The Effects of Different Feeding Program and Inclusion of Glycerol, Glucose or Sucrose in Broiler Starter Diets on Growth Performance and Intestinal Development

by

Anhao Wang

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
March 2014

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The easily utilized energy sources, glycerol, glucose and sucrose were used in broiler starter diets to improve growth performance. Trials investigated the effects of inclusion of easily utilized energy sources (EUES) on broiler duodenum and ileum histological developments. The effects of EUES on CHICKS delayed access to feed were investigated. In trial one, newly hatched chicks were randomly assigned to immediate (IA) or 36 hours delayed access to feed and water (DA) and fed 4 or 8% EUES during first 14 days post hatch. In trial two, males and female chicks were randomly assigned to IA or 48 hours DA, and fed 8% glycerol or glucose diets for a 14 days period. In both trials growth performance, duodenum and ileum developments were affected by dietary treatments. In conclusion, glycerol can be added into broiler starter diets up to 8%.

LIST OF ABBREVIATIONS USED

Apparent metabolic energy nitrogen corrected……...……AMEn
Atlantic Poultry Research Centre……APRC
Average body weight…….....BW
Average daily weight gain……ADG
Biological age……..BA
Chronological age…….CA
Compound symmetry……...CS
Corrected Akaike's information criterion……...AICC
Daily feed consumption…….DFC
Delayed access to feed and water…….……DA
Dietary energy source…… DES
Dietary energy source…….DES
Easily utilized energy source……...EUES
Energy source inclusion levels…….ESIL
Feed to gain ratio……...FGR
Gastrointestinal tract……...GIT
Gross energy……..…GE
Immediate feed and water access……..IA
National Research Council……NRC
Percentage of body weight……...…%BW
Post hatch……..PH
Relative humidity…….RH
Untrusted…….UN
Variance components……..VC

ACKNOWLEDGEMENTS

First I would like to express sincere thank to my supervisor, Dr. Derek Anderson for all his endless encouragement and guidance throughout my Masters's program and for providing me this great oppotunities to gain knowledge and skills. Thanks go to my supervisory committee members, Dr. Bruce Rathgeber, for giving me numerous great suggestions on the project design and Dr. Nancy Pitts, for reviwing the documents and providing great suggestions. Thanks to Janice MacIsaac for her help with my project. She has always been patient and kind, answering my questions regardless of the subject matter. Thank goes to the staff of the Atlantic Poultry Research Centre for making feed, day-to-day care of the chickens and help with data collection. My gratitude goes to those graduate and under-graduate students who gave me help during the data collection.

Last but not least, I would like to thank my parents and girlfriend for their continuous support and encouragement.

CHAPTER 1 INTRODUCTION

The annual broiler meat consumption per person increased from 4.0 kg in 1950 to around 40 kg in 2000 in the United States of America (Havenstein et al.2003), consumption is still increasing. Modern broiler chickens are selected for precocity. Broiler market age has been reduced by approximately one day every year (Willemsen et al. 2010) while feed efficiency has improved due to genetics selection. Along with the genetic selection, the understanding of broiler early nutrition is required to improve growth performance. Noy and Uni (2010) reported that broiler body weight at Day 7 post hatch will increase if highly digestible feed ingredients are provided during first 7days and this advantage can be can maintained through the entire production cycle (Noy and Uni 2010).

Modern broilers are marketed around 35 days of age therefore the starter stage (14 days) represents 40% of the chicks' life span. The nutrition and feeding strategies during the starter stage become increasingly important. During commercial production, up to 48 hours holding period may occur prior to access to feed (Willemsen et al. 2010 and Noy and Sklan 1998). During that time period, chicken do not have access to feed or/and water and bird body weight decreases and intestine and muscle developments are reduced (Noy and Uni 2010).

Starch digestion and absorption were low at hatch and increased with chick age (Noy and Sklan 1999a; 2001). Glucose, sucrose and glycerol may have potential to increase chicken performance. Glucose and sucrose are mono and disaccharide carbohydrates and can be easy utilized by chickens. Earlier research found that chicks acquire more energy from a glucose based diet than a corn based diet until day 21 post hatch (PH) (Batal and Parsons, 2002). Crude glycerol is a biodiesel by product. Chicks consuming a glycerol

containing diet showed better growth performance during a 21 day trial (Mclea et al. 2011). However, the optimal glycerol dietary inclusion level has not been determined.

In order to provide newly hatched chicks sufficient nutrition to meet their genetic potential, feed ingredients with excellent digestibility must be used during first few days post hatch. The effects of using different levels of glucose, sucrose and glycerol in broiler starter diets (first 14 days PH) combined with different feeding programs (immediate access (IA) to feed and water vs. delayed access (DA)) on broiler chicken growth performance and intestinal development have not been studied.

CHAPTER 2 LITERATURE REVIEW

2.1 GASTROINTESTINAL TRACT DEVELOPMENT

The basic functions of the gastrointestinal tract (GIT) include the digestion and absorption of nutrients. The development of the GIT is important for fast growing broilers to fully utilize dietary nutrients and achieve maximum muscle growth rate. After hatching, the GIT undergoes dramatic changes in enzyme production, nutrient transportation and glycosidation patterns in order to transition from utilization of nutrients from lipid and protein rich yolk to utilization of nutrients from carbohydrate and protein rich commercial diets (Perry, 2006). During the first week post hatch, intestinal weights increase more rapidly than the rest of the body (Perry, 2006; Uni et al. 1999; Sklan, 2001). Intestinal weight as percentage of body weight reaches a peak at about 6 days of age (Perry, 2006; Uni et al. 1999; Sklan, 2001). The small intestine weight at 14 days PH is approximately 5% of the total body weight (Mahmoud and Edens. 2012).

Post hatch GIT development includes structural and functional changes in broiler chickens. The small intestinal mucosa structures develop at different rates (Iji et al. 2001a; Sklan, 2001). By 7 days of age, the villi in the anterior section of small intestine, the duodenum, are almost completely developed (Sklan, 2001). The duodenal villus height increases two fold during first 2 days PH and reaches a plateau at 6 to 8 days PH (Sklan, 2001). Villi in the middle section, the jejunum, and the posterior section, the ileum, continue to develop even after day 14 PH (Uni et al. 1998a; Sklan, 2001). Uni et al. (1996) stated that cellular level developments of GIT are characterized by increasing

DNA content and increasing cell size. It is important to understand the stages of GIT development in order to achieve optimal broiler growth performance.

2.1.1 Structural developments of the gastrointestinal tract

The GIT of broiler chickens develop at different rates during the development of embryo in the incubation period (Uni et al. 2003, Perry; 2006) and PH (Uni et al. 1999, Iji et al. 2001a). Dramatic structural changes in the GIT were observed during the late incubation period and the early PH period.

The development of the intestine during incubation of broiler hatching eggs was summarized by Uni et al. (2003). The incubation of broiler eggs is a 21 day process. During incubation, the small intestine development is not constant. The intestinal weight at 17E is approximately 1% of body weight but increases to around 3.5% at hatch (Perry, 2006). Rapid development of villi is observed after 15 days of incubation (15E). On 15 E, villi start to develop through all sections of intestine, by 17E, two different development stages of villi are observed (figure 2. 1a). The larger, pear – shaped villi are about 1.5 times the size of smaller, narrower rocket – like shaped villi. This pattern is maintained until 19E. On 20E, three different development stages of villi are observed (Perry. 2006). The smallest villi start developing after 17 E and are about 65% the length of the second largest (figure 2. 1b). During the last day of incubation, the villi in all three development stages increase their size by two fold (Perry. 2006).

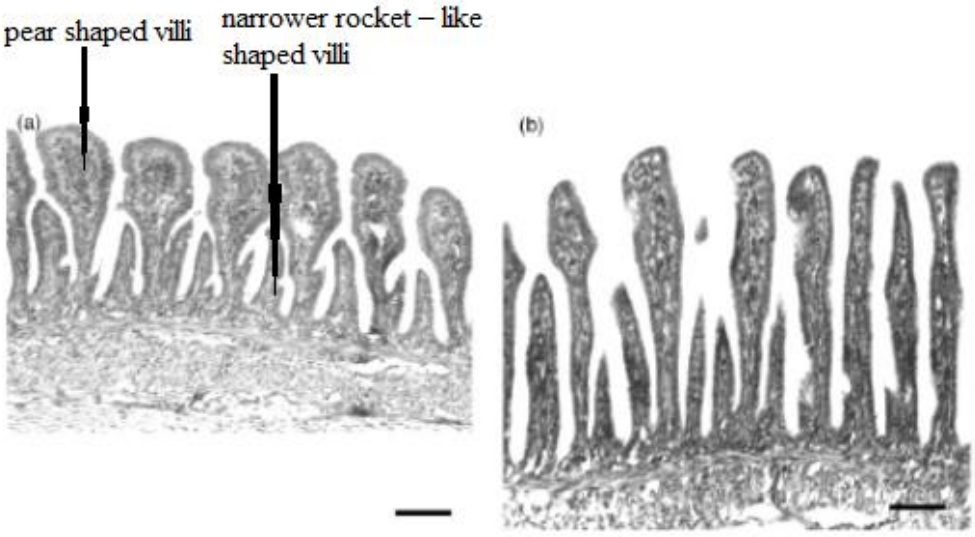

Figure 2.1. Intestinal villi development at different stages of incubation. a. Intestinal villi structure at Day 17 of incubation. b. Intestinal villi structure at Day 20 of incubation (Perry, 2006). (Permission to use requested)

Intestinal mucosa is structurally developed at hatch, but major changes still occur during the first days PH (Iji et al. 2001a). The structurally developed intestinal mucosa allows newly hatched chicks to utilize the dietary nutrients. During first three weeks post hatch, crypt depth and villi height increase as birds age. The mucosal structures are different in different GIT regions. The crypt depth, villus height and villus surface area decreases from the duodenum to the ileum region (Iji et al. 2001a).

The GIT develops rapidly after hatch. The villus surface areas are different for each section of small intestine at hatch. The duodenum has larger villi than the jejunum and ileum (Uni et al. 1998). The villus surface area in the jejunum, where carbohydrates digestion mainly occurs, is lower than duodenum during the first week PH (Uni and Ferket 2004). The jejunum develops more rapidly in comparison to other regions (Iji et

al. 2001a). Uni et al. (1998a) reported that, jejunum villi surface area is greater than that in the duodenum after 10 days PH.

The jejunum mucosa structures from 1 day old chicks and 21 day old chicks are shown in figure 2.2 (Iji et al. 2001a). At 21 days of age, jejunum villi are about three times longer than those at 1 day of age (Figure 2.2). Villus height at 14 days PH increased 9 to 11 fold compared to 14E (Uni et al. 1996). Enterocyte migration rates also increase between day 1 and day 14 PH (Iji et al. 2001a). Between day 1 and day 14 PH, the intestinal enterocytes migrate a longer distance from crypt to villus tip as chickens age (Iji et al. 2001a). The intestinal enterocytes' migration time remains the same (Iji et al. 2001a).

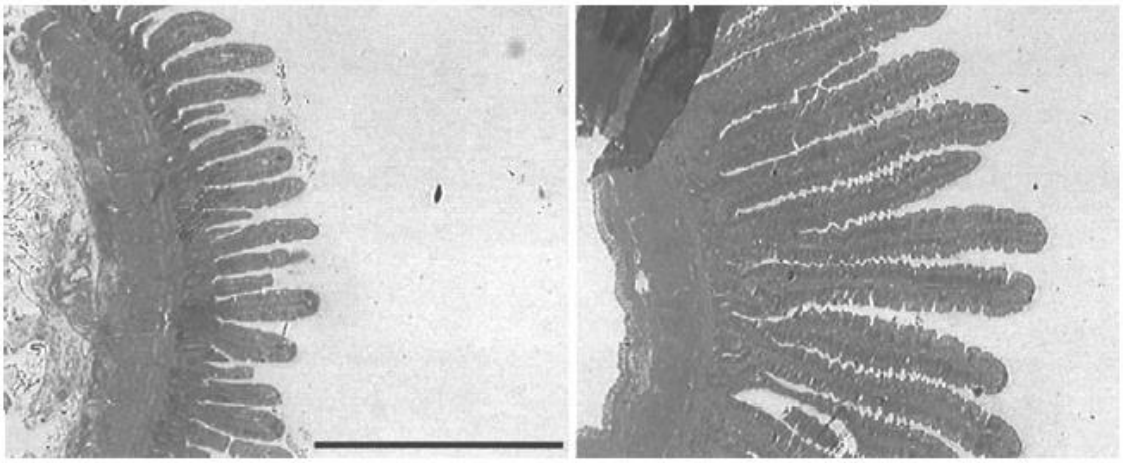

Figure. 2. 2 Jejunum mucosa structures on day 1 post hatch (left) and day 21 post hatch (right). Scale bar = 1000 μm (Iji et al. 2001a). (Permission to use requested)

At day 12 PH, small intestine is two to four times longer and seven to ten times heavier than that in newly hatched chicks (Perry, 2006). The differences and similarities for GIT development rates that occur between different sections of the small intestine are constant between commercial broiler chickens and layers (Uni et al. 1996; Yamauchi et al. 1992). The broiler chicken intestinal epithelial cells are larger and have a greater surface area than epithelial cells from layer chickens. Uni et al. (1996) and Yamauchi et al. (1992)

reported that for both types of chicken, the jejunum and the ileum has the similar length and two segments represent more than 82% of the total GIT length at day 21 PH (Iji et al. 2001a). In both types of chicken, cell development was stable stage by 60 days PH. The 2014 version of commercial broilers are marketed at around 31 to 35 days of age. Therefore, at market age, broiler intestinal cells are still developing.

The GIT post hatch development rates are not constant (Uni et al. 1999, Iji et al. 2001a). The duodenal villi develop more rapidly than the jejunal and the ileal villi. However, the duodenal villi development rates are different among studies. Uni et al. (1995; 1999) reported that the development of duodenal villi is almost complete on day 7 PH. Geyra et al. (2001b) reported that villus surface area increase steadily during first 12 days PH. The jejunal and the ileal villi will continue to develop until day 14 PH (Uni et al. 1995; 1999). During the first 14 days PH, the growth rate of villus surface area is changing. The villi surface increases more slowly after 4 days PH (Geyra et al. 2001b).

The total villi number and surface area of each intestinal cross section increase at different rates for each segment of the small intestine (Geyra et al. 2001b). In the duodenum and the jejunum, an increase in villi number was observed but not for the ileum. The total villi surface area increased at the same speed for each section of the small intestine during the first 3 days PH. After 3 days PH, the total villi surface area increases more rapidly in the jejunum than those in duodenum and ileum.

The crypts play an important role in the continuous renewal of the intestinal mucosa process (Perry, 2006). The cells located in crypts differentiate to enterocytes and migrate up to the villi tips. During the migration process, the enterocytes digestive functions,

including absorption and mucin secretion are developing (Traber et al. 1991). The enterocytes express the complete digestive functions when they reach the villi tip. Unlike mammals, where enterocytes proliferation only occurs in the crypts after birth, chicken enterocytes have the ability to proliferate in both the crypts and villi (Uni et al. 1998b). At hatch, the crypts are immature and only contain a few cells (Geyra et al. 2001b). Individual crypts will complete development around 48 hours PH, and crypt number per villi reaches a peak and remains stable approximately 72 hours PH. The crypt number per villus increases and reaches three to four crypts per villus by day 14 PH (Perry, 2006). The speed of enterocyte migration also decreases when chickens get older compared with 2 weeks old birds (Geyra et al. 2001b; Uni et al. 1998b). The depth of crypts varies among GIT segments. During the first 7 days post hatch, the duodenal and the jejunal crypt depth increased significantly, but no change was observed in ileum (Iji et al. 2001a).

The development of intestinal mucosa structures varies for different types of chicken. During the first 5 days PH, broiler chicks develop deeper crypts than layer chicks (Uni et al. 1996). The different GIT development rates between broilers and layers may be a result of differences in volumes of feed intake between these two types of bird.

2.1.2 The functional development of the GIT

The GIT in hatching chicks is anatomically complete, however, digestion and absorption functions are still developing (Uni et al., 1996). Pancreatic amylase and intestinal maltase were found to increase during the first 3 weeks PH (Uni et al. 1995). This is important for metabolizing dietary carbohydrates from plant-based feed post hatch. Batal and

Parsons (2002) found nitrogen corrected metabolizable energy (MEn) of a corn-soybean meal diet increased substantially with increasing chick age until day 21 PH. The chicken is able to digest around 96% of dietary starch by 21 days of age, however, the digestibility of starch is low at hatch (Jamroz et al. 2001). These finding suggest that there may be an increase in enzyme quantity or quality when chicks are growing.

The development of intestinal function, including digestion and absorption can be measured by tracking the expression of related genes (Perry, 2006). Sucrase-isomaltase is the enzyme complex responsible for digesting sucrose and isomaltose. Expression of the sucrase-isomaltase gene was detected by 15E in broiler embryos (Uni et al. 2003; Sklan et al. 2003) with expression continuing to increase until post hatch (Sklan et al. 2003). Expression for genes associated with other digestive enzymes such as protein digestive enzymes, also increased after 15E (Uni et al. 2003). The gene expression of sodium-glucose transporter was found 2 days before hatch and continued increasing following hatch (Sklan et al. 2003). Sodium-glucose transporter is responsible for glucose absorption in GIT. Generally, Perry (2006) reported gene expression of digestive enzymes and related transporters increased on approximately 17E and 19E (Perry, 2006).

The ability of chickens to digest and absorb disaccharides starts developing near the end of incubation and continues post hatch (Perry, 2006). Digestive capacity for disaccharides in the three different regions of the intestine varies with the jejunum having the highest capacity, the duodenum is the lowest and the ileum being intermediate (Uni et al. 1998a). Sucrase is the enzyme responsible for sucrose digestion. It breaks one molecule of sucrose into one molecule each of glucose and fructose. Sucrase is found in the form of a sucrose – isomaltase enzyme complex in the small intestine. The activity of this enzyme

complex is low in the duodenum and high in the jejunum and the ileum at hatch (Uni et al. 1998a). The sucrase activity increases during first 2 days PH and then decreases in the jejunum region (Uni et al. 1998a). The same publication reported that after day 7 PH, sucrase activity per unit of intestine is positively correlated to the number of enterocytes per villus. This suggests that enzyme activity per enterocyte does not change greatly with age.

Iji et al. (2001a) established that during the first two weeks PH, the number of enterocytes proliferating in the intestine increases in broiler chickens. The speed of migration of the enterocytes from the bottom of crypts to the tip of villi also increased (Iji et al. 2001a). The increased number of proliferating intestinal enterocytes was thought to be related to increased functional development of the small intestine. The RNA:DNA ratio in each individual enterocyte also increased during first three weeks PH (Iji et al. 2001a). The higher RNA level is the result of higher DNA expression. This evidence suggests an increased capacity for digestive function of individual enterocytes.

Enzyme production outside the digestive tract begins prior to the chick hatching. Pancreatic enzymes were found in the small intestine during the pre-hatch period by Uni et al. (2003). A number of reports indicated that the pancreatic and brush-border enzyme activities increase rapidly in the post hatch days (Sklan and Noy, 2000; Uni et al. 1999). Increasing enzymes activities indicates an increase in the capacity to digest nutrients for the growing chickens.

Previous studies suggest that starch in plant ingredient based feeds has a limited digestibility during first days post hatch. Carbohydrates in the form of starch are key

sources of dietary energy for growing birds. Providing another more easily utilized dietary energy source may provide access to more readily available supply of energy for growing chicks. Some non-traditional feed ingredients will be discussed as potential energy sources in the following sections.

2.2 Effects of early access to feed on growth performance

Broiler chickens have undergone intensive genetic selection for better growth performance during the last few decades (Shariatmadari, 2012). As the number of days required to reach market weight continues to decline, the first few days of life has become a greater portion of the whole life cycle of the broiler. This fact has magnified the importance of the getting the birds off to a good start. Early access to feed can provide advantages to growth that continue for up to 35 days PH (Bhanja et al. 2010). Noy and Sklan (1999a) conducted a series of studies to compare effects of immediate access to feed and 34 hours delayed access to feed on chicken growth performance. A 10% increase in breast muscle yield occurred at market age in broilers that accessed feed early (Noy and Sklan, 1999a). They also reported that mortality was reduced by early feeding treatments (Noy and Sklan, 1999a). Uni and Ferket (2004) stated that around 2 to 5% of newly hatched chicks did not survive because of limited body nutrition reserves and delayed access to feed and water during first week post hatch.

Under commercial conditions, the hatching process occurs over a 24 - 48 hour hatching window. As a result some early hatched chickens may be deprived of feed and water for up to 72 hours before they reach farms (Willemsen et al. 2010). Peebles et al. (2005) found that after a 72 hour holding period, newly hatched chicks lose approximately 19% of their body weight. Sklan et al. (2000) reported that chick body weight decreases

linearly at a rate of approximately 0.14 gram/hr post hatch if feed is unavailable. Several studies have been conducted to evaluate the impact a delay in access to feed for newly hatched chickens has on broiler growth performance. Bigot et al. (2003) found feed deprivation for 48 hours post hatch caused a 7% loss in body weight while body weight increased by 36% for early fed chicks. Juul-Madsen et al. (2004) reported that early fed chickens were 6.1% heavier at market age than those with a 48 hour delay in access to feed. Nir and Levanon (1993) found a 24 to 48 hour holding period without feed resulted in growth retardation and increased the market age by 1 to 2 days. A 72 hour delay in feeding was found to reduce satellite cell proliferation and muscle weight in both heavy and light strains of broiler chicken (Berri et al. 2007).

Varying the time that feed is available post hatch has different effects on chicken growth performances. Abed et al. (2011) compared broiler growth performance for chicks given immediate access or delayed access to feed for 16, 32 and 48 hours. Body weight at market age was lower for the group with a 48 hour delay but the chicks from the other treatments weighed the same. A 16 and 32 hour feed delay had negative effects on body weight until the birds were 21 days old compared to chicken which had immediate access to feed. By day 28 and 35 PH, there was no longer a difference in body weight. Rammouz et al. (2011) reported that a 6 to 12 hour post hatching delay in feed access did not affect chicken body weight at market age. Juul – Madsen et al. (2004) found that by delaying access to feed for 24 hours after hatching did not have impact on body weight at market age. Feed intake during the first 10 days post hatch as well as over the whole production cycle was improved by early access to feed. Feed to gain ratio (FGR) for the first 10 days post hatch was suppressed by a 48 hours delay in access to feed but not for

other delay times (Abed et al. 2011). The overall FCR was not affected by delay in first feeding (Abed et al. 2011).

The effects of delayed access to feed on different breeds of broiler chicken were studied. Kornasio et al. (2011) indicated that if chicks did not access to feed for more than 36 hours PH, the growth rate is irreversibly reduced compared to the chicks which access feed within 6 hours PH for both fast and slow growing broiler strains. If fasting growing broiler chicks (Ross 308) were subjected to 36 hours PH delayed access to feed and water, body weight and feed consumption was reduced during first 4 weeks of the production cycle (Cengiz et al. 2012). In the same experiment, birds were able to overcome disadvantages caused by delayed feeding. In some studies, water and saw dust were provided immediately post hatch (Noy and Sklan 1999a). Providing water and saw dust immediately post hatch resulted in heavier chicks compared with chicks that had delayed accessed to feed. However, this advantage of body weight was only maintained until 8 days PH for the chicks provided with water immediately after hatch and 14 days for the chicks provided with saw dust (Noy and Sklan 1999a).

The chicken growth rate was affected by delayed feed access. If feed is provided early, chicks can gain around 11g body weight during first 2 days PH (Mahmoud and Edens. 2012). Chicken body weight at 8 days PH was lower for the chicks which were held for a 48 hours period post hatch without feed (Richards et al. 2010). The reason was that muscle development corresponds with the time between hatch and availability of feed (Bigot et al. 2003). El-Husseiny et al. (2008) indicated that a decrease in body weight gains and higher feed to gain ratio, resulting from holding chicks without feed, continued to affect chicks up to 6 weeks PH. The current broiler production time in Atlantic Canada

is approximately 5 weeks. Therefore, any starvation induced growth disadvantage from delaying access to feed during the hatching process may not be overcome and body weight at market age may be reduced.

Timing of the first access to feed is important. The feed access time is calculated by the time spent in the hatchery after removal from the incubator in addition to transportation time (Willemsen et al. 2010). However, this calculation is not always accurate. In commercial hatchery operations, chicks hatch over a 24-48 hour period (Willemsen et al. 2010) and are subjected to hatchery treatments (sexing, vaccination etc.) following by transportation which may involve up to a 72 hour holding period for early hatched chicks (Willemsen et al. 2010). Two different age assessments (biological age vs. chronological age) of newly hatched chicks were compared by Careghi et al (2005). The biological age (BA) of a chick starts at the time it exits the eggshell. The chronological age (CA) is the time of end of incubation process of all eggs (Careghi et al. 2005; Willemsen et al. 2010). Late hatching groups only have a short period time of feed withdraw in comparison with early hatched group (Figure 2.3). The growth rate, body weight at hatch is higher in late hatching group (Careghi et al. 2005).

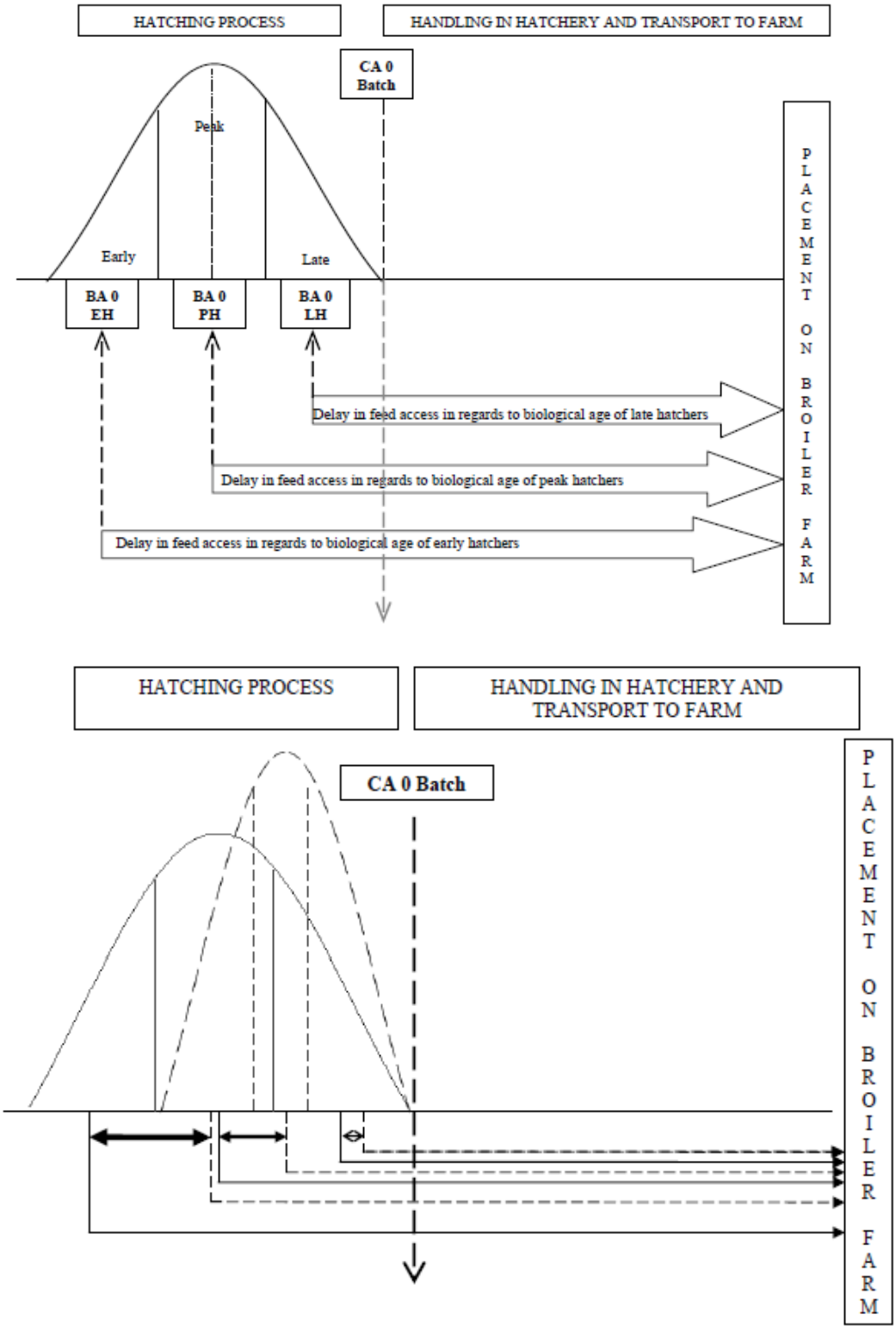

Figure 2.3. Different hatching time and feed withdraw of newly hatched chicks (Willemsen et al. 2010). (Permission to use requested)

Early feed access becomes more important when chick age is calculated by using BA than by using CA (Careghi et al. 2005). If feed is provided at the time of hatch, the early hatched chicks express a lower rate of growth compared to late hatched chicks, and this growth rate defference is maintained until 7 days of BA (Careghi et al. 2005). During the first 12 hours PH, the early hatch chicks which were subjected to conventional hatchery treatments lose body weight (Ven et al. 2013). If feed is provided at the hatchery, the early hatched chick body weight increased during the first 12 hours PH (Ven et al. 2013). The effects of early feed access on chicks' body weights are not significant for midterm and late hatch groups. An earlier study (Vargas et al. 2009) concluded that a 12 hours feed delay at hatch only had a negative effect on feed intake during first 10 days PH and did not affect other growth performance throughout the trial. Vargas et al. (2009) suggested that a longer period of fasting may be necessary in future studies to show difference.

