Comparison between the intermittent and ad libitum feeding with and without exogenous enzyme (xylanase and β- glucanase) supplementation on the digestive tract of broiler chickens fed barley based diet.

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Abstract

An experiment was conducted to study the comparison between the intermittent and ad libitum feeding supplemented with and without exogenous (xylanase and β-glucanase) enzyme on the anterior digestive tract of broiler chickens with barley based diet. In an experiment 144 male broiler chickens (Rose 308) were fed barley based diet supplemented with or without exogenous enzyme under intermittent and ad libitum feeding system in a 2 x 2 factorial arrangement with 16 hours of light and 8 hours of darkness for 7 to 35 days of age. Intermittent fed birds had access to feed five times a day with four 1 hour and one 2 hour feeding bout a day from 7 to 12 days of age and five 1 hour feeding bout a day from 12 to 35 days of age. Ad libitum feeding consisted of continuous access to feed. The experiment was terminated on 35 days of age. The sample collected on 34^{th} day of the experiment was used for the comparison between the intermittent and ad libitum feeding system on the anterior digestive tract. The birds were selected randomly and killed exactly after 3 hours of feeding commencement to collect the content of crop, proventriculus-gizzard, duodenum + jejunum and ileum for the determination of dry matter content and intestinal viscosity. No interaction was seen between the feeding regimen and exogenous enzyme in the performance characteristics of broiler chickens. Exogenous enzyme didn't affect the body weight (BW) gain and feed intake but significantly increased AME ($P<0.0001$) for 7-21 days of age. Enzyme supplementation reduced feed intake ($P=0.0003$) and improved feed utilization efficiency ($P=0.08$) but the body weight (BW) gain and intestinal viscosity was not affected for 21-35 days of age. The crop has been used as a storage organ in intermittent feeding system which helped in the compensatory body weight (BW) gain during the last days of the experiment. In the ad libitum feeding system large variation in the crop and gizzard among the chickens was seen.

In conclusion, the results from this experiment show no difference in the performance of chickens with or without exogenous enzyme in the feeding system. There is no effect of enzyme on the viscosity of digesta from small intestine which may be due to low soluble fiber concentration in barley. The chickens have ability to maintain growth rate during intermittent feeding due to storage capacity of crop.

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1. Introduction

Barley is the cereal used for animal feeding. It has been used in Norway for most animal feed because of its easy availability and cheap price. However, in poultry, mainly broiler chicks, has been traditionally restricted from barley due to low energy value and associated problems like sticky dropping (Teirlynck et al., 2009). Barley contains β-glucans and arabinoxylan, the major source of anti-nutritional factor in the cell wall of the aleurone and endosperm layers. β-glucans, non-starch polysaccharides (NSP) consists of D-glucopyranosyl residues β-1,4 or β-1,3 glycosidic linked which are associated with an increased gut viscosity that inhibits digestion and absorption in the digestive system. The use of enzymes that degrade soluble fiber have reduced this problem and increased the potential use of barley in poultry diets (Svihus & Gullord, 2002). The digestive enzymes can be categorized into two forms either as endogenous or exogenous enzymes. Endogenous enzymes are those produced by the animal themselves such as pancreatic lipase, which splits fat or lipid into glycerol and fatty acids. Exogenous enzymes, those added from outside to feed as a supplement (Khattak et al., 2006). The use of various supplements of exogenous enzymes has been studied in the feed industry from many years. These enzymes have been used in poultry diets to improve the feed utilization. The exogenous enzymes include β- glucanase, xylanase, and amylase, α- galactosidase, protease, lipase and phytase. These enzymes are used mainly for two reasons, either to correct the lack of specific endogenous enzymes for digesting certain nutrients in various feedstuffs or to hydrolyze anti-nutritional factors in feed ingredients (Annison, 1993; Bedford and Schulze, 1998; Simon, 1998; Sheppy, 2001). Non-starch polysaccharide degrading enzymes (NSP-ases) are being used in the diets of poultry. There are various fiber-degrading enzymes used. The β-glucanase which degrade β-(1-3) (1-4) - glucans and the xylanase which degrade arabinoxylans are the two most widely used enzymes in the current diets of poultry.

The benefits of using feed enzymes in poultry diets have been discussed in various scientific paper which can be abridged as reducing gut viscosity, reduction in digesta viscosity; improving Apparent Metabolizable Energy (AME) value of the diet, improving fat utilization; improvement in nutrient digestibility thereby increasing feed intake, weight gain and feed-gain ratio, reduced water intake, reduced water content of

excreta, reduced production of ammonia from excreta, reduced output of excreta, including N and P; and also health improvement with reduced beak impaction and vent plugging, decreased size of gastrointestinal tract, altered population of microorganisms in gastrointestinal tract (Khattak et al., 2006). The authors have also discussed that the supplementation of enzyme could affect the Dry Matter Digestibility (DMD) depending on the type of diet and animal used such as in poultry, increase in DMD range from 0.9 (Schutte et al., 1995) to 17 % (Annison and Choct, 1993).

Barley consists of non-starch polysaccharides β-glucan and arabinoxylan. Izydorczyk and Dexter (2008) reported these amounts to be 2.5-11.3 and 5.8% respectively. The authors have explained well about the structures of β-glucan and arabinoxylan: glucan consists of D-glucopyranosyl residues linked through 1,4 linkages, arabinoxylans consists of D-xylopyranosyl units linked through (1,4) glycosidic linkages with L-arabinofuranosyl residues. β-glucan polymer of 38-69% is reported to dissolve in 2 hours at 38 ^{o}C (Belitz et al., 2009) which is reported to be close to the deep body temperature of around 41.5 ^{o}C of broiler chickens (Lacey et al., 2000). The difference in type of feed ingredients can affect the proportions of soluble NSPs (Mathlouthi et al., 2002). The authors have described the levels of different feed ingredients (Rialto wheat, Scarlett barley, Maize, and Soybean meal) which are shown in table 1.

Table 1. Soluble NSP levels (g/kg DM) in four different ingredients (Modified from Mathlouthi et al. (2002)).

Item	Rialto Wheat	Scarlett barley	Maize	Soybean meal
Soluble arabinoxylans[1]	5.2	3	0.3	1.2
Soluble β-glucans	2.4	24.3	0.5	0.6

Arabinoxylans[1]: arabinose + xylose

The purpose of present study was to compare between the intermittent and ad libitum feeding, crop feed variation in the intermittent feeding and the effect of feeding program on the performance of broiler chicks in poultry fed barley based diet with and without enzymes.

