# Bone Composition in Male and Female Göttingen Minipigs Fed Variously Restrictedly and Near *ad Libitum*

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***Summary***

The current study evaluated the influence of restricted feeding at low and medium levels versus near *ad libitum* food intake, on the growth and bone development in male and female Göttingen minipigs aged 8 to 43 weeks fed two different types of diets. Diet 1 was a low fat, high fibre diet, whereas diet 2 was a high fat, low fibre diet. A higher level of feed intake led to a significant increase in the following parameters: body weight development, bone size (length and width of rib and femur), bone volume (rib), bone (rib) dry matter and ash content (mg), as well as bone density (femur) as measured by X-ray absorption. Diet 2 gave a significantly higher body weight, bone volume and bone density of the femur shaft (cortical bone density) as compared to diet 1, whereas feed conversion was significantly lower on diet 2. On either diet, female minipigs had a significantly higher body weight development, bone volume, and dry matter and ash content of the rib (mg) as compared to males. Also bone mineral concentrations in the femur, expressed as calcium, phosphorus and magnesium in mg/cm$^3$, were significantly higher in females as compared to males, as was the Ca:$P_i$ ratio. Bone density measurements of the femur's proximal and distal segment, and total femur bone density (g/cm$^2$) were significantly higher in females as compared to males. Feed conversion in females was significantly lower than in males. This study illustrates that female and male minipigs show distinct differences in body and bone metabolism. Bone densities, in contrast to bone mineral concentrations, were related to the level of feed restriction and may therefore be useful biomarkers to study the influence of nutrient intake on bone metabolism in Göttingen minipigs.

***Introduction***

As minipigs are generally fed restrictedly in order to prevent fast growth and obesity, it is essential that the amount of essential nutrients ingested is sufficient to guarantee good health and reliable research results. The nutrient requirements for minipigs have not been studied systematically before (*Ritskes-Hoitinga & Bollen, 1997; Bollen, 2001*), and those guidelines that are available have been based simply on practical experience (*GV-Solas, 1993*). The current experiment was part of a multitude of studies into the nutrient needs of Göttingen minipigs, with the goal to establish a scientific basis for the minipig's nutrient requirements and proper feed intake level. The influence of two types of diet fed restrictedly and near *ad libitum* in growing male and female Göttingen minipigs was measured. Parameters measured were body weight development, feed conversion, bone mineral content and bone density. Moreover, the usage of bone density and bone mineral content was evaluated for their usefulness as possible biomarkers for the detection of nutritional deficiencies.

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## *Materials and Methods*

### *Animals and housing*

Forty male (n=20) and female (n=20) Göttingen minipigs of 8 weeks of age (Ellegaard Göttingen Minipigs, DK-4261 Dalmose, Denmark) were housed in same-sex pairs in floor pens of 1.2 $m^2$ at the SPF facility of the breeder. The health status was defined for 29 pathogens, of which only porcine rotavirus was found positive (*Hansen et al., 1997*). There were no clinical signs of rotavirus infection. The environmental temperature was 20.7 +/- 1.7 °C, and the relative humidity 63 +/- 18% (means +/- sd). No bedding material was provided. Lights were switched on from 6.00 to 18.00 hours.

Animals were randomly divided into pairs of the same sex over the different feeding groups, but ensuring that littermates were not included in the same group. All feeding groups had a similar body weight distribution at the start of the experiment.

