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► **To cite this version:**

Robin Kalmendal, Klas Elwinger, Lena Holm, Ragnar Tauson. High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens. *British Poultry Science*, 2011, 52 (01), pp.86-96. 10.1080/00071668.2010.547843 . hal-00671644

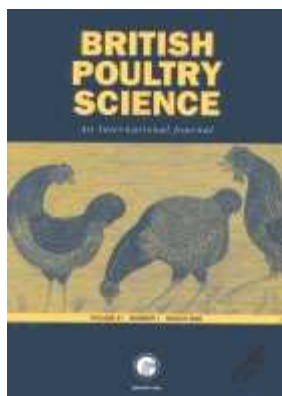
HAL Id: hal-00671644

<https://hal.science/hal-00671644>

Submitted on 18 Feb 2012

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Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2010-053.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	24-May-2010
Complete List of Authors:	Kalmendal, Robin; Department of Animal Nutrition and Management Elwinger, Klas; Department of Animal Nutrition and Management Holm, Lena; Department of Anatomy, Physiology and Biochemistry Tauson, Ragnar; Department of Animal Nutrition and Management
Keywords:	Feedstuffs, Insoluble fibre, Sunflower cake, Broilers, Gut health, Digestibility

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Manuscripts

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3 1 **High-fibre sunflower cake affects small intestinal digestion and health in broiler**
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5 2 **chickens**

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21 **Running title: HIGH-FIBRE SUNFLOWER AND INTESTINE**
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Accepted for publication 28th June 2010

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2
3 24 **Abstract** 1. An experiment was conducted to evaluate high-fibre sunflower cake (HF-SFC);
4
5 25 a feed ingredient distinguished by large amounts of crude fibre and insoluble non-starch
6
7 26 polysaccharides (i-NSP).
8
9
10 27 2. Broiler chickens (n=160) were fed on pelleted maize-based diets free from coccidiostats
11
12 28 and antibiotic growth promoters between 15 and 31 d of age. Diets included 0, 10, 20 or 30%
13
14 29 HF-SFC. Performance and small intestinal health were assessed.
15
16
17 30 3. In general, HF-SFC inclusion mediated significant linear increases in ileal digestibility of
18
19 31 fat and protein and significant linear decreases in ileal digestibility of dry matter, ash and
20
21 32 energy.
22
23
24 33 4. Weight gain increased linearly with HF-SFC inclusion. Feed conversion was negatively
25
26 34 affected by 30% HF-SFC but not by 20% HF-SFC.
27
28
29 35 5. In the jejunal lumen, inclusion of HF-SFC was associated with significant decreases in
30
31 36 colony counts of *Clostridium* spp.
32
33
34 37 6. HF-SFC inclusion resulted in significant linear reductions of villus height, thickness of
35
36 38 muscularis mucosa, and the circular and longitudinal layers of muscularis in the jejunum.
37
38 39 Crypt depth and submucosal thickness were not affected.
39
40
41 40 7. The data indicate that broiler chickens may thrive on feeds with insoluble fibre contents far
42
43 41 exceeding those used in practice, and that HF-SFC exerts some positive effects on digestion
44
45 42 and small intestinal health.
46
47

48 43 INTRODUCTION

49
50 44 Price fluctuations on the feedstuffs market and the progression of alternative poultry systems,
51
52 45 *e.g.* organic production, make it interesting to evaluate less-conventional feed ingredients
53
54 46 such as cold-pressed sunflower cake. Sunflower cake is a protein rich feedstuff distinguished
55
56 47 by a favourable amino acid profile. Given that the seeds are not decorticated, it also
57
58 48 constitutes a rich source of insoluble non-starch polysaccharides (i-NSP). In poultry nutrition,
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60

1
2
3 49 the early literature describes beneficial effects of i-NSP-rich feedstuffs on pullet health
4
5 50 (Bearse *et al.*, 1940), growth (Davis and Briggs, 1947) and digestibility in broiler chickens
6
7 51 (Rogel *et al.*, 1987). More recently, it was indicated that the addition of some i-NSP fraction
8
9 52 to the feed correlates with improved feed conversion (Hetland *et al.*, 2003; González-
10
11 53 Alvarado *et al.*, 2007; Jiménez-Moreno *et al.*, 2009b), increased AME_N of the feed
12
13 54 (González-Alvarado *et al.*, 2007; Hetland and Svihus, 2007; Amerah *et al.*, 2009; Jiménez-
14
15 55 Moreno *et al.*, 2009b) and enhanced digestibility of dry matter (DM), organic matter, nitrogen
16
17 56 and fat (González-Alvarado *et al.*, 2007; Jiménez-Moreno *et al.*, 2009a,b). Further, it has been
18
19 57 demonstrated that i-NSP influence gut microflora (Amerah *et al.*, 2009) and may reduce
20
21 58 necrotic enteritis lesions (Branton *et al.*, 1997). The mechanisms underlying these effects are
22
23 59 not fully understood, but feeds rich in i-NSP were shown to stimulate gizzard activity and the
24
25 60 secretion of amylase and bile acids in broiler chickens and layers (Hetland *et al.*, 2003) as
26
27 61 well as the activity of digestive enzymes bound to the apical membrane of small intestinal
28
29 62 enterocytes in newly weaned pigs (Hedemann *et al.*, 2006). We hypothesised that high-fibre
30
31 63 sunflower cake (HF-SFC) may exert beneficial effects on broiler performance and small
32
33 64 intestinal health. Hence, the present experiment was set up to study the effects of different
34
35 65 amounts of HF-SFC in the diet of broiler chickens between 15 and 31 d of age.
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67 MATERIALS AND METHODS

68 **Experimental design**

69 Day-old Ross 308 broiler chickens (n=160) of mixed sexes were randomly distributed to 20
70 pens (replicates) measuring 70 x 148 cm, in a fluorescent-lit temperature-controlled facility.
71 From d 1 to d 8, the chicks were fed on a commercial starter diet and a commercial grower
72 diet from d 8 to d 15. The fibrous residue of cold-pressed black-and-white sunflower seeds
73 was used to formulate maize-based diets free from antibiotic growth promoters and

