

## Methane excretion in the growing pig

BY KIRSTEN CHRISTENSEN\* AND GRETE THORBEC

National Institute of Animal Science, Rolighedsvej 25, DK-1958 Frederiksberg C,  
Denmark

(Received 9 September 1986 – Accepted 3 November 1986)

1. Total methane excretion ( $\text{CH}_4$  in breath + flatus) was measured in two experiments with thirty-six castrated male pigs (Danish Landrace) during the growth period from 20 to 120 kg live weight (LW). In Expt A, twenty-eight pigs were fed on a commercial diet alternately at high (HFL; metabolizable energy (ME) 1234 (SE 41) kJ/kg LW<sup>0.75</sup>) or low (LFL; ME 784 (SE 31) kJ/kg LW<sup>0.75</sup>) feed levels in different weight classes. In Expt B, eight pigs were constantly fed on a semi-purified diet at HFL without (–oil) or with 90 g soya-bean oil/kg diet (+oil) corresponding to daily intakes of ME of 1339 (SE 11) and 1413 (SE 8) kJ/kg LW<sup>0.75</sup> respectively.

2.  $\text{CH}_4$  excretion was measured during 24 h respiration trials in open-air-circulation chambers.

3. About 1 litre  $\text{CH}_4$  was excreted per day at 20–25 kg LW increasing to a maximum of 12 litres at 120 kg LW, which corresponded to no more than 1.2% of dietary gross energy.

4. In Expt A,  $\text{CH}_4$  excretion increased linearly with LW, while in Expt B the increase was linear until about 70 kg LW, when it reached a plateau. On average LFL reduced  $\text{CH}_4$  excretion by 23% compared with HFL. When related to dry matter (DM) intake, however, the pigs on LFL excreted 3.1 litres  $\text{CH}_4$ /kg dietary DM and those on HFL 2.5 litres  $\text{CH}_4$ /kg dietary DM, the difference being significant ( $P < 0.05$ ). In Expt B the inclusion of soya-bean oil in the basal diet (+oil) reduced  $\text{CH}_4$  excretion by 26% compared with the diet without oil (–oil). The pigs receiving the basal diet excreted 5.2 litres  $\text{CH}_4$ /kg DM and the pigs receiving soya-bean oil 4.3 litres  $\text{CH}_4$ /kg DM, the difference being highly significant ( $P < 0.001$ ). All differences between Expt A and B in  $\text{CH}_4$  excretion based on DM intake were highly significant ( $P < 0.001$ ).

5. The results are discussed in relation to gas production in ruminants, rats and humans. It is suggested that flatus production may not only be reduced by changing the composition of the dietary carbohydrates, but also by inclusion of a polyunsaturated oil in the diet of simple-stomached animals and humans.

Fermentation of carbohydrates in the hind-gut results in the formation of gases including hydrogen, carbon dioxide and methane. The main part is excreted in flatus, while a smaller part is absorbed into the bloodstream and excreted in expired air.  $\text{CH}_4$  production has been studied extensively in ruminants and in vitro, but to a lesser extent in the hind-gut in simple-stomached animals and in man.

In the rumen,  $\text{CH}_4$  is formed by reduction of  $\text{CO}_2$  and, to some extent, from formate (Hungate, 1968).  $\text{CH}_4$  excretion represents a loss of dietary energy as it contains 39.55 kJ/l (Brouwer, 1965). In growing calves the total loss of  $\text{CH}_4$  amounts to 50–100 litres daily depending on the size of the animal, feed composition, feed level and digestibility of the feed, and it constitutes a loss of energy corresponding to 4–11% of dietary gross energy (GE) (Thorbeck, 1980).

$\text{CH}_4$  production in the hind-gut of growing pigs amounts to only a few litres daily and represents an energy loss of about 0.2–1.0% of GE (Verstegen, 1971; Hoffmann *et al.* 1977; Jentsch & Hoffmann, 1977; van der Honing *et al.* 1982). In sows of about 200 kg live weight (LW) and partly fed on grass, the energy loss increased to a maximum of 3.5% of GE (Breirem, 1935). Generally the energy loss in  $\text{CH}_4$  is not taken into consideration when estimating feed values for pigs. In man, flatus production is becoming of interest, both in health and disease, and recently the pig was suggested as an appropriate model for studying fermentation in the human gut (Fleming & Wasilewski, 1984).

In studies on nitrogen and energy metabolism in growing pigs from 20 to 120 kg LW

\* Present address: Royal Veterinary and Agricultural University, Institute of Animal Physiology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark.

