The Effect of Intermittent Feeding on Performance of Broiler Chicken and Influence of Oat Hulls Structure on Diet Flow

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Master Thesis 30 credits 2013

Abstract

Two trials were carried out to study the effect of intermittent feeding on performance of broiler chicken and passage rate of diet. In addition, interaction effects among feeding regime, oat hulls (OH) structure and phytase supplementation were studied.

In trial one, broilers were fed, either ad libitum or intermittently, a phosphorus deficient pelleted diet with OH or without and either contained phytase or not. Ad libitum feeding consisted of continuous access to feed with 2x4h hours of complete dark periods, which separated by one hour lightening between 03.00 and 04.00. Intermittent feeding from 7d to 14d consisted of four 1h and one 2h feeding, where one feeding was when the light turned on and off between 03.00 and 04.00. From 14d to 21d, feeding consisted of five 1h feedings and with the same dark period as before.

Performance, features of the anterior digestive tract was evaluated in trial 1. In trial 2, passage rate of diet and starch content in the digestive tract at different times were assessed. One hypothesis was that combination of intermittent feeding, OH structure and phytase supplementation can improve the performance of broiler chickens. Another hypothesis was that birds can over-consume feed, and that this happens mainly when the gizzard is nonfunctioning due to lack of stimulation.

In trial 1, intermittent feeding did not improve the efficacy of the enzyme added. The crop dry matter content of the intermittent feeding birds, which had been slaughtered 3 hours after commencement of feeding, was higher than the crop dry matter content from ad libitum birds as expected. Intermittent feeding didn’t show a negative effect on feed/gain in spite of the lower weight gain. In trial 2, birds where the gizzard has not been stimulated by structural components seems to over consume after long time starvation, which can be regulated with inclusion of structure components.

In conclusion, broiler chickens quickly adapt to intermittent feeding with improvements in feed efficiency, but without improving the efficacy of the phytase used. Overload of feed can happen to long time starved birds when the diet is lack of structural components.

Key words: intermittent feeding, oat hulls, passage rate, starch digestibility

Acknowledgements

The cost of diet processing and experiment were funded by the company of Nutreco.

The thesis is devoted to my family who have been supporting me all the way in my life both spiritually and financially and my fiancee Alima Tshenghel and her family, who have been supporting my career planning since the first date of our relationship.

It is my honour to choose Prof. Dr. Birger Svihus as my supervisor, who was always ready to devote his valuable time to help me with problems not only relating to the thesis but also from other major related area. He provided wise guidance, appropriate encouragement combined with practical advice, which are all essential for completion of the thesis. I am more than grateful for his supervision.

I would like to express my immense gratitude to Dr. Adam Sacranie of his practical suggestions and guidance on description of results and scientific writing. I appreciate to Dejan Miladinovic and Ismet Nikqi for assisting me with feed processing. Frank Sundby and Nina Pedersen Asper guaranteed my laboratory work by sharing their excellent technical skills and meticulous attitude, which are respectful. I am also grateful to my fellow student partners Qi Ji, Sigrun Schumpa, Zhamuer Borjigen that worked together with me during the whole experiment and provided me with essential helps. I would like to sincerely thank Prof. Dr. Mingan Choct and teacher Li Ming, models of my ethnicity, who have been encouraging me to study overseas, thus enrich the knowledge of developed feed industry and contribute to my hometown in the future. I am deeply thankful to Dr. Tsechoe Dorji, who had been encouraging me since my study in Norway, and I will never forget the days we drank milk tea together.

Ås, August 2013,

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1. Introduction

The utilization of feed in broilers is influenced by different factors. As well as the age, sex and genetic differences, feeding regime and feed structure are considered to influence the performance of broiler chickens.

The growth rate of broilers was considered as one of the important factors of broiler performance, which increased dramatically these years. While the rapid growth of birds can induce some health problems, for example, leg weakness (Su et al., 1999).

Introduction of the intermittent feeding regime, however, was found to decrease this problem. . Restricted access to feed has also been shown to improve feed utilization through reducing maintenance requirements and spillage of feed (Buyse et al., 1996). In addition, it has been suggested that broilers reduce fat deposition through intermittent feeding (Jones and Farrell, 1992), which is an important aspect of carcass quality. Although the intermittent feeding was found to reduce the weight gain at the beginning because birds are adapting to the feeding regime, it normally followed by a high compensatory growth (Svihus et al., 2013).

The crop is a ventral diverticulum of the oesophagus, and contains longitudinal folds on the inner surface making it distensible. Although during ad libitum feeding, the crop is not used to its maximal capacity, it serves as the main food storage organ when intermittent feeding is applied (Svihus et al., 2010). The crop wall has no mucus-secreting glands. While when the feed stored in the crop during the intermittent feeding, may obtain sufficient time for fermentation by lactobacilli, the dominating microflora in the chicken crop, producing lactic acid, thereby reduce the crop pH (Guan et al., 2003, Hilmi et al., 2007). The reduced pH may benefit phytase activity, because many microbial phytases reach their optimal activity at between pH 4.0 and 6.0 (Simon and Igbasan, 2002). Exogenous enzyme supplements, such as phytase, are quite commonly used method to deal with the problems exist in monogastric animals (Bedford, 2000). Phytase is used to release phosphorous from phytic acid in raw materials hence increasing their feeding value. In addition, bacteria fermentation of non-starch carbohydrate in the crop also results in some short chain fatty acids, which may provide, though not accepted as common, the chicken with extra energy (Adil and Magray, 2012). The

oesophagus ends at the proventriculus, where the glands secrete pepsinogen and hydrochloric acid (HCl). The proventriculus has limited mobility and feed pass through it quickly to enter the gizzard. The gizzard is a muscular organ with inner ridges behind the proventriculus, which contracts rhythmically and grinds the wet feed into a smooth paste (McDonald, 2002).

Inclusion of structural components in the diet is found to increase gizzard size and increase the starch digestibility of the diet (Hetland et al., 2003). In addition, the retention time of the diet in the anterior digestive tract may also increase with exposure to structural components (Hetland et al., 2003). All feed particles will be grounded to a particular critical size and then leave the gizzard through pylorus activity (Moore, 1999, Ferrando et al., 1987), which may because well-functioned gizzard will lead to an improvement on grinding ability (Svihus, 2011). The diet retention time in the anterior digestive tract is vital for controlling the ratio at which these get in touch with digestive enzymes and absorptive surfaces (Vergara et al., 1989).

Consequently, utilization of nutrients may increase through getting larger surface area. Hetland et al. (2003) found that amylase activity and bile salt concentration increase in chyme following the intake of OH in broilers, which implies a mechanism for the improvement in starch digestibility due to OH addition including stimulation of secretion of pancreatic enzymes and bile. Consequently, protein degradation and emulsification of lipids may be facilitated (Hetland et al., 2003). Furthermore, intestinal villi height of broilers on day 21 and later was found (Sarikhan et al., 2010) to be increased as inclusion of insoluble fibre, which may increase nutrient absorption because of the increased surface area.

While the birds' ability to deal with intermittent feeding is not affected by adding structure components (Sacranie et al., 2012, Svihus et al., 2013), so there is a possibility that a combination of intermittent feeding and OH can increase enzyme efficacy.

Two separate trials were run in this study. Trial one was performed to study the influences of intermittent feeding on the performance of broilers and if there is an interaction effect between intermittent feeding, and structure/phytase activity. The following hypothesis was tested: Trial two was carried out to study the differences of diet flow through the anterior

digestive tract and small intestines in two groups of chickens fed diets with phytase either with or without OH and intermittent feeding. The following hypothesis was tested: does an inclusion of OH help to regulate the digesta flow through the anterior digestive tract by stimulating the gizzard, without decreasing feed intake and starch digestibility?

Effects of phytase in itself were investigated in another master thesis.