2. Literature

2.1. Enzyme activity at different pH & temperatures

The enzymes that are added to the diets must be active under the physiological conditions in the animal's digestive tract in order to function and also it is discussed that the enzyme in poultry diets must be sufficiently high to compensate the short transit time (Ao T, 2005). The enzyme activity is also influenced by the pH of the digesta. It was discussed that the presence of the crop may have contributed to the higher efficacy of the exogenous enzymes (Chesson, 1993; Dierick and Decuypere, 1996; Bedford and Schulze, 1998; Danicke et al., 1999; Partridge, 2001). The optimum pH of most exogenous enzymes is between 4.0 and 5.0. According to De Vries and Visser (2001) the enzyme Xylanase have a pH optimum between 4.0 and 6.0 but studies from Ding et al (2008) showed the range from pH 3.0 to 7.0 where more than 50 % of its maximum activity occurred at pH 6 (Wu et al,. 2005). Thacker and Baas (1996) demonstrated that the xylanase enzyme preparation when incubated at pH 6.5 or at pH 3.5 has low activity but showed higher activity at pH 4.5 and 5.5. While the optimum pH of many commercial β-glucanase to be 5.5, very low activity at pH 2.5 and 3.5 with considerably lower activity at pH 6.5. Ao et al. (2008) has investigated the β-glucanase and xylanase activity at different pH levels modelling the digestive tract of birds shown in fig. 1

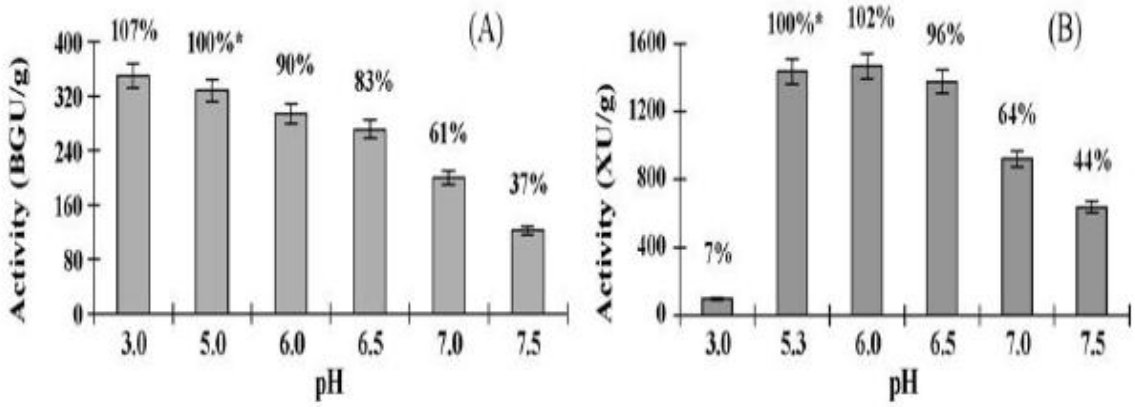

Figure 1. Fungal β-glucanase (A) and xylanase (B) activity. BGU denotes β-glucanase unit and XU denotes xylanse unit. Values on top of bars represent activity as a percentage of activity obtained at optimum pH as indicated by (*). The activities are expressed as units per gram enzyme product (Source: Ao et al. (2008)).

From the figure above, β-glucanase and xylanase seem to show 100% activity at pH 5 and 5.3 respectively. B –glucanase has shown to be active between pH 3.0- 7.0 with gradual reduction in activity with increase in pH. Xylanase had no enzyme activity at pH 3.0, but had high activity at pH levels 6.0-7.0. Xylanase activity is reduced when pH is decreased from 5.3 to 3.0

Enzyme activity is not only affected by pH but also by temperature and the most enzymes have a temperature optimum between 45 and 65^{o}c. De vries and Visser (2001) has reported the optimum temperature for β-glucanase and xylanase synthesised from *Aspergilli* strains range between 45-70^{o}C and 42-55^{o}C respectively. The deep body temperature of broiler chickens has been determined to be around 41.5 ^{o}C by Lacey et al. (2000). This is the temperature close to the optimum temperature of NSP degrading enzymes due to which a high relative enzyme activity could be expected in broilers digestive tract temperature wise. Wu et al. (2005) and Ding et al. (2008) have observed only small change in the enzyme activity with the increase of temperature from 40 to 50^{o}c.

2.2. Retention time

The passage of the matter in the growing chicken and the laying hen is faster, most of the studies have shown that the marker added to the feed will appear in the faeces within 2 to 2.5 hours after feeding and most of them will be exerted within 12 hours (Tuckey et al., 1958). According to Duke et al. (1968) the marker can be detected upto 72 hours after feeding but this may be due to the reason that a portion of the ingesta may enter in the caeca. Various experiments show that the average retention time in the digestive tract excluding the caeca is 4 to 8 hours (Shires et al., 1987; Vanderklis et al., 1990; Almirall and Esteve-Garcia, 1994; Danicke et al., 1999; Hetland and Svihus, 2001).

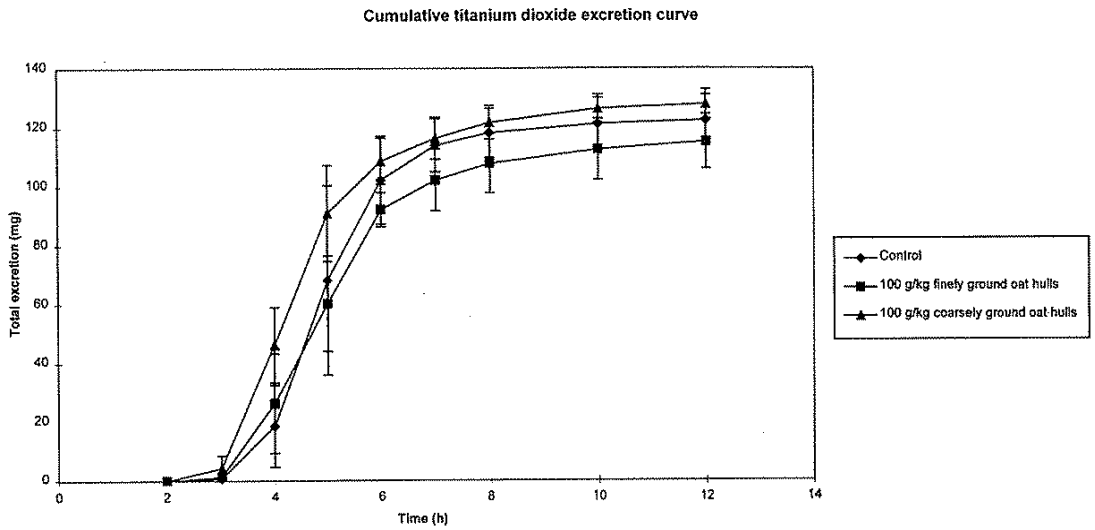

Figure. *Cumulative excretion curves of wheat diets without oat hull supplementation and with 100 g/kg inclusion of oat hulls at 2 different milling degrees. Bars indicate standard deviation for each point (n=4).*

