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Using different levels of glycerine, glucose or sucrose in broiler starter diets to overcome negative effects of delayed feed access on growth performance

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Two trials were conducted to evaluate the inclusion of glycerine, glucose or sucrose in broiler starter diets to overcome negative effects of delayed feed access. In trial 1, 2160 newly hatched chicks (mixed sexed) were randomly assigned to two feeding programs (immediate vs. 36h delayed feed access). Nine starter diets prepared with crude glycerine, glucose or sucrose fed at 0, 4 or 8% levels, were fed during the first 14 days post hatch. In trial 2, 720 male and 720 female newly hatched chicks were randomly assigned to two feeding programs (immediate vs. 48h delayed feed access). Starter diets fed during the first 14 days of feed access, consisted of a control, 8% glycerine or 8% glucose. In both trials, a common grower and finisher diet were fed during days 14-24 and 25-35 respectively. Body weight and feed consumed were measured on 7, 14, 24 and 35 days. In trial 1, all 8% diet fed birds were heavier compared to the control on day 35 post hatch (2259 vs. 2198 g, $P<0.01$). Birds fed glycerine starter diets had improved FCR during 22-35 d ($p<0.01$). In trial 2, glycerine diet resulted in heavier birds after 35 days of feed access compared to birds, fed control and glucose diets immediately after hatch (2373 vs. 2276 g, $P<0.01$). Dietary inclusion of 8% glycerine can assist newly hatched broiler chicks to overcome delayed feed access induced growth suppression.

Key words: delayed feed access, crude glycerine, glucose, sucrose, broiler growth performance

Introduction

Broiler chickens have undergone intensive genetic selection for improved growth performance, resulting in a broiler growth rate that has quadrupled and feed conversion ratio that has decreased approximately 50% during the last four decades (Shariatmadari 2012, Collins et al. 2014, Zuidhof et al. 2014). As the number of days required to reach market weight continues to decline, the first days of life have become an increasing portion of the total time of broiler production. Newly hatched chicks may not be able to access feed and water for 72 hours (h) under commercial conditions (Willemsen et al. 2010). Prolonged delayed feed and water access can result in an irreversible reduction in growth performance, increased disease susceptibility and mortality (Willemsen et al. 2010; Abed et al. 2011, Lamot et al. 2014). Providing high quality feed to newly hatched broiler chicks with a highly available energy source has potential to improve growth performance, enhance flock health and perhaps increase profitability.

Small grain cereals and corn, rich in starches, are used as primary energy sources in broiler diets. However, starch digestibility is low during first days post hatch (Noy and Sklan 1999a, 1999b; Sklan 2003). Glucose and sucrose are simple sugars with less complex structures compared to starch. Newly hatched chicks can use both of these simple carbohydrates efficiently (Batal and Parsons 2002, 2004; Perry 2006). Batal and Parsons (2002, 2004) have demonstrated that incorporation of glucose or sucrose into broiler diets results in heavier birds compared to starch based diets. However, in these studies, starch-rich cereals were completely replaced by glucose and sucrose (Batal and Parsons 2002, 2004). Complete replacement of starch-rich cereals with feed grade glucose and sucrose significantly increases the price of feed compared to corn or other cereal grain based diets. Partial replacement of starch with glucose or sucrose in broiler

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starter diets has potential to enhance bird performance and could be used as a tool to overcome negative effects of delayed feed access.

Crude glycerine is a major by-product of biodiesel production. Global growth in biodiesel production has resulted in a steady increase in supply of crude glycerine (Thompson and He 2006). Crude glycerine products have diverse compositions (Jung and Batal 2011a, 2011b; Alvarenga et al. 2012). Glycerol represents 80 to 90% of crude glycerine and the remaining 10 to 20% are represented by water, ash, fatty acids and traces of protein and methanol (Jung and Batal 2011a; Alvarenga et al. 2012). Crude glycerine products have relatively high metabolic energy (3331 to 4135 Kcal/kg, Dozier et al. 2011; Alvarenga et al. 2012). The differences in metabolic energy levels are due to variability in the presence of fatty acids and water in crude glycerine products (Dozier et al. 2011; Alvarenga et al. 2012). The increasing availability and high metabolic energy of crude glycerine make it a good candidate as a potential feed ingredient. Many studies were conducted to evaluate effects of feeding broilers crude glycerine, however, dietary inclusion levels and durations of experiments vary considerably (Schmidt and Zsédely 2010; Abd-Elsamee et al. 2011; Jung and Batal 2011b). Alvarenga et al. (2012) suggested that the optimal dietary inclusion level of crude glycerine is approximately 5% in broiler feed. Young chicks have accelerated metabolic rates where tissue can use glycerol more efficiently than older birds (Cerrate et al. 2006; McLea et al. 2011; Alvarenga et al. 2012). It is possible that, dietary inclusion levels of crude glycerine in young broiler diets can be higher than 5% and may result in enhanced growth performance in later development. In previous studies (Schmidt and Zsédely 2010; Abd-Elsamee et al. 2011; Jung and Batal 2011b), crude glycerine diets were provided throughout the entire growth period. To author's knowledge, there were no experiments reported that evaluated the effects of crude glycerine in young broilers, particularly newly hatched birds.