2. Materials and methods

2.1. Diet composition and processing

The diets were produced in the Centre of Feed Technology (FôrTek), at the University of Life Science in Ås, Norway. The diets were based on wheat with high protein and high fall number, grown and harvested in the Drammen area in Norway, in 2012.

Four wheat-based diets were processed. Table 1 shows the different diets, diet 1 and 3 were feed contained OH that were with or without phytase, and diet 2 and 4 were feeds without OH that were with or without phytase. Titanium dioxide was the marker.

The diets were made to meet nutritional requirements of experimental birds according to Ross 308 Broiler Management Manuel (2007), except that phosphorous was provided to a large extent in the form of phytic acid, and with a somewhat lower total provision.

Table 1: Feed composition, Diet 1 – 4:

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
	g/kg	g/kg	g/kg	g/kg
Wheat	529.5	529.5	529.5	529.5
Soybean meal	200	200	200	200
Rapeseed meal	80	80	80	80

Rice bran	60	60	60	60
Oat hulls	50		50	
90 cellulose + 10 wheat flour*		50		50
Soya oil	40	40	40	40
Limestone	14	14	14	14
Salt	1.8	1.8	1.8	1.8
Sodium bicarbonate	2.6	2.6	2.6	2.6
Mineral premix	1.3	1.3	1.3	1.3
Vitamin A	0.7	0.7	0.7	0.7
Vitamin D3	0.7	0.7	0.7	0.7
Vitamin E	0.4	0.4	0.4	0.4
Vitamin ADKB	0.8	0.8	0.8	0.8
DL-methionine	2	2	2	2
L-lysine	3	3	3	3
Titanium	5	5	5	5
L-threonine	2	2	2	2
Ground wheat (4.9) +				
Xylanase, Econase® XT 25	5	5	5	5
Choline chloride	1.2	1.2	1.2	1.2
Phytase, Quantum Blue®			0.028	0.028

*90 cellulose + 10 wheat flour because the starch in OH was estimated as 10%

Table 2: Calculated/analysed----diet composition

Metabolizable energy (MJ/kg)	11.8
Crude Protein (g/kg)	197.3
Calcium (g/kg)	7.1
Non-phytate Phosphorous (g/kg)	1.6

Wheat, soybean and rape seed were ground separately by a hammer mill (E-22115 TF, Muench-Wuppertal, Germany, under Bliss-USA, 18.5 kW and 2870 rpm) on a 3 mm sieve. The OH were sieved with a 1.4 mm sieve to filter out fine particles.

Four batches were produced continuously, each weighing 230 kg mixed in a 400 l mixer conditioner (Twin shaft paddle, Tatham of England, Forberg, Norway, 7.5 kW). The 2 diets without phytase were processed first to avoid contamination. Duration time of mixing for each batch was 2 minutes when micro ingredients together with OH or with cellulose were added. Then soy oil was sprayed on the mash with a pressure of 4 bar for 4 minutes and 45 seconds. The spray nozzle had capacity size of 6505 (angle 65, size 05, Unijet, spraying systems Co, Wheaton, Illinois, USA) and spraying capacity of 2.3 l/min (based on water viscosity). The mixing time after oil addition was 2 minutes.

Three samples from each diet were taken after mixing process from the “waiting hopper” (after mixing, before conditioning). The samples were taken directly from different places – representative samples – and then mixed together in a bucket and distributed into plastic bags. The feed mash was sent through the twin pass/double conditioner (Twin Pass, Muench, Germany, 1.2 t/h, 2 x 1.8m x 30cm). There was 4% steam added at 75℃ in 20-30 seconds (retention time) before it was processed in a pellet mill (Muench, Germany, 1.2t/h max. Capacity, 2 x 18.5 kW). Processing parameters of pelleting were recorded, shown in table 3. Temperatures of feed were measured manually immediately after the pelleting process, with a thermometer in an isolated box.

The pellets were cooled in a counter-flow cooling system for 30 minutes, which used ambient air to reduce the temperature of the products (Miltenz, New Zealand, capacity 1.2 t/h). Then each cooled pellet diet was packed in 1000 l bags containing the final product 200 $\pm$ 6 kg. Then 3 representative pellet samples from each diet were taken directly from the filled bags with grain sampler. Before each new diet was processed, the system was cleaned by 30kg of ground wheat to avoid contamination.

Table 3. Processing parameters:

Die Specification		
Conditioner temperature	ºC	74.8
Production capacity	kg/h	700.0
Die diameter	mm	3.0
Die length	mm	36/42
Knife distance	mm	6.6
Motor load	%	22.8
Amperes Motor 1	amp	13.6
Amperes Motor 2	amp	12.9
Average amperes motor	amp	13.3
Energy Consumption	kW	8.1
Specific Energy Cons.	kWh/kg	0.0116
Steam	kg / h	51.0
ISO - Box	ºC	79.2

2.2. Experimental animals and feeding

The experiment was performed between the 12th of October and 14th of November 2012 at the Animal Production Experimental Centre (Senter for Husdyrforsøket), UMB. There were 380 day-old female Ross 308 broiler chickens placed in brooder cages in a room with 23 h of light and a temperature of 32℃, and were fed on a commercial starter diet and water ad libitum till 7 days of age. Feed consumption and weight gain were recorded weekly as groups for all birds used in the experimental trials.

Trial 1 – Excreta collection and dissection

At 7 days of age, 4 randomly selected birds were weighed and placed in each of 48 mesh floor cages (50cm x 35cm x 20 cm). Two racks of cage (24 cages per rack) were placed such that

the intermittently fed birds (cage 13-36) did not have visual contact with ad libitum fed birds (cage 1-12 and 37-48) by placing the ad libitum birds facing the wall and intermittent facing each other. The four diets were given in rows of four and sequentially. A bucket was assigned to each cage and contained 5kg of feed. The gross weight of the buckets was recorded.

The ad libitum chickens had a 2x4h hours dark period (23.00 – 03.00 and 04.00 – 08.00). The intermittent feeding regime lasted from 7 to 14 days of age, while birds had access to feed consumption (ad libitum) from 08.00 – 09.00, 12.00 – 13.00, 16.30 – 17.30 and 21.00 until the light went off at 23.00 (adaptation period). From 14 days of age until termination of the experiment at 21 days of age, feed for the intermittently fed group were available for ad libitum consumption from 08.00 – 09.00, 13.00 – 14.00, 17.30 – 18.30 and 22.00 – 23.00. The temperature was reduced to 29 ℃ when chickens reached 7 days of age, and further reduced to 26 ℃ at 14 days of age.

At 17d of age, in preparation of excreta collection the birds and feed were weighed at 08.00 and the trays under cages were removed and cleaned. After 6 hours at 14.00, the clean trays were placed back under the cages. Cages were not cleaned because of human error, which may have influenced the final results.

At 17d of age, birds and feeds were weighted at 08.00. Excreta was collected quantitatively on d18 and 19. At 20d of age, the feed and birds and were not weighed at 08.00 but 12.00 due to human error and so the excreta were collected at 18.00 instead of 14.00.

At 21d of age, the lights were switched on at 04.00 and feed was removed from intermittently fed birds at 04.00. From 06.30 to 07.40 all birds and feed, intermittent fed birds then ad libitum fed birds, were weighed.

The intermittently fed birds in cage 13-15, 16-18, 19-21, 22-24 were given access to feed at 07:40h, 08:00h, 08:20h, 08:40h respectively. After 40 min of feeding, the feed were removed. The intermittently fed birds in cage 25-27, 28-30, 31-33, 34-36 were all given access to feed

at 07:00h-08:00 as before. At 11:40h, 12:00h, 12:20h, and 12:40 h the birds were given access to feed respectively. Birds were fed for 40 minutes and all the intermittently fed birds were killed exactly 3 hours after commencement of the feeding. According to the previous experiment, intermittent fed birds consume 88% of the feed during the first 40 min in 1-h feeding bout, assumed to be enough to meet the requirements of this trial (Svihus et al., 2010).

