	77.0	83.9
Pooled SEM	2.3	1.6	2.9	1.8	1.7	2.0	2.0	4.9	5.2	2.9	2.1

[1]Each least squares mean represents 8 observations.

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

[3]TIAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

0.05) and the SID of Gly decreased and then increased (quadratic, $P < 0.05$) whereas the SID of Ser decreased (linear, $P < 0.05$) over the six 2-h periods. However, no differences were observed in the SID of Pro, total dispensable AA, and CP over the six 2-h periods.

The SID values of AA in SBM (Tables 3 and 4) that were calculated for the 12-h collection period are in agreement with values reported by Goebel and Stein (2011), NRC (2012), and Baker et al. (2014). The SID of Lys and the concentration of Lys in the SBM that was used in this experiment indicate that the SBM was not heat damaged, because heat damage will reduce both the concentration and digestibility of Lys (Fontaine et al., 2007; González-Vega et al., 2011). The SID of Ile, Leu, Thr, Trp, and Val increased and then decreased (quadratic, $P < 0.05$) and the SID of His, Lys, Met, Phe, and total indispensable AA linearly decreased ($P < 0.05$) whereas the SID of Arg did not change over the six 2-h collection periods. The SID of Ala and Cys increased and then decreased (quadratic, $P < 0.05$) and the SID of Asp, Glu, Gly, Ser, Tyr, and CP linearly decreased ($P < 0.05$) over the six 2-h collection periods. However, no differences were observed in the SID of Pro and total dispensable AA over the six 2-h collection periods.

The SID values of AA in DDGS (Tables 5 and 6) are consistent with published values (Pahm et al., 2008; Urriola et al., 2009; NRC, 2012). The SID of Lys and the concentration of Lys in DDGS also indicate that the DDGS used in this experiment was not heat damaged (Fontaine et al., 2007; Pahm et al., 2008; Almeida et al., 2013). The SID of Ile, Leu, Lys, Phe, Trp, and Val decreased and then increased (quadratic, $P < 0.05$) whereas no differences were observed in the SID of Arg, His, Met, Thr, and total indispensable AA over the six 2-h collection periods. The SID of Cys linearly decreased ($P < 0.05$) and the SID of Gly and Pro decreased and

Table 2. Standardized ileal digestibility (%) of dispensable AA and CP calculated based on collection of ileal digesta during different time periods from pigs fed a corn-based diet[1,2], as-fed basis

