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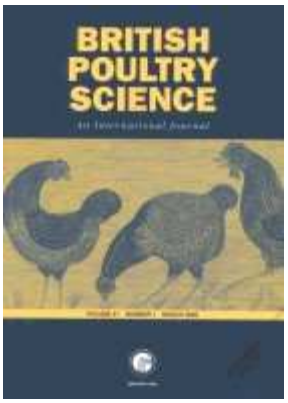

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High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens

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Abstract 1. An experiment was conducted to evaluate high-fibre sunflower cake (HF-SFC); a feed ingredient distinguished by large amounts of crude fibre and insoluble non-starch polysaccharides (i-NSP).

2. Broiler chickens (n=160) were fed on pelleted maize-based diets free from coccidiostats and antibiotic growth promoters between 15 and 31 d of age. Diets included 0, 10, 20 or 30% HF-SFC. Performance and small intestinal health were assessed.

3. In general, HF-SFC inclusion mediated significant linear increases in ileal digestibility of fat and protein and significant linear decreases in ileal digestibility of dry matter, ash and energy.

4. Weight gain increased linearly with HF-SFC inclusion. Feed conversion was negatively affected by 30% HF-SFC but not by 20% HF-SFC.

5. In the jejunal lumen, inclusion of HF-SFC was associated with significant decreases in colony counts of *Clostridium* spp.

6. HF-SFC inclusion resulted in significant linear reductions of villus height, thickness of muscularis mucosa, and the circular and longitudinal layers of muscularis in the jejunum. Crypt depth and submucosal thickness were not affected.

7. The data indicate that broiler chickens may thrive on feeds with insoluble fibre contents far exceeding those used in practice, and that HF-SFC exerts some positive effects on digestion and small intestinal health.

INTRODUCTION

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

the early literature describes beneficial effects of i-NSP-rich feedstuffs on pullet health (Bearse *et al*., 1940), growth (Davis and Briggs, 1947) and digestibility in broiler chickens (Rogel *et al*., 1987). More recently, it was indicated that the addition of some i-NSP fraction to the feed correlates with improved feed conversion (Hetland *et al*., 2003; González-Alvarado *et al*., 2007; Jiménez-Moreno *et al*., 2009*b*), increased AME_N of the feed (González-Alvarado *et al*., 2007; Hetland and Svihus, 2007; Amerah *et al*., 2009; Jiménez-Moreno *et al*., 2009*b*) and enhanced digestibility of dry matter (DM), organic matter, nitrogen and fat (González-Alvarado *et al*., 2007; Jiménez-Moreno *et al*., 2009*a*,*b*). Further, it has been demonstrated that i-NSP influence gut microflora (Amerah *et al*., 2009) and may reduce necrotic enteritis lesions (Branton *et al*., 1997). The mechanisms underlying these effects are not fully understood, but feeds rich in i-NSP were shown to stimulate gizzard activity and the secretion of amylase and bile acids in broiler chickens and layers (Hetland *et al*., 2003) as well as the activity of digestive enzymes bound to the apical membrane of small intestinal enterocytes in newly weaned pigs (Hedemann *et al*., 2006). We hypothesised that high-fibre sunflower cake (HF-SFC) may exert beneficial effects on broiler performance and small intestinal health. Hence, the present experiment was set up to study the effects of different amounts of HF-SFC in the diet of broiler chickens between 15 and 31 d of age.

MATERIALS AND METHODS

Experimental design

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

coccidiostats. Experimental diets of 0%, 10%, 20% and 30% of this high-fibre sunflower cake (HF-SFC) were pelleted (3 mm) and fed *ad libitum* from d 15 to d 31 to 5 replicates (pens) of 8 birds. Nutritional compositions of the experimental diets are shown in Table 1 and contents of non-starch polysaccharides (NSP) of the diets are given in Table 2. Feeds were iso-nitrogenous and formulated with fixed, calculated ratios between nitrogen-corrected apparent metabolisable energy (AME_N) and methionine and lysine. The AME_N value of the HF-SFC was assumed to be 6.7 MJ/kg, based on earlier experiments reviewed by Senkoylu and Dale (1999). The AME_N values of all other feed ingredients were predicted using the European table of energy values for poultry feedstuffs (Janssen, 1989). Using these predictions, the estimated AME_N values of feeds were equivalent to 12.8, 12.1, 11.4 and 10.7 MJ/kg for 0%, 10%, 20% and 30% HF-SFC respectively. Titanium dioxide (TiO_2) was used as an inert digestibility marker at 5 g/kg. Live weight and feed consumption was recorded weekly. On d 31 the chickens were stunned by a cranial blow and killed by cervical dislocation. Following slaughter, sex was determined and foot-pad health was examined. The small intestine was sampled within 5 min of death. All birds in all replicates were used for small intestinal sampling. In addition, one bird per replicate was used for histological examination. The trial was approved by the Uppsala Local Ethics Committee.

