FEED PROCESSING AND STRUCTURAL COMPONENTS AFFECTS STARCH DIGESTION DYNAMICS IN BROILER CHICKENS

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ABSTRACT

1. A 2x2 factorial design was used to test the hypothesis that impaired intestinal starch digestibility is attributable to rapid passage of digesta from the gizzard to the intestine, and that compared to steam pelleting, increasing the availability of starch through extrusion cooking may alleviate the potential negative effect of rapid digesta flow on starch utilisation.
2. Thus, 7-d-old broilers chickens were distributed to 48 cages and given a wheat-based (WB) pelleted diet containing either coarse oat hulls (**OH-Pel**) or fine cellulose (**Cel-Pel**) until d 19 to stimulate divergent development of the gizzard. Thereafter, both groups were further subdivided and challenged with a WB diet with cellulose in either pelleted (**Cel-Pel**) or extruded (**Cel-Ext**) form on d 20 and 22. Either excreta or intestinal contents were collected at time intervals after feeding and analysed for marker and starch.
3. OH-Pel increased gizzard size and holding capacity. No excessively high starch levels (max 25 g/kg) were detected in the excreta. However, 8 h feed-deprived birds given Cel-Pel and challenged with Cel-Pel exhibited higher starch-excretion and showed large individual variation during the first 135 min of collection.
4. Contrary to OH-Pel group, more digesta and starch passed to the jejunum at 1 and 2 h and ileum at 2 and 3 h after feeding for birds given Cel-Pel, resulting in lower jejunal and ileal starch digestibility.

5. Increased starch gelatinisation through extrusion processing significantly improved starch digestibility regardless of gizzard function. However, at 1, 2 and 3 h after feeding, more digesta was retained in the foregut of birds given Cel-Ext.
6. The current data showed that starch degradation rate is associated with the flow of digesta which is linked to gizzard development and that enzymatic hydrolysis of intact starch granules may be limited with more rapid feed passage through the gut.

Key words: starch digestion, structural component, feed processing, digesta flow, broiler

INTRODUCTION

Starch digestibility in wheat-based (WB) pelleted diets has been observed to be low or incomplete for broiler chickens (Wiseman et al. 2000, Svihus and Hetland 2001, Abdollahi et al. 2011), with values ranging from 0.69 to 0.84 for diets containing more than 600 g/kg wheat. Poor starch digestibility has generally been attributed to several grain- or processing-related factors including the soluble fibre-fraction present in wheat (Annison 1993), wheat hardness (Carré et al. 2002), resistant cell wall material (Meng et al. 2005) or a lower starch gelatinization degree (Zimonja and Svihus 2009). Fine grinding of hard wheat (Péron et al. 2005) or the addition of fibre-degrading enzymes to wheat diets only partially alleviated this problem (Svihus and Hetland 2001). For instance, starch digestibility in enzyme-supplemented wheat diets remained low compared to oat or barley-based diets without enzymes (Svihus 2001) and, in other studies, no relationship between grain hardness and starch digestibility was found (Rogel et al. 1987a, Amerah et al. 2007). Enzymatic degradation of starch granules may in some cases be rate limiting, nevertheless, extrusion cooking and gelatinization of starch has been shown to increase its susceptibility to amylase (Björck et al. 1984). Studies with broiler chickens however produced inconsistent results. Plavnik and Sklan (1995) observed no difference in the digestibility of starch between extruded and untreated wheat-based diets, while Zimonja and Svihus (2009) found that, compared to cold- or steam-pelleting, extrusion processing significantly improved ileal starch digestibility mainly as a consequence of increased gelatinisation. These inconsistencies suggested that other, possibly bird-related factors are interfering with starch digestion of wheat-based diets. The gizzard is the pace-maker of normal gut motility (Duke 1994) and the major site for particle size reduction and peptic proteolysis (Shires et al. 1987). Accordingly, shorter retention time in this compartment implies less physical and chemical breakdown of digesta and inadequate starch degradation along the intestinal tract (Svihus 2011b). It is well established that gizzard activity and size are highly influenced by diet structure. Numerous workers therefore have shown that feeding pelleted diets based on finely ground wheat reduced the grinding activity and the relative weight of the gizzard compared to diets containing coarse or large particles (Engberg et al. 2002, Engberg et al. 2004, Amerah et al. 2009). Moreover, Svihus (2006) observed that feed intake was negatively correlated with nutrient utilisation, particularly in birds fed diets that did not stimulate gizzard activity. In addition, Svihus (2011b) reported that starch digestibility of wheat diets correlated with the relative empty gizzard weight, as all birds with less developed gizzard exhibited low starch digestibility. In a previous study, Svihus and Hetland (2001) indicated that an overload of wheat starch in the ileum, due to high feed intake, was the cause of the reduced starch digestibility in birds given pelleted wheat diet as compared to those fed a diet with whole wheat. Accordingly, it was hypothesised that, a well stimulated gizzard may have a regulatory effect of feed flow through the digestive tract, thus on starch availability. The nutritional benefits of increasing gizzard activity using structural components in the diet is well documented (Rogel et al. 1987, Hetland et al. 2003, Amerah et al. 2009, Svihus 2011a), although the complete mechanism is yet to be elucidated.

Thus, the hypothesis that low intestinal starch digestibility may result from a rapid feed flow from the gizzard, was tested. The gizzard of broiler chickens on a wheat-based diet were divergently stimulated by including either oat hulls or cellulose powder, and digesta flow and starch digestion rate were assessed. Additionally, since extrusion as compared to pelleting generally increases starch digestibility, the birds with divergent gizzard development were fed either extruded or pelleted diets under the hypothesis that pelleted diets would have a more deleterious effect on starch digestibility than extruded diets.