Item	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	TDAA[3]	CP
2-h periods										
0800 to 1000 h	78.8	71.1	78.8	86.2	107.0**	100.8	81.1	84.7	86.6	77.6
1000 to 1200 h	80.4	71.5	78.2	84.8	75.8	87.6	78.1	82.6	81.4	70.1
1200 to 1400 h	87.3*	78.5*	83.8*	88.5	75.0	54.4*	82.2	87.6*	79.9	78.4
1400 to 1600 h	83.0	70.6	77.8	84.7	73.2	97.0	78.5	83.4	83.0	75.1
1600 to 1800 h	78.6	65.6*	74.5*	82.8	74.3	109.2	75.8	80.3	82.6	72.2
1800 to 2000 h	72.9**	61.2**	69.7**	79.1**	74.9	119.6	72.0**	75.1**	81.0	67.6**
Linear	0.09	0.02	<0.01	<0.01	<0.01	0.24	0.04	<0.01	0.56	0.15
Quadratic	<0.01	0.04	0.02	0.05	0.02	0.11	0.29	0.03	0.58	0.40
4-h periods										
0800 to 1200 h	79.2	69.6	77.3	84.6	78.4	97.5	77.5	82.0	82.6	70.8
1000 to 1400 h	85.1	76.1	81.6	87.1	77.2	75.6	80.9	85.6	82.1	76.1
1200 to 1600 h	86.2	75.5	81.7	87.1	76.7	80.8	81.1	86.1	83.0	78.1
1400 to 1800 h	82.2	69.7	77.3	84.6	75.1	103.5	78.3	82.9	83.9	75.5
1600 to 2000 h	77.2**	65.0**	73.3**	82.0**	74.5	110.9	75.2	79.0**	82.2	71.4
6-h periods										
0800 to 1400 h	83.5	74.3	80.5	86.7	79.7	87.4	80.2	84.7	83.5	75.9
1000 to 1600 h	85.5	75.5	81.3	86.9	78.3	86.8	81.2	85.7	83.9	77.5
1200 to 1800 h	84.3	73.2	80.1	86.2	75.5	86.2	80.0	84.9	82.7	76.9
1400 to 2000 h	81.7	69.9	77.1	84.5	75.8	103.2	78.5	82.5	83.7	75.4
8-h periods										
0800 to 1600 h	84.5	74.3	80.6	86.6	79.9	94.0	80.7	85.0	84.7	77.2
1000 to 1800 h	84.1	73.7	80.1	86.2	76.6	88.6	80.2	84.8	83.3	76.4
1200 to 2000 h	83.6	73.0	79.5	86.0	75.5	87.2	80.0	84.4	82.6	76.6
10-h periods										
0800 to 1800 h	83.3	72.8	79.6	86.0	77.7	93.0	79.9	84.3	83.7	76.2
1000 to 2000 h	83.4	73.1	79.5	85.9	76.3	88.9	80.0	84.3	82.9	76.0
SEM	1.3	1.6	0.7	0.6	7.7	23.3	1.7	0.6	4.5	2.1
12-h period										
0800 to 2000 h	82.6	72.2	79.0	85.6	77.0	92.8	79.6	83.8	83.2	75.6
Pooled SEM	2.5	3.5	2.4	1.7	6.9	20.3	2.9	2.1	4.2	3.6

[1]Each least squares mean represents 8 observations.

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

[3]TDAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

then increased (quadratic, $P < 0.05$) whereas the SID of Tyr increased and then decreased (quadratic, $P < 0.05$) over the six 2-h periods. However, no differences were observed in the SID of Ala, Asp, Glu, Ser, total dispensable AA, and CP over the six 2-h collection periods.

The observation that for almost all AA and regardless of ingredient, differences in SID among the six 2-h periods were observed indicates that diurnal variation in the SID of AA in pigs does exist, which is in agreement with previous data (Jørgensen et al., 1997). The maximum SID values were observed 4 to 8 h after the meal, and this observation is also consistent with the diurnal variation in the concentration of Cr in ileal samples collected in each of the six 2-h periods (Kim et al., 2016). 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, different diurnal variations were observed when pigs were fed different diets, which may be due to the different chemical composition of ileal digesta, which is affected by the type of diet (Low et al., 1978; Livingstone et al., 1980). In the present experiment, the average concentration of Cr and the average DM flow in ileal digesta collected over the entire 12-h period were also different among pigs fed the corn-based diet, the SBM-based diet, or the DDGS-based diet (Kim et al., 2016), which indicates that the flow of DM through the stomach and small intestine is influenced by the type of diet being fed.

When pigs were fed the corn-based diet, the SID of His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, and total indispensable AA were less ($P < 0.05$) if the ileal samples were collected from 1800 to 2000 h and the SID of His, Ile, Leu, Met, Phe, and Val tended to be greater ($P < 0.10$)

Table 3. Standardized ileal digestibility (%) of indispensable AA calculated based on collection of ileal digesta during different time periods from pigs fed a soybean meal–based diet[1,2], as-fed basis