(Thorbeck *et al.* 1984; Christensen, 1985), CH<sub>4</sub> excretion was measured simultaneously. The results of the measurements carried out in relation to the quality and quantity of feed are presented in the present paper.

## MATERIALS AND METHODS

### *Animals*

The present investigation included two experiments with castrated male pigs (Danish Landrace) from 20 to 120 kg LW. In the first experiment (Expt A) twenty-eight pigs were fed on a commercial diet at a level which was alternately high (HFL) or low (LFL) and measured in balance periods in different weight ranges from 20 to 120 kg LW. In the second experiment (Expt B) eight pigs were fed continuously on a semi-purified diet with (+oil) or without (–oil) a supply of soya-bean oil and measurements made in consecutive balance periods during the growth period.

The pigs, acquired at a mean age of 65 d and a mean LW of 18 kg, were initially adapted and were fed on barley+skim-milk powder and treated with Piperazin (Danmarks Apotekerforenings Kontrollaboratorium) for deworming, before they were allocated to their different diets and feed levels. The measurements in both experiments started with a mean initial LW of 25 kg and, except for two pigs, all animals were healthy and eating their stipulated rations. One pig in Expt A in the final weight range (105–115 kg) refused feed and the results were omitted. One pig in Expt B died suddenly before period 4. At autopsy it showed pale, soft and exudative muscle.

### *Diets*

A commercial feed mixture was used in Expt A, having a composition as shown in Table 1. Based on its composition it was calculated to contain 17 g fat and 48 g crude fibre/kg. From the measurements of energy metabolism (Thorbeck *et al.* 1984) the metabolizability (metabolizable energy (ME): gross energy (GE)) was found to be 0.78, and the content of ME in the feed mixture was 12.9 MJ/kg. The pigs were fed alternately at high (HFL) and low (LFL) feed levels. The daily intake of ME was 1234 (SE 41) and 784 (SE 31) kJ/kg LW<sup>0.75</sup> at HFL and LFL respectively.

In Expt B a semi-purified diet was given (Table 1) which contained 9.5 g fat and 38 g crude fibre/kg. The diet was given without (–oil) or with (+oil) the addition of 90 g soya-bean oil/kg. Measurements of energy metabolism (Christensen, 1985) showed that the ME content was 14.3 and 14.5 MJ/kg in the (–oil) and (+oil) diets respectively. The daily intake of ME was 1339 (SE 11) and 1413 (SE 8) kJ/kg LW<sup>0.75</sup> for pigs fed on the (–oil) and (+oil) diets respectively.

### *Experimental plan*

In Expt A, twenty-eight pigs were distributed in different LW ranges as shown in Table 2. Each pig was measured individually in their respective LW ranges; half the pigs started on LFL and the other half on HFL in order to obtain approximately the same age and LW in each range. Eight pigs were measured in the lower as well as in the higher LW ranges, while eight pigs were measured only in the higher LW ranges. In the intervening periods the pigs were kept in individual pens in the stable and fed on HFL. With one pig omitted from the final LW range a total of 126 measurements of the CH<sub>4</sub> excretion were carried out.

In Expt B, in which the pigs were maintained throughout on one of the experimental diets, four pigs were given the basal diet and four pigs the basal diet containing soya-bean oil. Measurements were made every fortnight in eight balance periods during the growth

Table 1. *Composition of experimental diets (g/kg diet)*

Expt A		Expt B	
Ingredients		Ingredients	
Barley	770	Maize starch	292
Oats	50	Cassava meal	185
Soya-bean meal	140	Glucose	65
Meat and bone meal	20	Sucrose	65
Mineral and vitamin mix	20	Cellulose	33
Analysis		Skim-milk powder	160
Dry matter	893	Soya-bean meal	80
Protein	165	Casein	80
Gross energy (MJ/kg)	16.48	Mineral and vitamin mix	40
		Analysis	
		Dry matter	900
		Protein	183
		Gross energy (MJ/kg)	15.76

Table 2. *Expt A. Distribution of animals in different live weight ranges*

Animal no.	Live wt range (kg)					
	20-25	25-35	50-60	65-75	90-100	105-120
1, 4	×	×	.	.	×	×
2, 3	×	×	.	.	.	.
6, 7	×	×	.	.	×	×
5, 8	×	×	.	.	.	.
9, 10, 11, 12	×	×	.	.	.	.
17, 18, 19, 20	×	×	.	.	.	.
21, 22, 23, 24	×	.	×	.	.	.
13, 14, 15, 16	.	.	×	×	.	.
25, 26*, 27, 28	.	.	.	.	×	×
Nos. of animals	20	16	8	4	8	7

Each animal was measured individually on high (HFL) and low (LFL) feeding levels in their respective live weight ranges.

