Abstract

An experiment was conducted to investigate the effect of intermittent feeding, exogenous enzyme supplementation (xylanase and β-glucanase) and their interaction on the performance of broiler chickens. Furthermore, possible selective retention of starch and/or protein as well as flow pattern of the nutrients in digestive tract was studied. 144 broiler chickens (Ross 308) were fed barley based diet supplemented or not with exogenous enzyme, under either *ad libitum* or intermittent feeding systems from 7 to 35 days of age in a 2 × 2 factorial design. *Ad libitum* fed birds were fed continuously, while intermittently 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 5 one hour feeding bout a day from 12 days of age until the end of experiment. In the last day of trial randomly selected intermittently fed birds were killed 40, 80,120,160,200 and 240 minutes after feeding commencement to collect the content of crop, proventriculus-gizzard, duodenum + jejunum and ileum for determination of starch, protein and titanium dioxide as well as intestinal viscosity. There was no interaction between feeding regimen and exogenous enzyme for any of performance parameters. From 7 – 21 days of age exogenous enzyme didn't affect body weight (BW) gain and feed intake but significantly increased AME *(P<0.0001).* For the period of 21-35 days of age enzyme supplementation reduced feed intake *(P=0.03)* and tended to improve feed utilisation efficiency *(P=0.08)* but intestinal viscosity and BW gain were not affected by the enzyme. Viscosity of digesta in the crop and duodenum +jejunum was seen to be extremely low. Intermittent feeding depressed BW gain, feed intake and feed utilisation efficiency until 21 days of age but thereafter the differences were not significant *(P>0.05).* There was no selective retention of nutrients in the crop but starch showed reduced concentration in the proventriculus-gizzard overtime. The pH significantly declined in the crop 4 hours after feeding was initiated *(P<0.05)* which is a result of fermentative activities of lactobacilli bacteria.

The result of this experiment showed that chickens are able to show compensatory growth after a period of depressed performance under intermittent feeding, most probably due to increased storage capacity of the crop. No effect of enzyme on viscosity may have been due to low soluble fibre concentration in barley. The effect of enzyme on AME, however, may partly be due to the ability of these enzymes to hydrolyse cell wall constituents or increase VFA (volatile fatty acids) production.

Sammendrag:

Forsøket ble utfør for å teste effekten av måltidsfôring, tilsatt enzym(xylanase og β-glucanase) og eventuelt samspillet mellom disse på slaktekylling. Muligheten for selektiv retensjon av stivelse og /eller protein og passasjehastighet av næringsstoffer i fordøyelseskanalen ble også undersøkt. 144 slaktekyllinger (Ross 308) ble fôret på en byggbasert diett med eller uten tilsatt enzym, og tildelt fôret enten ad libitum eller ved måltider fra 7-35 dagers alder i et 2 × 2 faktorial design. Kyllinger som fikk fôret ad libitum hadde kontinuerlig tilgang, mens de som fikk måltidsfôring fikk tilgang til fôret fem ganger daglig. Da hadde de tilgang i 1 time fire ganger og 2 timer en gang, fra 7-12 dagers alder og deretter fem perioder 1 timers tilgang fra 12 dagers alder til forsøket ble avsluttet. Den siste dagen i forsøket ble tilfeldig valgte kyllinger under måltidsregimet avlivet 40, 80, 120, 160, 200 og 240 minutter etter fôret ble fjernet og deretter ble innhold fra kro, kjertelmage med krås, duodeum +jejunum og ileum samlet for å bestemme stivelse, protein og titandioksid. Det ble også målt viskositet fra prøvene.

Det ble ikke funnet samspill mellom fôringsregimet og tilsatt enzym for noen av produksjonsparameterne. I perioden 7-21 dager var det ikke effekt av enzym på tilvekst eller fôropptak, men AME var signifikant høyere *(P<0.0001).* I perioden 21-35 dager var det redusert fôropptak *(P=0.03)* av dietten tilsatt enzym og en tendens til bedre fôrutnytting *(P=0.08)*, men tilsatt enzym hadde ikke effekt på tilvekst og viskositet målt i prøver fra tarm. Viskositet målt fra kro og duodenum + jejunum var ekstremt lavt. Måltidsfôring førte til lavest tilvekst, fôropptak og dårligst fôrutnytting fram til 21 dagers alder, men etter denne perioden var ikke forskjellen signifikant *(P>0.05).* Det var ingen selektiv retensjon av næringsstoffer i kro, men konsentrasjonen av stivelse i kjertelmage-krås sank med tiden. Det ble vist signifikant lavere pH i kro 4 timer etter tilgang på fôr *(P<0.05)* som et resultat av fermenteringsaktivitet av melkesyrebakterier.

Resultatene fra dette forsøket viser at slaktekyllinger kan vise kompensatorisk vekst etter en periode med redusert prestasjon under måltidsfôring, mest sannsynlig på grunn av økt lagringskapasitet i kro. Den fraværende effekten av enzym på viskositet kan skyldes lave konsentrasjoner av løselig fiber i bygg eller metoden som ble brukt for å måle viskositet. Effekten av enzym på AME kan skyldes at tilsatt enzym hydrolyserer celleveggens ikke-stivelses-polysakkarider (NSP) eller øker konsentrasjonene av kortkjedede fettsyrer i blindtarmene.

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Ås, May 2012,

Peyman Mosberian Tanha.

Table of Contents

1. Introduction

It is common to raise broiler chickens under continuous feeding regimen. However, managerial issues such as high mortality, occurrence of leg problems and feed overconsumption have been reported due to the broiler chickens' fast growth rate. Interruption in feeding during light phase has been argued to reduce leg problems (Petek *et al*., 2005), improve feed conversion ratio (Buyse *et al*., 1996b), and also seems to be closer to natural conditions of birds when ethical issues are taken in to consideration (Barash *et al*., 1992).

It has been demonstrated previously that intermittent programmes may also have beneficial effects on birds' performance (Buyse *et al*., 1996a; Svihus *et al*., 2010; Mahmud *et al*., 2011). The crop and gizzard-proventriculus capacity has been reported to increase when birds are fed intermittently which is a consequence of large feed intake and crop filling habit (Savory, 1980; Nielsen, 2004; Svihus *et al*., 2010). Prolonged retention of digesta in the crop could result in elevated lactobacilli fermentation and acidity of the upper digestive tract. This may improve the effectiveness of exogenous enzymes supplemented to feed. Svihus *et al*. (2010) observed increased exogenous phytase activity when birds were fed intermittently.

Soluble β – glucans and arabinoxylans are identified as major anti nutritional factors in barley which have shown to depress nutrient availability in broiler chickens (Villamide *et al*., 1997; Rodríguez *et al*., 2012). Increased feed and subsequently digesta viscosity has been suggested to diminish body weight (BW) gain and metabolisable energy (Yu *et al*., 1998; Svihus & Gullord, 2002; Rodríguez *et al*., 2012). In order to overcome the depressing effects of soluble fibers, exogenous β – glucanase are used in birds' diets. Decreased viscosity and improved performance of broiler chickens were observed by Mathlouthi *et al*. (2002) when exogenous xylanase and β-glucanase were added to the diet.

The present study was an attempt to investigate the effect of intermittent feeding programme on performance of broiler chickens, acidity of the crop and the effect it may have on the exogenous β-glucanase and xylanase activity. In addition, the flow of feed through the digestive tract and possible selective retention of starch and protein in the crop of intermittently fed birds were studied.

2. Literature

2.1. Intermittent feeding programmes in broiler chickens production:

Restrictions on access to feed are applied on broiler chickens in order to delay the growth and improve ethical and nutritional aspects of meat chickens management. Limited access to feed has been studied by application of either intermittent lighting or feed removal strategy. In an experiment the positive effect of intermittent access to feed was seen to be more pronounced than early feed restriction in broiler chickens (Su *et al*., 1999).

Intermittent programme may improve feed efficiency by reducing energy spent on maintenance due to decreased activities during darkness (Buyse *et al*., 1996a; Leeson and Summers, 1997). Buyse *et al*. (1997) observed increased Growth hormone (GH) secretion in male broiler chickens during compensatory growth period under intermittent lighting management, when compared with birds raised under continuous lighting regimes. Buyse *et al*. (1996a) also found that intermittent lighting improved nitrogen retention efficiency and reduced abdominal fat contents in birds.

In most cases intermittent programme is performed by introducing dark periods in to the light phase for varying length of times which is believed to reduce electricity costs and increase feed utilization efficiency (Appleby *et al*., 2004). Mahmud *et al*. (2011) reported that intermittent lighting program may lead to improved feed efficiency by significantly increasing weight gain without a major change in feed consumption in comparison with birds kept under continuous lighting program. On the other hand, Svihus *et al*. (2010) observed enhancement in feed utilization without improvement in nutrient availability and body weight gain among intermittently fed birds. Reduction in maintenance cost was suggested by the authors to be the cause of increased gain/ feed ratio.

Barash *et al*. (1992, 1993) showed that under meal feeding programme, longer retention time in the digestive tract could be expected as a result of crop filling behaviour of birds. The authors noticed that birds under *ad libitum* and 2-meal (2M) programme (fed twice daily, each meal lasted for two hours) excreted the entire marker within 2 and 6 hours respectively. While for 1-meal fed birds (1M) (fed once daily, feed available for two hours) excretion lasted for over 26 hours. Barash *et al*. (1993) observed increased crop and gizzard content

weight (Table 1) in meal fed birds during restoration, which could be a result of increased feed storage capability of the gastrointestinal tract (GIT). The authors recorded 50% of total daily feed intake was consumed within two hours of meal in 2-meal (2M) group of chicks. Severity of feed restriction seems to determine the extent to which the contents in GIT segments increase.

Table 1. Anterior GIT contents (g / kg body weight) of chicks under different feeding managements (Modified from Barash *et al*. (1993)).

		C[1]	2M[1]	1M[1]
Crop	Deprivation	0.7±0.06	0.6±0.02[*]	0.9±0.03[*]
	Restoration	1.2±0.05[c]	22.3±1[b]	63.3±7.3[a]
Proventriculus	Deprivation	0.3±0.06	0.5±0.1	0.7±0.1
	Restoration	1.1±0.2	0.4±0.08	0.4±0.08
Gizzard	Deprivation	2.7±0.8[b]	10.7±0.3[*a]	15.3±1.5[*a]
	Restoration	6.9±1.5[c]	18.2±2.3[b]	20.9±3.4[a]

[1] (C) *Ad libitum*, (2M) fed twice daily and each meal lasted for 2 hours, (1M) fed once daily and each meal lasted for 2 hours.
Within rows common letters indicate no significant difference *(P<0.05)*, while within columns (*) indicates significant difference between Deprivation and Restoration *(P<0.05)*.

