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The effects of fructo-oligosaccharides or whole wheat on the performance and the digestive tract of broiler chickens

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Short version of title: FOS and whole wheat

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Abstract 1. The objective of this experiment was to study two feeding methods, which could potentially act on the gut microflora, the structure and/or the function of the digestive tract and thereby improve the performance of broilers.

2. Four dietary treatments were studied: a negative control (wheat based) with no additives (C), a positive control with 0.01 g/kg avilamycin (AV), a treatment with 0.6 g/kg fructo-oligosaccharides (FOS) and a treatment with the same composition as treatment C but in which a part or all (400 g/kg) of the wheat was given as whole wheat and a concentrate complement (WW). The measurements were: the performance from 0 to 6 weeks, the bacterial counts at 3 weeks and 6 weeks, the digestive tract morphology and the activity of some intestinal enzymes at 3 weeks.

3. The birds fed AV had better daily live weight gain (DLWG) and FCR compared to treatment C. The birds fed FOS had a lower feed intake and a lower DLWG compared to the birds fed on treatment C, but their FCR was significantly improved. WW resulted in a numerically lower feed intake and a significant lower DLWG than treatment C. With AV, the number of aerobic mesophilic bacteria in the caeca was reduced at 3 weeks. With WW, gizzard and pancreas weights were higher and the surfaces of the ileal crypts were larger. An increased activity of leucine aminopeptidase (LAP) in the duodenum was found for treatments AV, FOS and WW.

4. In conclusion, in this study, treatments WW and FOS decreased the DLWG, which may be due to a lower feed intake during the whole period. With WW, the FCR was not affected maybe due to both positive and negative effects on digestive tract (higher gizzard and pancreas development and LAP activity; larger crypts). However, the FOS improved the FCR, which may be partly explained by the higher LAP activity.

INTRODUCTION

Since the 1950s subtherapeutical levels of antibiotic growth promoters (AGP) have been used in animal feed to improve the performance of animals by controlling the digestive microflora and thereby lower production costs (Thomke and Elwinger, 1998). However, the growing concern from consumers regarding antibiotic usage and the potential development of bacterial resistance, led to a ban of AGP from January 2006 in the European Union, which has resulted in a search for alternatives. Two potential alternatives in poultry production are fructo-oligosaccharides (FOS) and diets containing whole grains.

FOS are oligosaccharides, which are not hydrolysed by digestive enzymes, and may act as growth substrate for the intestinal flora (Monsan and Paul, 1995; Hartemink *et al.*, 1997). They are considered as prebiotics. They have been shown to have beneficial effects on the gut flora by stimulating the growth of beneficial bacteria such as bifidobacteria and lactobacilli, and by inhibiting potential pathogenic bacteria, i.e. *Salmonella* and *E. coli* (Bailey *et al.*, 1991; Waldroup *et al.*, 1993; Xu *et al.*, 2003). Furthermore, they stimulate the activity of some digestive enzymes. For example, Xu *et al.* (2003) found a higher activity of amylase and protease with the inclusion of FOS. The use of this prebiotic has also shown to improve the intestinal structure in broilers, by an increase in villus height in the ileum and a decrease in crypt depth in the jejunum and ileum (Xu *et al.*, 2003). The beneficial effects on the flora and the digestive physiology found with FOS could contribute to the observed improvements in the performance in poultry (Monsan and Paul, 1995; Orban *et al.*, 1997; Patterson and Burkholder, 2003; Xu *et al.*, 2003).

Another type of feeding, which potentially modifies the intestinal flora, is the inclusion of whole grains in the diet. A lower number of *E. coli* (Gabriel *et al.*, 2003b), a reduction in lactose negative enterobacteria and an increase in the number of certain lactobacilli have been reported (Engberg *et al.*, 2004). These modifications of the flora could be due to a reduction in the pH (0.5-1 unit) in the gizzard, caused by an increased secretion of hydrochloric acid in the proventriculus (Gabriel *et al.*, 2003a; Engberg *et al.*, 2004). The inclusion of whole wheat has also shown to improve the development and maturity of the intestinal mucosa (Gabriel *et al.*, 2007). These modifications may explain the improvement in the performance of broilers observed in several studies (Preston *et al.*, 2000; Hetland *et al.*, 2002; Plavnik *et al.*, 2002; Gabriel *et al.*, 2003a). Furthermore, the inclusion of whole grains is an attractive alternative. It meets the consumer requirements for a more "natural" production system and it reduces the feed costs due to less transport and processing and thereby lower production costs (Hetland *et al.*, 2002; Svihus *et al.*, 2004).

The objectives of this experiment were to study the effects of these two potential alternatives to AGP, FOS and whole wheat, on the performance, the gut flora, the intestinal structure and function of broiler chickens.

MATERIALS AND METHODS

Experimental diets

The birds were allocated to four dietary treatments: 1) a negative control (wheat based) with no additives (C), 2) a positive control containing 0.01 g/kg avilamycin (AV), 3) a treatment containing 0.6 g/kg of short chain fructo-oligosaccharides

(FOS) and 4) a treatment with the same composition as treatment C but in which a part or all (400 g/kg) of the wheat was given as whole wheat and a concentrate complement (WW).

