Effect of intermittent feeding and oat hulls to improve phytase efficacy and digestive function in broiler chickens

Short title: Phytase and broiler digestive function

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Abstract 1. Two trials were carried out to investigate the impact of intermittent feeding and oat hulls as methods to stimulate the anterior digestive tract.

2. In Trial 1, 7 d old broiler chickens were placed 4 per cage in 48 cages, and were either fed *ad libitum* or intermittent of a diet with 50 g/kg of either oat hulls or cellulose and either with or without 1000 FTU phytase, in a 2 x 2 x 2 factorial arrangement. *Ad libitum* fed birds had two 4-h dark periods with 1 h light in-between, while intermittently fed birds in addition had restricted access to feed through 4 1-h feeding bouts.

3. In Trial 2, 144 broiler chickens were divided amongst 12 pens which were intermittently fed the same diets as in Trial 1 in a 2 x 2 factorial arrangement. At 34 d of age, after 16 h starvation, birds were fed for 1 h, followed by dissection of 4 birds per treatment every hour for 9 h. Contents from different segments of the digestive tract were collected quantitatively to assess DM and starch flow.

4. In Trial 1, Phytase improved performance and nutrient availability, while oat hulls and intermittent feeding improved some measures of nutrient availability. Intermittent feeding augmented the effect of phytase on myo-inositol hexakisphosphate (IP6) degradation in the anterior digestive tract.

5. In Trial 2, birds filled up their crop and slowly passed these contents on to lower segments of the digestive tract. Already 1 h after commencement of feeding, the entire length of the small intestine was filled with DM, and no significant changes in DM content was observed before 4 to 5 h later, when the small intestine gradually became emptied. The tendency for a higher initial load of DM and starch in birds without oat hulls seems to support a hypothesis that one important function of the gizzard is feed flow regulation. ~~The small intestine appeared to be full already 1 h after commencement of feeding, and the passage appeared to be particularly rapid for birds without oat hulls in the diet, indicating a regulative effect of oat hulls on feed flow through the gizzard.~~

6. In conclusion, the anterior digestive tract seems to have an important regulative function in broiler chickens when stimulated by intermittent feeding or structural components. More research is needed in order to elucidate the role of the crop and gizzard for phytase function.

Key words: crop, gizzard, feed passage, structural components, performance

Introduction

New innovative methods to improve nutrient availability without compromising the performance of the bird is constantly evolving in the broiler industry. The addition of structural material in broiler diets is one way in which nutrient utilisation may be improved (Hetland and Svihus, 2001; Hetland *et al.*, 2004; Amerah *et al.*, 2009). Structure may be provided in the diet by cereal hulls or wood shavings, but improvements in performance have been particularly consistent in studies involving cereal hulls (Hetland and Svihus, 2001; Hetland *et al.*, 2005). Numerous studies have clearly illustrated the broiler´s impressive capacity to thrive on diets containing high levels of cereal hulls without compromising growth and often displaying significant improvements in feed conversion (Hetland and Svihus, 2001; Sacranie *et al.*, 2012). Structural components exert their positive impact on the bird by enhancing gizzard function, as a result of increased grinding activity, leading to improved nutrient digestibility (Rogel *et al.*, 1987; Hetland and Svihus, 2001).

The retention of feed along the chicken´s gastrointestinal tract is highly influential in determining the feeding value of the diet (Sacranie *et al.*, 2012; Sacranie *et al.*, 2013). While total tract retention times are insightful, they are not wholly illustrative. Rather, retention in specific sections of the digestive tract may be of higher importance. Perhaps of particular importance is the relative retention of feed in the foregut, avoiding an over-flow of nutrients into the small intestine (Sacranie *et al.*, 2013). Svihus *et al.* (2010) suggested that the modern broiler has a strong tendency to over-consume feed leading to a high feed intake, but low

apparent metabolisable energy (AME) value. This can perhaps be mitigated by stimulation of the foregut. Intermittent or meal feeding encourages the bird to store feed in its crop where it will become moistened, and such feeding has been shown to improve feed efficiency (Svihus et al., 2010; 2013). Likewise, stimulation of the gizzard through the use of structural components in the diet may also contribute to a better feed flow regulation, avoiding overfilling of the small intestine leading to poor digestibility (Svihus, 2001; Svihus *et al.*, 2010).

Promoting retention of feed in both compartments of the foregut is of particular relevance to exogenous phytase, which has to hydrolyse its phytate substrate myo-inositol hexakisphosphate (IP6) to its lower esters, before entering the duodenum where the released P can be absorbed. Phytate is impervious to hydrolysis when in salt form and the solubility and stability of phytate-mineral complexes are dependent on pH conditions (Tamim *et al.*, 2004). The majority of chelates attain maximum solubility in acidic conditions lower than pH 3.5, a result of ionic dissociation (Selle *et al.*, 2000; Selle and Ravindran, 2007).