2.2.1 Early feeding programs

As the time it takes for broiler chickens to reach market age is reduced, the importance of feeding broiler chicks at the early stage of life increases. At the end of incubation, chicks utilize yolk contents to meet the nutrient requirements for embryo development (Uni and Ferket, 2004). Additional nutrients can be provided during late incubation (Shariatmadari, 2012). '*In-OVO*' feeding involves providing extra nutrients using a similar technique developed to vaccinate to the developing embryo while it is still in the shell (Shariatmadari, 2012).

The remaining yolk sac contents provide nutrition during the post – hatch period (Uni and Ferket, 2004). The yolk sac content is the only nutrient source for the chick until feed is provided. After hatching, broilers are often given a 3 phase (starter, grower and finisher) feeding plan (Shariatmadari, 2012). The starter diet contains higher protein level and more easily digested energy sources. Sometimes, a pre-starter diet is provided during first week post hatch. Longo et al. (2007) conducted experiments where different carbohydrate and protein sources as feed ingredients were tested during the first 7 days post hatch. The feed ingredients included sucrose, corn gluten meal and blood meal. The chicks fed 20% sucrose diet had heavier body weight than chicks fed diets which contained 20% corn gluten meal or blood plasma meal (Longo et al. 2007). However, Sorbara et al. (2006) found using glucose and sucrose to replace starch as the energy source improved chick growth performance during the first 7 days post hatch. Providing nutrients early is important because newly hatched chicks have active satellite cells, responsible for muscle growth, that benefit from early access to nutrients. Feed restriction at an early age resulted in inhibition of satellite cell proliferation and differentiation (Yue et al. 2012). The satellite cells are able to respond to different nutritional factors that modify the size of muscle fibers (Longo et al. 2007). Providing easily utilized dietary nutrient at early stage may provide newly hatch chick with a growth advantage.

2.2.1.1 In – *OVO* feeding

In-OVO feeding involves injecting a feed solution into the embryonic amnion during late term incubation (Uni and Ferket, 2004). The developing embryo naturally consumes the amnion orally before the hatching process (Uni and Ferket, 2004). *In-OVO* feeding is able to improve hatching chick performance in many aspect (Shariatmadari, 2012) and

can be a solution to overcome the growth disadvantages caused by a 36 hour delay in feed access post hatch (Kornasio et al. 2011). Increasing chick performance with *in OVO* feeding includes up to 7% increase in body weight at hatch that is maintained throughout the 35 days production period. *In-OVO* fed chicks showed higher muscle weight and body weight gain compared to chicks denied feed for 36 hours PH (Kornasio et al. 2011). The myoblast proliferation was immediately promoted by *in-OVO* administered nutrient (Kornasio et al. 2011). The *in-OVO* feeding also promotes the intestinal developments. Uni and Ferket, (2004) reported that *in-OVO* fed chicks had intestinal functionality similar to 2 days old chicks given immediate feed access. *In-OVO* feeding can be a potential hatchery practice that improves the newly hatched chick qualities.

2.2.1.2 In hatchery feeding

Chicks normally hatch during a 24-48 hour hatching period (Willemsen et al. 2010) and may encounter different length of time between hatching and first access to feed. If feed is provided in the trays the birds were hatched in, chick body weight loss has been shown to decrease (Sklan et al. 2000). Sklan et al. (2000) reported that when chicks leave the hatchery they are 3 grams heavier on average than unfed birds. At day 21 PH, these chicks were still heavier than chicks that were not fed until placement. This was true for both sexes, male (718g vs. 660g) and female (689g vs. 633g) (Sklan et al. 2000). Feeding chicks in hatching trays is an option that can provide advanced growth performance especially for birds hatched early in the hatch period (Sklan et al. 2000).

2.3 Compensatory growth

Compensatory growth is an abnormally high growth rate relative to the same breed of animal during the same age (Zubair, and Leeson. 1996). When animals are under

unfavorable conditions, such as feed restriction or illness, the growth rate is often reduced (Zubair, and Leeson. 1996). After favorable conditions are provided again, the animals can exhibit an accelerated growth rate that will reduce the difference between these individuals with initially reduced growth rate and individuals with normal growth rate (Zubair and Leeson. 1996).

An example of a compensatory growth curve is demonstrated in figure 2.4 (Zubair, and Leeson. 1996). The lines denoted A, B, C and D represent the growth rates of different chickens. All four chickens reached the same body weight at the final age. Bird A had a higher growth rate at an earlier age than birds B, C and D. Birds C and D showed slower initial growth rates and an accelerated growth towards the market age. They exhibited a superior feed efficiency during the production period. The reasons for this are individuals with a lighter body weight have a reduced maintenance requirement in comparison with bird A (Zubair and Leeson. 1996). Many methods can be used to induce compensatory growth, such as physical and chemical methods of feed restriction, diet dilution and changing lighting programs (Zubair and Leeson. 1996; Leeson and Summers, 2001).

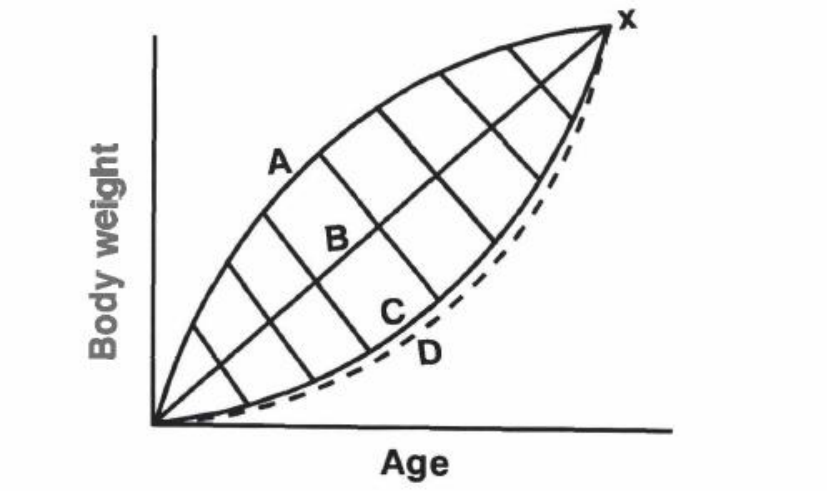

Figure 2.4. Different growth rates of chicken A, B, C and D at different age (Zubair, and Leeson. 1996).

Compensatory growth normally is induced by feed restriction during day 7 – 14 of age (Leeson and Summers, 2001). In a recent study, compensatory growth also induces the better carcass compositions (Jalal and Zakaria, 2012). The broilers exhibiting the compensatory growth had a smaller abdominal fat pad and reduced heart and liver weight compared with the control group (Jalal and Zakaria, 2012).

The compensatory growth may be able to provide overall advantages throughout the production period. In modern broiler chicken production system, the average time required for broiler chicken to reach a 2 kg body weight is becoming shorter due to genetic selection (Shariatmadari, 2012). Anoher study concluded that compensatory growth is not able to overcome the growth disadvantages caused by deprivation of feed and water in the 48 hours following hatching (Abed et al. 2011). The newly hatched chicks may not able to overcome the growth disadvantages caused by delayed feed access even when the chicken express compensatory growth.

2.4 Effects of early feed access on GIT developments

After hatching, chick internal organs continue to develop. The weight of the heart, lungs, and GIT increase and the weight of yolk sac contents decrease with both early feed access and conventional feeding methods (Ven et al. 2013). The major functional changes in the GIT include the increased expression and activity of brush border enzymes and transporters, increased crypt formation and an increase in the number of enterocytes (Uni and Ferket 2004). The changes that occur in the GIT post hatch provides the ability for newly hatched chicks to adapt to protein and carbohydrate rich diets.

Feed access affects the chicken intestinal weight. On day 4 post hatch, the jejunum weight increased by 3.3 fold compared with newly hatched chicks (Bigot et al. 2003). The jejunal weight only increased by 2 fold in the 48 hour delayed feed access chick (Bigot et al. 2003). The same study found, after 2 days of feed access, the 4 day old birds which did not access to feed during first 2 days PH and then were fed, had heavier intestinal weight than 2 day old chicks with early access to feed.

The influences of physical stimulation of feed on GIT developments were studied using feed ingredients with different nutritional value. Different types of feed were provided sawdust, standard starter feed, high protein, high fat, or high carbohydrate diets during first 24 hours post hatch (Bhanja et al. 2010). The chicks fed sawdust had heavier small intestines compared with fasted chicks at day 3 PH. The authors reported that small intestinal segment weights did not differ among dietary treatments but were heavier than those for chicks with a delay in access to feed (Bhanja et al. 2010). The authors suggested that sawdust provided the mechanical stimulation of GIT development (Bhanja et al. 2010). Other studies reported that a high protein level in starter diet may enable chicks to overcome disadvantages caused by a 48 hours delay in feed access (Gökceyrek and Ciftci, 2005).

2.4.1 Effects of feed access on yolk sac utilization

At hatch, the main energy source of chicks is from the contents within the yolk sac which can represent around 20% of the chick's body weight (Uni and Ferket, 2004). Yolk sac contents are approximately 50% lipid (Uni and Ferket, 2004). The contents of the yolk sac represent 22% of the energy and 30% of the protein requirement for the first 3 days

post hatch (Sulistiyanto et al. 1999). Utilization of yolk sac contents occurs by direct absorption through the yolk sac membrane.

Chicks which were fed during the first 24 hours post hatch utilized yolk sac contents more quickly than those which fasted for a 40-48 hour period (Bhanja et al. 2009). Chicks fed immediately post hatch were able to consume around 3g of yolk sac contents during first 24 hours and another 1.50g during the second day post hatch. During the same time, unfed chicks only consumed around 3g of yolk sac contents (Bhanja et al. 2009). After 4 days post hatch, yolk sac weight was around 0.4g in chicks with access to feed, and 1.5g for chicks subjected to a 2 day feed delay (Bhanja et al. 2009).

During first 4 days post hatch, the digestibility of lipid is higher than digestibility of carbohydrates (Noy and Sklan. 2001). The yolk free body weight for newly hatched chicks remains the same in delay feeding group during first 24 hours post hatch, while this yolk free body weight increases in the feed access group (Ven et al. 2013). The differences may be partly due to feed and water residues in GIT.

Not only is yolk sac content utilization greater in chicks that have access to feed early, but also organ weights increase more rapidly in chicks with early feed access. Different small intestine segments respond to the presence of early dietary nutrients differently. At day 7 PH, the jejunum was heavier in chicks with feed access during the first 24 hours post hatch, compared to chicks with a 32-48 hour delayed access to feed (Bhanja et al. 2009). Ileum weight was reduced by a 48 hours post hatch feed delay but not by 24-40 hours delayed feed access. The duodenum weight was not affected by delayed feed access (Bhanja et al. 2009).

2.4.2 Effects of feed access on GIT structure development

Different intestinal histological structures respond to delay feed access differently. The villi height, width, surface area and crypt depth were commonly measured when intestinal histological structure developments were studied. The duodenal and jejunal histological structures are more sensitive to post hatch starvation compared with ileal samples (Geyra et al. 2001b).

The effects of 36 hours post hatch delayed access to feed on chicken intestinal histological developments found smaller villi surface area three sections of small intestine in the birds subjected to 36 hours access to delayed feed (Uni et al. 1998a). The duodenal and jejunal villi volume in DA birds are approximately 80% the size of early feed access chicks. The reduced villi volumes in duodenum did not recover until 5 days PH and in the jejunum until 11 days PH (Uni et al. 1998a). The ileal villi in DA birds were approximately 90% the size of early feed access chicks. The ileal villi volume was similar to control treatment by day 14 post hatch (Uni et al. 1998a).

The duodenal, jejunal and ileal crypt depths were decreased by delayed feed access (Uni et al. 1998a). At 2 days PH, crypt depth in 36 hour DA chicks was approximately 80% the depth of the control group (Uni et al. 1998a). The ileal crypt depth reached control values at 5 days post hatch. The duodenal and jejunal crypt depths reached control values at 8 to 10 days post hatch. Chicks subjected to delay feeding for 48 hours had higher intestinal weight gains relative to body weight after feed was provided compared with the chicks with early access to feed (Mahmoud and Edens, 2012).

Delaying access to feeding during the first 2 days PH can lead to a decrease of the duodenal and the jejunal crypt size, the number of crypts per villi, crypt proliferation,

villus surface area and enterocyte migration rate (Geyra et al. 2001a). The decrease of villus surface area is due to a decrease in villus height but not villus width (Gökceyrek and Ciftci, 2005). A 36 hour delay in access to feed resulted in lower GIT weights, villi length and an increased incidence of epithelial degeneration (Cengiz et al. 2012). The villi surface area in the duodenum decreased in birds with delayed access to feed, but this disadvantage disappeared after 4 days from the start of feeding (Uni et al. 1998a; Uni and Ferket 2004). The crypt is responsible for renewing epithelial cells in villi. All the crypts epithelial cells undergo proliferation at hatch, but this decreases to 50 to 60% of the crypts by day 2 post hatch and further decreases to 10 to 20% by day 6 post hatch when it becomes stable (Geyra et al. 2001b).

The interactions between different hatching time and different post hatch feed access time were studied. Hatching time during the incubation did not affect intestinal weight at hatch (Ven et al. 2013). Chicks grouped as early, midterm and late hatching had the same intestinal weight, approximately 1.32g. In the same study, early feed access increased intestinal weight to 1.53g in the late hatching group and had no effects on early and midterm groups. However, only a 12 hour feed withdraw time was applied (Ven et al. 2013). Another study indicated that when a longer (48 hour) feed deprivation period was given effects on intestinal weight was observed (Abed et al. 2011). Duodenal weight was not affected by 16, 32 or 48 hours delay in feeding in the study by Abed et al. (2011). Jejunal and ileal weights decreased in chicks with 48 hours DA to feeding but not for 16 and 32 hours treatments (Abed et al. 2011).

Breeding hen age had interaction effects with feeding programs on GIT developments (Mahmoud and Edens 2012). The effects of different breeder ages (31 weeks, 40 weeks

or 63 weeks of age) subjected to a 48 hour extended time before access to feed on chicken GIT post hatch developments found that if chicks were provided feed immediately after hatch, intestinal weight increased around 2.3g in chicks from 31 weeks old parents and 2.7g when the breeders were 63 weeks old. If chicks were subjected to a delay in access to feed for 48 hour PH, chick intestinal development occurred at a higher rate compared with chicks provided immediate feed access for all ages of breeders (Mahmoud and Edens 2012). The villi height, crypt depth and villi surface area increased more rapidly in chicks from 40 weeks old hens. During the first week post hatch, a 48 hours delayed access to feed resulted the smaller villi and the shallower crypts in duodenum in the chicks from the 31 week old hens but had no effects on chicks from the 40 week old hens. Ileal villi and crypt developed at a higher rate in chicks from the 40 weeks old breeders compare to parent stocks of other ages. The effects of delayed feed access on intestinal structural development disappeared in all the chicks after 14 days PH.

2.5 Glucose and sucrose as energy sources in broiler starter diets

Carbohydrates are the main dietary energy source for chickens post hatch. The major dietary carbohydrate source is starch (Leeson and Summers, 2001). Easily utilized carbohydrate sources, such as, monosaccharides and disaccharides, are important for maximizing chick growth potential. Glucose is a monosaccharide that does not require digestion before absorption. Sucrose is a disaccharide, containing one molecule of glucose and one molecule of fructose. Sucrose digestion requires enzyme sucrase. Leeson and Summers (2001) reported that glucose supplementation significantly suppresses feed intake in broiler chicken but improves metabolic energy intake and water consumption.

Jiang et al. (2008) reported that providing broiler 8% glucose solution reduced chicken feed intake.

Starch digestion requires multiple enzymes compared to what is required for simple sugar digestion. The pancreatic enzyme alpha-amylase is secreted into the duodenum, hydrolyses alpha linkages on starch and yields smaller molecules, such as maltose and isomaltose for further digestion and absorption. The jejunum is the major digestion site for carbohydrates in broiler chickens (Leeson and Summers 2001). Maltose and isomaltose are further digested by maltase and isomaltase in the jejunum (Leeson and Summers 2001). Carbohydrate digestive enzymes sometimes are presented in the forms of an enzyme complex, such as the surcase – isomaltase complex.

Carbohydrate digestive enzymes activities are different among regions of intestine and for different age of chicks (Iji et al. 2001b). The sucrase and maltase specific activities (μmole product/mg protein/min) decrease as chickens age (Iji et al. 2001b). The highest sucrase specific activity was found in the ileum and was lowest in duodenum at hatch according to (Iji et al. 2001b). The highest sucrase specific activity was found in the jejunum, while the duodenum sucrase has lowest specific activity (Iji et al. 2001b). The ileum sucrase specific activity is intermediate at day 7 PH (Iji et al. 2001b). The jejunum's sucrase specific activity remains higher than duodenum but not different from the ileum (Iji et al. 2001b). Total sucrase and maltase activities (μmole product/ min) increases with age (Iji et al. 2001b).

2.5.1 Utilization of glucose and sucrose in chicken diet

The chicken is able to adjust feed intake over a range of nutrient density to maintain growth performance (Leeson and Summers 2001). Providing easy utilized dietary nutrients has the potential to reduce feed intake and feed cost. Glucose is absorbed by intestinal enterocytes through the Na^+/glucose co-transporter 1 (Moran et al. 2010). Fructose transportation is through a Na – independent transporter (Moran et al. 2010). Glucose absorption is accomplished by a secondary active transport mechanisms and homeostasis is maintained by active exclusion of Na^+ across the basolateral cell membrane by Na^+, K^+ ATPase (Sklan and Noy 2000). Studies have found that the active transport system for glucose and fructose start developing 1 h after feeding sucrose to 1 day old chicks (Sheshukova and Ozols 1986) and the mucosa responded to the adaptive changes in sucrase activity (Ozols and Sheshukova, 1985).

After absorption across the apical membrane, glucose and fructose are transported into blood stream across the cell basolateral membrane through another Na – independent monosaccharide transporter (Moran et al. 2010). The removal of glucose from intestinal enterocytes is a passive transportation process (Sklan and Noy, 2000). The glucose uptake is affected by feed access and Na^+ concentration. A study found that by Na^+, K^+ ATPase activities were low in unfed chicks and increased after feed access (Sklan and Noy, 2000). The uptake of glucose was low near hatch time (Noy and Sklan, 1999b) and this may due to the low level of Na^+ concentration (Sklan and Noy, 2000).

The chicken is able to digest around 85% (Noy and Sklan, 1999a; Noy and Sklan, 1999b) of dietary starch at day 4 PH and 96% at day 21 PH (Jamroz et al. 2001). However, the digestibility of starch is low at hatch (Jamroz et al. 2001). Glucose absorption increases

with age (Noy and Sklan 1998). This increase was found throughout the small intestine and particularly in the duodenum. Chicks acquire more energy from a glucose-based diet than a corn-based diet until day 21 PH (Batal and Parsons, 2002). Noy and Sklan (1999b) measured the chick glucose absorption during the first 4 days PH. If glucose was provided through a glucose solution, the glucose absorption was around 50% at hatch (Noy and Sklan, 1999b and 2001) and increased to about 76% at day 2 PH (Noy and Sklan, 1999b). Glucose absorption was reported around 90% at day 4 PH (Noy and Sklan, 1999b). If glucose was provided in solid form, glucose absorption showed a similar pattern, but higher at hatch, approximately 70% and lower at day 4 PH (80%) (Noy and Sklan, 1999b). The higher glucose absorption at hatch when glucose was provided in solid form is due to the hydrophobic environment in hatchling small intestines (Noy and Sklan, 2001). The hydrophobic environment is created by the present of yolk sac content in small intestine (Noy and Sklan, 2001). The lipid rich yolk will inhibit the water soluble glucose from reaching the brush border (Noy and Sklan, 2001).

Glucose absorption is a saturable process in the chicken intestine (Noy and Sklan 1998). At hatch, the chicken small intestine has excess glucose absorbability compared to the amount of glucose accrued from digestion of starch (Noy and Sklan, 1996). The glucose absorption increases after hatch and remains the same after day 4 PH (Sklan, 2003). The excess glucose absorbability suggests that providing glucose can partly replace traditional carbohydrate energy sources and increase AMEn compared to traditional diets.

Glucose absorption by chickens is affected by feed access. Noy and Sklan (2001) reported that glucose absorption in newly hatched chicks. At day 2 PH, glucose absorption was around 56% in DA bird compared with 61% in fed birds. At day 4 PH,

glucose absorption was approximately 76% in 48 hour DA chicks and more than 80% in early fed birds (Noy and Sklan, 2001). The glucose absorption remained low (59%) at day 4 post hatch, if the chicks were not fed (Noy and Sklan, 2001).

2.5.2 Effects of glucose and sucrose in chicken diet

The AMEn of glucose is lower than sucrose (3330 kcal/kg vs. 3750 kcal/kg) for chickens (Leeson and Summers 2001). However, this was measured after the chickens were fully developed. Glucose and sucrose have different effects on chicken performances when provided in diets. Sorbara et al. (2006) reported that the intestine was heavier in sucrose fed chicks than glucose fed chicks during first 7 days PH. Disaccharidase activity was higher in the sucrose fed chicks compared to those fed monosaccharides, such as, glucose and galactose. The developments of digestion and absorption function can be measured by analyzing the gene expression levels of related proteins, such as glucose transporters. The high glucose diet resulted in increased mRNA levels of the glucose transporters in chicken intestine (Suvarna et al. 2005). Higher mRNA levels suggest that the glucose absorption improves by the presence of glucose.

The effects of glucose on chicken digestive system developments were the subject of several publications. In some studies, glucose was provided in a water solution (0.5ml 20% glucose) after hatch (Takahashi and Akiba 2005). The pancreas weight at day 5 post hatch was heavier in glucose solution treatment (Takahashi and Akiba 2005). However, the pancreatic alpha-amylase activities were not affected by glucose solution (Takahashi and Akiba 2005). The effects of glucose on intestine development are small. This may bebecause glucose utilization in intestine does not require digestive enzymes and no stimulation of intestinal developments (Noy and Sklan 1998).

The effects of glucose and sucrose on chicken growth performances are not consistent among studies. Wei et al. (1984) reported that sucrose and glucose treated chicks did not show a significant differences in weight gain and feed conversion ratio. Jiang et al. (2008) indicated that providing 8% glucose solution did not affect chick weight gain during day 3 to day 10 PH. Another study reported that the glucose based diet showed a higher AMEn than corn based diet during the first week which may suggest that, the diet with glucose had better utilization than starch (Batal and Parson, 2004). Growth performances were better in glucose treated birds than sucrose fed birds (Batal and Parson, 2004). Batal and Parson (2004) indicated that reduced utilization of sucrose compared with dextrose might be due to the reduced levels of intestinal sucrase during first 3 weeks post hatch. An early study tried to determine the glucose TMEn in newly hatched and 3 days old chicks, but did not get any measurement due to sudden death of chicks soon after the feeding (Sulistiyanto et al. 1999). The chicks were force fed glucose by crop intubation in the experiment (Sulistiyanto et al. 1999). The chicks which were force fed dextrin or starch did not suffer the sudden death after the same feeding process. The TMEn of glucose for 10 day old broiler chicks was determined at 2914 kcal/kg and which is lower than for starch (3139 kcal/kg) (Sulistiyanto et al. 1999).

Dietary glucose and sucrose have to been found to affect body compositions of chicken (Jiang et al. 2008 and Longo et al. 2007). The breast and thigh muscle yield were not affected by glucose, the abdominal, cervical and thigh fat deposits were increased (Jiang et al. 2008). The carcass composition and yield at slaughter were not affected by feeding chicken sucrose based diet during first 7 days PH (Longo et al. 2007).

2.6 Glycerol as energy sources in broiler starter diets

Glycerol is a major by-product of biodiesel production. The steady increase in global biodiesel production has resulted in a steady increase of crude glycerol supply (Thompson and He 2006). It is possible to recover 10 kg of crude glycerol from every 125 liters of biodiesel produced (Thompson and He 2006). Glycerol can represent about 9% of broiler starter feed on a weight basis (Dozier et al. 2008). Crude glycerol is already widely used in the pharmaceutical and food industries after intensive purification process and can be used as a dietary energy source in poultry diets (Alvarenga et al. 2012).

The sources of glycerol vary and have different chemical composition (Thompson and He 2006; Dozier et al. 2011). Generally 80 to 90% of crude glycerin is glycerol, the remaining components are water, ash, fatty acids, methanol and a trace of protein (Alvarenga et al. 2012). In wheat-based chicken diets, addition of glycerol can improve the starch digestibility (McLea et al 2011). The best dietary inclusion level of glycerol has not been determined, but it has been suggested that it ranges from 5% to 10% for chickens (Swiatkiewicz and Koreleski 2009, Schmidt and Zsédely 2010, McLea et al. 2011). When included in the diet, glycerol not only provides dietary energy, but also improves the pellet stability (Swiatkiewicz and Koreleski 2009, McLea et al. 2011).

2.6.1 Apparent metabolizable energy (AMEn) of crude glycerol

The main component of crude glycerol is glycerin. Variability in the composition of crude glycerol products, means the AMEn values of different sources of crude glycerol are different. The AMEn of many feed ingredients including glycerol is affected by the age of chicken. Dozier et al. (2008) determined the AMEn of one source of glycerol (86.95% glycerin and 9.63% moisture) for broiler chickens at different age groups. For 7

to 10 day old broiler chicks, glycerol had an AMEn of 3,621kcal/kg. The AMEn was about 3,331 kcal/ kg for 21 to 24 days old broilers and 3,349 kcal AMEn/ kg for 42 to 45 days old broilers. Across three age groups, the AMEn of this glycerol was approximately 3,434 kcal/ kg.

The compositions of crude glycerol from 11 different sources across United States were measured (Table 2.1, Dozier et al. 2011). The percentage of glycerol ranged from 52.8% to 93.8%, methanol ranged from 0.03% to 15.0% and fatty acids from 0.01% to 38.4% (Dozier et al. 2011). This variation in composition for different sources of crude glycerol has been found by other researchers as well (Jung and Batal 2011). Jung and Batal (2011) reported crude glycerol has, on average, 78 to 86% glycerin and less than 0.05% methanol. The glycerol level can be as low as 44.6% and methanol can be up to 1.79% (Jung and Batal, 2011). The variability in composition of crude glycerol should be taken into a consideration during broiler diet formulation.

The AMEn of crude glycerol from 11 different sources across United States was measured (Dozier et al. 2011). The AMEn in USP- grade glycerol was 3662 kcal/ kg (Table 2.1). The average AMEn value of 11 samples was 3,646 kcal/ kg. These results suggest that crude glycerol can be used efficiently as an energy source. The AMEn value of crude glycerol can be calculated from following the multiple linear regression equation AMEn (kcal/kg) = 1,605 – (19.13 × % methanol) + (39.06 × % fatty acid) + (23.47 × % glycerin) (Dozier et al. 2011). Other factors that affect AMEn values were not included in the equation. This equation can be used to estimate the AMEn value but it may not be accurate enough for feed formulation (Dozier et al 2011).

Table 2. 1. Compositions of 11 different sources crude glycerol produced in United States (Dozier et al. 2011).

Sample[1]	Source[2]	Glycerol	Moisture	Methanol	Ash	Fatty Acids	AMEn (Kcal/kg)
USP	--	99.62	0.35	ND[3]	0.01	0.02	3662
ADM-MO	SB	83.88	10.16	0.01	5.83	0.12	3364
AGP-IA	SB	83.49	13.40	0.11	2.93	0.07	3849
REG-MN	SB	85.76	8.35	0.03	5.87	ND	3479
REG-R	SB	83.96	9.36	0.01	6.45	0.22	3889
REG-WL	SB	84.59	9.20	0.03	5.90	0.28	3644
WW-TX	SB	81.34	11.41	0.12	7.12	0.01	3254
WW-OH	TA	73.65	24.37	0.03	1.91	0.04	3256
IW-AC	YG	93.81	4.07	0.04	1.93	0.15	4100
IW-NA	YG	52.79	4.16	3.49	4.72	34.84	4135
USB-GA	PF	51.54	4.99	14.99	4.20	24.28	3476

[1]Sample: USP = USP-grade glycerin; ADM-MO = Archer Daniels Midland, Mexico, MO; AGP-IA = Ag Processing Inc., Sergeant Bluff, IA; REG-MN = Renewable Energy Group, Albert Lea, MN; REG-R = Renewable Energy Group, Ralston, IA; REG-WL = Renewable Energy Group, Wall Lake, IA; WW-TX = Westway Feed Products, Houston, TX; WW-OH = Westway Feed Products, Cincinnati, OH; IW-AC = Imperial Western Products acidulated glycerin, Coachella, CA; IW-NA = Imperial Western Products nonacidulated glycerin, Coachella, CA; USB-GA = US Biofuels, Rome, GA.
[2]Source: the origination sources of crude glycerol: SB = soybean oil, TA = tallow, YG = yellow grease, PF = poultry fat.
[3]ND = not detected

2.6.2 Utilization of crude glycerol

The chicken intestine can readily absorb glycerol (Dozier et al. 2011). There are two glycerol absorption pathways in small intestine. More than 70% of total glycerol absorption is through the Na dependent active transport system while the remaining is through the passive transportation (Alvarenga et al. 2012). After absorbed, glycerin can be metabolized into glucose or oxidized for energy production through glycolysis and the citric acid cycle (Dozier et al. 2011).

