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## **Low sanitary conditions increase energy expenditure for maintenance and decrease incremental protein efficiency in growing pigs**

**Y. van der Meer1,2, A.J.M. Jansman3 and W.J.J. Gerrits1**

*1Animal Nutrition Group, Wageningen University, Wageningen, 6708 WD, The Netherlands;*

*2De Heus Animal Nutrition, Ede, 6717 VE, The Netherlands;*

*3Wageningen Livestock Research, Wageningen, 6708 WD, The Netherlands*

Corresponding author: Walter Gerrits. E-mail: [walter.gerrits@wur.nl](mailto:walter.gerrits@wur.nl)

Animal journal

**Supplementary material S1**

*Vaccination program high sanitary condition pigs*

At the first week of age high sanitary condition (**HSC**) pigs were vaccinated against Mycoplasma hyopneumoniae (Porcilis M Hyo, MSD Animal Health, Boxmeer, The Netherlands) by subcutaneous injection in the neck.

Upon arrival and two days thereafter, HSC pigs received an antibiotic injection (Fenflor, AUV, Cuijk, The Netherlands, one mL per pig, *intramuscular* per time point). In week four of age HSC pigs were vaccinated against Mycoplasma hyopneumoniae, Porcine circovirus type 2, Porcine Reproductive and Respiratory Syndrome (PRRS), and Lawsonia intracellularis (Porcilis M Hyo, Porcilis Circo, Porcilis PRRS, all MSD Animal Health, Boxmeer, The Netherlands, and Enterisol Ileitis, Boehringer Ingelheim, Alkmaar, The Netherlands). At six weeks of age HSC pigs were vaccinated against Actinobacillus pleuropneumoniae, and Influenza A virus (Porcilis APP, MSD Animal Health, Boxmeer, The Netherlands, and Gripovac3, Merial, Velserbroek, The Netherlands); and at eight weeks of age HSC pigs were vaccinated against Actinobacillus pleuropneumoniae, and Influenza A virus (Porcilis APP, MSD Animal Health, Boxmeer, The Netherlands, and Gripovac3, Merial, Velserbroek, The Netherlands) by *subcutaneous* injection in the neck or in case of Enterisol by oral drench.

**Supplemental Table S1** *Energy balance parameters for ad libitum fed pigs kept under LSC or HSC and fed diets differing in amino acid composition1*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | LSC | | HSC | |  | *P* | | | | | | |
|  | AA-B | AA-S2 | AA-B | AA-S | SEM3 | SC | diet | batch | SC × diet | SC × batch | diet × batch | GE intake4 |
| BW, kg | 17.8 | 19.0 | 24.2 | 24.5 | 0.6 | <.0001 | 0.15 | <.0001 | 0.42 | 0.21 | 0.42 | - |
| ME:GE ratio, % | 79 | 81 | 85 | 84 | 1 | 0.003 | 0.68 | 0.52 | 0.44 | 0.22 | 0.18 | 0.30 |
| Parameter, kJ/(kg BW)0.6 per day |  |  |  |  |  |  |  |  |  |  |  |  |
| GE intake | 2 843 | 3 007 | 2 872 | 2 843 | 56 | 0.246 | 0.24 | 0.0008 | 0.10 | <.0001 | 0.06 | - |
| ME intake | 2 285 | 2 329 | 2 449 | 2 439 | 36 | 0.002 | 0.64 | 0.44 | 0.49 | 0.23 | 0.15 | 0.002 |
| Methane production | 3.8 | 4.8 | 9.2 | 10.5 | 1.1 | 0.0002 | 0.28 | 0.26 | 0.89 | 0.96 | 0.80 | 0.26 |
| Heat production | 1 373 | 1 368 | 1 429 | 1 401 | 20 | 0.04 | 0.43 | 0.05 | 0.58 | 0.24 | 0.61 | 0.10 |
| Energy retention total | 912 | 961 | 1 020 | 1 039 | 47 | 0.07 | 0.48 | 0.13 | 0.77 | 0.36 | 0.49 | 0.05 |
| Energy retention protein | 358 | 376 | 440 | 421 | 11 | <.0001 | 0.97 | 0.28 | 0.15 | 0.42 | 0.71 | 0.02 |
| Energy retention fat | 554 | 585 | 580 | 618 | 41 | 0.48 | 0.42 | 0.13 | 0.94 | 0.35 | 0.50 | 0.10 |

1LSC = low sanitary conditions; HSC = high sanitary conditions; SC = sanitary condition; AA-B = basal amino acid diet; AA-S = supplemented amino acid diet; GE = gross energy; ME = metabolizable energy.

2containing 20% more methionine, threonine, and tryptophan compared to the AA-B diet.

3 n = 6 per treatment group.

4The P-value for GE-intake represents the significance of the covariable GE-intake. All parameters except BW were adjusted to a similar level of feed intake by including GE intake as a covariate.