Ileal digestibility coefficients and AME_N

Contents of digesta between 5 cm posterior of Meckel´s diverticulum and the ileo-caecal-colonic junction were pooled within replicates, freeze dried and weighed prior to chemical analyses. Gross energy was determined by isoperibolic calorimetry (Parr 6300). Dry matter and ash were determined at 105°C for 6 h and 550°C for 3 h, respectively. Protein (Nx6.25) was determined with the Kjeldahl method as described by Nordic Committee on Food Analysis (1976) and fat (ether extract) as described in method B presented in the Official Journal of the European Communities (1984). TiO_2 was determined according to Short *et al.*

(1996). The ratios between TiO_2 and each parameter were used to calculate ileal digestibility coefficients (DC). AME_N was calculated by multiplying the gross energy with the DC of energy and corrected for N retention (Hill and Andersson, 1958). Crude fibre was determined by boiling samples in 200 ml H_2SO_4 (0.320 M) for 10 min, filtration with H_2O, boiling in 200 ml KOH (0.556 M) for 10 min, filtration with H_2O, and rinsing with acetone. Drying and ashing of crude fibre samples were performed for 16 h at 103ºC and 500ºC respectively. Total, soluble and insoluble NSP and their constituent sugars were determined as alditol acetates by gas–liquid chromatography for neutral sugars, uronic acids by a colorimetric method and Klason lignin by a gravimetric method using a modification of the Uppsala method (Theander *et al.,* 1995) previously described by Bach Knudsen (1997).

Histological examination

Five birds per treatment, *i.e.* one bird per replicate, were randomly selected for histological examination. In each selected bird, a segment (3 cm) of the jejunum (20 cm anterior of Meckel´s diverticulum) was excised and washed in physiological saline solution and fixed overnight in 2.5% glutaraldehyde in phosphate buffer (pH=7.2, 4°C). Following fixation, the samples were rinsed in phosphate buffer (1/15 M, pH=7.2), dehydrated with increasing concentrations of ethanol and embedded in a water-soluble glycol methacrylate (Leica Historesin, Heidelberg, Germany). Sections (2 µm) were cut with glass knives on a microtome (Leica RM 2165, Leica instruments GmbH, Heidelberg, Germany) and stained with haematoxylin-eosin and toluidine blue for light microscopic evaluation (Nikon Microphot-FXA imaging system, Bergström Instrument AB, Stockholm, Sweden) and image analyses (Eclipsenet 1.20.0, Nikon Instruments, Europe BV). For each bird, villus height (apical cell membrane of villus tip to apical membrane of deepest located cell between villi), crypt depth (basal membrane of deepest located crypt cell to the apical surface of deepest located cell between villi), and the thickness of muscularis mucosa, submucosa and the

longitudinal and circular layers of muscularis externa were measured in 5 intact villi, their respective crypts and tissue layers underneath these villi. All slides were coded and measurements were made by one individual using a ×4 objective lens.

Bacterial enumeration

Intact jejunal pieces of 5 cm were sampled aseptically 15 to 20 cm anterior of Meckel´s diverticulum. Within 3 h, the cold-stored samples were cut in smaller pieces, weighed and diluted 1:9 in sodium chloride followed by a 10-fold serial dilution in peptone water (Dilucups, LabRobot Products AB, Stenungsund Sweden). Analyses were performed at the Swedish National Veterinary Institute and bacterial numbers were expressed as $\log_{10}$ cfu/g intestine.

Clostridium spp. Between 10-15 ml of tryptose sulphite cycloserine (TSC) agar (45ºC) were poured into TSC dishes and mixed with samples (1 ml). After solidification, samples were anaerobically incubated for 24±2 h at 37.0±1ºC (Anaerogen, Oxoid). Post-incubation, plates were examined for typical and/or suspected colonies of *Clostridium* spp. At least 5 typical colonies were subcultured and confirmed by spread on two blood agar plates, one incubated aerobically and the other anaerobically, and spread on egg yolk agar (all incubated 24±2 h at 37.0±1ºC).

Escherichia coli. Between 10-15 ml of violet red bile agar (VRB) were mixed with samples (1 ml) in petri dishes and tempered to 45ºC. After solidification, a covering layer of VRB agar was poured. After solidification, samples were incubated (24±2 h at 37.0±1ºC) aerobically. Post-incubation, plates were examined for typical and/or suspected dark red colonies of *E. coli.* At least 5 typical colonies were confirmed by inoculation on brilliant green bile lactose broth. The tubes were incubated 24±2 h at 37.0±1ºC.

Lactobacillus spp. From each dilution, 0.1 ml was directly plated on horse blood agar plates and spreading was continued until the liquid was no longer visible on the agar. Plates

were incubated (24±2 h at 37.0±1ºC) and subcultured (Gram staining) and confirmed (catalase test) in 5 typical colonies.