### *Diets and feeding*

The minipigs were fed with two diets. Both diets were natural ingredient diets, custom-produced by Special Diets Services (SDS, Witham, CM8 3AD, Essex, UK), composed of cereals (barley, wheat, wheat feed), proteins (soya bean meal, sunflower seeds), fibre (oatmeal, soya hulls), energy (molasses) and supplements (vitamins, minerals). The diets were analysed for nutrients, chemical contaminants and microbiological contaminants. 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*). This diet could be characterized as a diet with a low metabolizable energy (ME) content (10.6 MJ/kg dry matter (DM)) and high fibre level (crude fibre was 150 g/kg DM), and was especially designed for minipigs. Diet 2 was a newly composed diet (*Ritskes-Hoitinga & Bollen, 1998*), based on the guidelines for swine from the US National Research Council (*NRC, 1988*). This diet had a high metabolisable energy content (12.0 MJ/kg DM) and a low fibre level (crude fibre content 100 g/kg DM), and was designed to meet the nutrient requirements of pigs. As part of the genetic background in Göttingen minipigs arises from the German Landrace pig, it was hypothesized that nutrient requirements for swine were applicable to minipigs as well. The physical form of both diets was an expanded pellet, with a diameter of 6 mm. During the entire experiment, diets from one production batch were used. The diets were stored at low temperature (5 °C).

The pair-housed animals were separated prior to feeding, and the meal fed from troughs at 9.00 and 14.30 hours. Feed intake (FI) was measured by weighing before feeding. The diets were offered to the animals for 30 minutes per feeding. Thereafter, the remains were removed and weighed again. Of diets 1 and 2, four males and four females received unrestricted amounts of feed (n=16 in total), and four males and four females received restricted amounts of feed (n=24 in total). The unrestricted amounts of feed were at 100% feeding level (*high*), based on the *ad libitum* FI (*Bollen et al., 2005*) and adjustments according to appetite. The restricted amounts of feed were aimed at 40% feeding level (*low*) compared to unrestricted feeding, and were adjusted according to the FI of the 100% feeding groups during the previous week. In addition, diet 2 was presented at 60% feeding level (*medium*). The duration of the experiment was 35 weeks.

### *Body weight*

The body weight (BW) of each animal was measured weekly. A table top balance (Mettler Toledo Spider 1, max. 60 kg, d= 20 g, CH-8606 Greifensee, Switzerland) was used for animals under 25 kg. Animals above 25 kg were weighed on a platform balance (max. 160 kg, d= 200 g, Danvaegt, DK-4000 Roskilde, Denmark).

### *Bone size*

After the end of the experiment, the minipigs were euthanized with an overdose of pentobarbital 20%, 100 mg/kg IV (Central Pharmacy, Royal Veterinary and Agricultural University, DK-1870 Frederiksberg, Denmark) after sedation with azaperone 4 mg/kg i.m. (Stresnil, Janssen Pharmaceutica, B-2340 Beerse, Belgium) and midazolam 1 mg/kg i.m. (Dormicum, Hoffmann-La Roche, CH-4070 Basel, Switzerland). The third right rib and left femur were isolated, cleaned free of for soft tissue and measured with a calliper gauge. The length of the rib was measured from the caput to the costochondral junction, and the width was measured on the widest part of the costal body. The measurements of the femur were taken from radiographs at a 1:1 magnification ratio. Radiographs were exposed for 0.1 seconds at 45 kV and 20mA (Philips BV22, A-1101 Vienna, Austria) on Fuji HRL film (FujiFilm, Bedford, MK42 0LF, UK) in a cassette with a CEA G Regular intensifying screen (CEA, S-64523 Strängnäs, Sweden). The length was measured from the greater trochanter to the lateral condyle, and the diameter was measured at the thinnest part of the shaft.

*Bone mineral content*

The ribs were boiled for 10 minutes under high pressure (1 kg/cm$^2$, Presto Deluxe, National Presto Ind., Inc., Eau Claire, WI 54703, USA) and remains of soft tissue were removed. Of the ribs, the wet weight and the weight under water were determined (Mettler H20T, max. 160 g, d= 0.01 mg, CH-8606 Greifensee, Switzerland). From this, the volume was calculated. The ribs were dried for 17 hours at 105 °C, and after weighing, the ribs were ashed for 17 hours at 500 °C in a muffle furnace (Hereaus Thermicon P, D-63405 Hanau, Germany). The dry weight of the ribs and ashes were determined (Sartorius AC210P, max 100 g, d= 0.1 mg, D-37075 Göttingen, Germany). The ashes were extracted in 6N HCl, and the bone mineral content (BMC), including calcium (Ca) and magnesium (Mg), was determined in the presence of 1% lanthanum chloride by atomic absorption spectrophotometry (Varian SpectrAA 250 Plus, Mulgrave Victoria 3170, Australia). Inorganic phosphorus (P) concentrations were determined by colorimetry (Cobas Bio, Roche Diagnostic Systems, CH-4070 Basel, Switzerland).