Broiler chickens under intermittent lighting were shown by Buyse *et al*. (1993) to have extremely more amount of dry matter in the crop and gizzard-proventriculus at the beginning of dark phase than that during the light phase. The authors reported maximum feed intake at the beginning of the both light and dark phases. They did not observe large amounts of feed in the upper digestive tract during 14 hours of light period. It was argued that retention time of digesta in the gizzard-proventriculus during light phase was shorter than that within the darkness. Feed consumption figure illustrated by Svihus *et al*. (2010) shows significant increase in feed intake among intermittently fed birds within the last feeding bout before the lights were switched off (Figure 1). During the time period in between the first and the last feeding bouts, intermittently fed birds consumed less feed than *ad libitum* counterparts; however, large feed intake in each feeding bout was observed among these birds. This could be an indication of high storage capacity of the anterior part of the digestive tract.

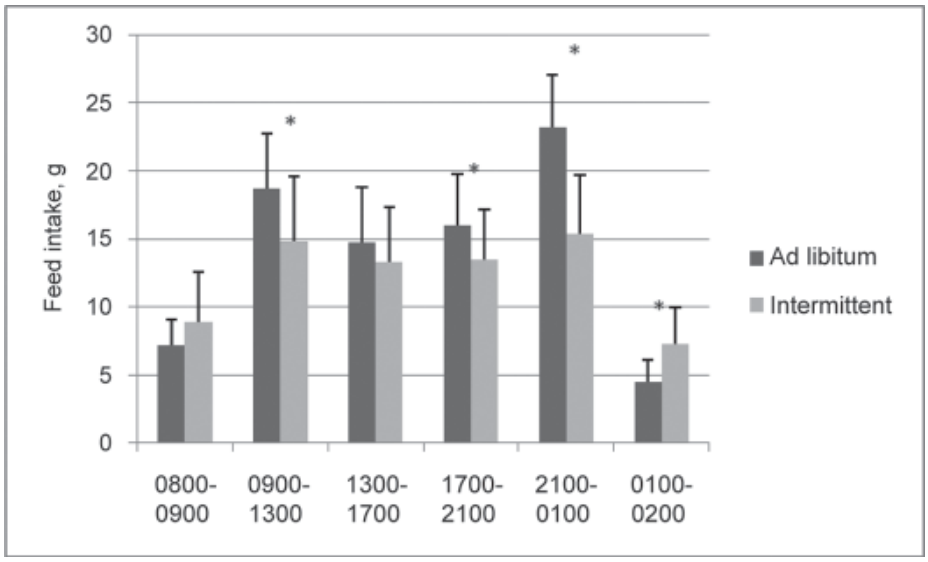

Figure 1. Average feed intake during different time periods. For intermittent feeding, feed was available for one hour within each different time period. Asterisks (*) indicate a significant difference between the feeding regimens *(P < 0.05)* (source: Svihus *et al*. (2010)).

2.2. Crop functionality under intermittent feeding regimen:

Crop is known to be a storage organ in birds and is a dilated part of oesophagus in the neck region. The activity of this segment of digestive tract is prominent when birds are fed intermittently and consequently they eat large amounts of feed in a short time to stock up feed in their crop (Savory, 1980; Nielsen, 2004; Svihus *et al*., 2010). This behaviour is also observed among birds raised on litter with low accessibility to particulate feed due to the tendency of birds to consume hard pieces of material (Appleby *et al*., 2004). However, in the crop apart from accumulation of digesta, some microflora activity has been observed which may have positive nutritional and hygienic outcomes. Guan *et al*. (2003) found that lactobacilli species which differ in proportion during the life of broiler chickens, dominated the microflora population in the crop content. Extended retention time in the crop as seen under intermittent programmes may give sufficient time to lactobacilli type of bacteria to produce more lactic acid through fermentation process. This bacterial activity in turn reduces the pH in the crop. The extent to which the pH in the crop is reduced depends on feed composition and ingredients; however, it has been reported by Bayer *et al*. (1978) that prolonged retention time in the crop may decrease the pH to 4.5. Increased acidification of crop may have two important outcomes, firstly, increased nutrient digestibility by possibly

escalating enzyme efficiency (Boling-Frankenbach *et al*., 2001) and secondly, prevention of pathogenic microorganisms (Jin *et al*., 1996).

The positive effect of increased retention time in the crop on exogenous phytase has been shown previously by Svihus *et al*. (2010) who observed improved efficiency of enzyme with lengthened retention time in the crop (Figure 2). They also detected reduced dry matter percentage with prolonged retention time in the crop of birds killed at different periods of time after feeding terminated.

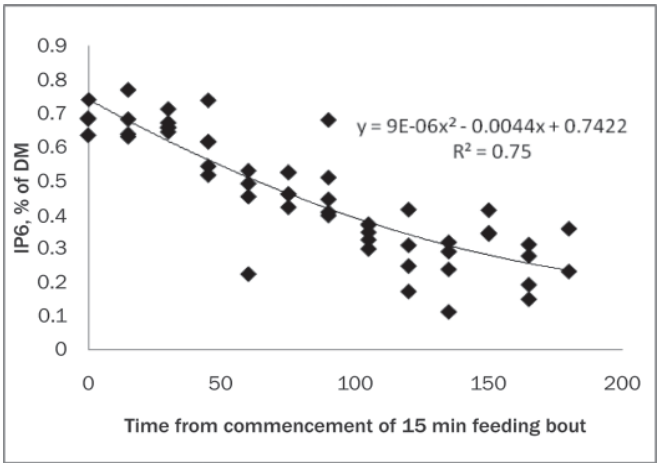

Figure 2. Percentage of inositol 6-phosphate (IP6) in the crop contents of broiler chickens that were killed at different time points after 15 minute feeding bout. The birds were meal-fed a wheat-based diet with added exogenous phytase (Source: Svihus *et al*. (2010)).

Diet composition is shown to affect lactobacilli strain population and composition in the crop. Hammons *et al*. (2010) found that the crop of birds fed maize and soybean based diets contained *lactobacilli agilis* type R5 as a dominant strain, which did not exist in the crop of birds fed the same diet but diluted with wheat middlings (high fibre content). The latter birds had significantly high ratio of *lactobacilli agilis* type R1 in their crop. Although the knowledge of nutritional effect of different lactobacilli strains activity is very limited, it has been shown that the dominance of lactobacilli reduces the proportion of unwanted and harmful bacteria throughout GIT (Mead, 2000).

The age of birds also has an impact on microbial population in the crop. Lactobacilli *cripatus* and *lactobacilli acidophilus* are shown to be the major species in the crop at 1 week of age, and *lactobacilli salivarius* was dominant at the age of 5 weeks (Guan *et al*., 2003).

2.3. Review on barley β – glucans and arabinoxylans and their nutritional effects:

Barley as well as other types of cereals contains non-starch polysaccharides (NSP) which are termed "dietary fibres" (excluding lignin) and include pentosans, cellulose, β-glucans and glucofructans (Belitz *et al*., 2009). They are classified as soluble and insoluble and have important nutritional effects. β – glucan and arabinoxylan with the amounts of 2.5-11.3 and 5.8%, respectively, are known to be the major non-starch polysaccharides in barley (Izydorczyk and Dexter, 2008). β – glucan consists of D-glucopyranosyl residues linked through β-1,3 and β-1,4 linkages (Izydorczyk and Dexter, 2008). 38-69% of the polymer is reported to dissolve in 2 hours at 38˚C (Belitz *et al*., 2009) which is close to the broiler chickens reported deep body temperature of around 41.5˚C (Lacey *et al*., 2000). Trisaccharide (DP3) and tetrasaccharide (DP4) units are released upon hydrolysis of β – glucan polymer via lichenase (Endo-1, 3(4) - β -D-Glucanase). According to Izydorczyk and Dexter (2008) DP3 and DP4 make up 90-95% and longer carbohydrates (DP ≥ 5) 5-10% of oligosaccharides (Figure 3). Arabinoxylans consists of D-xylopyranosyl units linked via (1, 4) glycosidic linkages as the main structure and L-arabinofuranosyl residues are attached to this main chain at specific positions (Izydorczyk and Dexter, 2008). The four structural components of arabinoxylans have been illustrated in Figure 4.

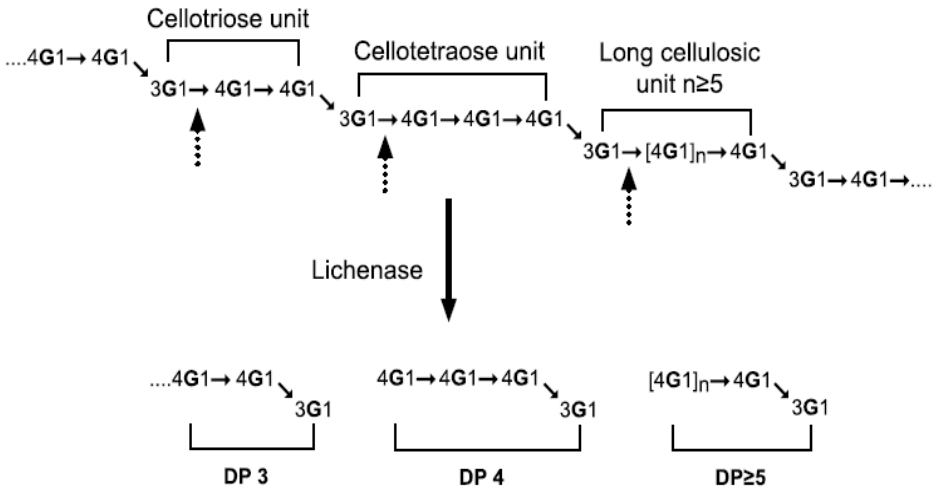

Figure 3. The structure of β – glucans and oligosaccharide units of the polymer liberated through lichenase hydrolysis (Source: Izydorczyk and Dexter (2008)).