1
2
3 74 coccidiostats. Experimental diets of 0%, 10%, 20% and 30% of this high-fibre sunflower cake
4
5 75 (HF-SFC) were pelleted (3 mm) and fed *ad libitum* from d 15 to d 31 to 5 replicates (pens) of
6
7
8 76 8 birds. Nutritional compositions of the experimental diets are shown in Table 1 and contents
9
10 77 of non-starch polysaccharides (NSP) of the diets are given in Table 2. Feeds were iso-
11
12 78 nitrogenous and formulated with fixed, calculated ratios between nitrogen-corrected apparent
13
14
15 79 metabolisable energy (AME_N) and methionine and lysine. The AME_N value of the HF-SFC
16
17 80 was assumed to be 6.7 MJ/kg, based on earlier experiments reviewed by Senkoylu and Dale
18
19 81 (1999). The AME_N values of all other feed ingredients were predicted using the European
20
21 82 table of energy values for poultry feedstuffs (Janssen, 1989). Using these predictions, the
22
23 83 estimated AME_N values of feeds were equivalent to 12.8, 12.1, 11.4 and 10.7 MJ/kg for 0%,
24
25 84 10%, 20% and 30% HF-SFC respectively. Titanium dioxide (TiO₂) was used as an inert
26
27 85 digestibility marker at 5 g/kg. Live weight and feed consumption was recorded weekly. On d
28
29 86 31 the chickens were stunned by a cranial blow and killed by cervical dislocation. Following
30
31 87 slaughter, sex was determined and foot-pad health was examined. The small intestine was
32
33 88 sampled within 5 min of death. All birds in all replicates were used for small intestinal
34
35 89 sampling. In addition, one bird per replicate was used for histological examination. The trial
36
37 90 was approved by the Uppsala Local Ethics Committee.
38
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42

43 **Ileal digestibility coefficients and AME_N**

44
45 92 Contents of digesta between 5 cm posterior of Meckel's diverticulum and the ileo-caecal-
46
47 93 colonic junction were pooled within replicates, freeze dried and weighed prior to chemical
48
49 94 analyses. Gross energy was determined by isoperibolic calorimetry (Parr 6300). Dry matter
50
51 95 and ash were determined at 105°C for 6 h and 550°C for 3 h, respectively. Protein (Nx6.25)
52
53 96 was determined with the Kjeldahl method as described by Nordic Committee on Food
54
55 97 Analysis (1976) and fat (ether extract) as described in method B presented in the Official
56
57 98 Journal of the European Communities (1984). TiO₂ was determined according to Short *et al.*
58
59
60

1
2
3 99 (1996). The ratios between TiO_2 and each parameter were used to calculate ileal digestibility
4
5
6 100 coefficients (DC). AME_N was calculated by multiplying the gross energy with the DC of
7
8 101 energy and corrected for N retention (Hill and Andersson, 1958). Crude fibre was determined
9
10 102 by boiling samples in 200 ml H_2SO_4 (0.320 M) for 10 min, filtration with H_2O , boiling in 200
11
12 103 ml KOH (0.556 M) for 10 min, filtration with H_2O , and rinsing with acetone. Drying and
13
14
15 104 ashing of crude fibre samples were performed for 16 h at 103°C and 500°C respectively.
16
17 105 Total, soluble and insoluble NSP and their constituent sugars were determined as alditol
18
19 106 acetates by gas-liquid chromatography for neutral sugars, uronic acids by a colorimetric
20
21 107 method and Klason lignin by a gravimetric method using a modification of the Uppsala
22
23 108 method (Theander *et al.*, 1995) previously described by Bach Knudsen (1997).
24
25

26 27 109 **Histological examination**

28
29 110 Five birds per treatment, *i.e.* one bird per replicate, were randomly selected for histological
30
31 111 examination. In each selected bird, a segment (3 cm) of the jejunum (20 cm anterior of
32
33 112 Meckel's diverticulum) was excised and washed in physiological saline solution and fixed
34
35 113 overnight in 2.5% glutaraldehyde in phosphate buffer (pH=7.2, 4°C). Following fixation, the
36
37 114 samples were rinsed in phosphate buffer (1/15 M, pH=7.2), dehydrated with increasing
38
39 115 concentrations of ethanol and embedded in a water-soluble glycol methacrylate (Leica
40
41 116 Historesin, Heidelberg, Germany). Sections (2 μm) were cut with glass knives on a
42
43 117 microtome (Leica RM 2165, Leica instruments GmbH, Heidelberg, Germany) and stained
44
45 118 with haematoxylin-eosin and toluidine blue for light microscopic evaluation (Nikon
46
47 119 Microphot-FXA imaging system, Bergström Instrument AB, Stockholm, Sweden) and image
48
49 120 analyses (Eclipsenet 1.20.0, Nikon Instruments, Europe BV). For each bird, villus height
50
51 121 (apical cell membrane of villus tip to apical membrane of deepest located cell between villi),
52
53 122 crypt depth (basal membrane of deepest located crypt cell to the apical surface of deepest
54
55 123 located cell between villi), and the thickness of muscularis mucosa, submucosa and the
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3 124 longitudinal and circular layers of muscularis externa were measured in 5 intact villi, their
4
5 125 respective crypts and tissue layers underneath these villi. All slides were coded and
6
7
8 126 measurements were made by one individual using a $\times 4$ objective lens.
9

10 127 **Bacterial enumeration**

11
12 128 Intact jejunal pieces of 5 cm were sampled aseptically 15 to 20 cm anterior of Meckel's
13
14
15 129 diverticulum. Within 3 h, the cold-stored samples were cut in smaller pieces, weighed and
16
17 130 diluted 1:9 in sodium chloride followed by a 10-fold serial dilution in peptone water
18
19
20 131 (Dilucups, LabRobot Products AB, Stenungsund Sweden). Analyses were performed at the
21
22 132 Swedish National Veterinary Institute and bacterial numbers were expressed as \log_{10} cfu/g
23
24
25 133 intestine.