Therefore, it is logical to assume that structural components and intermittent feeding combined will lengthen the retention of feed in the foregut providing more time for phytase to act on phytate, and promote more acidic conditions suiting both the enzyme activity and phytate solubility. However, Svihus *et al.* (2013) was not able to demonstrate an effect of intermittent feeding or structure on phytase efficacy. In that experiment, intermittent feeding assured that apart from a 6 h darkness period, time without feed was maximum 3 h. Also, no detailed assessment of phytate degradation was carried out. The fact that enzymes had no overall effect on P retention in that experiment also warrants further studies. In addition, the same research group demonstrated a considerable IP6 degradation in the crop when retention was stimulated through intermittent feeding (Svihus *et al.*, 2010).

The current trial was therefore conducted with longer feed withdrawal periods and a higher phytase concentration to investigate the hypothesis that phytase activity can be influenced by retention time in the foregut and the presence of structural components in the diet. In addition, phytate degradation in the anterior digestive tract was assessed. A smaller trial was also carried out to accurately determine gastrointestinal feed and starch flow dynamics in birds exposed to feed with and without structural components.

Material and Methods

Birds, feed and experimental design in Trial 1

Female Ross 308 broiler chicks which had been reared in a brooding cage on a commercial diet, were randomly assigned 4 to each of 48 metabolism cages with mesh flooring (50 cm x 35 cm x 20 cm) at 7 d of age. Six cages were allocated to each treatment combination of feeding regime, structure and phytase addition in a 2 x 2 x 2 factorial design. The 48 cages were arranged in such a way to ensure that intermittently fed birds did not have visual contact with *ad libitum* fed birds, so not to influence behaviour. Light was off for all birds from 23:00 to 03:00 and from 04:00 to 08:00, but feed was not removed in this period, allowing birds to eat between 03:00 and 04:00~~with access to feed in between.~~. The intermittent feeding regime from 7 to 14 d of age consisted of limiting~~*ad libitum*~~ access to feed to between the hours of 08:00 to 09:00, 12:00 to 13:00, 16:30 to 17:30 and 21:00 to 23:00. From 14 d of age until termination of the experiment at 21 d of age, the intermittently fed birds had access to feed between the hours of 08:00 to 09:00, 13:00 to 14:00, 17:30 to 18:30 and 22:00 to 23:00. The temperature was reduced from 32-34 to 29ºC when the birds reached 7 d of age, and was further reduced to 26ºC at 14 d of age. The experiment was carried out at the experimental facilities of Norwegian University of Life Sciences.

Diets either with or without 1000 FTU/kg phytase (Quantum Blue, ABVista, Marlborough, UK) and with either 50 g/kg unground oat hulls or 50 g/kg cellulose powder, were produced at Center for Feed Technology, Ås, Norway. The diet was designed to be deficient in available phosphorus, had no inorganic phosphorus added, and was formulated to have a rather high phytate phosphorus content due to the use of wheat, wheat bran and rape seed meal (Table 1). Coarse oat hulls was used as a source of gizzard-stimulating structure. To isolate the effect of structure through coarse oat hulls without exerting other confounding effects, a purified cellulose in powder form was used in diets without oat hulls. Due to the small particle size of this component, it was assumed to have no gizzard-stimulating structural effect. To compensate for a small estimated starch content in the oat hulls, one part wheat flour was mixed with 9 parts of the cellulose powder, All diets were conditioned in a double conditioner at 75°C for 60 s followed by pelleting through a 3 mm pellet press die (Muench–Wuppertal, Germany, PMP 350.100, 700 kg/h capacity) and cooling from a post-pelleting temperature of 78.9–81°C to ambient temperature in a counter flow cooler. Due to a failure during freezer storage, feed stored in a refrigerator for more than a year was used to analyse for phytase activity. However, the analysis confirmed phytase activity in accordance with supplementation and estimated storage loss.

Data and sample collection in Trial 1

At 17 d of age, birds and feed were weighed and trays under the cages were removed and cleaned. From 17 to 20 d of age, excreta was quantitatively collected and frozen per cage. At the end of the excreta collection period, birds and feed were again weighed.

On d 21, lights were switched on at 04:00 to give *ad libitum* fed birds sufficient feeding time before killing. Feed was removed from intermittently fed birds before switching on light. The intermittently fed birds were given 40 min access to feed after which feed was

removed. Two randomly selected birds from each cage were then killed by stunning followed by cervical dislocation. For intermittently fed birds, killing took place exactly 3 h after commencement of feeding. A plastic zip tie was placed on the bird's neck immediately after killing to prevent loss of crop contents. Contents of the crop, proventriculus + gizzard, duodenum + jejunum and ileum were collected and pooled per cage. All samples were immediately frozen in liquid nitrogen. The gizzard pH and empty gizzard weight were taken for all birds, while the crop pH was measured only for intermittently fed birds due to insufficient material in enough animals from *ad libitum* fed birds. Crop and gizzard pH were measured by inserting the sensor of pH meter (Hamilton, Tiptrode electrode, Bonaduz, GR, Switzerland) vertically into the targeted organ or directly tested in the sampling container when there was insufficient contents.