The ad libitum fed birds received access to feed when the light was switched on at 04:00 until dissection. Birds in cage 1-12 and 37-48 were killed 08.20h and 12.40h respectively. Two birds from each cage were killed in the afore mentioned order and a plastic strip wrapped around the bird's neck immediately to hinder crop content regurgitation, birds were then weighed. For the sampling, contents of the crop, proventriculus + gizzard, duodenum + jejunum, ileum were collected. All samples were frozen in liquid nitrogen. The gizzard pH and empty gizzard weight were taken for all the birds, while the crop pH was measured only for intermittently fed birds. Crop and gizzard pH were taken immediately 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.

Trial 2 – Performance data

At 7 days of age, 12 randomly selected birds were weighed and placed per pen in 12 group pens with rubber mats. All birds were fed intermittently, with three replicates for each diet, with and without OH and with and without phytase. The four diets were given in rows from pen 1-12 and successively.

The intermittent feeding regime lasted from 7 to 14 days of age (adaptation period), while birds received access to feed from 08.00 – 09.00, 12.00 – 13.00, 16.30 – 17.30 and 21.00 until the light went off at 23.00. From 14 days of age until termination of the experiment at 32 days of age, feed for the intermittently fed group were available for ad libitum consumption from 08.00 – 09.00, 13.00 – 14.00, 17.30 – 18.30 and 22.00 – 23.00. The temperature was

gradually reduced to 29 ℃ when chickens reached 7 days of age, and further reduced to 26 ℃ at 21 days of age.

At 33 days of age, in preparation of dissection, the birds were given access to feed from 13.00 to 16.00, and started starving for 16 hours. The lights were switched off from 22.00h to 07.00h on d34. At 34 days of age, the birds were given access to phytase feed with and without OH with marker (titanium) for 60 min at 08.00. Thereafter, 7-8 birds were killed every 60 minutes from 08.00 to 18.00h, 3-4 birds per structure diet.

The birds were killed by cervical dislocation and a plastic strip was wrapped around the neck immediately to hinder crop content regurgitation, before the birds were weighed. The whole weight of gizzard, small intestine with pancreas was taken. The contents of crop, gizzard, duodenum + jejunum and ileum were collected. All samples were frozen in liquid nitrogen. The crop content from 10.00 to 15.00 were tested for pH value, which followed the same pH meter and method as in experiment one.

The birds were cared for according to the laws and regulations ruling experiments with live animals in Norway (the Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning experiments with animals of January 15, 1996). Content from proventriculus + gizzard is simplified as the gizzard. Dry matter, duodenum + jejunum are represented below as DM and duo+jej.

2.3. Sample analysis

2.3.1 Digestive tract and excreta

Samples from both d 21 and d 34 were dried for DM. The contents of the four sections of the anterior digestive tract (crop, gizzard, duo+jej, ileum) at different hours were lyophilized in a freeze dryer (Beta 1-6, LMC-2, Christ, Osterode, Germany) at -56˚C and 25 mbars for 92 hours to obtain data in regards to a dry matter content without encountering any possible biochemical changes in the samples.

2.3.2 Chemical analysis:

Starch percentage and titanium dioxide (marker) from duo+jej and ileum based on freeze-dried matter were measured in samples from 09:00 to 16:00 for d 34. Samples at 08:00, 17:00 and 18:00 were not included because too small amount existed. Some other figures were rejected because of low weight of the birds and too little content of samples combined together, which was an indication of unhealthy situation. Starch and titanium analysis were executed at the Animal and aquaculture sciences department at the Norwegian university of life sciences, via AACCI Method 76-13.01.Total Starch Assay Procedure (Megazyme Amyloglucosidase/alpha-Amylase Method) and Short et al. (1996) method, respectively. Titanium dioxide equivalent to approximately 5 g for per kg feed.

2.3.3 Statistical analysis:

Data from experiment 1 were subjected to a three-way ANOVA (feeding regime ×diet structure × enzyme addition) and data in experiment 2 were subjected to a two-way ANOVA (feeding regime × time), followed by pair-wise comparisons using the Ryan-Einot-Gabriel-Welsh procedure when relevant, with $P < 0.05$ as the significance level (SAS Institute, 2006). The square root of mean square error in the analysis of variance (residual standard deviation, RSD) was used as a measure of random variation.

2.3.4 Definition

The data called g feed tract are based on titanium analyses of small intestinal content, which is an estimation of the amount of feed represented in the digestive tract. The "g feed tract" represents the sum of these estimates for the small intestine, plus DM data from crop and gizzard (figure 7).

3. Results

Trial 1

For the broilers of 21 days of age, no interaction effect was found between feeding regime and phytase for all performance data. As shown in table 4, feed intake and weight gain were lower

for both intermittent feeding (P<0.001) than ad libitum birds, and lower with OH (P<0.001 and P=0. 022, respectively) than without. Due to the fact that reduced feed intake (P=0. 038) and weight gain (P=0. 014) was observed only in intermittently fed birds, a significant interaction between feeding regime and structure was recorded for this parameter. The feed-/ gain was not influenced by intermittent feeding.

As shown in table 5, crop DM content at 21 days of age was lower (P＜0. 001) for ad libitum fed than intermittent fed birds. The crop DM percentage was lower for intermittent fed birds (P=0. 008) than ad libitum fed ones and birds exposed to OH (P=0. 037). The significant interaction effect (P=0. 016) between feeding regime and OH structure was based on the fact that the lower crop DM percentage of the birds given a diet with OH was only seen for ad libitum fed birds. Higher ileum DM percentage (P=0. 030) was seen for the birds given feed without OH than with. The significant interaction effect (P=0. 026) between feeding regime and structure was because the higher ileum DM percentage of the birds given feed without OH was only seen for ad libitum fed birds. The tendency (P= 0. 051) for an interaction effect between feeding regime and enzyme for empty gizzard weights was due to the trend for increased empty gizzard weights in only ad libitum fed birds exposed to phytase. Gizzard pH tended to decrease (P=0. 086) by intermittent feeding compared to ad libitum.

Toe ash, thigh ash and their percentages in the DM were also analysed, which, however, are not the main focus of this thesis and will not be commented here.

Table 4. Results from birds in 4-bird cages from 7 to 21 days of age[1]

Feeding Regime	Oat hulls Structure	Enzyme addition	Feed intake, g	Weight gain, g	Feed/gain	Toe ash, % of DM	Toe ash, g	Thigh ash, % of DM	Thigh ash, g
Ad libitum	Coarse	No	1003^{bc}	713^{bc}	1.41^{c}	10.3	0.026	31.9	0.75
Ad libitum	Fine	No	1021^{bc}	689^{c}	1.48^{a}	10.0	0.025	31.3	0.75
Ad libitum	Coarse	Yes	1094^{ab}	773^{ab}	1.42^{bc}	11.1	0.030	35.5	0.96
Ad libitum	Fine	Yes	1125^{a}	793^{a}	1.42^{bc}	11.4	0.029	34.7	0.95
Intermittent	Coarse	No	906^{d}	645^{c}	1.40^{c}	10.2	0.022	32.4	0.78
Intermittent	Fine	No	977^{cd}	672^{c}	1.45^{ab}	10.0	0.023	32.2	0.75
Intermittent	Coarse	Yes	982^{cd}	688^{c}	1.43^{bc}	11.7	0.025	34.1	0.88
Intermittent	Fine	Yes	1094^{ab}	775^{ab}	1.41^{bc}	11.2	0.027	35.7	0.98
√MSE			54.2	39.6	0.025	0.54	0.0037	1.69	0.097
Feeding regime									
Ad libitum			1061	742	1.43	10.7	0.027	33.3	0.85
Intermittent			990	695	1.42	10.8	0.024	33.6	0.85
Structure									
Fine			1054	732	1.44	10.7	0.026	33.5	0.86
Coarse			996	705	1.41	10.8	0.026	33.5	0.84
Enzyme									
No			977	680	1.44	10.1	0.024	31.9	0.76
Yes			1074	757	1.42	11.4	0.028	35.0	0.94
Main effects									
Feeding regime		NS	<0.001	<0.001	NS	NS	0.0095	NS	NS
Structure		NS	<0.001	0.022	<0.001	NS	NS	NS	NS
Enzyme		NS	<0.001	<0.001	0.0162	<0.001	0.0012	<0.001	<0.001
Feeding*Structure		NS	0.038	0.014	NS	NS	NS	NS	NS
Feeding*Enzyme		NS	NS	NS	NS	NS	NS	NS	NS
Structure*Enzyme		NS	NS	0.031	<0.001	NS	NS	NS	NS
Feed*Structure*Enzyme		NS	NS	NS	NS	NS	NS	NS	NS

