

Growth differences of male and female Göttingen minipigs during *ad libitum* feeding: a pilot study

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Summary

Even though minipigs have been used in biomedical research for nearly half a century now, no specific nutrient requirements are available. For that reason a series of studies into the nutrient requirements of Göttingen minipigs were carried out. Firstly, a pilot study was carried out to determine the *ad libitum* feed intake (FI) during growth, as a reference for later feed restriction studies. Four male and four female minipigs were fed two types of diet, one standard pig diet (20.6% crude protein; 11.7% crude fat; 13.5 MJ/kg DM metabolizable energy) and one diet specially designed for minipigs (12.0% crude protein; 2.9% crude fat; 11.9 MJ/kg DM metabolizable energy). When fed *ad libitum* for 13 weeks, female Göttingen minipigs developed a significantly ($P < 0.05$) higher body weight (BW) than males (27.4 vs 16.6 kg) on either diet. The large difference in growth between male and female Göttingen minipigs did not appear to be the result from differences in metabolizable energy intake. Metabolizable energy intake of male and female Göttingen minipigs could be predicted by $ME = 1877 \text{ kJ} \times BW^{0.61}$. Both male and female Göttingen minipigs became obese when fed *ad libitum*, defined by relative backfat thickness. Relative backfat thickness ranged from 5 to 13 cm/100 kg. Females had thicker relative backfat layers than males. Remarkably, no large changes in haematology and clinical chemistry occurred in *ad libitum* fed Göttingen minipigs as compared to reference values, and no abnormalities other than enlarged fat reserves were observed at necropsy. Apparently, Göttingen minipigs do not restrain FI voluntarily, and restricted feeding is therefore indicated to prevent obesity.

Keywords Minipigs; growth; blood values; pathology; obesity

Until now, no scientific investigations into the nutrient requirements of minipigs have been carried out. Specific recommendations for the composition of minipig diets for breeding and maintenance do exist

(GV-SOLAS 1993), but the scientific evidence is missing. Nutrient requirements for large pigs (ARC 1981, NRC 1988) have been based on extensive literature reviews on pig nutrition (Lewis 1993). Previously it was suggested that established nutrient levels for large pigs were recommended as a guide for miniature pigs until experimental data on the nutrient needs of miniature pigs

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became available (Cuhna 1966), and it was assumed that the nutritional requirements of miniature pigs did not differ from those of large pigs, although studies to define the requirements precisely were yet to be done (Dettmers 1968). But since nutrient requirements for pigs are based on *ad libitum* feeding in order to obtain maximum growth, the question was raised whether these recommendations were appropriate for minipigs (Ritskes-Hoitinga & Bollen 1997). Body weight (BW) development in growing Göttingen minipigs has been well described, however, without giving detailed information on dietary composition and applied feeding levels (Glodek *et al.* 1977, Glodek 1981, Holtz & Kallweit 1981, Li *et al.* 1988). Previously, minipigs have been fed with a commercial large pig diet at a feeding level allowing good fertility and growth, as well as preventing obesity (Smidt 1981, Oldigs 1986). However, it was recognized that this could lead to overnutrition (Glodek 1986). Presently, no combined information on dietary composition, feed intake (FI) and BW development is available for the Göttingen minipig. For that reason a series of studies into the nutrient requirements of Göttingen minipigs were carried out between 1998 and 2001. Firstly, a pilot study was carried out to determine the *ad libitum* FI during growth, as a reference for later feed restriction studies. Reference values for haematology and clinical chemistry during *ad libitum* feeding were established as well. The pilot experiment was completed with a necropsy.

Materials and methods

Four male and four female 8-week-old Göttingen minipigs were housed individually in cages of 0.8 m² at the SPF facility of the breeder (Ellegaard Göttingen Minipigs, DK-4261 Dalmose, Denmark) for a duration of 13 weeks. Visual and olfactorial social contact was possible continuously, and the animals were allowed to exercise in the corridors daily. The environmental temperature was 24.6 ± 1.0°C, and the relative humidity

67 ± 18% (means ± SD). No bedding material was provided. Lights were switched on from 06:00 to 18:00 h. Two males and two females were fed *ad libitum* with Diet 1, and two males and two females were fed *ad libitum* with Diet 2. Both diets were natural ingredient diets, custom-produced by Special Diets Services (SDS, Witham, Essex, UK), composed of cereals (barley, wheat, wheat feed), proteins (soy bean meal, sunflower seeds), fibre (oatmeal, soy hulls), energy (molasses) and supplements (vitamins, minerals). The diets were analysed for nutrients (moisture, crude protein, crude fat, crude fibre, ash, Ca, P, Na, Cl, K, Mg, Fe, Cu, Mn, Zn, Vit. A and Vit. E), chemical contaminants (F, NaNO₃, NaNO₂, Pb, As, Cd, Hg, Se, total PCB, total DDT, dieldrin, lindane, heptachlor and malathion) and microbiological contaminants (total viable organisms, mesophilic spores, salmonellae, enterobacteriae, *E. coli*, fungal units and antibiotic activity) at the laboratory of the producer's parent organization (Trouw Nutreco, Northwich, CW9 6DF, Cheshire, UK). Diet 1 was the standard diet used at the breeding facilities, and was based on best practical experience and guidelines from the German Society of Laboratory Animal Science (GV-SOLAS 1993), and Diet 2 was a newly composed diet (Ritskes-Hoitinga & Bollen 1998), based on guidelines for swine from the US National Research Council (NRC 1988). The physical form of both diets was an expanded pellet, with a diameter of 6 mm. The analysed composition of the diets is given in Table 1. Chemical and microbiological contamination was not detected or below tolerance levels, and is therefore not presented. Feed intake was monitored twice daily, at 09:00 and 14:30 h. The BW of each animal was measured weekly (Mettler Toledo Spider 1, max. 60 kg, d = 20 g, CH-8606 Greifensee, Switzerland). Monthly blood samples were collected for haematology and clinical chemistry. Samples were taken after overnight fasting, between 07:00 and 09:00 h, from the precaval sinus of conscious animals in dorsal recumbency with evacuated tubes (Venoject, Terume, B-3001 Leuven, Belgium). Volumes of 3 ml EDTA