Figure 2.Cummulative excretion rate through the broiler chicken (Hetland and Svihus, 2001)

The passage rate is affected by the feed intake and the holding capacity of the digestive tract. Therefore, high passage rate would facilitate a high feed intake which could be used for selecting different feeding system. There are various parameters that affects the retention time in different segments, such as water absorbing capacity and flow rate, holding capacity of the segment. Different fractions of the feed may pass through segments at different rates which were shown for the gizzard by Vergara et al. (1989). This is also affected by the feeding patterns, particular length of the pre-prandial fast which was shown by Chaplin et al.(1992) that feed would pass without entering the crop if the gizzard is empty. Same result was shown by Jackson and Duke (1995) for the gizzard of turkey, in which growing turkeys were fed a finely ground diet after 10 hours fast, the small intestine was filled with feed within 25 minutes from commencement of feeding. The average retention time in the crop was found to be approximately 50 minutes but it may vary (Danicke et al., 1999).

2.3. Crop functionality under intermittent and ad libitum feeding regimen

According to Nielsen (2004), commercial broilers on ad libitum feeding showed a semi-continuous way of eating feed and under such conditions, the crop was not used to its maximal capacity (Denbow, 1994). Negligible amounts of feed materials were found in the crop of ad libitum fed fast and slow growing broilers while significantly increased

crop contents in intermittent feeding (Boaamponsem et al., 1991). Thus, the crop is mainly thought to have a role as a storage organ for birds and is not involved in feed intake regulation under ad libitum feeding (Jackson and Duke, 1995). But the studies performed by Svihus et al. (2010) have confirmed that the ad libitum fed broiler chickens do not use the crop to any significant extent which indicates that the ad libitum fed birds will adapt a habit of letting feed bypass the crop. According to Barash et al (1992) birds that were adapted to 2-meal (2M) programme (fed twice per day) were able to consume approximately 40% of the daily intake of ad libitum fed birds during each meal which lasted for two hours and also the authors (1993) observed a significant increase in weight and feed- holding capacity of both crop and gizzard when chicks were fed meals one or two times per day instead of ad libitum fed. Buyse et al (1993) showed that broiler chickens use both the crop and the proventiculus/gizzard as storage organs for food when adapted to long periods of food deprivation and also found considerable amounts of feed in the crop of broiler chickens after 5 hours of last feeding. Broiler chickens which had access to feed only every fourth hour have also confirmed that birds are storing feed in the crop and that feed can be found in the crop at least 3 hours after last feeding (Svihus et al.,2002).

With intermittent feeding, broiler chickens were found to have extremely more amount of dry matter in the crop and gizzard-proventiculus at the beginning of the dark phase than that during the light phase (Buyse et al., 1993). The storage of large quantities of feed in the crop was also examined by Svihus et al. (2010). Savory (1980), Nielsen (2004) and Svihus et al. (2010) has reported that when birds are fed intermittently, they eat large amounts of feed in a short time and stock up in their crop. The accumulation of digesta for prolonged time may help the micro flora of the crop in the fermentation process. During this process more lactic acid is produced thus reducing the pH in the crop. The reduced pH in the crop depends on the feed composition and ingredients. However, Bayer et al. (1978) reported the decrease in the pH of the crop to 4.5 with prolonged retention time. Svihus et al. (2010) observed the improved efficiency of exogenous enzyme phytase with lengthened retention time in the crop. The authors detected reduced dry matter percentage in the crop of birds killed at different periods of time after feeding is terminated.

3. Materials and methods

3.1. Diet composition

Diets used for the whole experiment were formulated and processed at the Centre for Feed Technology (Fortek), Norwegian University of Life Sciences (UMB), Ås, Norway.

In the experiment, the Norwegian barley grains harvested in 2010 was used. Two diets were formulated: one with enzyme and other without enzyme. The enzyme used was a product of Danisco A/S, Denmark. Axtra XB 201 L type with 12200 U/g endo- 1,4-beta xylanase and 1520 U/g endo-1,3- beta glucanase activity was used in the diet with enzyme. The amount of enzyme added to the diet 2 (with enzyme) was discovered to be 2.5 times more than the recommended level of 200g/tone feed because of the human error during the time of processing. The marker used was Titanium dioxide and due to the human error; the amount added to the feed was found to be extremely lower than that in the diet formulation. Due to this error, the results regarding marker concentration and illeal digestibility of nutrients could not be used and is therefore not discussed in the current work. The diets were made according to the nutritional requirements of experimental birds given by Aviagen Group Ross 308 Broiler (2007).

Table 2.Composition of the diet

Ingredients	Diet 1 (g/kg)	Diet 2 (g/kg)
Barley	660	660
Fish meal	90	90
Soy Bean Meal	184	184
Soy oil	30	30
Lime stone	10	10
Mono Calcium Phosphate	10	10
DL-Methionine	2	2
L-Threonine	1	1
Salt	2.5	2.5
Mineral		
Premix.Fkøv	1.5	1.5
Vitamins		
A. Fkøv	0.5	0.5
ADKB. Fkøv	1	1
D3. Fkøv	0.8	0.8
E. Fkøv	0.5	0.5
Choline chloride	1.2	1.2
Titanium dioxide	0.5	0.5
Enzyme Axtra XB 201L	-	0.5

Table 3.Calculated diet composition (as feed-basis):-

Metabolizable energy (MJ/kg)	13
Crude Protein (g/kg)	206.4
Calcium (g/kg)	10.4
Available Phosphorus (g/kg)	5.8

3.2. Processing:-

Hammer mill (Model: E-2211 TF, Münch- Wuppertal, Germany, under Bliss-USA, 18.5 kW and 2870 rpm) of 3-mm sieve was used for the grounding of barley and soy bean meal. 250 kg of 3 batches each grounded mash was produced and mixed in the mixer conditioner (Twin Shaft Paddle, Tatham of England under license from Forberg, Norway, 400 lt, 4kW). The mixing was done for 2 minutes to each batches before the addition of soy oil. The soy oil was sprayed with the help of FôrTek made tank. After the soy oil was poured into the tank; air was pumped in so as to create the pressure of 4 bars. Then, the tank was put on the zeroed scale before spraying. The spraying was done until the amount of 7.5 kg was displayed on the scale screen (3% of each batch). The nozzle used had a capacity size of 6506 (angel 65, size 05, Unijet, Spraying Systems Co, Wheaton, Illinois, USA) and spraying capacity of 2.4 lit/min (based on water viscosity). After oil addition, the mash was mixed for 2 minutes. So, the total time used for the mixing of the mash was 8 minutes and 45 seconds. After mixing, samples were taken from all batches randomly and the final mixed mash was filled into two sample bags. Then the mash was inserted into the pellet press control panel for automatic calculation of the machine's capacity. The density was measured to be 608g/litre.