MATERIAL AND METHODS

This study was carried out in strict accordance with the laws and regulations governing experiments with live animals in Norway (the Animal Protection Act of 20 December 1974, and the Animal Protection Ordinance concerning experiments with animals of 15 January 1996).

Experimental diets and processing

Experimental diets were processed at the Centre for Feed Technology (Fôrtek), Norwegian University of Life Sciences, Ås, Norway, and were formulated to meet or exceed Ross 308 strain recommendations (Aviagen 2014) for major nutrients (**Table 1**). The diets consisted of a steam-pelleted WB diets containing 50 g/kg coarse oat hulls (**OH-Pel**) or fine cellulose powder (**Cel-Pel**). In addition, the WB diet with fine cellulose powder was also produced in extruded form (**Cel-Ext**). The above diets contained 5 g/kg Titanium dioxide, (TiO2) as a digestibility marker. The wheat used was ground in a Münch hammer mill (HM 21.115, Wuppertal, Germany) fitted with a 2 mm screen prior to any processing. The mash was steam-conditioned at 75°C in a double pass pellet-press conditioner (Münch-Edelstahl, Germany) prior to pelleting (Pellet press, Münch-Edelstahl, Germany, 1.2 t/h, 2×17 kW, RMP 350.100) through a 3 mm die with 42 mm thickness, at a production rate of 600 kg/h. The extruded diet was steam heated at 83°C in an extruder pre-conditioner (Bühler BCTC 10, Uzwil, Switzerland) prior to processing in a co-rotating twin-screw extruder (Bühler BCTG 62/20 D, 5 sections, 72 kW DC, Uzwil, Switzerland) fitted with 12 dies x 3 mm and with a feeder rate of 360 kg/h. A starch- and TiO_2 -free fine-mash diet comprising mainly dextrose and soybean protein concentrate was also produced by dry mixing the ingredients without any further processing. This diet served as a washout diet for birds prior to feed flow measurements, to avoid an excessively long starvation period.

Table 1 here

Birds, housing and management

A total of 120-day-old male broiler chicks were randomly allocated to four pens of 30 birds each, and fed on a commercial starter pelleted diet until 7 d. The pens were located in an environmentally controlled, continuously lit room at the experimental farm of the Norwegian University of Life Sciences (NMBU), Ås, Norway. Using two suspended heat lamps per pen, brooding temperature was maintained at approximately 32°C for the first five days and reduced to 30°C on d 7. Subsequently, room temperature was reduced by 4°C per week until an average of 22°C was reached by the end of the experiment at 22 d. The pens had wire mesh floors covered with sheets of newspaper. At 7 d, 24 birds from each pen were weighed and placed in pairs in 48 cages (width 50 cm× depth 35 cm× height 20 cm), so that the average weight was similar for each cage. Underweight birds were discounted. The cages had wire-mesh floor and an excreta collection tray. All birds were provided with feed and water *ad libitum* in two troughs attached along the front of each cage. From d 7 to d 19, the 48 cages were divided into two groups of 24 cages each and allocated to either **OH-Pel** or **Cel-Pel** to stimulate divergent development of the gizzard. Subsequently, to study the effect of gizzard manipulation and feed processing on digesta flow and starch utilization, birds in each of these dietary groups were further subdivided and subjected to two dietary treatments on day 20 and 22. Accordingly, the birds were challenged with a WB diet with fine cellulose in either pelleted (**Cel-Pel**) or extruded (**Cel-Ext**) form.

Excreta collection on day 20 (with feed deprivation)

In the evening of day 19, feed was withdrawn for two hours, then all birds (OH-Pel and Cel-Pel) were given the starch- and TiO_2-free mash diet for eight hours. This was done to ensure complete passage of previously ingested feed and that thus, the digestive tract did not contain starch or TiO_2. The fine-textured mash diet was hand-mixed with water at a ratio of 3:1 (w/v) immediately preceding feeding to avoid moisture loss and to encourage prompt consumption. Thereafter, the cages, were divided into subgroups of 12 cages each and subjected to either three or eight hours feed deprivation. Subsequently, the 12 cages were further subdivided into two groups of 6 cages each and given access to either Cel-Pel or Cel-Ext for 30 min, after which feed was withdrawn and water was made freely available. Thereafter, the two birds in each cage were separated using a cardboard to enable individual excreta collection, resulting in 12 replicate birds per combination of previous feeding (OH-Pel or Cel-Pel), feed deprivation (three or eight hours) and processing method (Cel-Pel or Cel-Ext). Fifteen min after feed removal, clean excreta trays were placed under each cage for the collection of droppings from each individual bird 90, 135, 180, 225 and 270 min after feed access. At the end of excreta collection, the birds were given access to their respective diets (OH-Pel or Cel-Pel) until the next day. Cecal droppings, identified as brown and watery,, were avoided. Excreta samples were frozen at −20°C until analysis. Due to a too small amount of droppings produced within the collection periods, the number of birds for each treatment with sufficient excreta during at least three time periods was only between four and six. To have an equal number of replicates of collection, four birds per treatment were chosen at random and included in the analysis.

Excreta collection on day 21 (without feed deprivation)

After 24 h continuous access to their respective diets, clean excreta trays were placed under each cage of the birds that were subjected to three hours feed deprivation on day 20.. After 5 hours, representative samples of droppings from each cage were then collected and frozen at −20°C until analysis. This was done to measure starch digestibility and determine AME in *ad libitum*-fed, unstressed birds.