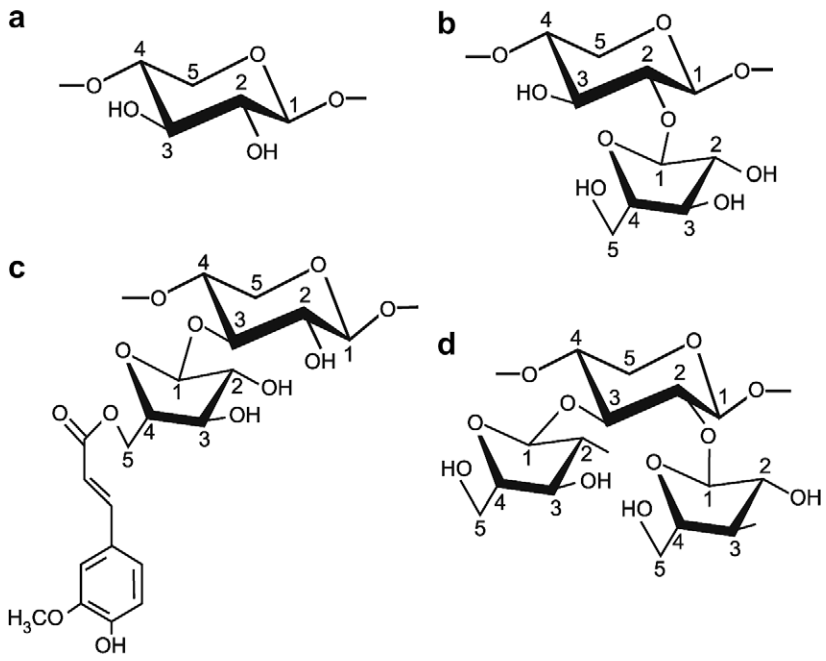

Figure 4. Components of arabinoxylans; (a) xylopyranosyl unit; (b) arabinofuranosyl attachment at position O-2; (c) arabinofuranosyl unit already esterified to ferulic acid is attached to the xylopyranosyl unit at position O-3 (d) two arabinofuranosyl residues attached to xylopyranosyl unit at positions O-2 and O-3 (Source: Izydorczyk and Dexter (2008)).

Water soluble fractions of β – glucan and arabinoxylan increase the viscosity of water solutions to a significant extent (Belitz *et al*., 2009).

Genotype of plants is a key factor determining the concentration of soluble β – glucans and arabinoxylans. Mathlouthi *et al*. (2002) have shown how difference in type of feed ingredients (Rialto wheat, Scarlett barley, Maize, and Soybean meal) can affect the proportion of soluble NSPs (Table 2).

Table 2. 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

[1] Arabinoxylans: arabinose + xylose.

Different varieties of barley also have been studied for the amount of non-starch polysaccharides and the severity of viscosity they may cause (Villamide *et al*., 1997), which clarifies the effect of genetics on the chemical composition and subsequently rheological behaviour of plant ingredients.

Table 3. NSP content and viscosity of barley cultivars based on dry matter (Modified from Villamide *et al*. (1997)).

	Spring				Winter			
	Two - rowed cultivars				Two - rowed cultivars		Six- rowed cultivars	
	Beka	Gabriela	Klaxon	Clerix	Alpha	Joline	Dobla	Hatif de Grignon
Total NSP (%)	12.37	11.97	10.2	16.51	13	11.79	15.64	19.9
Total β–glucans (%)	3.62	3.3	3.45	3.6	3.93	4.4	3.9	4.2
Viscosity(cst)*	4.93	4.83	6.75	4.32	5.77	7.03	3.6	4.82

*Kinematic viscosity, cst denotes centistokes and 1 cst = 1 cP/density of the fluid.

It has been shown that the increase in viscosity adversely affect the nutritional value of barley based diets (Yu *et al*., 1998; Svihus & Gullord 2002; Rodríguez *et al*., 2012).

Villamide *et al*. (1997) have demonstrated that AMEn is reduced as barley inclusion increases in a diet (Table 4).

Table 4. Decreased AMEn of barley based diet (Beka cultivar) as a result of increased level of barley inclusion (Modified from Villamide *et al*. (1997)).

Barley inclusion (%)	AMEn (kcal/kg DM)
30	3098.1
40	3068.8
50	3027.8
60	2998.1

Nutritional effect of high-viscosity barley-wheat based diet was compared with low-viscosity maize based diet by Rodríguez *et al*. (2012) who observed significant differences in feed intake, weight gain, AMEn and jejuna viscosity. However, feed conversion was shown to be equal in the birds fed wheat-barley based diets and those fed maize based diet. Reduced feed intake and declined AMEn as a result of higher viscosity of wheat-barley based diets was concluded to be the cause of declined weight gain (Table 5).

Table 5. Nutritional effects of Wheat-barley and Maize based diets (Modified from Rodríguez *et al*. (2012)).

Parameter	Maize based	Wheat-barley based
Weight gain (g)	1254[a]	1147[b]
Feed intake (g)	1906[a]	1749[b]
Feed conversion	1.52	1.52
AID of crude protein[1]	0.835	0.821
AID of starch	0.897[a]	0.882[ab]
AID of crude fat	0.823[a]	0.763[c]
AMEn (MJ/kg DM)	14.01[a]	13.38[bc]
Viscosity (m Pa.s)[2]	0.23[c]	1.83[ab]

Different letters indicate significant difference among means.
[1] AID denotes Apparent Ileal Digestibility.
[2] 1 cP = 1 mPa.s

Having discussed the effect of soluble non-starch polysaccharides (NSP) on the viscosity and subsequently nutritional value of barley containing diets, it seems to be of importance investigating different factors which may influence the amount of soluble and insoluble fractions of barley endosperm and cell wall. Genotype and environmental factors seem to be the most common causes of variation in non-starch polysaccharides macromolecules content. It has been reported that waxy and high amylose barley contains higher levels of β – glucan than those having normal starch, and moreover, barley grown in hot and dry conditions contain more β – glucan (Izydorczyk & Dexter 2008). Fuente *et al*. (1998) demonstrated that soluble NSPs (including β – glucan) content of a two-rowed winter barley (Beka cultivar) stored at room temperature for 0, 3, 6, 16, and 32 weeks after harvesting declined with storage time, while AMEn of diet increased. A reduction in jejunum viscosity was also observed. The authors argued that transformation from soluble to insoluble β -glucans in the barley during storage may result in decreased viscosity. The alteration was observed to occur

without changes in the total amount of β-glucans. Svihus *et al*. (1997) who studied changes in barley fibres during high moisture storage, however, discovered reduction of soluble fraction of β-glucans in steam treated and irradiated barley samples and concluded that endogenous enzymes and lactic acid bacteria are not necessarily responsible for the reduction of soluble β-glucans. The authors also found negative correlation between the amount of soluble and insoluble fractions of β-glucans with a small change in the total as a result of biochemical changes during storage. Thus, it was explained that changes in the level of β-glucans are related to alteration in the ratio of soluble to insoluble fractions. The cause of change in the ratio, referring to Miller & Fulcher (1995) report on oat fibre levels, was explained to be possibly a consequence of change in the level of non-covalent bonding with cell wall skeleton.

An experiment testing the effect of lactic acid fermentation, on soluble dietary fibre levels in the barley and wheat whole meal flours has shown that lactobacillus is capable of hydrolysing β-glucan significantly in barley and consequently enhance nutritional value of barley based diets (Table 6) (Skrede *et al*., 2003).

Table 6. The effect of lactic acid activity on fermentation parameters and carbohydrate composition of wheat and barley whole meal flour (WMF) used in chicken diets (Modified from Skrede *et al*. (2003)).

	Wheat WMF		Barley WMF	
Item	Untreated	Fermented	Untreated	Fermented
Lactic acid bacteria (CFU) [a]	2×10^3	1×10^9	1×10^3	1×10^9
pH	6.0	3.9	5.4	3.8
Dietary fibre (g/kg DM)				
Total	119	96	204	162
Soluble	12	6.0	38	23
(1,3)(1,4) β-glucan (g/kg DM)				
Total	4.9	4.8	36.9	29.3
Soluble	2.1	3.3	17.8	12.6
Total starch (g/kg DM)	651	625	556	576

[a] CFU g^{-1} WMF/water mixture.

2.4. Exogenous β – glucanase and xylanase functionality under intermittent feeding regimen:

The adverse nutritional effects of soluble non-starch polysaccharides have led to increased interest in application of exogenous β – glucanase and xylanase. There are numerous scientific publications demonstrating the positive outcome of these enzymes on broiler chickens performance. It has been proposed that exogenous β – glucanase and xylanase reduce viscosity of digesta in small intestine, which in turn improve performance of chickens (Mathlouthi *et al*., 2002; Ravindran *et al*., 2007).

Exogenous enzymes are usually obtained from bacterial or fungi sources and presented in the form of liquid or solid (powder). Within a particular species of microorganism, different strains produce enzymes which differ in optimal pI (Isoelectric point), pH and temperature. For example de Vries & Visser (2001) referring to many scientific papers demonstrated β – glucanase and xylanase synthesised by different *Aspergilli* strains to have optimum pH of between 2.5 – 7 and 2 – 6 respectively, many cases fall within 4 – 6. They also reported optimum temperature for β – glucanase and xylanase, from the same sources, range between 45 – 70 ˚C and 42 – 55 ˚C respectively. Deep body temperature of broiler chickens has been determined previously by Lacey *et al*. (2000) to be at around 41.5 ˚C, which is close to the optimum temperature of the NSP degrading enzymes, therefore, a high relative enzyme activity could be expected in broilers digestive tract temperature wise. Due to the fact that poultry are warm-blooded as other land based mono gastric animals are, the internal body temperature is maintained. However, there are factors affecting enzyme activities which vary under different circumstances such as pH and enzyme substrate concentration.

β – glucanase and xylanase activity at different pH levels modelling digestive tract of birds has been investigated in vitro by Ao *et al*. (2008) and shown in Figure 5.

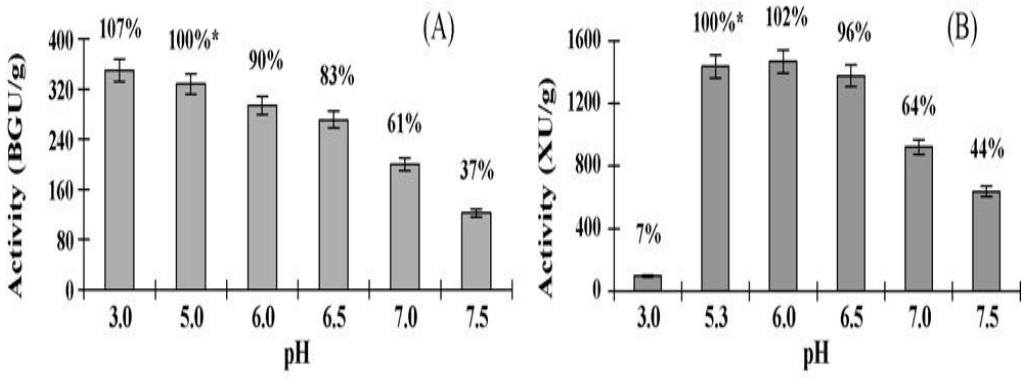

Figure 5. Fungal β – glucanase (A) and xylanase (B) activity. BGU denotes β – glucanase unit and XU denotes xylanase unit. Values on top of bars represent activity as a percentage of activity obtained at optimum pH as indicated by (*). The activities are expressed as units per gram enzyme product (Source: Ao *et al*. (2008)).