The mixed mass was conditioned in the twin pass conditioner (Twin Pass, Muench, Germany, 2t/h, 2 x 2m x 40cm) with steam at 75 ºC before processing in a pellet mill (Muench, Germany, 1.2 t/h max. capacity, 2 x 45 kw). The processing parameters of pellet production were recorded as in table 4.

Table 4. Processing parameters:

Temperature in the conditioner	(^{o}c)	75
Production Capacity	(kg/h)	600
Die diameter	(mm)	3.0
Die length	(mm)	42.0
Motor Load	(%)	31
Amperes Motor 1	(amp)	16
Amperes Motor 2	(amp)	15.0
Average Amperes motor	(amp)	15.50
Energy Consumption	(kW)	9.53
Specific Energy Cons.	(kWh/kg)	0.0159
Temperature of the pellet upon Discharge	(^{o}c)	86.8

The pellets upon discharge at the opening of pellet mill were collected in an insulated box so as to measure the temperature of the pelleted feeds. The temperature was read and recorded by the thermometer attached to the box.

Pelleted diets, after collected in a container were cooled in a counter-flow cooling system (Miltenz, New Zealand, capacity 2000 kg/h) for 30 minutes. Then the cooled pelleted diets total 650kg were packed in 26 bags each containing 25 kg pelleted feed. 26 Bags were distributed randomly in two groups of 13 bags for the equal distribution of feed production.

Diet 1 feed without enzyme was sprayed with 1.16kg of water for 45 seconds into the mixture. Diet 2 feed with enzyme was sprayed with a solution of water and enzyme. To make the same amount of liquid, 160 grams of liquid enzyme was diluted in one litre of water.

The same mixture tank (OPÜR F50 1”20”, capacity 2500 l/hour, temperature 0-80 °C, max pressure 10 bars, Model 2006, Teknisk vannservice AS, PO Box 5, Stovner, 0913 Oslo) was used for spraying process on the two diets. The nozzle capacity size was 6503.

While the pelleted feed was being released, separate samples were taken from both the diets representing diet 1 (without enzyme) and diet 2 (with enzyme).

3.3. Experimental animals and feeding

The experiment was performed at the Animal Production Experimental Centre (Senter for Husdyrforsøk), UMB from 17th of November to 22nd of December 2011.

The experimental birds were 200 day-old male broiler chickens (Ross 308). These were placed in brooder cages where light was on for 24 hours with temperature of 33^{o}C until 7 days of age and were fed on a commercial starter diet.

The birds were weighed at 7 days of age, 144 birds with the average weight of 182 grams were selected and the rest of the birds (approximately 30-35) were discarded. **S**elected birds were distributed randomly with 3 birds per cage (50cm x 35cm x 20cm) in 48 cages. The two racks of cage (24 cages per rack) were managed in such a way that the intermittently fed birds did not have visual contact with the ad libitum counterparts. The birds in cages with the odd numbers were given diet 1 (feed without enzyme) and the birds in cages with even number were given diet 2 (feed with enzyme). Each of the cages was assigned bucket filled up with 5 to 6 kg of feed. Feed weight was recorded along with the bucket. The ad libitum birds were assigned with the cage no. 1-12 and 37- 48 whereas intermittent birds were with cage no. 13-36.

3.3.1. Lightning and feeding system

Light was on for 24 hours until 7 days of age and fed on commercial diet. Temperature was reduced from 33^{o}c to 29^{o}c at 7 days of age, and to 26^{o}c at 16 days of age. From 7 to 34 days of age, dark period was applied for 8 hours twice a day from 23:00 to 03:00 and from 04:00 to 08:00. From 7 to 12 days of age, birds under intermittent feeding regimen were fed ad libitum from 03:00 to 04:00, 08:00 to 09:00, 12:00 to 13:00, 16:30 to 17:30 and 21:00 until lights were switched off at 23:00. The feed was removed from the trough during no access to feed except for 23:00 to 03:00 and 04:00 to 08:00 when access to feed was controlled by lighting system. From 12 until 34 days of age,

intermittently birds were fed 5 times with one hour feeding from 03:00 to 4:00, 08:00 to 09:00, 13:00 to 14:00, 17:30 to 18:30 and 22:00 to until light goes off at 23:00.

3.3.2. Feed and excreta record

On 18 days of age at 12:00 pm, the feed and excreta trays under the cages were cleaned. At the same time, the birds and feed weight was taken. The excreta between 18 – 21 days were collected in the white boxes from the cages after it was cleaned for feed and feathers. The boxes were kept frozen. These frozen samples were used for the determination of AME.

3.3.3. Moving and marking of birds to next room

At day 21, the birds were weighed in groups of three from the same cages and the buckets containing feed were also weighed. Then, 2 randomly selected birds from each cage were transferred into 12 pens (1-6 pens assigned to intermittently and the rest to ad libitum fed birds) in a room where temperature had been already set to 22^{o}c. There was no visual contact between the intermittent and the ad libitum fed birds. For the identification, the two birds from each cage were tied with a same coloured strap around their feet. Birds from four cages were placed in one pen (all together 8 birds in each cage), therefore using four different colours of straps.

3.3.4. Dissection

At day 22 the remaining birds in cages were killed by cervical dislocation and a strap was wrapped around the neck immediately to avoid crop content regurgitation. The birds were dissected to collect samples from crop, gizzard and small intestine quantitatively and freezed in liquid nitrogen. But the samples collected on this day were discarded and not used for analysis because the intermittently fed birds were supposed to be killed after three hours of having excess to feed but were killed approximately after one hour due to the human error.

At 34 days of age, the feed troughs were emptied for the intermittently fed birds before the light was switched on at 6:00 in the morning. All the intermittently fed birds and their feed troughs were weighed from 7:30 to 08:00 and from 08:00 to 08:30 the same procedure followed on ad libitum fed birds. The intermittently fed birds (Pen 1, 2, 3, 4,

5 and 6) were given access to feed from 08:00 to 08:40, 08:20 to 09:00, 08:40 to 09:20, 09:00 to 09:40, 09:20 to 10:00, and 09:40 to 10:20, respectively. At 08:40 feed was removed from pen 1, at 09:00 from pen 2, similarly feed from rest of the pen 3, 4, 5 and 6 were removed after 40 minutes of access to feed. At 11:40 exactly after 3 hours of feeding gap, 3 birds (randomly selected from different strap colour) from pen 1 were killed by cervical dislocation and strap was wrapped around the neck immediately. At 12:00, 4 birds from pen 2 were killed (one bird per colour). Same procedure was repeated for rest of the pens 3, 4, 5 and 6 until 13:20. For the ad libitum fed birds (Pen 7 to 12), at 09:00, one bird of each colour (4 in total) from each pen were killed. In this way birds were killed exactly 3 hours after feed was removed from them. Contents from the crop, gizzard, duodenum + jejunum and ileum were collected and frozen in liquid nitrogen. The samples collected were used for the comparison between the ad libitum and intermittent feeding and crop content variation in the intermittently fed birds.