Digesta collection on day 22

In the evening of day 21, feed was withdrawn for two hours, the birds were given the starch- and TiO_2-free mash diet for eight hours, and subsequently deprived of feed for five hours. Thereafter, the 24 cages in each prior feeding treatment (OH-Pel and Cel-Pel) were divided into two equal groups and given access to Cel-Pel or Cel-Ext for 30 min, after which feed was withdrawn. Twenty-four birds (6/treatment) were killed each time at 1, 2 and 3 h after feed access. Despite some unavoidable minor differences in pellet appearance between the pelleted and extruded diet, no differences in feed intake were detected between the treatments (data not shown). At the time of feeding, birds were observed with minimal disturbance, and lethargic or inactive birds (3 in total) not consuming any feed were excluded from the analysis. The crop and gizzard were dissected out with care to avoid material loss and stored at −20°C until analysis. The rest of the digestive tract with content (excluding colon and ceca), was placed in a zic-zac pattern over an aluminium foil on a rack, snap-frozen with liquid nitrogen and stored at −20°C for later analysis. A section from the posterior jejunum with content (5 cm from Meckel's diverticulum) was removed and stored at −80°C for later amylase activity analysis. The jejunum was defined as the segment from the end of the duodenal loop to Meckel's diverticulum, and the ileum as the section from Meckel’s diverticulum to the ileo-caecal junction.

Performance measurements

Body weights and feed intake per cage were recorded at 7, 14 and 21 d. Mortality was recorded as it occurred, and the three birds that died were weighed and feed per gain was corrected by dividing body weight gain of live plus dead birds by total feed intake.

Chemical analyses

Representative feed samples were ground on a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55743 Idar-Oberstein, Germany) through a 0.5 mm sieve. Gross energy was determined using an adiabatic bomb calorimeter (Parr 6400, Moline, USA) standardized with benzoic acid. Dry matter and ash content of the feed were determined after drying overnight at 105°C and after 12 h ashing at 550°C, respectively. Crude protein in the feed was determined by the Kjeldahl method. The degree of starch gelatinization (DG) (as a proportion of total starch) was measured by differential scanning calorimetry (DSC 823e Module, Mettler-Toledo, Switzerland) as described by Kraugerud and Svihus (2011) . Dry matter of the excreta, crop and gizzard content, jejunal and ileal digesta were determined after drying overnight at 105°C. Dried excreta and freeze-dried jejunal-ileal content were pulverized using a mortar and pestle for subsequent starch and TiO_2 analysis. TiO_2 content of feed, excreta, jejunal and ileal contents was determined as described by Short et al. (1996) . For starch analysis, 7-8 ml of 80% ethanol was added to each tube containing 100 ±5 mg sample of ground feed, pulverized dried excreta or freeze-dried intestinal content. The mixture was vortexed for 5-10 s and incubated for 5 min at 80°C, centrifuged for 10 min at 3000 rpm and supernatant containing mono-, di- and small oligosaccharides discarded. This procedure was repeated twice. Starch content was then determined enzymatically based on the use of thermostable α-amylase and amylo-glucosidase as described by McCleary et al. (1994) . Samples for amylase activity were prepared as described by Pérez de Nanclares et al. (2017) and assayed colorimetrically using amylase assay kit (Abcam- ab102523, Cambridge, UK) according to manufacturer's instructions. Activity of amylase was expressed as unit/g jejunal chyme on dry and wet basis. The amount of digesta passing to different section in the small intestine and starch digestibility were estimated on a dry matter basis and were calculated relative to the TiO_2 concentration.

Statistical analysis

All statistical analyses were conducted using the general linear models procedure of SAS (SAS INSTITUE 2004). Performance parameters and excreta data (from *ad libitum*-fed birds) on d 21 were compared using Student's t-test. Excreta data on d 20, digesta data and enzyme activity on d 22 were subjected to two-way analysis of variance with fibre particle size and processing method as main effects. The interaction between sampling time, fibre particle and processing were not analysed statistically due to the complexity of the statistical model, and so each sampling time was analysed separately. The significance of differences between groups was determined using the Ryan–Einot–Gabriel–Welsh *F*-test. Differences were considered significant at $P < 0.05$.

Results

Excreta analysis on day 20

Although no particularly high level of starch was found in the excreta (**Figure**), 8 h feed-deprived birds fed on cellulose-containing diet (Cel-Pel) during gizzard manipulation period and challenged with pelleted diet (Cel-Pel), exhibited higher ($p<0.05$) starch excretion (g/kg freeze-dried excreta collected) between the first 135 and 180 min after feeding. Independent of feed deprivation time, birds fed on oat hull-containing diet (OH-Pel) or challenged with extruded diet (Cel-Ext) showed a similar low starch excretion pattern, characterized by lower individual variation as compared to those given the Cel-Pel and challenged with Cel-Pel diet.

Figure here

Performance and excreta analysis on day 21

As shown in **Table 2**, birds fed on diet with fine cellulose (Cel-Pel) tended to consume more feed ($P=0.0945$) and were less efficient ($P<0.001$) in feed conversion than birds given the coarse oat hulls-containing diet (OH-Pel). Compared to OH-Pel, Cel-Pel feeding reduced ($p<0.001$) the AME value by 6.6% and dry matter digestibility by 7%. Moreover, although significantly different, starch levels were only 11 g/kg freeze-dried excreta, which was reflected by the nearly complete total tract starch digestibility in both groups.