26
27 134 *Clostridium* spp. Between 10-15 ml of tryptose sulphite cycloserine (TSC) agar (45°C)
28
29 135 were poured into TSC dishes and mixed with samples (1 ml). After solidification, samples
30
31 136 were anaerobically incubated for 24 ± 2 h at $37.0 \pm 1^\circ\text{C}$ (Anaerogen, Oxoid). Post-incubation,
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33
34 137 plates were examined for typical and/or suspected colonies of *Clostridium* spp. At least 5
35
36 138 typical colonies were subcultured and confirmed by spread on two blood agar plates, one
37
38 139 incubated aerobically and the other anaerobically, and spread on egg yolk agar (all incubated
39
40
41 140 24 ± 2 h at $37.0 \pm 1^\circ\text{C}$).

42
43 141 *Escherichia coli*. Between 10-15 ml of violet red bile agar (VRB) were mixed with
44
45 142 samples (1 ml) in petri dishes and tempered to 45°C. After solidification, a covering layer of
46
47
48 143 VRB agar was poured. After solidification, samples were incubated (24 ± 2 h at $37.0 \pm 1^\circ\text{C}$)
49
50
51 144 aerobically. Post-incubation, plates were examined for typical and/or suspected dark red
52
53 145 colonies of *E. coli*. At least 5 typical colonies were confirmed by inoculation on brilliant
54
55 146 green bile lactose broth. The tubes were incubated 24 ± 2 h at $37.0 \pm 1^\circ\text{C}$.

56
57 147 *Lactobacillus* spp. From each dilution, 0.1 ml was directly plated on horse blood agar
58
59
60 148 plates and spreading was continued until the liquid was no longer visible on the agar. Plates

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2
3 149 were incubated (24 ± 2 h at $37.0 \pm 1^\circ\text{C}$) and subcultured (Gram staining) and confirmed
4
5 150 (catalase test) in 5 typical colonies.
6
7

8 151 **Short chain fatty acids and pH**

9
10 152 Concentrations of acetate, butyrate, iso-butyrate, propionate and lactate were determined in
11
12 153 digesta between Meckel's diverticulum and 15 cm anterior of this position using HPLC. In
13
14 154 short, wet samples were centrifuged (1300 g, 5 min) and the liquid top layer was diluted with
15
16 155 internal standard solution (pivalic acid/ H_2SO_4). The internal standard solution (600 μl) was
17
18 156 mixed with samples (500 μl) and centrifuged (16000 g, 5 min). The liquid phase obtained was
19
20 157 decanted into glass vials and capped and analysed with HPLC as described by Andersson and
21
22 158 Hedlund (1983). Digesta samples used for HPLC were also used for pH determination
23
24 159 (Metrohm 654).
25
26
27
28

29 160 **Statistics**

30
31 161 Data were analysed with general linear models (SAS 9.1) and presented as least square means
32
33 162 and the appropriate standard error term. The linear regression and ANOVA models can be
34
35 163 described as

$$36 \quad 164 \quad y_i = \beta_0 + \beta_1 x_i + e_{ij}$$

37
38 165 where β_0 denotes intercept or mean, β_1 denotes slope or treatment effect and x_i denotes
39
40 166 amount of HF-SFC in the feed. At d 15, bird weight correlated with male:female ratio of the
41
42 167 pen ($r = 0.53$; $P = 0.02$) and unbalanced male:female ratios caused live weights to differ
43
44 168 significantly between treatments. The individual bird weights (g \pm standard deviation) were
45
46 169 587 (± 19), 615 (± 27), 588 (± 13) and 619 (± 21) and the male:female ratios were 0.56 (± 0.20),
47
48 170 0.58 (± 0.26), 0.48 (± 0.10) and 0.59 (± 0.16) for 0%, 10%, 20% and 30% HF-SFC respectively.
49
50 171 However, there was only a significant effect of male:female ratio on colony counts of
51
52 172 *Lactobacillus* spp. A significant effect of live weight at d 15 was found on acetic acid
53
54 173 concentration of ileal digesta. These parameters were consequently added as co-variates (β_2)
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1
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3 174 in the respective statistical models. Due to technical errors, birds in 10% HF-SFC treatment
4
5 175 were fed on 30% HF-SFC between d 15 to d 19. Data from this treatment were consequently
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7
8 176 excluded in analyses of weight gain, feed consumption and feed conversion ratio (FCR).
9
10 177 However, since all other parameters were assumed to mirror the birds' status at d 31, the error
11
12 178 of the first 4 d in the 10% HF-SFC treatment was regarded as insignificant and information
13
14
15 179 from this treatment was used.

180 RESULTS

181 **Bird performance**

182 In total, 3 birds died between d 15 and 31 and pen-wise means of final live weight ranged
183 from 1806 to 2144 g (data not shown). Performance figures are summarised in Table 3. Both
184 20% HF-SFC and 30% HF-SFC increased weight gain and feed consumption. The FCR was
185 impaired by 30% HF-SFC, but not by 20% HF-SFC. Birds effectively compensated for lower
186 dietary energy by adjusting feed intake and consumed equal amounts of AME_N, lysine,
187 methionine and threonine, irrespective of treatment. However, birds in 20% and 30% HF-SFC
188 treatment consumed significantly more protein, fat and ash than the control. Dry weight of
189 ileal digesta between Meckel's diverticulum and ileo-cecal junction increased linearly with
190 HF-SFC inclusion. On d 31, no effects on foot-pad health were seen in any treatment group.

191 **Table 3, Figures 1 and 2 near here**

192 **Digestibility coefficients and AME_N**

193 There were significant positive regressions of digestibility of fat and protein on HF-SFC
194 inclusion (Table 3). In parallel, HF-SFC inclusion resulted in a significant negative linear
195 regression of digestibility of ash, gross energy and DM on HF-SFC inclusion. The AME_N of
196 feeds decreased linearly with HF-SFC inclusion.