Feeding rest of the birds under intermittent system was carried out from 16:00 - 17:00 pm and 20:00 - 21:00 pm. Feed troughs belonging to intermittently fed birds were weighed at 21:00 pm after the lights were switched off. The experiment was terminated on 35 days of age.

At 35 days of age, light was switched back on for an hour at 01:00 – 02:00 am. After 5 hours of darkness at 07:00 am feed was removed from the pens 1 to 6 and then light was switched on. The weight of feed troughs were recorded and allowed to eat for 40 minutes from 08:00-08:40. The feed was removed from all the pens 1 to 6 and birds were randomly selected to kill in the following ways:-

At 08:40 one bird in pen 1 and two birds in pen 2,
At 09:20 two birds in pen 3 and 1 bird in pen 4,
At 10:00 two birds in pen 5 and two birds in pen 6
At 10:40 two birds in pen 1 and two birds in pen 2
At 11:20 two birds in pen 3 and two birds in pen 4
At 12:00 two birds in pen 5 and two birds in pen 6

All the killed birds were dissected to collect the contents from crop, gizzard, duodenum + jejunum and ileum and immediately frozen in liquid nitrogen. These samples were used for the starch, protein and marker (Titanium dioxide) analysis and also for the

investigation of material flow dynamics in digestive tract. But the results from this chemical analysis in not discussed in this paper.

3.4. Sample analysis

3.4.1. Digestive tract

Digesta that were collected from the four segments of digestive tract were lyophilized in a freeze dryer (Beta 1-6, LMC-2, Christ, Osterode, Germany) at -56^{o}c and 25 mbars for 92 hours to obtain dry matter content without encountering any possible biochemical changes in the samples.

3.4.2. Chemical analysis

The boxes of excreta samples were thawed at room temperature, and next day the gross weight of each box was measured. The contents of each box's were homogenized by mixer. Then, the representative samples were put in the crucibles and weighed immediately. After the weight was taken, samples were dried in the oven at 104^{o}c during night (16 hours) then cooled in the desiccators. The net weight of the dried samples was taken. Then the samples in the crucibles were put in the bomb calorimeter (Bomb calorimeter PARR 1281, Moline, Illinois, USA) to calculate the AME.

3.4.3. Viscosity and pH measurements

0.4 grams of freeze dried sample from each container of duodenum + jejunum were taken in a 2ml centrifuge tube. Then, 1.5 ml of distilled water maintained at temperature of 40^{o}c on shaking water bath (Julabo, Model SW22, Labortechnik GmbH Seelbach, Germany) was added into the centrifuge tube. After centrifugation for 5 minutes, the viscosity of supernatant was taken by a viscometer running at 60 rmp (Brookfield, DV-II, Brookfield Engineering Laboratories, Massachusetts, USA) followed by pH measurement. The pH sensor calibrated at pH=4 was used because the pH sensor could not be calibrated at pH=7.

3.4.4. Calculations

Apparent metabolisable energy (AME) was calculated using the following equation:

$$AME = \frac{(\text{Feed intake} \times GE_{\text{diet}}) - (\text{Excreta output} \times GE_{\text{excreta}})}{\text{Feed intake}}$$

GE denotes Gross energy

3.4.5. Data analysis

Statistical Analysis System (SAS, 2006) was used for data analysis of performance table with General Linear Model (GLM) in a 2x2 factorial design and Excel 2007 was used for the mean and graph calculation.

4. Results

Performance table shows the intermittent feeding has significantly increased feed: gain (F: G) ratio (*P=0.0158*) as well as feed intake (*P=0.0003*) and body weight gain (BW gain) (*P<0.0001*) from 7-21 days as compared to ad libitum feeding. Energy utilization was not affected by feeding regime (*P>0.05*).

The result for the period of 21-35 days of age shows the feed consumption was significantly increased when feed was supplemented without enzyme. There was no significant interaction (*P>0.*05) between the exogenous enzyme and feeding regime throughout the experiment which is shown in table 5.

Table 5. Growth performance, feed intake, feed efficiency and AME in broiler chicks during 7 to 21 days of age (7-21d) and 21 to 35 days of age (21-35d), fed barley based diet supplemented or not with exogenous enzyme.

	7-21d				21-35 d		
	Feed intake (g)	BW gain (g)	F:G	AME (MJ/kg)	Feed intake (g)	BW gain (g)	F:G
Item							
Treatment							
Ad libitum + Enzyme	1293[a]	990[a]	1.31	15.1[a]	2110	1333	1.58
Ad libitum	1260[a]	967[a]	1.30	14.9[b]	2178	1334	1.63
Intermittent + Enzyme	1216[b]	916[b]	1.33	15.1[a]	2015	1257	1.60
Intermittent	1233[b]	908[b]	1.36	14.9[b]	2199	1347	1.63
Root mean square error1	45.3	39.8	0.06	0.08	82.60	72.0	0.03
Feeding regimen							
Ad libitum	1277[a]	978[a]	1.31[b]	15	2144	1334	1.61
Intermittent	1225[b]	912[b]	1.35[a]	15	2107	1302	1.62
Enzyme supplementation							
With enzyme	1255	953	1.32	15.1[a]	2063[b]	1295	1.59
Without enzyme	1247	937	1.33	14.9[b]	2188[a]	1341	1.63
Main effect							
Enzyme	NS	NS	NS	<.0001	0.03	NS	0.08
Feeding regimen	0.0003	<.0001	0.0158	NS	NS	NS	NS
Feeding regimen X Enzyme	0.06	NS	NS	NS	NS	NS	NS

[a-b] Means with different superscript are significantly different *P*<0.05).
[1] n=48 for 7-21d and n=12 for 21-35d.
(Source:- Master thesis 2012 Peyman Mosberian Tanha , Master thesis 2012 Ragnhild Sandnes)

The dry matter (DM) content was higher in the crop of intermittent fed birds than the ad libitum fed birds. The highest amount of dry matter (DM) was found in the gizzaard of ad libitum fed birds. There was no difference in the viscosity of both ad libitum and intermittent fed birds (table 6).