*Bone density*

The bone density was determined on a dual emission X-ray absorption scanner using the lumbar spine mode (Hologic QDR-1000/W, Bedford, MA 01730, USA). The proximal and distal segments, representing cancellous bone, and the shaft, representing cortical bone were analysed.

*Statistics*

Statistical analyses were performed using the ANOVA procedure (STATA 5, College Station, TX 77840, USA), with sex, diet and feeding level as between-subjects factors. When significant differences ($P<0.05$) were found in the between-subjects factors, pairwise comparisons of means using the Tukey method (*Gleason, 1999*) were performed to identify significantly different pairs. Only relevant pairs were compared, i.e. males and females fed the same diet and feeding level, males or females fed different diets at the same feeding level, and males or females fed the same diet at different feeding levels.

*Ethical aspects*

The study was granted a licence from the Danish Animal Experimentation Inspectorate, and was in compliance with national and international regulations.

***Results***

*Diets*

The analysed composition of the diets is summarised in *Table 1*. Chemical and microbiological contamination was not detected or below tolerance levels, and is therefore not presented.

*Feed intake, body weight, growth, feed conversion*

The feed intake (FI) of the individual minipigs ranged from 110 to 1490 g/day, at ages from 8 to 42 weeks. The average FI for the entire experiment was 534 +/- 284 g/day (mean + sd). FI increased with age, females had a higher FI than males, and FI increased with higher feeding levels, in accordance with the experimental design. The average FI expressed in g/day, and in ME DM per day and the

Table 1. Analysed composition (g/kg) of diet 1 (batch 5379) and diet 2 (batch 5387)

| | Diet 1 | Diet 2 |
|---|---|---|
| Dry matter | 904 | 900 |
| Organic matter | 840 | 841 |
| Crude protein | 144 | 187 |
| Crude fat | 25 | 113 |
| Crude fibre | 150 | 100 |
| Ash | 64 | 59 |
| Calcium | 11.7 | 7.7 |
| Phosphorus | 5.9 | 6.2 |
| Sodium | 2.6 | 2.3 |
| Chloride | 5.8 | 4.1 |
| Potassium | 8.5 | 5.7 |
| Magnesium | 1.6 | 1.8 |
| Iron | 0.149 | 0.140 |
| Copper | 0.005 | 0.008 |
| Manganese | 0.044 | 0.033 |
| Zinc | 0.155 | 0.101 |
| GE (kJ/kg)* | 16226 | 18465 |
| ME DM (kJ/kg DM)** | 10561 | 11965 |

*) GE = 23.86*CP + 39.76*CF + 17.58*CHO and CHO = OM - CP - CF
**) ME DM = 0.72 * GE * (DM / 1000)

CP = crude protein; CF = crude fat; CHO = carbohydrates; GE = gross energy;
ME = metabolisable energy; DM = dry matter; OM = organic matter

percentage of FI as compared to the high level food intake over the entire experiment are summarised in *Table 2*. The actual FI expressed as % of near *ad libitum* intake deviated from the targeted levels of 60% for the medium group, and 40% for the low group. Body weight and growth was significantly influenced by sex, type of diet and level of feeding (ANOVA test, P<0.05). Females were heavier than males. Diet 2 led to a higher body weight than diet 1 did, and higher levels of feeding led to higher body weights.

Feed conversion (FC) is presented as g FI/ g growth/ day and kJ ME DM FI/ g growth/ day. Values (means +/- sd over the entire period) were 4.4 +/- 0.2 g/g/day and 50.2 +/- 1.5 kJ/g/day respectively. Feed conversion was significantly influenced by sex and diet (ANOVA, P<0.05): females have lower FC's than males, and diet 2 gave a lower FC than diet 1. FC expressed as g FI/ g growth/ day showed significant differences between males on the high FI level on diet 1 versus diet 2, and the same was seen for females on this high FI level. These significant differences however disappeared when FC was expressed as kJ ME DM FI/ g growth/ day.