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To fully understand the effects of early inclusion of crude glycerine in broiler diets, the performance of the bird from hatch to target weight should be monitored.

This study was conducted to evaluate dietary inclusion of glucose, sucrose and glycerine on broiler growth performance in 35-day feeding trials. Initial focus was to evaluate the partial replacement of starch-rich cereals grain in broiler starter diets (1-14d). The second trial investigated if partial replacement of starch-rich cereals grain with easily utilized dietary energy sources in starter diets could assist both male and female chicks in overcoming the negative effects of a delay in access to feed. We hypothesized that, (1) partial replacing corn with easily utilize energy sources can assist broiler chicks to overcome delayed feed access induced negative effect and (2) easily utilize energy sources (glucose or glycerine) affect male and female broiler differently.

Material and Methods

Animal Care and Animals

The protocols for both experiments were approved by the local animal care and use committee. All the procedures were carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009). In both trials, fertilized eggs (Ross 308) were purchased from a local hatchery. The average egg weight was 61.7 ± 0.7 g. The eggs were incubated in Chick Master® G90 incubators (Chick Master®, Medina, Ohio) at the Dalhousie University Hatchery in Truro, Nova Scotia (NS). During the first 18 days of incubation, the temperature was set at 37.5 C and at 75% relative humidity (RH). The eggs were automatically turned every 45 minutes. On day 18 of incubation, the eggs were transferred into hatching trays. The incubator conditions were monitored twice daily. The RH was reduced to 55% at 508 h of incubation and remained at

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55% for 2 h to allow newly hatched birds' feathers to properly dry out. The hatching process was terminated at 21 days plus 6 h (510 h). After hatch, broilers were feather sexed, weighed, vaccinated for Marek's disease (Intervet Canada Corp, Kirkland, Quebec) and transported to the poultry rearing facility at the Faculty of Agriculture, Dalhousie University, Truro, NS, Canada.

The birds were placed in floor pens in four environmentally controlled rooms with all treatments equally represented in each room. Environmental conditions were similar in all rooms. In trial 1, 2160 birds (1080 males and 1080 females) were placed in 72 floor pens with wood shaving bedding. Each pen held 15 birds of each sex. In trial 2, 720 males and 720 females were placed in 48 floor pens with a single gender of 30 birds in each pen.

Feeding Programs

In both trials, water was provided from 2 nipple drinkers per pen and diets were loaded into cardboard box tops during the first 7 days post hatch (PH) and into tube feeders for the entire experiment.

In trial 1, newly hatched chicks were randomly assigned to feed availability treatments. They were either provided immediate access (within 3 h after the hatching process was terminated) to feed and water (IA) or not allowed access to feed and water until 36 h after the hatching process was terminated, which will be known as delayed access (36DA) treatment. In trial 2, newly hatched chicks were randomly assigned to immediate access to feed and water (IA) or a treatment where 48 h elapsed before access to feed and water was allowed (48DA). In both trials, feed and water were provided *ad libitum* once feed access was initiated.