Short chain fatty acids and pH

Concentrations of acetate, butyrate, iso-butyrate, propionate and lactate were determined in digesta between Meckel´s diverticulum and 15 cm anterior of this position using HPLC. In short, wet samples were centrifuged (1300 *g*, 5 min) and the liquid top layer was diluted with internal standard solution (pivalic acid/H_2SO_4). The internal standard solution (600 µl) was mixed with samples (500 µl) and centrifuged (16000 *g*, 5 min). The liquid phase obtained was decanted into glass vials and capped and analysed with HPLC as described by Andersson and Hedlund (1983). Digesta samples used for HPLC were also used for pH determination (Metrohm 654).

Statistics

Data were analysed with general linear models (SAS 9.1) and presented as least square means and the appropriate standard error term. The linear regression and ANOVA models can be described as

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

where β_0 denotes intercept or mean, β_1 denotes slope or treatment effect and x_i denotes amount of HF-SFC in the feed. At d 15, bird weight correlated with male:female ratio of the pen (r = 0.53; P = 0.02) and unbalanced male:female ratios caused live weights to differ significantly between treatments. The individual bird weights (g ± standard deviation) were 587 (±19), 615 (±27), 588 (±13) and 619 (±21) and the male:female ratios were 0.56 (±0.20), 0.58 (±0.26), 0.48 (±0.10) and 0.59 (±0.16) for 0%, 10%, 20% and 30% HF-SFC respectively. However, there was only a significant effect of male:female ratio on colony counts of *Lactobacillus* spp. A significant effect of live weight at d 15 was found on acetic acid concentration of ileal digesta. These parameters were consequently added as co-variates (β_2)

in the respective statistical models. Due to technical errors, birds in 10% HF-SFC treatment were fed on 30% HF-SFC between d 15 to d 19. Data from this treatment were consequently excluded in analyses of weight gain, feed consumption and feed conversion ratio (FCR). However, since all other parameters were assumed to mirror the birds´ status at d 31, the error of the first 4 d in the 10% HF-SFC treatment was regarded as insignificant and information from this treatment was used.

RESULTS

Bird performance

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

Table 3, Figures 1 and 2 near here

Digestibility coefficients and AME_N

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

Histological evaluation

Morphometric data are shown in Table 4. Extensive lymphocyte infiltration of the lamina propria was seen in 3 out of 5 replicates in 0% HF-SFC. This phenomenon was associated with irregular and blunt shapes of villi, shown in Figure 1. As a comparison, regularly shaped and well-defined villi are shown in Figure 2. Morphometrical assessment was not possible in one of the 0% HF-SFC replicates because of villus irregularity. Villus height and the thickness of muscularis mucosa decreased significantly in a linear fashion with HF-SFC inclusion but treatments explained only about 25% of data variation. There were no differences in crypt depth or thickness of the submucosa, but HF-SFC inclusion resulted in significantly thinner circular and longitudinal muscularis.

Table 4, Figures 3 and 4 near here

Bacterial enumeration, short chain fatty acids and pH

Bacterial colony counts are shown in Figure 3. HF-SFC inclusion was associated with a significant linear reduction in colony counts of *Clostridium* spp. *Clostridium* spp. counts were significantly lower in 20% and tended to be lower in 30% HF-SFC (P = 0.05) *vs* control. *Lactobacillus* spp. counts were significantly lower in 10% HF-SFC *vs* control and 20% HF-SFC. No effects on *E.coli* were seen. Short chain fatty acid profiles of the small intestine are illustrated in Figure 4. A significant negative linear effect was seen in both acetic acid and propionic acid concentration on HF-SFC inclusion. Mean pH measured in all samples was 6.54 (SE 0.10) and not affected by treatment.

DISCUSSION

In this study, the i-NSP-rich residue of non-decorticated cold-pressed SFC was used to formulate broiler feeds that were given between 15 and 31 d of age. In general, performance and health was satisfactory throughout the trial and, despite fibre contents far exceeding what is used in practice, the chickens thrived on diets containing HF-SFC. Birds efficiently compensated for differences in dietary energy levels by adjusting their feed intake. Experimental feeds were formulated with fixed AME_N:methionine and AME_N:lysine ratios,