The feeding program consisted of four different diets for each treatment: a starter diet (from 1 to 11 days of age), a grower diet (from 12 to 25 days of age), a finisher diet (from 26 to 36 days of age) and a withdrawal diet (from 37 to 42 days of age). The composition of the basal diets (the negative control diets) is shown in table 1. The diets were steam pelleted (2.5 mm in diameter, at 55 to 66°C).

For the treatments FOS and AV, the fructo-oligosccharides and the avilamycin were incorporated at the expense of the same amount of maize. For treatment WW, a part or all of the ground wheat of the basal diets was replaced by the same amount of coarsely ground or whole wheat, and was mixed with pelleted concentrate complements. These complements were calculated from the basal diets without the part of wheat given as coarse particles or whole grains. To accustom the chickens to whole grain, the coarsely ground or the whole wheat was gradually incorporated in the diet. Until day 7 the birds allocated to treatment WW received the same diet as treatment C. On day 8 and 9, 200 g/kg coarsely ground wheat was mixed with a pelleted concentrate complement (basal diet without 200g/kg of wheat). On day 10 and 11, 200 g/kg whole wheat was incorporated to the same concentrate complement and from day 12 onwards 400 g/kg whole wheat was added to concentrate complements (basal diets without 400g/kg of wheat).

The feed and the water were supplied *ad libitum*.

Birds and housing

A total of 864 Ross PM3 male broiler chickens vaccinated against infectious bronchitis were obtained from a commercial hatchery (Sicamen, Volnay, France). The chickens were raised in 3 m^2 floor pens with a stocking density of 12 birds/m^2 with 6 replicates per treatment. From day 1 to 5 the lighting programme consisted of 23 hours light and 1 hour dark (0-1 am), from day 6 to 11, it consisted of 20 hours light and 4 hours dark (0-4 am) and from day 12 to the end of the experiment 18 hours light and 6 hours dark (0-6 am). The temperature was 32°C from day 1 to 6 , it was reduced to 31°C at day 7, 29°C at day 14, 28°C at day 21, 24°C at day 28, 22°C at day 37 and 18°C at day 42.

Experimental protocol and sample collection

The experiment was carried out in accordance with the specific guidelines for experiments on animals (Decree, 2001).

At arrival, the birds were randomly distributed with 36 birds in each pen with a similar weight per pen. After 6 hours of fasting, the birds were individually weighed on day 11, 25, 36 and 42. The feed intake in each pen was measured at the same age and the FCR calculated. The actual proportion of whole wheat intake was determined after measuring the whole grains in feed refusals. The mortality was checked daily. Feed intake was expressed as animal present each day (i.e. dead birds were not included). To calculate Daily Live Weight Gain (DLWG) any females and dead birds were taken out of the calculation, but they were included in the FCR calculation.

At 3 weeks of age, 6 chickens representative of their pens were selected (according to their weight) from each pen. They were killed by intravenous injection of sodium pentobarbital. For treatments C and WW, the gizzards were emptied, trimmed for

139 excess fat and weighed, and the pancreases were collected and weighed. The weights
140 were expressed as percentage of live weight.
141 For all 4 treatments, the digestive tract was removed from the beginning to the end of
142 the intestine. The small intestine was divided into three segments: the duodenum
143 (from gizzard to pancreo-biliary ducts), the jejunum (from pancreo-biliary ducts to
144 Meckel's diverticulum) and the ileum (from Meckel's diverticulum to ileo-caecal
145 junction). For histological analysis, the middle part (1.5 cm long) of the duodenum
146 and ileum was taken, from 3 of the 6 sampled animals per pen. The samples were
147 opened longitudinally, rinsed with cold saline (NaCl 9 g/l) and fixed in a buffered
148 formaline solution overnight. They were then rinsed and stored in ethanol/water
149 (70/30, v/v) and stored at 4°C until further analysis.
150 The cloacal content was obtained by abdominal pressure on the birds before they
151 were slaughtered. The content from the ileum and caeca was collected by gentle
152 pressure. These digestive content samples were pooled from the 6 animals per pen
153 and stored at -70°C until further microbial analysis.
154 For the determination of intestinal enzymatic activities, samples were taken from the
155 3 animals per pen used for histological analysis. The middle section (one third) of
156 each intestinal segment (duodenum, jejunum and ileum) was split longitudinally,
157 rinsed with cold saline, wiped on a paper towel and the mucosa scrapped off before
158 freezing in liquid nitrogen and stored at -70ºC.
159 At 6 weeks of age, 6 chickens representative of each pen were selected. The cloacal
160 contents were collected as previously described, then the birds were killed. For
161 treatments C and WW, the gizzard and pancreas were removed and weighed as
162 described at 3 weeks. For all 4 treatments, the ileal and caecal contents were sampled
163 and processed as described previously.

Histological analysis

The intestinal samples (duodenum and ileum) were analysed as described by Goodlad *et al.* (1991). A 0.5 cm sample was cut off and kept in ethanol/acetic acid (75/25, v/v) for 24 hours, followed by a rehydration in ethanol/water (50/50, v/v) and then in distilled water. Thereafter, the samples were stained by the Feulgen reaction: first a hydrolysis in hydrochloric acid 1 N at 60ºC for 6 minutes, then rinsed with distilled water and thereafter stained with Schiff reagent for 30 minutes. Finally, the samples were rinsed in distilled water and stored in acetic acid/water (45/55 v/v) at 4ºC until analysis.