Digesta samples were dried for 8 h at 105°C for DM with the exception of the duodenum and jejunum samples which were freeze dried. One thigh and one toe per bird were collected for tibia and toe ash analysis.

Trial 2

At 7 d of age, 144 birds were weighed and equally distributed amongst 12 pens, and presented with one of the 4 experimental diets in a 2 structures x 2 phytase factorial design. The pens had rubber mats to avoid interacting effects of wood shavings consumption. All birds were intermittently fed following the same procedures as in Trial 1. At 32 d of age all birds and feed were weighed for performance measurements. Birds were then given the same diets, except that only diets containing phytase were used. At 33 d of age, birds were given access to feed from 13:00 until commencement of starvation period at 16:00. The next day, birds were given access to their respective diets with or without oat hulls from 08:00 to 09:00.

Four birds were killed per treatment using the same procedure as in experiment 1 every 60 min from 08:00 to 18:00. Due to mortality ~~and/~~or signs of ascites~~abnormality~~, only three birds were ~~sometimes~~ killed per time and diet on eight occasions. Weight of empty gizzard was recorded, and contents from crop, gizzard, duodenum + jejunum and ileum (defined as beginning at Meckel's diverticulum) were quantitatively collected. All samples were frozen immediately in liquid nitrogen. The pH of crop content from 10:00 to 15:00 was measured following the same procedure as described for Trial 1.

Analyses

For DM determination, excreta collected from Trial 1 and digestive tract contents collected from both Trial 1 and 2 was homogenised and then dried at 105°C overnight. Experimental feeds, excreta and digesta samples were then ground in a IKA A11 (IKA Werke GmbH, Staufen, Germany) coffee--grinder type laboratory mill. Ground samples were analysed for titanium content following the procedure described by Short *et al.* (1996) and starch following the procedure describe by McCleary *et al.* (1997). Ground excreta samples from Trial 1 were analysed for Dumas-N following AOAC (2005) on a Leco FP 528, and gross energy was determined using a Parr 1281 isoperibol calorimeter (Parr Instrument Company, Moline, IL). AME was calculated by correcting to N balance based on N analyses of feed and excreta.

Feed samples and duodenum + jejunum contents from Trial 1 were freeze-dried, homogenized in a coffee grinder, and inositol phosphates were extracted using the method described by Carlsson *et al.* (2001). Briefly, the samples were extracted with 0.5 M HCl for 3 h under magnetic stirring. An aliquot (0.5 ml) of the extract was centrifuged at 12-000 x g for 10 min through a 30 kDa filter (Microcon YM–30, Millipore, Bedford, MA, USA). The inositol phosphates in the filtrate were separated on an Ultimate 3000 HPLC system (Dionex)

equipped with a Dionex CarboPac PA1 guard column and a CarboPac PA1 analytical column. A gradient with 1.5 M methanesulfonic acid and water was used as the mobile phase at 0.8 ml/min (Blaabjerg *et al.* 2010). The inositol phosphates were detected at 290 nm after a postcolumn reaction with 0.1% $Fe(NO_3)_3*9H_2O$ dissolved in 2% $HClO_4$, and quantified against an external IP6 standard curve using the Chromeleon software (Dionex).

Tibias were boiled and the soft tissue~~flesh~~ removed using a scalpel, and then both toes and tibias were dried for 24 h at 80°C and then weighed to calculate DM. Tibias and toes were then ashed for 24 h at 480~~°C~~°C, and the ash weighed.

Data from Trial 1 and 2 were subjected to 3-way and 2-way ANOVA, respectively, followed by pair-wise comparisons using the Duncan procedure, with $P<0.05$ as the significance level (SAS Institute, 2006).

Results

Trial 1

All main factors had an effect on feed intake (Table 2). A higher feed consumption was recorded in birds from *ad libitum* treatment, birds exposed to feed without structure, and with phytase ($P<0.001$). An interaction between feeding and structure was observed due to a further reduction in feed intake in birds exposed to oat hulls and intermittent feeding compared to intermittently fed birds consuming diets without structure ($P=0.038$). The reduced intake led to a similar trend in weight gain, with intermittent feeding generally giving lower gains ($P<0.001$). An interaction between feeding regime and structure was again observed for weight gain, with structure in the feed of intermittently fed birds further depressing growth ($P=0.014$). As opposed to when phytase was added to the diet without oat hulls, addition of phytase to the diet with oat hulls did not always result in weight gain, leading to a significant interaction between enzyme and structure ($P=0.031$). Feed/gain was

not affected by feeding regime for the period 7 to 21 d. Structure and phytase interacted in regards to effect on feed/gain (P<0.001). An improvement in feed/gain due to addition of oat hulls or phytase was only observed in the absence of the other factor.