197 **Histological evaluation**

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2
3 198 Morphometric data are shown in Table 4. Extensive lymphocyte infiltration of the lamina
4
5 199 propria was seen in 3 out of 5 replicates in 0% HF-SFC. This phenomenon was associated
6
7
8 200 with irregular and blunt shapes of villi, shown in Figure 1. As a comparison, regularly shaped
9
10 201 and well-defined villi are shown in Figure 2. Morphometrical assessment was not possible in
11
12 202 one of the 0% HF-SFC replicates because of villus irregularity. Villus height and the
13
14
15 203 thickness of muscularis mucosa decreased significantly in a linear fashion with HF-SFC
16
17 204 inclusion but treatments explained only about 25% of data variation. There were no
18
19 205 differences in crypt depth or thickness of the submucosa, but HF-SFC inclusion resulted in
20
21
22 206 significantly thinner circular and longitudinal muscularis. Table 4, Figures 3 and 4 near here

24 207 **Bacterial enumeration, short chain fatty acids and pH**

26 208 Bacterial colony counts are shown in Figure 3. HF-SFC inclusion was associated with a
27
28 209 significant linear reduction in colony counts of *Clostridium* spp. *Clostridium* spp. counts were
29
30 210 significantly lower in 20% and tended to be lower in 30% HF-SFC ($P = 0.05$) vs control.
31
32 211 *Lactobacillus* spp. counts were significantly lower in 10% HF-SFC vs control and 20% HF-
33
34 212 SFC. No effects on *E.coli* were seen. Short chain fatty acid profiles of the small intestine are
35
36 213 illustrated in Figure 4. A significant negative linear effect was seen in both acetic acid and
37
38 214 propionic acid concentration on HF-SFC inclusion. Mean pH measured in all samples was
39
40 215 6.54 (SE 0.10) and not affected by treatment.
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44

46 216 DISCUSSION

47
48 217 In this study, the i-NSP-rich residue of non-decorticated cold-pressed SFC was used to
49
50 218 formulate broiler feeds that were given between 15 and 31 d of age. In general, performance
51
52 219 and health was satisfactory throughout the trial and, despite fibre contents far exceeding what
53
54 220 is used in practice, the chickens thrived on diets containing HF-SFC. Birds efficiently
55
56 221 compensated for differences in dietary energy levels by adjusting their feed intake.
57
58 222 Experimental feeds were formulated with fixed AME_N:methionine and AME_N:lysine ratios,
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60

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3 223 and so the total intake of these parameters did not differ between treatments. Hetland and
4
5 224 Svihus (2001) indicated that feeding i-NSP increased gut capacity in broiler chickens. Thus, it
6
7
8 225 can be hypothesised that the i-NSP fraction of HF-SFC allowed for the large compensations
9
10 226 of feed intake seen in this experiment. Weight gain and feed consumption increased with 20%
11
12 227 and 30% HF-SFC, but FCR was only negatively affected by 30% HF-SFC. Interestingly,
13
14
15 228 Viveros *et al.* (2009) found significant improvements in both weight gain and FCR when 4%
16
17 229 sunflower seed hulls were added to a broiler diet. This effect was also seen with 3% oat hulls
18
19
20 230 (González-Alvarado *et al.*, 2007; Jiménez-Moreno *et al.*, 2009b). Thus it may be hypothesised
21
22 231 that the performance improvements in the present trial were, at least partly, mediated by the i-
23
24 232 NSP fraction of the HF-SFC. Improvements in feed utilisation were also reported in broiler
25
26 233 chickens fed on diets diluted with i-NSP-rich ingredients such as wood shavings (Amerah *et*
27
28 234 *al.*, 2009) and oat hulls (Hetland *et al.*, 2003; Jiménez-Moreno *et al.*, 2009b). In the present
29
30 235 study, the DM weight of small intestinal digesta was increased with HF-SFC inclusion. This
31
32 236 effect was also described, but not explained by Hetland and Svihus (2001). In the present
33
34 237 experiment, the effect of increased DM weight of digesta was not mediated by differences in
35
36 238 feed intake, since adding feed consumption as a co-variate to the ANOVA model increased
37
38 239 the initial R^2 value (0.84) by less than 1% (data not shown). Rather, because fat is known to
39
40 240 slow down digesta passage rate in the small intestine (Krogdahl, 1985), the elevated fat
41
42 241 intakes associated with HF-SFC inclusion might explain this finding. Another feature possibly
43
44 242 explaining the increased weight of digesta DM might be associated with small intestinal
45
46 243 motility. Hetland *et al.* (2003) demonstrated indications of increased gastroduodenal reflux
47
48 244 with i-NSP feeding. An increased reflux of digesta between gastrointestinal segments in
49
50 245 addition to an increased grinding and mixing activity of the gizzard have been suggested as
51
52 246 plausible explanations for the positive effects of i-NSP on digestion (Hetland *et al.*, 2003;
53
54 247 González-Alvarado *et al.*, 2007; Jiménez-Moreno *et al.*, 2009a).

1
2
3 248 In the present trial, DCs of DM, gross energy and ash declined in a linear fashion
4
5 249 with HF-SFC inclusion. This was expected since HF-SFC contributed with large amounts of
6
7
8 250 indigestible fibres. In contrast, protein and fat digestibility increased linearly with HF-SFC
9
10 251 inclusion. It was demonstrated by Hetland *et al.* (2003) that oat hulls increases the amount of
11
12 252 bile acids in the small intestine and, according to Krogdahl (1985), bile salt excretion
13
14
15 253 constitutes the first limiting factor in fat digestion in poultry. However, because several
16
17 254 important digestive adaptations occur in response to the fat content of the feed (Krogdahl,
18
19 255 1985), it cannot be out ruled that the positive effects on digestion associated with HF-SFC
20
21
22 256 were a consequence of elevated fat intakes. Although the design of the present study does not
23
24 257 allow for a separation of the effects of fat intakes from the effects of i-NSP, other recent
25
26 258 studies have shown positive results on total tract digestibility of dry and organic matter,
27
28 259 protein, fat and ash from adding 3% oat hulls (González-Alvarado *et al.*, 2007; Jiménez-
29
30 260 Moreno *et al.*, 2009a,b).

31
32
33 261 In weaned pigs, both i-NSP and soluble NSP (s-NSP) positively influence the activity of
34
35 262 enterocyte brush-border enzyme activity but, whereas s-NSP acted deleteriously on
36
37 263 performance, i-NSP did not (Hedemann *et al.*, 2006). Interestingly, in the work by Jiménez-
38
39 264 Moreno *et al.* (2009b), the improvements seen at ileal level by adding 3% oat hulls were not
40
41 265 seen with 3% sugar beet pulp; a feed ingredient distinguished by a relatively larger proportion
42
43 266 of s-NSP.