For histological measurements, villi with their attached crypts of Lieberkühn were individually dissected under a dissecting microscope then mounted between a slide and a cover slip in an aqueous mounting agent (Aquatex, Merck). They were measured under the magnification of 40 for crypts and 10 for villi, using an optical microscope (Leitz, Laborux), a camera (Scion corporation, CFW 1308C) and an image analysis software (Visilog 6.3, Noesis). The length and width of 10 villi and the depth and width of 20 crypts were measured from each segment of each bird. The surface area was calculated for each villi and crypt. An average value was calculated for each bird intestinal segment. Villus to crypt length and surface ratios were then calculated.

Enzyme activity assays

188 The intestinal samples (duodenum, jejunum and ileum) were analysed for enzymatic
189 activity of alkaline phosphatase (AP) (EC 3.1.3.1) and of the digestive enzymes
190 maltase (EC 3.2.1.20) and leucine aminopeptidase (LAP) (EC 3.4.11.2).
191 The frozen intestinal tissues were homogenised at a ratio of 50 mg/ml in phosphate
192 buffer saline (pH 7.4) using an Ultra-turrax® (IKA) for 3 x 10 seconds, and
193 centrifuged (10 000g, 15 min, 4°C). The supernatants were stored at -70°C until
194 further analysis.
195 For measuring the different enzymatic activities, continuous methods with 96-well
196 microplates were used. For the AP activity, the homogenate was diluted (1/20 for
197 duodenum and jejunum and 1/10 for ileum) and 0.1 ml of the dilution was mixed
198 with 0.2 ml of substrate (8.8 µmole of *p*-nitrophenyl phosphate (Sigma N 4645) per
199 ml of glycine buffer 93 mM containing 50 mM $MgCl_2$, pH 8.8). Readings were
200 carried out at 5 minutes intervals for 30 minutes with a multiscan spectrophotometer
201 (Argus 300 Microplate reader) at 405 nm (at 37°C) using a standard curve with *p*-
202 nitrophenol (Sigma N 7660).
203 For the LAP activity, the samples were diluted (1/2 for all segments) and 0.03 ml
204 was mixed with 0.25 ml of substrate (1 µmol of L-leucine *p*-nitroanalide (Sigma L
205 2158) per ml of phosphate buffer 0.1 M, pH 7.2). The plate was read at 405 nm
206 (37°C) at 2 minutes interval for 10 minutes. P-nitroaniline (Sigma N 2128) was used
207 for the standard curve.
208 Maltase was measured as described by Giorgi *et al.* (1992). The samples were diluted
209 (1/5 for all the samples). 0.05 ml of the sample was mixed with 0.15 ml of substrate
210 15 mM of maltose (Sigma M5885) in maleate buffer 60 mM containing 11 mM
211 $MgCl_2$ pH 6.8, 342 000 IU/l mutarotase (Biozyme, MUR1), 5 025 IU/l hexokinase
212 (Roche 11 426 362 001), 1.6 mmol/l ATP (Roche 10 519 979 001), 1.3 mmol/l

NADP (Roche 10 128 0314 001) and 1 200 IU/l glucose 6-phosphate dehydrogenase (Roche 10 127 671 001). The plate was read at 366 nm at 37°C at 2 minutes interval for 15 minutes. Glucose was used for the standard curve.

Bacteriology

The samples for bacterial analysis were successively diluted at 1/10 in 9 g/l NaCl and analysed for coliform, lactic acid bacteria and aerobic mesophilic bacteria. The lactic acid bacteria were counted after being plated onto MRS agar (Man, Rogosa, Sharpe) and incubated for 48 hours, the coliforms were plated onto Drigalski agar and incubated for 24 hours and the aerobic mesophilic bacteria on brain heart infusion agar and incubated for 48 hours. All the plates were incubated aerobically at 37°C. The results were expressed as $\log_{10}$ colony forming units (CFU)/g of digestive contents.

Statistical analysis

The data were analysed using Statview® software programme (Abacus Concepts, Berkeley, CA, USA) by one-way analysis of variance (ANOVA), and significant differences between treatments were determined by Student Newman-Keuls test ($P < 0.05$). The proportion of whole wheat for treatment WW was compared to the expected value with a one-tailed T-test ($P < 0.05$). These results were presented in the text as mean ± standard error.

RESULTS

Performance

During the whole experiment, the mortality was not significantly different between dietary treatments, 4.6% for C, 4.6% for AV, 3.2% for FOS and 4.7% for WW.

For the treatment AV, a significantly higher feed intake was seen from day 26 to 36 compared to the negative control treatment, a significantly higher DLWG was found at each period and throughout the experiment (day 1 to 42). A better FCR was also observed from day 26 to 36 and throughout the experiment (Table 2).

For the treatment FOS, the feed intake and the DLWG were significantly reduced from day 1 to 25 and for the whole period (Table 2). However, FCR was significantly improved for the treatment FOS compared to the control from day 26 to 36 and throughout the experiment.

The feed intake with the treatment WW was numerically lower during the whole experiment (-5 %). The actual proportion of whole wheat intake in the treatment WW, during the first two days of introduction (from day 10 to 11), was lower than the amount included in the feed, 138 ± 6 g/kg instead of the 200 g/kg, but thereafter the actual proportion of whole wheat intake was only slightly different than the targeted one (400 g/kg): 381 ± 3 g/kg from day 12 to 25 and 388 ± 3 g/kg from day 26 to 36, and 405 ± 1 g/kg from day 37 to 42. The DLWG was lower for the treatment WW compared to the control from day 12 to 36 and for the entire period (Table 2). The FCR was not significantly affected apart from day 1 to 11, where an improvement was observed with WW.