Intermittent feeding tended (P=0.06) to improve AME, while birds exposed to structure and phytase yielded higher AME values (P=0.001 and 0.019 respectively) (Table 2). However, the magnitude of improvement in AME with structure was larger for *ad libitum* fed birds than for intermittently fed birds, leading to an interaction between feeding and structure (P=0.045). Addition of oat hulls improved digestibility of starch (P<0.001). Intermittent feeding (P=0.002) and addition of phytase (P=0.03) improved N retention. Mineral retention as measured by ash in excreta improved with intermittent feeding as well as phytase addition (P<0.001), but was impaired by adding oat hulls (P=0.003) (Table 2).

Jejunal P digestibility increased with intermittent feeding as well as with phytase addition (P=0.003), while oat hulls had a negative effect on this parameter (P=0.024). Likewise, IP6 degradation was increased with both intermittent feeding and phytase (P=0.001 and 0.003, respectively), but the magnitude of improvement in IP6 degradation was larger when phytase was used under intermittent feeding (P=0.03). Birds exposed to phytase in the diet revealed higher levels of toe and thigh ash as proportion of DM (P=0.001).

Intermittently fed birds killed after 140 min starvation and birds not given structural materials had a higher crop DM content (P<0.001 and P=0.047, respectively) (Table 3). For DM proportion in crop content, the picture was less clear due to a significant interaction effect between feeding regime and structure (P=0.016). Crop pH was 5.3 in intermittently fed birds, and was not affected by structure or phytase (data not shown). Oat hulls in the diet resulted in a large increase in gizzard weight and gizzard DM content (P<0.001), reduced pH of contents (P<0.001), and increased DM proportion of gizzard contents (P=0.002). Enzyme

addition also increased gizzard weight (P<0.001). Oat hulls in the diet resulted in a lower DM content in the anterior small intestine (P<0.001).

Trial 2

The number of birds that diedMortality per pen varied between 0 and 4, but was high in average withwas high at 25 percent mortality in diets without hulls and 13 percent in diets with hulls (data not shown). Mortality was spread throughout the last 14 days of the experiment, with no dominant causes for death recorded. However, 4 of the dead birds had fluids accumulating in the abdomen, indicating that ascites was a problem. Eight birds were significantly smaller than normal at the time of death. but growth rate and feed intake was normal for the remaining birds. Birds with phytase in the diet had a higher feed intake and weight gain, and a reduced feed/gain (P<=0.00109, P<0.00102 and, P=0.014, respectively) (Table 4). Phytase did not affect mortality. A tendency (P=0.057) was observed for a reduced feed intake in birds exposed to structure in the diet. Oat hulls almost doubled relative gizzard weight (P<0.001). Birds trained to intermittent feeding were able to store large quantities of feed in their crop during the hour when feed was available (Figure 1). One bird had only 17 g feed DM in the crop, while the rest of the birds killed at 09:00 had between 33 and 50 g feed DM in their crop (data not shown).

The crop was gradually emptied, and none or very little feed was found in the crop of birds killed 5 h after withdrawal of feed (6 h after commencement of feeding). However, birds killed 5 h after withdrawal of feed all had between 3 and 17 g feed DM in their crop (data not shown). The pH remained between 6.6 and 6.8 the first three hours after commencement of feeding, but then slowly diminished. Crop contents of birds killed 5 h after commencement of feeding had pH values varying from 4.5 to 5.6, while all the values measured later had values varying between 4.6 and 5.2 (data not shown).

The gizzard contained significantly more DM for the diet with oat hulls ($P<0.05$), and as opposed to when no oat hulls was included, was never fully emptied (Figure 2). Feed passed very rapidly into both the anterior (Figure 3) and the posterior (Figure 4) digestive tract independent of diet, with the whole digestive tract appearing to be full already 1 h after commencement of feeding. In fact, when the diet was devoid of structural components, the duodenum and jejunum even contained significantly more DM than at later times except at 13:00 ($P<0.05$). More feed DM was found in the duodenum and jejunum of birds with no structural components than for birds with structural components 1 and 5 h after commencement of feeding ($P<0.05$), and although the picture was less clear for the ileum, the same tendency during the first 5 h was observed there.