44
45
46 267 However, when scrutinising the literature, it appears that there were important
47
48 268 differences between our study and others which make interpretation of our findings more
49
50 269 difficult. In our study and that of Hetland *et al.* (2003) birds were kept on the floor, but in
51
52 270 others, *e.g.*, González-Alvarado *et al.* (2007); Viveros *et al.* (2009) and Jiménez-Moreno *et al.*
53
54 271 (2009a,b), birds were kept in cages and therefore had no access to wood shavings or other i-
55
56 272 NSP rich litter. And so it remains to be discovered to what extent poultry digestion benefits

1
2
3 273 from i-NSP, apart from what might be considered as minimal fractions available in the feed or
4
5 274 litter.

6
7
8 275 In the gastrointestinal tract, both s-NSP and i-NSP display a bulk-forming capacity, but
9
10 276 it is not known to what extent this feature influences digestion. In the present trial, villus
11
12 277 height decreased linearly with HF-SFC inclusion. This contradicts the view of Moran (2006),
13
14 278 who suggested that small intestinal villi would lengthen if the plane of nutrition is decreased
15
16 279 by the inclusion of insoluble fibres. However, Baurhoo *et al.* (2007) reported that, despite
17
18 280 there being no differences in performance, broiler chickens given more insoluble fibres (2.5%
19
20 281 lignin) had significantly lower villi than 42-d-old birds receiving less insoluble fibres (1.25%
21
22 282 lignin). Whether the bulk forming capacity of fibres affects the morphometry of the small
23
24 283 intestine is not fully investigated. Since the inclusion of HF-SFC in the present trial mediated
25
26 284 a pronounced effect on digesta DM weight in the small intestinal lumen, it might be
27
28 285 hypothesised that such an increase in weight is associated with a corresponding increase in
29
30 286 volume. This increase would mediate an expansion and stretching of the intestinal tract,
31
32 287 possibly explaining why there was a linear decrease in thickness of muscularis mucosa and
33
34 288 the circular and longitudinal sections of muscularis in birds fed on HF-SFC.

35
36 289 Morphometrical effects similar to those observed in the present trial were also seen
37
38 290 when an antibiotic growth promoter (virginiamycin) was added to broiler diets (Miles *et al.*,
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40 291 2006), and so it cannot be ruled out that the histological effects on the small intestine were
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42 292 associated with changes in the bacterial community. Interestingly, in the present experiment
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44 293 reductions of villus height were paralleled by an increase, not a decrease, in weight gain and
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46 294 nutrient absorption. Microscopic evaluation of the small intestinal mucosa revealed villus
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48 295 disorientation and extensive lymphocyte infiltration of the lamina propria in 3 out of 5 birds
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50 296 fed on 0% HF-SFC. These findings indicate that the gut mucosa of birds fed on 0% HF-SFC
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3 297 was subjected to stress and it may be speculated that the integrity and function of the mucosa
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5 298 benefits from i-NSP.

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8 299 In the current experiment, colony counts of *Clostridium* spp. were intermediate between
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10 300 what is normally found in healthy flocks and what is seen in diseased flocks (Drew *et al.*,
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12 301 2004). This can partly be attributed the absence of coccidiostats (Elwinger *et al.*, 1998) in the
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14 302 present experimental diets. Regardless of this fact, colonies of *Clostridium* spp. were
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16 303 significantly suppressed in the 20% HF-SFC and tended to be lower in 30% HF-SFC *vs.*
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18 304 control. These results can be interpreted in light of the positive effects of HF-SFC on protein
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20 305 DC seen in this experiment. Because protein is an important substrate for growth of
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22 306 *Clostridium* spp. (Drew *et al.*, 2004), it may be assumed that in the present trial, the
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24 307 suppression of *Clostridium* spp. in 20% HF-SFC and 30% HF-SFC birds was a consequence
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26 308 of improved protein digestibility. A *Clostridia*-reducing effect of i-NSP was demonstrated by
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28 309 Branton *et al.* (1997), who recorded a significant reduction in necrotic enteritis lesions in
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30 310 broiler chickens following a substitution of wheat by 4% wood shavings. In the present trial,
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32 311 colony counts of *Lactobacillus* spp. were significantly decreased in 10% HF-SFC *vs.* control
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34 312 and 20% HF-SFC, with a tendency towards a decrease in 30% HF-SFC birds *vs.* control. The
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36 313 suppressive effect of high male:female ratios on *Lactobacillus* spp. colony counts remains
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38 314 enigmatic. Whether the observed effect is best explained by body weight or some other
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40 315 characteristics of the sexes needs to be studied further. HF-SFC inclusion also mediated a
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42 316 shift in the SCFA profile of the small intestinal lumen. Whereas acetic and propionic acid
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44 317 decreased with HF-SFC inclusion, there was a weak tendency towards an increase in lactic
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46 318 acid concentration in 20% HF-SFC *vs.* 30% HF-SFC. However, the variance of lactic acid
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48 319 concentrations was notably large. Despite differences between SCFA profiles, the pH
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50 320 remained unaltered irrespective of treatment.
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3 321 In conclusion, these results indicate that broiler chickens may thrive on feeds with
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5 322 insoluble fibre contents far exceeding what is used in practice. Furthermore, HF-SFC exerts
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8 323 some positive effects on performance and small intestinal health.
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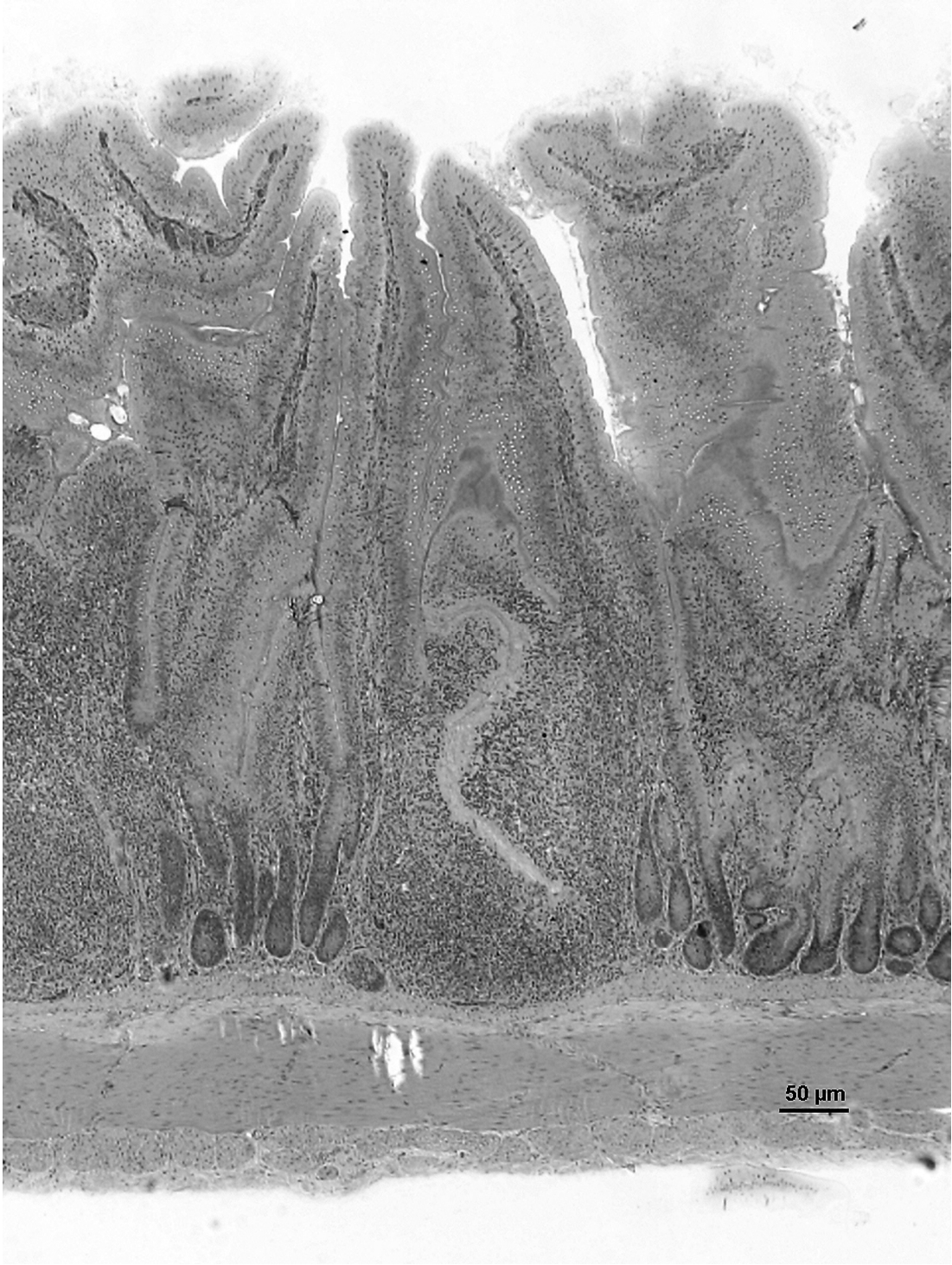
406 **Table 1.** *Broiler chicken diet composition (g/kg as fed) and nutrient contents (g/kg DM)*