Table 1 Analysed composition (g/kg) of Diet 1 (batch 3629) and Diet 2 (batch 3602)

	Diet 1	Diet 2
Dry matter (DM)	933	924
Organic matter	876	871
Crude protein	120	206
Crude fat	29	117
Crude fibre	129	76
Ash	57	53
Calcium	10.2	6.8
Phosphorus	6.2	5.8
Sodium	3.0	2.6
Chloride	5.6	4.4
Potassium	10.5	11.5
Magnesium	1.8	2.0
Iron	0.320	0.195
Copper	0.008	0.010
Manganese	0.042	0.041
Zinc	0.113	0.116
ME (MJ/kg DM)	11.9	13.5

ME = metabolizable energy

stabilized blood, and 10 ml non-stabilized blood were taken. Haematological parameters including red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), white blood count (WBC) and platelet count (PLT) were analysed automatically (Celltac α MEK-6108k, Nihon Kohden, Tokyo 161, Japan) in the EDTA stabilized samples. Non-stabilized blood samples were kept at room temperature for 1–2 h, before centrifugation at 4700 rpm (3500 g) for 10 min (Sigma 3–15, D-37507 Osterode, Germany). Clinical chemistry included serum cholesterol (CHOL), creatinine (CREA), glucose (GLUC), total protein (TPROT), triglyceride (TRIG) and urea (UREA), analysed colorimetrically (Cobas Mira, Roche Diagnostic Systems, CH-4070 Basel, Switzerland).

After the end of the experiment, the minipigs were euthanized by means of captive bolt pistol and exsanguinated from the axial blood vessels for pathological examination and isolation of organs. Organs included for empty weight measurement (Mettler Toledo SB8000, max. 8 kg, d = 1 g, and Mettler Toledo PB1501, max. 1.5 kg, d = 0.1 g, CH-8606 Greifensee, Switzerland) and histology were the stomach, ileum,

caecum, spiral colon, heart, liver and kidneys. The aorta was opened, and examined for macroscopically changes such as atherosclerotic plaques. The thickness of the cervical, thoracic and lumbar layers of subcutaneous fat was measured with a nomogram at the location of the vertebrae C4, T4 and L4. Statistical analyses were performed using the ANOVA procedure (STATA 5, College Station, TX 77840, USA), with gender and diet as between-subjects factors. Where repeated samples were analysed, the repeated measures ANOVA procedure (Gleason 1999a) was used, with age as within-subjects factor and gender and diet as between-subjects factors. When significant differences ($P < 0.05$) were detected in the between-subjects factors, pairwise comparisons of means using the Tukey method (Gleason 1999b) were performed to identify significantly different pairs.

A licence to perform this study was obtained from the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Results

The FI ranged from 213 to 1284 g/day. The average FI was 603 ± 248 g/day (mean \pm SD). The repeated measures ANOVA procedure detected significant differences within age ($P < 0.01$), between gender ($P < 0.01$) and between diets ($P < 0.05$). FI increased significantly with age, and FI on Diet 1 was significantly higher than on Diet 2. Also, females had a significantly larger FI than males. Age–gender interaction was observed, as well as age–diet interaction. FI increased more in females than males with age, and FI increased more on Diet 1 than on Diet 2 with age. Pairwise comparison of means identified significant differences ($P < 0.05$) between males on Diet 2 and females on Diet 1, from the age of 10 weeks, but no significant differences were identified anymore at the age of 13 weeks. Significant differences were also found between males and females on Diet 2, from the age of 14 weeks. From the age of 16 weeks, there were significant differences between males

on Diet 1 and males on Diet 2. At the ages of 16, 18 and 20 weeks, significant differences were identified between males and females on Diet 1, and at 15 and 18 weeks between males on Diet 1 and females on Diet 2. No significant differences were found between females on Diet 1 and females on Diet 2. FI expressed as gross energy (GE) intake ranged from 4080 to 21 567 kJ/day. The average GE intake was 10752 ± 4278 kJ/day (mean \pm SD). Figure 1 displays metabolizable energy (ME) intake, with regression lines for each gender–diet group. The repeated measures ANOVA procedure detected significant differences within age ($P < 0.01$), between gender ($P < 0.01$) and between diets ($P < 0.05$). ME intake increased significantly with age, and ME intake on Diet 1 was significantly larger than on Diet 2. Females had a significantly larger ME intake than males. Age–gender interaction was observed. ME intake increased more with age in females than in males. Pairwise comparison of means identified significant differences ($P < 0.05$) between males on Diet 2 and females on Diets 1 and 2, from the age of 14 weeks. At

the age of 16, 19 and 20 weeks, there were significant differences between males on Diet 1 and males on Diet 2. At the age of 15, 18 and 20 weeks, significant differences were identified between males on Diet 1 and females on Diets 1 and 2. Only at an age of 16 weeks, a significant difference was identified between males on Diet 1 and females on Diet 1. No significant differences were identified between females on Diet 1 and females on Diet 2.