\* No. 26 refused feed in the final weight range and the results were omitted.

period from 25 to 115 kg LW, giving a total of fifty-nine measurements of the CH<sub>4</sub> excretion, as one pig died before period 4.

At each feeding level all measurements were carried out with a 7 d preliminary period followed by a 7 d balance period in which a 24 h respiration experiment was placed in the middle of the balance period. The pigs were kept in individual pens in the stable during the preliminary periods and in individual metabolic crates in the balance periods. The temperature in the stable as in the respiration chambers was kept at 22° at the beginning of the experiments decreasing to 18° in the later periods. All pigs were fed twice daily and received water *ad lib*.

#### Instrumentation

The CH<sub>4</sub> excretion over 24 h was measured by means of a respiration unit working according to the open-air-circulation principle (Thorbeck, 1969). The unit, consisting of two independently-working respiration chambers, had an accuracy of 1.0-1.5% and was

frequently calibrated by means of CO<sub>2</sub>-test gas. The flow of the air through the respiration chambers was kept between 6000 and 6500 litres/h at normal conditions (760 mm, 0°). The volume of the outgoing air was measured continuously from the differential pressure over an orifice in the pipe by means of a Barton cell. The electrical signal from the instrument together with signals from the measurements of vacuum and temperature in the pipe and the barometric pressure were transformed to an analogue computer for calculation of the volume of the air at normal conditions.

The CH<sub>4</sub> concentration in the outgoing air was measured partly continuously for registration and partly in representative samples of the air by means of a gas analyser (URAS; Hartmann & Braun, Frankfurt), based on the absorption spectra of the gases from infrared radiation, working in the range 0–0.2% CH<sub>4</sub>. The instrument was frequently calibrated by means of CH<sub>4</sub>-test gas over the range found during animal studies. With the measurement of the total air flow over 24 h and the mean concentration of CH<sub>4</sub> in the air, the total amount of CH<sub>4</sub> excreted in flatus and expired air was calculated. The data obtained were analysed statistically by Student's *t* test, and ANOVA in accordance with Snedecor (1956).

#### RESULTS

The results from the measurements of the CH<sub>4</sub> excretion in Expts A and B are shown in Tables 3 and 4 respectively. The excretion amounted to a maximum of 14 litres CH<sub>4</sub>/24 h. The air flow was about 150000 litres/24 h in order to keep a CO<sub>2</sub> concentration below 1% in the chamber. Thereby the CH<sub>4</sub> concentration varied from 0.001% in the first period increasing to 0.01% in the later periods. The individual variation in CH<sub>4</sub> excretion in the different LW groups was great with coefficients of variation (CV) from 2 to 70%.

The daily CH<sub>4</sub> excretion in Expt A increased linearly with LW on both feed levels. The pigs on LFL showed a lower CH<sub>4</sub> excretion in all LW classes than the pigs being fed near the *ad lib.* level (HFL). On average the CH<sub>4</sub> excretion was 23% lower than that on HFL. A Student's *t* test on individual differences between HFL and LFL (paired *t* test) showed a mean difference of 0.8 litres CH<sub>4</sub>/d, being highly significant ( $P < 0.001$ ). However, in relation to the intake of dry matter (DM) the LFL group showed the greatest CH<sub>4</sub> excretion with a mean value of 3.1 (SE 0.21) litres/kg DM v. 2.5 (SE 0.14) litres/kg DM for the HFL group, the difference being significant ( $P < 0.05$ ).

The CH<sub>4</sub> excretion in Expt B increased linearly to about 70 kg LW when a plateau was reached for both diets, being about 12 litres/d for pigs fed on the –oil diet and about 10 litres/d for pigs fed on the +oil diet. On average the inclusion of oil reduced CH<sub>4</sub> excretion by 26% during the growth period. One-way ANOVA on the differences between groups fed on the +oil and –oil diets in eight periods showed a mean difference of 1.9 litres CH<sub>4</sub>/d, being highly significant ( $P < 0.001$ ). In relation to intake of DM the mean CH<sub>4</sub> excretion in periods 4–8 was 5.2 (SE 0.14) and 4.3 (SE 0.15) litres/kg DM for the –oil and +oil diets respectively, the difference being highly significant ( $P < 0.001$ ).