Digestive microflora

The microflora was not affected by dietary treatments at 3 weeks of age in the ileum and the cloaca. However, in the caeca the number of aerobic mesophilic bacteria was lower for the treatment AV, but none of the other treatments influenced the bacterial counts at this age (Table 3). At 6 weeks of age, none of the dietary treatments affected the number of aerobic mesophilic bacteria, lactic acid bacteria or coliform in the ileum, caeca and cloaca (data not presented).

Digestive tract morphology and enzyme activities

For the treatment WW, the gizzard and the pancreas weights (Figure 1) were significantly higher compared to treatment C, both at 3 and 6 weeks.

At 3 weeks of age, the different treatments did not affect the gut morphology in the duodenum. The villus height, width and surface were not affected by dietary treatments in the ileum. However, for treatment WW, a numerically higher crypt depth (+ 12 %) was found and a significantly larger crypt surface.

For the intestinal enzyme activity at 3 weeks of age, LAP was significantly higher for the treatments AV, FOS and WW in the duodenum, but no effect was observed in the other segments. The AP and the maltase activities were not significantly affected by dietary treatments in any of the intestinal segments. However, it should be noticed that a numerically higher level of LAP (+ 18 %) and maltase (+ 20 %) occurred for the treatment AV in the jejunum, and for maltase (+ 24 %) for the treatment FOS in the ileum (Table 5).

DISCUSSION

Effect of the AGP avilamycin

A significantly lower number of bacteria was observed in the caeca of birds fed on the treatment AV. This could be expected as AGP reduce the number of bacteria in the digestive tract (Thomke and Elwinger, 1998; Engberg *et al.*, 2000). Avilamycin in particular acts by interfering with the polypeptides-synthesizing functions and it is mainly active against gram positive bacteria (Wolf, 1973; Butaye *et al.*, 2003), the most numerous bacteria in the digestive tract (Gabriel *et al.* 2006). This reduction in the digestive flora may partly explain the improved performance observed with AV. Indeed, a decrease in the microflora may lead to a lower stimulation of the immune system (Gabriel *et al.*, 2006), which could prevent a depression in feed intake (Klasing *et al.*, 1987) as observed in our study. This increased feed intake may have contributed to the higher weight gain. Moreover the lower digestive microflora resulted in less competition for nutrients (Gabriel *et al.*, 2006) and could partly explain the improved FCR.

This improved FCR could also be due to an increased activity of the digestive enzyme LAP in the duodenum and the numerically higher level of maltase and LAP in the jejunum, which may have contributed to a better feed digestion.

AGP positively affect the intestinal structure. They reduce the weight of the small intestine by thinning the intestinal wall (Coates *et al.*, 1955; Jukes *et al.*, 1956), and this has been suggested to improve the nutrient absorption and thereby the performance. The changes in intestinal morphology (villus and crypt size) depend on the type of AGP (Miles *et al.*, 2006). With avilamycin, higher villus surface area in the jejunum and lower crypt depth in the jejunum and ileum were reported (Sarica *et al.*, 2005; Hernandez *et al.*, 2006). These modifications improve the intestinal

function. However, in the current experiment, the inclusion of avilamycin did not affect the gut morphology in the duodenum and the ileum, as previously reported by Catala-Gregori *et al.* (2007).

Effect of the prebiotic FOS

In the current study, FOS resulted in a lower feed intake. This has also previously been observed in broilers (Demir *et al.*, 2005) as well as in layers (Li *et al.*, 2007), but not in all studies. For example, Juskiewicz *et al.* (2006) reported no effects on the feed intake in turkeys, and Orban *et al.* (1997) reported a higher feed intake when including sucrose thermal oligosaccharide caramel, which is a complex mixture containing fructose-rich oligosaccharides and difructose di-anhydrides.

The lower feed intake observed in our study could have been caused by a stimulation of the intestinal immune system (Klasing *et al.*, 1987), as seen with FOS (Perrin *et al.*, 2001; Bornet and Brouns, 2002) due to bacterial stimulation. Indeed with FOS, a change in the digestive flora could be expected, as oligosaccharides increase the production of volatile fatty acids and lower the pH of the digestive content (Djouzi and Andrieux, 1997; Iji and Tivey, 1998; Perrin *et al.*, 2001; Bornet and Brouns, 2002), which promotes the growth of beneficial bacteria and suppresses the growth of certain pathogenic bacteria (Snel *et al.*, 2002). Thus, with conventional culturing methods Xu *et al.* (2003), when including 2 g/kg FOS, found an increase in the number of lactobacilli and a reduction in the number of *E. coli* in the caeca. With 4 g/kg FOS, they observed more differences in the digestive flora: an increase in the number of lactobacilli and bifidobacteria and a reduction in the number of *E. coli* in both the small intestine and the caeca. Similarly, Orban *et al.* (1997) reported an

increase in the number of bifidobacteria in the caeca of broilers, but a reduction in lactobacilli in one study and no effect on either of them in another when using a sucrose thermal oligosaccharide caramel. In their second study they also noticed a reduction in the number of coliforms in the caeca. In the current experiment the inclusion of FOS in the diet did not affect the bacterial counts as observed by Catala *et al*. (2007) with the same inclusion rate of FOS (0.6 g/kg). This low inclusion rate in these studies might explain the lack of response, especially since effects on the intestinal bacterial counts have been noticed with inclusion rates of 2.0 g/kg, but mainly with inclusion rates of 4.0 g/kg (Griggs and Jacob, 2005). However, with low inclusion levels of FOS, modifications of the microflora can occur. Thus, with molecular techniques, which are more exhaustive methods than the standard microbiological cultures, Massias *et al.* (2006) reported changes in the bacterial populations with FOS incorporated at 0.6 g/kg and in particular for lactobacilli.