Table 6. Digestive Characteristics at 34 day of age

	DM in Crop (g)	DM in Gizzard (g)	DM in Duodenum + Jejunum (g)	DM in Ileum (g)	DM in Crop (%)	DM in Gizzard (%)	DM in Duodenum + Jejunum (%)	DM in Ileum (%)	Viscosity D + J (cP)
Item									
Feeding regime									
Ad libitum	4.1	6.9	6.6	4.7	22.5	25.8	19.9	20.8	2.6
Intermittent	9.6	5.2	5.7	4.3	27.5	24.3	18.6	19.7	2.6

Value presented are the average of the dry matter content from the digestive tract .
n=24 for ad libitum fed birds and n= 23 for intermittently fed birds

In intermittently fed birds the dry matter content in the digestive tract from crop to ileum was decreased. More than double amount of dry matter was found in the crop of intermittent fed birds than the ad libitum ones. The amount of dry matter in the gizzard, duodenum + jejunum and ileum of ad libitum fed birds was higher than the intermittent fed birds (figure3).

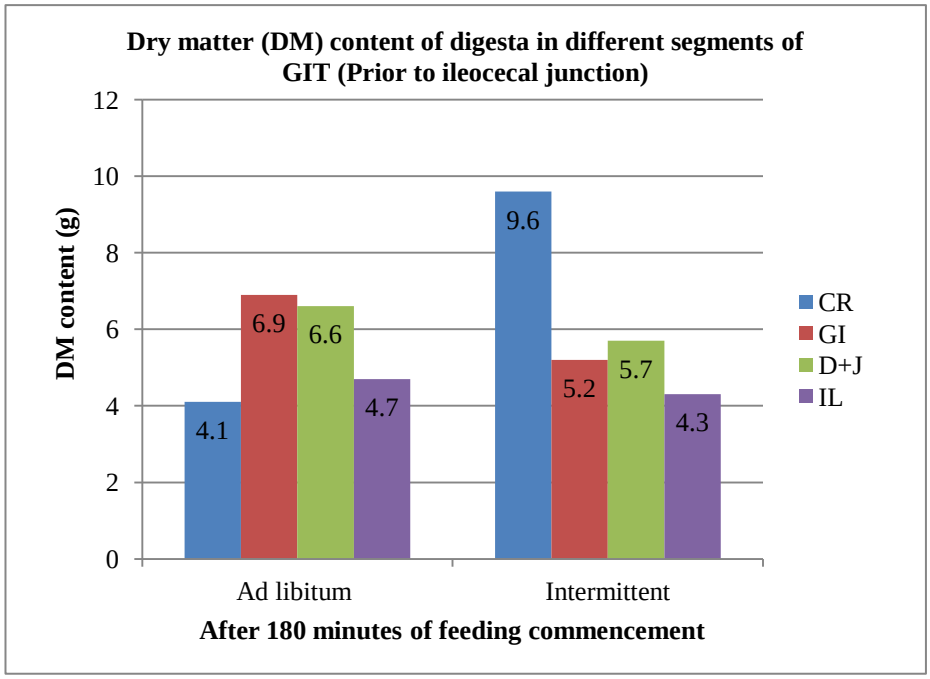

Figure 3. Comparison between the dry matter (DM) content of digesta in different segments of GIT (prior to ileocecal junction) of the intermittent and ad libitum fed birds after 180 minutes of feeding commencement. Values presented as mean ± standard error (SE). CR: Crop, GI: Gizzard-Proventriculus, D+J: Duodenum + Jejunum, IL: Ileum.

The highest percentage of dry matter was 27.5% and the lowest was 18.6% in the crop and duodenum + jejunum of intermittently fed birds respectively. The dry matter percentage of gizzard was higher in ad libitum fed birds (figure 4).

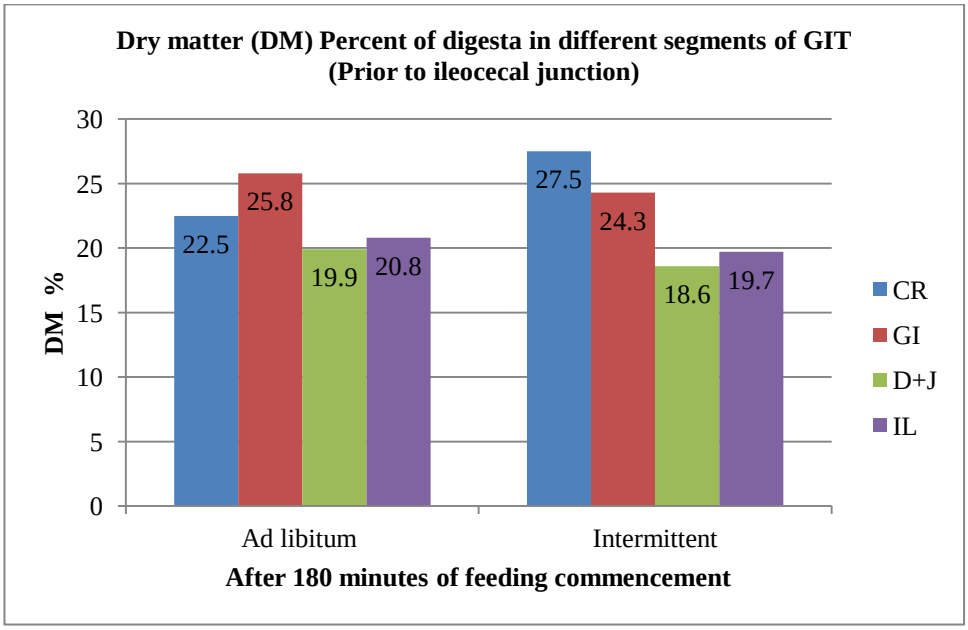

Figure 4. Percentage of Dry Matter (DM %) in different segments of GIT (Prior to ileocecal junction) of ad libitum and intermittent fed birds after 180 minutes of feeding commencement. CR: Crop, GI: Gizzard-Proventriculus, D+J: Duodenum + Jejunum, IL: Ileum.

Figure 5 shows there are large no. of birds (45.8%) with less than 1 g of dry matter (DM) in their crop when fed under ad libitum feeding system whereas no birds were found to have less than 1g of dry matter (DM) under intermittent feeding system. Many intermittently fed birds were examined to have more than 1g of dry matter in their crops; it was found that 30.4% of the intermittent fed birds were with 3-6g of dry matter in their crop. Few birds were found to have more than 15g dry matter content in the crop when fed with both the feeding system.