The CH<sub>4</sub> excretion on a DM basis was greater for the pigs receiving the semi-purified diet (Expt B) than for the pigs fed on the commercial diet (Expt A), and all differences between Expts A and B were highly significant ( $P < 0.001$ ).

#### DISCUSSION

Carbohydrate fermentation in the rumen yields volatile fatty acids, CO<sub>2</sub> and H<sub>2</sub> and most of the H<sub>2</sub> is used in the reduction of CO<sub>2</sub> to CH<sub>4</sub> (Hungate, 1968). In the hind-gut of pigs the proportions of individual volatile fatty acids were found to be similar to those in the rumen (Kidder & Manners, 1978), and it is generally accepted that qualitatively the

Table 3. *Expt A. Daily methane excretion in growing pigs fed on commercial mixtures at high (HFL) or low (LFL) feed levels\**

(Mean values with their standard errors)

Weight range (kg)	No. of measurements		Live wt (kg)				Dry matter intake (g)		CH <sub>4</sub> excretion (litres)			
			HFL		LFL				HFL		LFL	
	HFL	LFL	Mean	SEM	Mean	SEM	HFL	LFL	Mean	SEM	Mean	SEM
20-25	20	20	23.1	0.39	22.2	0.65	750	518	1.66	0.24	1.33	0.24
25-35	16	16	31.5	0.75	30.4	1.12	1115	759	2.42	0.18	2.16	0.28
50-60	8	8	57.2	1.26	54.9	1.81	1785	1071	4.11	0.46	3.18	0.54
65-75	4	4	70.8	3.57	69.5	2.47	2052	1339	5.80	1.46	3.47	1.15
90-100	8	8	95.8	1.59	93.2	2.40	2677	1339	7.69	0.86	5.23	0.40
105-120	7	7	110.9	2.15	109.6	2.50	2677	1785	8.50	0.95	7.78	0.68

\* For details of diets and levels of feeding, see p. 356 and Table 1.

Students *t* test on sixty-three individual differences between HFL and LFL (paired *t* test) showed a mean difference of 0.8 litres/d with a *t* value of 3.59 ( $P < 0.001$ ).Table 4. *Expt B. Daily methane excretion in growing pigs fed on semi-purified diets without (-oil) or with (+oil) addition of soya-bean oil*

(Mean values with their standard errors)

Balance period no.	No. of measurements		Live wt (kg)				Dry matter intake (g)	CH <sub>4</sub> excretion (litres)			
			-oil		+oil			-oil		+oil	
	-oil	+oil	Mean	SEM	Mean	SEM	Both groups	Mean	SEM	Mean	SEM
1	4	4	28.0	0.41	29.1	0.30	987	1.88	0.36	1.13	0.38
2	4	4	37.3	0.76	39.6	0.23	1257	3.76	0.40	2.66	0.95
3	4	4	47.6	0.78	51.9	0.15	1526	6.48	0.74	3.78	1.32
4	4	3	57.4	0.71	64.5	0.29	1795	9.12	0.07	6.49	0.49
5	4	3	68.9	1.09	77.0	0.70	2038	10.70	0.61	9.31	0.98
6	4	3	81.1	1.07	89.7	1.87	2304	12.19	1.06	9.99	0.49
7	4	3	91.2	0.92	101.7	1.77	2482	11.86	0.56	10.08	0.64
8	4	3	107.0	0.36	115.0	1.34	2127	12.23	0.66	9.66	0.31

\* For details of diets and levels of feeding, see p. 356 and Table 1.

One-way ANOVA on the differences between groups on -oil or +oil in eight periods showed a mean difference of 1.9 litres/d with an *F* value of 7.18 ( $P < 0.001$ ).

fermentative processes are the same as in the rumen. Both CH<sub>4</sub> and H<sub>2</sub> are expired in the pig, but breath CH<sub>4</sub> concentrations are about ten times greater than breath H<sub>2</sub> concentrations (Fleming & Wasilewski, 1984) indicating that H<sub>2</sub> is being used in the formation of CH<sub>4</sub>. In the present study all pigs (*n* 36) excreted CH<sub>4</sub>, but twelve pigs excreted very small amounts of CH<sub>4</sub> in the lower weight classes. From 20 to 25 kg LW the mean excretion was about 1 litre CH<sub>4</sub>/d, increasing to about 12 litres/d at 80-115 kg LW. These amounts of CH<sub>4</sub> represented a daily energy loss of 40-480 kJ, which corresponds to no more than 1.2% of daily GE intake. Under the dietary conditions used here it seems that this energy loss is not an important consideration for feed evaluation. Individual variations in CH<sub>4</sub> excretion as expressed by the CV were considerable, ranging from 2 to 70%. Considerable variation

in gas production was also noted in the response of humans to an individual diet (Marthinsen & Fleming, 1982).

