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Effect of sampling technique on ileal pH, short chain fatty acids concentration and digestibility of peas in pigs

Nuria Canibe, K. E. Bach Knudsen and B.O. Eggum

Danish Institute of Animal Science, Department of Nutrition,
Research Centre Foulum
P.O. Box 39
DK-8830 Tjele, Denmark

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ABSTRACT

Two experiments were performed with pigs fed peas. Pigs were fitted with a T-shaped cannula at the end of the ileum. The animals were fed three times daily and two series of ileal digesta collections were carried out during the day and night. The ileal samples were pooled in three intervals: morning, evening and night. In Experiment 1 the samples were collected and frozen every 2-3 h during the morning and evening intervals, and every 9 h in the night interval, whereas in Experiment 2, the samples were collected on a continuous basis and frozen immediately.

In Experiment 1, lactic acid and short chain fatty acid (SCFA) concentrations, and the digestibilities of dry matter and non-starch polysaccharides increased from morning to night. The molar ratio of SCFA was also affected by the interval. In Experiment 2, no differences were observed in the mentioned parameters between intervals.

Results of the experiments indicate that there are no differences between day and night in digestibility of nutrients when pigs are fed pea-based diet. The collection of ileal digesta during 8 h for digestibility measurements is representative for 24 h period when animals were fed at 8 h intervals. Ileal samples of digesta should be collected frequently and frozen when pH, SCFA are to be measured.

KEY WORDS: sampling technique, ileal digesta, pigs

INTRODUCTION

Many studies have been carried out in recent years to obtain more detailed information on the digestive processes and site of absorption of nutrients in pigs (Żebrowska et al., 1978; Sauer and Ozimek, 1986; Abrahamsson et al., 1993).

These authors have shown that the determination of amino acid digestibility by collection of digesta at the terminal ileum is more accurate than that determined by analysis of faecal samples because of the modifying action of the microflora in the large intestine. Ceratin oligo- and polysaccharides escape digestion by endogenous enzymes and pass to the large intestine where they are fermented. Depending on their chemical and physical structure the digestion of these polysaccharides may vary between the small and large intestines (Bach Knudsen and Hansen, 1991; Abrahamsson et al., 1993).

Analysis of digesta taken from the end of the small intestine has been widely used to differentiate between the digestion of nutrients by the animal's enzymes and microbial fermentation. Prerequisites for this method are that the animals are in a physiological state and that samples of ileal chyme are representative.

In many studies dealing with ileal measurements, samples from the distal ileum are taken during a certain interval, normally during the day. It could be questioned whether that interval is representative for the digestive processes occurring in the animal throughout 24 h. It has been shown, for example, that ileal digesta composition varies with time after feeding (Graham and Åman, 1986; Jørgensen et al., in press).

On the other hand, the sampling procedure can also affect the results obtained, and can be of importance depending on the parameters under investigation (Bach Knudsen and Hessov, 1995).

In the course of our investigations (Experiment 1) we obtained results that we thought should be further clarified. When ileal digesta was collected from pigs over 24 h periods or in intervals (morning, evening, night), differences between certain parameters among intervals were observed. These differences could be related to circadian variations in the digestibility of nutrients of to the sampling technique used.

In an attempt to investigate the reasons for the differences observed in the samples taken at different intervals, a study (Experiment 2) was planned in which special focus was on the sampling technique. This point is of interest for future planning of trials involving ileal measurements, where questions like, How long should we collect ileal digesta to get a representative sample or, How often should we collect and freeze the samples, may be raised.

MATERIAL AND METHODS

Diets and feeding

Diets were formulated with peas (*Pisum sativum* ssp. *hortense*), cultivar Solara as the only source of polysaccharides and nitrogen. The peas were either dried to

a moisture content of ~15% or toasted (pea temperature, 130°C) for 3-4 min. The composition of the diets on a dry matter (DM) basis was: 661 g/kg peas (dried or toasted), 129 g/kg sucrose, 123 g/kg dextrose, 58 g/kg soyabean oil, 29 g/kg vitamins and minerals, and 1 g/kg (Experimental 1) or 2 g/kg (Experiment 2) chromic oxide.

Experiment 1

The animals were fed daily an amount of feed (kg as is) equivalent to 0.09 of the animal's metabolic weight (kg $^{0.75}$) at the beginning of the experiment, with increases of 10 g/d until the end of the experiment. The feed was offered three times daily, at 07.00, 15.00 and 22.00 h, in equal meals.

Experiment 2

The pigs were offered daily and amount of feed (kg as is) equivalent to 0.09 of the animal's metabolic body weight at the beginning of each digesta collection and it was kept constant. The feed was given in three equal meals, at 07.00, 15.00 and 23.00 h.