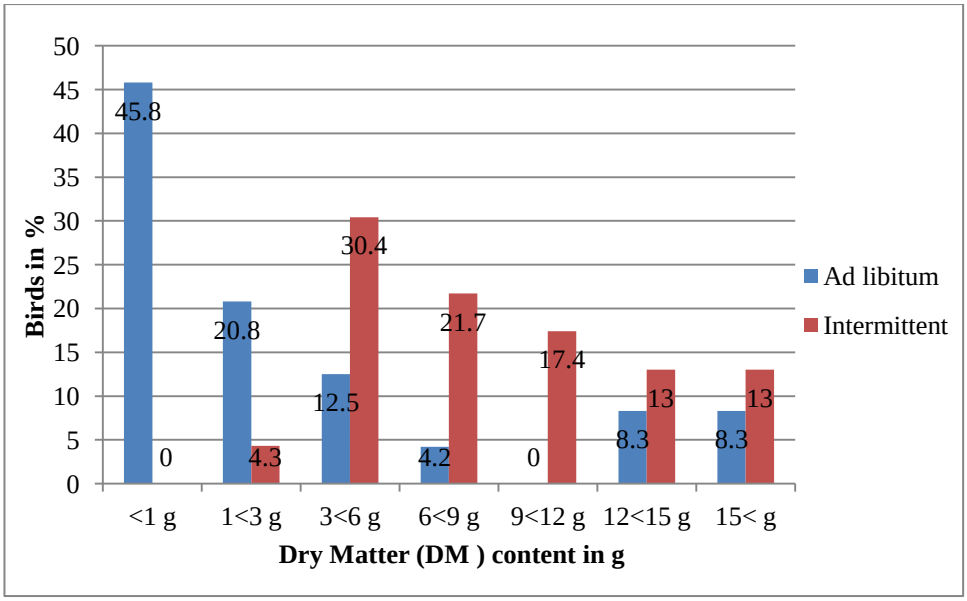

Figure 5. Dry Matter (DM) content variation in the crop of ad libitum and intermittent fed birds after 180 minutes of feeding commencement.

The result in the figure 6 shows 29.2% and 30.4% of the ad libitum and intermittent fed birds contained about 6-9g digesta in their gizzard. The maximum amount of digesta found was 12-15g with ad libitum fed birds and 9-12g with intermittent fed birds. Approximately 4.2% and 8.7% of ad libitum and intermittent fed birds were with less than 1g of digesta in their gizzard.

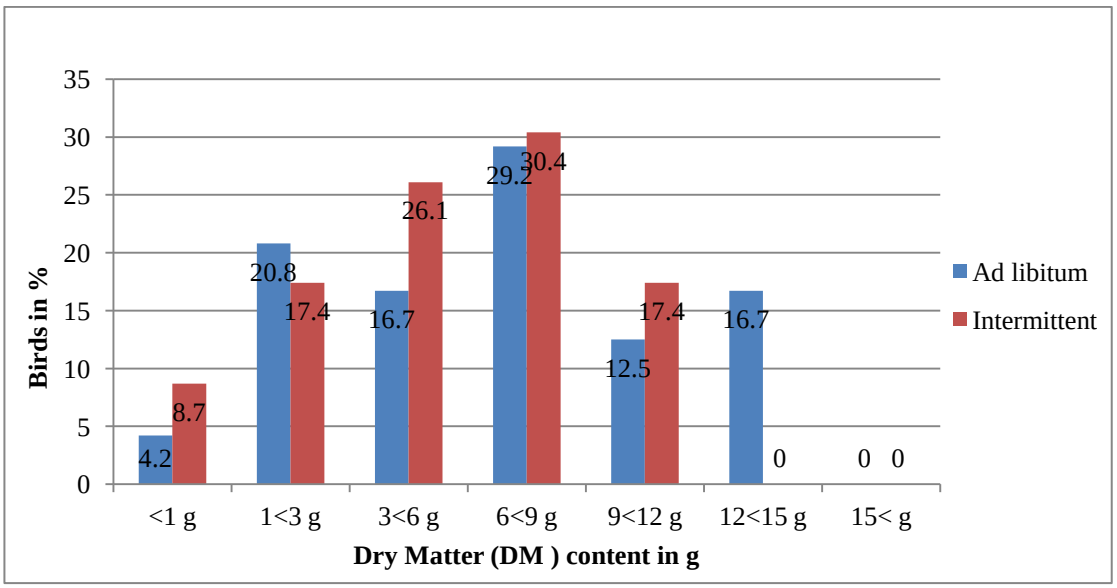

Figure 6. Dry Matter (DM) content variation in the gizzard of ad libitum and intermittent fed birds after 180 minutes of feeding commencement.

Approximately 54.2% and 65.2% of ad libitum and intermittent fed birds were with 1<3g of digesta (figure 7). The maximum content amount of digesta was between 6-9g in 20.8% of ad libitum fed birds whereas 3-6g in 34.8% of intermittent fed birds.

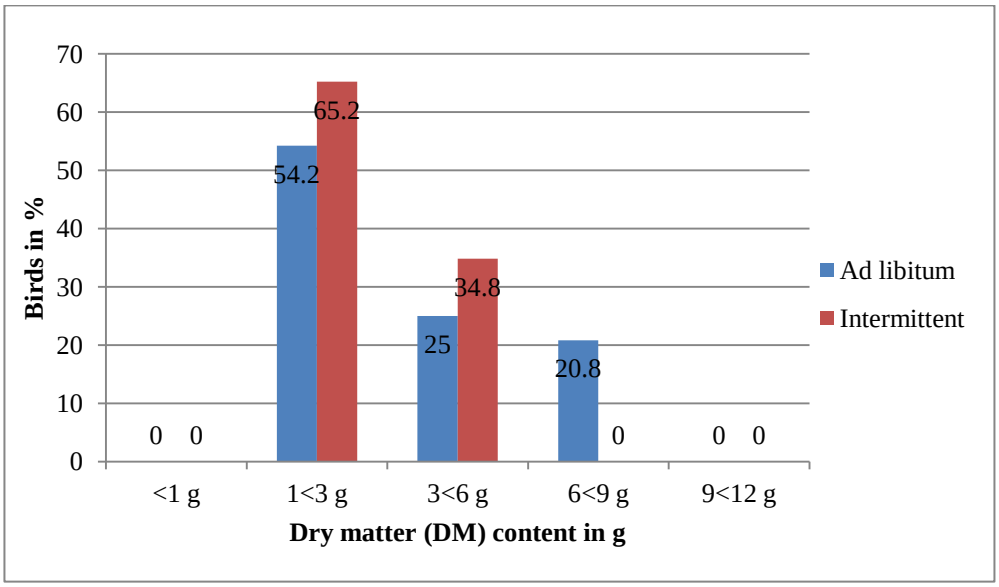

Figure 7. Dry Matter (DM) content variation in the duodenum + jejunum of ad libitum and intermittent fed birds after 180 minutes of feeding commencement.

In intermittent feeding 52.2% birds were with 1<3g digesta, 30.4% with less than 1g and 17.4% birds were with 3-6g of digesta (figure 8). 54.2% of ad libitum fed birds were with 3<6g of digesta and the maximum amount of digesta (6-9g) was found in 25% birds (figure 8).