*Bone size*

The length of the right third rib and left femur were significantly different at different feeding levels (P<0.05), as was the width of the right third rib and left femur (P<0.05). Bone size increased with higher feeding level. The bone size is given in *Table 3*.

*Bone mineral content*

BMC is given in *Table 4*. Bone volume was significantly different between sexes (P<0.05), diets (P<0.05) and feeding levels (P<0.01). Females had a larger bone volume than males, bone volume was larger on diet 2 than on diet 1, and larger at high feeding level than at medium and low feeding levels. DM and ash content expressed in mg were significantly different between sexes (P<0.01) and feeding levels (P<0.01), but when ash content was expressed as % of DM significant effects were no longer found. No significant sex, diet or feeding level effects were detected in Ca, P and Mg content expressed as % of DM and % of ash. Bone mineral parameters Ca, P, and Mg, expressed as $mg/cm^3$, were significantly different between sexes (P<0.01), with females expressing higher values than males. Pairwise comparisons revealed that females on the medium level of diet 2 had a significantly higher Ca density than males on the same feeding level of the same diet. Also the molar $Ca:P_i$ ratio was significantly different between sexes (P<0.01), with females having a mean Ca:P ratio between 1.91 and 2.14, and males having a mean $Ca:P_i$ ratio between 1.65 and 1.92. The $Ca:P_i$ ratio

Table 2. Feed intake (FI), body weight (BW), growth and feed conversion (FC) of male and female Göttingen minipigs (n= 40) fed at high, medium and low level.

| | Diet 1 | | | | Diet 2 | | | | | | SEM* | ANOVA** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | | Low | | High | | Medium | | Low | | | |
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | |
| n | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | |
| FI (g/day) | 611 | 992 | 345 | 426 | 478 | 899 | 384 | 581 | 277 | 390 | | nt |
| FI (kJ ME DM/day)* | 6453 | 10477 | 3644 | 4499 | 5719 | 10757 | 4595 | 6952 | 3314 | 4666 | | nt |
| FI (%) | 100 | 100 | 56 | 43 | 100 | 100 | 80 | 65 | 58 | 43 | | nt |
| BW (kg), start | 3.5 | 4.2 | 4.4 | 4.0 | 4.7 | 4.3 | 4.0 | 4.3 | 4.4 | 4.1 | 0.1 | ns |
| BW (kg), end | 28.9 $^{abc}$ | 48.7 $^{d}$ | 19.9 $^{ac}$ | 26.4 $^{abc}$ | 33.2 $^{bc}$ | 62.9 $^{e}$ | 27.3 $^{abc}$ | 47.5 $^{d}$ | 18.7 $^{a}$ | 30.2 $^{c}$ | 2.3 | S D L |
| Growth (g/day) | 106 $^{ab}$ | 187 $^{d}$ | 65 $^{bc}$ | 94 $^{abc}$ | 120 $^{a}$ | 251 $^{e}$ | 98 $^{ac}$ | 182 $^{d}$ | 60 $^{bc}$ | 109 $^{a}$ | 10 | S D L |
| FC (g FI/g growth/day) | 5.8 $^{a}$ | 5.3 $^{ac}$ | 5.3 $^{ac}$ | 4.5 $^{ab}$ | 4.0 $^{bc}$ | 3.6 $^{b}$ | 3.9 $^{bc}$ | 3.2 $^{b}$ | 4.6 $^{ab}$ | 3.6 $^{b}$ | 0.2 | S D |
| FC (kJ ME DM/g growth/day) | 60.9 | 56.0 | 56.1 | 47.9 | 47.7 | 42.9 | 46.9 | 38.2 | 55.2 | 42.8 | 1.5 | ns |

*) Pooled standard error of the mean
**) Analyses of Variance; significant differences in between subjects factors Sex, Diet and Level are indicated with S, D and L respectively if P<0.05. nt = not tested, ns = not significant.
a-b) Within each row, values with different superscripts are significantly different (P<0.05).

in females ingesting the medium level of diet 2 had a significantly higher value as compared to males on the same diet and FI level.