The effects of the inclusion of FOS in poultry diets on weight gain are not consistent. In our study, a lower weight gain was found, whereas Demir *et al.* (2005) reported no effects in broilers and Juskiewicz *et al.* (2006) in turkeys. On the contrary, Orban *et al.* (1997) reported a higher weight gain with sucrose thermal oligosaccharide caramel in broilers as did Catala-Gregori *et al.* (2007) with an inclusion of 0.6 g/kg FOS. These contradictory results, particularly between the current study and that of Catala-Gregori *et al.* (2007) could be explained by the rearing conditions of the birds, the effects of oligosaccharides are likely to be more beneficial when the chickens are raised in less than ideal conditions (Orban *et al.*, 1997). For example stocking density in Catala-Gregori *et al.* (2007) was 15 birds/m^2 and was only 12 birds/m^2 in our study, and the density was reduced during the experiment by the birds taking out for analyses. With 15 birds/m^2, their raising conditions were more compromised than

363 those in the current study (12 birds/m^2). In the current study, the lower weight gain
364 could have been caused by the lower feed intake.
365 The inclusion of FOS in the current study improved the FCR in agreement with other
366 studies in broilers (Ammerman *et al.*, 1988; Orban *et al.*, 1997; Xu *et al.*, 2003) or in
367 layers (Respondek and Rudeaux, 2005; Li *et al.*, 2007), while others have reported
368 no significant effects, for example Demir *et al.* (2005) in broilers and Juskiewicz *et*
369 *al.* (2006) in turkeys. The improved FCR observed in this study might partly be
370 explained by the increased intestinal enzymatic activity with the FOS (a higher LAP
371 activity in the duodenum and a numerically higher level of maltase activity in the
372 ileum). Higher enzymatic activity of protease and amylase has previously been
373 reported with FOS by Xu *et al.* (2003).
374 In the current study, the intestinal structure was not affected by the inclusion of FOS
375 in the diet, in agreement with Catala-Gregori *et al.* (2007). However, Xu *et al.* (2003)
376 reported higher villi in the ileum and shorter crypts depths in the jejunum and ileum
377 with the inclusion of 4 g/kg FOS. But with the inclusion of 2 g/kg FOS, these authors
378 only observed an increase in the ratio between the villus height:crypt depth in the
379 ileum. The lower inclusion rate of FOS used in the current study might explain the
380 lack of response, maybe due to lower modification of microflora as previously
381 explained.
382
383 **Effect of the diet structure: Whole wheat**
384
385 A lower weight gain after the introduction of whole wheat was observed with whole
386 wheat, which may have been caused by the numerically lower feed intake due to the
387 different structure of the feed compared to the control diet. The reduced feed intake

388 in the beginning of WW introduction may be due to a limited capacity for grinding
389 whole wheat grains in the gizzard and the resulting slower transit rate in the digestive
390 tract. Although, the gizzard adapted fast, as seen by the higher gizzard weight as
391 early as one week after whole wheat introduction, the lower feed intake in the young
392 bird led to a lower growth rate and thus a lower intake thereafter. Otherwise, a
393 reaction towards the new form of diet was noted by the lower proportion of whole
394 wheat grains intake in the first two days after their introduction (138 g/kg actually
395 eaten compared to 200 g/kg included in the diet). However, the animals adapted
396 quickly to this type of feeding in the experiment, as it was seen by the higher
397 proportion of whole wheat after the first two days of introduction, where the actual
398 intake was close to the amount mixed in the feed. A lower feed intake with whole
399 wheat has already been reported by Engberg *et al.* (2004) and Hetland *et al.* (2002),
400 who included moderate 125 to 300 g/kg or high 300 to 440g/kg rates of whole grains.
401 However, other studies showed no difference in feed intake (Preston *et al.*, 2000;
402 Plavnik *et al.*, 2002; Svihus *et al.*, 2002). Several studies have reported no effect on
403 weight gain (Preston *et al.*, 2000; Bennett *et al.*, 2002; Svihus *et al.*, 2004), some
404 have observed a higher weight gain (Plavnik *et al.*, 2002), and others as in the current
405 study have reported a lower weight gain (Hetland *et al.*, 2002).
406 Although whole wheat improved the FCR in the starting period (day 1 to 11), it was
407 not affected during the whole period. This is in agreement with previous studies
408 (Hetland *et al.*, 2002; Gabriel *et al.*, 2003a; Svihus *et al.*, 2004). However, Plavnik *et*
409 *al.* (2002) and Wu *et al.* (2004) have reported an improvement in FCR with the
410 inclusion of 200 g/kg whole wheat. On the contrary, a poorer FCR has been reported
411 particularly with high inclusion level of whole grain (Bennett *et al.*, 2002; Engberg *et*
412 *al.*, 2006).

