| Item                              | LOFLOP | LOFHIP  | HIFLOP   | HIFHIP   |
|-----------------------------------|--------|---------|----------|----------|
| Ingredients (g/kg as-fed basis)   |        |         |          |          |
| Wheat starch                      | 233    | 167     | 66       | 0        |
| Whole grain wheat (roller milled) | 150    | 150     | 150      | 150      |
| Wheat bran (finely milled)        | 125    | 125     | 125      | 125      |
| Enzyme-treated wheat bran         | -      | -       | 200      | 200      |
| Wheat gluten                      | 65     | 65      | 32       | 32       |
| Fish meal                         | 20     | 20      | 20       | 20       |
| Whey protein hydrolysate          | -      | 68      | -        | 68       |
| Fructose                          | 200    | 200     | 200      | 200      |
| Lard                              | 150    | 150     | 150      | 150      |
| Vitamins and minerals             | 57     | 56      | 57       | 56       |
| Chemical composition (g/kg DM)    |        |         |          |          |
| DM (g/kg as-fed basis)            | 913    | 913     | 919      | 919      |
| Ash                               | 62     | 65      | 73       | 76       |
| Crude protein (N $\times$ 6.25)   | 113    | 179     | 114      | 175      |
| HCL fat                           | 174    | 180     | 188      | 187      |
| Available carbohydrates           | 577    | 522     | 456      | 387      |
| Sugars                            |        |         |          |          |
| Fructose                          | 225    | 223     | 224      | 221      |
| Glucose                           | 1      | 1       | 7        | 7        |
| Sucrose                           | 7      | 7       | 9        | 8        |
| Starch                            | 344    | 292     | 216      | 150      |
| Dietary fibre <sup>1</sup>        | 100    | 106     | 191      | 205      |
| NSP (soluble NSP)                 | 69 (8) | 75 (12) | 136 (22) | 136 (15) |
| AX (SAX)                          | 44 (5) | 46 (7)  | 86 (16)  | 85 (12)  |
| AXOS                              | 5      | 3       | 12       | 17       |
| Fructans                          | 6      | 8       | 11       | 9        |
| Klason lignin                     | 19     | 20      | 30       | 41       |
| RS <sup>2</sup>                   | 2      | 1       | 1        | 1        |
| Gross energy (MJ/ kg DM)          | 20.7   | 21.5    | 21.3     | 21.7     |

Table S1 Ingredients and chemical composition of the experimental diets.

LOFLOP, low fibre low protein diet; LOFHIP, low fibre high protein diet; HIFLOP, high fibre low protein diet; HIFHIP, high fibre high protein diet. NSP, total non-starch polysaccharides; AX, arabinoxylan; RS, resistant starch; A:X, arabinose:xylose; AXOS, low molecular weight arabinoxylan-oligosaccharides.

<sup>1</sup>Dietary fibre = NSP + fructans + RS + AXOS + Klason lignin.

<sup>2</sup>Determined by enzymatic resistant starch assay (AOAC method 2002.02).