When starch content in the small intestine was quantified, an even more conspicuous intestinal overload was observed when diet contained no structural components (Figures 5 and 6). Throughout the length of the small intestine, the starch content was high 1 h after commencement of feeding, and were significantly different both from contents of birds given oat hulls in the diet and from later times in the day ($P<0.05$). Average starch concentration in both the duodenum + jejunum and ileum for diets with cellulose was 320 g/kg 1 h after commencement of feeding, while the values for diets with oat hulls was 26 and 20, respectively (data not shown).

Discussion

Despite the fact that feeding time was reduced from 16 to 5 h with a very short adaptation period, feed intake and weight gain was only modestly affected in intermittently fed birds in Trial 1. In Trial 2, where the birds had more time to adapt to this feeding regime, growth was similar to commercial standards for the breed despite the fact that birds were raised on rubber mats, which has been shown under similar conditions to result in poorer growth than when

297 wood shavings are used (Skånseng et al., 2013). The rubber mats and the resulting difficulty
298 in keeping the pen dry, could also have contributed to the high mortality, although abnormal
299 mortality levels had not been experienced previously when rubber mats were used. Neither
300 has similar intermittent feeding regimes resulted in abnormal mortality in previous
301 experiments, and this fact and the fact that mortality or low weight was not a problem in the
302 first part of Trial 2 also indicates that the mortality was not related to problems with adapting
303 to intermittent feeding. The high mortality calls for caution in interpretation of these data.
304 The fact that no single cause for mortality was identified and the fact that performance of the
305 remaining birds were normal, however, does not indicate a considerable health problem in the
306 flock as a whole. The normal performance results with intermittent feeding are in accordance
307 with previous findings (Svihus et al., 2010; Sacranie et al., 2012, Svihus et al., 2013), and
308 demonstrates the tremendous capacity of the broiler chicken to adapt to an intermittent
309 feeding regime. Large quantities of feed are rapidly consumed and stored in the crop, and
310 then slowly portioned out during the subsequent hours of feed restriction. Data from Trial 2
311 even indicate that the crop of 34 d old broiler chickens can hold close to 50 g feed; equivalent
312 to a quarter of daily feed intake. In previous studies it was shown that the majority of the feed
313 is consumed during the first 20 minutes of a one hour feeding bout, and only very little during
314 the last third of the hour (Svihus et al., 2010), indicating that feeding time could even have
315 been shortened further without affecting intake significantly.

316 Apart from an improvement in nitrogen retention and a tendency for improvement in
317 AME, intermittent feeding did not have a clear effect on macronutrient availability.
318 Conflicting results have also been obtained before, with improvements in feed efficiency
319 observed by Svihus et al., (2010), but not by Sacranie et al. (2012). Buyse et al. (1996),
320 reviewing the effect of lighting regime, concluded that improvements in feed efficiency was a

common feature, and attributed this to a reduced maintenance requirement over the whole production period as a result of a more concave-shaped pattern of growth.

An increased weight gain and feed efficiency with phytase is in accordance with results obtained before (Selle et al., 2009; Pirgozliev et al., 2010). As previously reported (Svihus et al., 2013), there was no interaction between phytase and feeding regime on most parameters. For P availability, however, the significantly higher IP6 digestibility for phytase with intermittent feeding compared to with *ad libitum* feeding indicates a beneficial effect of intermittent feeding on phytase efficiency. It has been demonstrated that IP6 is enzymatically degraded during retention in the crop (Svihus et al., 2010~~3~~; Zeller et al., 2016). Others have also concluded that the anterior digestive tract and the crop in particular, is the major site for phytase activity (Lan et al., 2010; Liebert et al., 1993). An elevated pH in the crop could be a limiting factor for phytase activity. Although pH is slowly reduced from around 6.5 in the feed to between 4 and 5, the current data shows that a retention time of more than 3 h is needed for pH to be reduced to such a level. Since most phytases, including those used in the current experiment, have a pH optimum of below 5.5 (Menesez-Blackburn *et al.*, 2015*)*, it is possible that pH is a major limitation for phytate breakdown in the crop. The tendency for a reduced gizzard pH with intermittent feeding, which is in accordance with results observed before (Sacranie et al., 2012), could also be a contributing factor, since the lower range pH optimum for the enzyme used is higher than the average gizzard values of 1.9 to 2.8 reported here.

Phytase also improved N and ash retention, P digestibility in the anterior digestive tract, toe ash and bone ash, but did not respond further under intermittent feeding. It must be kept in mind that the P and IP6 digestibility data were collected after 3 h retention in the crop for intermittently fed birds. Thus, these data could well overestimate the overall effect of intermittent feeding on phytase efficacy. Svihus et al. (2013) was not able to demonstrate any

346 interaction effects between intermittent feeding and phytase. Thus, more research is needed to
347 clarify the effect of intermittent feeding on phytase efficacy.