and so the total intake of these parameters did not differ between treatments. Hetland and Svihus (2001) indicated that feeding i-NSP increased gut capacity in broiler chickens. Thus, it can be hypothesised that the i-NSP fraction of HF-SFC allowed for the large compensations of feed intake seen in this experiment. Weight gain and feed consumption increased with 20% and 30% HF-SFC, but FCR was only negatively affected by 30% HF-SFC. Interestingly, Viveros *et al*. (2009) found significant improvements in both weight gain and FCR when 4% sunflower seed hulls were added to a broiler diet. This effect was also seen with 3% oat hulls (González-Alvarado *et al*., 2007; Jiménez-Moreno *et al*., 2009*b*). Thus it may be hypothesised that the performance improvements in the present trial were, at least partly, mediated by the i-NSP fraction of the HF-SFC. Improvements in feed utilisation were also reported in broiler chickens fed on diets diluted with i-NSP-rich ingredients such as wood shavings (Amerah *et al*., 2009) and oat hulls (Hetland *et al*., 2003; Jiménez-Moreno *et al*., 2009*b*). In the present study, the DM weight of small intestinal digesta was increased with HF-SFC inclusion. This effect was also described, but not explained by Hetland and Svihus (2001). In the present experiment, the effect of increased DM weight of digesta was not mediated by differences in feed intake, since adding feed consumption as a co-variate to the ANOVA model increased the initial R^2 value (0.84) by less than 1% (data not shown). Rather, because fat is known to slow down digesta passage rate in the small intestine (Krogdahl, 1985), the elevated fat intakes associated with HF-SFC inclusion might explain this finding. Another feature possibly explaining the increased weight of digesta DM might be associated with small intestinal motility. Hetland *et al.* (2003) demonstrated indications of increased gastroduodenal reflux with i-NSP feeding. An increased reflux of digesta between gastrointestinal segments in addition to an increased grinding and mixing activity of the gizzard have been suggested as plausible explanations for the positive effects of i-NSP on digestion (Hetland *et al*., 2003; González-Alvarado *et al*., 2007; Jiménez-Moreno *et al*., 2009*a*).

In the present trial, DCs of DM, gross energy and ash declined in a linear fashion with HF-SFC inclusion. This was expected since HF-SFC contributed with large amounts of indigestible fibres. In contrast, protein and fat digestibility increased linearly with HF-SFC inclusion. It was demonstrated by Hetland *et al*. (2003) that oat hulls increases the amount of bile acids in the small intestine and, according to Krogdahl (1985), bile salt excretion constitutes the first limiting factor in fat digestion in poultry. However, because several important digestive adaptations occur in response to the fat content of the feed (Krogdahl, 1985), it cannot be out ruled that the positive effects on digestion associated with HF-SFC were a consequence of elevated fat intakes. Although the design of the present study does not allow for a separation of the effects of fat intakes from the effects of i-NSP, other recent studies have shown positive results on total tract digestibility of dry and organic matter, protein, fat and ash from adding 3% oat hulls (González-Alvarado *et al*., 2007; Jiménez-Moreno *et al*., 2009*a*,*b*).

In weaned pigs, both i-NSP and soluble NSP (s-NSP) positively influence the activity of enterocyte brush-border enzyme activity but, whereas s-NSP acted deleteriously on performance, i-NSP did not (Hedemann *et al*., 2006). Interestingly, in the work by Jiménez-Moreno *et al*. (2009*b*), the improvements seen at ileal level by adding 3% oat hulls were not seen with 3% sugar beet pulp; a feed ingredient distinguished by a relatively larger proportion of s-NSP.

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

from i-NSP, apart from what might be considered as minimal fractions available in the feed or litter.

In the gastrointestinal tract, both s-NSP and i-NSP display a bulk-forming capacity, but it is not known to what extent this feature influences digestion. In the present trial, villus height decreased linearly with HF-SFC inclusion. This contradicts the view of Moran (2006), who suggested that small intestinal villi would lengthen if the plane of nutrition is decreased by the inclusion of insoluble fibres. However, Baurhoo *et al*. (2007) reported that, despite there being no differences in performance, broiler chickens given more insoluble fibres (2.5% lignin) had significantly lower villi than 42-d-old birds receiving less insoluble fibres (1.25% lignin). Whether the bulk forming capacity of fibres affects the morphometry of the small intestine is not fully investigated. Since the inclusion of HF-SFC in the present trial mediated a pronounced effect on digesta DM weight in the small intestinal lumen, it might be hypothesised that such an increase in weight is associated with a corresponding increase in volume. This increase would mediate an expansion and stretching of the intestinal tract, possibly explaining why there was a linear decrease in thickness of muscularis mucosa and the circular and longitudinal sections of muscularis in birds fed on HF-SFC.

Morphometrical effects similar to those observed in the present trial were also seen when an antibiotic growth promoter (virginiamycin) was added to broiler diets (Miles *et al.*, 2006), and so it cannot be out ruled that the histological effects on the small intestine were associated with changes in the bacterial community. Interestingly, in the present experiment reductions of villus height were paralleled by an increase, not a decrease, in weight gain and nutrient absorption. Microscopic evaluation of the small intestinal mucosa revealed villus disorientation and extensive lymphocyte infiltration of the lamina propria in 3 out of 5 birds fed on 0% HF-SFC. These findings indicate that the gut mucosa of birds fed on 0% HF-SFC

was subjected to stress and it may be speculated that the integrity and function of the mucosa benefits from i-NSP.