[ab]Means within a column not sharing a common superscript differ at P<0.05. [1]Each treatment combination had either 6 or 12 replicate

Table 5. Results from birds in 4-bird cages from 7 to 21 days of age[1]

Feeding Regime	Oat hulls Structure	Enzyme addition	Crop DM, g	Crop DM %	Gizzard DM, g	Gizzard DM %	Duo+jej DM, g	Duo+jej DM %	Ileum DM, g	Ileum DM %	Empty gizzard weight, g	Crop pH	Gizzard pH
Ad libitum	Coarse	No	1.37^{b}	28^{b}	2.07^{abc}	29	1.79^{ab}	19	1.50^{b}	20^{ab}	20.0^{b}	-	1.9
Ad libitum	Fine	No	3.57^{ab}	40^{ab}	1.38^{abc}	20	2.30^{ab}	19	2.27^{ab}	20^{a}	12.6^{c}	-	2.8
Ad libitum	Coarse	Yes	2.70^{ab}	36^{ab}	2.64^{a}	29	1.90^{ab}	17	1.66^{ab}	18^{b}	24.6^{a}	-	2.2
Ad libitum	Fine	Yes	2.67^{ab}	48^{a}	1.34^{abc}	27	2.59^{a}	19	2.30^{ab}	19^{ab}	14.5^{c}	-	3.3
Intermittent	Coarse	No	3.83^{ab}	31^{b}	2.43^{ab}	28	1.75^{ab}	18	1.77^{ab}	19^{ab}	22.2^{ab}	5.2	2.1
Intermittent	Fine	No	4.78^{a}	31^{b}	0.96^{bc}	23	2.04^{ab}	18	2.09^{ab}	19^{ab}	12.5^{c}	5.3	2.5
Intermittent	Coarse	Yes	3.83^{ab}	31^{b}	2.56^{a}	28	1.64^{b}	18	1.64^{ab}	19^{ab}	23.2^{a}	5.6	1.9
Intermittent	Fine	Yes	4.84^{a}	30^{b}	0.74^{c}	21	2.55^{a}	19	2.44^{a}	20^{ab}	13.5^{c}	5.3	2.7
√MSE			1.707	9.2	0.839	5.9	0.493	1.1	0.454	0.9	2.72	0.48	0.69
Feeding regime													
Ad libitum			2.58	38	1.86	27	2.15	18	1.93	19	17.9	-	2.5
Intermittent			4.34	31	1.67	25	2.00	18	1.99	19	17.8	-	2.3
Structure													
Fine			3.97	37	1.10	23	2.37	19	2.27	20	13.3	5.4	2.8
Coarse			2.96	32	2.42	29	1.77	18	1.64	19	22.5	5.3	2.0
Enzyme													
No			3.41	33	1.71	25	1.97	18	1.91	20	16.8	5.2	2.3
Yes			3.51	36	1.82	26	2.17	18	2.01	19	18.9	5.4	2.5
Main effects													
Feeding regime		NS	<0.001	0.008	NS	NS	NS	NS	NS	NS	NS	-	0.086
Structure		NS	0.047	0.037	<0.001	0.002	<0.001	0.007	<0.001	0.030	<0.001	NS	<0.001
Enzyme		NS	NS	NS	NS	NS	NS	NS	NS	0.037	<0.001	NS	NS
Feeding*Structure		NS	NS	0.016	NS	NS	NS	NS	NS	0.026	NS	-	NS
Feeding*Enzyme		NS	NS	NS	NS	NS	NS	NS	NS	NS	0.051	-	NS
Structure*Enzyme		NS	NS	NS	NS	NS	NS	0.028	NS	NS	NS	NS	NS
Feed*Structure*Enzyme		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	NS

[ab]Means within a column not sharing a common superscript differ at P<0.05.[1]Each treatment combination had either 6 or 12 replicates.

Trial 2

For the broilers of 34 days of age, from the passage data table, except a significant amount of content in the gizzard of birds that had been given diets with OH since d 7, little content was found after 16 hours starvation both in the anterior digestive tract and small intestine.

The birds were in group in pens, so no individual feed intake was recorded and feed consumption of each bird was assumed to be the same within groups. Average feed consumption for the two groups was similar, which were as high as up to around 55 g. The birds fed diet with OH showed a higher weight of gizzard but a lower weight of the small intestine than the birds fed diet without oat hulls.

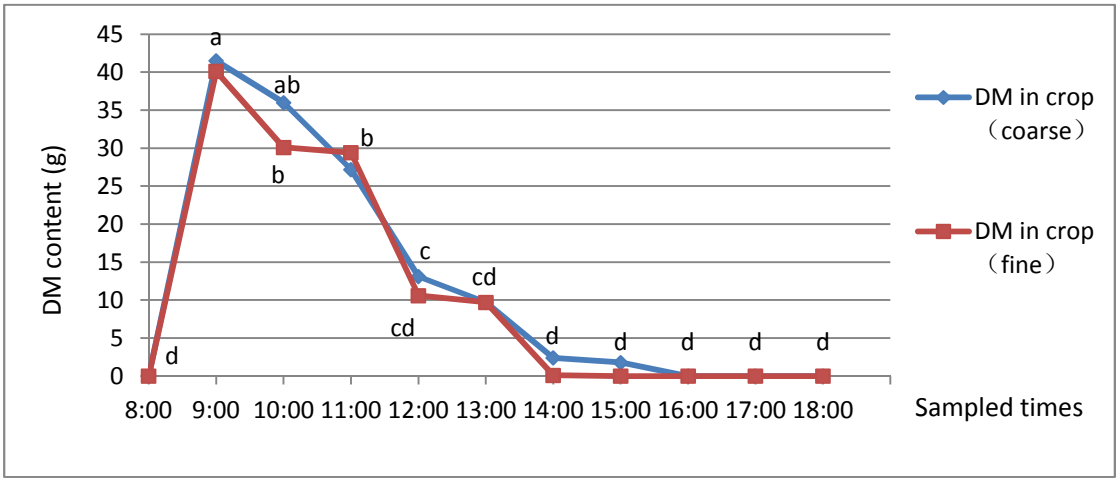

Figure 1. Crop DM content from different sampled times.
Points with different letters are significantly different (p<0.05).

From figure 1, no significant difference was found between crop DM content from birds fed diet without OH and with oat hulls. After one hour's feeding, high amounts of DM (up to 40 g) were found in the crop from both groups of birds. Then the content gradually decreased until 14:00, when almost no feed was found.

Furthermore, as shown in figure 2, dry matter content in the gizzard of the birds fed diet with OH remained relatively constant during the 10 hours of observations, while less amount was found in birds given a diet without OH, and with the gizzard being

empty at periods.