348 Coarse structure in the form of oat hulls increased size and holding capacity of the
349 gizzard concurrently with reducing gizzard pH, and this resulted in significant improvements
350 in feed efficiency and nutrient availability, as shown numerous times before. The fibre
351 content in the basal diet was rather high due to the soybean meal, rape seed meal and rice
352 bran used in the experiment. Thus, the beneficial effect of the unground oat hulls added was
353 probably mainly due to the large particle size, and not to fibre effects *per se*. The negative
354 effect of structure on P digestibility and ash retention, however, was surprising, and also
355 conflicted with IP6 digestion data. Jiménes-Moreno *et al.* (2013) neither observed such an
356 effect, and even found soluble ash digestibility to increase when oat hulls were added to the
357 diet.

358 No beneficial interactions between oat hulls and phytase were observed. In fact,
359 phytase only improved feed efficiency for diets without oat hulls, even indicating a negative
360 interaction. This is surprising considering the assumed prominent role of the gizzard for
361 phytase activity (Liebert et al., 1993), and the fact that gizzard volume and thus retention time
362 increases proportionately to an increase in empty gizzard weight (Svihus, 2011). Svihus et al.
363 (2013) neither found any interactions between structure and phytase activity. One possibility
364 is that oat hulls made the digestive tract less conducive to phytase activity, e.g. due to
365 excessively acidic conditions in the gizzard as discussed above. It is possible that the
366 beneficial effect of increased retention time is counterbalanced by a more unfavourable pH
367 for phytase activity and a larger extent of phytase degradation in the gizzard. After all,
368 activity level of the enzyme at pH 2 is only one-third of maximum activity for the enzyme
369 used in this experiment (Menesez-Blackburn *et al.*, 2015).

Measurement of feed flow in Trial 2 demonstrated significant differences between diets depending on level of structural components. As expected, the holding capacity of the gizzard was considerably increased for diets with oat hulls. Interestingly, the gizzard of birds receiving oat hulls was not emptied even after 16 hours of starvation. This fits with observations by Hetland et al. (2003), who demonstrated that coarse particles are retained in the gizzard until they are grinded down. The most striking observation, however, was the contrasting pattern of passage of feed material from the gizzard to the small intestine. Firstly, it was shown that material passes very rapidly into the small intestine of starved birds. This has been observed before (Svihus et al., 2002), but the magnitude of this passage was surprising. Already 1 h after commencement of feeding, the entire length of the small intestine was filled with DM, and no significant changes in DM content was observed before 4 to 5 h later, when the small intestine gradually became emptied. The tendency for a higher initial load of DM and starch in birds without oat hulls seems to support a hypothesis that one important function of the gizzard is feed flow regulation. Although total tract starch digestibility was not measured in this experiment, the high amount of starch in the ileum indicates that when birds are starved and then refed, feed passed too fast through the small intestine in birds that lacked structural components in their diet. Thus, this could explain the consistent improvement in starch digestion observed before in trials involving structural components (Svihus, 2011). However, the possibility cannot be excluded that reflux would allow for a complete digestion, and it must be noted that although structure significantly affected starch digestibility in Trial 1, even diets without structural components exhibited very high faecal starch digestibility. Basha and Duke (1999) have demonstrated that reflux is particularly prevalent in galliformes, and Sacranie *et al.* (2012) demonstrated that the extent of flow of indigestible markers from the small intestine to the foregut was increased when the

gizzard was stimulated by structural components in the diet. More data is clearly needed to understand intestinal function in broiler chickens.

In conclusion, broiler chickens adapt to intermittent feeding by temporary storing large quantities of feed in the crop, and then slowly releasing these contents during times without feed. This appears to have some beneficial effects on feed utilisation, although more data is needed in regards to interactions with efficacy of an added phytase. The gizzard also acts as a feed flow regulator, but seems to be dependent on proper development through structural components to act effectively as an impediment of a too rapid flow of feed into the small intestine after starvation.

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Table 1 Composition and nutrient content of the basal diet used in Trial 1 and 2 (g/kg unless otherwise stated)

Ingredient	
Wheat	52930.57
Soybean meal, 440 g of CP/kg	200.0
Rapeseed meal, 380 g of CP/kg	80.0
Rice bran	60.0
Oat hulls or cellulose powder[1]	50.0
Soy oil	40.0
Limestone	14.0
Salt	1.8
Sodium bicarbonate	2.6
Mineral premix[2]	1.3
Vitamin premix[2]	2.6
L-lysine, 780 g/kg	3.0
DL-methionine, 990 g/kg	2.0
L-threonine, 985 g/kg	2.0
Titanium dioxide	5.0
Enzyme premix [3]	5.0
Choline chloride	1.2
Nutrient content	
Calculated AMEn (MJ/kg)	11.8
Analysed crude protein	188
Analysed starch	345
Calculated lysine	12
Calculated methionine	9
Calculated threonine	6
Calculated calcium	7.1
Analysed P	4.4
Analysed IP6	12.5
Calculated non-phytate P	1.6

[1] The oat hulls had 850 g/kg particles larger than 2 mm and no particles smaller than 0.8 mm. The cellulose powder had no particles larger than 0.1 mm. Based on earlier analysis, oat hulls was estimated to contain 48 to 123 g/kg starch, 25 to 46 g/kg crude protein and 777 to 847 g/kg insoluble dietary fibre. The cellulose powder (Cellulose MN 100, Macherey-Nagel GmbH & Co, Düren, Germany) was a purified cellulose powder. The cellulose was mixed with 100 g/kg wheat flour before inclusion into the diet.