In the current experiment, colony counts of *Clostridium* spp. were intermediate between what is normally found in healthy flocks and what is seen in diseased flocks (Drew *et al*., 2004). This can partly be attributed the absence of coccidiostats (Elwinger *et al*., 1998) in the present experimental diets. Regardless of this fact, colonies of *Clostridium* spp. were significantly suppressed in the 20% HF-SFC and tended to be lower in 30% HF-SFC *vs*. control. These results can be interpreted in light of the positive effects of HF-SFC on protein DC seen in this experiment. Because protein is an important substrate for growth of *Clostridium* spp. (Drew *et al*., 2004), it may be assumed that in the present trial, the suppression of *Clostridium* spp. in 20% HF-SFC and 30% HF-SFC birds was a consequence of improved protein digestibility. A *Clostridia*-reducing effect of i-NSP was demonstrated by Branton *et al*. (1997), who recorded a significant reduction in necrotic enteritis lesions in broiler chickens following a substitution of wheat by 4% wood shavings. In the present trial, colony counts of *Lactobacillus* spp. were significantly decreased in 10% HF-SFC *vs*. control and 20% HF-SFC, with a tendency towards a decrease in 30% HF-SFC birds *vs.* control. The suppressive effect of high male:female ratios on *Lactobacillus* spp. colony counts remains enigmatic. Whether the observed effect is best explained by body weight or some other characteristics of the sexes needs to be studied further. HF-SFC inclusion also mediated a shift in the SCFA profile of the small intestinal lumen. Whereas acetic and propionic acid decreased with HF-SFC inclusion, there was a weak tendency towards an increase in lactic acid concentration in 20% HF-SFC *vs.* 30% HF-SFC. However, the variance of lactic acid concentrations was notably large. Despite differences between SCFA profiles, the pH remained unaltered irrespective of treatment.

In conclusion, these results indicate that broiler chickens may thrive on feeds with insoluble fibre contents far exceeding what is used in practice. Furthermore, HF-SFC exerts 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
Analysed contents				
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

407 [1]The high-fibre sunflower cake had the following composition (g/kg DM): ash 43.2, crude
408 protein 230.3, crude fibre 370.3, fat (ether extract) 140.1 and dry matter 937.0 g/kg as fed.
409 [2]Premix declaration: retinol 3.6 mg, cholecalciferol 0.125 mg, DL-α-tocopheryl 80 mg,
410 vitamin B1 3mg, vitamin B2 8mg, vitamin B3 60mg, vitamin B6 5mg, vitamin B12 0,02mg,
411 vitamin B7 0,3mg, vitamin K3 3mg, vitamin B9 2mg, vitamin B5 20mg, choline chloride
412 1600 mg, iodine 1mg, selenium 0,35mg, manganese 100mg, zinc 120mg, copper 16mg, iron
413 30mg.
414