Glycerol is metabolized by enzyme glycerol kinase into glyceraldehyde-3- phosphate after it has been absorbed (Alvarenga et al. 2012). Glycerol kinase which is found in intestinal epithelium cells and liver cells, is the enzyme which convert glycerol to

glycerol-3-phosphate (Bickerstaffe and Annison 1969). Liver serves as the major organ for glycerol metabolism and is responsible for more than 75% of glycerol metabolism in mammals, the same process is believed to exist in avian species (Alvarenga et al. 2012). The glyceraldehyde-3- phosphate is a substrate for lipid synthesis, gluconeogenesis and oxidation for energy in intestinal epithelium cells and liver cells (Figure 2.5). In other tissues, such as muscle and kidney, glycerol can be directly utilized. The kidney can represent around 20% of total glycerol usage. Glycerol is also found in urine (Alvarenga et al. 2012).

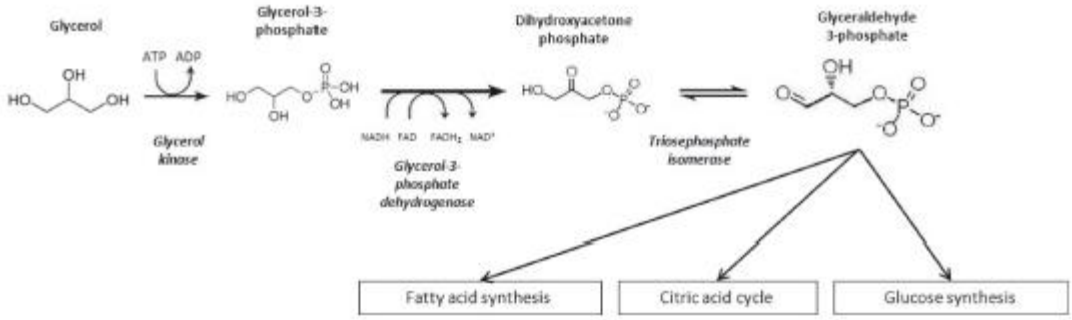

Figure 2.5. Glycerol pathway in animal cell. Glycerol kinase produces glyceraldehyde-3-phosphate from glycerol. ATP involves in this process. Glyceraldehyde- 3-phosphate is used for fatty acid synthesis, citric acid cycle and glucose synthesis (Alvarenga et al. 2012). (Permission to use requested)

Glycerol metabolism is controlled primarily by two hormones: insulin and glucagon (Alvarenga et al. 2012). In avian species, glucagon is a lipolytic hormone and insulin has the positive function of increasing blood glycerol levels. Glucagon stimulates the release of glycerol and fatty acids from adipose tissue and increases the rate of gluconeogenesis (Alvarenga et al. 2012). When glycerol blood concentration is high, the lipolytic catecholamines, are produced that trigger the use of glycerol for lipid synthesis (Alvarenga et al. 2012).

The age of the chicken influences the glycerol utilization. Cerrate et al. (2006) reported that growth performance was not affected by feeding up to 10% glycerol during first 14 days PH but it was suppressed by 10% glycerol after 35 days of age. It appears that glycerol utilization decreases as chickens age (Cerrate et al. 2006, McLea et al. 2011). The glycerol digestibility remained high when fed at 10% (Schmidt and Zsédely 2010, McLea et al. 2011).

2.6.3 Inclusion levels of crude glycerol

Suggested inclusion level for crude glycerol in broiler diets varies among studies. The AMEn for diet did not differ when glycerol was included at 3.3, 6.7 or 10% level. Schmidt and Zsédely (2010) reported that glycerol at 5% and 10% dietary inclusion levels can increase the feed intake and improve the nitrogen free extract digestibility.

Chicken feed intake was depressed by providing 15% glycerol included in the diet (Schmidt and Zsédely 2010). Margetyal et al. (2009) reported that feeding glycerol at 5% level for the first 10 days PH did not affect feed to gain ratio. Abd-Elsamee et al. (2011) reported that during the first 7 days PH, 2% glycerol suppressed chick growth and feed to gain ratio. However, Margetyal et al. (2009), Schmidt and Zsédely (2010) and Mclea et al. (2011) found that feeding glycerol at 5% to 6.7% level resulted in a superior feed to gain ratio without any negative effect on chick growth performance. Jung and Batal (2011) disagreed with both Abd-Elsamee et al. (2011) and McLea et al. (2011) findings. Weight gain and feed consumption were depressed by glycerin at 2.5, 5 and 7% levels. Jung and Batal (2011) suggested that the performance decrease may have resulted from a high level of methanol present in glycerin. The glycerin inclusion level should not exceed

5% in the broiler diet. The variability of the composition of crude glycerol products should be considered prior to feed formulation.

2.7 Knowledge gap

Crude glycerol has the potential to be a widely used ingredient in broiler diet formulation. However, recommended dietary inclusion level and the effects of crude glycerol are not fully determined. The effects of partially replaced dietary starch with EUES (glucose, sucrose or glycerol) in chicken starter diets on chicken growth performance and intestinal development are not well documented.

The effects of easily utilized dietary energy sources on growth performance and intestinal development in broiler chicks that have DA to feed need to be studied. The effects of partial replacement of dietary starch with glucose, sucrose or glycerol on chicken growth performance and intestinal development need to be studied. The results from such studies may provide new information that will facilitate the use of these feed ingredients in the formulation of broiler diets to overcome the growth disadvantages caused by a DA to feed following hatching.

CHAPTER 3 THE EFFECTS OF DIFFERENT FEEDING PROGRAM AND INCLUSION LEVELS OF GLYCEROL, GLUCOSE OR SUCROSE IN BROILER STARTER DIETS ON GROWTH PERFORMANCE AND INTESTINAL DEVELOPMENT

3.1 Abstract

This trial investigated the effects of inclusion of easily utilized energy sources (EUES, glucose, sucrose or glycerol) and delayed access to feed and water on broiler growth performance and intestinal development. In this trial, 2160 newly hatched chicks were randomly assigned to immediate (IA) or 36 hours delayed access (DA) to feed and water and fed 0, 4 or 8% EUES during first 14 days post hatch. On Day 35 post hatch, the IA birds were heavier than DA birds (2309.9±99.6g vs. 2147.6±66.7g; $p<0.05$). The 8% EUES starter diet fed birds were heavier than 0% EUES starter diet fed birds (2259.0±128.7g vs. 2198.2±93.9g; $p<0.05$). On Day 7 post hatch, 4% sucrose diet fed birds had higher duodenum villi surface areas (0.202±0.032 mm^2) than the birds fed 8% sucrose (0.179±0.039 mm^2). Ileal villi were higher in 4% sucrose diet fed birds than the birds fed glucose or glycerol treatments. On Day 14 post hatch, duodenal histological developments were not affected by treatments. Ileal villi developments were not affected by treatments. The 8% EUES diets fed IA birds had deeper crypts (137.7±9.4 μm) than DA birds subjectd to same diets (125.0±7.4 μm; $p<0.05$).

3.2 Introduction

Broiler chickens have undergone intensive genetic selection for better growth performance during the last few decades (Shariatmadari, 2012). As the number of days required to reach market weight continues to decline, the first few days of life has become an increasingly significant portion of the life term of the broiler chicken.

Carbohydrates are the main dietary energy source for chickens post hatch. The major dietary carbohydrate source is starch (Leeson and Summers, 2001). However, starch digestion and absorption were low at hatch and increased with chick age (Noy and Sklan 1999; 2001). Providing easily utilized energy sources immediately after hatch have potential to improve chicken growth performance. During commercial production, up to 48 hours holding period may occur prior to first access to feed (Willemsen et al. 2010). During that time period, body weight decreases and intestine and muscle developments are reduced (Noy and Uni 2010).

Glucose and sucrose are mono and disaccharide carbohydrates respectively and can be easily utilized by chickens. The effects of glucose and sucrose on chicken growth performances are not consistent among studies. Wei et al. (1984) reported that sucrose and glucose fed chicks did not show significant differences in weight gain and feed conversion ratio. Jiang et al. (2008) indicated that providing an 8% glucose solution did not affect chick weight gain during day 3 to day 10 PH. Feed intake was reduced by providing glucose to newly hatched birds (Leeson and Summers 2001; Jiang et al. 2008). Batal and Parson (2004) reported that growth performance was better in glucose fed birds compared to sucrose fed birds.Glycerol is easily abosrped by the small intestine (Dozier et al. 2011). The steady increase in global biodiesel production has resulted in a steady increase of crude glycerol supply (Thompson and He 2006). However, recommended dietary inclusion level for crude glycerol varies among studies (Abd-Elsamee et al. 2011; Margetyal et al. 2009 and Mclea et al. 2011).

The effects of partially replacing dietary starch with EUES (glucose, sucrose or glycerol) in chicken starter diets on growth performance and intestinal developments are not well

documented. In this experiment, the effects of dietary inclusion different levels of glycerol, glucose or sucrose on growth performance and intestinal developments were studied when birds were subjected to IA or DA to feed.

3.3 Objectives

To determine the effects of different feeding program and 4% or 8% dietary inclusion of glycerol, glucose or sucrose in broiler starter diets on growth performance of broiler chicks

To determine the effects of different feeding program and 4% or 8% dietary inclusion of glycerol, glucose or sucrose in broiler starter diets on small intestinal developments of broiler chicks

3.4 Hypotheses

It is hypothesized that inclusion of glycerol, glucose or sucrose in broiler chicken starter diet will improve growth performances and overcome the effects of delayed access to feed and water.

Birds receiving glycerol, glucose or sucrose supplied in diets will have more developed intestines than control birds. The glycerol, glucose or sucrose supplied will promote intestinal histological developments.

3.5 Materials and methods

3.5.1 Hatchery practice

3.5.1.1 Incubation environment

Fertilized eggs (Ross 508) were purchased from a local hatchery. The average egg weight was 61.66±0.73g. The eggs were stored at the Faculty of Agriculture, Dalhouise

University Hatchery in Truro, NS. The storage temperature was 18°C at 75% relative humidity (RH) for 3 days.

The eggs were placed in the setting racks with the small end down. There were 132 eggs per rack and 6 trays were placed in Chick Master® G90 incubators (Chick Master®, Medina, Ohio). The incubators were preheated to 37.5°C and RH was set at 55% for 24 hours before eggs were set. The incubation condition schedule was listed in Table 3.1.

The total length of incubation was 21 days plus 6 hours. During the first 18 days of incubation, the temperature was set at 37.5°C and at 55% RH. The eggs were automatically turned very 45 minutes. On day 18 of incubation, the eggs were removed from setting racks and transferred into the hatching trays. At two hours before the end of hatching period, the RH was reduced to 55% RH. The incubators were monitored twice daily at 0800h and 1600h. During the incubation process, no abnormal temperature or RH were recorded.

Table 3.1: The incubator temperature and relative humidity (RH) during hatching process.

Time of Incubation	Temperature (°C)	Relative Humidity (%)
Day 1-18	37.5	55%
Day 20	37.5	64%
Day 20.5	37.5	72%
Day 21	37.5	82%
2 hours before hatch pull	37.5	55%

3.5.1.2 Post hatching treatment and feather sexing

After completion of the hatching process, the chicks were feather sexed (Figure 3.1). The male chicks have similar length of primary feathers and covert feathers (Figure 3.1 left). The female chicks have the shorter covert feathers than primary feathers (Figure 3.1

right). The male and female chicks were placed into different containers for the further hatchery processing.

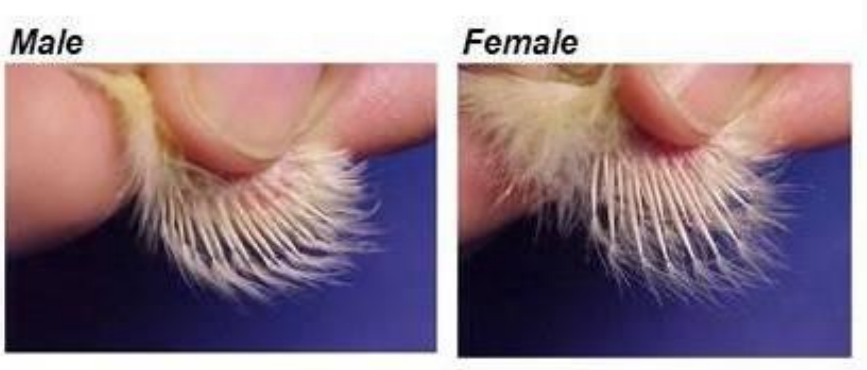

Figure 3.1. The feather sexing of newly hatched broiler. Left: male. Right: female.

The chicks were vaccinated for Marek's disease. The vaccine was produced by Intervet Canada Corp (Kirkland, Quebec). A 0.2 ml of vaccine was delivered by Socorex® 187 self-refilling syringes (Socorex® Swiss, Lausanne Switzerland) subcutaneously in the back of the neck. After vaccination, chicks were placed in the transport trays and delivered to Atlantic Poultry Research Centre (APRC) in Truro, NS.

3.5.2 Animals and general rearing environment

After hatch, one thousand and eighty male and one thousand and eighty female Ross 308 broilers were randomly placed into 72 floor pens in 4 similar climate controlled rooms (18 pens /room) in the APRC. The pen was 2.13m x 0.93m with stocking densities of 0.5kg/m^2 on day 0 and 21.02kg/m^2 on day 35. In each pen, there were 15 male and 15 female chicks. Lighting and temperature schedules are described in Table 3.2. The temperature was measured electronically using Raytek Mini temp gun twice daily during health checks throughout the trial.

Feed and water were provided ad libitum once feeding was initiated. Water was provided from nipple drinkers and experimental diets were provided in cardboard boxes,

measuring 53.3 cm x 43.2 cm x 5.1cm during the first 7 days of placement as well as feed was provided from tube feeder for the entire experiment. After day 7 of placement, feed were provided through the tube feeders. All the procedures were carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009).

Table 3.2. Lighting and temperature schedules for broilers housed at Atlantic poultry research center during experiment.

Days post hatch	Temperature (^{o}C)	Light Hours	Light Intesity
0-1	32	24	20
2-3	31	23	20
4	30	23	20
5	30	16	15
6	29	16	15
7-8	29	16	10
9-10	28	16	5
11-12	27	16	5
13-15	26	16	5
16-17	25	16	5
18-19	24	16	5
20-22	23	16	5
23-26	22	16	5
27	21	16	5
28-31	21	17	5
32-35	21	18	5

3.5.3 Timing of access to feed

There are two different timings of first feed and water access. The newly hatched chicks were randomly assigned to immediate feed and water access (IA) or 36 hours delay in access to feed and water (DA).

All the IA birds were placed near the drinker, had their beaks dipped in water in the drinker. The feed and water was available on the cardboard box lid immediately after birds arrived the APRC. The starter diets were provided from cardboard box lid for first 7 days PH and from tube feeder from day 1 to 14 PH. The grower diet was provided from

tube feeders from day 15 to 24 PH. The finisher diet was provided from tube feeder from day 25 to 35 PH.

For the DA birds, after 36 hours without feed and water, all the birds had their beaks dipped in water in the drinker and in the feed in the cardboard box lid to introduce them to where the water and feed were available. The grower diet was provided from tube feeders from day 15 to 24 PH. The finisher diet was provided from tube feeder from day 25 to 35 PH.

3.5.4 Experimental diets

All diets throughout the trial were formulated to be isonitrogenous and isocaloric within phases. The starter mash diets were formulated to contain 23% crude protein and 3050 kcal AMEn/kg and were fed during first 14 days PH (Table 3.3). The starter diets contained 0, 4 and 8% level of glucose, sucrose or glycerol. The glycerol used in this experiment was REG Glycerin – 98 (Renewable Energy Group®, Ames, IA). This crude glycerol contained 98% glycerin and 0.8% moisture.The calculated and analyzed values of nutrient compositions of starter diets were shown in Tables 3. 4 and 3.5, respectively.

Table 3.3. Diet formulations for chicks receiving glucose, sucrose or glycerol in diets during the first 14 days post hatch.

Feed	Control	Glucose		Sucrose		Glycerol	
Ingredients %		4%	8%	4%	8%	4%	8%
Corn	44.59	38.90	34.59	39.67	33.64	38.50	33.79
Soybean meal	38.72	39.72	40.47	39.72	40.61	39.79	40.61
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Tallow-grease blend	3.05	3.23	2.79	3.23	2.07	3.56	2.79
Glucose	0	4	8	0	0	0	0
Sucrose	0	0	0	4	8	0	0
Glycerol	0	0	0	0	0	4	8
Limestone	1.65	1.66	1.65	1.66	1.62	1.66	1.65
Mono-Dicalcium phosphate	0.59	0.86	0.88	0.86	0.65	0.86	0.88
Methionine Premix[a]	0.64	0.64	0.65	0.64	0.65	0.64	0.65
Mineral & Vitamin Premix[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Iodized salt	0.431	0.431	0.434	0.431	0.434	0.431	0.434
Lysine 98%	0.040	0.040	0.021	0.040	0.021	0.039	0.021
Amprolium[c]	0.013	0.013	0.013	0.013	0.013	0.013	0.013
BMD[d]	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Total	100	100	100	100	100	100	100

[a] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

[b] Vitamin/Mineral premix, supplied per kg diet: vitamin A, 9750 IU; vitamin D3, 2000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.95 mg; thiamine, 2.91 mg; manganese, 70. 2 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg.

[c] Amprolium -- AMPROL® 25% FEED MIX Huvepharma AD, Bio Agri Mix LP, Mitchell, ON, Canada (amprolium 25% w/w)

[d] BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne-1 mixed feed)

Table 3.4. The calculated nutrient composition of diets where chicks received a glucose, sucrose or glycerol supplement during the first 14 days post hatch.

Feed	Control	Glucose		Sucrose		Glycerol	
		4%	8%	4%	8%	4%	8%
Calculated analysis							
AMEn (kcal/kg)[a]	3050	3050	3050	3050	3050	3050	3050
Crude Protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Calcium (%)	1.00	1.05	1.05	1.05	1.05	1.05	1.05
Crude Fiber (%)	2.54	2.45	2.37	2.45	2.37	2.45	2.35
Lysine (%)	1.43	1.43	1.43	1.43	1.43	1.43	1.43
Methionine + cysteine (%)	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Histidine (%)	0.61	0.61	0.62	0.61	0.62	0.61	0.62
Linoleic Acid (%)	1.87	1.79	1.60	1.79	1.60	1.87	1.75
Arginine (%)	1.66	1.66	1.67	1.66	1.67	1.66	1.67
Total phosphorus (%)	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Magnesium (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Selenium(ppm)	0.38	0.39	0.37	0.39	0.37	0.38	0.36
Thiamin (ppm)	5.51	5.51	5.46	5.51	5.46	5.51	5.45
Methionine (%)	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Copper (ppm)	33	33	33	33	33	33	33
Zinc (ppm)	118	118	118	118	118	118	118
Potassium (%)	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Dry Matter (%)	90	90	90	90	90	90	90

[a] AMEn is apperant metabolic energy nitrogen corrected.

Table 3.5. The analyzed nutrient compositions of diets where chicks received a glucose, sucrose or glycerol supplement during the first 14 days post hatch.

Feed		Glucose		Sucrose		Glycerol	
	Control	4%	8%	4%	8%	4%	8%
Crude Protein (%)	23.2	23.1	23.0	23.1	23.2	23.3	23.0
Calcium (%)	0.96	1	1	0.99	0.92	1.03	0.94
Potassium (%)	0.94	0.99	1.05	0.96	0.95	0.94	0.94
Magnesium (%)	0.19	0.19	0.19	0.19	0.18	0.19	0.18
Phosphorus (%)	0.61	0.58	0.61	0.64	0.59	0.58	0.61
Sodium (%)	0.22	0.19	0.20	0.22	0.19	0.20	0.20
Copper (ppm)	30	27	29	33	28	28	29
Manganese (ppm)	104	97	106	105	103	101	95
Zinc (ppm)	110	104	107	110	106	106	104
Crude fat (%)	5.99	5.28	4.92	5.45	5.39	5.66	4.88

On day 15 PH, one common grower pelleted diet was provided to all treatments. The grower diet formulated to contain 20% crude protein and 3150 kcal AMEn/kg (Table 3.6) was provided from the day 15 to day 24 PH. From the Day 25 - 35 PH, one common finisher pelleted diet was provided to all treatments. This diet was formulated to contain 18% crude protein and 3200 kcal ME/kg (Table 3.6). The grower or finisher diet did not contain any EUES. The calculated and analyzed nutrient compositions of grower and finisher diets were shown in the Table 3.7.

All diets met or exceeded the National Research Council (NRC, 1994) requirements for broiler growth within each growth stage. For all phases, feed was weighed in as needed and feed weigh backs occurred at the end of each dietary phase, or as mortality occurred.

Table 3.6. The diet formulations for chicks receiving common diets during day 15-24 and day 25-35 post hatch.

Feed Ingredients %	Grower	Finisher
Corn	49.70	38.90
Soybean meal	31.40	39.72
Wheat	10.00	10.00
Tallow-grease blend	4.61	3.23
Limestone	1.43	1.66
Mono-Di calcium Phosphate	0.72	0.86
Methionine premix[a]	0.59	0.64
Mineral and Vitamin Premix[b]	0.5	0.5
Iodized salt	0.40	0.40
Lysine 98%	0.111	0.123
Amprolium[c]	0.05	0
BMD[d]	0.004	0
Pel-stik[e]	0	0.05
Total	100	100

[a] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.
[b] Vitamin/Mineral premix, supplied per kg diet: vitamin A, 9750 IU; vitamin D3, 2000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.95 mg; thiamine, 2.91 mg; manganese, 70. 2 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg.
[c]Amprolium -- AMPROL® 25% FEED MIX Huvepharma AD, Bio Agri Mix LP, Mitchell, ON, Canada (amprolium 25% w/w)
[d]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne-1mixed feed)
[e]Pel – stik -- Pellet Binder – Uniscope Inc., Johnstown, CO, USA.

Table 3.7. The calculated and analyzed nutrient compositions of the common diets during day 15-24 and day 25-35 post hatch.

Feed Ingredients %	Grower	Finisher
Calculated analysis		
AMEn (kcal/kg)[a]	3150	3200
Protein (%)	20	18
Calcium (%)	0.92	0.85
Lysine (%)	1.24	1.09
Methionine + cysteine (%)	0.95	0.86
Potassium (%)	0.92	0.83
Sodium (%)	0.18	0.18
Crude fiber (%)	2.47	2.47
Histidine (%)	0.53	0.47
Linoleic acid (%)	2.45	2.37
Arginine (%)	1.4	1.23
Methionine (%)	0.45	0.41
Total phosphorus (%)	0.55	0.52
Magnesium (%)	0.16	0.15
Zinc (ppm)	115	113
Copper (ppm)	32.12	31.51
Selenium(ppm)	0.43	0.45
Thiamin (ppm)	5.41	5.33
Dry matter (%)	90	90
Analyzed level		
Crude Protein (%)	20.1	18.9
Calcium (%)	0.89	0.82
Potassium (%)	0.93	0.82
Magnesium (%)	0.16	0.15
Total Phosphorus (%)	0.53	0.49
Sodium (%)	0.17	0.16
Copper (ppm)	31	28
Manganese (ppm)	88	77
Zinc (ppm)	136	87
Crude fat (%)	6.65	5.77

[a] AMEn is apperant metabolic energy nitrogen corrected.

3.5.5 Data collection

3.5.5.1 Growth performance

On days 7, 14, 24 and 35 PH, all birds in the pen were batch weighed and their feed was weighed back and recorded. Feed added was measured and recorded each day at feeding. When mortalities occurred, the feed weigh back was conducted and recorded.

Using this data, daily feed consumption (DFC), average body weight (BW), average daily weight gain (ADG), and feed to gain ratio (FGR) on a per bird basis and % mortality were calculated for each period of growth.

On each weight sampling date, all birds were batch weighed using a balance equipped with live weight capability (Mettler PM 34-K Delta Range, Mississauga ON). Before weighing, the containers were tared on the balance. The birds were placed into the container (number of birds was determined by the number of the birds that can easily stand on the bottom of the container) then the container was placed on the balance. The weight and number of the birds were recorded. After weighing, the birds were placed back into the pen. When mortality occurred, the dead bird was weighed and sent to a veterinary pathologist for necropsy.

3.5.5.2 Intestinal sampling

On day 7, 14 and 35 PH, one male and one female from each pen were randomly selected and euthanized by cervical dislocation. Each bird was weighed and the ileum, jejunum, and duodenum removed following the sampling procedure in Figure 3.2. Procedure outline, notes and the sampling image were created by Jennifer Dobson based on modifications from Klasing (1998). The intestinal sections of each bird were weighed using a top pan balance (Mettler Toledo, Thermo Fisher Scientific Inc.) and the length of the ileum, jejunum and duodenum measured separately using a standard metric ruler.

After measuring the weights and length, a 2.5 – 3 cm length segment was taken from each intestinal section (Figure 3.2) and stored in scintillation vials with 10% buffered neutral formalin for subsequent histological analysis.

1. Remove proventriculus, cut open and clean then weigh.
2. Remove gizzard, cut open and clean then weigh.

Duodenum

3. Cut the intestine parallel with the end of the pancreas to remove the duodenum.
4. Make a slice through the intestine just after the loop of the duodenum.
5. Remove the pancreas….CAREFUL not to switch the ends of the duodenum!
6. Weigh the duodenum… CAREFUL not to switch the ends of the duodenum!
7. Measure the total length of the duodenum (will be in two sections)
8. Cut a 1cm sample section of the duodenum from the slice after the loop. Put sample 1 in formalin.

Ileum

9. Find the end of the digestive tract and cut the intestine just above the ileum/cecal junction (below where the open ends of the ceca are attached).
10. Cut the intestine above where the blind pouch ends of the ceca are attached.
11. Remove the ceca from both sides.
12. Weigh the ileum and measure the length.
13. Cut a 1cm sample section from the intestine just above where the open ends of the ceca are attached. Put sample 2 in formalin.

Jejunum

14. Remove the rest of the remaining intestine tract, this is the jejunum.
15. Remove the mesentery, weigh the jejunum and measure the length.
16. Measure 15cm from the end of the jejunum that was closest to the duodenum.
17. Take a 1cm sample from the intestine at this point. Put sample 3 in formalin.

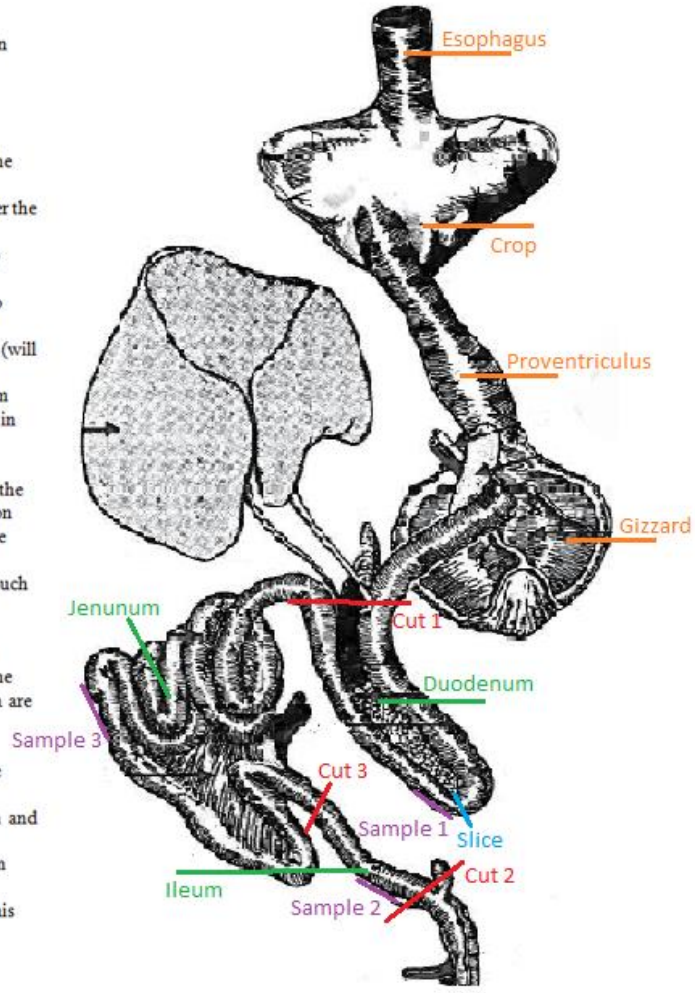

Figure 3.2. Dissection protocol prepared by Jennifer Dobson, modified from Klasing (1998) for intestinal sampling of broilers.

3.5.5.3 Intestinal histomorphology

3.5.5.3.1 Preparation of histological slices

The duodenum and ileum histomorphological analysis was conducted by following the procedure described by Budgell (2008). The intestinal tissue samples were removed from the formalin, then three slices were cut from each sample. If the sample had poor tissue integrity or was small then fewer divisions were made. The samples were then placed in plastic cassettes.

The cassettes were placed in the Tissue-Tek® VIP™ (Sakura Finetek USA Inc., Torrance CA) for the dehydration process. The dehydration was conducted in a series of alcohol solutions ranging from 70% to 100%. After dehydration, three tissue sections were then fixed in a paraffin wax block together after being permeated with xylene.

After the fixing process, the samples were placed and then imbedded in paraffin wax using a Tissue-Tek® TEC™ (Sakura Finetek USA Inc., Torrance CA). The slices were made by a microtome (Leica RM2255, Nussloch Germany). The slice was 0.5μm in thickness and was placed in a 35.5ºC water bath to be transferred onto a slide. The slices were stained by the Tissue-Tek® DRS™ (Sakura Finetek USA Inc., Torrance CA) using haematoxylin and eosin staining following the procedure of Drury and Wallington (1980). The slides were covered by using an automated coverslipper (Thermoscientific Clearvue, Waltham MA). All slides were prepared at the Hancock Veterinary Building (Dept. of Agriculture, Government of Nova Scotia, Truro, NS).