| Item   | Q1 mass | Q3 mass | DP   | EP  | CE  | CXP |
|--|---------|---------|------|-----|-----|-----|
| Acetic acid quantifier                                 | 193.8   | 151.9   | -80  | -15 | -15 | -15 |
| Acetic acid qualifier                                  | 193.8   | 46.0    | -80  | -15 | -50 | -15 |
| $^{13}C_2$ acetic acid quantifier                      | 196.0   | 152.0   | -100 | -15 | -24 | -13 |
| $^{13}C_2$ acetic acid qualifier                       | 196.0   | 121.8   | -100 | -15 | -24 | -10 |
| Propionic acid quantifier                              | 208.0   | 136.9   | -20  | -10 | -24 | -9  |
| Propionic acid qualifier                               | 208.0   | 46.0    | -20  | -10 | -50 | -20 |
| <sup>13</sup> C <sub>1</sub> propionic acid quantifier | 209.0   | 137.0   | -20  | -10 | -25 | -9  |
| <sup>13</sup> C <sub>1</sub> propionic acid qualifier  | 209.0   | 165.1   | -20  | -10 | -18 | -11 |
| Isobutyric acid quantifier                             | 221.9   | 178.9   | -100 | -2  | -18 | -15 |
| Isobutyric acid qualifier                              | 221.9   | 42.0    | -60  | -2  | -70 | -19 |
| Butyric acid quantifier                                | 221.9   | 178.9   | -100 | -2  | -18 | -15 |
| Butyric acid qualifier                                 | 221.9   | 42.0    | -60  | -2  | -70 | -19 |
| <sup>13</sup> C <sub>2</sub> butyric acid quantifier   | 224.0   | 205.8   | -100 | -10 | -20 | -12 |
| $^{13}C_2$ butyric acid qualifier                      | 224.0   | 180.0   | -100 | -10 | -18 | -15 |
| Succinic acid quantifier                               | 387.1   | 234.1   | -30  | -10 | -25 | -12 |
| <sup>13</sup> C <sub>2</sub> succinic acid qualifier   | 387.1   | 97.8    | -30  | -10 | -47 | -10 |
| <sup>13</sup> C <sub>2</sub> succinic acid quantifier  | 389.1   | 99.9    | -145 | -10 | -40 | -10 |
| Succinic acid qualifier                                | 389.1   | 236.1   | -145 | -10 | -24 | -15 |
| Isovaleric acid quantifier                             | 235.9   | 137.0   | -15  | -15 | -30 | -15 |
| Isovaleric acid qualifier                              | 235.9   | 46.0    | -15  | -15 | -40 | -15 |
| Valeric acid quantifier                                | 235.9   | 137.0   | -15  | -15 | -30 | -15 |
| Valeric acid qualifier                                 | 235.9   | 46.0    | -15  | -15 | -40 | -15 |
| <sup>13</sup> C <sub>3</sub> valeric acid quantifier   | 239.0   | 136.9   | -120 | -10 | -27 | -15 |
| <sup>13</sup> C <sub>3</sub> valeric acid qualifier    | 239.0   | 151.8   | -120 | -10 | -24 | -12 |

**Table S2** Compound-dependent LC-MS/MS parameters, declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CXP).

|              | 0 5               | 1                |                  |                 |                              |       |              |
|--------------|-------------------|------------------|------------------|-----------------|------------------------------|-------|--------------|
| Itom         | Diet <sup>1</sup> |                  |                  |                 | <i>P</i> -value <sup>2</sup> |       |              |
| Item         | LOFLOP            | LOFHIP           | HIFLOP           | HIFHIP          | F                            | Р     | $F \times P$ |
| Caecum       |                   |                  |                  |                 |                              |       |              |
| Total SCFA   | $9.1 \pm 1.5$     | $8.1 \pm 1.6$    | $9.7 \pm 1.3$    | $13 \pm 1.3$    | 0.077                        | 0.29  | 0.13         |
| Acetate      | $6.2 \pm 1.0$     | $5.3 \pm 1.1$    | $6.3\pm0.89$     | $8.8\pm0.93$    | 0.091                        | 0.29  | 0.10         |
| Propionate   | $1.6\pm0.29$      | $1.5 \pm 0.31$   | $1.4\pm0.27$     | $2.3\pm0.27$    | 0.29                         | 0.090 | 0.080        |
| Butyrate     | $1.1\pm0.23$      | $0.97\pm0.25$    | $1.4 \pm 0.21$   | $1.6\pm0.22$    | 0.048                        | 0.80  | 0.37         |
| BCFA         | $0.11\pm0.02$     | $0.11\pm0.02$    | $0.08\pm0.02$    | $0.11\pm0.02$   | 0.28                         | 0.26  | 0.33         |
| Entire colon |                   |                  |                  |                 |                              |       |              |
| Total SCFA   | $29^{b} \pm 5.0$  | $27^{b} \pm 5.0$ | $29^{b} \pm 4.7$ | $50^a \pm 4.7$  | 0.023                        | 0.052 | 0.024        |
| Acetate      | $18^{b} \pm 3.0$  | $17^{b} \pm 3.0$ | $18^{b} \pm 2.8$ | $30^a \pm 2.8$  | 0.032                        | 0.064 | 0.023        |
| Propionate   | $5.0 \pm 1.2$     | $4.7 \pm 1.2$    | $5.9 \pm 1.1$    | $10 \pm 1.1$    | 0.007                        | 0.092 | 0.058        |
| Butyrate     | $4.5 \pm 1.2$     | $4.3 \pm 1.2$    | $5.7 \pm 1.1$    | $7.6 \pm 1.1$   | 0.040                        | 0.41  | 0.32         |
| BCFA         | $0.68 \pm 0.12$   | $0.73 \pm 0.12$  | $0.52 \pm 0.11$  | $0.98 \pm 0.11$ | 0.68                         | 0.028 | 0.072        |