In our study, the inclusion of whole wheat in the diet did not significantly affect the bacterial count in the intestine. However, other studies have shown a decrease in the number of aerobic mesophilic bacteria, coliforms and lactose-negative enterobacteria and higher counts of some *Lactobacillus* species (Gabriel *et al.*, 2003b; Engberg *et al.*, 2004; Gabriel *et al.*, 2007). Although no changes in the microflora were observed in the current study with conventional cultivation methods, other bacterial population could have been modified. This may be observed by using molecular tools, as previously explained for the FOS. These modifications of the digestive flora could be due to a decreased pH in the gizzard (Gabriel *et al.*, 2003a). Moreover, the higher activity of this organ, as indicated by its higher weight observed in our study and in previous studies (Jones and Taylor, 2001; Plavnik *et al.*, 2002; Gabriel *et al.*, 2003a; Engberg *et al.*, 2004), may increase digestion of all dietary compounds. The higher pancreas weight observed in this study and in previous studies (Banfield *et al.*, 2002; Engberg *et al.*, 2004; Wu *et al.*, 2004) may be responsible for the increased amylase activity in the jejunum content, which may contribute towards a higher ileal starch digestibility (Svihus and Hetland, 2001; Svihus *et al.*, 2004). This higher digestibility of nutrients leads to less available substrate for the microflora.

In the duodenum, in the current experiment, the feeding of whole wheat had no effect on morphological parameters, contrary to results obtained in a previous study (Gabriel *et al.*, 2007) showing a reduction in the crypt depth. However, an increased intestinal enzyme activity was observed in this experiment as well as in the previous study. Thus in our study a higher activity of LAP was observed, and in the previous study, a higher activity of AP.

In the ileum, although previous studies showed no effect of feeding whole wheat on the intestinal structure or enzymatic activity (Wu *et al.*, 2004; Gabriel *et al.*, 2007),

438 we observed larger crypt surfaces. It may be related to an increase of the cellular
439 renewal, as shown by the relation between the crypt depth and the activity of cellular
440 proliferation (Brunsgaard and Eggum, 1995). This higher cell turn-over may lead to
441 lower enterocyte maturity. However, no difference in AP activity, used as an
442 indicator of enterocyte maturity (Weiser, 1973), was observed in our study. The
443 increased crypt surfaces may also be due to a higher number of goblet cells
444 particularly concentrated in the crypt, which can result in increased mucus secretion
445 (Langhout *et al.*, 1999). The higher mucus production can decrease the nutrient
446 absorption. In both the cases, the increase of cellular turn-over or the mucus
447 production, this represents an increase of energy requirement for gut maintenance,
448 which means the animal uses the nutrients for the functioning of the digestive tract
449 instead of its growth.
450 Positive effects of whole wheat feeding were observed at the beginning of the
451 digestive tract (increase development of gizzard and pancreas, increase enzymatic
452 activity in the duodenum), whereas a negative effect was observed at the end of the
453 intestine (higher crypt development in the ileum). This may explain the lack of effect
454 on FCR during most of the experiment.
455 In conclusion, the inclusion of avilamycin improved the performance of broilers,
456 which could be explained by the lower bacterial load in the caeca and the increased
457 activity of the digestive enzymes. With the inclusion of FOS in the diet, a reduction
458 in weight gain was observed which may be explained by the lower feed intake.
459 However, the FCR was improved, which might be due to the contribution of higher
460 intestinal enzymatic activities. With whole wheat feeding, the effects both positive
461 (increase development of gizzard and pancreas, increase enzymatic activity) and
462 negative (higher crypt development) on digestive tract may explained the lack of

463 effect on FCR during most of the experiment. The reduction of weight gain with this
464 treatment may be explained by the numerically lower feed intake due to the different
465 structure of the feed.
466
467 **ACKNOWLEDGEMENTS**
468
469 This work was financed by the European programme “Poultryflorgut” (2005-2008).
470 The authors also wish to thank Harold Rigoreau for animal care, Jean-Marc Hallouis,
471 Anne-Marie Chagneau, Angelique Travel and Bertille Tharrault for helping with the
472 sampling, and Nathalie Meme for the help with the bacterial analysis.
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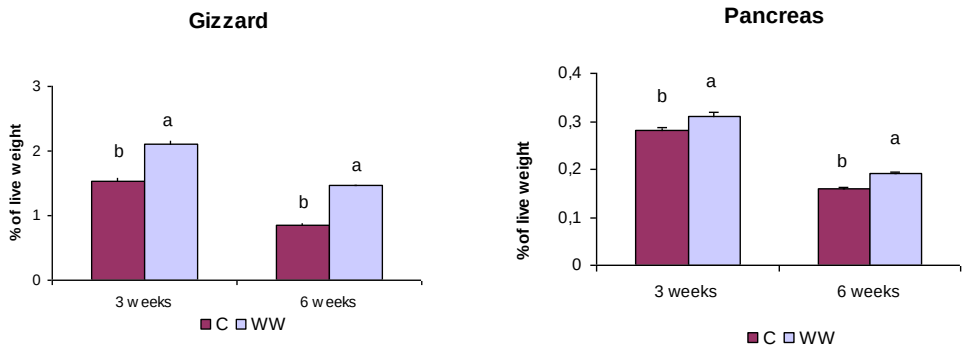