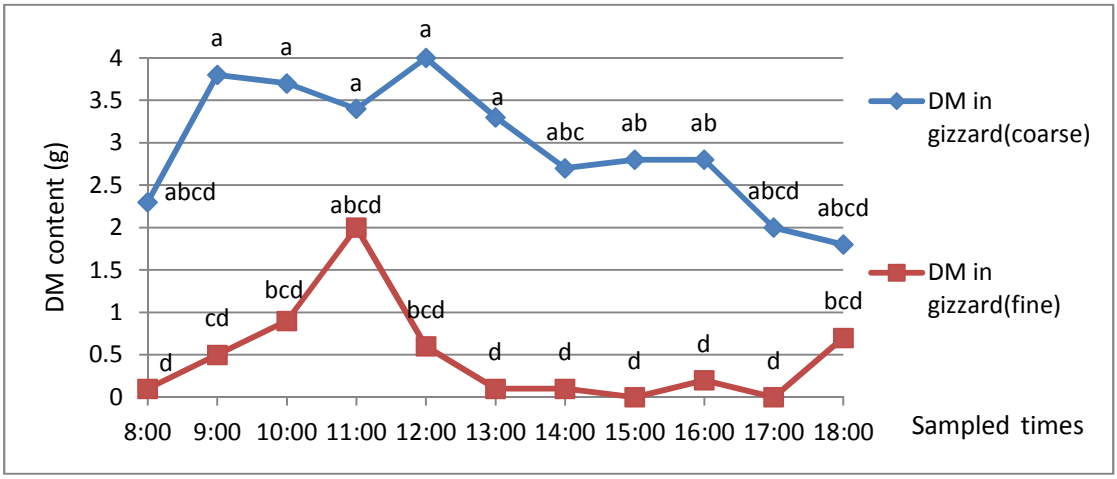

Figure 2. Gizzard DM content from different sampled times.
Points with different letters are significantly different (p<0.05).

For the birds given a diet with OH (figure 3), the DM content in the duo+jej was rather stable after feeding until 13:00, and then gradually decreased. In contrast, for the birds fed diet without oat hulls, the content varied a lot. Significantly higher amount of DM was found in duo+jej for the birds fed diet without OH at 09:00 and 13:00 compared with the data from birds given diet with oat hulls.

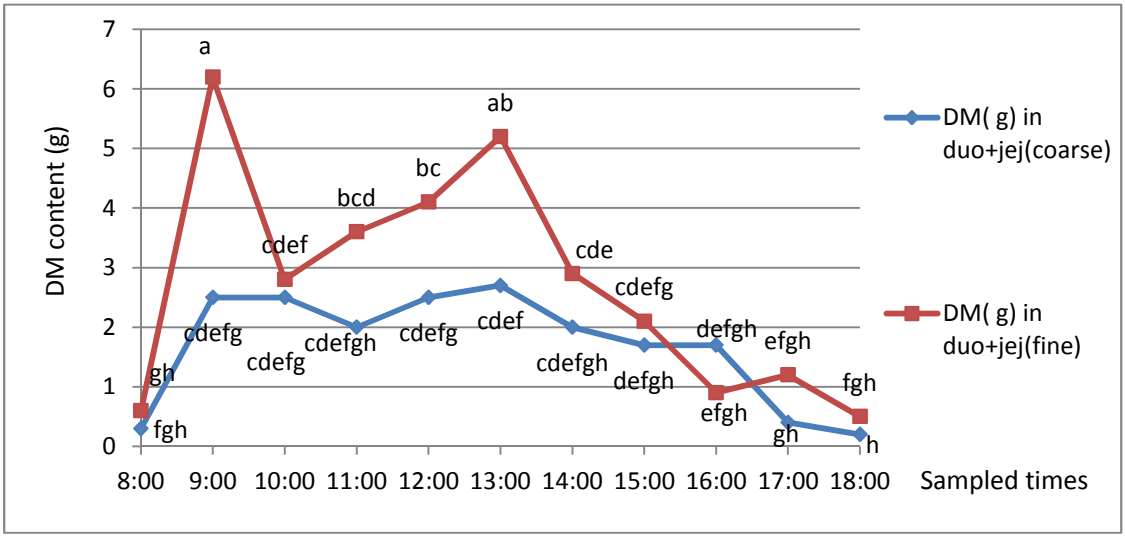

Figure 3. Duo+jej DM content at different times.
Points with different letters are significantly different (p<0.05).

DM content in the ileum (Fig 4) at different times were similar for two groups of birds, but the diet flow seems more even for the birds fed OH containing feed. The content

increased quickly between 08:00 and 09:00, and then gradually decreased from around 14:00 for both groups of birds.

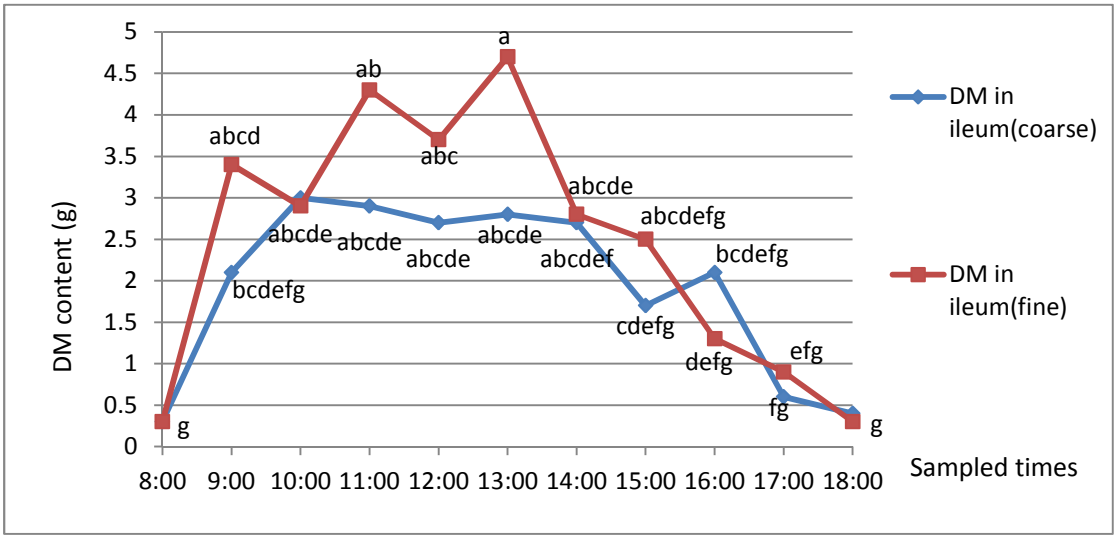

Figure 4. Ileum DM content at different times.
Points with different letters are significantly different (p<0.05).

Starch content from duo+jej (Fig 5) and ileum (Fig 6) were significantly higher for birds fed diet with OH than without at 9:00, and then stayed similar for two groups of birds. Because there was almost no DM content in the small intestine at 08:00 and after 16:00, starch content was not tested for these times.

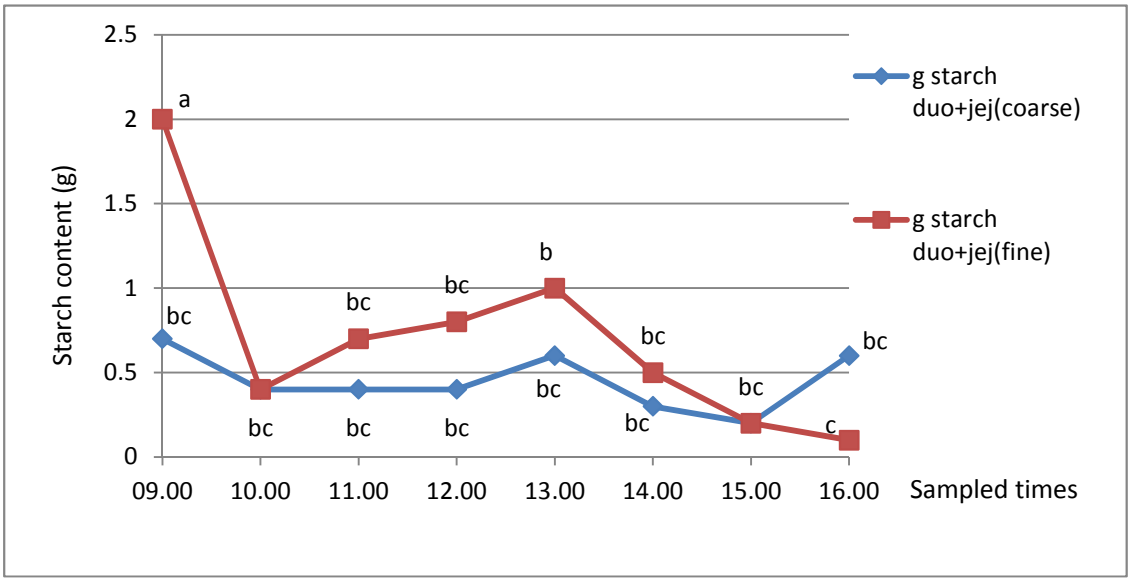

Figure 5. Amount of starch in duo+jej at different times.
Points with different letters are significantly different (p<0.05).