3.5.5.2. Intestinal histomorphology measurements

Before the analysis was conducted, the three cuts of each intestinal segment on each slide were assessed using scales described by Budgell (2008) Table 3.8, Figure 3.3). The clearest of the three slices on a slide was used for measurements. Villi height was measured from the top of the villi to the start of the crypt. Crypt depth was measured from the bottom end of the villi to the start of the mucosa. Villi width was measured equidistant from the top of the villus to the start of the crypt. Villi apparent area was calculated via the imaging software from the sum of calibrated pixel units within the defined region of each villi. Six to ten measurements were taken per slide and then averaged to give the measurement for each bird.

Table 3.8. Scoring the quality of broilers intestinal cross-sections slices for histomoporphological measurement.

Villus Defects	Scores
Few villi unreadable in cross section (0-25%)	1
Several villi unreadable in cross section (26-50%)	2
Numerous villi unreadable in cross section (51-75%)	3
Severe unreadability in cross section (>75%)	4
Cross section completely unreadable (100%)	5

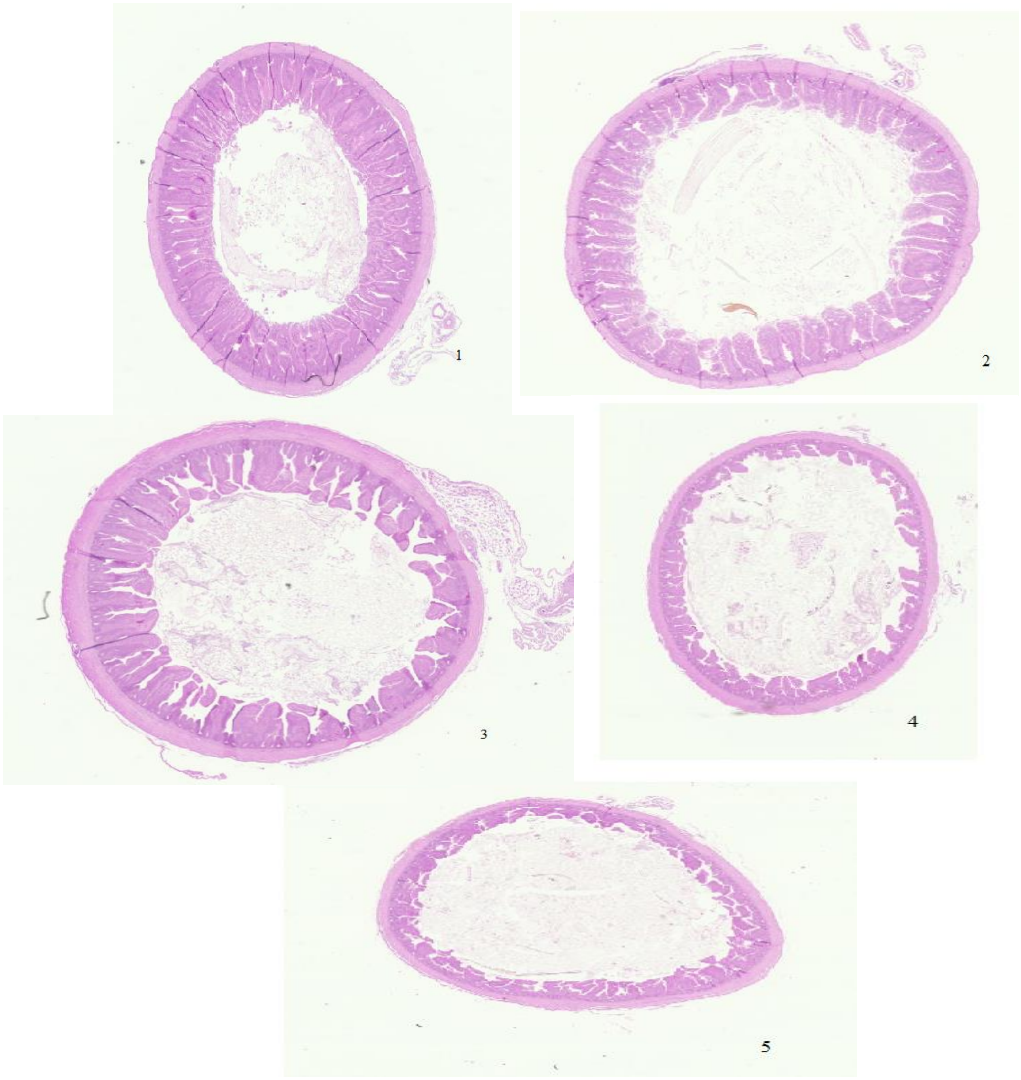

Figure 3.3: Intestinal readability scoring: Top Left: 0 – 25% villi unreadable in cross section (1), Top Right: 26 – 50% villi unreadable (2), Middle left: 51 – 75% villi unreadable (3), Middle Right: Above 76% villi unreadable (4), Bottom: Cross section completely unreadable (5).

After samples were prepared for image analysis, slides were scanned onto the computer using a Nikon Super CoolScan 400ED (Nikon Inc., Japan). Measurements were then taken using SigmaScan Pro 5 (SPSS Inc., Chicago, IL). Before measurements were conducted, the imaging software was calibrated using a 1.00mm calibration slide. The calibrating slice was also scanned into the computer using the Nikon Super Coolscan (Nikon Inc., Japan).

The villus height was measured from the top of the villi to the top of the crypt (Figure 3.4). The crypt depth was measured from the top of the crypt to the muscularis mucosae (Figure 3.4). The villus width was measured at the villus midpoint (Figure 3.4). The villus surface area was measured by SigmaScan Pro 5 (SPSS Inc., Chicago, IL) as the sum of the calibrated pixel units in a defined region (Figure 3.4). For each cross-section, six to ten measurements were taken. The number of measurements depended on the quality of each slice.

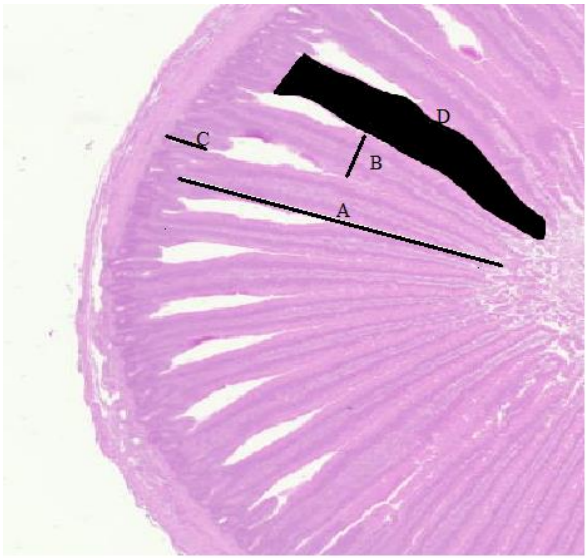

Figure 3.4 histology measurements of the cross-sections A: villus height, B:villus mid width C: crypt depth, D: villus surface area

3.5.6 Statistical analysis

The trial was a 2*3*3 blocking factorial design with easily utilized energy source (EUES), energy source inclusion levels (ESIL) and feed and water access program as main factors. The pen was used as the experimental unit. The room was used as blocking factor. There were four blocks involved in experiment. The factor dietary energy source has three levels: glucose, sucrose and glycerol. The factor inclusion level has three levels: 0, 4 and 8%. The factor feed and water access program has two levels: DA or IA.

3.5.6.1 Statistical analysis of growth performance data

The FI, BW, ADG and FGR were analyzed as repeated measures when time was a factor. The growth performances data were analyzed using ANOVA by the Proc Mixed procedure (Littell et al., 1996) was used in in SAS 9.3 (SAS Institute Inc., Cary, NC). Three covariance structures, compound symmetry (CS), untrusted (UN) and variance components (VC) were compared. The covariance structure which provided the smallest corrected Akaike's information criterion (AICC) was used to conduct the ANOVA test. The covariance structures CS was selected. If high order interactions were significant, the lower order interaction and main effects that involved in high order interaction were ignored. Where interactions with time were significant (α=0.05) data was sliced by time and analyzed separately. Any significant main or interaction effects (α =0.05) were analyzed using the Tukey-Kramer test to differentiate the means. The statistical model for repeated measures analysis was:

$$\gamma_{ijklm} = \mu + \alpha_i + \beta_j + \delta_k + \alpha\beta_{ij} + \alpha\delta_{ik} + \beta\delta_{jk} + \alpha\beta\delta_{ijk} + \zeta_l + \alpha\zeta_{il} + \beta\zeta_{jl} + \delta\zeta_{kl} + \alpha\beta\zeta_{ijl} + \alpha\delta\zeta_{ikl} + \beta\delta\zeta_{jkl} + \alpha\beta\delta\zeta_{ijlk} + \theta_m + \varepsilon_{ijklm}$$

Where γ_{ijklm} = response, μ = population mean, α = factor 1 or dietary energy source, i = levels of factor 1 (glucose, sucrose or glycerol) (i = 1-3), β = factor 2 or dietary inclusion levels, j = levels of factor 2 (0, 4 or 8%) (j = 1-3), δ = factor 3 or feed and water access program, k = levels of factor 3 (IA or DA) (k = 1-2), ζ = time factor, k = sampling days (day 7, 14, 24 and 35 days post hatch) (k = 1-4), θ = blocking factor, m = levels of blocking factor, (m = 1 - 4), ε = uncontrollable factors.

3.5.6.2 Statistical analysis of intestinal data

The intestinal data were analyzed by ANOVA using the Proc Mixed procedure of SAS (Littell et al., 1996). The statistical model was:

$$\gamma ijkl = \mu + \alpha_i + \beta_j + \delta_k + \alpha\beta_{ij} + \alpha\delta_{ik} + \beta\delta_{jk} + \alpha\beta\delta_{ijk} + \theta_l + \varepsilon_{ijkl}$$

Where γ_{ijkl} = response, μ = population mean, α = factor 1 or dietary energy source, i = levels of factor 1 (glucose, sucrose or glycerol) (i = 1-3), β = factor 2 or dietary inclusion levels, j = levels of factor 2 (0, 4 or 8%) (j = 1-3), δ = factor 3 or feed and water access program, k = levels of factor 3 (IA or DA) (k = 1-2), θ = blocking factor, l = levels of blocking factor, (l = 1 - 4), ε = uncontrollable factors.

3.6 Results and discussion

3.6.1 Growth performance

The p value for main factors, or their interactions on growth performances, are shown in Table 3.9. If the ANOVA result showed significant differences ($P<0.05$) for any of the parameters, the effects were analyzed for different sampling days. Table 3.10 shows the ANOVA result of the main factor effects for each sampling day.

During the different growth stages, the body weight was affected by the three main effects (Table 3.9). The ADG was affected by the different feeding programs (Table 3.9). The EUES and ESIL affected the ADG significantly during the different growth stages (Table 3.9). ESIL and EUES affected the DFC (Table 3.9).The DFC was also affected by

Table 3.9. The P values for the effects of energy source, inclusion level and feeding program and their interactions on broiler chicken growth performance through a 35 day production period.

Growth Performance Effect	Body Weight	Average Daily Gain	Daily Feed Consumption	Feed to Gain Ratio
Level	0.005	0.059	0.003	0.982
Energy Source	<0.001	0.097	0.010	0.525
Level* Energy Source	0.831	0.828	0.072	0.061
Program	<0.001	<0.001	<0.001	0.504
Level*Program	0.494	0.794	0.377	0.904
Energy Source* Program	0.684	0.442	0.756	0.770
Level* Energy Source* Program	0.981	0.829	0.952	0.333
Time	<0.001	<0.001	<0.001	<0.001
Time*Level	0.005	0.040	0.342	0.018
Time* Energy Source	0.003	0.034	0.064	0.004
Time* Level* Energy Source	0.733	0.505	0.313	0.627
Time* Program	<0.001	0.354	0.002	0.632
Time*Level*Program	0.652	0.975	0.420	0.040
Time*Energy Source*Program	0.389	0.227	0.614	0.095
Time*Level* Energy Source* Program	0.960	0.753	0.689	0.127

If the $p<0.05$, the effects are significant.

Table 3.10. The P values for the effects of energy source, inclusion level and feeding program and their interactions on broiler chicken growth performance for different ages.

Growth performance	Age	Level	Energy source	Feeding program
Body Weight	7	0.939	0.930	0.029
	14	0.323	0.281	<0.001
	24	0.030	<0.001	<0.001
	35	<0.001	0.005	<0.001
Average Daily Gain	7	0.900	0.881	0.006
	14	0.291	0.267	0.002
	24	0.098	0.005	<0.001
	35	0.003	0.083	0.002
Daily Feed Consumption	7	0.939	0.947	0.002
	14	0.014	0.137	0.002
	24	0.143	0.853	<0.001
	35	0.019	<0.001	<0.001
Feed to Gain Ratio	7	0.644	0.299	0.303
	14	0.708	0.464	0.513
	24	0.020	0.036	0.427
	35	0.045	0.005	0.883

If the $p<0.05$, the effects are significant at the age.

the feeding program during the different growth stages. The FGR were affected by the ESIL and the feeding program interactions, as well as the main effects of the ESIL during the different growth stages (Table 3.9). The feeding program had an impact on body weight and DFC throughout the experiment (Table 3.10). The ESIL and the EUES did not affect growth performance during the starter stage (days 1 – 14 PH) ($P>0.05$). During the grower stage (days 15 – 24 PH), body weight and FGR were affected by the ESIL and the EUES. The ADG was only affected by the EUES but not by the ESIL. During the finisher period (days 25 – 35 PH), body weight, ADG and DFC were affected by inclusion levels and the EUES.

3.6.1.1 Body weight

At day 35 PH, birds on all treatments had reached the same body weight (2228±117.0g). On day 24 PH, chickens fed glycerol starter diet had heavier body weight (1142.3±65.9g) than glucose (1089.7±68.2g) or sucrose (1075.5±70.0g) fed chickens (Table 3.11). On day 35 PH, chicken which were fed with glycerol, glucose and sucrose starter diets reached body weights of 2254.8±132.8g, 2216.0±124.5g and 2215.5±55.9g, respectively (Table 3.11).

Table 3.11. The effects of different energy sources fed in starter diets (day 1 –14) on broiler body weight (g) during the 35 day period.

Energy Source	Days			
	7	14	24	35
Glycerol	136.6±15.6e	376.9±31.3d	1142.3±65.9b	2254.8±132.8a
Glucose	133.2±17.6e	357.9±32.1d	1089.7±68.2c	2216.0±124.5a
Sucrose	131.4±16.6e	357.7±30.0d	1075.5±70.0c	2215.5±55.9a

a -- e means different letters differ significantly ($P<0.05$).
The differences of body weight during grower and finisher stage were identified from the ANOVA ($P < 0.01$,Table 3.10), but the Tukey – Kramer test did not differentiate the means ± SEM ($P>0.05$).

The effects of inclusion of glycerol, glucose and sucrose on body weight were identified from the ANOVA ($P < 0.01$), but the Tukey – Kramer test did not differentiate the body weight differences on day 35 PH (Table 3.11). The Tukey – Kramer test controls the experimentwise error rate at the selected level and it is preferred by statisticians (Montogomery, 2013).

Inclusion of any of the energy sources at 8% of the starter diet resulted in the highest body weights (2259.0±128.7g), in comparison to chickens raised on the control diet (2198.2±93.9g) during the same time period (Table 3.12). The effects of different inclusion levels on the body weights were identified from the ANOVA ($P = 0.03$) at day

24 post hatch (Table 3.10), but the Tukey – Kramer test did not differentiate the means (Table 3.12).

Table 3.12. The effects of different levels of glucose, glycerol or sucrose in starter diet (day 1 –14) on broiler body weight during the entire 35 day period.

Inclusion Level (%)	Days			
	7	14	24	35
0	132.6±16.3e	354.9±30.2d	1106.5±74.2c	2198.2±93.9b
4	132.1±14.1e	362.3±28.0d	1082.3±88.5c	2229.1±122.5ab
8	136.5±13.4e	375.4±29.0d	1118.7±82.3c	2259.0±128.7a

a -- e means different letters differ significantly ($P<0.05$).
The differences of body weight during grower stage were identified from the ANOVA ($P = 0.03$, Table 3.10), but the Tukey – Kramer test did not differentiate the means ± SEM ($P>0.05$).

The studies of the effects of dietary glucose on chicken body weight are inconclusive. Wei et al. (1984) reported that providing newly hatched chicks with 5% sucrose or glucose for two weeks did not affect body weight. When 8% glucose solution was provided, chicken body weights were the same during the first 7 days PH (Jiang et al. 2008). Noy and Pinchasov (1993) reported that providing 0.5ml 50% glucose solution immediately after hatch increased chicken body weight on day 21 PH.

The IA birds were heavier than the DA birds at all the sampling days except day 7 (Table 3.13). On day 35 PH, the IA chickens reached a heavier body weight of 2309.9±99.6g, compared to DA birds 2147.6±66.7g (Table 3.13).

Research has shown that the weight of the first 7 days PH has a linear relationship with the slaughter weight (Saki, 2005). The differences in body weight caused by delayed feed access remained throughout the current experiment. A previous study concluded that a 36 hour PH fasting reduced chicken growth rate irreversibly (Kornasio et al. 2011) and early feed access provided growth advantages up to 35 days PH (Bhanja et al. 2010). The

current experiment supports these previous studies. Conversely, Cengiz et al. (2012) reported that, Ross 308 birds were able to reach the same final body weight after a 36 hour delayed access to feed and water.

Table 3.13. The effects of different feeding programs on broiler body weight during the 35 day post hatch.

Program	Days			
	7	14	24	35
Immediate Access	146.1±7.7g	389.7±14.8e	1159.1±56.7c	2309.9±99.6a
Delay access	121.4±7.5g	338.7±16.0f	1045.9±62.3d	2147.6±66.7b

a -- g means different letters differ significantly ($P<0.05$).

There is no previous study comparing the effects of glycerol, glucose and sucrose on chicken growth performance. The AMEn of glucose is lower than sucrose (3330 kcal/kg vs. 3750 kcal/kg) for chickens (Leeson and Summers 2001). However, this was measured after the birds were fully developed. An earlier study tried to determine the glucose MEn in newly hatched chicks, but no data was measured due to sudden death of chicks soon after they were force fed glucose into their crops (Sulistiyanto et al. 1999). Glycerol has the 3,621kcal/kg AMEn for 7 to 10 days old broilers (Dozier et al. 2008) and this energy content is higher than AMEn of glucose (3330 kcal/kg; Leeson and Summers 2001). The higher AMEn value for glycerol during the chicken starter phase may provide the development advantage for body weight during the grower period. However, in the current study, at market age, inclusion of 8% of all EUES in the starter diet resulted in heavier body weight than the control group.

3.6.1.2 Average daily gain

The DA to feed had negative effects on the ADG throughout the experiment. The IA birds gained 60.2±0.4g per day. The DA chickens only gained 55.8±0.4g per day. This

agreed with other research where the chicks subjected to DA to feed, expressed a slower growth rate than the birds which were immediately fed. The effects of delayed access to feed carried throughout the experiments (Abed et al. 2011; Bhanja et al. 2010; El-Husseiny et al. 2008 and Mahmoud and Edens. 2012). In the current study, the ADG did not differ during the grower phase (Days 15 – 24 PH) and finisher phase (Days 25 – 35 PH, Table 3.9). Abed et al. (2011) reported that if chickens were subjected to a 16 or 32 hour delayed feed access, the growth rate increased during says 21 to 28 PH. Cengiz et al. (2012) reported that Ross 308 chickens expressed a higher growth rate during the last week of production.

During the grower stage, the birds fed glycerol starter diet had the ADG of 76.54g/day, the birds fed glucose starter diet had the ADG at 71.75g/day (Table 3.14). The effects of glycerol, glucose and sucrose on the ADG were identified from the ANOVA ($P = 0.005$, Table 3.10), although the Tukey – Kramer test did not differentiate the means (Table 3.14). During the first 3 weeks PH, glucose fed chickens did not express a higher growth rate than chickens fed a corn soybean meal based diet (Batal and Parson 2002). The glucose fed chickens had a higher growth rate than the sucrose fed birds during the first week post hatch (Batal and Parson 2004). It should be noted that, in the study by Batal and Parson (2004), the glucose and sucrose completely replaced the corn starch at 40% level in the diet.

Table 3.14. The effects of different energy sources fed in starter diet (day 1 – 14) on broiler average daily gain during the 35 day of production.

	Days			
	0-7	8-14	15-24	25-35
Energy Source				
Glycerol	13.32±1.99d	34.32±2.95c	76.54±4.98b	111.25±8.50a
Glucose	12.87±2.03d	32.10±2.00c	71.75±7.39b	114.05±8.23a
Sucrose	12.57±2.09d	32.33±2.63c	73.20±4.62b	111.05±±8.90a

a -- d means different letters differ significantly (P<0.05).
The differences of ADG during grower stage were identified from the ANOVA (P <0.01, Table 3.10), but the Tukey – Kramer test did not differentiate the means ± SEM (P>0.05).

The effects of different inclusion levels on the ADG were identified from the ANOVA at day 35 post hatch (P = 0.003, Table 3.10), but the Tukey – Kramer test did not differentiate the means (Table 3.15). The chickens fed control diet had ADG at 109.19g, while, the chickens fed EUES at 8% inclusion level had ADG at 114.03g (Table 3.15).

Table 3.15. The effects of different levels of energy source fed in starter diet (day 1 –14) on broiler average daily gain during the 35 day of production.

	Days			
	0-7	8-14	15-24	25-35
Inclusion Level (%)				
0	12.68±2.69d	31.76±2.59c	75.15±5.36b	109.19±6.69a
4	12.77±1.95d	32.88±2.63c	72.02±6.74b	113.13±7.08a
8	13.31±1.88d	34.12±2.50c	74.33±5.82b	114.03±9.88a

a -- d means different letters differ significantly (P<0.05).
The differences of ADG during finisher stage were identified from the ANOVA (P < 0.01, Table 3.10), but the Tukey – Kramer test did not differentiate the means ± SEM (P>0.05).

An earlier study reported that 5% dietary glucose and sucrose did not affect chickens ADG during the first 2 weeks PH (Wei et al. 1984). The best dietary inclusion level of glycerol has not been determined, but it has been suggested that it ranges from 5% to 10% (Schmidt and Zsédely 2010). Conversely, other studies reported that chicken weight gain was suppressed by 2% dietary inclusion of glycerol (McLea et al. 2011and Jung and Batal 2011). McLea et al. (2011) reported that the weight gain during the second week

post hatch was reduced by dietary glycerol at 3.3, 6.7 or 10% level. Jung and Batal (2011) reported that broiler weight gain was depressed by dietary glycerin inclusion at 2.5, 5 and 7.5% levels.

In this experiment, there is no EUES and ESIL interaction effect on ADG. This is supported by previous studies. Jiang et al. (2008) reported that feeding 8% glucose during the starter period did not affect body weight gain. The starch digestibility was 93% on Day 4 PH (Batal and Parson 2002). During the first 14 days post hatch, chicks fed both 5% and 10% glycerol included diets had the same weight gain during the first 14 days PH (Cerrate et al. 2006).

3.6.1.3 Daily feed consumption

The EUSE and ESIL affected daily feed consumptions (Table 3.9). When the three energy sources were included at 8% of the diet, the average consumption was 90.46±0.68g feed/day throughout the experiment. The consumption was higher than that of the birds on the control diets, which only consumed 87.01±0.69g feed/day. The chickens fed the 4% inclusion level of EUES during the starter period consumed around 88.62±0.69g feed feed/day throughout the production period. Inclusion of glycerol resulted in the highest DFC at 90.38±0.69g feed/day. Sucrose inclusion resulted in lowest DFC which was 87.35±0.69g feed/day. Birds fed glucose during the starter period consumed around 88.35±0.69g feed/day and this number is intermediate among the three treatments. The feeding program affected DFC throughout the experiment. The IA birds had higher DFC at all sampling period in comparison with DA birds (Table 3.16).

Table 3.16. The effects of 36 hours delay in feed and water access compared to immediate feed and water access post hatch on average daily feed consumption during the 35 day period.

	Periods			
First Feeding	0-7d	8-14d	15-24d	25-35d
36h Delay	12.96±0.66h	46.25±4.29f	98.52±7.65d	182.60±9.36b
Immediate	17.55±1.09g	50.78±4.39e	109.57±4.81c	191.33±12.54a

a -- h means different letters differ significantly ($P<0.05$).

A previous study indicated that 5% and 10% glycerol fed chicks had the same feed consumption during first 14 days post hatch (Cerrate et al. 2006). Another study reported that 3.3, 6.7 and 10% dietary crude glycerol did not affect chicken feed consumption during the first 3 weeks post hatch (McLea et al. 2011). Feed intake was reduced by the glucose based diet (Batal and Parson, 2004). The sucrose based and starch based control diets did not affect feed intake (Batal and Parson, 2004). However, in the study by Batal and Parson (2004), the glucose completely replaced the starch. Glucose was used at 40% of diet (Batal and Parson 2004). In this current experiment, glucose was used at 8% level. The lower dietary glucose inclusion level may be the reason for different results between studies.

3.6.1.4 Feed to gain ratio

During days 25 – 35 PH, the birds fed glycerol in the starter diet had FGR at 1.73, while the chickens fed glucose and sucrose starter diets had FGR at 1.63 (Table 3.17). During the days 15 – 24 PH, the chickens fed glycerol in the starter diet had FGR at 1.38 (Table 3.17). During the same period, the birds fed glucose starter diets had FGR at 1.46 and birds fed the sucrose starter diets had FGR at 1.42 (Table 3.17). The ANOVA indicated that there were differences among EUES treatments during the grower ($P = 0.036$) and finisher ($P = 0.005$) phases, but the Tukey – Kramer test did not differentiate the means during those periods (Table 3.17).

Table 3.17. The effects of different energy sources in starter diet (day 1 – 14) on broiler feed to gain ratio during the 35 day period.

Energy Source	Periods			
	0-7d	8-14d	15-24d	25-35d
Glycerol	1.19±0.13c	1.47±0.10b	1.38±0.08b	1.73±0.12a
Glucose	1.23±0.08c	1.48±0.08b	1.46±0.20b	1.63±012a
Sucrose	1.23±0.14c	1.44±0.07b	1.42±0.09b	1.63±0.12a

a -- c means different letters differ significantly ($P<0.05$).
The differences of FGR during grower ($P = 0.03$) and finisher ($P <0.01$) stage were identified from the ANOVA (Table 3.10), but the Tukey – Kramer test did not differentiate the means ± SEM ($P>0.05$).

Feed efficiency was improved by replacing corn starch with glucose in the diet (Jiang, et al., 2008). Batal and Parson (2004) reported that glucose based diets resulted in a better FGR than sucrose based and starch based diets. An earlier study reported that 5% dietary glucose or sucrose did not affect chicken feed to gain ratio (Wei et al. 1984). When inclusion level was less than 10%, glycerol showed the improved FGR without any negative effect on weight gain and final body weight (Margetyal et al. 2009).

The ESIL and feeding program interaction affected FGR during the grower and finisher periods. During the finisher phase, the DA chickens fed 4% EUES starter diets had FGR at 1.63, the IA chicken fed same diets had FGR at 1.61 (Table 3.18). During the finisher phase, the effects were identified from the ANOVA, but the Tukey – Kramer test did not differentiate the means (Table 3.18).

Table 3.18. The effects of feeding programs and different levels of energy source interactions in starter diet on feed to gain ratio during the 35 day period.

Feeding program	36 hours delayed access to feed and water			Immediately access to feed and water		
Inclusion Level (%)	0	4	8	0	4	8
Time						
0-7	1.26±0.09ef	1.19±0.07f	1.16±0.14f	1.20±0.02f	1.25±0.02ef	1.24±0.02ef
8-14	1.47±0.07bcd	1.48±0.09bcd	1.47±0.09bcd	1.44±0.07d	1.45±0.08cd	1.49±0.10bcd
15-24	1.32±0.08def	1.47±0.16bcd	1.44±0.16d	1.44±0.12cd	1.47±0.13bcd	1.39±0.12de
25-35	1.69±0.13a	1.63±0.07ab	1.67±0.19a	1.72±0.09a	1.61±0.09abc	1.68±0.14a

a --f means different letters differ significantly ($P<0.05$).

The differences of FGR during finisher stage were identified from the ANOVA, but the Tukey – Kramer test did not differentiate the means ± SEM ($P>0.05$).

3.6.2 Intestinal length and weight

3.6.2.1 Intestinal length

By day 7 PH, the duodenal lengths were not affected by treatments (Table 3.19). The jejunal and the ileal lengths were affected by the feeding program (Table 3.19). By day 14 PH, duodenal length was affected by different ESIL (Table 3.19). The jejunal length was affected by interaction of ESIL and the feeding program (Table 3.19). The ileal length was influenced by feeding program (Table 3.19). By day 35 PH, the duodenal length was affected by interaction of ESIL and different EUES (Table 3.19). The jejunal lengths were not influenced by treatments, while the ileal lengths were (Table 3.19).

By day 7 PH, the average duodenal length was 17.3 cm. The IA birds had longer jejuna (38.3±2.9 cm) and ilea (40.8±1.9 cm) than the same sections compared to DA birds (35.8±2.7 cm and 38.0±2.6 cm, respectively, Table 3.20). Bhanja et al. (2009) reported that by day 7 PH, IA chicks had longer jejuna and ilea compared with the 32-48 hours fasted chicks. The current study supports these findings.