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	TIAA[3]
2-h periods											
0800 to 1000 h	98.1	95.6**	91.4	90.6	94.9	92.2	92.4	86.1	92.8	89.9	92.7
1000 to 1200 h	97.7	95.8**	93.9**	93.5**	97.2**	93.7**	94.6**	91.0**	95.4**	93.2**	94.9**
1200 to 1400 h	97.9	94.2	91.3	90.8	95.0	92.0	92.0	86.5	94.3	90.2	92.7
1400 to 1600 h	97.4	93.9	90.4	89.9	93.8	91.9	91.4	84.7	93.2	89.1	91.8
1600 to 1800 h	97.2	92.2**	86.9**	85.8**	90.5**	89.1**	88.3**	77.1**	90.4**	84.8**	88.6**
1800 to 2000 h	96.8	92.6**	87.1**	86.1**	91.3**	88.5**	88.6**	78.1**	89.7**	85.2**	88.8**
Linear	0.23	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.80	0.89	0.05	0.03	0.08	0.07	0.12	0.03	<0.01	0.04	0.07
4-h periods											
0800 to 1200 h	97.5	95.5**	93.0**	92.5**	96.4**	93.2	93.8**	89.1**	94.3	92.0**	94.0**
1000 to 1400 h	98.5	95.3*	93.0**	92.4**	96.2**	93.2	93.5**	89.2**	94.9**	92.0**	94.1**
1200 to 1600 h	97.7	94.1	90.9	90.4	94.4	92.0	91.7	85.7	93.8	89.7	92.3
1400 to 1800 h	97.3	93.5	89.6*	88.9*	92.9**	91.2	90.7*	82.8*	92.5	88.1	91.0*
1600 to 2000 h	97.3	92.5**	87.2**	86.3**	91.0**	89.0**	88.7**	78.1**	90.3**	85.3**	88.9**
6-h periods											
0800 to 1400 h	98.2	95.2	92.6**	92.0**	95.9**	92.9	93.2*	88.4**	94.4*	91.6*	93.8**
1000 to 1600 h	98.2	94.8	92.2	91.6	95.5	92.8	92.8	87.8	94.4*	91.1	93.4
1200 to 1800 h	97.4	93.8	90.3	89.6	93.7	91.6	91.2	84.3	93.2	88.9	91.7
1400 to 2000 h	97.2	93.4	89.2**	88.5**	92.7**	90.8	90.4**	82.1**	92.0*	87.7**	90.7**
8-h periods											
0800 to 1600 h	98.0	94.8	92.0	91.4	95.3	92.7	92.7	87.4	94.1	90.9	93.2
1000 to 1800 h	97.8	94.5	91.5	90.9	94.8	92.3	92.3	86.4	93.8	90.3	92.8
1200 to 2000 h	97.3	93.7	90.0	89.3	93.5	91.2	91.0	83.6	92.8	88.6	91.4
10-h periods											
0800 to 1800 h	97.7	94.4	91.4	90.8	94.7	92.2	92.2	86.1	93.6	90.2	92.6
1000 to 2000 h	97.6	94.3	91.1	90.5	94.5	92.0	92.0	85.7	93.4	89.9	92.4
12-h period											
0800 to 2000 h	97.5	94.3	91.0	90.4	94.5	91.9	91.9	85.5	93.3	89.8	92.3
Pooled SEM	0.8	0.6	0.7	0.8	0.7	0.8	0.7	1.4	0.6	0.9	0.7

[1]Each least squares mean represents 8 observations.

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

[3]TIAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

if the ileal samples were collected from 1200 to 1400 h compared with the SID values for the entire 12-h period. Compared with the SID values when ileal samples were collected over the entire 12-h period, the SID of Ile, Leu, Met, Phe, and Val were less ($P < 0.05$) and the SID of Thr, Trp, and total indispensable AA tended to be less ($P < 0.10$) when the ileal samples were collected from 1600 to 2000 h. For the dispensable AA, the SID of Ala, Asp, Cys, Glu, Ser, Tyr, and CP were less ($P < 0.05$) if the ileal samples were collected from 1800 to 2000 h and the SID of Ala, Asp, Cys, Glu, and Tyr were less ($P < 0.05$) if the ileal samples were collected from 1600 to 2000 h but the SID of Gly was greater ($P < 0.05$) if the ileal samples were collected from 0800 to 1000 h compared with the SID value when the ileal samples were collected over the entire 12-h period. No differences were observed in SID values from the ileal samples collected in 6-, 8-, or 10-h periods compared with the ileal samples collected over the entire 12-h period, indicating that 6 h of ileal sample collection starting right after feeding or 2 or 6 h after feeding may provide representative samples of ileal digesta from pigs fed a corn-based diet.