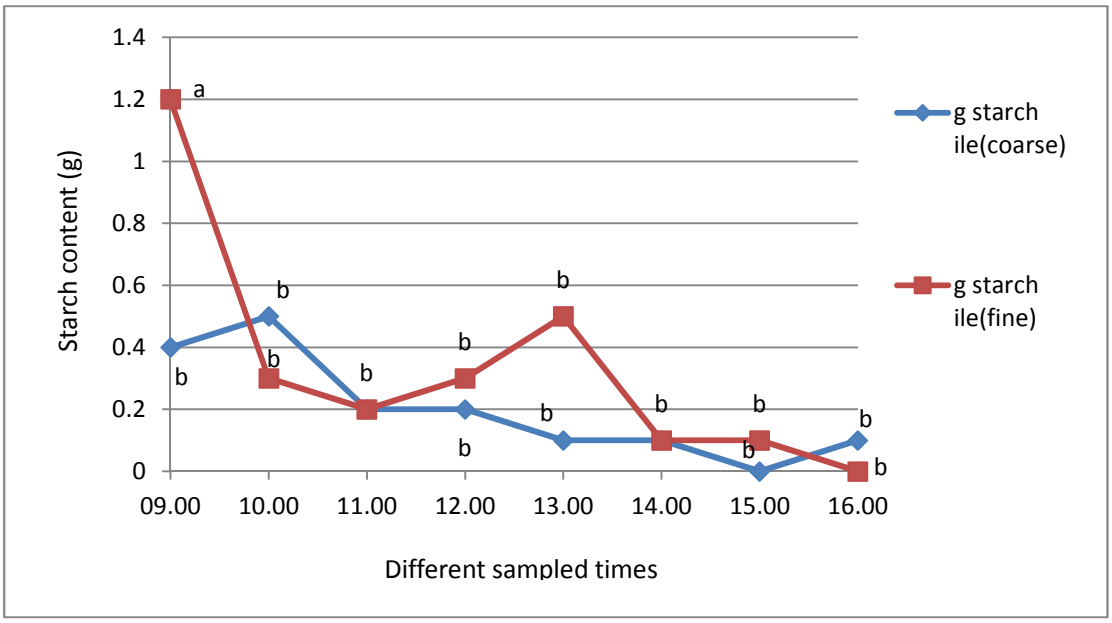

Figure 6. Amount of starch in ileum at different times.
Points with different letters are significantly different (p<0.05).

As shown in Fig 7, estimated DM content data on whole digestive tract indicates that significant amount of feed had not passed the small intestine before at 12.00.

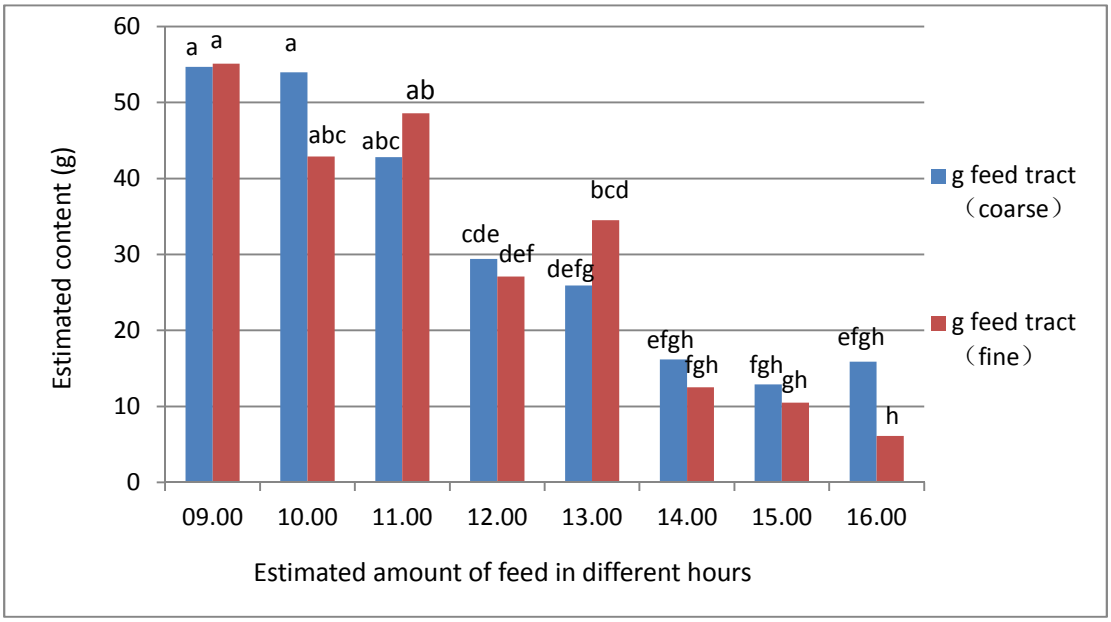

Figure 7. Estimated amount of feed from whole digestive tract.
Points with different letters are significantly different (p<0.05).

4. Discussion

Trial 1

There was no interaction between phytase and feeding regime, which denotes that retention time in the crop is not a limiting factor for phytase efficacy. The results are in accordance with a recent study (Svihus et al., 2013).

Significantly higher crop DM content in intermittent feeding birds even after over two hour starvation is in accordance with previous finding (Barash et al., 1993, Svihus et al., 2013), which indicating that birds are easily adapted to intermittent feeding through store the food temporarily in the crop. In the well functioning gizzard, the gizzard will squeeze the grounded chyme into duodenum through regular muscle contract (Svihus, 2011).

Intermittent feeding reduced the weight gain during this period, which has shown the same before (Svihus et al., 2010), but did not improve the feed/gain results as seen before. The possible reason is due to less feed intake and short adaptation period. The fact that intermittent feeding did not show a negative effect on feed/gain in spite of lower weight gain may indicate a better utilization, because the ratio of the maintenance requirement is relatively increased with reduced weight gain. The intermittent feeding tented to decrease gizzard pH, from 2.5 to 2.3, which is not as strong as previous findings (Svihus et al., 2013, Sacranie et al., 2012), where gizzard pH is reduced by intermittent feeding. This may be because of the rise of DM content in anterior digestive tract induced by intermittent feeding could increase the fermentation of diet, thus lead to more acidic digesta pouring into the proventriculus and gizzard (Sacranie et al., 2012). This pH reduction may be unfavourable for phytase activity, as many phytases show optimal activity at pH between 4 and 6 (Simon and Igbasan, 2002).

Structural components such as OH generally improved feed/gain as shown previously

(Hetland et al., 2003), but reduced weight gain when combined with intermittent feeding during this period has been observed (Svihus et al., 2013). This is likely due to the short time of adaptation, and the feed intake-limiting effect of the structural components as indicated by the significant interaction effect.