Ingredients	0 % HF-SFC	10 % HF-SFC	20 % HF-SFC	30 % HF-SFC
Maize	637.1	560.2	483.4	406.6
Maize gluten meal	60.0	60.0	60.0	60.0
High-fibre sunflower cake ¹	0.00	100.0	200.0	300.0
Peas	150.0	150.0	150.0	150.0
Potato protein	99.6	78.7	57.8	36.8
Calcium carbonate	18.9	18.1	17.3	16.5
Monocalcium phosphate	19.6	19.1	18.6	18.0
Sodium chloride	3.7	3.0	2.3	1.6
DL-methionine	1.4	1.1	0.8	0.5
L-lysine	2.4	2.3	2.4	2.4
Premix ²	2.5	2.5	2.5	2.5
TiO ₂	5.0	5.0	5.0	5.0
<i>Analysed contents</i>				
Dry matter (g/kg)	896.0	895.0	916.0	917.0
Gross energy (MJ)	18.1	19.0	19.3	19.3
Crude protein	223.0	220.0	226.0	218.0
Fat (ether extract)	46.0	55.0	69.0	76.0
Ash	61.0	58.0	63.0	63.0
Methionine	5.8	5.2	5.1	4.7
Cystine	4.1	4.1	4.1	4.0
Lysine	14.1	13.0	12.6	11.9
Threonine	10.0	9.4	9.0	8.6
TiO ₂	4.91	4.77	5.16	4.84
Ca	12.9	12.9	12.7	12.5
P	9.8	10.5	11.2	11.7
Mg	1.9	2.4	2.8	3.2
K	4.8	5.6	6.0	6.6
Na	1.5	3.1	3.1	3.0
S	3.0	3.1	3.1	3.0

¹The high-fibre sunflower cake had the following composition (g/kg DM): ash 43.2, crude protein 230.3, crude fibre 370.3, fat (ether extract) 140.1 and dry matter 937.0 g/kg as fed.

²Premix declaration: retinol 3.6 mg, cholecalciferol 0.125 mg, DL- α -tocopheryl 80 mg, vitamin B1 3mg, vitamin B2 8mg, vitamin B3 60mg, vitamin B6 5mg, vitamin B12 0,02mg, vitamin B7 0,3mg, vitamin K3 3mg, vitamin B9 2mg, vitamin B5 20mg, choline chloride 1600 mg, iodine 1mg, selenium 0,35mg, manganese 100mg, zinc 120mg, copper 16mg, iron 30mg.