If pigs were fed the SBM-based diet, the SID of all indispensable AA except Arg was greater ($P < 0.05$) when the ileal samples were collected from 1000 to 1200 h but less ($P < 0.05$) when the ileal samples were collected from 1600 to 1800 h or from 1800 to 2000 h compared with the SID values from the ileal samples collected over the entire 12-h period. Therefore, the SID of almost all indispensable AA were greater ($P < 0.05$) if the ileal samples were collected from 0800 to 1200 h, from 1000 to 1400 h, or from 0800 to 1400 h but less ($P < 0.05$) if samples were collected from 1600 to 2000 h or from 1400 to 2000 h compared with the SID values from samples collected over the entire 12-h period. The SID of almost all dispensable AA were greater ($P < 0.05$) if samples

Table 4. Standardized ileal digestibility (%) of dispensable AA and CP calculated based on collection of ileal digesta during different time periods from pigs fed a soybean meal–based diet[1,2], as-fed basis

Item	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	TDAA[3]	CP
2-h periods										
0800 to 1000 h	86.5	91.0**	88.2	94.4**	99.6**	111.5	90.7*	92.9*	94.5	91.6
1000 to 1200 h	92.3**	92.1**	91.0**	94.4**	90.3	95.0	92.6**	94.4**	93.2	92.4*
1200 to 1400 h	89.2	89.4	89.3	92.5	91.4	97.5	88.4	91.6	91.5	89.9
1400 to 1600 h	87.8	87.4	87.6	91.7	87.8	101.0	87.5	91.0	90.5	88.7
1600 to 1800 h	83.8**	83.9**	82.1**	90.1**	84.6	129.1**	84.2**	87.9**	90.8	85.8**
1800 to 2000 h	83.1**	85.0**	82.8**	91.1**	85.6	123.9*	85.2**	88.2**	90.9	86.6**
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	0.07	<0.01	<0.01	0.10	<0.01
Quadratic	0.02	0.71	0.02	0.38	0.20	0.12	0.93	0.37	0.38	0.92
4-h periods										
0800 to 1200 h	90.6	91.6**	89.8*	94.2**	89.8	98.0	91.5**	93.6**	92.9	91.4
1000 to 1400 h	92.0**	91.2**	90.5**	93.7*	93.0	107.5	91.1**	93.2**	94.0	92.2*
1200 to 1600 h	88.7	88.5	88.6	92.2	90.0	100.7	88.0	91.4	91.2	89.5
1400 to 1800 h	86.7	86.5**	86.3	91.4*	87.1	107.3	86.9	90.3	90.6	88.1
1600 to 2000 h	83.9**	84.6**	82.8**	90.7**	86.4	128.6**	85.1**	88.4**	91.3	86.6**
6-h periods										
0800 to 1400 h	91.0	91.1**	89.9*	93.7*	92.1	105.6	90.7*	93.0*	93.5	91.6
1000 to 1600 h	90.7	90.0	89.6	93.0	91.2	105.1	89.9	92.5	92.8	91.1
1200 to 1800 h	87.6	87.7	87.5	91.8	88.3	101.8	87.4	90.8	90.7	88.7
1400 to 2000 h	86.1	86.4**	85.8**	91.4*	86.9	109.6	86.7*	90.0**	90.6	87.8
8-h periods										
0800 to 1600 h	90.2	90.0	89.3	93.1	90.8	104.5	89.8	92.4	92.7	90.9
1000 to 1800 h	89.5	89.2	88.6	92.7	89.5	103.7	89.2	91.9	92.0	90.1
1200 to 2000 h	87.1	87.4	87.0	91.8	88.0	103.5	87.3	90.6	90.7	88.5
10-h periods										
0800 to 1800 h	89.1	89.2	88.4	92.7	89.4	103.9	89.1	91.9	92.0	90.0
1000 to 2000 h	88.8	88.8	88.1	92.5	88.9	103.4	88.8	91.6	91.7	89.7
12-h period										
0800 to 2000 h	88.5	88.9	88.0	92.6	88.8	103.8	88.8	91.6	91.7	89.6
Pooled SEM	1.5	0.9	1.1	0.6	2.5	10.8	1.1	0.8	1.8	1.4