The BW of the minipigs ranged from 3.4 to 28.6 kg. The average BW was 12.7 ± 6.5 kg (mean \pm SD). Table 2 gives the mean BW at the beginning and end of the experiment, as well as growth during the entire experiment, including ANOVA analyses. Figure 2 displays BW, with regression lines for male and female minipigs. The repeated measures ANOVA procedure detected significant differences within age ($P < 0.01$), and between gender ($P < 0.01$). BW increased with age, and females had a significantly larger BW than males. Interaction was observed between age and gender. BW increased more in females than males with age. The ANOVA procedure detected

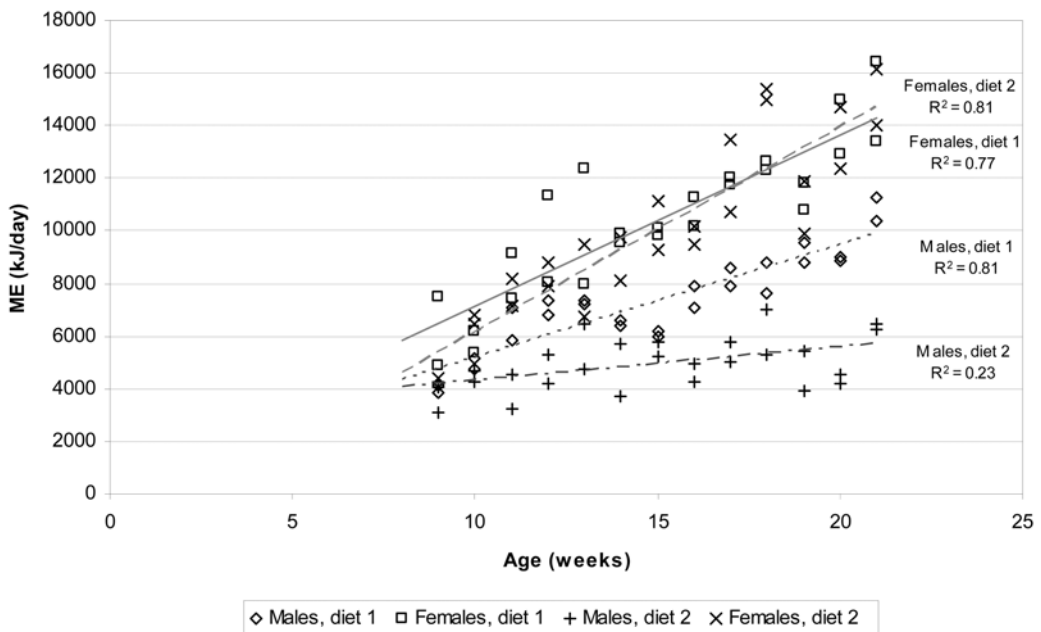


Fig 1 Metabolizable energy (ME) intake of male and female Göttingen minipigs fed *ad libitum* on two types of diet ($n = 2$ per group)

Table 2 Body weight (BW), growth and feed intake (FI) of male and female Göttingen minipigs ($n=8$) fed *ad libitum* on two types of diet for 13 weeks

	Age (weeks)	Diet 1		Diet 2		SEM*	ANOVA**
		♂	♀	♂	♀		
BW (kg), start	8	4.0	3.8	3.9	3.6	0	ns
BW (kg), end	21	16.9 ^a	27.4 ^b	16.4 ^a	27.4 ^b	2	S
Growth (g/day)		142 ^a	259 ^b	138 ^a	261 ^b	2	S
FI (g/day)		574 ^a	814 ^b	337 ^c	689 ^{ab}	2	SD

*Standard error of the mean (pooled). **Analyses of variance; significant differences in between-subjects factors sex and diet are indicated with S and D, respectively if $P < 0.05$. ns = not significant. ^{a-b}Within each row, values with different superscripts are significantly different ($P < 0.05$)

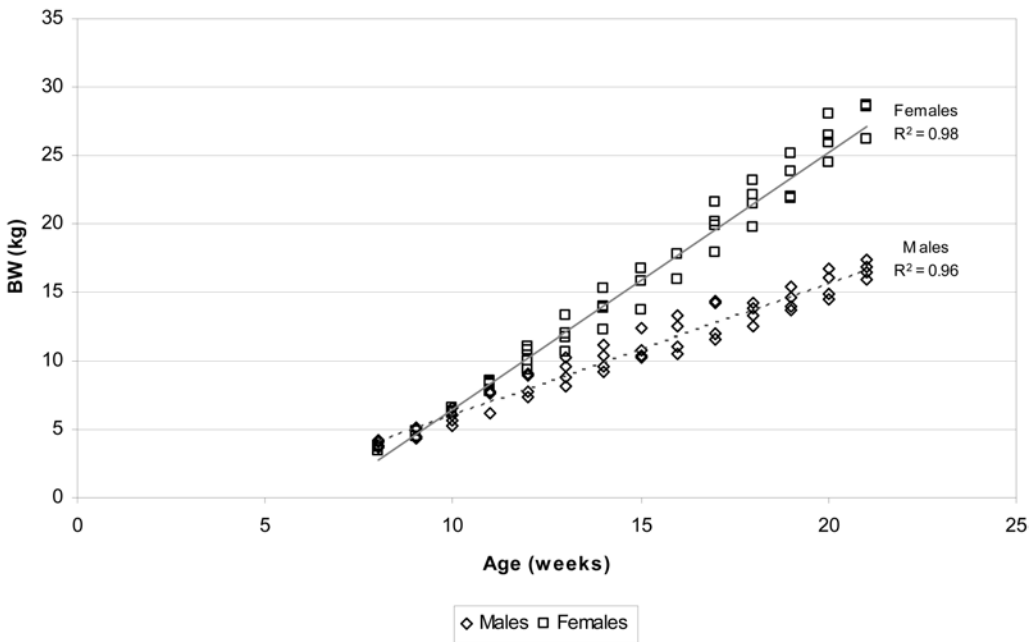


Fig 2 Body weight (BW) of male and female Göttingen minipigs fed *ad libitum* on two types of diet. Females ($n=4$) and males ($n=4$) are pooled, since no significant diet differences within each sex were found