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416 **Figure 1.** *Irregular and blunt villi infiltrated with lymphocytes.*



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418 **Figure 2.** *Regularly shaped and well-defined villi.*

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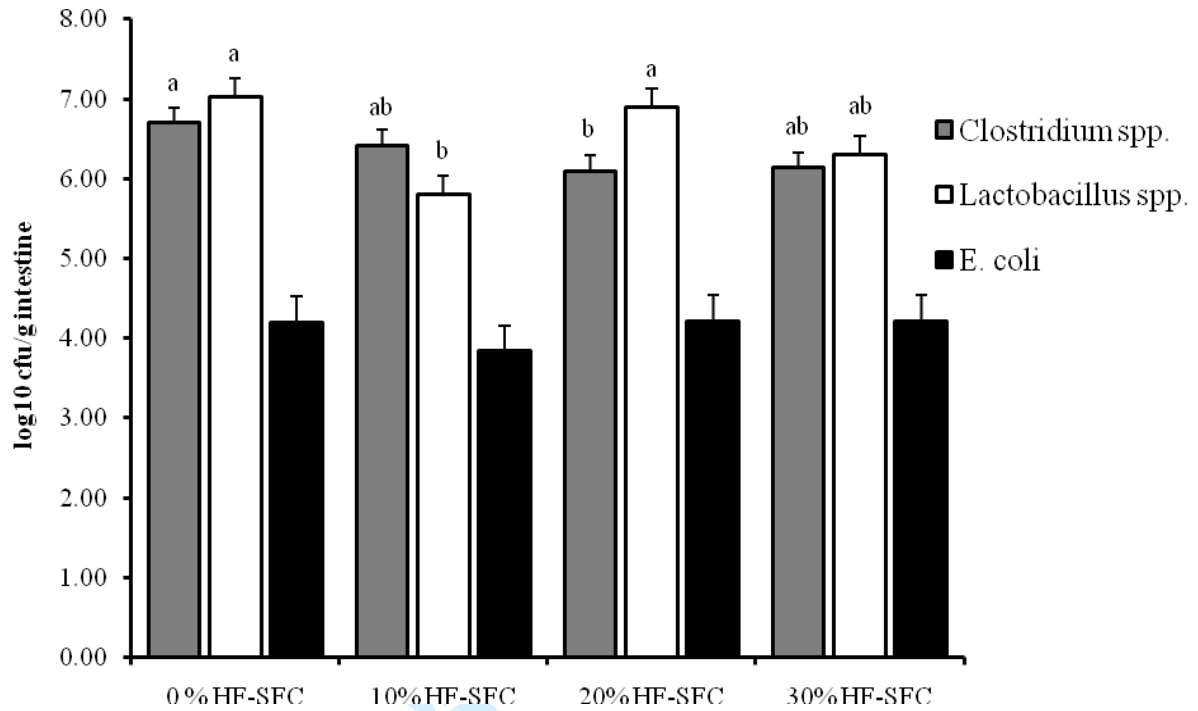
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420 **Figure 3.** Bacterial colony counts in jejunum, presented as LS means (+SEM). Superscripts

421 indicate significant differences between treatments at $P < 0.05$.

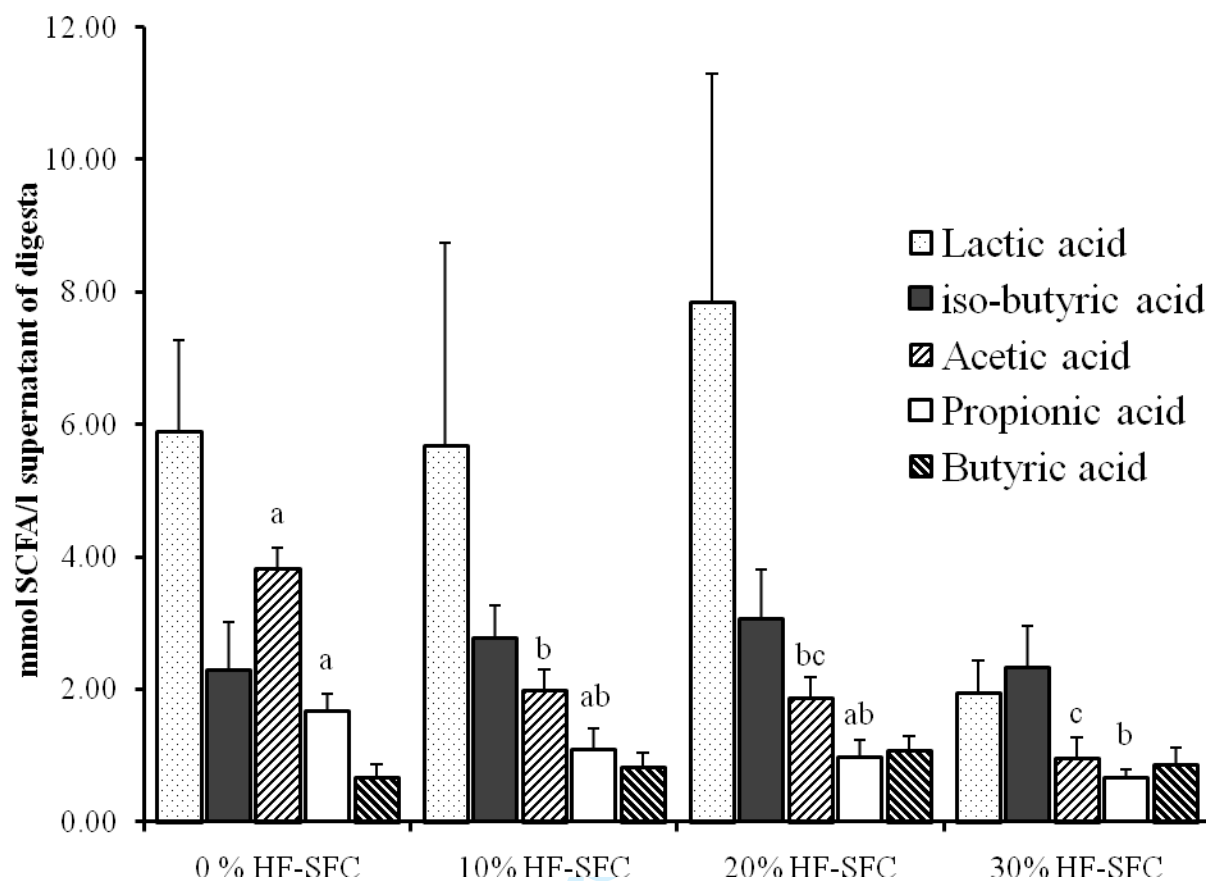


Figure 4. Short chain fatty acid profiles of jejunum, presented as LS means (+SEM).

Superscripts indicate significant differences between treatments at $P < 0.05$.