[1]Each least squares mean represents 8 observations.

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

[3]TDAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

were collected from 0800 to 1000 h, from 1000 to 1200 h, from 0800 to 1200 h, or from 1000 to 1400 h but less ($P < 0.05$) if samples were collected from 1600 to 1800 h, from 1800 to 2000 h, or from 1600 to 2000 h compared with the SID values from ileal samples collected over the entire 12-h period. However, the SID of Pro was greater ($P < 0.05$) if the ileal samples were collected from 1600 to 1800 h or from 1600 to 2000 h compared with the SID of Pro from the ileal samples collected over the 12-h period. No differences were observed in SID values from the ileal samples collected in 8- or 10-h periods compared with the ileal samples collected over the entire 12-h period, regardless of the start of the collection period. These observations indicate that 6 h of ileal sample collection starting 4 to 6 h after feeding may provide representative samples of ileal digesta from pigs fed a SBM-based diet. However, if sample collection is initiated right after feeding, collection for at least 8 h is needed.

When pigs were fed the DDGS-based diet, the SID of all indispensable AA except Arg was less ($P < 0.05$) if the ileal samples were collected from 1800 to 2000 h but the SID of Arg was greater ($P < 0.05$) if the ileal samples were collected from 0800 to 1000 h compared with values for samples collected over the entire 12-h period. For the dispensable AA, the SID of Gly, Pro, Ser, and total dispensable AA was greater ($P < 0.05$) if samples were collected from 0800 to 1000 h but the SID of Asp, Cys, Glu, and Tyr was less ($P < 0.05$) if the ileal samples were collected from 1800 to 2000 h compared with the entire 12-h period. No differences were observed in SID values from the ileal samples collected in 4-, 6-, 8-, or 10-h periods compared with collection over the entire 12-h period, regardless of the time sample collection started, which indicates that 4 to 6 h of ileal sample collection starting any time during the day provides representative samples of ileal digesta from pigs fed a DDGS-based diet.

Table 5. Standardized ileal digestibility (%) of indispensable AA calculated based on collection of ileal digesta during different time periods from pigs fed a diet based on distiller's dried grains with solubles[1,2], as-fed basis