Trial 2

The anterior digestive tract and small intestine were successfully emptied after 16 hours starvation. Structural components results in a significant increase of gizzard size, which is in accordance with previous findings (Hetland and Svihus, 2001, Sacranie et al., 2012).

Significantly higher amount of DM in the duo+jej at the first hour after commencement of feeding and the significantly higher amount of starch in both the duo+jej and the ileum indicate that the birds where the gizzard has not been stimulated by structural components seems to overload their small intestine immediately after re-feeding.

Dietary starch is the main energy source for broiler chickens, and digestibility of starch in the anterior digestive tract and small intestine is important for feed utilization. Svihus (2001) found that the increase in gizzard size with whole wheat indicates that gizzard may be the key site for prevention of starch overload in the small intestine. From the previous study of Svihus and Hetland (2001), poor starch digestibility for some broilers can happen when cold pelleted Norwegian wheat based diet are used, which can either relate to starch load in the digestive tract and/or gizzard function. Significant increase in starch digestibility was found (Svihus and Hetland, 2001) when the diet was crushed and fed in mash form, which, however, resulted in reduction of feed intake. Starch digestibility was also increased significantly by adding OH in the cold pelleted diet for broilers (Hetland et al., 2003), which did not affect weight gain and increased feed conversion efficiency when corrected for insoluble fibre contents. As mentioned in the beginning, all feed

particles will be grounded to a particular critical size and then leave the gizzard (Hetland et al., 2003, Moore, 1999), so pelleted feed with structure component can be a favourable choice to increase feed utilization without affecting the final performances of birds.

Non-starch components, such as fat and protein, in the starch granule may hinder digestion both directly by reducing contact of digestive enzymes and starch, and indirectly through a reduced swelling of the starch granule (Svihus et al., 2005). Therefore, breaking down the protein part can also facilitate starch digestibility. Pepsin is generally accepted as the most important enzyme for decomposing protein. Péron et al. (2007) found proventricular pepsin has an optimal activity at pH between 2 and 3, which is accordance with gizzard pH results from experiment one, when the same diet but younger birds were tested. Furthermore, adding OH in the feed stimulate the gizzard function, which make pepsin work more efficiently than the birds with poor-functioning gizzard, because of the increased retention time (Sacranie et al., 2012).

As digestibility increased enough, there is a limited amount of starch and nitrogen resident in the terminal small intestine, which limits the available undigested nutrients to the bacteria habited in the large intestine (Bedford and Cowieson, 2012), thus benefiting the host animal.

Thus present study suggests that the feed over-loading problem of the birds may be reduced through increasing the retention time of feed in the gizzard by adding structure components in the diet, which can stimulate the gizzard to provide more even flow of chyme into the small intestine.

Contrarily, estimated g feed in small intestine (fig 7) indicates that significant amount of feed had not passed the small intestine before at 12.00, which tends to play down the significance of DM data (fig 3).

Besides, anti- peristaltic wave of the digestive tract can be an important factor that influences diet digestibility, as it can prolong the digestion of nutrients through increasing retention time. From a previous research (Sacranie et al., 2012), a sufficient magnitude of reverse peristaltic contractions exhibited in broilers to propel the marker from the cloaca to the gizzard, which alludes that the diet flow in the digestive tract of broilers is not flowing evenly in one way direction. Therefore, more details about the movement of diet in the digestive tract should be provided in future.

Generally, the DM content of the anterior digestive and the small intestine were more stable with OH containing feed. Except the first hour after commencement of feeding, similar starch content was found from the small intestine at different times between the birds given a diet with OH and without, which may indicate that when the content from anterior digestive tract decrease as time passes, the utilization of the diet without OH is increased. This shows the advantage of adding structural component in the diet during intermittent feeding is obvious when the birds show their maximum feed intake capacity.

DM content in the crop of the birds fed diet with OH indicating that birds will need about 4 to 5 hours to empty the crop. Consequently, duo+jej and ileum will be emptied around 6 hours after commencement of feeding. Therefore, it may reasonable to arrange the interval between each feeding for broilers under a combination of intermittent feeding and OH structure to 6 hours in order to provide broilers with continuous nutrient supply.

5. Conclusion

5.1 Broiler chickens adapt to intermittent feeding rapidly, but no interaction between feeding regime and phytase efficacy was found.

5.2 Feed over-loading problem can happen to the long time starved broilers, which may be decreased through increasing the retention time of feed in the gizzard by

adding structure components in the diet during intermittent feeding. Hourly feed consumption and quantitative excreta collection are needed in later research to get a more detailed understanding of the diet flow rate.