As it is illustrated β – glucanase and xylanase seem to show 100% activity at pH 5 and 5.3 respectively. Xylanase is shown to be sensitive to reduction of pH from 5.3 to 3. Based on data obtained by Jiménez-Moreno *et al*. (2009) from chickens fed different sources of fibre *ad libitum*, pH in the crop ranged between 4 -5 indicating possible activity of β – glucanase in this segment of digestive tract (Figure 5).

Intermittent feeding program in comparison with *ad libitum* regimen could be expected to decrease the crop pH due to increased retention time and elevated lactic acid synthesis. The higher acidity may result in increased exogenous enzyme activity.

One of the most important factors affecting catalytic activity of enzyme is substrate concentration, which at very low level may cause relevant enzyme to be even non-functional (Matthews & van Holde, 1996) (Figure 6).

The effect of substrate concentration on enzyme kinetics has been also investigated by De Gende & Alonso (1983) and also Rees (1984), who found that product accumulation tended to be zero when substrate concentration was too low.

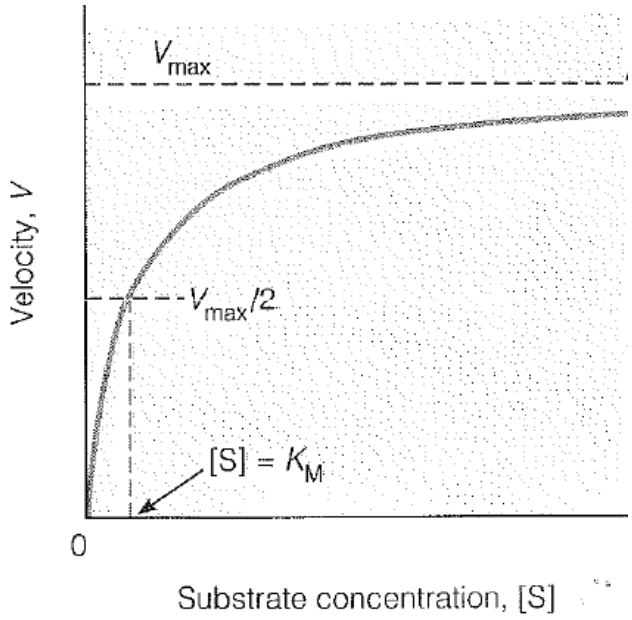

Figure 6. The relationship between velocity (V) and substrate concentration [S]; Km is the concentration of substrate that leads to half-maximal velocity (Source: Matthews & van Holde, 1996).

Exogenous xylanase and β-glucanase have been shown to reduce intestinal viscosity by degrading soluble fibres. This functionality may improve availability of nutrients and increase digestive enzymes diffusion. Mathlouthi *et al*. (2002) discovered improved energy availability (AMEn) along with increased crude protein and fat digestibility coefficients in broiler chickens from 4 to 20 days of age. In their experiment wheat-barley based diets with or without xylanase and β-glucanase supplementation was compared with maize based diet (Table 7). The nutritional improvements were seen to happen in conjunction with decreased viscosity and increased feed intake. It was then suggested by the authors that wheat-barley based diets containing high amount of fibre could be substituted with maize based diets if enzymes are included.

Table 7. The effect of cereal type and enzyme addition to wheat-barley based diets on broilers performance, small intestine physicochemical characteristics and nutrient digestibility from 4 to 20 days of age (Modified from Mathlouthi *et al*. (2002)).

Variable	Corn based diet (A)	Wheat and Barley-based diet (B)	Wheat and Barley-based diet+E[1] (C)	Level of significance		
				A,B	A,C	B,C
Weight gain (g)	605±57	438± 98	619± 67	P<0.0001	P=0.6765	P<0.0001
Feed intake (g)	899 ± 71	714± 138	924± 89	P=0.0002	P=0.5841	P=0.0002
Feed/gain ratio	1.489± 0.046	1.645± 0.104	1.495± 0.030	P<0.0001	P=0.8538	P=0.0001
Digestibility coefficients (%)						
Crude protein	86.34± 1.22	82.21± 3.71	86.81± 3.95	P=0.0018	P=0.7057	P=0.0015
Crude fat	81.19± 1.72	77.88± 3.15	82.75± 1.34	P=0.0007	P=0.0879	P<0.0001
AMEn[2] (MJ/kg DM)	13.56± 0.19	12.91± 0.33	13.32± 0.16	P<0.0001	P=0.0507	P=0.0014
Viscosity[3](relative viscosity mPa.s)	1.074±0.169	1.683±0.270	1.306±0.131	P<0.0001	P= 0.0271	P=0.0008
pH (duodenum+Jejunum	6.33±0.17	6.06±0.19	6.00±0.22	P=0.0010	P<0.0001	P=0.4223

Level of significance *(P≤0.05).*
[1] Xylanase and β-glucanase.
[2] Apparent metabolizable energy corrected to a zero nitrogen balance.
[3] viscosity of supernatant of small intestine content. It was measured as Natural logarithm (Ln) of relative viscosity (viscosity of the solution to the viscosity of the buffer used). 1 mPa.s = 1 cP

It has been reported that dry matter content of small intestine is increased and water intake by animals decreased upon application of NSPase enzyme to diets of broiler chickens (Hesselman & Åman., 1986; García *et al*., 2008). This may have occurred due to degradation of β-glucans and arabinoxylans to low molecular weight polymers resulting in more fluidity of material in small intestine and higher accessibility of digesta water to animals. However, García *et al*. (2008) did not observe significant change in excreta dry matter content when diet was supplemented with enzyme.

Exogenous xylanase and β-glucanase enzymes have been observed to reduce the pH of the crop in chickens fed barley or oat based diets (Józefiak *et al*., 2006) which were expressed to be a result of stimulation of lactic acid producing organisms in this segment of digestive tract (Table 8). Changes in the concentration of volatile fatty acids in the crop indicate significant changes in microbial fermentation as a result of cereal type used in the diet.

Table 8. Lactate and short fatty acid concentrations in the crop of broiler chickens fed barley and oat based diets with or without enzyme (Modified from Józefiak *et al*. (2006)).

Fatty acids (µmoles/g digesta)	Barley		Oats		Effect of treatment (P- value)		Interactions (P-value)
	-	+	-	+	Cereal	Enzyme	Cereal×Enzyme
Acetate	7.06^{b}	11.40^{ab}	10.10^{ab}	13.66^{a}	0.2067	0.0664	0.85
Propionate	n.d.	n.d.	n.d.	n.d.	-	-	-
Butyrate	n.d.	n.d.	n.d.	n.d.	-	-	-
Lactic acid	13.25^{c}	41.20^{b}	27.73b^{c}	64.13^{a}	0.0171	0.0001	0.5683
Total	20.32^{c}	52.61^{ab}	38.28b^{c}	77.94^{a}	0.0241	0.0004	0.6886
pH	5.42^{a}	4.76^{c}	5.12^{b}	4.62^{c}	0.0061	0.0001	0.2695

(-) and (+) indicate without and with exogenous xylanase and β-glucanase inclusion in the diet respectively.
(n.d) denotes "Not determined".
Values with different letters are significantly different.

3. Materials and methods:

3.1. Diet composition and processing:

Diets were processed at the Centre for Feed technology (FôrTek), Norwegian University of Life sciences (UMB) located in Ås, Norway.

Norwegian barley grains harvested in 2010 was used in the current experiment due to unusual heavy and frequent precipitation during the year 2011. Two barley based diets, one with and the other without enzyme were processed. The enzyme used in the experiment was Axtra XB 201 L type with 12200 U/g endo- 1,4-beta xylanase and 1520 U/g endo-1,3- beta glucanase activity which was a product of Danisco A/S, Denmark. It was discovered that the amount of enzyme added to the diet 2 was 2.5 times as much as maximum recommended level of 200 g/tonne feed due to human error at the time of processing. Titanium dioxide was used as a marker. However, it was also found out that the amount of titanium dioxide added to the feed was extremely lower than that in diet formulation due to human error; therefore the results regarding marker concentration and illeal digestibility of nutrients could not be used and are not discussed in the current work. The diets were made to meet nutritional requirements of experimental birds according to Aviagen Group Ross 308 Broiler (2007).

Table 9. Composition of the diet.

Ingredients	Diet 1 (g/kg)	Diet 2 (g/kg)
Barley	660	660
Fish meal	90	90
SBM[1]	184	184
Soy oil	30	30
Lime stone	10	10
MCP[2]	10	10
DL- Metionin	2	2
L- Threonin	1	1
Salt	2.5	2.5
Mineral[3]		
premix.FKØV	1.5	1.5
Vitamins[4]		
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

[1]SBM denotes soy bean meal.
[2]MCP denotes mono calcium phosphate.
[3]Mineral premixes supplied the following per kg of diet: Fe 50 mg; Mn 40 mg; Zn 70 mg; Cu 10 mg; I 0.5 mg; Se 0.2 mg.
[4]Vitamin and mineral premixes supplied the following per kg of diet: retinol 3.4 mg; cholecalciferol 0.062 mg; tocopherol 55 mg; menadione 6.6 mg; pyridoxine 4.4 mg; riboflavin 17.6 mg; pantothenic acid 18.25 mg; biotin 0.286 mg; thiamine 2.75 mg; niacin 55 mg; cobalamine 0.022 mg; folic acid 2.75 mg.

Table 10. Calculated diet composition (as-fed basis).

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

Barley and soy bean meal were ground by hammer mill (Model: E-22115 TF, Münch-Wuppertal, Germany, under Bliss-USA, 18.5 kW and 2870 rpm) separately on a 3-mm sieve. 3 batches each weighing 250 kg were produced consecutively and mixed in the mixer conditioner (Twin Shaft Paddle, Tatham of England under license from Forberg, Norway, 400 lt, 4kW). Duration of mixing was 2 minutes for each batch from the moment micro ingredients were added to the mixer until the addition of soy oil. FôrTek made tank was used to spray soy oil on the batches in the mixer. Soy oil was poured into the tank and then air was pumped in to create the pressure of 4 bars. The tank then was put on the scale which was zeroed before spraying. Soy oil was sprayed on the mash due to pressure release until the amount of -7.5 kg was displayed on the scale screen (3% of each batch). The nozzle used for this process 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). The mash was mixed for two minutes after oil addition, thus, total mixing time being 8 minutes and 45 seconds. Samples were taken after mixing from all batches randomly. The samples from each batch placed in a bucket and mixed. Final representatives of mixed mash poured into two sample bags.