686

687 **Figure 1**. *Empty weight of gizzard and pancreas for broiler chickens (3 and 6 weeks*
688 *old) fed the control (C) or the whole wheat (WW) treatments. Means ± SE with*
689 *different letters for an age or an organ are significantly different (n=36 birds,*
690 *P<0.05).*
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693

694 **Table 1.** *Composition of basal diets (g/kg)*

	Starter	Grower	Finisher	Withdrawal
Period (days)	**1-11**	**12-25**	**26-36**	**37-44**
Ingredients				
Wheat	400.0	400.0	400.0	400.0
Soyabean meal	368.7	281.0	276.5	276.5
Maize	133.8	217.0	227.9	228.1
Rapeseed oil	59.0	50.0	49.0	49.0
Maize gluten meal		17.4	14.5	14.5
Dicalcium phosphate	16.4	14.4	13.8	13.8
Calcium carbonate	12.9	9.7	10.2	10.2
Vitamin/mineral premix[1]	4.0	4.0	4.0	4.0
Sodium chloride	3.0	3.0	3.0	3.0
Lysine	0.50	1.70		
Methionine	1.50	1.60	0.95	0.95
Anticoccidian (Clinacox ™)	0.2	0.2	0.2	
Calculated nutrient analysis				
ME [2] (MJ/kg)	12.6	12.8	12.8	12.8
Crude protein	220.0	200.0	195.0	195.0
Lysine	12.0	11.0	9.5	9.5
Methionine + cystine	8.5	8.2	7.5	7.5
Calcium	11.0	9.0	9.0	9.0
Available phosphorus	4.2	3.8	3.7	3.7

695 [1]The composition of the vitamin/mineral premix was (per kg diet): Co 0.6 mg, Cu 20 mg, I 2 mg, Se
696 0.2 mg, Zn 90 mg, Fe 50 mg, Mn 80 mg, retinyl acetate 5.2 mg, cholecalciferol 125 µg, D,L-α-
697 tocopheryl acetate 100 mg, thiamine mononitrate 5 mg, menadione 5 mg, riboflavin 8 mg, pyridoxine
698 7 mg, cyanocobalamine 0.02 mg, calcium pantothenate 25 mg, folic acid 3 mg, biotin 0.3 mg, choline
699 chloride 550 mg, niacin 100 mg, butylated hydroxy toluene 125 mg.

700 [2] ME = metabolisable energy

701

Table 2. *Performance of broiler chickens fed the experimental diets from 1 to 42 days*

	Treatment					
	C[1]	**AV**[2]	**FOS**[3]	**WW**[4]	**S.E.M.**[5]	**P**
Daily feed intake (g/animal/day)[6]						
Day 1-11	24.7^{ab}	25.6^{a}	22.5^{c}	23.7^{b}	0.36	<0.001
Day 12-25	79.7^{ab}	84.3^{a}	70.3^{c}	75.3^{b}	1.67	<0.001
Day 26-36	133.9^{b}	143.6^{a}	126.2^{b}	127.7^{b}	3.13	0.003
Day 37-42	181.8^{ab}	191.7^{a}	173.7^{b}	174.9^{b}	3.69	0.009
Day 1-42	91.1^{ab}	96.1^{a}	84.3^{c}	86.6^{bc}	1.82	0.001
Daily live weight gain (g/animal/day)[7]						
Day 1-11	19.9^{b}	21.2^{a}	17.8^{c}	20.1^{b}	0.22	<0.001
Day 12-25	53.6^{b}	58.7^{a}	49.3^{c}	50.1^{c}	0.65	<0.001
Day 26-36	78.4^{b}	84.3^{a}	76.0^{b}	72.5^{c}	0.95	<0.001
Day 37-42	100.1^{b}	106.9^{a}	97.3^{b}	97.8^{b}	1.41	<0.001
Day 1-42	58.8^{b}	63.5^{a}	55.7^{c}	55.8^{c}	0.59	<0.001
Feed conversion ratio[6]						
Day 1-11	1.25^{bc}	1.22^{ab}	1.28^{c}	1.19^{a}	0.013	<0.001
Day 12-25	1.50^{ab}	1.46^{a}	1.45^{a}	1.53^{b}	0.013	0.002
Day 26-36	1.77^{c}	1.73^{b}	1.69^{a}	1.80^{c}	0.013	<0.001
Day 37-42	1.85	1.83	1.81	1.79	0.018	NS
Day 1-42	1.64^{b}	1.61^{a}	1.60^{a}	1.65^{b}	0.006	<0.001

[a, b, c] = Means in the same row with no common superscript differ significantly ($P < 0.05$).

[1] C = negative control treatment.

[2] AV = positive control treatment containing 0.01 g/kg avilamycin.

[3] FOS = treatment containing 0.6 g/kg fructo-oligosaccharides.

[4] WW = treatment in which wheat is given as coarsely ground or whole grains.

[5] S.E.M. = standard error of the mean.

[6] Data represent the mean value of 6 replication pens.

[7] Data represent the mean value of 6 replication pens with 36 birds in each from the beginning of the experiment until the first slaughtering of birds (3 weeks old), and with 30 birds in each pen after first slaughtering of birds.