P values < 0.05 from 12 weeks of age, and P values < 0.01 from 15 weeks of age. Figure 3 plots ME intake against BW. Males on Diet 2 are plotted separately, since their ME intake was significantly lower than that of males on Diet 1. The equations of the regression curves for all females and males on Diet 1 is given by $ME = 1877 \text{ kJ} \times BW^{0.61}$, whereas the equation for males on Diet 2 is given by $ME = 2206 \text{ kJ} \times BW^{0.34}$.

Blood values are given in Table 3. Significant age effects were detected in RBC,

HGB, HCT, WBC and PLT ($P < 0.01$). RBC, HGB and HCT increased with age, whereas WBC and PLT decreased with age. A diet effect was detected in PLT ($P < 0.05$), but pairwise comparison of means identified no significant different pairs. Animals on Diet 2 had larger PLT values than animals on Diet 1. No significant differences were detected in GLUC. Significant age effects were detected in TPROT ($P < 0.01$) and TRIG ($P < 0.05$). TPROT and TRIG increased with age. Significant gender differences were

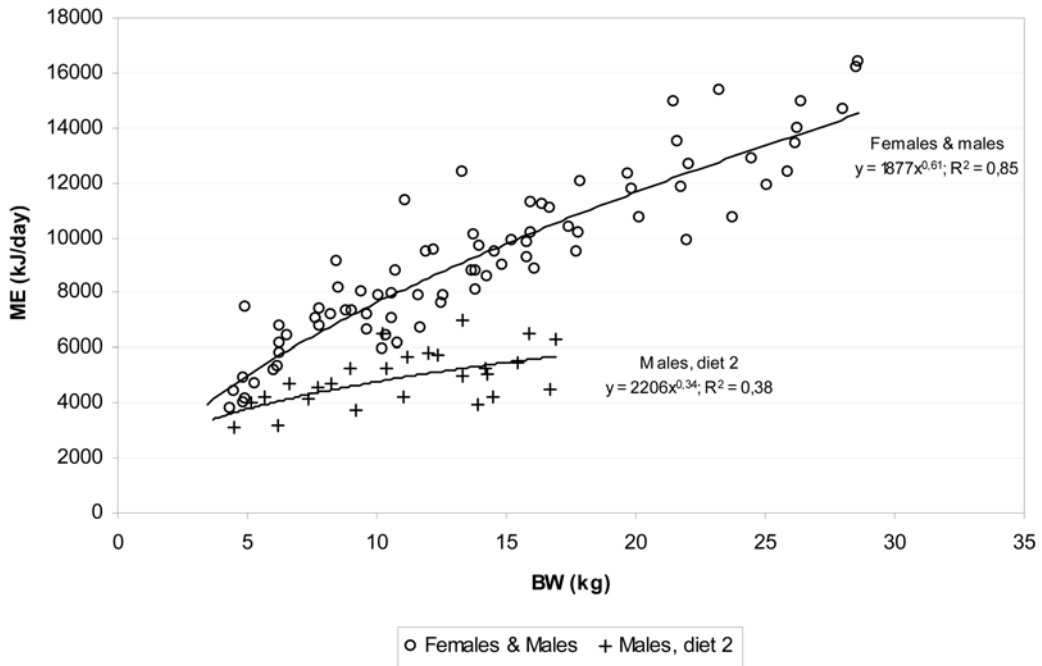


Fig 3 Metabolizable energy (ME) intake as a function of body weight (BW) for male and female Göttingen minipigs on two types of diet ($n=6$). Males on diet 2 ($n=2$) are depicted separately, since they significantly differed from the other groups

detected in CREA ($P < 0.01$), and in CHOL and TPROT ($P < 0.05$), but pairwise comparison of means identified only significant differences in CHOL and TPROT, at the age of 21 weeks. Females had larger CREA, CHOL and TPROT values than males. Significant diet effects were detected in CREA ($P < 0.01$) and UREA ($P < 0.05$). Pairwise comparison of means identified significant different pairs in UREA at the age of 21 weeks, but not in CREA. UREA values were larger on Diet 2, whereas CREA values were larger on Diet 1. Gender–diet interaction was observed for CREA and UREA, and age–gender interaction for CHOL. CREA was larger in females at the age of 8 weeks, but vice versa at the ages of 13 and 21 weeks. This difference was larger for those on Diet 1 than on Diet 2. UREA was larger in males than females on Diet 1, but vice versa on Diet 2. CHOL decreased with age in males and increased with age in females. Macroscopic examination during necropsy showed only minor changes in organs.