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Appendix

Hourly sample data of the trial 2

Diet	Time	Live weight	Gizzard weight	Intestine weight	DM in crop	DM in gizzard	DM in duo+jej	DM in ileum	Tot DM in tract	DM % in crop	DM % in gizzard	DM % in duo+jej	DM % in ileum	Starch % duo	Starch % ileum	g starch duo+jej	g starch ileum	g starch int	g feed duo+jej	g feed ileum	g feed tract
Coarse	08.00				0^{d}	2.3^{abcd}	0.3^{gh}	0.3^{g}	2.9^{f}	0^{h}	30^{a}	12^{bc}	10^{c}								
Coarse	09.00				41.5^{a}	3.8^{a}	2.5^{cdefg}	2.1^{bcdefg}	50.0^{a}	51^{a}	31^{a}	18^{abc}	24^{a}	26^{ab}	20^{b}	0.7^{bc}	0.4^{b}	1.1^{bc}	4.3^{abc}	5.1^{bc}	54.7^{a}
Coarse	10.00				36.0^{ab}	3.7^{a}	2.5^{cdefg}	3.0^{abcde}	45.2^{ab}	42^{abcd}	28^{ab}	16^{abc}	20^{ab}	15^{bc}	16^{bc}	0.4^{bc}	0.5^{b}	0.9^{bc}	4.6^{abc}	9.7^{bc}	54.0^{a}
Coarse	11.00				27.2^{b}	3.4^{a}	2.0^{cdefgh}	2.9^{abcde}	35.5^{b}	37^{abcde}	30^{a}	15^{bc}	18^{abc}	19^{abc}	6^{d}	0.4^{bc}	0.2^{b}	0.5^{bc}	3.5^{bc}	8.7^{bc}	42.8^{abc}
Coarse	12.00				13.1^{c}	4.0^{a}	2.5^{cdefg}	2.7^{abcde}	22.3^{c}	31^{cde}	29^{ab}	17^{abc}	19^{abc}	18^{abc}	7^{d}	0.4^{bc}	0.2^{b}	0.6^{bc}	4.6^{abc}	7.7^{bc}	29.4^{cde}
Coarse	13.00				9.7^{cd}	3.3^{a}	2.7^{cdef}	2.8^{abcde}	18.6^{cde}	30^{cde}	30^{a}	16^{abc}	18^{abc}	20^{abc}	3^{d}	0.6^{bc}	0.1^{b}	0.6^{bc}	4.0^{abc}	8.8^{bc}	25.9^{defg}
Coarse	14.00				2.4^{d}	2.7^{abc}	2.0^{cdefgh}	2.7^{abcdef}	9.7^{def}	22^{efg}	27^{ab}	14^{bc}	16abc	14^{bc}	2^{d}	0.3^{bc}	0.1^{b}	0.3^{bc}	3.4^{c}	7.8^{bc}	16.2^{efgh}
Coarse	15.00				1.8^{d}	2.8^{ab}	1.7^{defgh}	1.7^{cdefg}	8.0^{ef}	13^{fgh}	27^{ab}	14^{bc}	15^{abc}	12^{bc}	2^{d}	0.2^{bc}	0.0^{b}	0.3^{c}	3.0^{c}	5.3^{bc}	12.9^{fgh}
Coarse	16.00				0^{d}	2.8^{ab}	1.7^{defgh}	$2.1b^{cdefg}$	6.6^{f}	0^{h}	29^{ab}	15^{bc}	17^{abc}	23^{ab}	3^{d}	0.6^{bc}	0.1^{b}	0.7^{bc}	4.1^{abc}	8.8^{bc}	15.9^{efgh}
Coarse	17.00				0^{d}	2.0^{abcd}	0.4^{gh}	0.6^{fg}	2.9^{f}	0^{h}	27^{ab}	11^{c}	18^{abc}								
Coarse	18.00				0^{d}	1.8^{abcd}	0.2^{h}	0.4^{g}	2.4^{f}	0^{h}	26^{ab}	12^{bc}	12^{bc}								
Fine	08.00				0^{d}	0.1^{d}	0.6^{fgh}	0.3^{g}	1.1^{f}	0^{h}	3^{d}	17^{abc}	13^{bc}								
Fine	09.00				40.1^{a}	0.5^{cd}	6.2^{a}	3.4^{abcd}	50.2^{a}	47^{ab}	23^{abc}	22^{a}	25^{a}	32^{a}	32^{a}	2.0^{a}	1.2^{a}	3.1^{a}	8.0^{a}	6.6^{bc}	55.1^{a}
Fine	10.00				30.1^{b}	0.9^{bcd}	2.8^{cdef}	2.9^{abcde}	36.6^{b}	46^{ab}	22^{abc}	16^{abc}	19^{abc}	14^{bc}	10^{cd}	0.4^{bc}	0.3^{b}	0.7^{bc}	4.2^{abc}	7.6^{bc}	42.9^{abc}
Fine	11.00				29.4^{b}	2.0^{abcd}	3.6^{bcd}	4.3^{ab}	39.3^{ab}	46^{abc}	30^{a}	16^{abc}	17^{abc}	19^{abc}	4^{d}	0.7^{bc}	0.2^{b}	0.9^{bc}	5.3^{abc}	11.9^{ab}	48.6^{ab}
Fine	12.00				10.6^{cd}	0.6^{bcd}	4.1^{bc}	3.7^{abc}	19.0^{cde}	33^{bcde}	24^{abc}	18^{ab}	19^{abc}	18^{abc}	7^{d}	0.8^{bc}	0.3^{b}	1.0^{bc}	6.0^{abc}	9.9^{bc}	27.1^{def}
Fine	13.00				9.7^{cd}	0.1^{d}	5.2^{ab}	4.7^{a}	19.7^{cd}	26^{def}	11^{cd}	17^{abc}	18^{abc}	19^{abc}	9^{cd}	1.0^{b}	0.5^{b}	1.5^{b}	7.7^{ab}	17.0^{a}	34.5^{bcd}
Fine	14.00				0.1^{d}	0.1^{d}	2.9^{cde}	2.8^{abcde}	6.0^{f}	23^{efg}	11^{cd}	15^{bc}	16^{abc}	14^{bc}	4^{d}	0.5^{bc}	0.1^{b}	0.6^{bc}	4.2^{abc}	8.0^{bc}	12.5^{fgh}
Fine	15.00				0^{d}	0^{d}	2.1^{cdefg}	$2.5^{abcdefg}$	4.6^{f}	13^{fgh}	3^{d}	14^{bc}	17^{abc}	11^{bc}	3^{d}	0.2^{bc}	0.1^{b}	0.3^{bc}	3.7^{bc}	6.8^{bc}	10.5^{gh}
Fine	16.00				0^{d}	0.2^{d}	0.9^{efgh}	1.3^{defg}	2.4^{f}	8^{gh}	11^{cd}	13^{bc}	13^{bc}	5c	3^{d}	0.1^{c}	0.0^{b}	0.1^{c}	2.0^{c}	3.8^{c}	6.1^{h}
Fine	17.00				0^{d}	0^{d}	1.2^{efgh}	0.9^{efg}	2.1^{f}	0^{h}	7^{d}	12^{bc}	12^{bc}								
Fine	18.00				0^{d}	0.7^{bcd}	0.5^{fgh}	0.3^{g}	1.6^{f}	0^{h}	14^{abc}	12^{bc}	17^{abc}								
Diet																					
Coarse		1877	45.1^{a}	75.6^{b}	10.7	2.92^{a}	1.65	1.9^{b}	17.2	19.4	28.5^{a}	14.5^{b}	16.5	18	7	0.4^{b}	0.2^{b}	0.6^{b}	3.9^{b}	7.7^{b}	30.4
Fine		1929	19.5^{b}	84.2^{a}	11.1	0.52^{b}	2.81	2.55^{a}	16.9	18.2	14.7^{b}	16.1^{a}	16.9	16	9	0.7^{a}	0.3^{a}	1.0^{a}	5.2^{a}	9.2^{a}	29.6
Time																					
08.00					0^{d}	1.2	0.5	0.3^{e}	2.0^{d}	-	17	15^{bcd}	11^{c}								
09.00					40.8^{a}	2.2	4.4	2.8^{abc}	50.1^{a}	49^{a}	27	20^{a}	24^{a}	29^{a}	26^{a}	1.3^{a}	0.8^{a}	2.1^{a}	6.1^{a}	5.8^{c}	54.9^{a}
10.00					33^{b}	2.3	2.7	2.9^{abc}	40.9^{b}	44^{ab}	25	16^{bc}	20^{ab}	14^{b}	13^{b}	0.4^{bc}	0.4^{b}	0.8^{bc}	4.4^{abc}	8.7^{bc}	48.4^{a}
11.00					28.3^{b}	2.7	2.8	3.6^{a}	37.4^{b}	35^{bc}	30	15^{bcd}	18^{b}	19^{b}	5^{c}	0.5^{bc}	0.2^{bc}	0.7^{bc}	4.4^{abc}	10.3^{ab}	45.7^{a}
12.00					11.8^{c}	2.3	3.3	3.2^{ab}	20.7^{c}	29^{c}	26	18^{ab}	18^{b}	18^{b}	7^{c}	0.6^{bc}	0.2^{bc}	0.8^{bc}	5.3^{abc}	8.8^{bc}	28.2^{b}
13.00					9.7^{c}	1.7	4	3.8^{a}	19.2^{c}	27^{cd}	20	17^{abc}	18^{b}	20^{b}	6^{c}	0.8^{b}	0.3^{bc}	1.1^{b}	5.9^{ab}	12.9^{a}	30.2^{b}
14.00					1.4^{d}	1.6	2.4	2.7^{abc}	8.1^{d}	18^{de}	20	14^{bcd}	16^{bc}	14^{b}	3^{c}	0.4^{bc}	0.1^{bc}	0.4^{bc}	3.7^{abc}	7.9^{bc}	14.6^{c}
15.00					1.1^{d}	1.6	1.8	2.0^{bc}	6.6^{d}	11^{e}	17	14^{bcd}	16^{bc}	12^{b}	3^{c}	0.2^{c}	0.1^{bc}	0.3^{c}	3.3^{bc}	6.0^{c}	11.9^{c}
16.00					0^{d}	1.5	1.3	1.7^{cd}	4.5^{d}	-	20	14^{bcd}	15^{bc}	13^{b}	3^{c}	0.3^{bc}	0.1^{c}	0.4^{bc}	2.9^{c}	6.0^{c}	10.3^{c}
17.00					0^{d}	1.3	0.6	0.7^{de}	2.7^{d}	-	20	12^{d}	16^{bc}								
18.00					0^{d}	1.4	0.3	0.4^{e}	2.0^{d}	-	21	12^{cd}	14^{bc}								
Main effects																					
Diet					NS	<0.001	<0.001	0.009	0.06	0.065	<0.001	0.023	NS	NS	0.082	0.003	0.018	0.002	0.007	0.074	NS
Time					<0.001	0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001
Diet*Time					NS	NS	<0.001	NS	NS	NS	<0.001	NS	NS	0.036	0.002	<0.001	0.009	<0.001	0.026	0.001	0.056