Table 2 here

Dissection results on day 22:

As presented in **Table 3a**, **b** and **c**, the content of the crop decreased with time. At 1 h following feeding, there was a trend ($p=0.1083$) for higher DM content in the crop of birds given the extruded diet (Cel-Ext). At 2 and 3 h after feeding, birds given the Cel-Ext had significantly more material in the crop than the birds given the pelleted diet (Cel-Pel). At 1 and 2 h after feeding, a higher ($p<0.05$) dry matter content was found in the gizzard of birds given the Cel-Ext. As expected, oat hulls had a large ($p<.0001$) stimulating effect on gizzard development and holding capacity, expressed as relative empty weight and dry matter content, respectively. There was an increase in the amount of dry matter flowing to the jejunum at 1 h ($p=0.001$) and 2 h ($p=0.0236$), and to the ileum at 2 h ($p=0.0568$) and 3 h ($p=0.0883$) for birds given the cellulose-containing diet (Cel-Pel). The pattern of starch-flow closely followed that of dry matter at the jejunal and ileal level. Accordingly, jejunal starch concentration was lower in birds fed on diet with coarse structure (OH-Pel) at 2 and 3 h ($p=0.0007$ and $p=0.0998$) respectively. A significant ($p=0.0295$) interaction was observed at 1 h between fibre structure and processing method on starch content in the jejunum. As a result, birds given the Cel-Pel during gizzard manipulation period had higher concentration of starch in the jejunum only when challenged with pelleted diet (Cel-Pel). Ileal starch concentration was also lower at 2 and 3 h ($p=0.0089$ and $p=0.0223$) respectively for OH-Pel group. This resulted in higher starch digestibility at both jejunal (at 1 h, $p=0.0447$ and 2 h, $p=0.0004$) and ileal (at 2 h, $p=0.0101$) level. The effect of fibre structure on ileal starch digestibility was less obvious ($p=0.0957$) at 3 h after feeding, even though starch concentration was significantly lower in the OH-Pel group.

A significant main effect of feed processing on digesta flow into the intestine was also observed. Accordingly, lower content of digesta entered the jejunum and ileum at 1 h ($p=0.0037$ and $p=0.0228$, respectively) and the ileum ($p=0.0438$) at 3 h for birds receiving the extruded diet (Cel-Ext). Starch content (g/kg freeze-dried jejunal and ileal contents) was consistently and significantly lower for birds challenged with Cel-Ext as compared with those challenged with the Cel-Pel at all killing times. Consequently, extrusion resulted in significantly higher starch digestibility and tended (p=0.1073) to alleviate the negative effect of lack of oat hulls (i.e., gizzard stimulation) on ileal digestibility.

Table 3a, b, c here

Amylase activity

As shown in **Table 4**, jejunal amylase activity was not affected by feed processing method (P>0.1). However, there was a tendency (P=0.0963) for a higher amylase activity in birds given the OH-Pel as compared to Cel-Pel (Table 4). When expressed as unit per gram of dry chyme, the tendency was higher but not to a level of significance (P=0.0797).

Table 4 here

Discussion

The current experiment demonstrated the occurrence of rapid passage of digesta from the gizzard into the intestine when the gizzard was insufficiently stimulated. In addition, compared to pelleting, starch digestibility in the extruded diet seemed to be less affected by gizzard function. This initially supports the hypothesis put forward earlier of negative consequence of rapid passage of digesta on more digestion-resistant components, i.e. in pelleted diets. Before incorporation, wheat was finely-ground (2 mm screen size) to avoid any confounding effect of coarse grain grinding on gizzard stimulation (Svihus 2011a) or grain hardness on starch accessibility (Péron et al. 2005). In addition, diets were supplied with fibre-degrading enzymes to eliminate any potential effect of the soluble fibre fraction in wheat on digesta viscosity (Choct et al. 1996). The ability of the avian gizzard to exhibit rapid phenotypic responses to dietary stimuli was previously demonstrated (Starck and Rahmaan 2003). Thus, the stimulatory effect of OH-Pel on gizzard development in this experiment was expected and is in line with previous reports (Hetland et al. 2003, Sacranie et al. 2012).

Excreta analysis showed no sign of high starch levels (max 25 g/kg) being excreted independently of the lengths of feed deprivation used in this experiment. Comparable levels of starch in the excreta were also detected by Svihus and Hetland (2001), although no feed deprivation was used. Accordingly, they reported values ranging from 20 to 47 g/kg for a cellulose-diluted (10%) or undiluted pelleted WB diet respectively. Similarly, but with mash diets, cereal grains had an undigested starch fraction between 20 and 60 g/kg freeze-dried excreta (Weurding et al. 2001). It is worth mentioning that the individual variation and starch levels were higher at the beginning of excreta collection (135 min) particularly for birds with smaller gizzards and challenged with pelleted diet after 8-h feed deprivation. This suggests that, the combination of a rapid passage of digesta into the intestine, due to inadequate stimulation of gizzard function, and an insufficient degradation of starch may be the cause for the higher amount of starch lost in excreta. Nevertheless, the magnitude was lower than expected. The very small amount of starch in the excreta indicate that starch digestibility was very high or nearly complete (data not shown). It should be also noted that a fraction of starch may be lost in the lower digestive tract due to microbial fermentation in the ceca (Svihus et al. 2013) . Thus, total tract digestibility values may in some cases (Marron et al. 2001) give an inaccurate picture of starch digestibility (Svihus and Hetland 2001). Therefore, analysing ileal content allowed for more precise assessment on the fate of starch and confirmed that starch was highly digestible even in stress conditions such as feed deprivation.