715 **Table 3**. *Digestive flora (log $_{10}$ CFU/g intestinal content) of broiler chickens (3*

716 *weeks old) fed the experimental diets* [1]

	Treatment					
	C[2]	**AV**[3]	**FOS**[4]	**WW**[5]	**S.E.M.**[6]	**P**
Ileum						
Aerobic mesophilic	7.52	6.62	8.06	7.60	0.358	0.065
Lactic acid bacteria	7.58	6.78	8.04	7.63	0.419	NS
Coliform	3.20	3.67	3.74	3.87	0.311	NS
Caeca						
Aerobic mesophilic	10.25[a]	8.78[b]	10.23[a]	10.09[a]	0.261	0.002
Lactic acid bacteria	10.67	10.53	10.72	10.15	0.169	NS
Coliform	6.70	6.85	6.92	6.90	0.154	NS
Cloaca						
Aerobic mesophilic	8.24[ab]	7.54[b]	9.15[a]	8.31[ab]	0.349	0.032
Lactic acid bacteria	8.34	7.83	9.18	8.36	0.401	NS
Coliform	5.35	5.38	5.40	5.06	0.237	NS

717 [a, b] = Means in the same row with no common superscript differ significantly ($P < 0.05$).

718 [1] Data represent the mean value of 6 replication pens with pools of 6 birds in each.

719 [2] C = negative control treatment.

720 [3] AV = positive control treatment containing 0.01 g/kg avilamycin.

721 [4] FOS = treatment containing 0.6 g/kg fructo-oligosaccharides.

722 [5] WW = treatment in which wheat is given as coarsely ground or whole grains.

723 [6] S.E.M.= standard error of the mean.
724

725

726 **Table 4**. *Histological measurements of the intestinal wall of broiler chickens (3*
727 *weeks old) fed the experimental diets* [1]

		Treatment					
		C[2]	**AV**[3]	**FOS**[4]	**WW**[5]	**S.E.M.**[6]	**P**
Duodenum							
Villus	Height (µm)	1548	1516	1441	1507	37.5	NS
	Width (µm)	681	670	663	643	24.5	NS
	Surface (µm^2)	1 055 137	1 035 221	955 297	976 309	48 393	NS
Crypt	Depth (µm)	118	114	121	120	2.6	NS
	Width (µm)	61	61	61	63	1.2	NS
	Surface (µm^2)	7 234	6 939	7 378	7 487	262.5	NS
Villus/crypt	Height	13.24	13.23	12.05	12.80	0.437	NS
	Surface	148	147	133	135	8.2	NS
Ileum							
Villus	Height (µm)	420	412	445	442	15.6	NS
	Width (µm)	504	505	503	471	17.6	NS
	Surface (µm^2)	212 527	208 967	226 681	209 866	12 582	NS
Crypt	Depth (µm)	102	102	105	114	3.8	0.094
	Width (µm)	70	71	72	75	1.6	NS
	Surface (µm^2)	7 258^{b}	7 207^{ab}	7 716^{ab}	8 684^{a}	403.6	0.042
Villus/crypt	Height	4.15	4.11	4.31	3.91	0.159	NS
	Surface	29.8	29.8	30.2	24.9	1.63	0.073

728 [a, b] = Means in the same row with no common superscript differ significantly ($P < 0.05$).

729 [1] Data represent the mean value of 18 birds (6 pens of replication x 3 birds/pen).

730 [2] C = negative control treatment.

731 [3] AV = positive control treatment containing 0.01 g/kg avilamycin.

732 [4] FOS = treatment containing 0.6 g/kg fructo-oligosaccharides.

733 [5] WW = treatment in which wheat is given as coarsely ground or whole grains.

734 [6] S.E.M. = standard error of the mean.

735

Table 5. *Enzyme activity (U/g tissue) in the intestine of broiler chickens (3 weeks old) fed the experimental diets* [1]

	Treatment					
	C[2]	**AV**[3]	**FOS**[4]	**WW**[5]	**S.E.M.**[6]	**P**
Duodenum						
AP[7]	4.29	4.85	4.14	5.19	0.350	NS
LAP[8]	2.68[b]	3.36[a]	3.14[a]	3.41[a]	0.156	0.006
Maltase	3.55	3.84	3.73	3.44	0.211	NS
Jejunum						
AP	2.69	3.13	2.46	3.18	0.256	NS
LAP	2.76	3.25	2.85	2.77	0.153	0.087
Maltase	4.10	4.94	3.79	3.99	0.307	0.054
Ileum						
AP	0.60	0.58	0.65	0.58	0.036	NS
LAP	2.52	2.63	2.62	2.45	0.131	NS
Maltase	1.80	1.62	2.24	1.98	0.177	0.092

[a, b] = Means in the same row with no common superscript differ significantly ($P < 0.05$).

[1] Data represent the mean value of 18 birds (6 replicate pens x 3 birds/pen).

[2] C = negative control treatment.

[3] AV = positive control treatment containing 0.01 g/kg avilamycin.

[4] FOS = treatment containing 0.6 g/kg fructo-oligosaccharides.

[5] WW = treatment in which wheat is given as coarsely ground or whole grains.

[6] S.E.M. = standard error of the mean

[7] AP = alkaline phosphatase

[8] LAP = leucine aminopeptidase