By day 14 PH, the DA chicken fed 4% EUES starter diets had shorter jejuna (45.2±2.1cm) than IA birds fed same diet (49.3±2.6 cm, Table 3.21). The duodenum lengths of other treatments were intermediate (Table 3.21). The DA birds had shorter ilea by day 14 PH (45.8± 3.9 cm) than IA birds (48.4±3.6 cm) (Table 3.20). The information of intestinal lengthes by day 14 PH is limited. Jamroz et al. (2001) reported that on day 12 PH, intestines were two to four times longer than those of newly hatched chicks. The effects of delayed feed or water access on intestinal length may carry over during the first 14 days PH.

Table 3.19. The P values for the effects energy source, inclusion level and feeding program and their interactions on broiler chicken intestinal length on day 7, 14 and 35 post hatch.

Day	7			14			35		
Intestinal Section Effects	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Level	0.649	0.133	0.954	0.040	0.204	0.123	0.522	0.640	0.919
Energy Source	0.981	0.789	0.697	0.791	0.506	0.456	0.208	0.546	0.731
Level* Energy Source	0.806	0.792	0.065	0.571	0.271	0.263	0.032	0.918	0.996
Program	0.052	<0.001	<0.001	0.157	0.062	0.005	0.340	0.505	0.024
Level*Program	0.167	0.508	0.306	0.053	0.049	0.310	0.907	0.781	0.275
Energy Source* Program	0.304	0.174	0.819	0.107	0.055	0.490	0.996	0.192	0.124
Level* Energy Source* Program	0.528	0.217	0.384	0.518	0.684	0.450	0.060	0.167	0.295

If the $p<0.05$, the effects are significant at the age.

Table 3.20. The effects of the easily utilized energy source, energy source inclusion level and feeding program on chicken intestinal section length (cm) on day 7, 14 and 35 post hatch.

	Age	Day 7		
	Intestinal Section	Duodenum	Jejunum	Ileum
Effect	Level			
Inclusion Level (%)	0	17.2±1.3	36.1±3.2	39.5±2.9
	4	17.5±1.2	37.5±2.6	39.4±2.5
	8	17.4±0.9	37.6±3.1	39.3±2.8
Energy Source	Glycerol	17.3±1.2	37.3±3.3	39.2±2.5
	Glucose	17.4±1.0	37.1±2.8	39.7±2.5
	Sucrose	17.3±1.2	36.8±3.1	39.3±3.0
Feeding Program	Delay Access	17.6±1.2	35.8±2.7b	38.0±2.6b
	Immediate Access	17.1±1.1	38.3±2.9a	40.8±1.9a
	Age	Day 14		
Inclusion Level (%)	0	19.8±1.5b	48.9±3.9	48.4±4.7
	4	20.9±1.4a	47.2±3.1	46.7±3.8
	8	20.6±1.5ab	48.0±3.4	46.3±4.4
Energy Source	Glycerol	20.5±1.5	48.6±3.5	47.7±3.7
	Glucose	20.3±1.3	47.5±2.7	46.3±4.3
	Sucrose	20.6±1.8	47.5±4.1	47.4±5.1
Feeding Program	Delay Access	20.2±1.6	47.3±3.0	45.8±4.7b
	Immediate Access	20.7±1.4	48.8±3.8	48.4±3.6a
	Age	Day 35		
Inclusion Level (%)	0	27.3±2.1	68.2±1.14	69.7±1.07
	4	28.0±2.7	67.2±1.14	70.3±1.07
	8	27.4±2.5	68.6±1.14	70.2±1.07
Energy Source	Glycerol	28.1±2.3	68.9±1.14	69.9±1.07
	Glucose	27.0±2.7	67.3±1.14	70.7±1.07
	Sucrose	27.6±2.1	67.8±1.14	69.5±1.07
Feeding Program	Delay Access	27.3±2.5	67.5±0.93	68.6±0.87b
	Immediate Access	27.8±2.3	68.4±0.93	71.5±0.87a

a -- b means different letters differ significantly ($P<0.05$).

Table 3.21. The effects of feeding program and energy source inclusion levels in starter diets on broiler jejunal length (cm) on day 14 post – hatch.

Feeding Program	Inclusion Level		
	0	4	8
Delay Access	49.3±2.7ab	45.2±2.1b	47.6±3.0ab
Immediately Access	48.7±4.8ab	49.3±2.6a	48.5±3.9ab

a -- b means different letters differ significantly (P<0.05).

By day 35 PH, the birds fed 4% glycerol starter diet had duodenal length at 30.1±2.2 cm (Table 3.22). The duodenal length from the 4% sucrose starter diet fed birds was 26.5±1.7 cm (Table 3.22). The differences were identified from the ANOVA (P = 0.032), but the Tukey – Kramer test did not differentiate the means (Table 3.22). The IA birds had longer ilea (71.5±0.87 cm) than DA birds (68.62±0.87cm) (Table 3.20).

Table 3.22. The effects of feeding program and energy source inclusion levels in starter diets on broiler duodenal length (cm) on day 35 post – hatch.

Energy Source	Inclusion Level		
	0	4	8
Glycerol	27.4±1.9	30.1±2.2	26.9±1.7
Glucose	26.7±1.9	27.4±2.9	26.8±3.4
Sucrose	27.8±2.4	26.5±1.7	28.5±1.9

The differences of duodenum length were identified from the ANOVA (P = 0.03,Table 3.19), but the Tukey – Kramer test did not differentiate the means ± SEM (P>0.05).

The effects of the 24, 48 and 72 hour delayed feed or water access on the chicken small intestine length was studied (Maiorka et al. 2003). The duodenal length was not affected by post hatch delayed feed or water access (Maiorka et al. 2003). The chicken jejunal and ileal lengths were shortened by a 48 or a 72 hour delayed feed and water access (Maiorka et al. 2003). Maiorka et al. (2003) did not measure the small intestinal lengths after day 3 PH.

Branton et al. (1988) reported that at market age (49 days PH), intestinal length was correlated with chicken body weight. On days 14 and 35 PH, IA birds had heavier body

weight than DA birds (389.7±14.8g vs. 338.7±16.0g and 2309.9±99.6g vs. 2147.6±66.7g; Table 3.11) espectively. The longer jejuna and ilea in IA birds may be related to their heavier body weight.

3.6.2.2 Intestinal weight

By day 7 post hatch, the feeding program had affected the duodenal, jejunal weights (Table 3.23). Jejunal weight was affected by ESIL (Table 3.23). The three way interactions affected ileum weight (Table 3.23). IA birds had heavier (3.54±0.30g) duodena than DA birds (3.19±0.32g, Table 3.24). The jejuna were heavier in IA birds (7.50±0.74g) than in DA birds (6.69±0.89g, Table 3.24). When the EUES was added at 4% level, jejunal weight was heavier (7.32±0.77g) than the control birds (6.70±0.91g, Table 3.24). By day 14 PH, duodenal and jejunal weights were not affected by the treatments (Table 3.23). Ileal weight was affected by the feeding program (Table 3.23). By day 35 PH, duodenal, jejunal and ileal weights were affected by different feeding programs (Table 3.23).

The responses differed for the small intestine sections with delayed feed access. Jejunal lengths were not affected by treatments (Table 3.19). By day 7 PH, the jejuna were lighter in chicks that did not have feed access for 32 hours post hatch, compared to those fed immediately (Bhanja et al. 2009). The ileal and duodenal weights were not affected by 40 hours delayed feed access (Bhanja et al. 2009). Intestinal weight on day 6 and 9 PH was heavier in the birds received a 30% extra dietary protein (Wijtten et al. 2010).

Table 3.23. The P values for the effects energy source, inclusion level and feeding program and their interactions on broiler chicken intestinal weight on day 7, 14 and 35 post hatch.

Day	7			14			35		
Intestinal Section Effects	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Level	0.341	0.040	0.088	0.784	0.302	0.177	0.062	0.879	0.954
Energy Source	0.870	0.826	0.104	0.539	0.315	0.884	0.395	0.400	0.578
Level* Energy Source	0.993	0.326	0.732	0.718	0.911	0.977	0.727	0.813	0.460
Program	<0.001	<0.001	<0.001	0.158	0.075	<0.001	0.006	0.004	0.004
Level*Program	0.304	0.243	0.163	0.618	0.407	0.851	0.535	0.363	0.270
Energy Source* Program	0.112	0.068	0.156	0.315	0.248	0.283	0.864	0.685	0.803
Level* Energy Source* Program	0.772	0.376	0.029	0.293	0.723	0.433	0.148	0.604	0.803

If the $p<0.05$, the effects are significant at the age.

Table 3.24. The effects of the easily utilized energy sources, energy source inclusion levels and feeding programs on chicken intestinal section weight (g) on day 7, 14 and 35 post hatch

	Age	Day 7		
	Intestinal Section	Duodenum	Jejunum	Ileum
Effect	Level			
Inclusion Level (%)	0	3.29±0.39	6.70±0.91b	5.69±0.64
	4	3.43±0.32	7.32±0.77a	5.82±0.52
	8	3.38±0.34	7.17±0.99ab	6.01±0.59
Energy Source	Glycerol	3.34±0.37	7.11±1.11	5.80±0.61
	Glucose	3.39±0.32	7.15±0.63	6.01±0.57
	Sucrose	3.36±0.40	7.02±0.92	5.70±0.70
Feeding Program	Delay Access	3.19±0.32b	6.69±0.89b	5.63±0.62
	Immediate Access	3.54±0.30a	7.50±0.74a	6.05±0.48
	Age	Day 14		
Inclusion Level (%)	0	6.60±0.77	9.25±1.73	7.03±0.75
	4	6.77±1.01	9.84±1.75	6.66±0.88
	8	6.67±0.88	9.23±1.25	7.04±0.91
Energy Source	Glycerol	6.77±0.98	9.71±1.47	6.84±0.67
	Glucose	6.53±0.82	9.05±1.87	6.95±0.87
	Sucrose	6.74±0.85	9.56±1.41	6.92±1.03
Feeding Program	Delay Access	6.54±0.89	9.11±1.59	6.44±0.74b
	Immediate Access	6.82±0.86	9.77±1.56	7.37±0.72a
	Age	Day 35		
Inclusion Level (%)	0	14.22±1.93	27.06±3.39	22.22±3.53
	4	15.17±2.05	26.82±3.85	22.45±3.04
	8	14.00±1.69	26.55±3.50	22.24±2.67
Energy Source	Glycerol	14.32±1.83	26.73±3.48	21.87±2.83
	Glucose	14.20±1.96	26.16±3.62	22.76±2.96
	Sucrose	14.86±2.03	27.54±3.53	22.28±3.43
Feeding Program	Delay Access	13.86±1.62b	25.58±2.47b	21.26±2.75b
	Immediate Access	15.06±2.06a	28.05±4.02a	23.35±3.00a

a -- b means different letters differ significantly ($P<0.05$).

By day 7 PH, the heaviest ileal weights were observed from 8% glucose starter diet fed IA birds (6.46±0.18g) and DA birds which were fed 8% glycerol starter diet (6.28±0.67g, Table 3.25). The DA birds fed 0% sucrose starter diet had the lightest ileal weights (4.91±0.50g, Table 3.25). The ileal weights from other treatments were

Table 3.25. The three way interaction effects of feeding program, easily utilized energy sources and different energy source levels in chicken starter diet on ileal weight (g) on day 7 post hatch.

program	36 h delayed access to feed and water			Immediately access to feed and water		
Level (%)	0	4	8	0	4	8
Source						
Glycerol	5.44±0.73ab	5.32±0.29ab	6.28±0.75a	5.78±0.30ab	6.15±0.64ab	5.83±0.25ab
Glucose	5.61±0.50ab	5.65±0.61ab	5.97±0.67ab	6.28±0.67a	6.11±0.38ab	6.46±0.18a
Sucrose	4.91±0.50b	5.99±0.29ab	5.50±0.41ab	6.10±0.13ab	5.68±0.56ab	6.05±0.74ab

a -- b means different letters differ significantly ($P<0.05$).

intermediate (Table 3.25). The effects of different nutrients and feed access on newly hatched chicks' intestinal weights were studied. Different carbohydrates, fats, protein sources and sawdust were provided immediate after hatch (Bhanja et al. 2010). There was no intestinal weight difference reported among dietary treatments by day 3 and 21 PH (Bhanja et al. 2010). The chicks fed saw dust had heavier intestines than unfed birds by day 3 PH. Bhanja et al. (2010) concluded that sawdust provided the mechanical stimulations of the GIT growth.

By day 14 PH, the DA resulted in lower ileal weights (6.44 ± 0.74g) than those from IA birds (7.37 ± 0.72g) (Table 3.24). By day 35 post hatch, IA birds had heavier duodena, jejuna and ilea than DA birds (Table 3.24). Corless and Sell (1999) reported that a 54-hour delay in the provision of feed and water to poults resulted in a delay in the development of the intestinal tract, causing reduced weight of the small intestine.

3.6.2.3 Intestinal weight as percentage of body weight

By day 7 PH, duodenal weight as percentage body weight (% BW) was affected by the feeding program (Table 3.26). The duodenal weight was 2.32±0.18% BW in DA birds and it was higher than that for IA birds (2.22±0.17%, Table 3.27).

Jejunal and ileal weights, as % BW, were affected by three way interactions by day 7 post hatch (P = 0.026). The jejunal weights, as % BW for all treatments, are shown in Table 3.27. The Tukey – Kramer test did not differentiate the treatment effects. The 8% glycerol starter diet fed DA birds (5.38±0.80% BW, Table 3.28) had similar jejunal weights as % BW as the 0% glycerol starter diet fed IA birds (4.44±0.39 % BW).

Table 3.26 The P values for the effects of energy sources, inclusion levels and feeding programs and their interactions on broiler chicken intestinal weight as percentage of body weight on day 7, 14 and 35 post hatch.

Day	7			14			35		
Intestinal Section Effects	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Level	0.534	0.120	0.834	0.834	0.561	0.166	0.063	0.926	0.870
Energy Source	0.182	0.475	0.477	0.477	0.644	0.412	0.187	0.127	0.853
Level* Energy Source	0.467	0.103	0.550	0.550	0.199	0.620	0.722	0.525	0.193
Program	0.015	0.071	<0.001	<0.001	0.008	0.211	0.691	0.605	0.562
Level*Program	0.638	0.439	0.185	0.185	0.795	0.452	0.919	0.624	0.411
Energy Source* Program	0.529	0.121	0.853	0.853	0.367	0.275	0.720	0.520	0.650
Level* Energy Source* Program	0.273	0.026	0.007	0.007	0.790	0.787	0.174	0.502	0.845

If the $p<0.05$, the effects are significant at the age.

Table 3.27. The effects of the easily utilized energy sources, energy source inclusion levels and feeding programs on chicken intestinal section weight as percentage of body weight on day 7, 14 and 35 post hatch.

	Age	Day 7		
	Intestinal Section	Duodenum	Jejunum	Ileum
Effect	Level			
Inclusion Level (%)	0	2.27±0.18	4.69±0.47	3.93±0.31
	4	2.30±0.16	4.92±0.42	3.95±0.39
	8	2.25±0.20	4.76±0.54	3.89±0.48
Energy Source	Glycerol	2.26±0.20	4.80±0.65	3.87±0.50
	Glucose	2.24±0.17	4.71±0.40	3.92±0.36
	Sucrose	2.32±0.17	4.84±0.35	3.99±0.32
Feeding Program	Delay Access	2.32±0.17a	4.87±0.53	4.09±0.32
	Immediate Access	2.22±0.18b	4.70±0.41	3.75±0.39
	Age	Day 14		
Inclusion Level (%)	0	1.76±0.18	2.45±0.40	1.88±0.22
	4	1.77±0.25	2.57±0.42	1.81±0.29
	8	1.71±0.22	2.37±0.28	1.85±0.29
Energy Source	Glycerol	1.75±0.26	2.51±0.37	1.89±0.36
	Glucose	1.72±0.19	2.38±0.46	1.83±0.21
	Sucrose	1.77±0.20	2.50±0.29	1.82±0.22
Feeding Program	Delay Access	1.81±0.23a	2.52±0.41	1.84±0.29
	Immediate Access	1.68±0.19b	2.41±0.34	1.85±0.25
	Age	Day 35		
Inclusion Level (%)	0	0.64±0.07	1.22±0.11	1.00±0.11
	4	0.68±0.08	1.21±0.14	1.01±0.11
	8	0.63±0.08	1.20±0.13	1.01±0.09
Energy Source	Glycerol	0.65±0.08	1.21±0.12	1.00±0.09
	Glucose	0.63±0.06	1.17±0.12	1.01±0.08
	Sucrose	0.67±0.09	1.25±0.13	1.01±0.13
Feeding Program	Delay Access	0.65±0.07	1.20±0.11	1.00±0.10
	Immediate Access	0.65±0.09	1.22±0.14	1.01±0.10

a -- b means different letters differ significantly ($P<0.05$).

Table 3.28. The interaction of feeding program easily utilized energy sources and inclusion levels in starter diets on jejunal weight as percentage of body weight on day 7 post hatch.

	Feeding program					
	36 h delayed access to feed and water			Immediate access to feed and water		
Level (%) Source	0	4	8	0	4	8
Glycerol	4.51±0.77	4.92±0.48	5.38±0.80	4.44±0.39	5.11±0.37	4.46±0.67
Glucose	5.14±0.36	4.76±0.56	4.74±0.23	4.50±0.29	4.68±0.46	4.47±0.20
Sucrose	4.78±0.50	5.07±0.47	4.54±0.17	4.76±0.14	4.92±0.25	4.97±0.33

The jejunum weight as percentage of body weight differences were identified from the ANOVA (P = 0.026, Table 3.26), but the Tukey – Kramer test did not differentiate the means ± SEM.

The ileum weight, as % BW, was lower in 8% glycerol fed IA birds (3.34±0.64% BW) than in DA birds receiving the same diet (4.36±0.41%, Table 3.29).

Table 3.29. The interaction of feeding program easily utilized energy sources and inclusion level in chicken starter diets on ileal weight as percentage of body weight on day 7 post hatch.

	Feeding program					
	36 h delayed access to feed and water			Immediate access to feed and water		
Level (%) Source	0	4	8	0	4	8
Glycerol	4.01±0.19ab	3.81±0.07ab	4.36±0.41a	3.58±0.26ab	4.12±0.57ab	3.34±0.64b
Glucose	3.97±0.39ab	3.96±0.46ab	4.03±0.19ab	3.79±0.20ab	3.79±0.24ab	3.61±0.26ab
Sucrose	3.97±0.16ab	4.33±0.31a	4.08±0.28ab	3.90±0.13ab	3.71±0.33ab	3.93±0.40ab

a -- b means different letters differ significantly (P<0.05).

By day 14 PH, jejunal and ileal weights, as % BW, were not affected by the treatments (Table 3.26). The duodenal weight % BW was affected by different feeding programs (Table 3.26). The duodenal weight was 1.68±0.19% BW in IA birds and it was less than that in DA birds (1.81±0.23% BW, Table 3.27). By day 35 PH, all intestinal section weights, as % BW, were not affected by the treatments (Table 3.26). The intestinal section weights, as % BW, in Table 3.27 showed the duodenum was at 0.65±0.07% of body weight, while jejunum and ileum were at 1.21±0.13% and 1.01±0.10% respectively.

Intestinal weights increased more rapidly than other parts of the body. The intestinal weight as % BW reached a peak at about day 7 PH (Uni et al. 1999). By day 12 PH, chicken intestines were seven to ten times heavier than on the day of hatch. Maiorka et al. (2003) reported that duodenum weight as % BW was reduced by delayed feed access. This supports the results of the current study. The jejunal and ileal weight as percentage of body weight were not affected by delayed feed access (Maiorka et al. 2003; Bhanja et al. 2009). In the current study, the jejunal and ileal weights, as % BW, were affected by three way interactions of the main effects.

The DA in feed and water resulted in shorter and lighter intestine compared with IA birds. The differences may be caused by lighter DA birds' BW (Table 3.11).

3.6.3 Intestinal histological development

3.6.3.1 Duodenal histological development

By day 7 PH, the duodenal villus heights were not affected by the main effects or their interactions (Table 3.30). The average duodenal villus height of all treatments was 1111±27 µm. The villus width was affected by the interactions of ESIL and EUSE (Table 3.30). The crypt depths were affected by two way interactions of EUSE and the feeding program (Table 3.30). The villi surface areas were affected by the interactions between EUES and ESIL and the interactions between EUES and the feeding program (Table 3.30). The main effects on duodenum histological development are shown in Table 3.31.

Table 3.30. The P values for the effects of energy sources, inclusion levels and feeding programs and their interactions on broiler chicken duodenal histological development on day 7 and 14 post hatch.

	Day	7				14			
	Histological Structure	Villus Height	Villus Width	Crypt Depth	Villus Surface Area	Villus Height	Villus Width	Crypt Depth	Villus Surface Area
Effects									
Level		0.735	0.008	0.950	0.232	0.219	0.902	0.271	0.656
Energy Source		0.666	0.111	0.058	0.534	0.615	0.721	0.293	0.476
Level* Energy Source		0.887	0.007	0.793	0.027	0.112	0.272	0.078	0.116
Program		0.992	0.077	0.213	0.289	0.332	0.723	0.966	0.512
Level*Program		0.364	0.694	0.869	0.390	0.189	0.183	0.924	0.167
Energy Source* Program		0.131	0.117	0.021	0.033	0.026	0.842	0.816	0.146
Level* Energy Source* Program		0.169	0.153	0.129	0.166	0.969	0.727	0.042	0.660

If the $p<0.05$, the effects are significant at the age.

Table 3.31. The effects of the easily utilized energy sources, inclusion levels and feeding programs on chicken duodenal histological development on day 7 and 14 post hatch.

	Age	7			
	Histological structure	Villus Height (µm)	Villus Width (µm)	Crypt Depth (µm)	Villus Surface Area (mm^2)
Effects	Level				
Inclusion Level (%)	0	1102.7±10.4	174.5±21.5	156.0±15.4	0.207±0.004
	4	1102.1±13.4	184.0±26.1	154.3±21.9	0.220±0.004
	8	1129.2±15.2	163.2±29.9	156.6±22.8	0.205±0.005
Energy Source	Glycerol	1104.3±13.9	179.7±19.5	153.9±20.3	0.217±0.004
	Glucose	1098.4±12.9	175.5±22.7	151.6±21.1	0.208±0.003
	Sucrose	1130.2±12.6	167.7±35.7	163.5±17.7	0.206±0.005
Program	Delay Access	1111.1±13.1	169.4±27.9	153.6±19.1	0.215±0.004
	Immediate Access	1111.1±13.2	179.5±25.3	159.0±20.9	0.206±0.004
	Age	14			
Inclusion Level (%)	0	1619.9±17.8	214.9±34.9	176.4±29.2	0.351±0.003
	4	1617.2±18.9	210.4±37.8	172.2±32.1	0.347±0.006
	8	1538.9±20.0	214.2±42.9	169.7±34.2	0.336±0.009
Energy Source	Glycerol	1595.3±19.8	216.2±39.8	175.2±34.0	0.348±0.006
	Glucose	1616.7±17.9	217.1±37.6	174.5±35.7	0.354±0.007
	Sucrose	1564.1±19.6	209.1±38.2	168.7 ±24.4	0.332±0.007
Program	Delay Access	1613.3±18.7	213.4±41.6	174.1±30.8	0.343±0.007
Inclusion Level	Immediate Access	1570.8±19.3	212.2±35.4	171.5±32.7	0.346±0.006

When glycerol, glucose or sucrose was added into the starter diet at 4% level, the duodenal villi were wider (184.0±26.1µm) than in 8% sucrose fed birds (137.1±24.1 µm, Table 3.32). The birds receiving 8% glycerol diet had wider (187.9±14.9 µm) villi than 8% sucrose treatment birds (Table 3.32).

Table 3.32. The effects of energy source inclusion levels and easily utilized energy sources in starter diets on broiler duodenal villus width (µm) on day 7 post hatch.

Inclusion Level (%)	Energy Sources		
	Glycerol	Glucose	Sucrose
0	172.3±16.0ab	178.7±21.7a	174.3 ±26.7a
4	181.1±23.8a	182.4±19.9a	188.6±35.2a
8	187.9±14.9a	165.2±25.2ab	137.1±24.1b

a -- b means different letters differ significantly (P<0.05).

The IA birds received sucrose diet had deeper crypts (169.6±14.3µm) than DA birds which received glucose diet (143.6±13.0µm, Table 3.33). The crypt depths in other treatments were intermediate (Table 3.33). The duodenal crypts were found to be shallower in the 36 hour delayed feed access chicks during the first 5 day PH, but the difference disappeared by day 8 PH (Uni et al. 1998a). In the current study, the IA birds fed sucrose diet had deeper crypts than DA birds fed glucose diet. The crypt depth difference may be caused by the delayed access to feed and water.

Table 3.33. The effects of feeding program and easily utilized energy sources in starter diets on broiler duodenal crypt depth (µm) on day 7 post hatch.

Feeding Program	Energy Sources		
	Glycerol	Glucose	Sucrose
Delay Access	159.9±21.0ab	143.6±13.0b	157.5±19.2ab
Immediate Access	147.9±18.9ab	159.6±25.0ab	169.6±14.3a

a -- b means different letters differ significantly (P<0.05).

The birds fed the 4% sucrose diet had the biggest villi surface area among the treatments (0.226±0.055 mm^2, Table 3.34). The birds subjected to 8% sucrose diet had the smallest villi surface area (0.179±0.039mm^2, Table 3.34).

Table 3.34. The effects of inclusion levels and easily utilized energy sources in starter diets on broiler duodenal villus surface area (mm^2) on day 7 post hatch.

Inclusion Level (%)	Energy Sources		
	Glycerol	Glucose	Sucrose
0	0.204±0.034ab	0.215±0.030ab	0.202±0.032ab
4	0.221±0.032ab	0.201±0.029ab	0.226±0.055a
8	0.226±0.050ab	0.208±0.042ab	0.179±0.039b

a -- b means different letters differ significantly ($P<0.05$).

When birds were subjected to different feeding programs and received glycerol, glucose and sucrose diets, the duodenum villi surface areas were different ($P = 0.03$), but the Tukey – Kramer test did not differentiate the means (Table 3.35). The villi surface areas for glycerol starter diet fed DA birds were (0.226±0.009 mm^2) and this number did not differ ($P=0.07$) from sucrose starter diet fed DA birds (0.189±0.009 mm^2).

Table 3.35. The effects of feeding program and easily utilized energy sources in starter diets on broiler duodenal villus surface area (mm^2) on day 7 post hatch.

Feeding Program	Energy Sources		
	Glycerol	Glucose	Sucrose
Delay Access	0.226±0.041	0.204±0.026	0.189±0.031
Immediate Access	0.208±0.034	0.213±0.040	0.224±0.056

The differences of duodenal villi surface area were identified from the ANOVA ($P = 0.03$, Table 3.30), but the Tukey – Kramer test did not differentiate the means ± SEM ($P > 0.05$).

The differences of duodenal villi surface areas may be due to different absorption and utilization of glycerol, glucose and sucrose. The glycerol and glucose absorptions in the small intestine did not require the digestive process. Glucose is absorbed by intestinal enterocytes through the Na^+/glucose co-transporter 1 (SGLT 1) (Moran et al. 2010). More than 70% of total glycerol absorption is through the Na dependent active transport system and the rest is through passive transportation (Alvarenga et al. 2012). The sucrose requires sucrase before it can be absorbed (Iji et al. 2001b). After the sucrose molecule is broken down into glucose and fructose, then the absorption occurs. The Tukey – Kramer did not differentiate the treatment means. The Tukey- Kramer test controls the

experimentwise error rate at a selected level and is preferred by the majority of statisticians (Montogomery, 2013). A larger sample sizes may be required to identify the treatment effects. The duodenal villi height and crypt depth were improved by higher (22.5%) dietary protein level (Laudadio et al. 2010).

The effects of delayed feed access on chicken villi surfaces areas were studied. Uni et al. (1998a) reported that 36 hour delay in access to feed resulted in smaller villi volume in the duodenum during the first 5 days PH. The previous studies concluded that the development of duodenal villi was almost completed on day 7 PH (Uni et al. 1995; 1999). The other study indicated that villus surface areas increased steadily during the first 12 days PH (Geyra et al. 2001b).

By day 14 PH, the villus heights were affected by EUES and feeding program interactions (Table 3.30). The difference of villus heights were identified from the ANOVA (P=0.026), but the Tukey – Kramer test did not differentiate the treatment means (Table 3.36). The glucose starter diet fed IA birds had the same duodenal villus height as sucrose fed DA birds.

Table 3.36. The interaction effects of different feeding programs and easily utilized energy sources in starter diets on broiler duodenal villi height (µm) on day 14 post hatch.

Feeding Program	Energy Sources		
	Glycerol	Glucose	Sucrose
Delay Access	1659.3±19.7	1560.6±19.5	1492.4±15.2
Immediate Access	1531.4±19.4	1672.7±15.6	1635.7±22.0

The differences of duodenal villi height were identified from the ANOVA (P = 0.03, Table 3.30), but the Tukey – Kramer test did not differentiate the means ± SEM (P > 0.05).

The duodenal villus width and villus surface area were not affected by treatments (Table 3.30). The average villus width of all treatments was 213±7 µm. The average villus surface area of all treatments was 0.345±0.013 mm^2. The main factor effects on villus

width and villus surface areas are shown in Table 3.31. Multiple studies suggested that duodenal villi development is completed before day 14 PH (Uni et al. 1995; 1998a; 1999; Geyra et al. 2001b).

The crypt depths were affected by the three way interactions on day 14 PH (Table 3.30). Crypt depth differences were identified from the ANOVA (P = 0.02), but the Tukey – Kramer test did not differentiate the crypt depths among treatments (Table 3.37). The 4% glycerol starter diet fed IA birds had crypt depths around 203±10 µm and they were the same as the 4% sucrose starter diet fed IA birds (167±10 µm, P = 0.59). A larger sample size may be required to identify the treatment effects on crypt depths.