Two main observations can be drawn from dissection results: First, differences in digesta flow and amount of starch recovered in the small intestine were likely influenced by the rate at which feed was leaving the gizzard. Independent of the processing method, digesta passed into the intestine faster for birds with smaller gizzards. Accordingly, more starch reached the jejunum or ileum, which caused a reduction in starch digestibility in the respective intestinal segment. On the contrary, due to oat hulls inclusion, larger gizzards were able to hinder the fast flow of digesta into the jejunum at 1 and 2 h and into the ileum at 2 and 3 h after feeding. The current results are in line with recent findings. Already 1 h after feeding, Sacranie et al. (2017) found higher ($P < 0.05$) load of DM and starch in the small intestine of 16 h-starved birds, adapted to, and re-fed a diet with fine cellulose as compared with coarse oat hulls. Also, using whole wheat as gizzard-stimulating components, Svihus et al. (2010) reported that the jejunum and ileum of birds killed 1 h after re-feeding, contained less ($P = 0.01$) DM for the whole wheat diet compared with the ground wheat diet. This was accompanied with a concomitant reduction ($P < 0.001$) in the ileal concentration of starch and improvement ($P < 0.001$) in total tract starch digestibility for the whole-wheat diet.

In the current experiment, the challenge diets contained the same source of fibre (fine cellulose powder) and thus, only differed in the way they were processed (pelleted vs extruded). In the aforementioned studies, the challenge diets given to feed-deprived birds contained different structural components, as already mentioned. Using the same source of fibre, this experiment eliminated the potential confound of coarse or fine structure on digesta passage, and clearly demonstrated the ability of a well-functioning gizzard in modulating the flow of feed, even when lacking structural components. The above observations emphasize the importance of the gizzard as a feed-flow regulator (Svihus 2014, Classen et al. 2016, Sacranie et al. 2017) and seem to validate the hypothesis that the gizzard may be the key site for prevention of starch overload in the digestive tract (Svihus and Hetland 2001).

Secondly, the more vigorous conditions in the extrusion processing are generally sufficient to cause complete disruption of starch granule structure (Skoch et al. 1983, Svihus et al. 2005), which is expected to increase the susceptibility of starch to enzymatic hydrolysis (Björck et al. 1984, Sun et al. 2006) . Our results are in accordance with those reported by Zimonja and Svihus (2009), where higher gelatinization degree of starch in the extrusion processing significantly increased starch digestibility in wheat diets. However, it was observed during dissection that the content of the crop and gizzard differed in physical appearance between the extruded and pelleted diets. Crop and gizzard digesta appeared lumpy with intact and swollen pellets for the extruded diet, while it was watery with no apparent intact pellets for the pelleted diet. Hilton et al. (1981) reported similar observations and attributed this to the higher water stability of the extruded diets which increases its retention time in the upper gut compartments. This is consistent with our results, where more DM was found in the crop and gizzard for birds given the extruded diets at least in the first two hours after feeding. With such characteristics, extruded diet tends to have a slower passage rate than the pelleted diet, and interaction between feed processing, feed flow and starch availability may exist. The longer time required to moisturise the extruded feed in the upper gut would be a potential confounding factor affecting starch availability. An improved nutrient digestibility and feed efficiency have been associated with slower digesta transit time caused by longer retention of the feed in the crop (Svihus 2014, Classen et al. 2016) and gizzard (Sacranie et al. 2012). Therefore, care must be taken before drawing firm conclusion regarding the cause of the high digestibility of starch in the extruded diet.

A combination of factors in this experiment may have contributed to the high starch digestibility even in pelleted diets such as the fine grinding of the wheat and NSP-ase addition. However, the latter variables were held constant for both groups except for gizzard stimulation. Moreover, contrary to the finding of Hetland et al. (2003), no difference in amylase activity was observed which also explain the high starch digestibility in all treatments. And, although starch excretion/digestibility were statistically different between treatments, the difference was smaller than expected. As a result, birds fed on a diet without structure and challenged with pelleted diet were also able to cope with the stress and surprisingly exhibited high starch digestibility. In this case, improved gizzard function does not solely explain this high starch availability and thus, certainly other mechanism must be involved. Unlike mammals, vigorous gut refluxes are normal in birds (Ferket and Veldkamp 1999), and as Basha and Duke (1999) stated, intestinal refluxes are uniquely avian. Sacranie et al. (2007) even found that intestinal reflux or the retrograde movement of digesta, occurs throughout the digestive tract of both fasted and fed chickens. Reflux therefore, serves to re-expose intestinal digesta to gastric secretion thereby extending the digestive and absorptive processes to compensate for the lack of food and short intestinal segments (Ferket and Veldkamp 1999, Sacranie et al. 2005). The small amount of starch excreted despite higher starch content in ileal digesta seems to support this postulation.

In conclusion, the current data showed that the rapid passage of digesta to the small intestine resulted in reduced starch digestibility, particularly with lower degree of starch gelatinisation. This suggests that starch degradation rate is associated with the flow of digesta which is linked to gizzard development and that, enzymatic accessibility of intact starch granules can be limiting with more rapid feed passage through the gut.