Yellow–grey discoloured regions in the pars oesophagea of the stomach in three female and one male minipig were found. In the liver, grey focal areas were observed in two females. Pale kidneys were seen in four minipigs, and grey focal spots were observed in the kidneys of one female minipig. Microscopic examination of the lesions revealed that the discoloured regions of the pars oesophagea of the stomach were caused by hyperkeratosis of the epithelium. No inflammatory cells were observed. The grey focal spots on the liver and kidneys were areas with interstitial cell infiltrates, mainly lymphocytes. No microscopic changes were found in the pale kidneys. Empty organ weights (g) and relative organ weights (g/kg BW) are given in Table 4, including BW on the day of pathological examination. No significant differences were detected in the organ weights of the ileum, liver, kidneys and heart, and in the relative organ weights of the ileum, liver and kidneys. Significant gender effects were detected in the organ

Table 3 Blood values of male and female Göttingen minipigs ($n = 8$) fed *ad libitum* on two types of diet for 13 weeks

	Age (weeks)	Diet 1		Diet 2		SEM*	ANOVA**
		♂	♀	♂	♀		
HGB (mmol/l)	8	7.10	9.95	6.55	7.00	0.08	} A
	13	7.45	6.25	6.85	7.25	0.20	
	21	8.10	7.75	7.80	8.50	0.16	
HCT (%)	8	38.0	37.5	36.5	37.5	0.3	} A
	13	38.0	32.5	36.5	37.0	0.8	
	21	40.0	39.5	40.0	41.5	0.6	
RBC ($10^{12}/l$)	8	6.88	7.54	7.11	7.66	0.23	} A
	13	7.06	6.45	7.19	7.11	0.15	
	21	7.99	7.99	8.82	8.52	0.18	
WBC ($10^9/l$)	8	13.25	10.55	12.55	12.65	1.16	} A
	13	10.60	8.25	8.95	9.90	0.41	
	21	10.70	10.75	8.80	10.25	0.58	
PLT ($10^9/l$)	8	596	708	765	842	38.5	} AD
	13	478	542	558	618	27.9	
	21	481	294	510	589	56.0	
CREA ($\mu\text{mol}/l$)	8	77.0	79.0	74.5	81.0	1.8	} SD
	13	85.5	66.5	59.5	65.5	4.4	
	21	85.5	68.5	86.0	75.0	3.3	
UREA (mmol/l)	8	2.0	1.9	1.4	2.3	0.2	} D
	13	2.7	2.0	1.9	3.4	0.4	
	21	2.1 ^{ab}	1.0 ^a	2.4 ^{ab}	4.5 ^b	0.5	
CHOL (mmol/l)	8	2.3	2.5	2.8	2.8	0.1	} S
	13	2.2	2.6	2.5	4.2	0.3	
	21	1.7 ^a	2.8 ^a	1.7 ^a	5.2 ^b	0.5	
TRIG (mmol/l)	8	0.81	0.56	0.59	0.60	0.06	} A
	13	0.54	0.45	0.55	0.62	0.04	
	21	0.56	0.63	0.90	1.12	0.12	
TPROT (g/l)	8	51.2	55.4	52.6	52.2	0.8	} AS
	13	57.8	62.0	52.4	57.3	1.9	
	21	58.4 ^a	64.7 ^{bc}	54.5 ^{ab}	60.7 ^c	1.4	

Non-significant results are not presented. *Standard error of the mean (pooled). **Analyses of variance; significant differences in within-subject factor age and in between-subjects factors sex and diet are indicated with A, S and D, respectively if $P < 0.05$. ns = not significant. ^{a-b}Within each row, values with different superscripts are significantly different ($P < 0.05$). HGB = haemoglobin concentration; HCT = haematocrit; RBC = red blood cell count; WBC = white blood count; PLT = platelet count; CREA = creatinine; UREA = urea; CHOL = cholesterol; TRIG = triglyceride; TPROT = total protein

weights of the caecum and spiral colon ($P < 0.01$), and the stomach ($P < 0.05$). Females had a larger stomach, caecum and spiral colon than males. There was a tendency ($P < 0.10$) towards a higher ileum weight in females than in males. Significant diet effects were detected in the caecum ($P < 0.01$) and stomach ($P < 0.05$). The stomach and caecum were largest on Diet 1. Pairwise comparison of means identified significant differences in the stomach, caecum and spiral colon weights. In the relative organ weights, significant gender effects were detected in the spiral colon and

heart ($P < 0.05$). The relative weight of the spiral colon was larger in females than in males, but the relative weight of the heart was smaller in females than in males. Significant diet effects were detected in the relative organ weights of the stomach and caecum ($P < 0.01$), and in the spiral colon ($P < 0.05$). The relative weights of these organs were larger for those on Diet 1 than those on Diet 2. Pairwise comparison of means identified significant differences in the relative organ weights of the stomach, caecum, spiral colon and heart. Gender-diet interaction was observed for the relative

Table 4 Organ weights (g) and relative organ weights (g/kg) of male and female Göttingen minipigs ($n = 8$) fed *ad libitum* on two types of diet for 13 weeks

	Diet 1		Diet 2		SEM*	ANOVA**
	♂	♀	♂	♀		
BW (kg)	17.3 ^a	26.9 ^b	17.1 ^a	28.3 ^b	2.0	S
<i>Organ weights</i>						
Stomach	180 ^{ab}	252 ^b	106 ^a	166 ^{ab}	21	SD
Caecum	21 ^{ac}	41 ^b	18 ^a	27 ^c	3	SD
Spiral colon	198 ^a	486 ^b	151 ^a	312 ^{ab}	52	S
<i>Relative organ weights</i>						
Stomach	10.37 ^a	9.32 ^{ab}	6.10 ^b	5.82 ^b	0.80	D
Caecum	1.21 ^{ab}	1.51 ^b	1.05 ^a	0.94 ^a	0.08	D
Spiral colon	11.41 ^{ab}	18.12 ^b	8.63 ^a	10.93 ^{ab}	1.46	SD
Heart	4.39 ^{ab}	3.27 ^{ab}	5.54 ^a	3.16 ^b	0.40	S

Non-significant results are not presented. *Standard error of the mean (pooled). **Analyses of variance; significant differences in between-subjects factors sex and diet are indicated with S and D, respectively if $P < 0.05$. ns = not significant. ^{a-b}Within each row, values with different superscripts are significantly different ($P < 0.05$)

organ weight of the caecum. Compared to males, females had a higher relative caecum weight on Diet 1, but a lower relative caecum weight on Diet 2.