[2] Mineral premix provided the following per kg diet: Fe, 75 mg; Mn, 60 mg; Zn, 105 mg; Cu, 15 mg; I, 0.75 mg; Se, 0.3 mg. Vitamin premix provided the following per kg diet: vitamin A (retinyl acetate), 2.7 mg; vitamin D3 (cholecalciferol), 0.14 mg; vitamin E (DL-α tocopheryl acetate), 66 mg; menadione, 9.0 mg; pyridoxine, 6.0 mg; riboflavin, 24.0 mg; Ca-pantothenate, 26.3 mg; biotin, 0.39 mg; thiamine, 3.75 mg; niacin, 75 mg; cobalamin, 0.03 mg; folic acid, 3.75 mg.

[3]Xylanase (Econase XT25 (32 000 XBU/kg) was used in all diets, while Quantum Blue Phytase (1000 FTU/kg) was only added to half the diets, both provided by AB Vista, Marlborough, UK.

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517 Table 2. Performance and nutrient retention of 7 to 21 d old broiler chickens (Trial 1)

Feeding Regime[1]	Structure	Phytase	Feed intake, g	Weight gain, g	Feed/gain, g/g	AME, MJ/kg	Faecal starch dig.	N retention	Ash retention	P dig.[2]	IP6 dig.[2]	Toe ash, g/kg DM	Tibia ash, g/kg DM
Ad libitum	Oat hulls	No	1003^{bc}	713^{bc}	1.41^{c}	13.8^{abc}	0.99	0.65	0.35	0.05	0.36^{b}	103	319
Ad libitum	Cellulose~~No hulls~~	No	1021^{bc}	689^{c}	1.48^{a}	13.5^{d}	0.98	0.65	0.37	0.13	0.34^{b}	100	313
Ad libitum	Oat hulls	Yes	1094^{ab}	773^{ab}	1.42^{bc}	14.1^{a}	0.99	0.66	0.40	0.15	0.37^{b}	111	355
Ad libitum	Cellulose~~No hulls~~	Yes	1125^{a}	793^{a}	1.42^{bc}	13.7^{cd}	0.98	0.65	0.40	0.26	0.40^{b}	114	347
Intermittent	Oat hulls	No	906^{d}	645^{c}	1.40^{c}	13.8^{abc}	0.99	0.66	0.37	0.19	0.43^{b}	102	324
Intermittent	Cellulose~~No hulls~~	No	977^{cd}	672^{c}	1.45^{ab}	13.7^{bcd}	0.98	0.67	0.40	0.22	0.37^{b}	100	322
Intermittent	Oat hulls	Yes	982^{cd}	688^{c}	1.43^{bc}	14.0^{ab}	0.99	0.67	0.40	0.26	0.60^{a}	117	341
Intermittent	Cellulose~~No hulls~~	Yes	1094^{ab}	775^{ab}	1.41^{bc}	13.7^{abcd}	0.98	0.67	0.42	0.33	0.53^{ab}	112	357
√MSE			54.2	39.6	0.025	0.19	0.004	0.014	0.017	0.108	0.106	5.4	16.9
Feeding regime													
Ad libitum			1061	742	1.43	13.7	0.99	0.65	0.38	0.15	0.37	107	333
Intermittent			990	695	1.42	13.8	0.99	0.67	0.40	0.25	0.48	108	336
Structure													
Cellulose~~No hulls~~			1054	732	1.44	13.6	0.98	0.66	0.40	0.24	0.41	107	335
Oat hulls			996	705	1.41	13.9	0.99	0.66	0.38	0.16	0.44	108	335
Phytase													
No			977	680	1.44	13.7	0.99	0.66	0.37	0.15	0.38	101	319
Yes			1074	757	1.42	13.8	0.99	0.67	0.41	0.25	0.48	114	350
P-values													
Feeding regime			<0.001	<0.001	NS	0.063	NS	0.002	<0.001	0.003	0.001	NS	NS
Structure			<0.001	0.022	<0.001	<0.001	<0.001	NS	0.003	0.024	NS	NS	NS
Phytase			<0.001	<0.001	0.016	0.019	NS	0.033	<0.001	0.003	0.003	<0.001	<0.001
Feeding x Structure			0.038	0.014	NS	0.045	NS	NS	0.074	NS	NS	NS	NS
Feeding x Phytase			NS	NS	NS	NS	NS	NS	0.094	NS	0.030	NS	NS
Structure x Phytase			NS	0.031	<0.001	NS	NS	NS	NS	NS	NS	NS	NS
Feeding x Structure x Phytase			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]*Ad libitum* fed birds had two 4-h dark periods during a 9-h night, while intermittently fed birds in addition had access to feed only during four 1-h periods spread throughout the day.