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Table 1. *Experimental diets composition, calculated and analysed nutrient content (g/kg as fed)*

Ingredients	OH-Pel*	Cel-Pel / Cel-Ext*
Wheat	671.5	671.5
Fish meal (72% CP)	149	149
Soybean concentrate (68% CP)	70.1	70.1
Soybean oil	26	26
Ground limestone	12	12
L-Lysine	1	1
DL-Methionine	2.5	2.5
L-Threonine	2.5	2.5
Mineral & Vitamin premix [1]	6.4	6.4
Choline chloride	2	2
Titanium dioxide	5	5
Oat hulls (unground)	50	-
Cellulose (fine powder) [2]	-	50
Enzyme (Rovabio) [3]	1.5	1.5
Calculated nutrient content		
Metabolisable energy (MJ/kg)	12.89	12.89
Dig. Lysine	13.2	13.2
Dig. Methionine	6.8	6.8
Dig Threonine	10.3	10.3
Calcium (g/kg)	11	11
Available phosphorous (g/kg)	4.8	4.8
Analysed nutrient content		
Gross energy (MJ/kg)	17.0	17.0 / 17.1
DM (g/kg)	908	883 / 893
Starch (g/kg)	419	419 / 429
Crude Protein (g/kg)	223	223 / 224
Starch gelatinization [4]	318	318 / 975

***OH-Pel**: Pelleted diet with oat hulls; **Cel-Pel**: Pelleted diet with cellulose; **Cel-Ext**: Extruded diet with cellulose;

[1] Mineral and vitamin premix provided the following per kg diet: Fe, 53 mg; Mn, 125 mg; Zn, 83 mg; Cu, 15 mg; I, 0·75 mg; Se, 0·30 mg; retinyl acetate, 5.75 mg; cholecalciferol, 0.18 mg; dl-α-tocopheryl acetate, 80 mg; menadione, 10 mg; thiamine, 6 mg; riboflavin, 26 mg; niacin, 35; calcium pantothenate, 26 mg; pyridoxine, 15 mg; cobalamin, 0.04 mg; biotin, 0.6 mg; folic acid, 5 mg.

[2] Cellulose powder: Product Sanacel 150 from CFF GmbH & Co.KG.

[3] Enzyme Rovabio Excel Ap T-Flex, Adisseo, France provided the following per kg diet: Endo-1,4-β-xylanase: 33 000 visco units; Endo-1,3(4)-β-glucanase: 45 000 visco units; Endo-1,4-β-glucanase (cellulase) >9600 DNS units + 16 other enzyme activities obtained from a fermentation broth of Penicillium funiculosum.

[4] Starch gelatinization (g/kg of total starch)

Table 2. *Performance and results of excreta analysis for male broilers fed on a wheat-based pelleted diet containing either coarse or fine fibre structure*

Gizzard manipulation diet [1]	Production performance (7-21 d)			Excreta analysis [2] on d 21			
Fibre structure	Feed intake	Weight gain	Feed per gain	AME [3]	DM [3] digest.	Starch [3] digest.	Starch g/kg
OH-Pel* (coarse)	1261.5 ± 26.17	903.4 ± 27.79	1.40 ± 0.025	12.99 ± 0.07	0.703 ± 0.01	0.995 ± 0.00	7.69 ± 0.41
Cel-Pel* (fine)	1312.7 ± 11.90	851.6 ± 13.99	1.54 ± 0.026	12.13 ± 0.13	0.653 ± 0.01	0.988 ± 0.00	11.34 ± 0.82
P-values	0.0945	0.1147	<0.001	<0.001	<0.001	<0.001	0.0013

[1] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel).

[2] After 24 h of open access to feed, clean excreta trays were placed under each cage for 5 h, then representative samples of droppings from each cage were collected, oven-dried and analysed.

[3] Apparent metabolisable energy (AME) MJ/kg DM, total tract dry matter (DM) and starch digestibility were calculated using marker techniques.

[*] Values are means ± SEM, (n= 12 replicate cages of 2 birds each) and are significantly different ($P < 0.05$).