Table 3.37. The interaction effects of different feeding programs, inclusion levels and easily utilized energy sources in starter diets on broiler duodenal crypt depth (µm) on day 14 post hatch.

program	36 h delayed access to feed and water			Immediately access to feed and water		
Level	0%	4%	8%	0%	4%	8%
Source						
Glycerol	172.6±29.3	186.2±14.5	194.6±13.2	187.5±44.3	202.6±42.8	173.6±28.7
Glucose	213.9±34.2	171.9±14.5	178.5±12.3	189.7±34.3	180.2±30.1	183.4±47.1
Sucrose	179.5±14.9	185.9±15.3	170.5±13.2	189.2±22.8	166.8±20.3	179.1±23.8

The differences of duodenum crypt depth were identified from the ANOVA (P = 0.04,Table 3.30), but the Tukey – Kramer test did not differentiate the means ± SEM (P > 0.05).

3.6.3.2 Ileum histological development

By day 7 post hatch, the ileal villus height was affected by feeding program and interactions between EUES and ESIL (Table 3.38). The ileal villus width, crypt depth and villus surface area were not affected by treatments by day 7 PH (Table 3.38).

The villus height width, surface area and crypt depth by Day 7 PH of main effects are shown in Table 3.39. The ileal villi were higher in IA birds (344.4±83.3 µm) than DA birds (318.5±41.2 µm, Table 3.39). The chickens fed 8% sucrose diet (374.5±66.5 µm, Table 3.40) had longer villi than other fed glycerol or glucose diet. The average villus

Table 3.38. The P values for the effects of energy sources, inclusion levels and feeding programs and their interactions on broiler ileal histological development on day 7 and 14 post hatch.

Day	7				14			
Histological Structure	Villus Height	Villus Width	Crypt Depth	Villus Surface Area	Villus Height	Villus Width	Crypt Depth	Villus Surface Area
Effects								
Level	0.622	0.332	0.886	0.492	0.149	0.337	0.444	0.108
Energy Source	0.191	0.464	0.443	0.213	0.145	0.840	0.079	0.217
Level* Energy Source	<0.001	0.646	0.142	0.084	0.304	0.681	0.046	0.582
Program	0.026	0.994	0.113	0.067	0.363	0.138	0.199	0.066
Level*Program	0.969	0.247	0.136	0.714	0.588	0.995	0.012	0.540
Energy Source* Program	0.909	0.597	0.934	0.806	0.520	0.404	0.054	0.319
Level* Energy Source* Program	0.817	0.762	0.903	0.994	0.959	0.525	0.500	0.824

If the $p<0.05$, the effects are significant at the age.

Table 3.39. The effects of the easily utilized energy sources, energy source inclusion levels and feeding programs on chicken duodenum histological development on day 7 and 14 post hatch.

	Age	7			
	Histological structure	Villus Height (µm)	Villus Width (µm)	Crypt Depth (µm)	Villus Surface Area (mm^2)
Effects	Level				
Inclusion Level	0	337.0±59.6	146.9±19.9	87.6±11.7	0.053±0.001
	4	324.2±60.4	150.8±18.8	87.8±12.4	0.051±0.001
	8	334.7±70.3	157.0±28.4	89.2±14.8	0.055±0.002
Energy Source	Glycerol	318.5±41.2	147.0±20.4	85.6±12.2	0.049±0.001
	Glucose	332.9±57.2	155.4±25.5	88.8±12.7	0.055±0.002
	Sucrose	344.4±83.3	152.4±22.6	90.2±14.7	0.054±0.001
Program	Delay Access	318.8±70.3b	151.6±22.0	85.8±14.7	0.050±0.001
	Immediately Access	345.1±52.4a	151.6±24.0	90.7±11.2	0.055±0.002
	Age	14			
Inclusion Level	0	584.8±14.5	196.0±26.8	135.1±9.9	0.118±0.042
	4	560.2±6.6	183.3±27.2	133.3±13.0	0.105±0.043
	8	548.9±11.1	191.7±22.5	131.3±10.4	0.110±0.040
Energy Source	Glycerol	546±13.3	187.8±24.4	129.4±11.0	0.106±0.040
	Glucose	566±12.8	190.6±25.6	135.2±10.4	0.111±0.043
	Sucrose	582±8.3	192.6±26.8	135.2±10.5	0.117±0.041
Program	Delay Access	558±13.5	185.1±24.3	131.6±11.3	0.107±0.032
Inclusion Level	Immediately Access	572±8.9	195.5±25.2	134.8±11.0	0.116±0.035

The means ± SEM with different letters (a -- b) differ significantly (P<0.05).

Table 3.40. The effects of energy source inclusion levels and different energy sources in starter diets on broiler ileal villus height (μm) on day 7 post hatch.

Inclusion Level (%)	Energy Sources		
	Glycerol	Glucose	Sucrose
0	331.4±36.6ab	379.3±58.1a	300.3 ±57.9b
4	302.4±49.0b	295.7±28.6b	374.5±66.5a
8	321.9±58.1b	323.9±49.1b	358.5±67.4ab

a -- b means different letters differ significantly ($P<0.05$).

width of all treatments was 152.4±22.6μm. The average villus surface area of all treatments was 0.053±0.002mm^2. The average crypt depth of all treatments was 88.3±13.0μm.

Ileal villi continue to develop until day 14 PH (Uni et al. 1995; 1998a; 1999). The crypts play an important role in the continuous renewal of the intestinal mucosa process (Perry, 2006). The cells in crypts differentiate to enterocytes and migrate up to the villus tips (Perry, 2006). In the current study, the villi surface areas and crypt depths did not differ among treatments on day 7 PH. By day 7 PH, ileal villi may still be developing. The treatment effects may not be detected. Ileal villi hight was improved by 22.5% dietary protein (Laudadio et al. 2010). The villi cell sizes were improved by dietary supplement of threonine (Horn et al. 2009). However, the villi surface area did not affect by dietary protein treatments (Laudadio et al. 2010).

Previous information on effects of dietary glycerol on chicken intestinal mucus development was limited. Kim et al. 2013 reported that dietary inclusion of crude glycerol at 5% level did not affect chicken intestinal transit time and nutrient utilization. The apparent digestibility of each dietary nutrient was not affected by dietary inclusion of 5% glycerol (Kim et al. 2013). The dietary inclusion of 5% glycerol may not change intestinal mucus structures.

By day 14 PH, villi heights, widths and surface areas were not affected by treatments (Table 3.38). The crypt depths were affected by ESIL and feeding program interactions (Table 3.38). The IA birds which received 8% energy sources diets had deeper crypts (13.77±0.30 µm) than DA birds that received the same diet (125±3 µm, Table 3.41). The crypt depths were affected by interactions of ESIL and EUES on day 14 PH (P = 0.046, Table 3.38), but the Tukey – Kramer test did not differentiate the means (Table 3.42). The chicken fed 0% glycerol and 0% sucrose had the same crypt depth on day 14 PH (P=0.09).

Table 3.41. The effects of feeding programs and easily utilized energy sources in starter diets on broiler ileal crypt depth (µm) on day 14 post hatch.

Feeding Program	Dietary Inclusion Level		
	0	4	8
Delay Access	133.9±9.8ab	136.1±13.2ab	125.0±7.4b
Immediate Access	136.2±10.5ab	130.6±12.6ab	137.7±9.4a

a -- b means different letters differ significantly (P<0.05).

Table 3.42. The effects of inclusion levels and easily utilized energy sources in starter diet on broiler ileal crypt depth (µm) on day 14 post hatch.

Inclusion Level (%)	Energy Sources		
	Glycerol	Glucose	Sucrose
0	126.3±3.6	137.6±7.5	141.3±9.4
4	127.0±13.5	138.0±15.3	135.0±10.3
8	134.7±8.7	130.0±13.8	129.4±7.6

The difference were identified from the ANOVA (P = 0.046), but the Tukey – Kramer test did not differentiate the means ± SEM (P > 0.05).

By day 14 PH, ileal villi may be completely developed (Uni et al. 1995; 1998a; 1999). The effects of delayed feed access on ileal villi development may not be detected. Geyra et al. (2001b) reported that the ileal villus surface area increases more slowly after day 4 PH. The IA birds may have larger villi surface area during the first 4 days PH, but slower growth rate after day 4 PH may minimize the differences.

In the current study, the villi heights were affected by ESIL and EUES interactions by day 7 PH. By day 14 PH, the crypt depths were affected by the same interactions. The cells in crypts differentiate to enterocytes and migrate up to the villi tips (Perry, 2006). This may suggested that the higher crypt cells differentiation rate occurred during the second week post hatch. The crypt development was reduced by 36 hour delayed feed access and recovered on day 7 PH (Uni et al. 1998a). The effects of the feeding program cannot be determined because of the significant interaction effects of the feeding program and ESIL.

3.7 Trial 1 Conclusions

The effects of supplementing the newly hatched chicken with glycerol, glucose and sucrose did not overcome the post hatch delay in acces to feed and water which caused growth disadvantages.

Crude glycerol can be added into broiler chicken starter diet at 8% level, without negative effects on growth performance and small intestine development.

The effects of added glycerol, glucose and sucrose in chicken starter diet at 4 or 8% level on post hatch small intestine histological developments remain unclear. A larger sample size and sampling frequencies may be necessary for the future studies.

CHAPTER 4 THE EFFECTS OF DELAYED ACCESS TO FIRST FEED AND DIETARY GLYCEROL OR GLUCOSE IN BROILER STARTER DIETS ON GROWTH PERFORMANCE AND INTESTINAL DEVELOPMENT

4.1 Abstract

This trial investigated the effects of inclusion of dietary energy source (DES, 8% glucose, or 8% glycerol) and delayed access to feed and water on broiler growth performance and intestinal development through a 35 days growth period. In this trial, 720 male and 720 female newly hatched chicks were randomly assigned to immediate (IA) or 48 hours delayed access to feed and water (DA) and fed DES diets during first 14 days of access to feed and water. After 35 days of access, the glycerol starter diet fed male birds had heavier body weight (2459.0±73.4g) than all the female and male glucose starter diet fed birds (2266.3±76.9g; $P<0.05$). During the grower period (Day 15 – 25 of access), the glucose fed DA birds had significantly higher average daily gain (ADG) (78.8±5.7g/day) compared with glycerol fed IA birds (68.0±4.4g/day). During the finisher period (day 25 – 35 of feed access), the glucose fed IA chickens had higher ADG (121.5±5.6g/day) than glucose fed DA birds (110.2±9.5g/day; $P<0.05$). After the 7 days of access, the DA females fed the glucose diet had longer and heavier jejuna than the glycerol fed DA females ($P<0.05$). The female IA chicks fed glucose starter diet had longer ilea (41.6±2.1cm) than fed glycerol starter diet IA males (35.3±1.0cm) and all DA females (35.9±2.0cm). The DA males fed the glucose diet had shorter ilea (36.3±1.4cm) than female IA chicks fed glucose starter diet.

4.2 Introduction

Glucose and glycerol have simple molecular structures and can be easy absorbed in the small intestine (Moran et al. 2010 and Alvarenga et al. 2012). Glucose and glycerol have

very close AMEn value for broiler. The AMEn value of glucose is 3330 kcal/kg (Leeson and Summers 2001). Glycerol has an AMEn of 3,434 kcal/ kg (Dozier et al. 2011). The effects of partially replacing dietary starch with glucose or glycerol in broiler starter diets on growth performance and intestinal developments are not well documented. The effects of DA to feed on broiler growth performances were studied. However, the DA access birds were fed for less than 35 days (Kornasio et al. 2011; Bhanja et al. 2010). Therefore, the time on feed was different.

In this experiment, both DA and IA birds were each fed for 35 days. The effects of 8% glycerol or glucose in starter diet on growth performance and intestinal developments were studied.

4.3 Objectives

To determine the effects of a 48 hours delay before PH feed access in combination with dietary glycerol or glucose in broiler starter diet on growth performance of newly hatched broiler chicks.

To determine the effects of a 48 hours delayed before PH feed access in combination with dietary glycerol or glucose in broiler starter diet on small intestinal length and weight**.**

4.4 Hypotheses

It is hypothesized that after the 35 days feed and water access period, the DA birds will have same growth performances as those IA birds.

The birds receiving glycerol starter diet will have the same intestinal histological developments after 7 and 14 days of access compared to birds receiving glucose starter diet.

4.5 Materials and Methods

4.5.1 Hatchery practice

The incubation environment and PH treatments followed those described in Chapter 3.5.1.

4.5.2 Animals and general rearing environment

Following 21.5 days of incubation, chicks were removed from the hatcher. After hatch, 720 male and 720 female Ross 308 broilers were randomly placed into 48 unisex groups in floor pens in 2 similar climate controlled rooms (24 pens /room) with similar conditions in the APRC. The post hatch animal rearing environments were as described in Chapter 3.5.2.

4.5.3 Feeding program

There were two different feed and water access programs involved in this trial. The newly hatched chicks were randomly assigned to IA or 48 hours DA.

All the IA birds were placed near the drinker, had their beaks dipped in water in the drinker. The feed was available on the cardboard box lids and in the tube feeder immediately after birds arrived in the APRC.

For the DA birds, the feed and water were not provided until 48 hours post placement. After 48, all the birds had their beaks dipped in water and feed was made available in the cardboard box lids and in the tube feeder.

4.5.4 Experimental diets

All diets throughout the trial met or exceeded the NRC, 1994 requirements for broiler growth in each phase of production. All the diets throughout the trial were formulated to be isonitrogenous and isocaloric within phases. The starter mash diets were formulated

to contain 23% crude protein and 3050 kcal AMEn/kg and were fed during first 14 days of feed access (Table 4.1). The starter diets were control, 8% glucose and 8% glycerol diet. The calculated and analyzed values of nutrient compositions of starter diets were shown in Table 4. 2.

Table 4.1. Diet formulations for chicks receiving a glucose or glycerol diet during the first 14 days of feeding.

Feed Ingredients (%)	Control	8% Glucose	8% Glycerol
Corn	44.59	34.59	33.79
Soybean meal	38.72	40.47	40.61
Wheat	10.00	10.00	10.00
Tallow-grease blend	3.05	2.79	2.79
Glucose	0	8.00	0
Glycerol	0	0	8.00
Limestone	1.65	1.65	1.65
Mono-dicalcium Phosphate	0.59	0.89	0.89
Methionine Premix[a]	0.64	0.65	0.65
Mineral and vitamin premix[b]	0.50	0.5	0.5
Iodized salt	0.43	0.43	0.43
Lysine 98%	0.043	0.021	0.021
Amprolium[c]	0.013	0.013	0.013
BMD[d]	0.004	0.004	0.004
Total	100	100	100

[a] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.
[b] Vitamin/Mineral premix, supplied per kg diet: vitamin A, 9750 IU; vitamin D3, 2000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.95 mg; thiamine, 2.91 mg; manganese, 70. 2 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg.
[c]Amprolium -- AMPROL® 25% FEED MIX Huvepharma AD, Bio Agri Mix LP, Mitchell, ON, Canada (amprolium 25% w/w)
[d]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne-1mixed feed)

Table 4. 2. The calculated and analyzed compositions of diets where chicks received a glucose or glycerol supplement during the starter period, the first 14 days of feeding.

Feed	Control	8% Glucose	8% Glycerol
Calculated analysis			
AMEn (kcal/kg)[a]	3050	3050	3050
Crude protein (%)	23.00	23.00	23.00
Calcium (%)	1.00	1.05	1.05
Crude fiber (%)	2.54	2.37	2.35
Lysine (%)	1.43	1.43	1.43
Methionine + cysteine (%)	1.07	1.07	1.07
Methionine (%)	0.69	0.69	0.69
Sodium (%)	0.19	0.19	0.19
Histidine (%)	0.61	0.62	0.62
Linoleic acid (%)	1.87	1.60	1.75
Arginine (%)	1.66	1.67	1.67
Total phosphorus (%)	0.61	0.61	0.61
Magnesium (%)	0.17	0.17	0.17
Selenium(ppm)	0.38	0.37	0.36
Thiamin (ppm)	5.51	5.46	5.45
Copper (ppm)	33	33	33
Zinc (ppm)	118	118	118
Potassium (%)	1.06	1.06	1.06
Dry matter (%)	90	90	90
Analyzed compositions			
Crude protein (%)	23.20	23.00	23.00
Calcium (%)	0.96	1.00	0.94
Potassium (%)	0.94	1.05	0.94
Magnesium (%)	0.19	0.19	0.18
Phosphorus (%)	0.61	0.61	0.61
Sodium (%)	0.22	0.20	0.20
Copper (ppm)	30	29.00	30
Manganese (ppm)	104	106	95
Zinc (ppm)	110	107	104
Crude fat (%)	5.99	4.92	4.88

[a] AMEn is apperant metabolic energy nitrogen corrected.

At the day 15 of feed access, one common pelleted grower diet was provided to all treatments. The grower diet did not contain any glucose, sucrose or glycerol. This diet was formulated to contain 20% crude protein and 3150 kcal AMEn/kg (Table 4. 3). The grower diet was provided from the day 15 to 24 of feed access. From the day 25 to 35 of feed access, one common pelleted finisher diet was provided to all birds. The finisher diet

did not contain any glucose, sucrose or glycerol. This diet was formulated to contain 18% crude protein and 3200 kcal AMEn/kg (Table 4.3). The calculated and analyzed nutrient compositions of grower and finisher diets were shown in the Table 4. 4. The feed was weighed in as needed and feed weigh backs occurred at the end of each dietary phase, or as mortality occurred.

Table 4. 3. The diet formulations for chicks receiving a common diet during the grower period from day 15-24 and during the finisher period from day 25-35 of feeding.

Feed Ingredients %	Grower	Finisher
Corn	49.70	38.90
Soybean meal	31.40	39.72
Wheat	10.00	10.00
Tallow-grease blend	4.61	3.23
Limestone	1.43	1.66
Mono-dicalcium phosphate	0.72	0.86
Methionine premix[a]	0.59	0.64
Mineral and vitamin premix[b]	0.50	0.50
Iodized salt	0.40	0.40
Lysine 98%	0.111	0.123
Amprolium[c]	0.05	0.00
BMD[d]	0.004	0.00
Pel-stik[e]	0.00	0.05
Total	100	100

[a] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.
[b] Vitamin/Mineral premix, supplied per kg diet: vitamin A, 9750 IU; vitamin D3, 2000 IU; vitamin E, 25 IU; vitamin K, 2.97 mg; riboflavin, 7.6 mg; Dl Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3 mg; pyridoxine, 4.95 mg; thiamine, 2.91 mg; manganese, 70. 2 mg; zinc, 80.0 mg; copper, 25 mg; selenium, 0.15 mg; ethoxyquin, 50 mg.
[c]Amprolium -- AMPROL® 25% FEED MIX Huvepharma AD, Bio Agri Mix LP, Mitchell, ON, Canada (amprolium 25% w/w)
[d]BMD – Bacitracin Methlyene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne-1mixed feed)
[e]Pel – stik -- Pellet Binder – Uniscope Inc., Johnstown, CO, USA.

Table 4. 4. The calculated and analyzed nutrient compositions of the common diet during the grower period from day 15-24 and during the finisher period from day 25-35 of feeding.

Feed Ingredients %	Grower	Finisher
Calculated analysis		
AMEn (kcal/kg)[a]	3150	3200
Protein (%)	20	18
Calcium (%)	0.92	0.85
Lysine (%)	1.24	1.09
Methionine (%)	0.45	0.41
Methionine + cysteine (%)	0.95	0.86
Potassium (%)	0.92	0.83
Sodium (%)	0.18	0.18
Crude Fiber (%)	2.47	2.47
Histidine (%)	0.53	0.47
Linoleic Acid (%)	2.45	2.37
Arg. (%)	1.4	1.23
Total phosphorus (%)	0.55	0.52
Magnesium (%)	0.16	0.15
Zinc (ppm)	115.3	113.4
Copper (ppm)	32.12	31.51
Selenium(ppm)	0.43	0.45
Thiamin (ppm)	5.41	5.33
Dry Matter (%)	90	90
Analyzed compositions		
Crude Protein (%)	20.1	18.9
Calcium (%)	0.89	0.82
Potassium (%)	0.93	0.82
Magnesium (%)	0.16	0.15
Phosphorus (%)	0.53	0.49
Sodium (%)	0.17	0.16
Copper (ppm)	30	28
Manganese (ppm)	88	77
Zinc (ppm)	135	87
Crude Fat (%)	6.65	5.77

[a] AMEn is apperant metabolic energy nitrogen corrected.

4.5.5 Data collection

4.5.5.1 Growth performance

At day 7, 14, 24 and 35 of feed access, all the birds in the pen were batch weighed and their feed was weighed back and recorded. Using this data, BW, FI, ADG, and FGR on a

per bird basis were calculated for each period of growth. The weight sampling followed the procedures described in Chapter 3.5.5.1.

4.5.5.2 Intestinal sampling

At day 7, 14 and 35 of feed access, two birds from each pen were randomly selected and euthanized by cervical dislocation. Each bird was weighed and the ileum, jejunum, and duodenum were collected by following the methods described in chapter 3.5.5.2.

4.5.6 Statistical analysis

The trial was a 3*2*2 factorial design with 4 replications. The dietary energy source (DES), feed access and gender of birds were the main factors. The pen was used as the experimental unit. The factor DES has three components: control, 8% glucose, and 8% glycerol. The factor gender has two options: male or female. The factor different feeding program has two options: IA or 48 hours DA. The chicks' body weight at first feed access was used as a covariate.

4.5.6.1 Growth performance data

The FI, BW, ADG and FGR were analyzed as repeated measures when time was a factor. Growth performances were analyzed using ANCOVA by the Proc Mixed procedure (Littell et al., 1996) of SAS 9.3 (SAS Institute Inc., Cary, NC). Three covariance structures, CS, UN and VC were compared. The covariance structure which provided the smallest AICC was used to conduct the ANCOVA test. The covariance structures CS was selected. If high order interactions were significant, the lower order interaction and main effects involved in high order interaction were ignored. Where interactions with time were significant (α=0.05) data was sliced by time and analyzed separately. Any

significant main or interaction effects (α =0.05) were analyzed using Tukey-Kramer test to differentiate the means. The statistical model for repeated measures analysis was:

$$\gamma_{ijklm} = \mu + \alpha_i + \beta_j + \delta_k + \alpha\beta_{ij} + \alpha\delta_{ik} + \beta\delta_{jk} + \alpha\beta\delta_{ijk} + \zeta_l + \alpha\zeta_{il} + \beta\zeta_{jl} + \delta\zeta_{kl} + \alpha\beta\zeta_{ijl} + \alpha\delta\zeta_{ikl} + \beta\delta\zeta_{jkl} + \alpha\beta\delta\zeta_{ijlk} + \theta x_{ijk} + \varepsilon_{ijklm}$$

Where γ_{ijklm} = response, μ = population mean, α = factor 1 or dietary energy source, i = levels of factor 1 (control, 8% glycerol or 8% glucose) (i = 1-3), β = factor 2 or gender, j = levels of factor 2 (male or female) (j = 1-2), δ = factor 3 or feed and water access program, k = levels of factor 3 (IA or 48 hours DA) (k = 1-2), ζ = time factor, k = sampling days (day 7, 14, 24 and 35 days post hatch) (k = 1-4), θ = covariate, x_{ijk} = the average body weight at feed access of each experimental unit, m = replication (m = 1-4), ε = uncontrollable factors.

4.5.6.2 Intestinal data

The intestinal data were analyzed by ANOVA using the Proc Mixed procedure of SAS (Littell et al., 1996). The statistical model was:

$$\gamma_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + \alpha\beta_{ij} + \alpha\delta_{ik} + \beta\delta_{jk} + \alpha\beta\delta_{ijk} + \varepsilon_{ijkl}$$

Where γ_{ijkl} = response, μ = population mean, α = factor 1 or dietary energy source, i = levels of factor 1 (control, glycerol or glucose) (i = 1-3), β = factor 2 or gender, j = levels of factor 2 (male or female) (j = 1-2), δ = factor 3 or feed and water access program, k = levels of factor 3 (IA or 48 DA) (k = 1-2), θ = blocking factor, l = replication, (l = 1 - 4), ε = uncontrollable factors.

4.6 Results and Discussion

4.6.1 Body weight at first access to feed

The average body weight of chicks at hatch was 41.93 ±0.24g and this number did not differ among treatment ($p>0.05$). The chicks body weights at first access to feed was affected by different feeding program ($P<0.05$). The IA birds had heavier body weights compared with 48 hours DA birds (41.93g vs. 39.87g).

Chick body weight linearly decreases at a rate of approximately 0.14 gram/hour post hatch if feed is unavailable (Sklan et al. 2000). Bigot et al. (2003) found feed deprivation for 48 hours post hatch can induce a 7% body weight loss. The studies reported that if the feed was provided immediately after hatch, chicks can consume around 4.5g of yolk sac contents during first 48 hours post hatch (Bhanja et al. 2009). During the same time, unfed chicks only consumed around 3g of yolk sac contents (Bhanja et al. 2009).

4.6.2Growth performance

4.6.2.1 Analysis of covariance results

The BW were affected by gender and DES interaction, DES and feeding program interaction and gender and feeding program interaction at different growth period (Table 4.5). The ADG and DFC were affected by gender and the interaction between feeding program and DES at different growth period (Table 4.5). The FGR was affected by different DES and gender interactions indifferent growth periods (Table 4.5).

The differences of BW and ADG were found during grower and finisher periods ($P<0.05$). The DFC was affected by gender at both the grower and finisher periods ($P<0.05$). The feeding program and DES interaction affected DFC during finisher period ($P<0.05$). The FGR was affected by different feeding program during the second week of

Table 4.5. The P values of the effects of dietary energy source, gender and feeding program and their interaction on broiler growth performance through a 35 days production period.

Growth Performance	Body Weight	Average Daily Gain	Daily Feed Consumption	Feed to Gain Ratio
ANCOVA				
Weight at access[a]	0.875	0.868	0.510	0.796
Gender	<0.001	<0.001	0.001	0.625
Energy Source	0.469	0.679	0.975	0.810
Gender* Energy Source	0.001	0.236	0.117	0.522
Program	0.012	0.061	0.223	0.292
Gender*Program	0.204	0.881	0.101	0.177
Energy Source* Program	0.009	0.042	0.563	0.102
Gender* Energy Source* Program	0.712	0.340	0.058	0.165
Time	<0.001	<0.001	<0.001	<0.001
Time*Gender	<0.001	<0.001	<0.001	0.772
Time* Energy Source	<0.001	0.178	<0.001	0.702
Time* Gender* Energy Source	<0.001	0.099	0.423	0.019
Time* Program	<0.001	<0.001	0.004	0.009
Time*Gender*Program	<0.001	0.160	0.610	0.282
Time*Energy Source*Program	<0.001	0.002	<0.001	0.109
Time*Gender* Energy Source* Program	0.514	0.760	0.441	0.202

If the $p<0.05$, the differences were considered significant.

[a] means the chicks' body weight at first access to feed was used as a covariance

access to feed and water. The FGR was affected by DES and gender interaction ($P<0.05$). But within each period, the FGR did not differ among treatments.

4.6.2.2 Body weight

During first two weeks of feed access, BW was not affected by threatments (Table 4.6, 4.7 and 4.8). On day 35 PH, the DA birds were lighter than IA birds (2146.4±132.3g vs. 2293.8±128.0g; Appendix I).

At the end of grower period, male chicks fed glycerol starter diet (1209.5±73.5g) were heavier than all the female treatments (Table 4.6). The DA male had significantly heavier body weight (1236.4±41.4g) than other treatments (Table 4.7). The glucose fed DA birds had heavier body weight (1208.9±76.3g) than IA birds (Table 4.8).

After 35 days feed access, the glycerol starter diet fed male birds had heavier body weight (2459.0±73.4g) than all the female birds and male glucose starter diet fed birds (2266.4±95.9g, Table 4.6). The male DA birds had the same body weight as IA male (Table 4.7). The males had heavier body weight than females (Table 4.7). The glycerol fed DA birds had heavier body weight (2373.2±139.6g) than the glycerol or control diet fed IA birds (Table 4.8). The glycerol fed DA birds were also heavier than glucose fed DA birds (2191.8±89.5g; Table 4.8).

The chicken growth performance was not affected by up to 10% dietary glycerol during first 14 days PH (Cerrate et al. 2006). Wei et al. (1984) reported that providing newly hatched chicks with 5% glucose for two weeks did not affect body weight. In pervious previous study, 8% dietary inclusion of glycerol, glucose or sucrose resulted in heavier birds on day 35 PH (Table 3.12). In this experiment, after 35 days access to feed, the DA

Table 4.6. The effects of gender and dietary energy sources on body weight (g) at different periods during a 35 days period of access to feed and water.

	Gender	Male			Female		
Days	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
7		121.3±9.9g	153.3±7.1g	153.4±8.8g	145.6±7.2g	144.5±10.8g	149.0±8.2g
14		400.2±26.2f	424.4±22.3f	413.8±21.5f	388.2±24.7f	408.4±24.8f	410.2±32.0f
24		1153.9±46.3cde	1186.72±82.0cd	1209.5±73.5c	1108.6±44.5de	1090.3±44.3e	1120.2±56.7de
35		2382.5±60.9a	2459.0±73.4a	2266.4±135.5b	2260.4±95.9b	2252.9±89.3b	2182.6±83.1b

a -- g means different letters differ significantly (P<0.05).

Table 4.7. The effects of gender and feeding program on body weight (g) at different periods during a 35 days period of access to feed and water.