For all three locations where backfat was measured, significant gender effects ($P < 0.01$) were detected (Table 5). Females had thicker backfat layers than males. Pairwise comparison of means identified significant differences in the cervical backfat layers between males and females on Diet 2, but not between males and females on Diet 1. Gender–diet interaction was observed in thoracic and lumbar backfat. The difference between males and females was larger for those on Diet 2 than those on Diet 1.

Discussion

The *ad libitum* FI of Göttingen minipigs increased with age, and was different between gender and diets. However, when FI was converted to ME intake, differences between the two diets no longer existed in females. This is consistent with the general pattern of *ad libitum* FI of homeothermic animals, which adjust feed consumption according to their metabolic rate and the energy density of the diet (Kleiber 1979). Kleiber referred to the classical experiment by Adolph in 1947, in which rats fed with diluted diets adjusted their FI accordingly to achieve an energy intake similar to that of a concentrated diet, and cited Hill and

Table 5 Backfat thickness (cm) of male and female Göttingen minipigs ($n = 8$) fed *ad libitum* on two types of diet for 13 weeks

	Diet 1		Diet 2		SEM*	ANOVA**
	♂	♀	♂	♀		
Cervical	2.22 ^a	2.84 ^{ab}	1.90 ^a	3.35 ^b	0.22	S
Thoracic	1.49 ^a	2.39 ^b	0.95 ^a	3.17 ^b	0.33	S
Lumbar	1.09 ^a	2.28 ^b	0.78 ^a	2.96 ^b	0.34	S

*Standard error of the mean (pooled). **Analyses of variance; significant differences in between-subjects factors sex and diet are indicated with S and D, respectively if $P < 0.05$. ns = not significant. ^{a-b}Within each row, values with different superscripts are significantly different ($P < 0.05$)

Dansky's experiments in 1954, with chickens by quoting: 'The rate of feed consumption was determined primarily by the energy level of the ration' (Kleiber 1979). Also Blaxter referred to Adolph and cited him by quoting 'rats eat for energy' (Baxter 1989). In the present study, ME intake was different between gender. Females had a ME intake which was approximately 1.5 times larger than that of males on Diet 1, and approximately 2.2 times larger than that of males on Diet 2. It is known that obese pigs have a higher FI, and spent more time per day on eating than did lean pigs during *ad libitum* feeding, although obese pigs have a slower rate of eating than lean pigs (Wangsnæs *et al.* 1980). This suggests that the difference in FI between genders in the present study may depend on a different predisposition for obesity in male and female Göttingen minipigs. It is also known that gonadal steroids have an appetite-suppressing action. Feed intake is less in boars than in barrows (castrates). High levels of testosterone may even cause the intake of feed to cease, as observed in wild boars during the mating season. But also oestradiol has an inhibiting action on FI, as sows have a reduced FI during oestrus, when oestradiol levels are high (Claus & Weiler 1994). In contrast to the results from Göttingen minipigs, large male pigs had a larger FI than females, both when lean and obese (Klindt *et al.* 1995).

The marked difference in growth between genders in the present study was unexpected, since such large differences between males and females have not been reported before. In published growth curves for the Göttingen minipig during the years 1974–1975, no gender differences in weight development were present from birth to 120 days of age. The BW of animals was reported to be 9.8 kg at the age of 120 days, but no information on diet type and feeding regimen were given (Glodek *et al.* 1977). Growth curves for the Göttingen minipig during the years 1980–1986 reported differences between gender from the age of 100 days. Females were found to be larger than males, with a BW of 11.3 kg vs 10.5 kg for males. This developed to 18.2 kg for females

and 16.0 kg for males at the age of 150 days, the age of female sexual maturity. But also here, no information on diet type and feeding regimen was given (Li *et al.* 1988). Differences between males and females were explained as the result of a stronger selection on BW for male minipigs. Nevertheless, it was recognized that the effect of nutrition on BW is important. Body weight in a research population of Göttingen minipigs (Oldigs 1986) was found to be much larger than in the breeding population (Glodek *et al.* 1977). Body weight at the age of 120 days was 18.3 kg, but it was observed that they were fed far too intensively, with a ration of twice the maintenance requirement (Glodek 1986). In this case minipigs were fed *ad libitum* from weaning to the age of 95 days, whereafter they were fed restrictedly at twice the maintenance requirement, based on requirements for large pigs, with an available commercial pig diet (Oldigs 1986). However, a comparison of gender was not included. In the present study, significant differences in BW ($P < 0.05$) between gender were detected from 12 weeks, or 84 days of age, and strongly significant differences ($P < 0.01$) were detected from 15 weeks, or 105 days of age, under true *ad libitum* conditions. BW in the present study is larger than has been reported before, with females being much heavier than males. In large pigs fed near *ad libitum*, no significant differences in BW were reported for males and females less than 40 kg, but males had a larger BW than females when BW exceeded 40 kg (Campbell *et al.* 1988). With respect to FI and BW, Göttingen minipigs are probably more similar to Vietnamese Sway-back pigs than to large pigs. In Vietnamese Sway-back pigs fed near *ad libitum*, females had a larger FI and BW than males. ME intake of females was 1.2 times larger than in males, and BW was 32.5 kg in females and 20.0 kg in males, at the age of 6–7 months (Derno *et al.* 1997). Vietnamese pigs are part of the genetic background of the Göttingen minipig (Glodek *et al.* 1977).