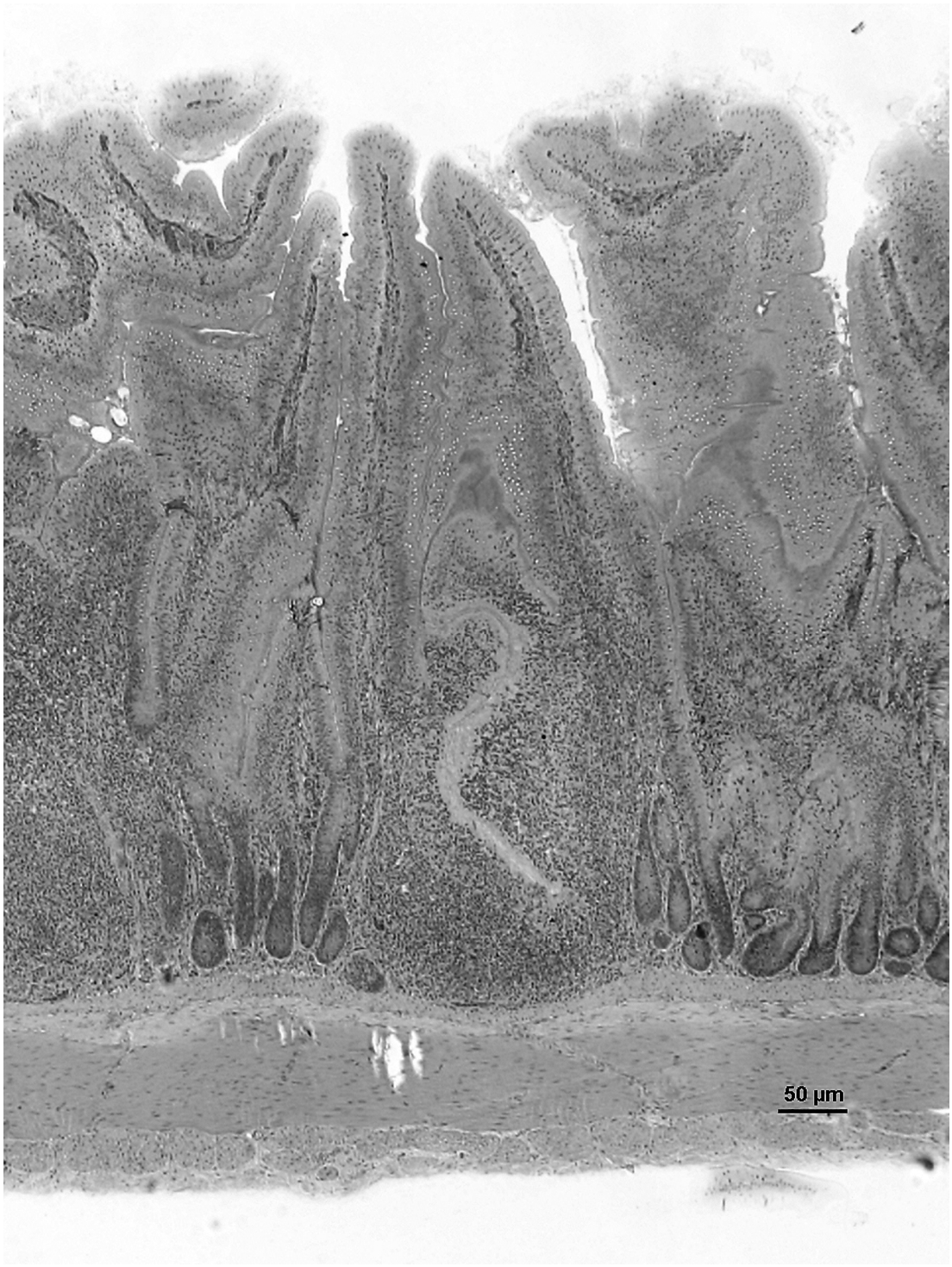


Figure 1. *Irregular and blunt villi infiltrated with lymphocytes.*