Experimental procedure

Experiment 1

Eight pigs, two litters of 4 pigs, weighing 35±6 kg, were fitted with a T shaped-cannula approximately 15 cm anterior to the ileo-caecal junction. The cannula was as described by Larsen and Sandstrm (1993). After a two-week recovery period, each litter was randomly divided into groups of two animals and each group was fed one of the experimental diets. The animals were offered the diets for a 10- day preliminary period, after which ileal digesta was collected for two days (2 x 24 h), on days 11 and 14. Ileal effluents were collected using plastic tubes leading to a container with ice. The tubes were fitted to the cannulas of the animals at 07.00 h the day of collection. The samples were collected at 09.00, 11.00, 13.00, 15.00, 17.00, 19.00, 22.00, and 07.00 h, frozen and stored at −20°C. After a second 10-day preliminary period, ileal digesta was collected following the same procedure as in the first collection period. At the end of the experiment, toluene was added to the samples to stop microbial activity, the collections were thawed overnight and subsamples taken for analyses.

Experiment 2

Eight randomly chosen pigs, with an initial weight of 35 ± 4 kg, were fitted with a simple T shaped-cannula as in Experiment 1. After a recovery period of two weeks, the animals were randomly divided into two groups of four pigs and each group was fed one of the experimental diets for 10 days.

Ileal effluents were collected during 24 h on two consecutive days, i.e. from 07.00 to 15.00 h (morning interval) on day 11, and from 23.00 to 07.00 h (night interval) and from 15.00 to 23.00 h (evening interval) on day 12. The samples were collected using plastic tubes attached to the cannula, removed and stored at -20^0C as soon as the tube was partially filled with digesta.

Two days later, a plastic tube was attached to the cannula of each animal after the morning meal, and when the tube was filled with digesta, the cannula was closed so that no more ileal effluent could come into the tube. At the time of closing the cannula (Time 0), the pH of the digesta in the collection tube was measured, a sample taken and the rest poured back in the collection tube. The tube with the remaining digesta was attached again to the closed cannula. In this way the ileal effluent could be in contact with the warm body of the animal, and we could mimic what occurs when the digesta samples are not removed. The same procedure was repeated at 90 (Time 90), 180 (Time 180) and 360 min (Time 360).

The pigs were crossed over to the other diet and after a preliminary period of 10 days, the same procedure was repeated.

The ileal digesta obtained from the morning, evening and night intervals was thawed quickly by immersing the container in hot water, and subsamples were taken for analyses. In this way microbial activity was prevented.

Analytical methods

Organic acids and chromic oxide were assayed in wet samples, remaining analyses were performed on freeze-dried material. Short chain fatty acids (SCFA) were determined according to a modification of the method of Fussell and McCalley (1987), and total lactic acid was analyzed enzymatically according to Noll (1984). Chromic oxide was measured using the procedure of Schürch et al. (1950). Non-starch polysaccharides (NSP) and their constituent sugars were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by a colorimetric method for uronic acids using a modification of the Uppsala and Englyst procedures as described by Bach Knudsen et al. (1993).

Statistical analysis

Experiment 1

The two experimental diets were randomized among the four littermates, with two littermates in each diet. The same was done with the two litters, litters then, represented blocks. The ileal samples collected during 48h (2x24 h) in each period were pooled in 3 intervals, i.e. 07.00-15.00 h, 15.00-22.00 h and 22.00-07.00 h, so that one value from each interval was obtained. The samples from the second collection period were treated in the same way. The average values obtained from each pig and interval in the two periods were used for the calculations.

The results were subjected to the following model to the test the effect of interval:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\delta\beta\gamma)_{ljk} + (\alpha\beta)_{ij} + \varepsilon_{ijkl}$$

where: Y = observed response; μ = overall average, α_i = effect of interval, i = 1,2,3; β_j = effect of diet; j = 1,2; γ_k = effect of block, k = 1,2; $(\delta\beta\gamma)_{ljk}$ = effect of interaction between pig, diet and block; $(\alpha\beta)_{ij}$ = effect of interaction between interval and diet; ε_{ijkl} = normally distributed random variable.