The results (Table 3) from pigs fed on a commercial diet (Expt A) showed that the daily  $\text{CH}_4$  excretion increased linearly with increasing LW. The highest values were measured on HFL (near *ad lib.* intake), probably because a greater amount of undigested material reached the hind-gut. As the increase in  $\text{CH}_4$  excretion was linear both on HFL and LFL, the microbes apparently were able to ferment the probably greater amounts of undigested material being transported to the hind-gut of animals fed on HFL. The results (Table 4) from pigs receiving the semi-purified diet (Expt B) showed that on this diet there was an upper limit in the capacity of the actual microbial population to ferment the material, as the  $\text{CH}_4$  excretion remained constant above an intake of 2100 g DM daily.

On a DM basis  $\text{CH}_4$  excretion in Expt A was 3.1 and 2.5 litres/kg DM on LFL and HFL respectively, which may be due to differences in transit times through the gastrointestinal tract. The  $\text{CH}_4$  excretion in Expt B for periods 4–8 was 5.2 and 4.3 litres/kg DM on –oil and +oil diets respectively which, compared with the results from pigs on a commercial diet (Expt A), indicate that apparently more of the carbohydrates from cassava meal and maize starch escaped enzymic degradation and absorption in the small intestine, leaving more material for fermentation in the hind-gut. The transit time may also be greater for this feed.

The results from Expt B showed that addition of 90 g soya-bean oil/kg diet (+oil) reduced  $\text{CH}_4$  excretion by an average of 26% during the growth period compared with the oil-free diet. The depressive effect of relatively large amounts of unsaturated fat or fatty acids on microbial activity and hence on  $\text{CH}_4$  excretion is well known in ruminants (Czerkawski *et al.* 1966; Rohr & Okubo, 1968; Czerkawski, 1976). Full-fat soya-bean meal produced less flatus than defatted soya-bean meal in humans (Steggarda *et al.* 1966). The inclusion of 350 g beef fat or safflower oil/kg in a basal, purified, low-fat diet significantly reduced the number of caecal bacteria and decreased most of the microbial enzyme activities in the caecum of rats (Mallett *et al.* 1983). Thus, there seem to be similarities in the depression of fat on microbial fermentation and hence on flatus production in ruminants, rats, humans and pigs.

The pig has been of interest as a model to study flatulent factors in humans as it is similar to man in its nutritional gastrointestinal physiology (Pond & Houpt, 1978). In humans intestinal fermentation always yields  $\text{H}_2$ , but apparently  $\text{CH}_4$  is not a universal component. The proportion of healthy adults excreting  $\text{CH}_4$  in the breath varied from 22 to 70% (Calloway & Murphy, 1968; Bond *et al.* 1971; Pitt *et al.* 1980).

Cancer of the colon (Haines *et al.* 1977; Piqué *et al.* 1984) and abdominal arterial disease (McKay *et al.* 1983) have been reported to be associated with a high incidence of  $\text{CH}_4$  excretion. In man, the biochemical pathway of  $\text{CH}_4$  production seems to be different from that in the rumen, as it is not entirely dependent on the concentration of  $\text{H}_2$  (Calloway, 1966; McKay *et al.* 1981). Human breath contains twice as much  $\text{H}_2$  as pig breath, and only about one-twentieth of pig breath  $\text{CH}_4$  concentrations (on quite different diets), but changes due to dietary manipulations seemed to be qualitatively similar (Fleming & Wasilewski, 1984). In human breath,  $\text{H}_2$  concentrations did not provide an adequate predictor of flatus gas excretion, but breath and flatus  $\text{CH}_4$  excretions were highly correlated (Marthinsen & Fleming, 1982). Lewitt (1985) concluded that the only feasible method to reduce flatus production at the present time appears to be the use of a diet that reduces the quantity of carbohydrate reaching the colon, namely a lactose-free, low-wheat diet. The present findings and those of others suggest that a daily intake of a polyunsaturated oil might also be helpful in reducing the amount of intestinal gas.