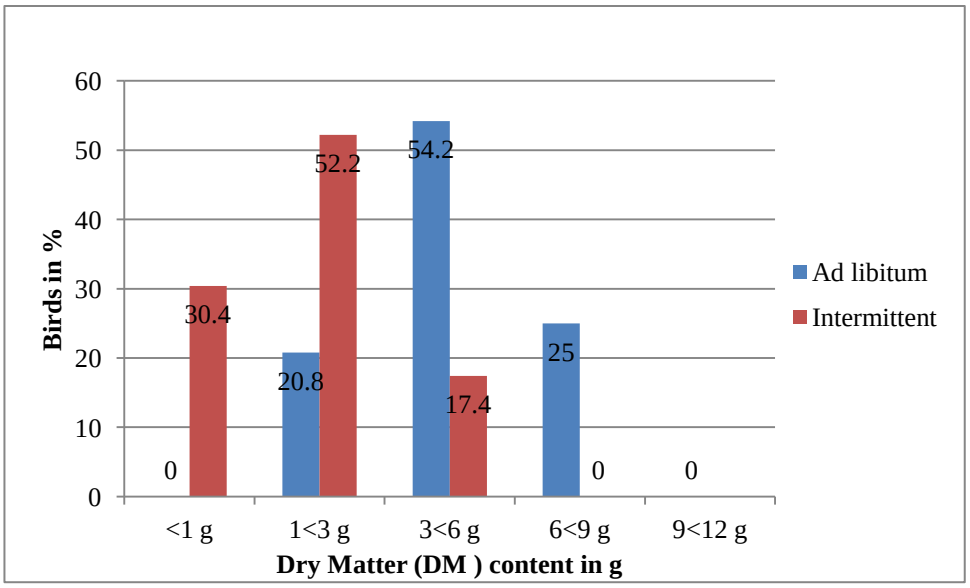

Figure 8. Dry Matter (DM) content variation in the ileum of ad libitum and intermittent fed birds after 180 minutes of feeding commencement.

5. Discussion

The feed efficiency as seen in this experiment was very high for both the ad libitum and intermittent fed birds in comparison to the producer predicted figures for high performance under continuous feeding regimen (Aviagen Group Ross 308 Broiler: Performance Objectives, 2012). As compared to the manual Rose 308 for the period of 7-21 days; feed intake and body weight gain was higher in this experiment. But for the period of 21-35 days feed intake was lower and the body weight gain was not affected. The F: G (feed: gain) shows improvement during 21-35 days which may be due to the significant reduction in feed intake (*P=0.08*) while body weight gain remained unchanged. This shows that the feed was well utilized in this experiment during the last 14 days. During 7-21 days of age, poorer feed efficiency was seen among the intermittently fed birds when compared to the ad libitum counterparts. This may be due to the reason that the birds demanded longer time to become accustomed to the feeding system. Later from 21-35 days of age, no significant difference was seen in any performance parameters between the ad libitum and intermittent fed birds. This indicates that the birds were able to adapt to the feeding system. Similar result was recorded by Sacranie et al. (2012), in which the authors recorded no difference in feed utilization efficacy and body weight (BW) gain between ad libitum and intermittent feeding regimens from 17 to 33 days of age.

There was no difference in the feeding regimens and the intestinal viscosity was not affected with or without enzyme. However, Svihus et al. (2010) reported the improvements in feed efficiency when exogenous enzyme phytase was used with intermittent feeding regimen. The AME increased when the enzyme was added and decreased when there was no enzyme in the diet: the significant effect of enzyme was observed on AME (*P<0.0001*) in this experiment. The viscosity of the digesta in duodenum + jejunum of ad libitum and intermittently fed birds were not affected by the enzyme supplementation. The viscosity was found to be lower when compared to other

studies in Yu et al. (1998) and Garcia et al. (2008). Lower viscosity in the small intestine has been reported to increase the performance of broiler chicks by improving digestibility and availability of nutrients (Svihus & Gullord, 2002; Rodriguez et al., 2012).

The digestive characteristics table showed that the dry matter (DM) content in the crop of intermittent fed birds was more than ad libitum fed birds. This suggests that the crop has been used to stock up large amounts of feed in short time when birds are fed intermittently and that may be used later when there is no feeding. There are various experiments which supports that crop have been used as a storage organ (Savory, 1980; Nielsen, 2004; Svihus, 2010). The positive effect of increased retention time in the crop was also shown previously on exogenous phytase by Svihus et al. (2010). In this experiment 45.8% of the birds were with less than 1g of material in their crop and 29.2% were with 6-9g dry material in their gizzard when fed ad libitum. This shows that the ad libitum fed birds have not used their crop for storing of feed as the feed is available continuously. So, the feed is passed to the gizzard. Gizzard has been used for the storage of feed and further digestion which shows gradual reduction in dry matter after 180 minutes of feeding commencement. In the case of intermittently fed birds, after hours of feeding gap the feed may have bypassed the crop to the gizzard as there is demand of feed after long hour of starvation. When the gizzard is full then only crop has been used for the storage of feed in short time. So, there was not so much difference in the dry matter (DM) percentage of crop and gizzard in the intermittently fed birds.

Large variation was examined in the dry matter content of crop after 180 minutes of feeding commencement which may be explained that many birds have used their crop for storing feed in the intermittent feeding system while only some birds have used in ad libitum feeding. High percentage of birds with more amount of dry matter content in the gizzard shows that gizzard has been used for the digestion of feed. This organ is specially used for the mastication of feed for digestion. The less amount of digesta in the posterior digestive tract suggests that the digestion was good enough for the absorption of the nutrient availability of the birds. This more amount of digesta in the gizzard shows that it has been well utilized for the digestion of feed which may have led

the birds to gain weight. The high amount of feed in the crop, and gradual reduction of digesta in the digestive tract may suggest the good utilization, digestion and absorption of nutrients from the feed source: this induces the weight gain of the birds.

6. Conclusion

The study indicates that the supplementation of exogenous enzyme: arabinoxylanase and β-glucanase did not affect the digesta viscosity and feeding regimen but increased AME significantly. This may be the result of cell wall non- starch polysaccharides (NSP) degradation and release of entrapped nutrients and also may be the reason that in this experiment, the barley used was harvested a year prior to the trial. This could be explained that ratio of soluble NSP was too low to see enzyme effect on the viscosity.

In intermittent feeding system, chicken were able to maintain the growth by using the storage feed in the crop. Thus, crop has been used as a storage organ for the compensatory growth during the experiment. The variation in the crop content of ad libitum fed chicken shows that some birds were using crop for storage but most of the birds used gizzard. However, there was no difference in their growth under both feeding regimen.

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