[2]Measured on contents collected from the duodenum and jejunum. IP6=inositol hexaphosphate.

[a-d]Means within a column not sharing the same superscript are significantly different (P < 0.05).

Table 3. Digestive tract results for 21 d old broiler chickens (Trial 1)[1]

Feeding Regime[2]	Structure	Phytase	Crop content, g DM	DM, g/kg crop content	Gizzard weight[3]	Gizzard content, g DM	DM, g/kg gizzard content	Gizzard pH	Intestine content,g DM[4]
Ad libitum	Oat hulls	No	1.37	280[b]	21.9	2.07	290	1.9	1.79
Ad libitum	Cellulose~~No hulls~~	No	3.57	400[ab]	14.0	1.38	200	2.8	2.30
Ad libitum	Oat hulls	Yes	2.70	360[ab]	25.2	2.64	290	2.2	1.90
Ad libitum	Cellulose~~No hulls~~	Yes	2.67	480[a]	14.6	1.34	270	3.3	2.59
Intermittent	Oat hulls	No	3.83	310[b]	24.8	2.43	280	2.1	1.75
Intermittent	Cellulose~~No hulls~~	No	4.78	310[b]	14.0	0.96	230	2.5	2.04
Intermittent	Oat hulls	Yes	3.83	310[b]	25.6	2.56	280	1.9	1.64
Intermittent	Cellulose~~No hulls~~	Yes	4.84	300[b]	13.6	0.74	210	2.7	2.55
√MSE			1.707	92	2.64	0.839	59	0.69	0.493
Feeding regime									
Ad libitum			2.58	380	18.9	1.86	270	2.5	2.15
Intermittent			4.34	310	19.5	1.67	250	2.3	2.00
Structure									
Cellulose~~No hulls~~			3.97	370	14.1	1.10	230	2.8	2.37
Oat hulls			2.96	320	24.3	2.42	290	2.0	1.77
Phytase									
No			3.41	330	18.7	1.71	250	2.3	1.97
Yes			3.51	360	19.7	1.82	260	2.5	2.17
P-values									
Feeding regime			<0.001	0.008	NS	NS	NS	0.086	NS
Structure			0.047	0.037	<0.001	<0.001	0.002	<0.001	<0.001

Phytase	NS	NS	0.049	NS	NS	NS	NS
Feeding x Structure	NS	0.016	NS	NS	NS	NS	NS
Feeding x Phytase	NS	NS	0.052	NS	NS	NS	NS
Structure x Phytase	NS	NS	0.075	NS	NS	NS	NS
Feeding x Structure x Phytase	NS	NS	NS	NS	NS	NS	NS

[1] Intermittently fed birds were killed 3 h after commencement of a 40 min feeding bout. [2]*Ad libitum* fed birds had two 4-h dark periods during a 9-h night, while intermittently fed birds in addition had access to feed only during four 1-h periods spread throughout the day. [3]Weight in g per kg bird weight. [4]Contents from duodenum and jejunum.
[a-d]Means within a column not sharing the same superscript are significantly different (P < 0.05).

Table 4. Performance of 7 to 32 d old intermittently fed broiler chickens (Trial 2)[1]

Structure	Phytase	Feed intake, g	Weight gain, g	Feed/gain, g/g	Gizzard weight[2]
Oat hulls	No	2417	1475	1.64	19.3
Cellulose~~No hulls~~	No	2512	1525	1.65	9.20
Oat hulls	Yes	2661	1712	1.55	18.6
Cellulose~~No hulls~~	Yes	2801	1762	1.59	9.90
√MSE		90.7	65.0	0.04	0.106
Structure					
Cellulose~~No hulls~~		2656	1643	1.62	9.60
Oat hulls		2539	1594	1.60	19.0
Phytase					
No		2464	1500	1.64	14.3
Yes		2731	1737	1.57	14.2
P-values					
Structure		0.057	NS	NS	<0.001
Phytase		<.001	<0.001	0.014	NS
Feeding*Structure		NS	NS	NS	NS

[1]Birds had two 4-hour dark periods during a 9-h night, and in addition had access to feed only during four 1-h periods spread throughout the day.
[2]Empty w~~W~~eight in g per kg bird weight.

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