Table 2. Fibre contents (g/kg DM) of HF-SFC and experimental feeds

Fraction	HF-SFC		0 % HF-SFC		10 % HF-SFC		20 % HF-SFC		30 % HF-SFC	
	NSP	NSP	NSP	NSP	NSP	NSP	NSP	NSP	NSP	NSP
	Soluble ¹	Insoluble	Soluble ¹	Insoluble	Soluble ¹	Insoluble	Soluble ¹	Insoluble	Soluble ¹	Insoluble
Klason lignin	161.4		31.3		40.9		48.1		72.3	
Crude fibre	370.3		23.0		54.2		79.6		109.9	
Ramnose	1.21	2.41	0.0	0.0	0.0	0.0	0.45	0.72	0.0	0.0
Fucose	0.14	0.81	0.0	0.0	0.0	0.0	0.0	0.51	0.0	0.0
Arabinose	8.25	16.94	3.57	14.63	4.86	14.63	4.27	14.53	5.73	14.70
Xylose	5.13	90.51	1.55	17.74	1.99	25.75	-0.61	32.19	-0.44	41.45
Mannose	1.41	9.11	1.04	1.83	1.29	2.60	0.95	3.21	0.95	4.00
Galactose	3.40	6.31	1.90	3.86	2.26	4.13	2.09	4.29	2.50	4.56
Glucose	4.41	164.3	1.92	25.0	3.25	41.1	-3.20	54.33	-1.87	70.72
Uronic acid	18.7	26.8	3.4	26.8	4.1	8.6	6.8	9.2	8.6	11.0
Total NSP	42.65	317.17	13.38	68.76	17.75	96.78	10.75	118.98	15.47	146.43

¹Determined as the difference between total NSP and insoluble NSP

438 **Table 3.** Broiler chicken production performance (d 15-31), mean ileal digestibility coefficients and AME_N (d 31), presented as LS means¹ (SE)

	ANOVA				Linear regression		
	0 % HF-SFC	10 % HF-SFC ²	20 % HF-SFC	30 % HF-SFC	Prediction equation	H ₀ : β ₁ ≠ 0	R ²
Weight gain d 15-31 (g)	1260 ^a (20)		1375 ^b (20)	1417 ^b (20)	β ₀ = 1262 (18.5) β ₁ = 5.31 (0.89)	P<0.0001	0.73
Feed consumed d 15-31 (g)	2163 ^a (42)		2329 ^b (42)	2520 ^c (42)	β ₀ = 2148 (41.4) β ₁ = 11.4 (1.99)	P<0.0001	0.72
FCR d 15-31 (g/g)	1.716 ^a (0.018)		1.701 ^a (0.018)	1.784 ^b (0.018)	β ₀ = 1.703 (0.02) β ₁ = 0.002 (0.002)	P=0.0928	0.20
Digestibility coefficients							
Dry matter	0.77 ^a (0.01)	0.70 ^b (0.01)	0.66 ^c (0.01)	0.61 ^d (0.01)	β ₀ = 0.761 (0.01) β ₁ = -0.005 (0.000)	P<0.0001	0.89
Gross energy	0.79 ^a (0.01)	0.73 ^b (0.01)	0.70 ^c (0.01)	0.64 ^d (0.01)	β ₀ = 0.787 (0.01) β ₁ = -0.005 (0.000)	P<0.0001	0.87
Crude Protein	0.81 ^a (0.01)	0.81 ^{ab} (0.01)	0.84 ^b (0.01)	0.83 ^{ab} (0.01)	β ₀ = 0.809 (0.01) β ₁ = 0.001 (0.000)	P<0.05	0.22
Fat (ether extract)	0.87 ^a (0.01)	0.86 ^a (0.01)	0.91 ^b (0.01)	0.91 ^b (0.01)	β ₀ = 0.865 (0.01) β ₁ = 0.002 (0.000)	P<0.001	0.49
Ash	0.32 ^a (0.02)	0.25 ^b (0.02)	0.23 ^b (0.02)	0.23 ^b (0.01)	β ₀ = 0.297 (0.02) β ₁ = -0.003 (0.000)	P<0.01	0.35
AME _N of feeds (MJ/kg DM)	14.65 ^a (0.19)	13.75 ^b (0.19)	13.32 ^b (0.19)	12.35 ^c (0.19)	β ₀ = 14.62 (0.15) β ₁ = -0.073 (0.008)	P<0.0001	0.81

439 ¹: Different superscripts within rows indicate significant treatment effects (P<0.05) in ANOVA test440 ²: Missing data as described in materials and methods section.

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442 **Table 4.** Morphometric data of broiler chicken jejunum (d 31). All values are presented as LS means¹ (SE) in μm

	ANOVA				Linear regression		
	0 % HF-SFC	10 % HF-SFC ¹	20 % HF-SFC	30 % HF-SFC	Prediction equation	H ₀ : $\beta_1 \neq 0$	R ²
Villi height	1430 (80)	1375 (72)	1303 (72)	1203 (72)	$\beta_0 = 1442 (61)$ $\beta_1 = -7.60 (3.17)$	$P < 0.05$	0.25
Crypt depth	163 (13)	179 (11)	154 (11)	171 (11)	$\beta_0 = 167 (10)$ $\beta_1 = -0.02 (0.54)$	n.s.	<0.01
Thickness of Muscularis mucosa	54 (6)	52 (5)	42 (5)	40 (5)	$\beta_0 = 55 (4)$ $\beta_1 = -0.54 (0.23)$	$P < 0.05$	0.24
Submucosa	30 (5)	35 (4)	25 (4)	24 (4)	$\beta_0 = 33 (4)$ $\beta_1 = -0.30 (0.20)$	n.s.	0.12
M. circular	169 ^a (12)	164 ^{ab} (11)	131 ^{cd} (11)	110 ^d (11)	$\beta_0 = 176 (10)$ $\beta_1 = -2.14 (0.51)$	$P < 0.001$	0.51
M. longitudinal	61 ^{ab} (6)	67 ^a (5)	53 ^{ab} (5)	47 ^b (5)	$\beta_0 = 66 (5)$ $\beta_1 = -0.59 (0.26)$	$P < 0.05$	0.23

443 ¹: Different superscripts within rows indicate significant treatment effects ($P < 0.05$) in the ANOVA.

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