Experiment 2

The pig were randomly divided in two groups, each group was fed one of the experimental diets and crossed over to the other diet in the second collection period. The effect of interval (morning, evening, night) and sampling time (0, 90, 180, 360 min) were statistically analyzed following the model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\delta\beta\gamma)_{ljk} + \delta_l + (\alpha\beta)_{ij} + \varepsilon_{ijkl}$$

where: Y = observed response; μ = overall average, α_i = effect of diet, i = 1,2; β_j = effect of collection period, j = 1,2; δ_k = effect of pig, k = 1,...,8; $(\alpha\beta\gamma)_{ijk}$ = effect of interaction between diet, collection period and pig; δ_l = effect of interval, l = 1,2,3, or sampling time, l = 1,2,3,4; $(\alpha\beta)_{il}$ = effect of interaction between diet and interval or diet and sampling time; ε_{ijkl} = normally distributed random variable.

The results are presented as least square means with standard errors. In cases where the overall effect was significant, the means were compared using Fisher's least significant difference procedure (Milliken and Johnson, 1984). All calculations were performed with the GLM procedure (SAS, 1988).

RESULTS

Experiment 1

The concentration of lactic acid was similar in the morning (67 mmol/kg digesta) and evening (70 mmol/kg) intervals, whereas it was significantly higher during the night (93 mmol/kg) (Table 1). Total SCFA concentration was increased from morning to night, with values of 37, 53 and 73 mmol/kg during the morning, evening and night intervals, respectively. The molar proportion of acetic acid was lower in the evening (70%) and night (64%) compared to the morning (78%). The proportion of propionic acid was similar in the morning (13%) and evening intervals (17%), and significantly higher during the night interval (22%). No changes were observed for butyric acid its values were between 7 and 10%. The digestibility of DM increased from morning to night with the dried pea-containing diet ($p=0.001$) and the same tendency was observed with the toasted pea-containing diet, although the values were not significantly different ($p=0.18$). The interaction diet x interval was significant. The digestibility of NSP showed a similar trend, the values were – 7% in the morning, 15% in the evening and 27% in the night interval.

TABLE 1

Concentrations of organic acids (OA), lactic acid (LA), short chain fatty acids (SCFA) (mmol/kg digesta) and molar proportions of acetic, propionic and butyric acid in ileal effluents, and apparent ileal digestibilities of dry matter (DM) and non-starch polysaccharides (NSP) in the morning, evening and night intervals (Experiment 1)

	Morning	Evening	Night	RMSE[1]
OA				
LA, mmol/kg	67^{A}	70^{A2}	93^{B}	19.1
SCFA, mmol/kg	37^{A}	53^{B2}	73^{C}	12.0
Molar proportion, %				
acetic acid	78^{A}	70^{B2}	64^{B}	5.5
propionic acid	13^{A}	17^{A2}	22^{B}	4.1
butyric acid	7	10^{2}	9	2.7
DM digestibility[3], %	70.8	74.7	77.6	1.90
NSP digestibility, %	-7^{A}	15^{B}	27^{B}	12.0

[1] – root mean square error ($n=24$)

[2] – $n=7$

[3] – the interaction diet x interval was significant. Look at the text for details

values are least square means

means within a row followed by different letters differ $P<0.05$

Experiment 2

The concentration of lactic acid was similar in all intervals 80, 75 and 76 mmol/kg digesta in the morning, evening and night intervals, respectively (Table 2). Total SCFA concentration was between 10 and 13 mmol/kg digesta in the three intervals. The proportions of acetic acid was 82% in the morning and lower (77%) during the evening and night. The proportion of propionic acid amounted to 14-18% and that of butyric acid to 4-5% in all the intervals. The digestibility of DM and NSP was similar in the three intervals, with values of 71.5-72.6% for DM and 29-35% for NSP.

TABLE 2

Concentrations of organic acids (OA), lactic acid (LA), short chain fatty acids (SCFA) (mmol/kg digesta) and molar proportions of acetic, propionic and butyric acid in ileal effluents, and apparent ileal digestibilities of dry matter (DM) and non-starch polysaccharides (NSP) in the morning, evening and night intervals
(Experiment 2)

	Morning	Evening	Night	RMSE[1]
OA				
LA, mmol/kg	80	75	76	15.2
SCFA, mmol/kg	10	13	11	3.6
Molar proportion, %				
acetic acid	82[A]	77[B]	77[B]	5.7
propionic acid	14	18	17	6.6
butyric acid	4	4	5	2.5
DM digestibility[3], %	72.4	72.6	71.5	2.89
NSP digestibility, %	32	35[2]	29	13.6

[1] – root mean square error (n = 48)
[2] – n = 15
[3] – n-14
values are least square means
means within a row followed by different letters differ $P < 0.05$

Table 3 shows values obtained when digesta was left in the collection tubes and samples taken at different times. The concentration of lactic acid was 65 mmol/kg at Time 0, increasing to 72 mmol/kg by Time 90 min, to 81 mmol/kg by Time 180 min, and to 99 mmol/kg by Time 360 min. The concentration of SCFA also increased, from 13 mmol/kg at Time 0 min to 16 mmol/kg at Time 90 min. At Time 360 min, the value was 27 mmol/kg. The proportion of acetic acid decreased from 82% at Time 0 min to 78% at Time 90 min, to 76% at Time 180 min and to 73% at Time 360 min.