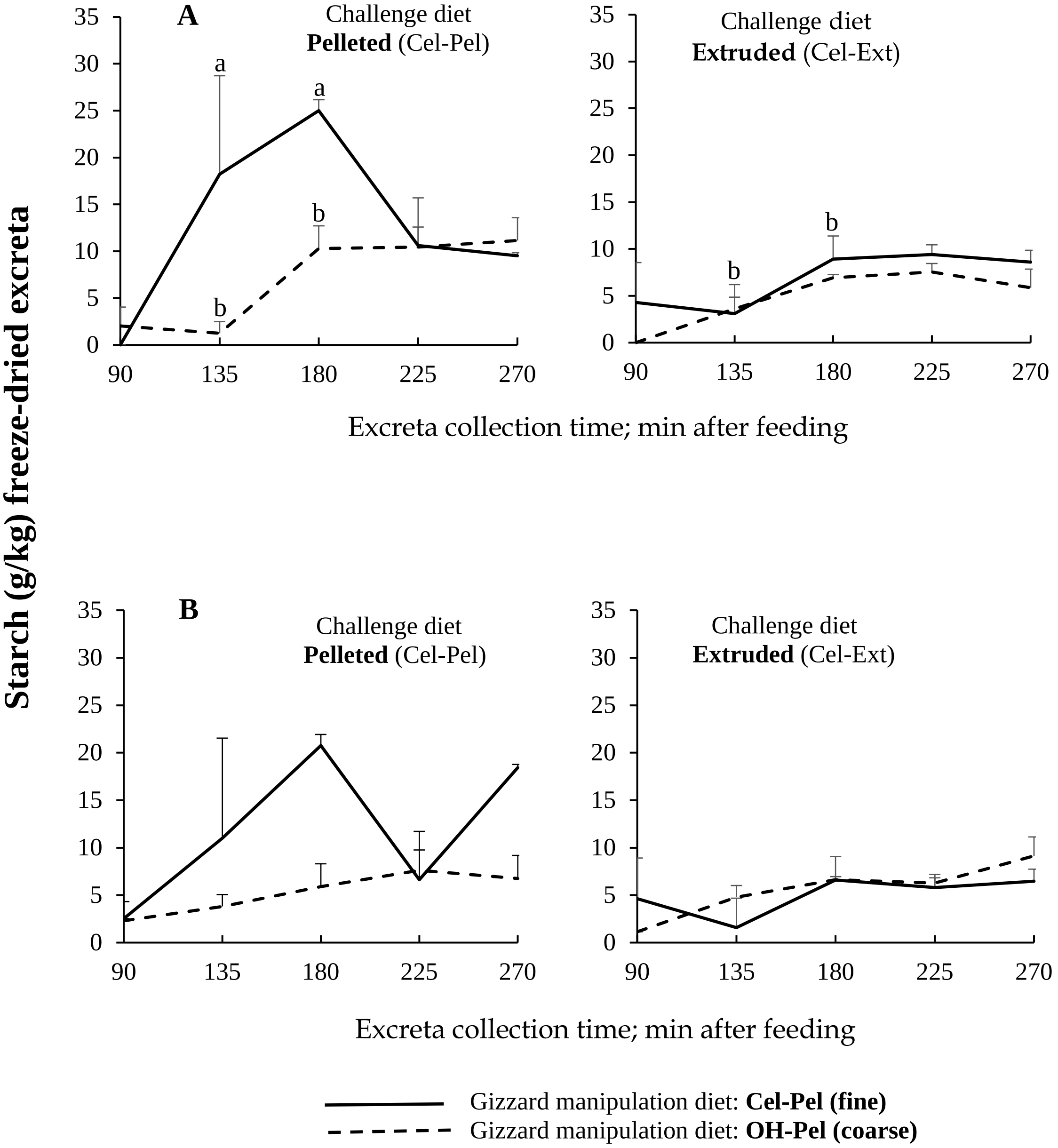

Figure. Starch content in excreta (g/kg dried excreta) on day 20, mean ±SEM (n= 4): 8- or 3-h feed deprivation, followed by 30 min access to either pelleted or extruded wheat-based diets. Excreta were collected 90 min after feeding and four times every 45 min thereafter. Treatment means within time with different letters are significantly different (*P* < 0.05).

Figure (A) 8-hour feed deprivation. Figure (B) 3-hour feed deprivation.

Table 3 a. *The influence of fibre structure and processing method on the weight of crop and gizzard contents, relative weight of empty gizzard, weight of digesta (expressed on a DM basis) passing to the jejunum and ileum, starch content in freeze-dried jejunal and ileal contents and starch digestibility in broilers killed at different times*

From 7-21 days	At 22 days	Killed at 1 hour after feeding[1]								
Gizzard manipulation diet[2]	Challenge diet[2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	**Processing** method	DM g	DM g	Rel. w. g/kg	Digesta[3] DM g	Starch g/kg	Starch digest.	Digesta[3] DM g	Starch g/kg	Starch digest.
OH-Pel (coarse)	Cel-**Ext**	14.8	2.0	15.2	2.0	23.4 c	0.971	0.8	7.5	0.989
Cel-Pel (fine)	Cel-**Ext**	15.8	1.1	9.5	2.7	40.8 c	0.952	0.7	9.5	0.987
OH-Pel (coarse)	Cel-**Pel**	12.2	1.4	17.1	2.6	129.2 b	0.802	1.3	80.1	0.861
Cel-Pel (fine)	Cel-**Pel**	10.6	0.6	11.0	3.3	224.1 a	0.690	1.5	99.9	0.849
	√MSE[4]	5.49	0.62	2.82	0.43	39.40	0.082	0.66	45.19	0.064
Fibre										
Coarse		13.5	1.7 a	16.2 a	2.3 b	76.3	0.887 a	1.1	40.5	0.925
Fine		13.0	0.8 b	10.3 b	3.0 a	140.8	0.821 b	1.1	54.8	0.918
Processing										
Extrusion		15.3	1.6 a	12.6	2.3 b	31.3	0.962 a	0.8 b	8.5 b	0.988 a
Pelleting		11.4	1.0 b	14.0	3.0 a	176.6	0.746 b	1.4 a	90.0 a	0.855 b
P-value										
Fibre		0.8947	0.0030	<.0001	0.0010	0.0029	0.0447	0.8242	0.5859	0.7907
Processing		0.1083	0.0368	0.1694	0.0037	<.0001	<.0001	0.0228	0.0008	<.0001
Fibre x Processing		0.5760	0.8002	0.8792	0.9927	0.0295	0.1276	0.5619	0.6624	0.7377

[1] Values are means of 3 replicate-cages of 2 birds each.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel); Challenge diet: WB diet with fine cellulose in extruded (Cel-Ext) or pelleted (Cel-Pel) form.

[3] The weight of digesta passing into the jejunum and ileum were estimated on a DM basis and calculated relative to the TiO_2 concentration in freeze-dried digesta.

[4] √MSE: square root of means square error in the analysis of variance.

[a, b, c] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 3 b.