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	TIAA[3]
2-h periods											
0800 to 1000 h	88.0**	82.0	77.3	86.2	64.2	83.7	83.4	70.7	80.2	76.0	80.3
1000 to 1200 h	80.3*	79.4	76.4	85.2	66.4	82.7	83.2	67.9	78.5*	75.4	78.7
1200 to 1400 h	82.2	81.3	78.7	86.3	67.9	81.9	84.5	69.7	81.2	77.7	80.3
1400 to 1600 h	83.8	81.2	78.0	86.3	66.9	84.0	84.2	71.7	82.9	76.9	80.4
1600 to 1800 h	86.1	81.1	77.7	86.4	64.0	84.1	84.1	71.3	84.3**	76.7	80.4
1800 to 2000 h	85.3	77.6**	72.5**	83.4**	59.0**	81.1**	80.2**	64.4**	78.1**	71.6**	76.5**
Linear	0.75	0.08	0.05	0.12	0.04	0.40	0.09	0.24	0.51	0.08	0.17
Quadratic	0.09	0.24	0.01	0.05	0.01	0.49	0.01	0.10	0.05	<0.01	0.10
4-h periods											
0800 to 1200 h	83.3	80.1	76.5	85.3	65.9	83.1	83.1	68.3	78.6*	75.4	79.1
1000 to 1400 h	81.6	80.3	77.5	85.7	67.1	82.2	83.7	68.7	79.7	76.5	79.5
1200 to 1600 h	83.0	81.3	78.4	86.3	67.6	82.9	84.4	70.8	82.0	77.4	80.4
1400 to 1800 h	84.9	81.2	77.9	86.3	65.5	84.1	84.1	71.5	83.6**	76.8	80.4
1600 to 2000 h	86.1	79.9	75.9	85.4	62.2*	83.1	82.7	68.9	82.2	74.9	79.1
6-h periods											
0800 to 1400 h	83.2	80.6	77.3	85.7	66.6	82.4	83.6	68.7	79.7	76.3	79.6
1000 to 1600 h	82.4	80.6	77.7	85.9	67.2	82.8	83.9	69.7	80.7	76.6	79.8
1200 to 1800 h	83.8	81.2	78.2	86.4	66.5	83.3	84.3	70.9	82.8	77.1	80.4
1400 to 2000 h	85.3	80.5	76.9	85.8	64.1	83.6	83.4	70.3	82.6	75.8	79.7
8-h periods											
0800 to 1600 h	83.4	80.7	77.5	85.9	66.7	82.9	83.8	69.6	80.5	76.5	79.9
1000 to 1800 h	83.1	80.7	77.7	86.0	66.5	83.1	83.9	70.1	81.5	76.6	79.9
1200 to 2000 h	84.2	80.8	77.4	86.0	65.3	83.0	83.8	70.2	82.2	76.4	79.9
10-h periods											
0800 to 1800 h	83.7	80.8	77.5	86.0	66.2	83.1	83.8	69.9	81.2	76.5	79.9
1000 to 2000 h	83.5	80.4	77.2	85.8	65.6	82.9	83.6	69.7	81.2	76.1	79.6
12-h period											
0800 to 2000 h	84.0	80.5	77.1	85.7	65.4	83.0	83.5	69.5	81.0	76.0	79.6
Pooled SEM	1.9	1.0	1.0	0.7	1.8	0.8	0.8	1.6	1.2	1.1	1.0

[1]Each least squares mean represents 8 observations.

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

[3]TIAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

The reason for the differences that were observed among diets for collection times are likely due to the high concentration of fiber in DDGS compared with corn and SBM (NRC, 2012), because increased fiber concentration in the diet may increase rate of passage and reduce transit time (Ehle et al., 1982; Kuan et al., 1983) and, therefore, increase the flow of DM to the distal ileum of pigs (Kim et al., 2016). Therefore, it appears that a more constant flow of AA reaches the distal ileum in pigs fed high-fiber diets compared with pigs fed diets containing less fiber. As a consequence, choosing the correct collection times relative to feeding of test diets is more critical if diets with a lower concentration of fiber are fed compared with high-fiber diets. It is, however, important to note that only collection times relative to feeding were considered in the present experiment. Other possible factors that may or may not impact the outcome of digestibility experiments include different digestion patterns during the nocturnal period due to changes in pancreatic enzyme secretions (Thaela et al., 1995, 1998) or diurnal and nocturnal patterns of endocrine secretions (Bodosi et al., 2004). Under the conditions of the present experiment, possible impacts of these factors could not be determined. However, to reduce day-to-day variations in digesta flow, digesta were collected over 3 consecutive days and results, therefore, represent the data for the average of 3-d collections.