Prior to feed processing following a normal procedure, density of mixed mash was measured and inserted into the pellet press control panel for automatic calculation of the machine's capacity. A one litre container was filled with mixed mash after its weight was zeroed on scale, then it was weighed again to obtain the net weight of the mixed mash. Density was then measured to be 608 g/litre.

Feed mash was mixed and conditioned with steam at 75 ºC which usually lasts for 20 to 30 seconds in the twin pass conditioner (Twin Pass, Muench, Germany, 2t/h, 2 x 2m x 40cm) before it was processed in a pellet mill (Muench, Germany, 1.2 t/h max. capacity, 2 x 45 kw). In the beginning of pellet production the processing parameters were recorded once and are presented in Table 11.

Table 11. Processing parameters:

Temperature in the conditioner	(ºC)	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 Ampers motor	(amp)	15.50
Energy Consumption	(kW)	9.53
Specific Energy Cons.	(kWh/kg)	0.0159
Temperature of the pellet upon discharge	(ºC)	86.8

In order to measure pelleted feed temperature upon discharge, an insulated box was used and filled up with pellets at the opening of the pellet mill. The temperature was read from thermometer attached to the box.

Pelleted diets were cooled in a counter-flow cooling system for 30 minutes which uses ambient air to reduce temperature of the product (Miltenz, New Zealand, capacity of 2000 kg/h). Cooled pelleted products were packed in 26 bags each containing 25 kg pelleted feed, total feed production calculated to be 650 kg. 13 bags were chosen randomly prior to enzyme

application. Selection of the bags in a random procedure was done to equally distribute the effects of feed processing operation between the two diets (with and without enzyme).

Pelleted feed without enzyme was first put into the mixer while the equipment was running. 1.16 kg of water was then sprayed into the mixer for 45 seconds. The same tank and nozzle were used for spraying process on the two diets. The nozzle capacity size was 6503. The tank used for the process had the following specifications:

Opür, Model 2006, F50 1" 20"
Capacity 2500 lit/hour
Range of working temperature 0 – 80 ºC
Maximum pressure 10 bars.
Company Teknisk Vannservice A.S
PO Box 5 Stovner Oslo.

Diet 2 was sprayed on for exactly 45 seconds with a solution of water and enzyme. 160 grams of liquid Axtra XB201L enzyme was diluted in one litre of water prior to spraying process. Thus, the same amount of liquid was added to both diets.

Separate samples were taken from both diets representing diet 1 (without enzyme) and diet 2 (with enzyme) while they were being discharged into 500 kg bags.

3.2. Experimental animals and feeding:

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

200 day-old male broiler chickens (Ross 308) were placed in brooder cages and fed on a commercial starter diet. At 7 days of age, the birds were weighed, animals weighing between 160 to 210 grams (average individual weight of 182 grams) were selected and the rest (approximately 30-35 birds) were discarded. The selected birds were distributed randomly among 48 mesh floor cages, three birds per cage (50cm×35 cm×20 cm). Two racks of cage (24 cages per rack) were placed such that the intermittently fed birds did not have visual contact with the *ad libitum* counterparts. Diet 1 (without enzyme), were given to cages with odd number. Diet 2 (with enzyme) was given to cages with even numbers. A bucket was

assigned to each cage and was filled with 5 to 6 kg of feed. The gross weight of the buckets was recorded.

Until 7 days of age lights were on for 24 hours a day, and from 7 to 34 days of age, lights were switched off from 23.00 to 03.00 and from 04.00 to 08.00. The birds under intermittent feeding regimen, from 7 to 12 days of age were fed *ad libitum* from 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. From 12 until 34 days of age, intermittently fed birds were fed *ad libitum* from 08.00 to 09.00, 13.00 to 14.00, 17.30 to 18.30 and 22.00 to 23.00. The feed was removed from the birds during the period of 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.

Temperature was reduced from 33 °C to 29 °C at 7 days of age, and to 26 °C at 16 days of age. At 18 days of age the amount of feed intake and excreta were recorded for 3 days and excreta was stored in a freezer to determine AME. At 21 days of age the birds were weighed in groups of three from the same cages and the buckets containing feed were weighed. Two randomly selected birds from each cage were transferred into 12 pens (6 pens assigned to intermittently and the other 6 to *ad libitum* fed birds) in a room where temperature had been already set at 22 °C. The intermittently fed birds were placed such that they did not have visual contact with the *ad libitum* fed birds. The two birds from each cage were identified by wrapping a strap around their feet. Birds from four cages were placed in one pen, therefore using four different colours of straps. Intermittently and *ad libitum* fed animals were allocated to pens 1–6 and 7-12 respectively.

The remaining birds in cages were killed on the following day and were dissected to collect samples from crop, gizzard and small intestine. A strap was wrapped around the neck of the birds immediately following cervical dislocation to avoid crop content regurgitation, the digestive tract was removed and the contents from crop, gizzard, duodenum + jejunum and ileum were collected quantitatively and following freezing in liquid nitrogen. Intermittently fed birds were supposed to be sacrificed after 3 hours having access to the feed but due to human error they were killed approximately one hour after having access to the feed, thus, the samples taken on the day were discarded and were not used for further analysis.

At the age of 34 days, the lights were switched on at 06.00 in the morning, after the feed troughs were emptied for the intermittently fed birds. From 07.30 to 08.00 all intermittently

fed birds and their feed troughs were weighed and from 08:00 to 08:30 the same procedure followed on *ad libitum* fed birds. Birds in pens 1, 2,3,4,5 and 6 were given access to feed from 08.00 to 08.30, 08.20 to 08.50, 08.40 to 09.10, 09.00 to 09.30, 09.20 to 09.50, and 09.40 to 10.10, respectively. At 09.00, one bird of each colour (4 in total) from each of pen 7 to 12 were killed and dissected by cervical dislocation. At 11.30 feed was removed from pen 1 and then 4 birds (randomly selected based on strap colour as explained earlier) in the pen were killed, and a strap was immediately wrapped around bird's neck to avoid regurgitation, there after 4 animals were sacrificed under the same procedure from each of pens 2,3,4,5 and 6 every 20 minutes until 01.10 pm. In this way all the birds were killed exactly 3 hours after feed was taken away from them. Contents from the crop, gizzard, duodenum + jejunum and ileum were collected and frozen in liquid nitrogen. Chemical analysis from these samples could not be executed due to shortage of time. Feeding the rest of birds under intermittent system was carried out from 16:00 to 17:00 pm and 20:00 to 21:00 pm the night before termination of the experiment. Feed troughs belonging to intermittently fed birds were weighed at 21:00 pm after the lights were switched off.

Lights were turned back on for an hour from 01:00 to 02:00am and after five hours of darkness at 07:00am feed was removed from pens 1 to 6 and then lights were turned back on. The weight of feed troughs was recorded before feeding all intermittently fed birds in the first six pens started at 08:00 am. Birds were allowed to eat for 40 minutes then at 08:40 am feed was taken away from pens 1 to 6 and animals were randomly selected and killed as follows: at 08:40 am one bird in pen 1 and two birds in pen 2, 09.20 am two birds in pen 3 and 1 birds in pen 4, 10.00 am two birds in pen 5 and two birds in pen 6, 10.40 am two birds in pen 1 and two birds in pen 2, 11.20 am two birds in pen 3 and two birds in pen 4, 12.00 pm two birds in pen 5 and two birds in pen 6.

Dissection executed on all birds to collect contents of crop, gizzard, duodenum + Jejunum and ileum followed by freezing in the liquid nitrogen which were used later for starch, protein and marker (Titanium dioxide) analysis and also investigation of material flow dynamics in digestive tract.

3.3. Sample analysis:

3.3.1. Digestive tract and excreta:

Contents collected from the four segments of digestive tract 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 dry matter content without encountering any possible biochemical changes in the samples.

On day 18 at 12.00h, feed and birds were weighed and cleaned trays replaced under the cages. On day 19 and 20 of the trial excreta was collected from the cages after it was cleaned for feed and feathers and then put in white boxes. The collection procedure was followed in the same order. The boxes were kept frozen between collections. On day 21, all birds and feed were weighed before two randomly selected birds from each cage were placed in the experimental pens.

3.3.2. Chemical analysis:

The buckets containing excreta samples thawed over night at room temperature and the gross weight of each bucket was measured, then each bucket`s content was homogenized by mixer. Representative samples were taken and put in the crucibles which were weighed immediately after. Samples then were dried in the oven at 104˚C during night (16 hours) then cooled in the desiccators for 30 minutes following weight measurement to obtain net weight of the dried samples. The samples in the crucibles then were put in the bomb calorimeter (Bomb calorimeter PARR 1281, Moline, Illinois, USA). The result was recorded to calculate AME.

In order to determine starch, protein and Titanium dioxide (marker) levels in dried material, all samples taken on the last day of experiment from intermittently fed birds were ground by mortar/pestle after freeze drying process. Due to the existence of large particles in the crop and gizzard samples, collected material from these two segments was ground by Retsch centrifugal mill (Model ZM 100, Retsch Technology GmbH., Haan, Germany) on a 0.5mm sieve. Starch, protein and marker concentrations were also determined on both feeds. Each of feed samples taken during the experiment were divided in to two portions and each portion was split into two pieces and one piece as a representative sample was ground by Retsch centrifugal mill on a 0.5mm sieve before chemical analysis. Starch, protein and Titanium dioxide were determined at Animal and aquaculture sciences department – Norwegian university of life sciences, by AACC Method 76-11, Kjeldahl digestion and Short *et al*. (1996) method, respectively.

3.3.3. Viscosity and pH measurements:

The amount of 0.4 grams of freeze dried sample was taken from each container holding crop and duodenum + jejunum contents (collected on the last day of experiment from intermittently fed birds) and placed in a centrifuge tube (2mL capacity) and 1.5 mL of distilled water maintained at temperature of 40˚C by a shaking water bath (Julabo, Model SW22, Labortechnik GmbH Seelbach, Germany) was added into the centrifuge tube to obtain a wet sample. The viscosity of the supernatant after centrifugation for 5 minutes determined by a viscometer running at 60 rpm (Brookfield, DV – II, Brookfield Engineering Laboratories, Massachusetts, USA) followed by pH measurement on crop samples (Hamilton, Tiptrode electrode, Bonaduz, GR, Switzerland). The pH sensor could not be calibrated at pH=7 but the calibration was successful at pH=4 in the beginning of the process.