**Supplemental Table S2** *Energy balance parameters for restricted fed pigs kept under LSC or HSC and fed diets differing in amino acid composition1*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | LSC | | HSC | |  | *P* | | | | | | |
|  | AA-B | AA-S2 | AA-B | AA-S | SEM3 | SC | diet | batch | SC × diet | SC × batch | diet × batch | GE intake4 |
| BW, kg | 21.6 | 23.4 | 29.3 | 29.5 | 0.6 | <.0001 | 0.13 | <.0001 | 0.20 | 0.83 | 0.74 | - |
| ME:GE ratio, % | 82 | 83 | 85 | 84 | 1 | 0.04 | 0.65 | 0.75 | 0.26 | 0.09 | 0.33 | 0.52 |
| Parameter, kJ/(kg BW)0.6 per day |  |  |  |  |  |  |  |  |  |  |  |  |
| GE intake | 1 819 | 1 934 | 2 035 | 1 933 | 49 | 0.05 | 0.89 | 0.007 | 0.04 | 0.001 | 0.24 | - |
| ME intake | 1 592 | 1 601 | 1 636 | 1 615 | 11 | 0.03 | 0.57 | 0.74 | 0.25 | 0.06 | 0.41 | <.0001 |
| Methane production | 4.6 | 5.2 | 10.3 | 10.3 | 0.8 | <.0001 | 0.61 | 0.62 | 0.74 | 0.67 | 0.46 | 0.71 |
| Heat production | 1 162 | 1 159 | 1 153 | 1 142 | 17 | 0.49 | 0.64 | 0.008 | 0.83 | 0.77 | 0.97 | 0.02 |
| Energy retention total | 430 | 442 | 483 | 473 | 21 | 0.08 | 0.94 | 0.01 | 0.64 | 0.41 | 0.72 | <.0001 |
| Energy retention protein | 244 | 249 | 261 | 254 | 6 | 0.11 | 0.82 | 0.08 | 0.39 | 0.21 | 0.06 | <.0001 |
| Energy retention fat | 185 | 193 | 222 | 219 | 18 | 0.13 | 0.88 | 0.02 | 0.79 | 0.62 | 0.98 | 0.0002 |

1LSC = low sanitary conditions; HSC = high sanitary conditions; SC = sanitary condition; AA-B = basal amino acid diet; AA-S = supplemented amino acid diet; GE = gross energy; ME = metabolizable energy.

2containing 20% more methionine, threonine, and tryptophan compared to the AA-B diet.

3 n = 6 per treatment group.

4The P-value for GE-intake represents the significance of the covariable GE-intake. All parameters except BW were adjusted to a similar level of feed intake by including GE intake as a covariate.

**Supplemental Table S3** *Nitrogen balance parameters for ad libitum fed pigs kept under LSC or HSC and fed diets differing in amino acid composition1*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | LSC | | HSC | |  | *P* | | | | | | |
|  | AA-B | AA-S2 | AA-B | AA-S | SEM3 | SC | diet | batch | SC × diet | SC × batch | diet × batch | GE intake4 |
| BW, kg | 17.8 | 19.0 | 24.2 | 24.5 | 0.6 | <.0001 | 0.15 | <.0001 | 0.42 | 0.21 | 0.42 | - |
| N intake, g/(kg BW)0.6 per day | 1.42 | 1.38 | 1.27 | 1.24 | 0.01 | <.0001 | 0.02 | <.0001 | 0.55 | 0.96 | 0.26 | <.0001 |
| N retention, g/(kg BW)0.6 per day | 0.73 | 0.75 | 0.80 | 0.76 | 0.02 | 0.09 | 0.81 | 0.02 | 0.21 | 0.38 | 0.53 | 0.05 |
| N retention/N intake | 0.51 | 0.55 | 0.63 | 0.61 | 0.02 | 0.0001 | 0.69 | 0.42 | 0.14 | 0.46 | 0.66 | 0.93 |

1LSC = low sanitary conditions; HSC = high sanitary conditions; SC = sanitary condition; AA-B = basal amino acid diet; AA-S = supplemented amino acid diet; N = nitrogen; GE = gross energy.

2containing 20% more methionine, threonine, and tryptophan compared to the AA-B diet.

3 n = 6 per treatment group.

4The P-value for GE-intake represents the significance of the covariable GE-intake. All parameters except BW were adjusted to a similar level of feed intake by including GE intake as a covariate.

**Supplemental Table S4** *Nitrogen balance parameters for restricted fed pigs kept under LSC or HSC and fed diets differing in amino acid composition1*

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | LSC | | HSC | |  | *P* | | | | | | |
|  | AA-B | AA-S2 | AA-B | AA-S | SEM3 | SC | diet | batch | SC × diet | SC × batch | diet × batch | GE intake4 |
| BW, kg | 21.6 | 23.4 | 29.3 | 29.5 | 0.6 | <.0001 | 0.13 | <.0001 | 0.20 | 0.83 | 0.74 | - |
| N intake, g/(kg BW)0.6 per day | 0.89 | 0.86 | 0.81 | 0.79 | 0.00 | <.0001 | 0.002 | <.0001 | 0.93 | 0.50 | 0.06 | <.0001 |
| N retention, g/(kg BW)0.6 per day | 0.46 | 0.47 | 0.45 | 0.44 | 0.01 | 0.15 | 0.64 | 0.006 | 0.43 | 0.19 | 0.17 | 0.0007 |
| N retention/N intake | 0.52 | 0.54 | 0.56 | 0.55 | 0.01 | 0.11 | 0.48 | 0.09 | 0.35 | 0.26 | 0.02 | 0.15 |

1LSC = low sanitary conditions; HSC = high sanitary conditions; SC = sanitary condition; AA-B = basal amino acid diet; AA-S = supplemented amino acid diet; N = nitrogen; GE = gross energy.

2containing 20% more methionine, threonine, and tryptophan compared to the AA-B diet.

3 n = 6 per treatment group.

4The P-value for GE-intake represents the significance of the covariable GE-intake. All parameters except BW were adjusted to a similar level of feed intake by including GE intake as a covariate.