From 7-21 days	At 22 days	Killed at 2 hours after feeding [1]								
Gizzard manipulation diet [2]	Challenge diet [2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	**Processing** method	DM g	DM g	Rel. w. g/kg	Digesta [3] DM g	Starch g/kg	Starch digest.	Digesta [3] DM g	Starch g/kg	Starch digest.
OH-Pel (coarse)	Cel-**Ext**	13.7	2.1	14.8	1.8	13.2	0.986	3.3	6.4 b	0.995
Cel-Pel (fine)	Cel-**Ext**	11.4	0.8	9.9	2.7	53.9	0.928	3.9	18.6 b	0.985
OH-Pel (coarse)	Cel-**Pel**	7.4	1.8	15.3	2.1	60.5	0.931	2.3	12.2 b	0.987
Cel-Pel (fine)	Cel-**Pel**	7.5	0.1	8.8	3.0	101.5	0.867	3.9	62.6 a	0.947
	√MSE [4]	4.84	0.51	2.15	0.88	24.31	0.037	1.29	25.71	0.023
Fibre										
Coarse		10.8	2.0 a	15.1 a	1.9 b	34.7 b	0.959 a	2.8	9.0 b	0.991 a
Fine		9.4	0.5 b	9.4 b	2.9 a	77.7 a	0.898 b	3.9	40.6 a	0.966 b
Processing										
Extrusion		12.6 a	1.4 a	12.4	2.3	33.5 b	0.957 a	3.6	12.5 b	0.990 a
Pelleting		7.5 b	0.9 b	12.1	2.6	82.8 a	0.899 b	3.1	39.7 a	0.967 b
P-value										
Fibre		0.5917	<.0001	<.0001	0.0236	0.0007	0.0004	0.0568	0.0089	0.0101
Processing		0.0208	0.0462	0.7235	0.4394	0.0001	0.0007	0.3386	0.0315	<.0001
Fibre x Processing		0.5754	0.3390	0.3828	0.9822	0.9914	0.8401	0.4181	0.0918	0.1073

[1] Values are means of 3 replicate-cages of 2 birds each.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel); Challenge diet: WB diet with fine cellulose in extruded (Cel-Ext) or pelleted (Cel-Pel) form.

[3] The weight of digesta passing into the jejunum and ileum were estimated on a DM basis and calculated relative to the TiO_2 concentration in freeze-dried digesta.

[4] √MSE: square root of means square error in the analysis of variance.

[a, b, c] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 3 c.

From 7-21 days	At 22 days	Killed at 3 hours after feeding [1]								
Gizzard manipulation diet [2]	Challenge diet [2]	Crop	Gizzard		Jejunum			Ileum		
Fibre structure	**Processing** method	DM g	DM g	Rel. w. g/kg	Digesta [3] DM g	Starch g/kg	Starch digest.	Digesta [3] DM g	Starch g/kg	Starch digest.
OH-Pel (coarse)	Cel-**Ext**	5.6	1.4	18.4	2.6	30.8	0.968	3.7	13.7	0.989
Cel-Pel (fine)	Cel-**Ext**	2.5	0.4	10.9	2.9	39.9	0.957	3.9	19.5	0.985
OH-Pel (coarse)	Cel-**Pel**	2.6	1.4	16.8	2.7	81.9	0.920	4.1	20.6	0.981
Cel-Pel (fine)	Cel-**Pel**	1.4	0.1	10.4	3.9	111.6	0.888	5.9	48.1	0.964
	√MSE [4]	2.09	0.59	2.89	1.06	26.14	0.036	1.31	16.02	0.013
Fibre										
Coarse		4.1 a	1.4 a	17.6 a	2.7	56.3	0.944	3.8	17.2 b	0.985
Fine		2.0 b	0.3 b	10.7 b	3.2	75.8	0.923	4.8	32.5 a	0.975
Processing										
Extrusion		4.1 a	0.9	14.6	2.7	34.9 b	0.963 a	3.8 b	16.6 b	0.987 a
Pelleting		2.0 b	0.8	13.9	3.2	95.4 a	0.904 b	5.0 a	33.1 a	0.973 b
P-value										
Fibre		0.0198	0.0002	<.0001	0.1405	0.0998	0.1402	0.0883	0.0223	0.0957
Processing		0.0268	0.5726	0.4043	0.2674	<.0001	0.0006	0.0438	0.0158	0.0218
Fibre x Processing		0.2953	0.5421	0.6731	0.3279	0.3702	0.4607	0.1566	0.1219	0.3103

[1] Values are means of 3 replicate-cages of 2 birds each.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls (OH-Pel) or fine cellulose (Cel-Pel); Challenge diet: WB diet with fine cellulose in extruded (Cel-Ext) or pelleted (Cel-Pel) form.

[3] The weight of digesta passing into the jejunum and ileum were estimated on a DM basis and calculated relative to the TiO_2 concentration in freeze-dried digesta.

[4] √MSE: square root of means square error in the analysis of variance.

[a, b, c] Means within column followed by different letters are significantly different ($P < 0.05$).

Table 4. *Amylase activity in the jejunum of 22-day old broilers as influenced by fibre structure and processing method*

From 7-21 days	At 22 days		Amylase activity [1]	
Gizzard manipulation diet	Challenge diet		Wet chyme	Dry chyme
Fibre structure	**Processing** method		Unit/g	Unit/g
OH-Pel (coarse)	Cel-**Ext**		118.6	563.0
Cel-Pel (fine)	Cel-**Ext**		90.6	415.8
OH-Pel (coarse)	Cel-**Pel**		99.3	447.8
Cel-Pel (fine)	Cel-**Pel**		73.4	337.6
		√MSE [3]	35.01	159.14
Fibre				
Coarse			108.9	505.4
Fine			82.8	380.0
Processing				
Extrusion			103.3	482.5
Pelleting			86.3	392.7
P-value				
Fibre			0.0963	0.0797
Processing			0.2501	0.1870
Fibre x Processing			0.9483	0.7914

[1] Values are means of 3 replicate-cages of 2 birds each and killed 2 h after feeding.

[2] Gizzard manipulation diet: WB pelleted diet with coarse oat hulls OH-Pel or fine cellulose Cel-Pel; Challenge diet: WB diet with fine cellulose in extruded Cel-Ext or pelleted Cel-Pel form.

[3] √MSE: square root of means square error in the analysis of variance.