3.4. Calculations:

Passage rate through the crop per hour was calculated using the following equation:

$$\text{Passage rate (gram digesta per hour)} = \frac{(\text{DM intake for 40 minutes} - \text{DM left in the Crop}) \times 60}{\text{Time from feeding to dissection (minutes)}}$$

DM denotes dry matter in the equation.

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

$$\text{AME} = \frac{(\text{Feed intake} \times \text{GEdiet}) - (\text{Excreta output} \times \text{GEexcreta})}{\text{Feed intake}}$$

GE denotes Gross energy.

3.5. Data analysis:

Statistical Analysis System (SAS, 2006) was used for data analysis with General Linear Model (GLM) in a 2×2 factorial design.

4. Results:

For the period of 7-21 days of age intermittent feeding 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)* when compared to *ad libitum* feeding (Table12). However, energy utilisation was not affected by feeding regimen *(P>0.05).* Exogenous enzyme supplementation had only a significant effect on AME *(P<0.0001)* and combination of feeding regimen and exogenous enzyme did not influence any variables *(P>0.05).*

For the period of 21-35 days of age, feed consumption was reduced significantly as a result of enzyme supplementation *(P=0.03).* In contradiction of the previous period (7-21 days of age) none of the parameters were affected by feeding regimen *(P>0.05).* Exogenous enzyme showed a tendency to improve feed utilisation efficiency *(P=0.08).*

No significant interaction between exogenous enzyme and feeding regimen was observed throughout the experiment *(P>0.05).*

Enzyme supplementation did not affect the viscosity of the digesta in crop and duodenum + jejunum of intermittently fed birds as demonstrated in Table 13. Prolonged retention time in both segments did not change the viscosity of digesta significantly. Water viscosity at 40 ˚C was measured to be 0.9 cP which is very close to the crop content viscosity up to 240 minutes after feeding (Table 13).

Table 12. 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				21-35d		
	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 error[1]	45.3	39.8	0.06	0.08	82.6	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 × 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.

Table 13. The effect of enzyme supplementation on viscosity (cP) [1] of crop and duodenum + jejunum content of intermittently fed birds. The differences are not significant *(P>0.05).*

	Crop		D+J[2]	
Time after feeding (minutes)	+	-	+	-
40	1.0	1.21	1.55	1.75
80	1.02	1.10	2.15	2.11
120	1.0	1.15	1.83	1.88
160	1.0	1.01	2.55	2.04
200	1.04	1.10	2.27	2.44
240	1	1.15	2.25	1.94
Average	1,0	1,1	2,1	2,0

(+) and (-) denote with and without enzyme supplementation respectively.
[1] cP = centipoise.
[2] D+J indicate duodenum + Jejunum.

The dry matter content in the crop obtained at different times after feeding started, shows a large variation, however, the dry matter percentage of the segment decreased to 28% after 200 min (Figure 7). It was also noticed that the crop dry matter content exhibits gradual reduction over time (Figure 8).

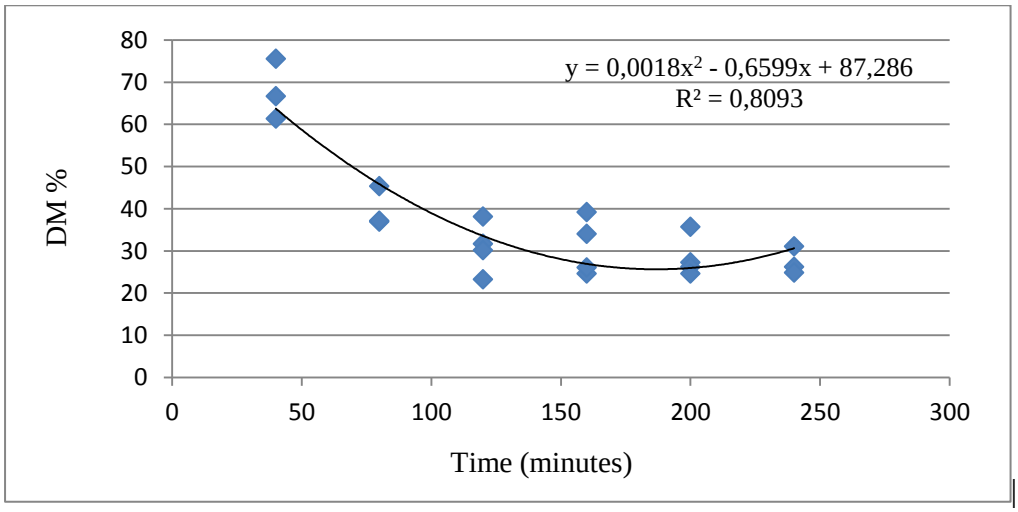

Figure 7. Percentage of dry matter (DM %) in the crop at different times (minutes) after feeding initiation.

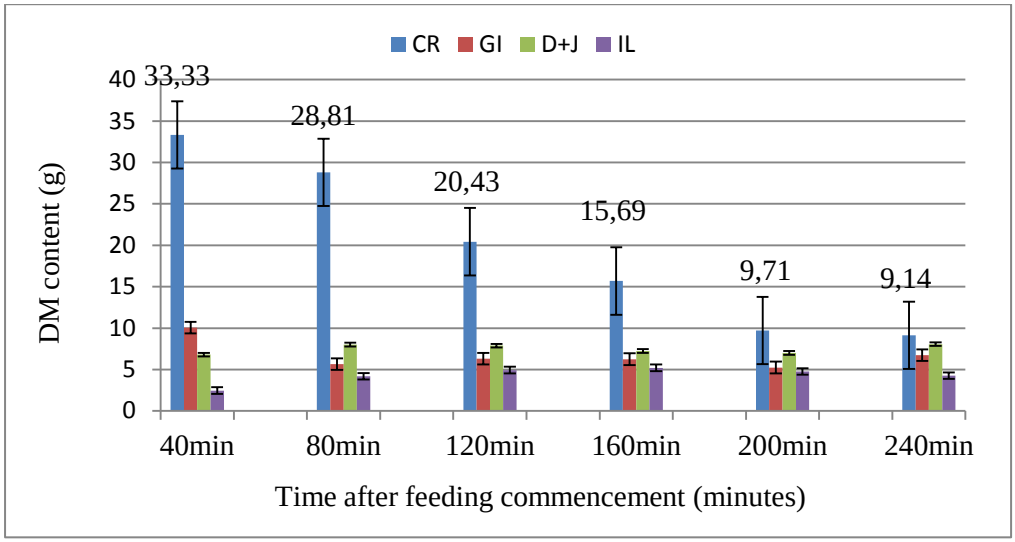

Figure 8. Gradual reduction in crop content at different times after feeding commencement and dry matter (DM) content of digesta in different segments of GIT (prior to ileocecal junction). Values presented as mean ± standard error (SE). CR: Crop, GI: Gizzard-Proventriculus, D+J: duodenum + Jejunum, IL: Ileum.

The passage rate of dry matter (DM) through the crop showed reduction over time (Figure 9) and dry matter percentage of the digesta was seen to increase along with the passage rate. It was assumed that all the birds from the same pen had consumed the same amount of feed during a 40 minute feeding bout.

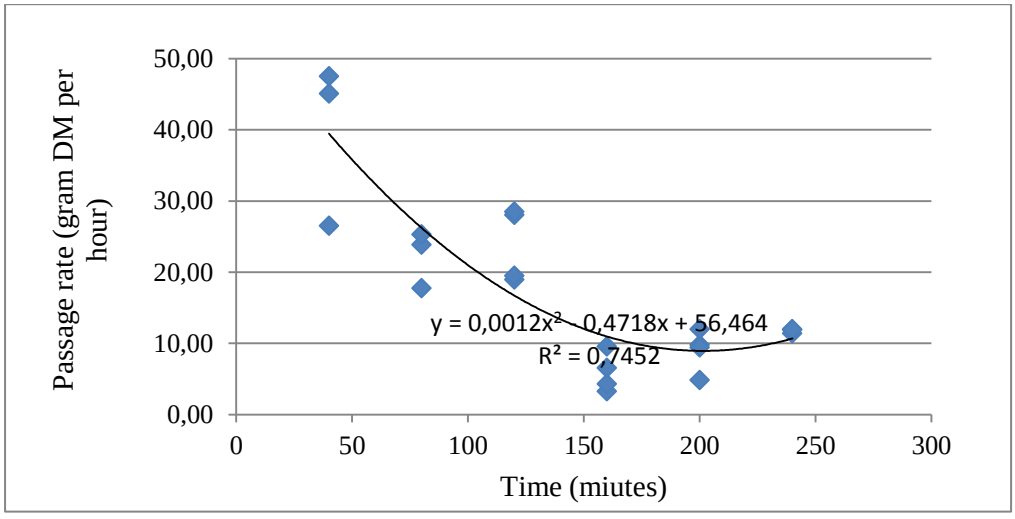

Figure 9. Passage rate (gram DM per hour) through the crop over time (minutes).

The pH of the experimental feed was measured to be 5.5 and the average pH of the crop at different dissection intervals are shown in Figure 10. After 240 minutes of retention in the crop the pH was significantly reduced to 4.7.

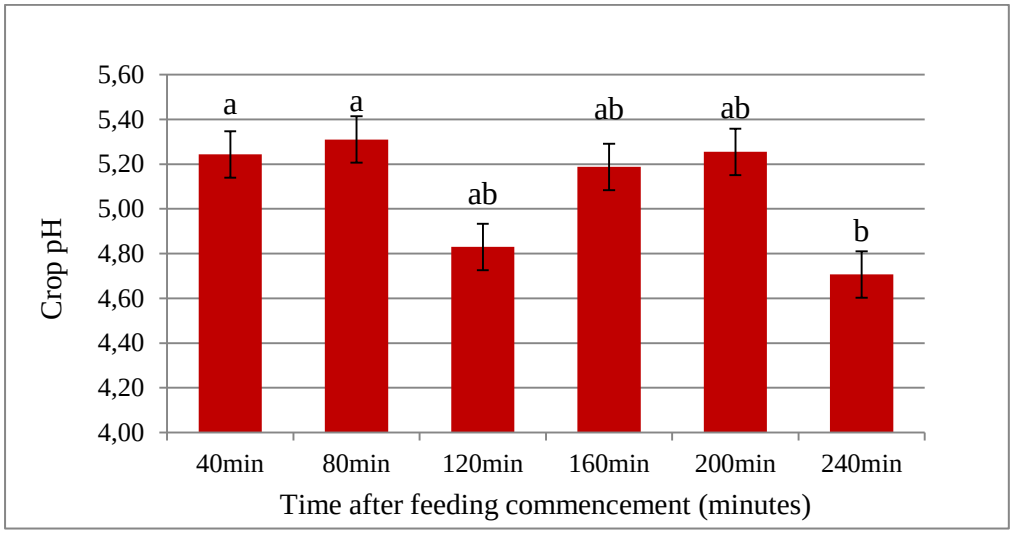

Figure 10. Crop pH measured at different times of sampling. Values presented as mean ± standard error (SE). Different letters are indication of significant difference *(P<0.05)*.