The propionic acid proportion increased over the same time with values from 15% at Time 0 to 22% after 360 min. The proportion of butyric acid was not

TABLE 3

Concentrations of organic acids (OA), lactic acid (LA), short chain fatty acids (SCFA) (mmol/kg digesta), molar proportions of acetic, propionic and butyric acid pH, and dry matter (DM) digestibility at the different sampling times

	Time				
	0	90 min	180 min	360 min	RMSE[1]
OA					
LA, mmol/kg	65^{A}	72^{B2}	81^{C2}	99^{D3}	6.2
SCFA, mmol/kg	13^{A}	16^{B2}	19^{B2}	27^{C2}	4.3
Molar proportion, %					
acetic acid	82^{A}	78^{Bb}	76^{C2}	73^{D2}	2.5
propionic acid	15^{A}	17^{B2}	20^{C2}	22^{D2}	2.3
butyric acid	3^{A}	4^{AB2}	4^{AB2}	4^{B2}	0.7
pH	6.4^{A}	6.1^{B}	5.9^{C}	5.4^{D2}	0.2
DM digestibility[3], %	67.7	65.7^{2}	68.0^{2}	68.8^{2}	6.1

[1] – root mean square error (n = 64)

[2] – n = 15

[3] – n = 14

values are least square means

means within a row followed by different letters differ $P < 0.05$

affected by time until the digesta had been in the tube for 360 min, where the value was slightly higher (4%) compared to Time 0 min (3%) pH decreased with time. Already after 90 min (6.1), it was significantly lower than at Time 0 (6.4). DM digestibility was not affected by time.

DISCUSSION

In an experiment with donkeys, Knapka et al. (1967) suggested that when these animals had access to unlimited physical activity, chromic oxide passed without restriction through the gastrointestinal tract of the animals, whereas when the donkeys were confined to metabolism cages, lack of exercise might have affected the passage of this indicator. Since there is a positive correlation between chromium and DM concentration in duodenal and ileal digesta (Graham and Åman, 1996), chromic oxide can be expected to follow the flow of DM through the gastrointestinal tract. Digesta transit time would then be affected by physical activity, too. Longer retention time of digesta in the gastrointestinal tract may result in higher digestibility and fermentation of nutrients (van Soest et al., 1982; Stephen et al., 1987; Varel et al., 1988). Pigs sleep more and are less active during the evening and night, when no personnel is around them, than during the day.

This could result in a longer retention time of the digesta in the stomach and small intestine of the animal and lead to higher digestibility of nutrients (Hologate and Read, 1983) and/or higher concentration of SCFA in the digesta caused by enzymes of the host and microflora. The previous could partly explain the higher digestibility of DM and higher SCFA concentrations measured during the evening and night intervals compared to the morning interval. However, in Experiment 2, where pigs were fed three times daily in 8 h intervals and ileal digesta was collected over 24 h, no effect of interval on nutrient digestibility or organic acid concentrations was observed. Other studies have also been unable to reveal differences in nutrient digestibility between day and night collections (Livingstone et al., 1980; Jørgensen et al., in press). It is important to mention that when ileal digesta is collected at short intervals during the night, the animals are disturbet and probably more active (they frequently rise when the samples are taken) than when they are left alone the whole night. The effect of activity of the animals in relation to transit time of digesta on digestibility and organic acid concentration is difficult to text in experiments like these.

The sampling procedure followed during the day and night collections in Experiments 1 and 2 was different, and it can, at least partly, explain the results. Although the plastic tubes used for ileal collections in Experiment 1 led to an ice container, the digesta did not reach the ice and the microbial activity was not stopped until the samples were collected and frozen. During the morning and evening, the samples were collected and frozen every 2 or 3h, whereas during the night they were left in the plastic tube for up to 9 h (digesta stayed in the bag for 4 1/2 h on average) before it was collected and frozen. The higher lactic acid and SCFA concentrations measured in intervals with less frequent collections (night) compared with more frequent collections (morning, evening) agree with the results obtained by Bach Knudsen and Hessov (1995). These autors collected samples from human ileostomy subjects every 2 h during the day, whereas digesta from the night stayed in the tube for 9 h. Higher lactic acid (7.3 vs. 10.4) and SCFA (51.7 vs. 83.9) concentrations (mmol/l) were measured in the night samples than in the day samples. As seen in the results obtained in Experiment 2 (Table 3), already after 90 min, pH values were significantly lower than at Time 0 and SCFA concentration higher. Furthermore, molar proportions of acetic and propionic acids were also affected by time. The former can explain the higher organic acid concentrations obtained during the night collections, suggesting that microbial fermentation continued in the collection tubes (outside the intestine of the animals) and affected the results obtained in this interval. On the other hand, the changes in molar ratios of some SCFA could be attributed to alterations in the substrates becoming available to, or being used by, the bacteria with time (Mathers and Fotso Tagny, 1994). The previous discussion indicates that if organic acid concentrations are to be measured, ileal digesta should be