	Program	Immediate access to feed and water		48 hours delayed access to feed and water	
Days	Gender	Male	Female	Male	Female
7		145.5±6.2f	140.9±7.3f	139.8±12.3f	151.9±7.6f
14		405.3±27.2e	394.0±28.0e	420.3±18.5e	410.3±19.1e
24		1130.4±48.7d	1074.9±41.2d	1236.4±41.4c	1137.8±37.0d
35		2388.5±73.5a	2197.7±99.7b	2350.1±74.9a	2266.3±76.9b

a -- f means different letters differ significantly (P<0.05).

Table 4.8. The effects of feeding program and dietary energy sources on body weight (g) at different periods during a 35 days period of access to feed and water.

	Program	Immediate access to feed and water			48 hours delayed access to feed and water		
Days	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
7		140.4±5.3h	143.7±10.3h	145.4±4.2h	126.5±11.9h	154.1±8.1h	157.0±8.6h
14		385.6±32.0g	410.5±33.3g	403.0±32.4g	402.8±12.7g	422.0±13.0g	421.0±14.4g
24		1089.8±39.9f	1097.2±66.8ef	1120.9±48.9ef	1165.4±34.5def	1187.2±66.0de	1208.9±76.3d
35		2276.0±113.3bc	2268.3±180.3bc	2335.0±82.3ab	2359.4±75.4ab	2373.2±139.6a	2191.8±89.5c

a -- h means different letters differ significantly ($P<0.05$).

birds had biological age 37 days old. They are 2 days older than IA birds. The 37 days old birds were expected to by heavier (Appendix II & III).

In the current study, after 35 days feed and water access, the DA males and females had the same body weight as IA treatments. In previous study (Chapter 3), the DA birds were lighter than IA birds. A previous study concluded that a 36 PH fast period was found to reduce chicken growth rate irreversibly (Kornasio et al. 2011). Early feed access can provide growth advantages up to 35 days PH (Bhanja et al. 2010). In those studies (Kornasio et al. 2011; Bhanja et al. 2010), the delayed feed access birds were fed for less than 35 days. Another study reported that Ross 308 chickens were able reach the same body weight after a 36 hour of PH fasting period (Cengiz et al. 2012). After 35 days feed and water access, both male and female birds had body weight higher than breeding company suggested value (Appendix II & III). Male chickens are expected to be heavier than female (Appendix II & III).

In this study, the male birds fed glycerol or control starter diets were heavier than those fed glucose starter diet (Table 4.6). In earlier study (Chpater 3), the BW differents were were identified from the ANOVA ($P < 0.01$), but the Tukey – Kramer test did not differentiate means (Table 3.11).Wei et al. (1984) found that 5% glucose diet did not affect chicken body weight at market age. An 8% glucose solution did not increase birds' body weights during the first 7 days PH (Jiang et al. 2008). In the current study, glycerol was added into diet at an 8% inclusion level. Cerrate et al. (2006) reported that growth performance was not affected by feeding up to 10% glycerol during the first 14 days PH. This agrees with the results of the current study. In both IA and DA treatments, body weights did not differ between control and glycerol fed birds.

4.6.2.3 Average daily gain

The ADG did not differ between male and female during the starter period. During the grower period, male gained 77.1±6.3g/day and female gained 70.4±4.1g/day (Table 4.9). During the finisher period, males had heigher ADG (118.5±7.9g/day) than females (112.6±7.2g/day, Table 4.9).

Table 4.9. The effects of gender on chicken average daily gain (g/day) at different periods during 35 days period of access to feed and water.

Gender Days	Male	Female
7	20.4±3.4f	20.9±1.3f
14	38.6±4.7e	36.5±3.7e
24	77.1±6.3c	70.4±4.1d
35	118.5±7.9a	112.6±7.2b

a -- f means different letters differ significantly ($P<0.05$).

The ADG were not affected by feeding program and DES during the starter period (Table 4.10). During the grower period, the glucose fed DA birds had significantly higher ADG (78.8±1.8g/day) compared with glycerol fed IA birds (68.0±4.4g/day) (Table 4.5). During the finisher period, the glucose fed IA chickens had higher ADG (121.5±5.6g/day) than glucose fed DA birds (110.2±9.5g/day, $P<0.05$). The differences of weight gain between male and female birds during the grower and finisher period agreed with breeding company suggested gains (Appendix II & III). Males are expected to grow faster during grower and finisher periods (Appendix II & III).

A 5% to 10% glycerol dietary inclusion level was suggested for growing broilers (Schmidt and Zsédely 2010, McLea et al. 2011). In the current study, 8% glycerol starter diet did not affect chicken ADG in both IA and DA treatment groups. This agreed with previous chapter (Table 3.14). However, Jung and Batal (2011) suggested that the glycerol inclusion level should not exceed 5%.

4.6.2.4 Daily feed consumptions

Gender affected DFC during the grower and the finisher period. Males had higher daily feed consumption than females (Table 4.11). During the grower period, males consumed 110.1±1.4g feed/day while females consumed 103.6±9.1g feed/day. During the finisher period, male consumed 204.5±1.6g feed/day and female consumed 192.2±17.2g/day. The DFC of males and females were the same during the first 14 days of feed and water access (Table 4.11).

The DFC was affected by feeding program and DES interaction during the finisher period. The DA birds which were fed the control diet had higher DFC (215.2±13.0g/day) than IA chickens and DA chickens fed glucose diet (191.6±13.4 g/day, Table 4.12). The glycerol fed DA birds had intermediate DFC during the finisher period (201.3±10.9g/day, Table 4.12).

During the first 14 days post hatch, up to 10% dietary glycerol inclusion did not affect broiler feed intake (Cerrate et al. 2006). The 5% and 10% glycerol fed chicks had the same growth performances at day 14 PH (Cerrate et al. 2006). Another study reported that 3.3, 6.7 or 10% dietary crude glycerol did not affect feed consumption during the first 3 weeks PH (McLea et al. 2011). The study reported that glucose diets showed higher AMEn than the control diet during the first week PH which may suggest that, the diet with the glucose based diet had been better utilized (Batal and Parson, 2004). Feed intake was reduced in glucose fed birds compared to starch fed birds (Batal and Parson, 2004). However, in this study, the glucose completely replaced the starch at 40% of dietary inclusion level. In this experiment glucose was used at 8% level. The lower dietary glucose inclusion level maybe the reason for different results.

Table 4.10. The effects of feeding program and dietary energy sources on chicken average daily gain (g/day) at different periods during 35 days period of access to feed and water.

	Program	Immediately feed and water access			48 hours delayed feed and water access		
Days	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
7		20.3±0.8f	20.6±1.5f	20.9±0.6f	17.9±3.8f	21.8±1.2f	22.4±1.2f
14		35.1±3.9e	38.1±4.5e	36.8±4.4e	39.40±2.2e	38.20±1.3e	37.7±1.7e
24		71.2±3.8cd	68.0±4.4d	71.8±7.2cd	76.17±3.9cd	76.43±6.3cd	78.8±5.7c
35		117.8±9.3ab	112.3±9.5b	121.5±5.6a	119.6±5.6ab	118.5±8.4ab	110.2±9.5b

a -- f means different letters differ significantly (P<0.05).

Table 4.11. The effects of gender on chicken average daily feed consumption (g/day) at different period during a 35 days period of access to feed and water.

Gender Days	Male	Female
7	22.8±2.6f	21.9±2.1f
14	50.2±3.2e	49.4±3.6e
24	110.1±4.9c	103.6±9.1d
35	204.5±12.1a	192.2±17.2b

a -- f means different letters differ significantly (P<0.05).

Table 4.12. The effects of feeding program and dietary energy sources on average daily feed consumption (g/day) at different period during a 35 days period of access to feed and water.

	Program	Immediate access to feed and water			48 hours delayed access to feed and water		
Days	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
7		22.3±2.3e	22.1±2.2e	23.2±2.2e	19.8±3.3e	22.9±2.9e	23.9±1.8e
14		49.5±2.8d	51.2±3.1d	51.1±2.5d	47.8±5.4d	48.5±3.4d	50.8±3.5d
24		105.4±6.4c	104.2±8.7c	105.3±5.5c	108.3±2.2c	108.2±5.2c	109.8±6.1c
35		195.1±12.7b	192.3±18.4b	191.6±13.4b	215.2±13.0a	201.3±10.9ab	191.0±12.6b

a -- e means different letters differ significantly ($P<0.05$).

4.6.2.5 Feed to gain ratio

The FGR was affected by feeding program throughout the experiment. During the second week of access to feed and water, the FGR differences were detected by ANCOVA (P=0.044), but the Tukey-Kramer test did not differentiate means within each sampling day. The FGR during each sampling period were shown in Table 4.13. During the finisher period, the chickens expressed a higher FGR than during other periods (Table 4.13). During the first week of access to feed and water, the birds expressed the lowest FGR than any other period (Table 4.13).The gender and DES interactions affected FGR during the experiment. However, during each sampling period, the FGR did not differ among treatments (P>0.05). This supported by previous study (Table 3.17). The FGR during each sampling period were shown in Table 4.14. During the finisher period, glucose diet fed males and control diet fed males had the same FGR (1.78±0.09, Table 4.14). In previous study, glycerol, glucose or sucrose included diets had effects on FGR (Table 3.17). But the Tukey – Kramer test did not differentiate means.

At hatch, the main energy source for the chick is from the yolk sac contents which can represent around 20% of body weight (Uni and Ferket, 2004). Yolk sac contents are approximately 50% lipid (Uni and Ferket, 2004). Newly hatched chicks use yolk sac contents as a primary energy source during first 3 days PH (Uni and Ferket, 2004). The better FGR observed during the first week of feed access may due to the chick utilization of the yolk sac contents to meet their growth requirements. During the first week of access to feed and water, the FGR did not differ among treatments in the current study.

Table 4.13. The effects of feeding program feed to gain ratio at different period during a 35 days period of access to feed and water.

	Day of feed and water access			
Program	7	14	24	35
Immediately Access	1.10±0.03d	1.40±0.03bc	1.49±0.07b	1.66±0.13a
Delay access	1.07±0.03d	1.32±0.03c	1.42±0.10bc	1.73±0.19a

a -- d means different letters differ significantly (P<0.05).

Table 4.14. The effects of gender and dietary energy sources on feed to gain ratio at different period during a 35 days period of access to feed and water.

	Program	Male			Female		
Days	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
7		1.11±0.07fg	1.08±0.04g	1.10±0.11g	1.04±0.02g	1.15±0.10fg	1.09±0.04g
14		1.30±0.14fg	1.39±0.14ef	1.38±0.09ef	1.39±0.14ef	1.30±0.14fg	1.36±0.14ef
24		1.46±0.10cde	1.48±0.11cde	1.37±0.08ef	1.45±0.08de	1.50±0.05bcd	1.48±0.11cde
35		1.63±0.11abc	1.68±0.14abc	1.78±0.09a	1.72±0.12ab	1.70±0.08ab	1.64±0.10abc

a -- g means different letters differ significantly (P<0.05).

The FGR was improved by supplying newly hatched chicks with 40% dietary glucose during the same period (Batal and Parson, 2004). Birds were able to acquire more AMEn from the glucose included diet than from the corn starch based diet up to day 21 PH (Batal and Parson, 2004). The different dietary inclusion level of glucose may be the reason for the differences between the studies.

Effects of dietary glycerol on chicken FGR were not consistent among studies. Margetyal et al. (2009) reported that during the first 10 days PH, 5% dietary glycerol did not affect FCR. Abd-Elsamee et al. (2011) found that during first 7 days PH, 2% glycerol suppressed chick growth and FGR. In the current study, adding glycerol into starter diet at 8% level did not have negative effects on chicken FGR at each sampling day throughout the experiment (Table 4.14).

The glycerol used in this experiment is REG Glycerin – 98, produced by Renewable Energy Group® (REG®, Ames, IA). This crude glycerol contains 98% glycerin and 0.8% moisture. The same growth performances between control and glycerol diets fed chickens may due to the low methanol level in the glycerol. This result agreed with previous experiment. At 8% level, glycerol inclusion diet did not affect chicken FGR compared with control diet (Table 3.17; 3.18). Cerrate et al. (2006) concluded that the growth performance was not affected by feeding 0 – 10% glycerol during the first 14 days PH. Our results supported this conclusion up to 8% inclusion level.

4.6.3 Intestinal length and weight

4.6.3.1 Intestinal length

After 7 days of feed and water access, duodenum lengths were affected by gender and feeding program (Table 4.15). Jejunal and ileal length were affected by the three way interactions (Table 4.15). After 14 days of feed and water access, the duodenal length was affected by feeding programs (Table 4.15). The jejunal and ileal length were affected by main factor gender (Table 4.15). After the 35 days of feed and water access, duodenal and ileal length were affected by gender (Table 4.15). Jejunal length was affected by the interaction between gender and feeding program (Table 4.15).

After 7 days access to feed and water, male birds had shorter duodena (15.7±1.1cm) than females (16.4±1.1cm, Table 4.16). The IA birds had shorter duodena (15.6±0.9cm) than DA birds (16.5±1.1cm, Table 4.16). The DA females which were fed the glucose diet had longer jejuna (38.7±2.2cm) than the glycerol fed DA females (32.8±2.2cm, Table 4.17). The female IA chicks fed glucose starter diet had longer ilea (41.6±2.cm) than those fed glycerol starter diet IA males (35.3±1.0cm) and all DA females (35.9±0.9cm). The DA males that fed the glucose diet had shorter ilea (36.3±1.4cm) than female IA chicks fed same diet.

After 14 days of access to feed and water, the DA birds had longer duodena (22.53±0.40 cm) than IA birds (20.04±0.36 cm, Table 4.16). The male chicks had longer jejuna (51.87±1.03 cm vs. 48.67±0.93 cm) and ilea (53.14±1.10 cm vs. 49.25±1.00 cm) than females, respectively (Table 4.18). After 35 days of access to feed and water, the male had longer duodena (31.04±0.75cm) than females (28.63±0.66cm, Table 4.16). The ileum is longer in male birds (73.69±2.06cm) than females (65.96±1.90cm, Table 4.16). The

Table 4.15 The P values for the effects energy sources, gender and feeding programs and their interactions on broiler chicken intestinal length after 7, 14 and 35 days of access to feed and water.

Day	7			14			35		
Intestinal Section Effects	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Gender	0.026	0.537	0.047	0.088	0.028	0.013	0.021	0.429	0.009
Energy Source	0.408	0.304	0.245	0.973	0.778	0.669	0.324	0.259	0.507
Gender* Energy Source	0.286	0.154	0.017	0.299	0.514	0.368	0.767	0.637	0.644
Program	0.003	0.015	0.094	<0.001	0.130	0.448	0.350	0.002	0.599
Gender*Program	0.158	0.188	0.098	0.084	0.286	0.643	0.099	0.009	0.331
Energy Source* Program	0.920	0.071	0.600	0.626	0.304	0.185	0.971	0.822	0.996
Gender* Energy Source* Program	0.907	0.035	<0.001	0.189	0.544	0.765	0.895	0.353	0.499

If the $p<0.05$, the effects are significant at the age.

Table 4.16. The effects of the easily utilized energy sources, gender and feeding programs on chicken intestinal section length (cm) after 7, 14 and 35 days of access to feed and water.

	Intestinal Section	Duodenum	Jejunum	Ileum
Effect	Level			
	Day		7	
Gender	Male	15.7±1.1b	36.5±2.7	38.0±2.1
	Female	16.4±1.1a	36.9±2.3	39.1±2.8
Energy Source	Control	16.0±1.2	37.0±2.3	39.0±2.1
	Glycerol	15.8±1.0	36.0±2.3	38.8±2.3
	Glucose	16.3±1.3	37.1±2.9	37.9±3.1
Feeding Program	Immediate Access	15.6±0.9b	37.5±1.7	39.0±2.7
	Delayed Access	16.5±1.1a	35.9±3.0	38.1±2.2
	Day		14	
Gender	Male	21.8±2.0	51.9±4.4a	53.1±4.2a
	Female	20.8±2.4	48.7±4.7b	49.3±5.3b
Energy Source	Control	21.3±2.2	50.9±3.8	52.2±5.0
	Glycerol	21.4±2.3	49.7±5.0	50.6±4.8
	Glucose	21.2±2.4	50.2±5.4	50.8±5.8
Feeding Program	Immediate Access	20.0±1.6b	49.2±4.7	50.6±5.2
	Delayed Access	22.5±2.1a	51.4±4.7	51.8±5.1
	Day		35	
Gender	Male	31.0±3.6a	69.9±7.7	73.7±8.9a
	Female	28.6±2.6b	68.5±6.1	66.0±8.7b
Energy Source	Control	30.1±2.8	71.1±6.2	70.3±11.6
	Glycerol	28.8±3.3	69.1±7.4	71.6±9.3
	Glucose	30.6±3.7	67.4±6.9	67.6±8.0
Feeding Program	Immediate Access	30.3±3.4	66.1±6.1	70.6±9.9
	Delayed Access	29.4±3.1	72.2±6.5	69.1±9.3

a -- b means different letters differ significantly ($P<0.05$).

Table 4.17. The three way interaction effects of feeding program, gender and dietary energy sources on jejunal length (cm) after 7 days access to feed and water.

	Program	Immediate access to feed and water			48 hours delayed access to feed and water		
Gender	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
Male		38.3±0.7a	37.8±1.8ab	37.3±1.4ab	35.0±3.5a	35.6±1.4ab	35.3±3.8ab
Female		36.6±2.4ab	38.0±2.7ab	37.3±1.3ab	38.1±1.3a	32.8±2.2b	38.7±2.2a

a – b means different letters differ significantly (P<0.05).

Table 4.18. The three way interaction effects of feeding program, gender and dietary energy sources on ileal length (cm) after 7 days access to feed and water.

	Program	Immediate access to feed and water			48 hours delayed access to feed and water		
Gender	Energy Source	Control	Glycerol	Glucose	Control	Glycerol	Glucose
Male		37.9±1.3abc	35.3±1.0d	38.5±1.8abc	38.8±1.6abc	38.9±1.1abc	36.3±1.4bcd
Female		36.6±3.0ab	40.8±1.7ab	41.6±2.1a	38.9±2.3abc	35.9±2.0cd	39.8±2.1abc

a – d means different letters differ significantly (P<0.05).

DA male had longer jejuna (75.44±1.92cm) than IA males (64.36±1.83cm) and DA females (67.92±1.73 cm, Table 4.19).

Bhanja et al. (2009) reported that jejunum and ileum were longer on day 7 PH in chicks fed during the first 24 hours period compared to those were not fed until 32-48hours after hatch. The chicken jejunal and ileal lengths were shortened by a 48 hours delayed feed and water access (Maiorka et al. 2003). In the current study, DES, gender and feeding program three way interactions made it impossible to determine the effects of delayed feed access on jejunal and ileal lengths after 7 days of access to feed and water. The jejunal and ileal length were affected by different feeding program on day 7 PH, but not by interaction effects (Table 3.19). The longer delayed feed access time may have more effects of intestinal length. Branton et al. (1988) reported that intestinal length is correlated with chicken body weight. After the 35 days of access to feed and water, the males had higher body weight than female (Table 4.6 & 4.7). The longer intestine length in male is most likely the result of heavier body weight.

4.6.3.2 Intestinal weight

The duodenal weight was affected by different feeding programs (Table 4.20). Jejunal and ileal weight were affected by the three way interactions of main factors (Table 4.20). After 14 days access to feed and water, the gender and feeding programs affected the duodenum weight (Table 4.20). The feeding programs affected chicken jejunal weight (Table 4.20). Ileal weight was affected by gender after 14 days of feed and water access (Table 4.20). After 35 days access to feed and water, chicken intestinal weight were affected by gender and feeding program interaction (Table 4.20).

Table 4.19. The effects of interactions between feeding programs and gender on jejunal length (cm) after 35 days access to feed and water.

Effect Feeding Program	Immediate access to feed and water	48 hours delayed access to feed and water
Gender		
Male	64.4±6.0b	75.4±4.9a
Female	67.9±6.0b	69.0±6.3ab

a -- b means different letters differ significantly (P<0.05).

Table 4.20. The P values for the effects energy source, gender and feeding program and their interactions on broiler chicken intestinal weight after 7, 14 and 35 days of access to feed and water.

Day	7			14			35		
Intestinal Section	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Effects									
Gender	0.132	0.134	0.281	0.033	0.083	0.013	0.021	0.078	0.034
Energy Source	0.289	0.036	0.171	0.824	0.322	0.127	0.414	0.300	0.665
Gender* Energy Source	0.671	0.013	0.171	0.678	0.682	0.127	0.353	0.729	0.420
Program	<0.001	0.300	0.251	0.004	0.010	0.771	0.114	<0.001	0.182
Gender*Program	0.160	0.463	0.277	0.712	0.397	0.293	0.034	0.002	0.003
Energy Source* Program	0.273	0.013	0.883	0.687	0.694	0.071	0.896	0.456	0.536
Gender* Energy Source* Program	0.526	0.035	0.005	0.783	0.878	0.946	0.247	0.898	0.926

If the p<0.05, the effects are significant at the age.

After 7 days access to feed and water, the IA bird duodenum is heavier (3.47±0.07g) than in the DA bird (3.10±0.06g, Table 4.21). This is supported by previous study (Table 3.24). The DA female chicks fed the glucose diet had heavier jejunum (8.06±0.27g) than the DA female chicks which fed the glycerol diet (6.30±0.27g, Table 4.22). In previous study, intestinal weights were not affected by EUES (Table 3.24). The differences of ileal weight among treatments were identified from the ANOVA (P=0.005), but Tukey – Kramer test did not differentiate the means (Table 4.23). The IA female fed the glucose starter diet (6.30±0.57g) and IA male fed the glycerol diet (5.21±0.23g) had similar (P=0.30) ileum weights (Table 4.23).

After the 14 days access to feed and water, the male birds had heavier jejuna (7.35±0.94g) than females (6.78±0.83g, Table 4.21). The IA birds had lighter jejuna (6.67±0.63g) than DA birds (7.46±1.01g). The IA birds had lighter jejuna (9.87±1.93g) than the DA birds (11.47±1.95g, Table 4.21). The males had heavier ileal weights (8.16±1.23g) than females (7.11±1.51g, Table 4.21). In previous study, jejunal weights were not affected by treatments on day 14 post hatch (Table 3.23).

In a study by Bhanja et al. (2010), the intestinal weight was not affected by different nutrients at day 3 and 21 PH. In that study, different carbohydrates, fats and protein sources (1.7 kcal AMEn/bird) were initiated into the crop of the newly hatched chicks. In the current study, the jejunal weights were the same among IA males, IA females and DA males. In DA females those, glycerol diet fed chicks had lighter jejunum than chicks fed the glucose diet. The DA females fed glycerol had lighter jejunums than chicks fed glucose. Information of the effects of glycerol on chicken intestinal weight is limited. The small intestine weight from glucose fed birds was reported to be lighter than in the birds

Table 4.21. The effects of the easily utilized energy sources, gender and feeding programs on chicken intestinal section weight (g) after 7, 14 and 35days of access to feed and water.

Effect	Intestinal Section Level	Duodenum	Jejunum	Ileum
	Day		7	
Gender	Male	3.22±0.40	7.07±0.67	5.79±0.46
	Female	3.36±0.32	7.32±0.69	5.94±0.58
Energy Source	Control	3.19±0.35	7.15±0.71	5.86±0.40
	Glycerol	3.36±0.32	7.48±0.66	5.71±0.62
	Glucose	3.32±0.43	6.96±0.63	6.03±0.50
Feeding Program	Immediate Access	3.47±0.31a	7.11±0.60	5.78±0.52
	Delayed Access	3.10±0.32b	7.28±0.76	5.94±0.53
	Day		14	
Gender	Male	7.35±0.94a	11.19±1.83	8.16±1.23a
	Female	6.78±0.83b	10.15±2.22	7.11±1.51b
Energy Source	Control	7.02±0.52	10.81±1.79	8.13±1.29
	Glycerol	7.18±1.05	11.13±1.87	7.11±1.44
	Glucose	7.00±1.09	10.06±2.51	7.68±1.57
Feeding Program	Immediate Access	6.67±0.63b	9.87±1.93b	7.70±1.54
	Delayed Access	7.46±1.01a	11.47±1.95a	7.58±1.38
	Day		35	
Gender	Male	19.69±3.56	34.11±7.37	25.23±6.67
	Female	17.62±2.39	31.32±4.90	21.76±4.39
Energy Source	Control	19.12±3.40	34.04±6.51	24.26±6.07
	Glycerol	17.86±3.76	31.17±6.49	23.66±5.84
	Glucose	18.98±1.81	32.93±5.80	22.57±5.64
Feeding Program	Immediate Access	17.96±3.13	35.65±6.33	22.42±4.18
	Delayed Access	19.35±3.03	29.78±4.91	24.57±7.01

a -- b means different letters differ significantly ($P<0.05$).

Table 4.22. The three way interaction effects of feeding program, gender and dietary energy sources on jejunal weight (g) after 7 days of access to feed and water.

Gender	Program Energy Source	Immediately feed and water access			48 hours delayed feed and water access		
		Control	Glycerol	Glucose	Control	Glycerol	Glucose
Male		7.05±0.88abc	6.96±0.87abc	6.76±0.25abc	6.48±0.51bc	7.38±0.27abc	7.78±0.48ab
Female		7.39±0.61abc	7.18±0.46abc	7.31±0.53abc	7.66±0.40ab	6.30±0.49c	8.06±0.30a

a -- c means different letters differ significantly (P<0.05).

Table 4.23. The three way interaction effects of feeding program, gender and dietary energy sources on ileal weight (g) after 7 days of access to feed and water.

Gender	Program Energy Source	Immediate access to feed and water			48 hours delayed access to feed and water		
		Control	Glycerol	Glucose	Control	Glycerol	Glucose
Male		6.03±0.25	5.21±0.23	5.66±0.35	5.75±0.38	6.21±0.60	5.87±0.76
Female		5.55±0.48	6.00±0.72	6.30±0.57	6.10±0.31	5.45±0.41	6.28±0.21

The differences of ileum weight were identified from the ANOVA (P = 0.005, Table 4.20), but Tukey – Kramer test did not differentiate the means ± SEM.

fed other carbohydrate sources (sucrose, corn starch, cassava starch and lactose) fed chicken at day 7 PH (Sorbara et al. 2006).

After the 35 days of access to feed and water, the male DA chickens had heavier duodena (21.33±0.91g) than IA females (17.87±0.82g) and DA females (17.36±0.82g, Table 4.24). The male DA chickens had heavier jejuna (39.68±1.62g, Table 4.25) than other treatments. The DA males had heavier ileum (28.83±1.67g) than IA males (21.64±1.59g) and DA females (20.30±1.51g, Table 4.26). In previous study, the IA birds had heavier intestinal weights than DA birds (Table 3.24).

Table 4.24. The effects of feeding program, gender and dietary energy sources on duodenal weight (g) after 35 days of access to feed and water.

Effect Gender	Feeding Program	Immediately Access	Delayed Access
Male		18.04±4.01ab	21.33±1.99a
Female		17.87±2.26b	17.36±2.59b

a -- b means different letters differ significantly (P<0.05).

Table 4.25. The effects of feeding program, gender and dietary energy sources on jejunal weight (g) after 35 days of access to feed and water.

Effect Gender	Feeding Program	Immediately Access	Delayed Access
Male		28.54±5.43b	39.68±3.93a
Female		31.02±4.25b	31.62±5.65b

a -- b means different letters differ significantly (P<0.05).

Table 4.26. The effects of feeding program, gender and dietary energy sources on ileal weight (g) after 35 days of access to feed and water.

Effect Gender	Feeding Program	Immediately Access	Delayed Access
Male		21.64±4.33b	28.83±6.87a
Female		23.21±4.07ab	20.30±4.38b

a -- b means different letters differ significantly (P<0.05).

4.6.3.3 Intestine weight as percentage of body weight

The main effects on chicken intestine weight as % BW were shown in Table 4.27. After 7 days access to feed and water, the duodenum and ileum weight as % BW did not differ

Table 4.27 The P values for the effects energy source, gender and feeding program and their interactions on broiler intestinal weight as percentage of body weight after 7, 14 and 35 days of access to feed and water.

Day	7			14			35		
Intestinal Section	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Effects									
Gender	0.343	0.407	0.713	0.887	0.532	0.414	0.429	0.539	0.306
Energy Source	0.764	0.050	0.186	0.614	0.146	0.044	0.525	0.190	0.795
Gender* Energy Source	0.518	0.024	0.748	0.515	0.949	0.176	0.919	0.400	0.389
Program	0.061	0.166	0.301	0.418	0.140	0.077	0.792	0.115	0.919
Gender*Program	0.405	0.774	0.613	0.354	0.185	0.646	0.728	0.163	0.029
Energy Source* Program	0.779	0.044	0.458	0.581	0.890	0.408	0.992	0.522	0.450
Gender* Energy Source* Program	0.651	0.967	0.536	0.449	0.550	0.626	0.656	0.267	0.948

If the $p<0.05$, the effects are significant at the age.

among the treatments (Table 4.27). The jejunum weight as % BW was affected by interaction of DES and gender and the feeding program and DES interactions (Table 4.27). After 14 days access to feed and water, duodenum and jejunum weight as % BW did not differ among the treatments (Table 4.27). The ileal weight % BW was affected by different DES (Table 4.27). After 35 days of access to feed and water, the ileal weight as % BW was affected by the interactions between feeding program and gender (Table 4.27).

The main effects of intestinal section weights as % BW were shown in Table 4.28. After 7 days of feed and water access, jejunal weight as % BW was higher in control diet fed females (4.94±0.34) than glycerol fed females (4.32±0.48, Table 4.29). Control diet fed IA birds had higher jejunal weight as % BW (4.91±0.44% BW) than glycerol diet fed DA birds (4.32±0.38, Table 4.30). In previous study, jejunal weight as % BW was affected by three way interactions (Table 3.26) but the Tukey – Kramer test did not differentiate the means (Table 3.28).