The starch and protein concentrations of the digesta in the crop did not show changes from that in the feed over time (Figure 11) , however, starch concentration was reduced in the proventriculus-gizzard and the reduction was significant from 40 to 240 minutes after feeding started (Figure 12). Protein ratio did not show a significant difference over time in proventriculus-gizzard (Figure 13).

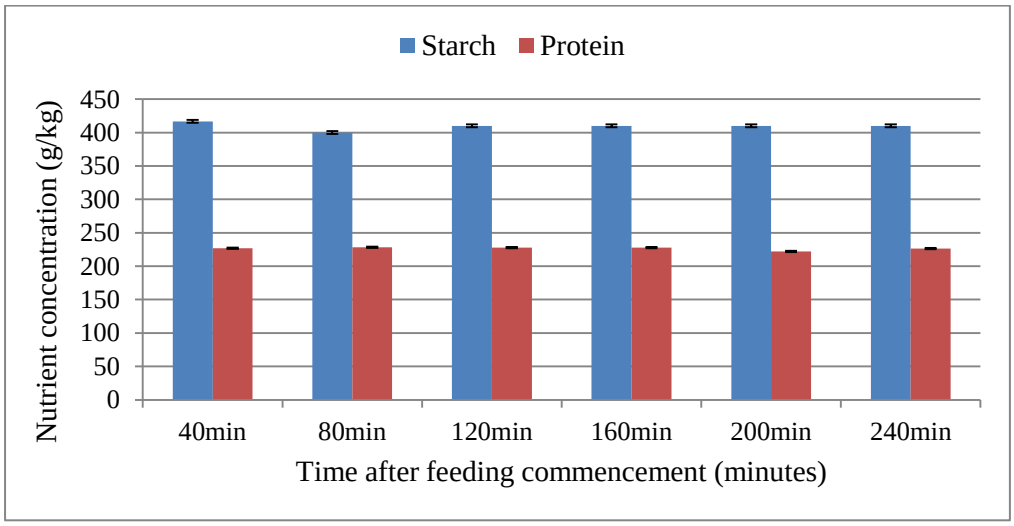

Figure 11. Concentration of starch and protein in the crop at different times after feed was given. Values presented as mean ± standard error (SE). No significant difference was observed in the ratio of starch and protein over time *(P>0.05).*

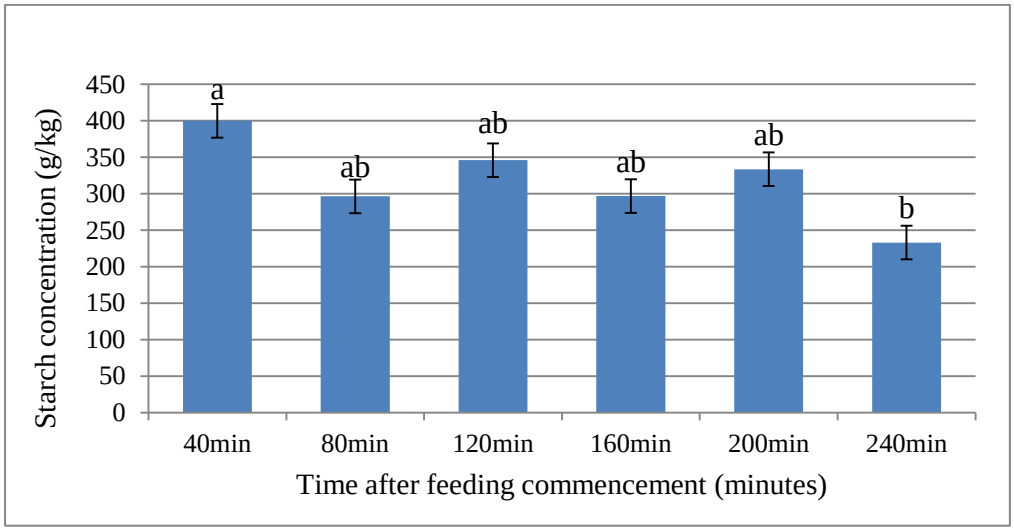

Figure 12. Concentration of starch in the gizzard- proventriculus content at different dissection times. Values presented as mean ± standard error (SE). Bars headed with different letters are significantly different *(P<0.05).*

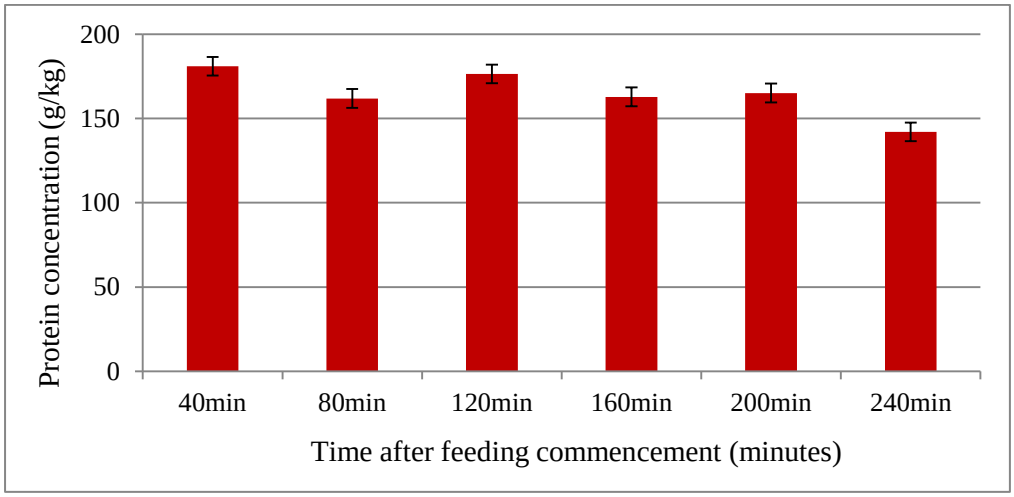

Figure 13. Concentration of protein in the proventriculus-gizzard content at different dissection times. Values presented as mean ± standard error (SE). Bars without head letters are not significantly different *(P>0.05).*

In duodenum + jejunum protein concentration was observed to be higher than that in the feed at all dissection times (Figure 14); on the other hand, proportion of starch was much lower

(more than 50% of that in the feed). The changes in concentration of both nutrients over time, however, were not significant.

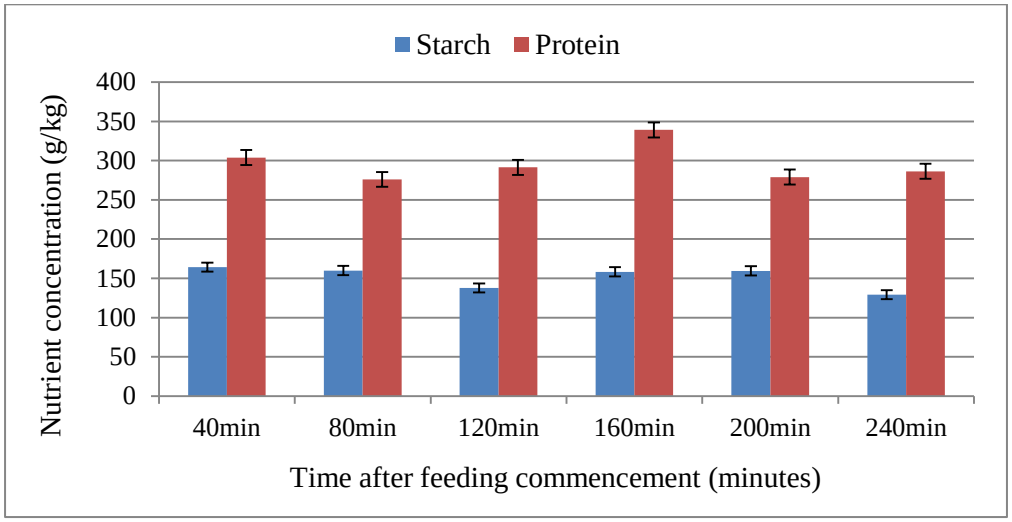

Figure 14. Starch and protein concentration in duodenum + jejunum. Values presented as mean ± standard error (SE). No significant difference in the nutrients concentrations over time *(P>0.05).*

Figure 15 shows that changes in the nutrients ratio over time are not significant and demonstrate that the percentage of protein and starch for all dissection times are lower than that in the duodenum + jejunum.

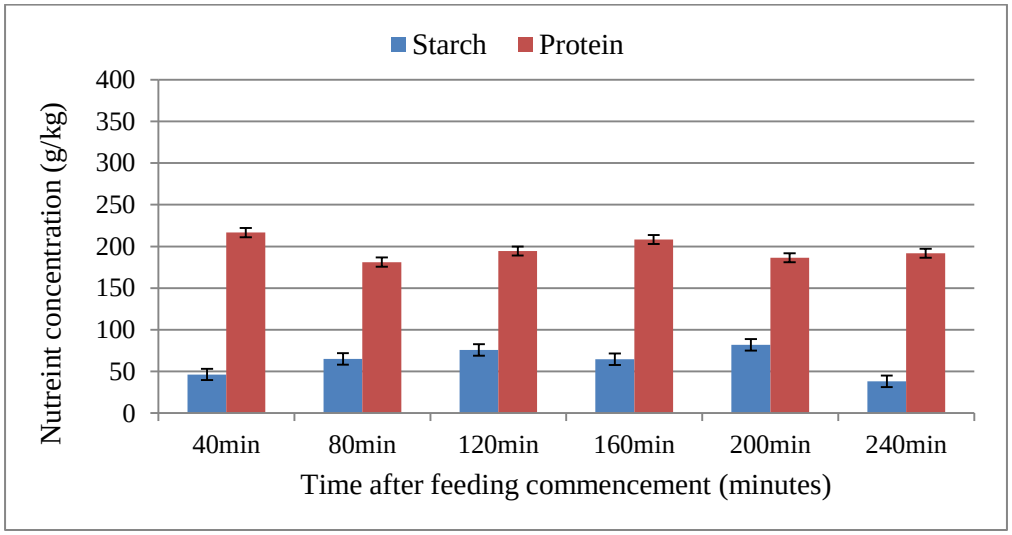

Figure 15. Starch and protein concentration in ileum. Values presented as mean ± standard error (SE). No significant difference in the nutrients concentrations over time *(P>0.05).*

5. Discussion:

The lack of significant effect of exogenous enzyme on the performance of broiler chickens from 7 to 21 days of age is contrary to various observations (Mathlouthi *et al*., 2002; Svihus & Gullord 2002; Lazaro *et al*., 2003; García *et al*., 2008). However, feed efficiency was very high for *ad libitum* and intermittently fed birds when compared to the producer predicted figures for high performance under continuous feeding regimen (Aviagen Group Ross 308 Broiler: Performance objectives, 2012).