*Bone density*

Bone density of the proximal and distal segments of the femur was significantly different between sexes (P<0.01) and feeding levels (P<0.01), but not between diets. Pairwise comparisons revealed that males and females on the low FI level of diet 2 had a significantly different bone density in the proximal femur segment. Males on the low FI level on diet 2 turned out to have a significantly lower bone density of the proximal femur segment than males on the high FI level on the same diet. Females on the high FI level of diet 1 had a significantly higher bone density in the distal femur segment as compared to females on the low FI level of the same

Table 3. Length and width (cm) of right third rib and left femur of male and female Göttingen minipigs fed at high, medium and low level.

| | Diet 1 | | | | Diet 2 | | | | | | SEM* | ANOVA** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | | Low | | High | | Medium | | Low | | | |
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | |
| Rib length | 11.4 | 12.5 | 11.3 | 10.6 | 12.3 | 12.8 | 10.7 | 10.6 | 10.8 | 11.1 | 0.2 | L |
| Rib width | 1.40 | 1.45 | 1.28 | 1.34 | 1.52 | 1.42 | 1.35 | 1.31 | 1.37 | 1.44 | 0.02 | L |
| Femur length | 13.7 | 13.5 | 12.9 | 13.1 | 14.4 | 14.2 | 13.6 | 13.8 | 12.9 | 13.0 | 0.1 | L |
| Femur width | 1.41 | 1.49 | 1.36 | 1.38 | 1.56 | 1.56 | 1.49 | 1.52 | 1.34 | 1.41 | 0.02 | L |

*) Pooled standard error of the mean
**) Significant differences in between subject factors Sex, Diet and Level are indicated with S, D and L respectively if P<0.05. ns = not significant.

Table 4. Bone mineral content of right third rib of male and female Göttingen minipigs fed at high, medium and low level.

| | Diet 1 | | | | Diet 2 | | | | | | SEM* | ANOVA** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | | Low | | High | | Medium | | Low | | | |
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | |
| Volume (cm$^3$) | $2.90^{ab}$ | $4.10^{bc}$ | $2.19^{a}$ | $2.43^{a}$ | $3.71^{b}$ | $4.25^{c}$ | $2.91^{ab}$ | $2.95^{ab}$ | $2.38^{a}$ | $2.96^{ab}$ | 0.13 | S D L |
| Dry matter (DM, mg) | $2937^{ab}$ | $4609^{cd}$ | $2225^{a}$ | $2710^{ab}$ | $3829^{bc}$ | $4761^{d}$ | $2685^{ab}$ | $3305^{abc}$ | $2337^{a}$ | $3134^{ab}$ | 155 | S L |
| Ash (mg) | $1825^{ab}$ | $2924^{c}$ | $1385^{a}$ | $1713^{ab}$ | $2404^{bc}$ | $2958^{c}$ | $1624^{ab}$ | $2043^{ab}$ | $1465^{a}$ | $1930^{ab}$ | 98 | S L |
| Ash (% of DM) | 62.2 | 63.5 | 62.2 | 63.0 | 62.8 | 62.1 | 60.6 | 61.9 | 62.8 | 61.5 | 0.3 | ns |
| Calcium (% of DM) | 23.6 | 23.7 | 23.3 | 23.9 | 23.8 | 22.8 | 22.7 | 23.8 | 23.4 | 23.0 | 0.1 | ns |
| Phosphorus (% of DM) | 9.7 | 9.9 | 10.1 | 9.9 | 10.3 | 9.3 | 9.9 | 10.1 | 9.7 | 9.9 | 0.1 | ns |
| Magnesium (% DM) | 0.41 | 0.41 | 0.40 | 0.41 | 0.41 | 0.38 | 0.40 | 0.40 | 0.41 | 0.39 | 0.00 | ns |
| Calcium (% of ash) | 37.9 | 37.3 | 37.4 | 37.8 | 37.9 | 36.7 | 37.5 | 38.5 | 37.3 | 37.4 | 0.1 | ns |
| Phosphorus (% of ash) | 15.6 | 15.6 | 16.3 | 15.8 | 16.4 | 15.0 | 16.3 | 16.4 | 15.5 | 16.1 | 0.1 | ns |
| Magnesium (% of ash) | 0.65 | 0.65 | 0.64 | 0.66 | 0.65 | 0.62 | 0.66 | 0.64 | 0.65 | 0.64 | 0.00 | ns |
| Calcium (mg/cm$^3$) | $241.0^{ab}$ | $265.2^{b}$ | $235.9^{ab}$ | $265.8^{b}$ | $244.7^{ab}$ | $257.5^{ab}$ | $210.6^{a}$ | $268.0^{b}$ | $232.6^{ab}$ | $243.2^{ab}$ | 3.9 | S |
| Phosphorus (mg/cm$^3$) | 99.2 | 110.8 | 102.7 | 110.7 | 105.7 | 105.0 | 91.7 | 113.5 | 96.7 | 104.9 | 1.7 | S |
| Magnesium (mg/cm$^3$) | 4.1 | 4.6 | 4.1 | 4.6 | 4.2 | 4.3 | 3.7 | 4.5 | 4.0 | 4.1 | 0.1 | S |
| Ca:Pi ratio | $1.92^{ab}$ | $2.08^{b}$ | $1.81^{ab}$ | $2.07^{b}$ | $1.85^{ab}$ | $2.14^{b}$ | $1.65^{a}$ | $2.06^{b}$ | $1.86^{ab}$ | $1.91^{ab}$ | 0.02 | S |