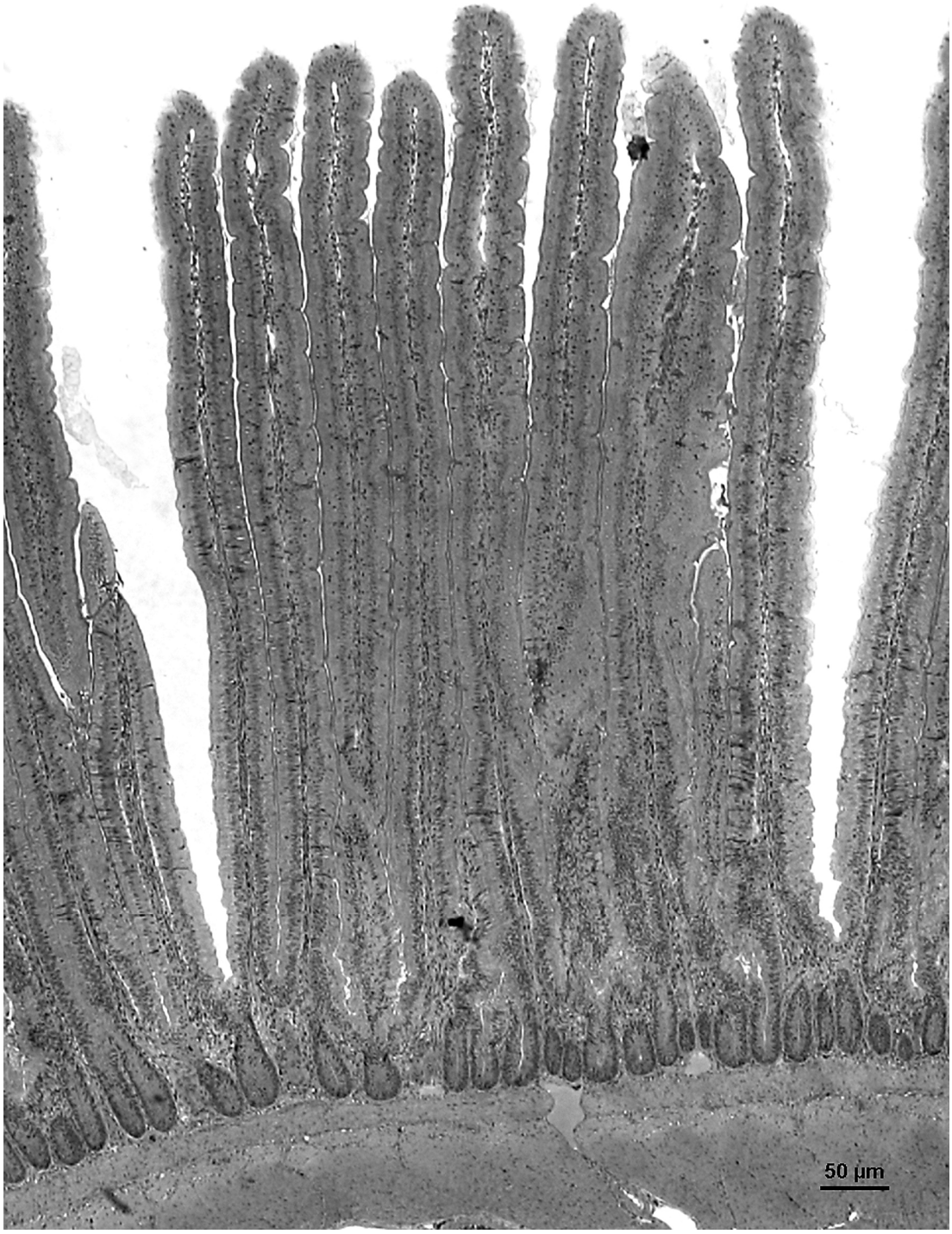
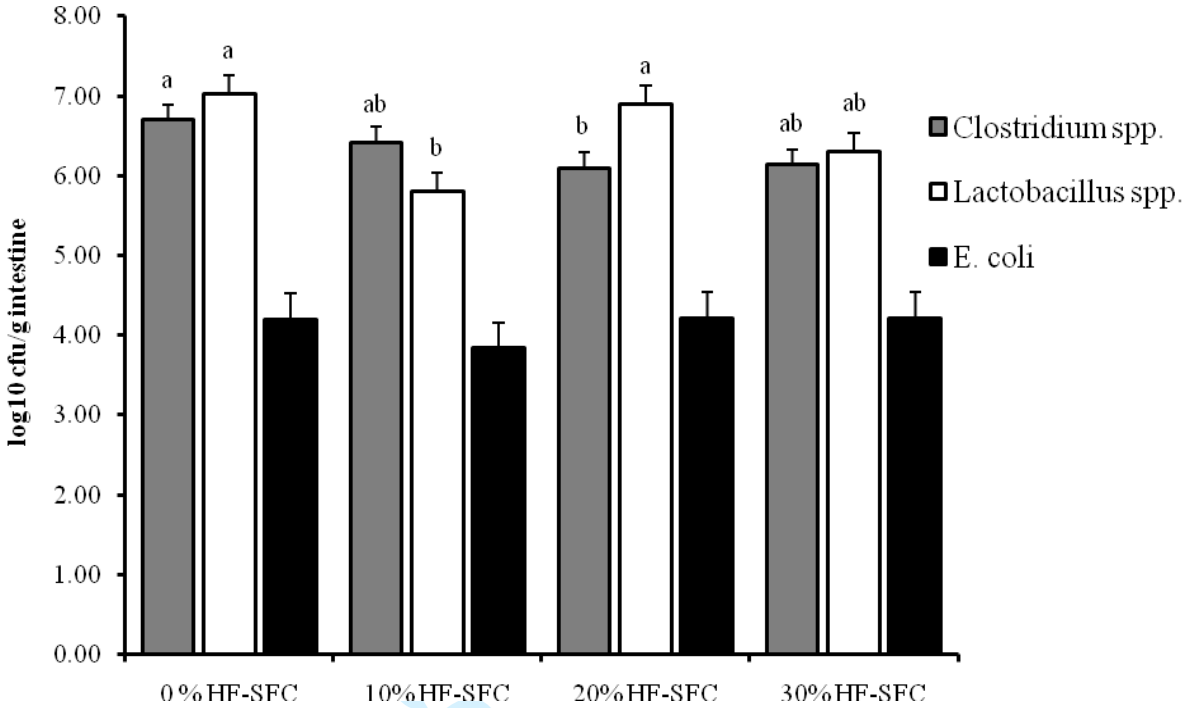