The F:G (feed per gain ratio) reached in this experiment has also been found to be lower than that in many other reports when barley based diets had been used (Yu *et al*., 1998; Mathlouthi *et al*., 2002; García *et al*., 2008). It may be assumed that the birds' genetic capacity in this trial was greater than those used in cited experiments or the environment was more favourable to the animals. However, significant effect of enzyme was observed on AME *($P<0.0001$)* up to 21 days of age, despite the fact that intestinal viscosity was not affected by such an enzyme. It is thought that the effect of xylanase and β-glucanase is not limited to only viscosity reduction. NSP in the cell wall of cereals are believed to entrap nutrients and as a result of this, so called "cage effect", the digestibility of starch and protein is decreased. Application of xylanase and β-glucanase may hydrolyse cell wall constituents and consequently increase availability of nutrients (Campbell & Bedford, 1992; Bedford & Schulze, 1998). Additionally, production of monosaccharide or fermentable sugars because of NSP hydrolysis and subsequently release of volatile fatty acids (VFAs) in the cecum may contribute to the change in AME. Elevated ratio of VFAs was observed in cecum of chickens upon application of commercial glycanase (with arabinoxylanase, β-glucanase and pectinase activity) by Choct *et al*. (1996). Furthermore, Mathlouthi *et al*. (2002) found that intestinal pH of chickens fed wheat-barley based diet supplemented with NSP degrading enzyme remained lower than that of chickens fed maize based diets and argued that this was a result of produced fermentable sugars and subsequently VFA accumulation. The contribution of VFAs in the cecum to metabolisable energy has been explained and demonstrated in some reports (Engberg *et al*., 2002; Moran, 2006).

Tendency to improve FCR during the last 14 days of experiment was observed with the application of enzyme *($P = 0.08$).* This may have occurred due to significant reduction in

feed intake *(P = 0.03)* while BW gain remained unchanged. Increased AME when enzyme was added to the diet might have led to the decreased feed intake during the last days of trial. Negative correlation between feed intake and AME of barley based diet when NSP degrading enzyme was used has been demonstrated previously (Svihus & Gullord, 2002). The lack of interaction between feeding regimen and exogenous enzyme during the experiment contradicts the report from Svihus *et al*. (2010) who observed increased efficiency of exogenous phytase among intermittently fed birds. In this trial the severity of intermittent feeding was even slightly more than that in the experiment performed by Svihus *et al*. (2010); therefore, longer retention of digesta in the crop could be expected. Possibly the ratio of soluble fibre and consequently viscosity of the diet was too low to see any significant interaction between enzyme and intermittent system.

The viscosity in duodenum + jejunum of intermittently fed birds shows greater figures than that in the crop. This may be due to the absorption of nutrients in small intestine which could have increased the proportion of soluble fibres. However, when compared to a number of other studies (Yu et al., 1998; Józefiak et al., 2006; García et al., 2008), viscosity in duodenum + jejunum was found to be low.

Less viscosity in the small intestine of broiler chicks has been demonstrated by several authors to increase performance of birds by improving digestibility and availability of nutrients (Hesselman & Åman, 1986; Svihus & Gullord, 2002; Rodríguez *et al*., 2012).The method used for viscosity measurements on dry samples, the barley variety and environmental factors affecting barley before harvesting might be the cause. For example different cultivars of barley have been reported previously to have diverse viscosity properties (Villamide *et al*., 1997; Izydorczyk & Dexter, 2008). The environment in which barley is grown has been shown to affect concentration of soluble NSPs and subsequently viscosity of the grain through polymers cross-links and interactions (Izydorczyk & Dexter, 2008). Bacterial and fungal fermentative activities before harvesting the barley under particular environmental conditions may result in reduced concentration of soluble fraction of fibres. It has been argued that the microbial source of exogenous enzyme may determine its hydrolysing activity on a particular substrate (Bedford & Schulze, 1998). Moreover, within a specific source, degradation activity could largely differ on variety of substrates. The barley used in this experiment was harvested in the previous year; therefore it could be assumed that long storage time led to decreased concentration of soluble NSPs. Reduction in the ratio of

soluble fibres in barley as a consequence of prolonged storage time was shown by Fuente *et al.* (1998).

Poorer feed efficiency up to 21 days of age among intermittently fed birds when compared to *ad libitum* counterparts could be as a result of severity of the intermittent system applied in this experiment. Svihus *et al*. (2010) found increased gain: feed ratio among intermittently fed birds which had access to feed 6 hours a day from 16 to 25 days of age. Availability of feed to intermittently fed birds in this trial was 5 hours a day and perhaps they demanded longer time to become accustomed to a new feeding regimen. It is most likely that any interruption in feed intake could have depressed weight gain and feed efficiency due to increased maintenance requirement. From 21 to 35 days of age, there was no significant difference in any performance parameters between intermittent and *ad libitum* fed birds, which indicates that birds were able to adapt to the intermittent programme and show compensatory growth. Similarly, depressed BW gain and feed intake were reported by Buyse *et al*. (1996a) for chickens under intermittent lighting system at early age, but it was followed by compensatory growth during finisher period. Sacranie *et al*. (2012) also recorded no difference in feed utilisation efficacy and BW gain between *ad libitum* and intermittent feeding regimens from 17 to 33 days of age.

Our average feed consumption data indicate that each intermittently fed bird at the age of 35, consumed 59 grams of feed during a 40 minute feeding bout. This is equivalent to 30% of daily *ad libitum* feed intake predicted by the birds' producer for that age. This may justify the ability of animals to compensate for reduced feed intake observed before 21 days of age as a result of crop filling. Capacity of the crop has shown to be a key factor affecting feed consumption in intermittently fed birds (Barash *et al*., 1993; Svihus *et al*., 2010).

Higher passage rate of digesta through the crop during the first hours of retention indicates that birds under intermittent programme, consumed large amount of feed when it was offered to fill their crop as formerly shown by Buyse *et al*. (1993). The large consumption instantly after feeding started may have forced the feed to pass through the crop more rapidly while containing a higher percentage of dry matter, however, feed intake is reduced over time and subsequently the passage rate is declined.

The pH of the feed (5.5) in this experiment falls within the range previously reported (Bolton, 1965; Yi & Kornegay, 1996; Carlson and Poulsen, 2003; Partanen *et al*., 2007; Ao *et al*.,

2008; Svihus, 2010). Significant reduction in the crop pH was observed 4 hours after feeding commenced. This reflects the importance of digesta retention time in the crop for effective fermentative activities of lactobacilli to occur and subsequently the pH to decline.

Unchanged concentration of starch and protein in the crop (Figure 11) in comparison with that in the feed over time is against the hypothesis that there might be selective retention in the crop based on nutrients concentration.

The reduction in percentage of starch in the proventriculus-gizzard after 240 minutes as shown in Figure 12 could have occurred due to the extraordinary responsibility of this segment in holding large particles, such as insoluble fibres. Retention in the gizzard seems to be of importance in order to reduce particles size until they are small enough to pass through the gizzard (Hetland *et al*., 2002; Hetland *et al*., 2003; Amerah *et al*., 2008). This functionality could have led to decreased concentration of starch in this segment by prolonged retention of some particles (e.g. insoluble fibre) or may have resulted in starch being washed out of the segment. However, the probable back flow of bile acids from duodenum in to the gizzard which has been observed in previous works (Hetland *et al*., 2003) might have been furthermore a cause to changes in the nutrient concentration in the proventriculus-gizzard. Protein concentration, however, did not show a significant change within 4 hours of retention. Perhaps, this nutrient requires longer time in the proventriculus-gizzard to show reduced concentration and may be pepsin secretion in this segment has contributed to the stability of nitrogen proportion up to 240 minutes after feeding started.

Higher percentage of protein in duodenum + jejunum in comparison with that in the feed at all dissection intervals could be related to the secretion of pancreatic juice and intestinal enzymes and also it could be assumed that amino acid absorption may not be high if any in duodenum + jejunum. Starch concentration, however, shows reduction at all dissection times which may be due to absorption of the glucose in this segment. Riesenfeld *et al*. (1980) reported that 48 and 97% of starch was absorbed as glucose in duodenum and the end of ileum, respectively. However, bile acids and pancreatic juice secretion may have contributed to the reduced starch concentration.

Further reduction in starch and protein proportions within ileum at different sampling times when compared to that in the previous segment, as illustrated in Figure 15 is expected to be a result of absorption of the nutrients in this part of small intestine.

6. Conclusion:

The result of the current study indicates that under intermittent feeding chickens are capable of exhibiting compensatory growth because of the increased crop storage capacity. The stage at which birds are able to adapt to feed restriction seems to be dependent on the severity of intermittent system.

Inclusion of exogenous arabinoxylanase and β-glucanase did not affect digesta viscosity but increased AME significantly which may be a result of cell wall NSP degradation and consequently release of entrapped nutrients. The effect of enzyme degradation activity on caecal microflora fermentation could possibly justify the increased energy availability as well.

Since the barley in the current experiment was harvested a year prior to the trial it could be imagined that the ratio of soluble NSP was too low to see any effect of enzyme on viscosity of digesta in crop and duodenum + jejunum.

Apart from increased storage capacity in the crop under intermittent programme, results from this experiment indicate that prolonged retention of the feed in the segment leads to higher fermentative activity of lactobacilli and subsequently reduced pH. The increased acidity of the crop content, however, did not improve the enzyme effect on any of performance parameters throughout the experiment.

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