After 14 days of access to feed and water, the control diet resulted a higher ileal weight as % BW (1.93±0.38) than glycerol treatment (1.59±0.32, Table 4.28). In previous study, the ileal weight as % BW was not affected by treatments on day 14 PH (Table 3.26).

After 35 days of access to feed and water, the differences of ileum weight as % BW among treatments were identified from the ANOVA (P = 0.029), but the Tukey – Kramer test did not differentiate the means. The ileum weights % BW were the same (p=0.11) between the DA male chicken (1.10±0.23) and the DA female chicken (0.88±0.21, Table 4.31). In previous study, ileal weight as % BW was not affected by treatments on day 35 PH (Table 3.26).

Table 4.28. The effects of the easily utilized energy sources, gender and feeding program on chicken intestinal section weight as percentage of body weight (%) after 7, 14 and 35 days of access to feed and water.

Effect	Intestinal Section Level	Duodenum	Jejunum	Ileum
	Day	7		
Gender	Male	2.08±0.21	4.58±0.37	3.75±0.27
	Female	2.15±0.24	4.67±0.44	3.79±0.37
Energy Source	Control	2.09±0.20	4.70±0.44	3.85±0.21
	Glycerol	2.10±0.25	4.44±0.44	3.64±0.39
	Glucose	2.15±0.23	4.74±0.24	3.82±0.33
Feeding Program	Immediate Access	2.05±0.20	4.70±0.42	3.82±0.36
	Delayed Access	2.18±0.23	4.55±0.38	3.72±0.28
	Day	14		
Gender	Male	1.63±0.15	2.49±0.32	1.81±0.30
	Female	1.63±0.20	2.42±0.45	1.72±0.42
Energy Source	Control	1.66±0.15	2.55±0.38	1.93±0.38a
	Glycerol	1.63±0.16	2.53±0.31	1.59±0.32b
	Glucose	1.60±0.21	2.29±0.45	1.77±0.34ab
Feeding Program	Immediate Access	1.65±0.17	2.37±0.39	1.86±0.42
	Delayed Access	1.61±0.18	2.54±0.38	1.67±0.26
	Day	35		
Gender	Male	0.82±0.10	1.42±0.23	1.02±0.21
	Female	0.78±0.17	1.38±0.17	0.96±0.22
Energy Source	Control	0.83±0.12	1.49±0.23	1.02±0.19
	Glycerol	0.77±0.12	1.34±0.15	0.96±0.17
	Glucose	0.81±0.18	1.37±0.27	0.99±0.28
Feeding Program	Immediate Access	0.81±0.13	1.35±0.23	0.99±0.16
	Delayed Access	0.79±0.16	1.45±0.21	0.99±0.25

a -- b means different letters differ significantly ($P<0.05$).

Table 4.29. The effects of gender and feeding program interaction on chicken jejunal weight as percentage of body weight (%) after 7 days of feed and water access.

Effect Gender	Energy Source	Control	Glycerol	Glucose
Male		4.45±0.42ab	4.56±0.38ab	4.73±0.30ab
Female		4.94±0.34a	4.32±0.48b	4.75±0.21ab

a -- b means different letters differ significantly (P<0.05).

Table 4.30. The effects of gender and dietary energy sources interaction on chicken jejunal weight as percentage of body weight (%) after 7 days of feed and water access.

Effect Feeding Program	Energy Source	Control	Glycerol	Glucose
Immediately Access		4.91±0.44a	4.56±0.48ab	4.62±0.27ab
Delayed Access		4.49±0.36ab	4.32±0.38b	4.85±0.15ab

a -- b means different letters differ significantly (P<0.05).

Table 4.31. The effects of gender and dietary energy sources on chicken ileal weight as percentage of body weight (%) after 35 days of feed and water access.

Effect Gender	Feeding Program	Immediately Access	Delayed Access
Male		0.95±0.08	1.10±0.23
Female		1.03±0.20	0.88±0.21

The differences of ileum weight as percentage of body weight were identified from the ANOVA (Table 4.25), but Tukey – Kramer test did not differentiate the means ± SEM.

Intestinal weight increased more rapidly than rest of body after hatch, the intestinal weight percentage of body weight reaching a peak at about day 7 post hatch (Uni et al. 1999). The duodenal weight as % BW was reduced by delayed feed access (Maiorka et al. 2003). The ileal weight as % BW was not affected by delayed access (Maiorka et al. 2003; Bhanja et al. 2009). In current study, feeding program did not affect ileal weight as % BW after 7 and 14 days feed access. After 35 days of feed and water access, the Tukey – Kramer test did not differentiate the means (p=0.11). A larger sample size maybe required to identify the treatment effects if they truly exist.

4.7 Trial 2 conclusions

The effects of dietary inclusion of 8% glycerol did not affect male or female chicken growth performance. A delay in access to feed for 48 hours post hatch did not affect body weight once birds had access to feed and water for 35 days. The broiler chicken is able to overcome the growth disadvantages caused by delayed access to feed. But delayed feed access should be avoided in the commercial practices. However, at the biological age of the birds at day 35 PH, the DA birds were ligeter than IA birds (2146.4±132.3g vs. 2293.8±128.0g). The PH delayed access to feed should be avoided.

CHAPTER 5 CONCLUSIONS

The dietary inclusion of 8% glycerol in broiler starter diet did not affect broilers growth performances (body weight, average daily weight gain, daily feed consumption and feed to gain ratio). The intestinal histological developments were not affected by dietary inclusion of glycerol up to 8% in starter diets. The crude glycerol can be included up to 8% level during first 14 days of feeding. Both male and female broiler had the similar growth response to 8% dietary glycerol.

The dietary inclusion of 4% or 8% glucose and sucrose did not affect bird growth performances. On day 7 and 14 post hatch, the small intestinal histological developments were not affected by glucose or sucrose feeding.

Other factors, such as, dietary protein level and amino acids profiles may promote the intestinal histological developments during first two weeks PH. Further researches may evaluate the effects of additional essential amino acids with glucose, glycerol or sucrose on broiler intestinal development.

In order to determine the effects of glucose, sucrose or glycerol on broiler performances, future studies should examine the carcass composition. The small intestine histological samples should be collected and on day 2, 4, 7, 12 and 14 post hatch. Glucose, glycerol transporter gene expressions should be examined. The intestinal digestive enzymes actives should be analyzed.

REFERENCES

Abd-Elsamee, M., Abdo, Z. M. A., El-Manylawi, M. and Salim, I. H. 2011. Use of crude glycerin in broiler diets. Egypt. Poult. Sci, **31: 407-407.**

Abed, F., Karimi, A., Sadeghi, G., Shivazad, M., Dashti, S., and Sadeghi-Sefidmazgi, A., 2011. Do broiler chicks possess enough growth potential to compensate long-term feed and water depravation during the neonatal period? S. Afr. J. Anim. Sci. **41**:33-39.

Alvarenga, R., R., Lima, E. M. C., Zangeronimo, M. G., Rodrigues, P. B., and Bernardino, V. M. P. 2012. Use of glycerine in poultry diets. Worlds Poult. Sci. J. **68**:637-644.

Aviagen®, 2012. Ross 308 broiler: performance objectives. Page 07 – 11. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross308BroilerPerfObj2012R1.pdf

Batal, A., B. and Parsons, C., M. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. **81:** 400-407.

Batal, A., B. and Parsons, C., M. 2004. Utilization of various carbohydrate sources as affected by age in the chick. Poult. Sci. **83**:1140–1147.

Berri, C., Godet, E., Hattab, N., H. and Duclos. M., J. 2007. Effect of genotype and early nutrition on the number and activity of satellite cells in chicken Pectoralis major muscle. Archiv für Tierzucht **50**:32-33.

Bhanja, S., K., Anjali Devi, C., Panda, A., K. and Shyam Sunder, G. 2010. Effect of post-hatch nutrient intubation on performance, intestinal growth, meat yield and immune response in broiler chickens. Asian-Aust. J. Anim. Sci. **23**:515-520.

Bhanja, S., K., Devi, C., A., Panda, A., K. and Sunder, G., S. 2009. Effect of post hatch feed deprivation on yolk-sac utilization and performance of young broiler chickens. Asian- Asian-Aust. J. Anim. Sci **22**:1174-1179.

Bickerstaffe, R. and Annison, E., F. 1969. Glycerokinase and desaturase activity in pig, chicken and sheep intestinal epithelium. Compar Biochem Physiol:47-54.

Bigot, K., Tesseraud, S., Picard, M. and Mignon-Grasteau, S. 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. Poult. Sci. **82**:781-788.

Branton, S., L., Lott, B., D., Morgan, G., W. and Deaton. J., W. 1988. Position of Meckel's diverticulum in broiler-type chickens. Poult. Sci. **67**:677-679.

Budgell, K. 2008. *Saccharomyces cerevisiae* cell wall extracts as alternatives to antibiotics in broiler chicken production. M.Sc. Thesis. Dalhousie University. Halifax, NS.Page 45.

Canadian Council on Animal Care (CCAC). 2009. Guidelines on: the careand use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON.

Careghi, C., Tona, K., Onagbesan, O., Buyse, J., Decuypere, E. and Bruggeman, V. 2005. The effects of the spread of hatch and interaction with delayed feed access after hatch on broiler performance until seven days of age. Poult. Sci. **84**:1314-1320.

Cengiz, O., Koksal, B. H., Tatli, O., Sevim, O., Avci, H., Epikmen, T., Beyaz, D., Buyukyoruk, S., Boyacioglu, M., Uner, A. and Onol, A. G. 2012. Influence of dietary organic acid blend supplementation and interaction with delayed feed access after hatch on broiler growth performance and intestinal health. Veterinární Medicína **57**:515-528.

Cerrate, S., Yan, F., Wang, Z., Coto, C., Sacakli, P. and Waldroup, P.W. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. Int. J. Poult. Sci., **11**: 1001–1007.

Corless, A., B., and Sell. J., L. 1999. The effects of delayed access to feed and water on the physical and functional development of the digestive system of young turkey. Poult. Sci. **78**:1158-1169.

Dozier, I., W., A., Kerr, B., J. and Branton, S., L. 2011. Apparent metabolizable energy of crude glycerin originating from different sources in broiler chickens. Poult. Sci. **90:** 2528-2534.

Dozier, I., W.A., Kerr, B., J., Corzo, A., A., Kidd, M., T., Weber, T., E., and Bregendahl, K., K. 2008. Apparent metabolizable energy of glycerin for broiler chickens. Poult. Sci. **87:** 317-322.

Drury, R., and Wallington, E. 1980. Carleton's histological techniques. 5th ed. Oxford University Press, Oxford. Pp. 57-68, 131-135.

El-Husseiny, O., M., El-Wafa, S., A. and El-Komy, H., A. 2008. Influence of fasting or early feeding on broiler performance. Int. J. Poult. Sci., **7:** 263-271.

Geyra, A., Uni, Z. and Sklan, D. 2001a. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr. **86:** 53-61.

Geyra, A., Uni, Z. and Sklan, D. 2001b. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. **80**:776-782.

Gökceyrek, D. and Ciftci, I. 2005. Effects of delayed access to feed and different crude protein levels in broiler chicks after hatching 3. Morphological development of small intestine segments. Proceedings of the 15th European Symposium on poultry nutrition, Balatonfüred, Hungary, 25-29.

Havenstein, G. B., Ferket, P. R., and Qureshi, M. A. 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. **82:** 1500-1508.

Horn, N. L., S. S. Donkin, T. J. Applegate, and O. Adeola. 2009. Intestinal mucin dynamics: Response of broiler chicks and White Pekin ducklings to dietary threonine. Poult. Sci. **88**:1906-1914.

Iji, P., A., Saki, A., and Tivey, D., R. 2001a. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. Br. Poult. Sci. **42**:505-513.

Iji, P., A., Saki, A., and Tivey, D., R. 2001b. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. Br. Poult. Sci. **42**:514-522.

Jalal, M., A., R. and Zakaria, H., A. 2012. The effect of quantitative feed restriction during the starter period on compensatory growth and carcass characteristics of broiler chickens. Pakistan J. of Nutrition **11**:1049-1054.

Jamroz, D., Jakobsen, K., Orda, J., Skorupinska, J. and Wiliczkiewicz, A. 2001. Development of the gastrointestinal tract and digestibility of dietary fibre and amino acids in young chickens, ducks and geese fed diets with high amounts of barley. Comp. Biochem. Physiol., A, **130:** 643-652.

Jiang, K., Jiao, H., Song, Z., Yuan, L., Zhao, J. and Lin, H. 2008. Corticosterone administration and dietary glucose supplementation enhance fat accumulation in broiler chickens. Br. Poultry Sci. **49**: 625-631.

Jung, B., B. and Batal, A., B. 2011. Nutritional and feeding value of crude glycerin for poultry. 2. Evaluation of feeding crude glycerin to broilers. J. Appl. Poultry Res., **20:** 514-527.

Juul-Madsen, H. R., Su, G. and Sørensen, P. 2004. Influence of early or late start of first feeding on growth and immune phenotype of broilers. Br. Poult. Sci. **45**:210-222.

Karrow, J., Widowski T. and Duncan, I. 1998. Starve-out syndrome in turkey poults: effects of a 48 hour delay in the hatcher on growth, physiology and behaviour. Poult. Sci. **77**:1842-1845.

Kim, J., H., Seo, S., ,Kim, C., H., Kim, J., W,. Lee, B., B., Lee, G., I., Shin, H., S., Kim, M., C. and Kil. D., Y. 2013. Effect of dietary supplementation of crude glycerol or tallow on intestinal transit time and utilization of energy and nutrients in diets fed to broiler chickens. Livest. Sci. **154**:165-168.

Klasing, K. 1998. Anatomy and physiology of the digestive system. *In* Comparative avian nutrition. CAB International. Wallingford UK. Pages 9-32.

Kornasio, R., Halevy, O., Kedar, O. and Uni. Z. 2011. Effect of in ovo feeding and its interaction with timing of first feed on glycogen reserves, muscle growth, and body weight. Poult. Sci. **90**:1467-1477.

Laudadio, V., L. Passantino, A. Perillo, G. Lopresti, A. Passantino, R. U. Khan, and V. Tufarelli. 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult. Sci. **91:**265-270**.**

Leeson, S. and Summers, J. D. 2001. Nutrition of the chicken, 4th edition. M.L. Scott and Associates, Ithaca, N.Y. Pp. 41.

Littell, R., Milliken, G., Stroup, W., and Wolfinger, R. 1996. SAS system for mixed models. SAS Institute, Inc., Cary, NC.

Longo, F., Menten, J., Pedroso, A., Figueiredo, A., Racanicci, A. and Sorbara, J. 2007. Performance and carcass composition of broilers fed different carbohydrate and protein sources in the prestarter phase. J. Appl. Poult. Res. **16:**171-177.

Mahmoud, K., Z. and Edens, F., W. 2012. Breeder age affects small intestine development of broiler chicks with immediate or delayed access to feed. Br. Poult. Sci. **53**:32-41.

Maiorka, A., E., Santin, F., Dahlke, I., C., Boleli, Furlan, R., L. and Macari. M. 2003. Posthatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. J. Appl. Poult. Res. **12**:483-492.

Margetyal, C., C., Launay, C., C. and Arveux, P., P. 2009. Impact of glycerin use on broilers' growth until 35 days of age. / Impact de l'utilisation de glycerine sur les performances des poulets de 1 a 35 jours. World Poultry Science Association (WPSA), Proceedings of The 8th Avian French Research Days, St Malo, France, 25-26 March 2009, 29.

McLea, L., Ball, M. E. E., Kilpatrick, D. and Elliott, C. 2011. The effect of glycerol inclusion on broiler performance and nutrient digestibility. Br. Poult. Sci. **52:** 368-375.

McLea, L., Ball, M., E., E., Kilpatrick, D. and Elliott, C. 2011. The effect of glycerol inclusion on broiler performance and nutrient digestibility. Br. Poult. Sci. **52:** 368-375.

Montogomery, D., 2013. Design and analysis of experiments. 8th ed. John Wiley & Sons, Inc. Pp. 98.

Moran, A., W., Ionescu, C., Bravo, D., Shirazi-Beechey, S., Coulter, E., A., Al-Rammahi, M., Arora, D. K. and Batchelor, D. J. 2010. Expression of Na+/glucose co-transporter 1 (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. Br. J. Nutr. **104**:647-655

Nir, I., I. and Levanon, M., M. 1993. Research note: effect of posthatch holding time on performance and on residual yolk and liver composition. Poult. Sci. **72:** 1994-1997.

National Research Council 1994, Nutrient requirements of poultry. 9th rev. ed. Washington. D.C.: National Academy Press.

Noy ,Y. and Sklan. D. 2001. Yolk and exogenous feed utilization in the posthatch chick. Poult. Sci. **80**:1490–1495.

Noy, Y. and Pinchasov, Y. 1993. Effect of a single posthatch intubation of nutrients on subsequent early performance of broiler chicks and turkey poults. Poult. Sci. **72**:1861-1866.

Noy, Y. and Sklan, D. 1995. Digestion and absorption in the young chick. Poult. Sci. **74:** 366-373.

Noy, Y. and Sklan, D. 1996. Uptake capacity for glucose, methionine and oleic acid in the proximal small intestine of posthatch chicks. Poult. Sci. **75**:998–1002.

Noy, Y. and Sklan, D. 1998. Metabolic responses to early nutrition. J. Appl. Poult. Res. **7**: 437-451.

Noy, Y. and Sklan, D. 1999. Energy utilization in newly hatched chicks. Poult. Sci. **78:** 1750-1756.

Noy, Y. and Sklan, D. 1999a. Different types of early feeding and performance in chicks and poults. J. Appl. Poult. Res. **8**:16-24.

Noy, Y. and Sklan, D. 1999b. Energy utilization in newly hatched chicks. Poult. Sci. **78:** 1750-1756.

Noy, Y. and Uni, Z. 2010. Early nutritional strategies. World Poult. Sci. J. **66:** 639-646.

Noy. Y. and Sklan. D. 2001. Yolk and exogenous feed utilization in the posthatch chick. Poult. Sci **80**:1490–1495.

Ozols, A. and Sheshukova, T. 1985. The role of the duodenum in adaptive changes in sucrose digestion in the small intestine of chicks. Comp. Biochem. Physiol., A, **80**: 343-347.

Pedroso, A., N., Figueiredo, A., M., Racanicci, C. and Gaiotto. J. B. 2003. Effects of different carbohydrates in the feed of newly hatched chicks on performance and organs allometric growth. Poult. Sci. **82**(Suppl. 1):67. (Abstr.)

Peebles, E., D., Keirs, R., W., Bennett, L., W., Cummings, T., S., Whitmarsh, S., K. and Gerard, P., D. 2005. Relationships Among Prehatch and Posthatch Physiological Parameters in Early Nutrient Restricted Broilers Hatched from Eggs Laid by Young Breeder Hens. Poult. Sci. **84**:454-461.

Perry, G., C. 2006. Avian gut function in health and disease. Poultry science symposium series; 28 Wallingford, UK page 30 - 43.

Rammouz, R., E., Said, S., Abboud, M., Yammine, S. and Jammal, B. 2011. Effect of Post-hatch Early Feeding Times Starter Supplemented with Egg Yolk and White of Boiled Chicken Eggs (Rhod Island Red) on Growth Performance, Viscera Development, and Immune Response in Broiler Chickens. Aust. J. Basic & Appl. Sci. **5**:660-671.

Richards, M., Proszkowiec-Weglarz, M., Rosebrough, R., McMurtry, J. and Angel, R. 2010 Effects of early neonatal development and delayed feeding immediately post-hatch on the hepatic lipogenic program in broiler chicks. Comp. Biochem. Physiol., B. **157:** 374-388.

Saki, A., A. 2005. Effect of post-hatch feeding on broiler performance. Int. J. Poult. Sci. **4**: 4-6.

Schmidt, J. and Zsédely, E. 2010. Importance of glycerol in the nutrition of monogastric animals: 1. Dietary glycerol for broiler chicken. / Glicerin felhasználása a monogasztrikus állatok takarmányozásában: 2. Glicerin a pecsenyecsirke hizlalásban. Állattenyésztés és Takarmányozás **59:** 457-469.

Shariatmadari, F. 2012. Plans of feeding broiler chickens. Worlds Poult. Sci. J. **68**:21-30.

Sheshukova, T., A. and Ozols, A., Y. 1986. A shift towards sucrose in carbohydrate transport in the small intestine of chickens of various ages. Assimilyatsiya pitatel'nykh veshchestv v organizme zhivotnykh. 203-214

Sklan, D. 2001. Development of the digestive tract of poultry. Worlds Poult. Sci. J. **57**:415–428.

Sklan, D. 2003. Fat and Carbohydrate Use in Posthatch Chicks. Poult. Sci. **82**:117-122.

Sklan, D. and Noy. Y. 2000. Hydrolysis and absorption in the small intestines of posthatch chicks. Poult. Sci. **79**:1306-1310.

Sklan, D., Geyra, A., Tako, E., Gal-Gerber, O. and Uni. Z. 2003. Ontogeny of brush border carbohydrate digestion and uptake in the chick. Br. J. Nutr. **89**:747-753.

Sklan, D., Noy, Y., Hoyzman, A. and Rozenboim. I. 2000. Decreasing weight loss in the hatchery by feeding chicks and poults in hatching trays. J. Appl. Poultry Res. **9**:142-148.

Sorbara, J., O., B., Menten, J., F., M., Longo, F., A., Pedroso, A., A., Racanicci, A., M., C., Gaiotto, J. B., Figueiredo, A., N. and Murakami, A., E. 2006. Allometric growth of the gastrointestinal tract of broilers from 1 to 7 day-old. EPC 2006 - 12Th European Poultry Conference, Verona, Italy. 10-14**.**

Sulistiyanto, B., Akiba, Y. and Sato, K. 1999. Energy utilisation of carbohydrate, fat and protein sources in newly hatched broiler chicks. Br. Poult. Sci. **40**:653-659.

Suvarna, S., S., Christensen, V., L., Ort, D., T. and Croom, W., R. 2005. High levels of dietary carbohydrate increase glucose transport in poult intestine. Comp. Biochem. Physiol., A. **141:** 257-263.

Swiatkiewicz, S., and J. Koreleski. 2009. Effect of crude glycerin level in the diet of laying hens on egg performance and nutrient utilization. Poult. Sci. **88**:615-619.

Swiatkiewicz, S., S. and Koreleski, J., J. 2009. Effect of crude glycerin level in the diet of laying hens on egg performance and nutrient utilization. Poult. Sci. **88(3)**: 615-619.

Takahashi, K. and Akiba, Y. 2005. Single administration of xylitol to newly hatched chicks enhances growth, digestive enzyme activity and immune responses by 12 d of age. Br. Poult. Sci. **46**:635-640.

Thompson, J. C. and He, B. B. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks [electronic resource]. Appl. Eng. Agric., **22**: 261-265.

Thompson, J., C. and He, B., B. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. Appl. Eng. Agric., **22**: 261-265.

Traber, P., G., Gumucio, D., L. and Wang, W. 1991. Isolation of intestinal epithelial cells for the study of differential gene expression along the crypt-villus axis. Am. J. Physiol. **260**:G895-G903.

Uni, Z. 1999. Functional development of the small intestine in domestic birds: cellular and molecular aspects. Poultry and Avian Biology Reviews **10**:167-179.

Uni, Z. and Ferket, R. 2004. Methods for early nutrition and their potential. World Poult. Sci. J. **60**:101-111.

Uni, Z., Ganot, S. and Sklan, D. 1998a. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci. **77**: 75-82.

Uni, Z., Geyra, A., Ben-Hur, H. and Sklan, D. 2000. Small intestinal development in the young chick: crypt formation and enterocyte proliferation and migration. Br. Poult. Sci. **41**:544-551.

Uni, Z., Noy, Y. and Sklan, D. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. **74**: 1622-1629

Uni, Z., Noy, Y. and Sklan, D. 1996. Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poult. Sci. **37**:63-71.

Uni, Z., Noy, Y. and Sklan, D. 1999. Posthatch development of small intestinal function in the poult. Poult. Sci. **78**: 215 – 222.

Uni, Z., Platin, R. and Sklan, D. 1998b. Cell proliferation in chicken intestinal epithelium occurs both in the crypt and along the villus. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology **168**:241-247.

Uni, Z., Tako, E., Gal-Garber, O. and Sklan, D. 2003. Morphological, Molecular, and Functional Changes in the Chicken Small Intestine of the Late-Term Embryo. Poult. Sci. **82**:1747-1754.

Vargas, F., S., C., Baratto, T., R., Magalhães, F., R., Maiorka, A. and Santin, E. 2009. Influences of breeder age and fasting after hatching on the performance of broilers. J. Appl. Poult. Res.**18**:8-14.

Ven, L., J., F., Wagenberg, A., V., Decuypere, E., Kemp, B. and Brand, H. 2013. Perinatal broiler physiology between hatching and chick collection in 2 hatching systems. Poult. Sci. 92:1050-1061.

Wijtten, P. J. A., Hangoor, E., Sparla, J. and Verstegen, M. 2010. Dietary amino acid levels and feed restriction affect small intestinal development, mortality, and weight gain of male broilers. Poult. Sci. **89:**1424-1439.

Wei, J., Coon, C. and Swanson, B. 1984. Weight gain and feed efficiency of chicks fed sucrose fatty acid esters. Poult. Sci. **63:** 378-380.

Willemsen, H., Debonne, M., Swennen, Q., Everaert, N., Careghi, C., Han, H., Bruggeman, V., Tona, K. and Decuypere, E. 2010. Delay in feed access and spread of hatch: importance of early nutrition. World Poult. Sci. J. **66:** 177-188.

Yamauchi, K., Isshiki, Y. and Iida, S. 1992. Post-hatching developmental changes in the ultrastructure of the duodenal absorptive epithelial cells in 1, 10 and 60-D-old chickens, with special reference to mitochondria. Br. Poult. Sci. **33**:475-488.

Yue, L., XiaoJing, Y., YingDong, N., Decuypere, E., Buyse, J., Everaert, N., Grossmann, R. and RuQian, Z. 2012. Early-age feed restriction affects viability and gene expression of satellite cells isolated from the gastrocnemius muscle of broiler chicks. Journal of Animal Science and Biotechnology **3**:33.

Zubair, A., K. and Leeson, S. 1996. Compensatory growth in the broiler chicken: a review. Worlds Poult. Sci. J. 52:**189-201**.

APPENDIX I: The effects of different feeding programs on body weight (g) during the first 35 days post hatch in Trial 2.

Program	Days			
	7	14	24	35
Immediate Access	143.8±7.1	400.3±32.8e	1159.1±52.5c	2293.8±128.0a
Delay access	*	333.3±21.1f	996.8±53.8d	2146.4±132.3b

a – f means different letters differ significantly (P<0.05).
*data was not collected at day 7 post hatch.

APPENDIX II: The growth performances of male Ross 308 during first 35 days post hatch (Aviagen®, 2012).

Age (Day)	Body Weight (g)	Daily gain (g)	Daily feed intake (g)	Feed to Gain Ratio
Hatch	42			
1	56	14	12	0.22
2	71	15	16	0.39
3	89	18	19	0.53
4	109	20	23	0.64
5	132	23	27	0.73
6	157	26	31	0.81
7	186	29	35	0.87
8	218	32	39	0.92
9	253	35	44	0.97
10	291	39	49	1.00
11	333	42	54	1.04
12	379	46	60	1.08
13	428	49	65	1.11
14	481	53	71	1.13
15	537	56	77	1.16
16	596	60	83	1.18
17	660	63	90	1.20
18	726	67	96	1.23
19	796	70	103	1.25
20	869	73	109	1.27
21	945	76	116	1.29
22	1025	79	123	1.31
23	1107	82	130	1.33
24	1191	85	136	1.35
25	1278	87	143	1.37
26	1368	89	150	1.39
27	1459	92	156	1.41
28	1553	94	163	1.43
29	1649	95	169	1.45
30	1746	97	175	1.47
31	1844	99	181	1.49
32	1944	100	187	1.51
33	2045	101	192	1.53
34	2147	102	198	1.55
35	2250	103	203	1.57
36	2353	103	208	1.59
37	2457	104	213	1.61

Appendix III: The growth performances of female Ross 308 during first 35 days post hatch (Aviagen®, 2012).

Age (Day)	Body Weight (g)	Daily gain (g)	Daily feed intake (g)	Feed to Gain Ratio
Hatch	42			
1	56	14	14	0.26
2	72	15	18	0.45
3	89	18	21	0.59
4	109	20	24	0.71
5	132	23	27	0.79
6	157	25	31	0.86
7	185	28	34	0.92
8	216	31	38	0.96
9	250	34	42	1.00
10	287	37	47	1.03
11	327	40	51	1.06
12	371	43	56	1.09
13	417	46	61	1.12
14	466	49	66	1.14
15	518	52	71	1.16
16	573	55	77	1.19
17	631	58	82	1.21
18	691	60	88	1.23
19	753	63	93	1.25
20	818	65	99	1.27
21	888	67	104	1.29
22	955	69	110	1.31
23	1026	71	115	1.34
24	1099	73	120	1.36
25	1174	75	126	1.38
26	1250	76	131	1.40
27	1327	77	136	1.42
28	1406	79	141	1.44
29	1485	80	146	1.46
30	1566	80	150	1.48
31	1647	81	155	1.50
32	1729	82	159	1.52
33	1811	82	163	1.55
34	1894	83	168	1.57
35	1977	83	171	1.58
36	2060	83	175	1.61
37	2143	83	179	1.63