The voluntary FI of pigs with continuous access to feed can be described as four times the maintenance requirement (ARC 1981).

From this it can be calculated that the voluntary ME intake in growing pigs with continuous access to feed can be given by $ME = 2876 \text{ kJ} \times BW^{0.63}$. The ME intake found in the present study lies far below the expected ME intake based on this. For homeothermic animals with continuous access to feed, voluntary ME intake can similarly be predicted by $ME = 1416 \text{ kJ} \times BW^{0.75}$, based on their maintenance requirement (Blaxter 1989). The equation for homeothermic animals corresponds more closely to the experimentally obtained data, than do the equations for pigs. This indicates that the FI of Göttingen minipigs is regulated as in homeothermic animals rather than as in modern production pigs. It remains unclear why the male Göttingen minipigs on Diet 2 had a much lower ME intake.

Haemoglobin, HCT, RBC and WBC were within published reference ranges (Jain 1986, Radin *et al.* 1986, Schmidt & Tumbleson 1986, Rispat *et al.* 1993, Ellegaard *et al.* 1995, Jorgensen *et al.* 1998). PLT in minipigs on Diet 2 were elevated compared to the reference range. Whereas reference values for PLT are 532 and $556 \times 10^9/l$ in 7-week-old male and female minipigs, respectively; 513 and $490 \times 10^9/l$ in 3-month-old and 349 and $365 \times 10^9/l$ in 6-month-old animals, respectively (Jorgensen *et al.* 1998); PLT values of minipigs on Diet 2 were 765 and $842 \times 10^9/l$, 558 and $618 \times 10^9/l$, and 519 and $589 \times 10^9/l$, respectively for males and females of 8, 13 and 21 weeks. PLT values of minipigs on Diet 1 were not elevated. Hypercholesterolaemia is associated with increased platelet reactivity, as was found in Göttingen minipigs fed a standard diet with added 2% cholesterol. CHOL levels were increased (7.83 mmol/l), and PLT values were elevated ($628 \times 10^9/l$). However, the mean platelet volume (MPV) had decreased significantly (6.91 vs 7.50 fl), which demonstrated an altered platelet function during hypercholesterolaemia (Garcia-Bolao *et al.* 1996). Incidentally, platelet dysfunction has been found in Göttingen minipigs fed at the recommended feeding level by the breeder (www.minipigs.com). Clinically, this manifested itself as thrombocytopenia purpura,

with low PLT findings (Carrasco *et al.* 2003). When comparing the *ad libitum* FI found in this study to the recommended FI, it can be found that the recommended FI corresponds to approximately 40% of the *ad libitum* FI. A milder level of restriction or adding more dietary fat to the diet may prevent thrombocytopenia purpura in Göttingen minipigs, by increasing PLT values. UREA and CHOL were elevated for females on Diet 2 compared to reference values (Parsons & Wells 1986, Tumbleson & Schmidt 1986, Ellegaard *et al.* 1995, Jorgensen *et al.* 1998, Loeb & Quimby 1999), but were not significantly different between groups. Also TRIG values for females on Diet 2 were elevated compared to reference values, although these were not significantly different between groups. Since females had the largest FI, and Diet 2 had the largest crude protein and crude fat content, elevated levels of UREA, CHOL and TRIG are consequential. Although GLUC levels all exceeded reference values, no significant differences were detected between groups. Reference values were based on analyses of full blood, and the data from the pilot experiment on analyses of serum. Therefore higher values are to be expected in serum samples, since pig red blood cells contain almost no glucose (Larsen *et al.* 2001). CREA and TPROT levels were within reference ranges.

The yellow-grey discoloured regions in the pars oesophagea of the stomach were caused by hyperkeratotic changes of the mucosa. This is frequently found in Göttingen minipigs (Madsen *et al.* 1998), as in other pig breeds, and is due to finely ground rations (Dobson *et al.* 1978) and pelleted feed (Pocock *et al.* 1969). This is consistent with the dietary formulation used in the pilot study, which was a finely ground, heat-expanded pellet. It was not specifically related to Diets 1 or 2. The grey focal areas in the liver and kidneys were inflammatory spots. Focal inflammation is regularly found in several organs, mainly consisting of interstitial mononuclear cell infiltrates, but is generally low in frequency in barrier-bred Göttingen minipigs compared to other species (Madsen *et al.* 1998,

Svendsen *et al.* 1998). Pale kidneys were likely to be the result of exsanguination directly after euthanasia.