In conclusion, diurnal variations in calculated values for SID of AA were observed, but the pattern of variation depends on the diet being fed. The SID values are less variable during the day if pigs are fed a DDGS-based diet compared with a corn-based diet or a SBM-based diet. However, regardless of diet being fed, it appears that collection of ileal digesta for 6 h starting approximately 4 h after feeding will result in values for SID of CP and AA that are representative

Table 6. Standardized ileal digestibility (%) of dispensable AA and CP calculated based on collection of ileal digesta during different time periods from pigs fed a diet based on distiller's dried grains with solubles[1,2], as-fed basis

Item	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	TDAA[3]	CP
2-h periods										
0800 to 1000 h	79.8	71.4	76.4	83.9*	78.4**	81.8**	81.5**	84.8	80.5**	73.6
1000 to 1200 h	78.7	68.4	73.8	81.8	55.0	51.7	75.9	84.5	71.9	68.8
1200 to 1400 h	79.9	70.1	75.2	82.9	59.0	53.6	76.6	85.3	73.4	70.0
1400 to 1600 h	79.9	70.4	75.6	82.9	59.7	61.0	78.8	85.0	74.9	71.5
1600 to 1800 h	80.3	69.8	75.6	83.2	62.0	66.3	79.1	84.4	76.0	72.8
1800 to 2000 h	77.8	64.8**	70.1**	80.1**	62.3	74.7	75.7	81.1**	74.8	68.7
Linear	0.68	0.07	0.05	0.07	0.11	0.86	0.19	0.03	0.52	0.52
Quadratic	0.46	0.27	0.17	0.35	<0.01	0.04	0.51	0.03	0.16	0.83
4-h periods										
0800 to 1200 h	79.4	69.3	74.5	82.3	61.3	67.5	77.5	84.4	75.5	70.4
1000 to 1400 h	79.4	69.1	74.3	82.3	57.4	55.4	76.2	84.8	73.1	69.6
1200 to 1600 h	80.0	70.3	75.5	82.9	59.6	57.6	77.8	85.2	74.3	70.9
1400 to 1800 h	80.1	70.1	75.6	83.1	60.9	63.6	79.1	84.8	75.5	72.2
1600 to 2000 h	79.6	68.1	73.7	82.2	62.5	70.1	78.0	83.4	75.8	71.5
6-h periods										
0800 to 1400 h	79.7	69.5	74.6	82.5	60.7	63.9	77.0	84.7	75.0	70.4
1000 to 1600 h	79.6	69.6	74.8	82.5	58.3	57.4	77.2	84.9	73.8	70.3
1200 to 1800 h	80.0	70.1	75.6	83.0	60.0	59.3	78.2	85.0	74.6	71.4
1400 to 2000 h	79.8	69.2	74.6	82.6	61.7	67.1	78.6	84.2	75.7	71.7
8-h periods										
0800 to 1600 h	79.8	69.8	74.9	82.6	60.5	63.4	77.6	84.8	75.1	70.8
1000 to 1800 h	79.7	69.6	75.0	82.6	58.9	58.6	77.6	84.8	74.1	70.8
1200 to 2000 h	79.8	69.4	74.9	82.7	60.7	62.2	78.0	84.6	74.8	71.2
10-h periods										
0800 to 1800 h	79.8	69.7	75.1	82.7	60.3	62.7	77.8	84.7	75.0	71.0
1000 to 2000 h	79.6	69.2	74.6	82.4	59.5	60.7	77.6	84.5	74.3	70.7
12-h period										
0800 to 2000 h	79.6	69.3	74.7	82.5	60.6	63.8	77.7	84.4	75.0	70.9
Pooled SEM	1.2	1.5	1.3	0.8	3.7	9.0	1.3	0.8	2.3	1.7

[1]Each least squares mean represents 8 observations.

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

[3]TDAA = total indispensable AA.

*Means different from the value for the 12-h period ($0.05 < P < 0.10$); **Means different from the value for the 12-h period ($P < 0.05$).

for the entire 12-h period between meals. By using a shorter collection schedule, the labor requirement for ileal digestibility experiments may be reduced.

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