**Table S3** Pool size (mmol) of short chain fatty acid in caecum and entire colon of Göttingen Minipigs fed diets low or high in dietary fibre and protein.

<sup>1</sup>The individual minipig was regarded as the experimental unit, n = 10 for low fibre low protein diet (LOFLOP), n = 10 for low fibre high protein diet (LOFHIP), n = 12 for high fibre low protein diet (HIFLOP) and n = 11 for high fibre high protein diet (HIFHIP). Data are shown as mean  $\pm$  SEM. <sup>2</sup>F, fibre effect; P, protein effect; F × P, fibre × protein interaction. Different superscript letters in a row are presented for the significant interaction (P < 0.05) after adjustment for multiple comparisons by the Tukey–Kramer post hoc test.



Fig. S1 The representative MRM chromatogram of SCFA standards.



**Fig. S2** Concentrations of total SCFA, acetate, propionate, butyrate and BCFA (A) and distribution of total SCFA (B) in faeces of Göttingen Minipigs. Data are shown as mean  $\pm$  SEM represented by vertical bars. The individual minipig was regarded as the experimental unit, n = 10 for low fibre low protein diet (LOFLOP), n = 10 for low fibre high protein diet (LOFHIP), n = 12 for high fibre low protein diet (HIFLOP) and n = 11 for high fibre high protein diet (HIFHIP). Only significant *P* - values are presented in the figure.



**Fig. S3** Alpha rarefaction curves for each diet, showing the Faith's phylogenetic diversity (faith\_ph) (y axis) as a function of sequencing depth (x axis). Samples were rarefied to 25322 sequences for caecum (A), 31787 sequences for mid colon (B) and 25806 for faecal samples (C), which means the minimum sequence per sample depth in the dataset. The individual minipig was regarded as the experimental unit, n = 10 for low fibre low protein diet (LOFLOP), n = 10 for low fibre high protein diet (LOFHIP), n = 12 for high fibre low protein diet (HIFLOP) and n = 11 for high fibre high protein

diet (HIFHIP). One digesta sample from mid colon in LOFLOP group (n = 9) was omitted due to low-quality reads. Only significant *P* -values are presented in the figure.



**Fig. S4** Principal coordinate analysis plot of the weighted Unifrac metric. The grey, yellow, red and blue line with points represented caecum (A), mid colon (B) and faecal (C) communities of individual minipig after feeding low fibre low protein diet (LOFLOP, n = 10), low fibre high protein diet (LOFHIP, n = 10), high fibre low protein diet (HIFLOP, n = 12) and high fibre high protein diet (HIFHIP, n = 11), respectively. One digesta sample from mid colon in LOFLOP group (n = 9) was omitted due to low-quality reads. Only significant fibre effect was observed in principal coordinate analysis plot of caecum, colon and faecal communities (P < 0.01).



**Fig. S5** Identification of the most differentially abundant genera in caecum (A), mid colon (B) and faecal samples (C) in Göttingen Minipigs fed low or high fibre and protein diets. The plot was generated from Linear Discriminant Analysis Effect Size (LEfSe) analysis with CSS-normalized OTU table and displays taxa with LDA scores above 2.0 and *P*-values below 0.01. Genera enriched

in the samples with low fibre (LOF) or low protein (LOP) are indicated with green bars, and genera enriched in the samples with high fibre (HIF) or high protein (HIP) are indicated with a red bars. The individual minipig was regarded as the experimental unit, n = 20 for LOF, n = 23 for HIF, n = 22 for LOP and n = 21 for HIP. One digesta sample from mid colon in LOFLOP group (n = 19 for LOF) was omitted due to low-quality reads.