No references on organ weights of Göttingen minipigs of 21 weeks of age were available, but reference values of adult (16 to 20-month-old) Göttingen minipigs were available (Holtz & Kallweit 1981). Relative weights of the stomachs of animals on Diet 1 were similar to those of adult Göttingen minipigs, but the relative weights of the stomachs of animals on Diet 2 were markedly lower. The relative organ weights of the ileum, caecum and spiral colon could not be compared, since Holtz and Kallweit (1981) reported weights of non-sectioned small and large intestines. The weight of the heart of males and females was alike, but the BW of females was larger than the BW of males, resulting in a significantly larger relative weight of the heart of male minipigs. It is known that obese pigs have a significant smaller heart in relation to BW than do lean pigs. The relative weight of the heart of obese pigs of 40 kg has been reported to be 3.48 g/kg, whereas lean pigs of 40 kg had a relative heart weight of 3.73 g/kg (Koong *et al.* 1983). Female minipigs had a relative heart weight close to this (3.16–3.27 g/kg), but the relative heart weight of males was markedly higher (4.39–5.54 g/kg). Since female minipigs could be characterized as obese, the results are in line with the finding of Koong *et al.* (1983). The absolute and relative weights of the spiral colon were larger in females than in males, as were the absolute weights of the stomach and caecum. The weights and relative weights of stomach and caecum were larger in animals on Diet 1 than on Diet 2, as was the relative weight of the spiral colon. The fact that females had a larger FI than males, and that the crude fibre content of Diet 1 was larger than the crude fibre content of Diet 2, are thought to be responsible for these effects. The effect of crude fibre on the intestines is well documented (Varel *et al.* 1982, Pond *et al.* 1988, Varel *et al.* 1988). Six-month-old lean and obese pigs, fed with high and low fibre diets, had a relative caecum weight (0.94–1.72 g/kg) similar to those of the Göttingen minipigs (0.94–1.51 g/kg), but

large pigs had a lower relative colon weight (6.95–13.20 g/kg) than Göttingen minipigs (8.63–18.12 g/kg) (Pond *et al.* 1988). Relative weights of both colon and caecum were significantly influenced by the type of diet. Animals fed the high fibre diet (Diet 1) had larger relative colon and caecum weights than animals fed the low fibre diet (Diet 2). Dietary effects can also be detected in relative weights of the stomach, liver and kidneys (Pond *et al.* 1988). In Göttingen minipigs this was found in the stomach, but not in liver and kidneys. The stomach of minipigs (5.82–6.10 g/kg) fed Diet 2 (low fibre) was approximately similar to that of pigs (4.03–6.34 g/kg), but minipigs on Diet 1 had markedly larger values (9.32–10.37 g/kg), which may indicate that the hypertrophic effect of a high fibre diet is stronger in minipigs than in pigs. The relative weight of the kidneys was approximately similar in minipigs than in lean and obese pigs (2.33–3.70 g/kg vs 2.04–2.42 g/kg), but the heart was larger in minipigs (3.16–5.54 g/kg vs 2.16–3.30 g/kg). The relative weight of the liver of minipigs (20.76–24.26 g/kg) was substantially larger than in pigs (8.09–9.08 g/kg).

Backfat thickness is larger in obese than in lean pigs (Buhlinger *et al.* 1978, Etherton 1980, Mersmann & Leymaster 1984, Seideman *et al.* 1989, Hauser *et al.* 1997). Buhlinger *et al.* (1978) compared age- and weight-matched lean Yorkshire pigs, with obese Ossabaw pigs, and found that weight-matched animals (45 kg) had different backfat thickness (2.0 vs 3.3 cm), but age-matched animals (170 days) had similar backfat thickness (3.1 vs 3.3 cm). Age-matched lean pigs were twice as heavy (90.4 kg vs 45.2 kg). Etherton (1980) also compared Yorkshire to Ossabaw pigs, but corrected for BW. He found a close to five-fold difference in backfat thickness between lean and obese pigs (1.5 vs 6.8 cm/100 kg). Mersmann and Leymaster (1984) also found that lean pigs had less backfat than obese pigs, and found that subcutaneous fat consists of an outer and an inner layer, of which the inner layer is most responsive to fat deposition in obese pigs. Backfat in pigs is largest over the shoulder region and

thinnest in the midback region. The lumbar region has a backfat thickness equal or greater than the midback region. Seideman *et al.* (1989) found backfat thicknesses of 3.10 cm in lean pigs, and 6.93 cm in obese pigs of approximately 110 kg. These results are in line with the backfat thickness of Göttingen minipigs. Females became obese after *ad libitum* feeding, whereas males did not, which is expressed by the significant difference between gender in backfat thickness. When correcting backfat thickness for weight for male and female Göttingen minipigs, values of 5–13 cm/100 kg (range) were found, which could be classified as obese compared to the results from pigs. In Göttingen minipigs, backfat thickness reduced from the cranial towards the caudal, contrary to the findings of Mersmann and Leymaster (1984). However, when analysing the results of Mersmann and Leymaster (1984) more closely, it can be found that their conclusion was based on the results from (lean) crossbred pigs, and that genetically-obese pigs had reduced backfat thickness from the cranial toward the caudal, similar to Göttingen minipigs.

It can be concluded that female Göttingen minipigs develop a significantly higher BW than males during growth, when fed *ad libitum*. The large difference in growth between male and female Göttingen minipigs during *ad libitum* FI does not appear to result from differences in ME intake. Metabolizable energy intake of Göttingen minipigs can be predicted by $ME = 1877 \text{ kJ} \times BW^{0.61}$, corresponding closely to the energy intake of homeothermic animals, but not to the energy intake of modern production pigs during *ad libitum* FI. Therefore, minipigs are considered to be different from large pigs, and nutrient requirements are thought to be different in both types of animals. Both male and female Göttingen minipigs became obese when fed *ad libitum*, as defined by relative backfat thickness and relative heart weight. Remarkably, no large changes in haematology and clinical chemistry occurred in *ad libitum* fed Göttingen minipigs, and no pathology other than enlarged fat reserves occurred. Apparently,

Göttingen minipigs are not able to restrain FI, and restricted feeding is therefore indicated to prevent obesity. The level of restriction is examined in our studies which follow.

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