## REFERENCES

- Bond, J. H., Engel, R. R. & Lewitt, M. D. (1971). *Journal of Experimental Medicine* **133**, 572–588.
- Breirem, K. (1935). *National Institute of Animal Science Report* no. 162, 0–269. Copenhagen: Landhusholdningsselskabet Forlag.
- Brouwer, E. (1965). In *Proceedings of the 3rd Symposium on Energy Metabolism. European Association of Animal Production Publication* no. 11, pp. 441–443. London: Academic Press.
- Calloway, D. H. (1966). *Gastroenterology* **51**, 383–389.
- Calloway, D. H. & Murphy, E. L. (1968). *Annual of the New York Academy of Sciences* **150**, 82–95.
- Christensen, K. (1985). *National Institute of Animal Science Report* no. 577, 0–158. Copenhagen: Landhusholdningsselskabet Forlag.
- Czerkawski, J. W. (1976). In *Proceedings of the 7th Symposium on Energy Metabolism. European Association of Animal Production Publication* no. 19, pp. 69–72. Clermont-Ferrand, France: G. de Bussac.
- Czerkawski, J. W., Blaxter, K. L. & Wainmann, F. W. (1966). *British Journal of Nutrition* **20**, 485–494.
- Fleming, S. E. & Wasilewski, M. M. (1984). *Nutrition Reports International* **30**, 825–834.
- Haines, A., Metz, G., Dilawari, J., Blendis, L. & Wiggins, M. (1977). *Lancet* **ii**, 481–483.
- Hoffmann, L., Jentsch, W., Klein, M. & Schiemann, R. (1977). *Archiv für Tierernährung* **27**, 421–438.
- Hungate, R. E. (1968). In *Handbook of Physiology*, vol. 5, pp. 2725–2745 [C. F. Code, editor]. Washington, DC: American Physiological Society.
- Jentsch, W. & Hoffmann, L. (1977). *Archiv für Tierernährung* **27**, 491–507.
- Kidder, D. E. & Manners, M. J. (1978). *Digestion in the Pig*. Bristol: Sciencetechnica.
- Lewitt, M. D. (1985). *Proceedings of the Nutrition Society* **44**, 145–146.
- McKay, L. F., Brydon, W. G., Eastwood, M. A. & Housley, E. (1983). *Atherosclerosis* **47**, 77–81.
- McKay, L. F., Brydon, W. G., Eastwood, M. A. & Smith, J. H. (1981). *American Journal of Clinical Nutrition* **34**, 2728–2733.
- Mallett, A. K., Rowland, I. R. & Wise, A. (1983). *Proceedings of the Nutrition Society* **43**, 7A.
- Marthinsen, D. & Fleming, S. E. (1982). *Journal of Nutrition* **112**, 1133–1143.
- Piqué, J. M., Pallarés, M., Cosó, E., Vilar-Bonet, J. & Gassull, M. A. (1984). *Gastroenterology* **87**, 601–605.
- Pitt, P., DeBruijn, K. M., Beeching, M. F. & Goldberg, E. (1980). *Gut* **21**, 951–959.
- Pond, W. G. & Houpt, K. A. (1978). *Biology of the Pig*. New York: Comstock Publisher Association.
- Rohr, K. & Okubo, M. (1968). *Milchwissenschaft* **23**, 608–614.
- Snedecor, G. W. (1956). *Statistical Methods Applied to Experiments in Agriculture and Biology*. Iowa: The Iowa State College Press.
- Steggarda, F. R., Richards, E. A. & Rackis, J. F. (1966). *Proceedings of the Society of Experimental Biology and Medicine* **121**, 1235–1239.
- Thorbek, G. (1969). *National Institute of Animal Science Report* no. 373, pp. 0–46. Copenhagen: Landhusholdningsselskabet Forlag.
- Thorbek, G. (1980). *National Institute of Animal Science Report* no. 498, pp. 0–95. Copenhagen: Landhusholdningsselskabet Forlag.
- Thorbek, G., Chwalibog, A. & Henckel, S. (1984). *National Institute of Animal Science Report* no. 563, pp. 0–114. Copenhagen: Landhusholdningsselskabet Forlag.
- Van der Honing, Y., Jongbloed, A. W., Smits, B. & Wieman, B. J. (1982). In *Proceedings of the 9th Symposium on Energy Metabolism. European Association of Animal Production Publication* no. 29, pp. 202–205. Ski, Norway: Informasjonsteknikk A/S.
- Verstegen, M. V. A. (1971). *Mededelingen van de Landbouwhoogeschool te Wageningen* **71–2**, 0–115.