*) Pooled standard error of the mean

**) Significant differences in within subjects factor Age, and between subjects factors Sex, Diet and Level are indicated with A, S, D and L respectively if P<0.05. ns = not significant.

a-b) Within each row, values with different superscripts are significantly different (P<0.05).

diet. Also on diet 2, females on the low FI level had a significantly lower bone density in this femur segment as compared to females on the high FI level of diet 2.

The bone density of the shaft was significantly different between feeding levels (P<0.01) and diets (P<0.05), diet 2 gave a higher density than diet 1. The total bone density of the whole femur was significantly different between sexes (P<0.01) and feeding levels (P<0.01). Females had a larger bone density than males, and bone density was larger at high feeding level than at medium and low level. Males on the low level of diet 2 had a significantly lower bone density as compared to males on the high FI level of diet 2. Females on diet 1 had a significantly higher total bone density on the high FI level as compared to the low FI level. The same result accounts for females on diet 2. The bone density is given in *Table 5*.

## *Discussion*

### *Level of feed intake*

This study shows that a lower level of feed intake in growing Göttingen minipigs is associated with a reduced body weight, growth, bone size, total DM and ash content of the bone and bone density. In rats it has been demonstrated that energy restriction at 60% of *ad libitum* food intake will lead to a reduced body weight and decreased femoral bone mineral density (*Talbott et al., 2001*). Banu et al. (*2001*) found that food restricted rats (at 60% of *ad libitum* intake) had a decreased bone mineral content. Moreover, a limited energy supply impaired bone mineralization in minipigs (*Maier & Kreis, 2005*). It appears that these effects on the bone minerals may be mediated through a reduced energy intake as well as a reduced mineral intake as a result of a reduced total food intake.