Figure 2. *Regularly shaped and well-defined villi.*



419

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

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

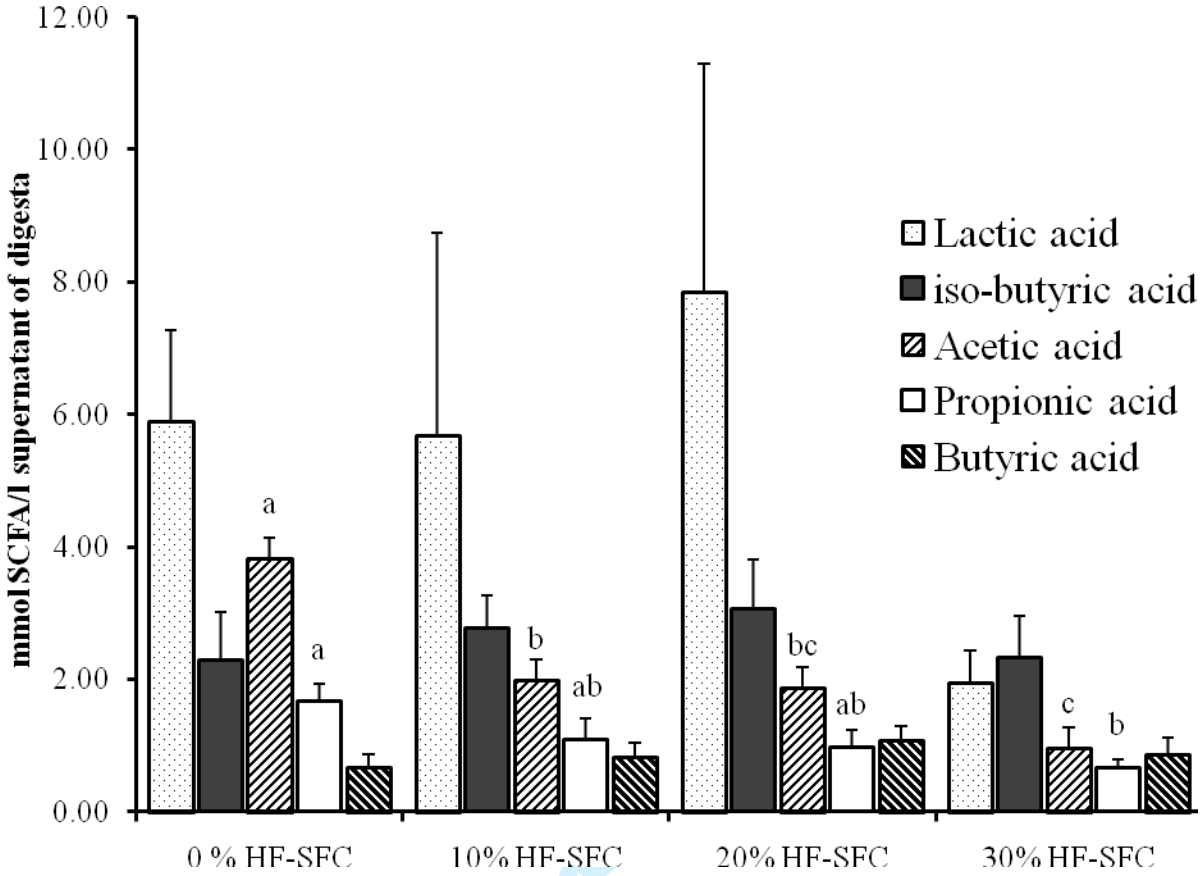


Figure 4. *Short chain fatty acid profiles of jejunum, presented as LS means (+SEM). Superscripts indicate significant differences between treatments at P <0.05.*

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	
Klason lignin	161.4		31.3		40.9		48.1		72.3	
Crude fibre	370.3		23.0		54.2		79.6		109.9	
	NSP		NSP		NSP		NSP		NSP	
	Soluble[1]	Insoluble	Soluble[1]	Insoluble	Soluble[1]	Insoluble	Soluble[1]	Insoluble	Soluble[1]	Insoluble
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

[1]Determined as the difference between total NSP and insoluble NSP

Table 3. *Broiler chicken production performance (d 15-31), mean ileal digestibility coefficients and AME_N (d 31), presented as LS means[1] (SE)*

	ANOVA				Linear regression		
	0 % HF-SFC	**10 % HF-SFC[2]**	**20 % HF-SFC**	**30 % HF-SFC**	**Prediction equation**	**H_0: $\beta_1 \neq 0$**	**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

[1:] Different superscripts within rows indicate significant treatment effects (*P*<0.05) in ANOVA test

[2]: Missing data as described in materials and methods section.

Table 4. *Morphometric data of broiler chicken jejunum (d 31). All values are presented as LS means[1] (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)	β_0= 1442 (61) β_1= -7.60 (3.17)	*P*<0.05	0.25
Crypt depth	163 (13)	179 (11)	154 (11)	171 (11)	β_0= 167 (10) β_1= -0.02 (0.54)	n.s.	<0.01
Thickness of							
Muscularis mucosa	54 (6)	52 (5)	42 (5)	40 (5)	β_0= 55 (4) β_1= -0.54 (0.23)	*P*<0.05	0.24
Submucosa	30 (5)	35 (4)	25 (4)	24 (4)	β_0= 33 (4) β_1= -0.30 (0.20)	n.s.	0.12
M. circular	169^{a} (12)	164^{ab} (11)	131^{cd} (11)	110^{d} (11)	β_0= 176 (10) β_1= -2.14 (0.51)	*P*<0.001	0.51
M. longitudinal	61^{ab} (6)	67^{a} (5)	53^{ab} (5)	47^{b} (5)	β_0= 66 (5) β_1= -0.59 (0.26)	*P*<0.05	0.23

[1]: Different superscripts within rows indicate significant treatment effects (*P*<0.05) in the ANOVA.