collected and frozen (or microbial actvity stopped by other means) at short intervals. That is, shorter than 3 h, which corresponds to the 90 min sample, if we assume that digesta passes continuously with time.

The higher DM and NSP digestibility values observed during the night in Experiment 1 cannot be explained by the results obtained in Experimental 2 (Table 3), since the values were unchanged with time. The higher SCFA concentration and the molar proportions of acetic and propionic acids observed in the evening interval compared to the morning are difficult to explain. The last evening collection, corresponding to a 3 h sample (from 19.00 to 22.00h), could have affected the results since, as it has been shown here, after 90 min there were evident changes in the pH and SCFA concentration. However, the extent of such an effect on the total evening collection is difficult to quantify.

There was a significant difference between the present investigations in the ileal SCFA concentration of samples (37 mmol/kg in Experiment 1 vs. 10 mmol/kg in Experiment 2). As has been previously described, the sampling techniques were different, which probably was one of the reasons for the results obtained. In Experiment 1, it is likely that there was production of SCFA in the collection tubes (2-3h) and further when the samples were thawed overnight, while in Experiment 2 the bacterial fermentation responsible for it was stopped by freezing the samples as they came out of the animal and by thawing the digesta very quickly when subsamples were taken for analyses. The type of cannula employed is also worth discussing here. The cannula used in Experimental 1 was somewhat bigger than that in Experiment 2, and, due to its shape, it is likely that some fermentation took place around it, resulting in higher SCFA values in the ileal digesta collected. This is supported by the higher ileal digestibility of α-galactosides measured when using this type of cannula compared to a sample T-shapped cannula (Canibe and Bach Knudsen, in press).

CONCLUSION

We cannot be creatin whether during the night, when the animals are less active, the retention time of the digesta in the stomach and small intestine is prolonged and ileal digestibility values increase. However, the results of the present studes indicate that there are no differences between day and night in digestibility of nutrients when pigs are fed pea-based diets; that collection of ileal digesta during 8h for digestibility measurements is representative for the 24h period when animals are fed at 8 h intervals; that ileal samples should be collected and frozen frequently when pH, SCFA concentrations or proportions of the individual acids, and probably other parameters, are to be investigated.

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STRESZCZENIE

Wpływ techniki pobierania treści jelita biodrowego świń na wyniki pomiaru pH, stężenia kwasów tłuszczowych i współczynniki strawności

Przeprowadzono dwa doświadczenia (1 i 2), na 8 świniach w każdym, z kaniulami do końca jelita biodrowego. Zwierzęta żywiono trzy razy dziennie grochem. Dawką, w której groch był jedynym źródłem wielocukrów i azotu pobierano po dwie serie dobowych, w trzech przedziałach czasowych, prób treści jelita biodrowego. W doświadczeniu 1 próbki pobierano i zamrażano w przedziałach 2-3 godzinnych w godzinach rannych i popołudniowych, a 9 godzinnych w nocnych. W doświadczeniu 2 natomiast pobrane próby mrożono natychmiast po ich pobraniu w ciągu całej doby. Stężenie kwasu mlekowego i LKT oraz strawność SM i węglowodanów nieskrobiowych były większe w godzinach nocnych niż porannych. Stosunek stężeń molarnych kwasów był również zróżnicowany w zależności od pory dnia. W doświadczeniu 2 natomiast takich zmian nie stwierdzono.

Wyniki doświadczeń wskazują, że różnice w strawności składników pokarmowych u świń żywionych dietą, której podstawą był groch, w ciągu dnia lub nocy nie były istotne i, że 8 godzinna kolekcja prób treści jelita biodrowego w celu określenia strawności daje dobre wyniki jak 24 godzinna kolekcja przy karmieniu zwierząt co 8 godzin. Próby treści jelita biodrowego do oznaczeń wartości pH i kwasów powinny być pobierane często i natychmiast zamrażane aż do wykonania analiz.