According to Pastoor et al. (*1995*) a restriction in

Table 5. Bone density (g/cm$^2$) of left femur of male and female Göttingen minipigs fed at high, medium and low level.

| | Diet 1 | | | | Diet 2 | | | | | | SEM* | ANOVA** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High | | Low | | High | | Medium | | Low | | | |
| | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | | |
| Proximal segment | $0.742^{abc}$ | $0.847^{c}$ | $0.676^{ad}$ | $0.739^{abc}$ | $0.788^{bc}$ | $0.839^{c}$ | $0.708^{ab}$ | $0.765^{bcd}$ | $0.642^{a}$ | $0.760^{bcd}$ | 0.012 | S L |
| Shaft | 0.839 | 0.761 | 0.714 | 0.767 | 0.850 | 0.904 | 0.771 | 0.823 | 0.713 | 0.794 | 0.013 | D L |
| Distal segment | $0.869^{abc}$ | $1.010^{cd}$ | $0.800^{ae}$ | $0.850^{ab}$ | $0.955^{acd}$ | $1.031^{d}$ | $0.853^{ab}$ | $0.910^{bde}$ | $0.774^{a}$ | $0.879^{abc}$ | 0.015 | S L |
| Total | $0.829^{abc}$ | $0.920^{c}$ | $0.726^{ad}$ | $0.782^{abd}$ | $0.823^{bc}$ | $0.919^{c}$ | $0.777^{abd}$ | $0.829^{abc}$ | $0.711^{d}$ | $0.807^{abd}$ | 0.013 | S L |

*) Pooled standard error of the mean

**) Significant differences in between subject factors Sex, Diet and Level are indicated with S, D and L respectively if P<0.05. ns = not significant.

a-h) Within each row, values with different superscripts are significantly different (P<0.05).

phosphorus (P) intake to half the recommended amount in cats, resulted in a slight but systematically reduced body weight gain and shorter bones. By restricting feed intake in the current study, P intake was restricted as well, which probably contributed to reduced body weight and bone size. In the experiments of Pastoor et al. (*1995*) cats were fed isocaloric diets, with only levels of phosphorus varying. In the present experiment, minipigs were fed restrictedly, resulting in lower intake levels of all nutrients, including energy. For pigs of 10-20 kg, NRC (*1988*) recommends a daily mineral intake of 6.6 g for calcium (Ca), 5.7 g for phosphorus (P), and 0.4 g for magnesium (Mg). In the current experiment, the levels of intake of Mg exceeded these recommended levels in all groups. For Ca, only males and females fed at high level on diet 1, and females fed at high level on diet 2 had intakes exceeding the recommended level, whereas only females fed at high level on diet 1 had intakes of P exceeding the recommended levels. All other minipigs had levels of Ca and P intake lower than recommended for pigs. This reduced Ca and P intake may have contributed to the reduced body weight and bone parameters. However, the precise nutrient requirements for minipigs are currently not known. As nutrient requirements for farm pigs are aiming at maximum growth for animals that have been selected for a large growth potential, these may not be relevant for minipigs with a reduced growth potential and low adult body weight. Other results have indicated that nutrient requirements for swine are not appropriate for minipigs (*Bollen, 2001; Bollen et al., 2005*).

When phosphorus intake in rats was restricted to 50% of recommended levels, the length of the tibia and bone mineralization parameters were not affected (*Adams et al., 1989*). In the present study the length of the femur and rib were influenced by the level of feed intake. The differences between these studies can be related to experimental design, the species and the bone selected for measurements. Bone mineralization parameters (mg/cm$^3$) were not influenced by feeding level in the current study, which is in accordance to the rat study by Adams et al. (*1989*), where 50% P intake restriction did not adversely affect bone mineralization. In minipigs, a low phosphorus intake combined with an elevated level of calcium intake gave no reduced growth, as compared to diets with elevated phosphorus levels, and diets with elevated phosphorus and calcium levels (*Filer et al., 1966*). Although phosphorus is a limiting factor for growth, bone growth was sustained when P feeding levels were lower than recommended (*Adams et al., 1989; Filer et al., 1966*). The differences in the lengths and widths of the right third rib and left femur of minipigs fed at different feeding levels may therefore depend on other nutritional factors than calcium or phosphorus intake, e.g. energy, or a combination of the influence of various dietary factors. The level of FI had no differential influence on the food conversion, bone mineral composition as expressed in $mg/cm^3$ and $Ca:P_i$ ratio. These parameters appear to be kept stable, independent of the level of food intake. Bone density measurements ($g/cm^2$), however, revealed that this was influenced by the level of feed intake and may therefore be a more sensitive parameter to use for evaluating the dietary influence on bone mineralisation.

Schanler et al. (*1991*) found a significantly lower mineral content in the tibia and thoracic vertebrae of minipigs receiving calcium and phosphorus at 20% of the recommended levels, as compared to intakes of 60% and 100%. The phoshorus content of the cranium was also significantly lower at 20% restriction, as compared to 100%. In the current study no significant effects of feed restriction up to 40% of near ad libitum intake on certain bone mineral parameters (ash as % of DM, Ca, P, Mg as % of DM or ash) were measured. Schanler et al. (*1991*) used young Pitman-Moore/Hanford minipigs of unknown gender during a feeding period of only 14 days, where only Ca and P intake was reduced to 20%. In our study total FI was restricted to maximally 40% of ad libitum during a feeding period of 35 weeks, which can explain the different findings. As there was a clear sex effect on many parameters in our study, it is essential that gender is taken into account. In rats it was found that a restricted intake of phosphorus at 25% of the recommended level, resulted in impaired bone mineralization (femur), compared to intakes of 50%, 75% and 100% of the recommended level (*Schoenmakers et al., 1989*), which is in accordance with the results from Schanler et al. (*1991*).

Our data on % Ca of DM in the rib are in accordance with the published data by Roschger et al. (*1997*) for female Hanford minipigs. The % ash of dry bone weight in farm pigs (*Aerssens et al., 1998*) also approaches the values for minipigs in our study. These parameters do not appear to be discriminating factors for detecting species/strain differences and the influence of dietary food intake levels.

*Dietary composition*

Dietary composition significantly influenced body weight, food conversion, bone volume and bone density in femur shaft. Diet 2 caused a higher body weight, bone volume and bone density of femur shaft, whereas it caused a lower food conversion as compared to diet 1. As diet 2 is composed on the basis of the nutrient requirements for swine, it can be expected to lead to a faster and more efficient growth. However, as minipigs do not and should not have this large growth potential, diet 2 is not recommended for minipigs (*Bollen, 2001; Bollen et al., 2005*).

*Gender*

There were significant differences between the sexes for the following parameters: body weight, body weight development, food conversion, bone volume, bone DM (mg), bone ash content (mg), Ca ($mg/cm^3$), P ($mg/cm^3$), Mg ($mg/cm^3$), $Ca:P_i$ ratio, bone density ($g/cm^2$) of proximal, and distal femur segment and total bone density. All values were higher in females as compared to males, except for the food conversion, which was lower in females. Already during the execution of a pilot study it has become clear that there are major gender differences in Göttingen minipigs (*Bollen et al., 2005*). This gender difference was also found in archaeological samples from early domesticated pigs (*Ioannidou, 2003*).

The level of FI significantly influenced the bone density measurements ($g/cm^2$), in contrast to bone mineral concentrations ($mg/cm^3$). This indicates that bone density measurements are a more sensitive parameter for detecting the influence of feed restriction on bone physiology in Göttingen minipigs, than bone mineral concentration analyses. Bone density measurement may therefore be useful as an early biomarker for bone development. In osteoporosis research, low bone density is achieved by combining calcium restriction with ovariectomy in minipigs and other species. Calcium restriction alone did not lead to osteoporosis in minipigs (*Mosekilde et al., 1993*). Reduction in bone minerals could be achieved in minipigs by treatment with glucocorticoids (*Ikeda et al., 2003*). In our study a reduced bone density in males and females was achieved by dietary restriction, and feed restriction may therefore be an appropriate supplemental tool to induce osteoporosis in minipigs, in combination with ovariectomy in females (*Mosekilde et al., 1993*) or treatment with glucocorticoids (*Ikeda et al., 2003*). As BMC were not differentially influenced by different feeding levels, bone density measurements appear a better tool for detecting the differential influence of dietary modifications on bone composition.

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