Diets

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The diets used in both trials were formulated to meet or exceed the nutrient requirements for broilers during each developmental stage (Aviagen®, 2014). In trial 1, 9 starter diets were prepared that contained 0, 4 or 8% glucose, sucrose or crude glycerine (Table 1). The glycerine used in this experiment was REG Glycerin – 98 (Renewable Energy Group®, Ames, IA). This crude glycerine contained 98% glycerol and 0.8% moisture. The starter mash diets were formulated to contain 23% crude protein and 3050 kcal AME/kg and were fed during first 14 days PH. On day 15 PH, a common pelleted grower diet was provided to all treatment groups. The grower diet was formulated to contain 20% crude protein and 3150 kcal AME/kg (Table 2) and was provided from the day 15 to day 24 PH. From the day 25 - 35 PH, a pelleted common finisher diet was fed to all treatment. This diet was formulated to contain 18% crude protein and 3200 kcal AME/ kg (Table 2). In trial 2, three starter diets were prepared. The starter diets were control, 8% glucose or 8% crude glycerine. The crude protein and energy levels of the diets were identical to trial 1 (Table 1). The starter mash diets were provided during first 14 days of feed access. From 15 to 24 days of feed access, a common pelleted grower diet was provided to all treatments. From 25 to 35 days of feed access, a common pelleted finisher diet was provided to all treatments. The formulations of grower and finisher diet were similar with trial 1 (Table 2).

Growth performance data collection

In trial 1, all birds in the pen were batch weighed with feed consumption measured and recorded on 7, 14, 24 and 35 days PH. 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 conversion ratio (FCR, gram feed to gram of weight gain) on a per bird basis

and % mortality were calculated for each period of growth. In trial 2, the same performance measurements (BW, DFC, ADG and FCR) were measured on 7, 14, 24 and 35 days following feed access.

Statistical Analysis

Trial 1 was a 3*3*2 factorial design with 4 replications. The energy source (ES, glucose, sucrose or glycerine), inclusion levels (IL, 0, 4 or 8%) and feed and water access program (IA or DA) were main factors. The contrast analysis was conducted to investigate the relationship of growth performance and inclusion levels. Trial 2 was a 3*2*2 factorial design with 4 replications. The dietary energy sources (control, 8% glucose or 8% glycerine), feed and water access program (IA or DA) and gender (male vs. female) were main factors. In both trials the pen was considered the experimental unit.

Bird growth performance was analyzed as repeated measures when time was a factor. The growth performance data was analyzed by ANOVA using the Proc Mixed procedure (Littell et al. 1996) was used in SAS 9.3 (SAS Institute Inc., Cary, NC). If significant main effects or interactions were found ($P \leq 0.05$) the Tukey-Kramer procedure was used to compare differences among the least-square means at $\alpha = 0.05$ (Gbur et al. 2012).

Results

Trial 1:

At hatch, average body weight was 42.3 ± 0.2 g and did not differ among treatments. On each sampling day, the main factors ES, IL and feed and water access time influenced BW (Table 3). There were no interactions between main effect treatments for BW. Birds fed

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glycerine were heavier than those fed glucose or sucrose on day 35 PH (2254 vs. 2215 g). On 35 day PH, the birds fed the three ES starter diets with 8% of the ingredient were heavier than birds fed control (2259 vs. 2198 g). Birds with early access to feed were heavier than delayed feed access birds through the trial (Table 3). Daily feed consumption (DFC) was improved with increasing inclusion levels of dietary energy sources (Table 4). Daily feed consumptions did not differ among glucose, sucrose and glycerine treatments (Table 4). Throughout trial 1, the IA birds consumed more feed than DA birds from start to finish (Table 4). Birds fed crude glycerine starter diets had a lower FCR during the finisher stage (25 – 35 d PH) than the birds fed glucose or sucrose (Table 5). The average FCR for birds provided the ES at 4% was improved compared with control diets. During the grower phase (15 – 24 d PH), DA birds fed 4% ES had the same FCR as IA birds fed control diets (Table 6).

Trial 2:

At hatch, average body weight was 41.9 ± 0.2 g and were similar among treatments. At 48 h after hatch, the IA birds were heavier than 48 DA birds (41.9 vs. 39.9 g). During the first 14 days of feed access, BW did not differ among treatments. After 35 days of feed access, male broilers (2388.5 ± 73.5 g) were heavier than females (2266.3 ± 76.9 g). Both males and females in the DA group birds reached the same body weight after 35 days of feed access (37 day PH) as the IA group (35 day PH) (Table 7). After 35d of feed access, birds fed glycerine in the starter diet in the DA group (37 day PH) were heavier than both birds fed glucose with DA and the birds fed glycerine or control diets in the IA group (35 day PH). On 24 day of feed access, dietary energy did not affect BW within male or female birds. On 35 day of feed access, male birds fed the glycerine diet were heavier than all other treatments.

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Average daily gains (ADG) were affected by feeding program and dietary energy source interactions. During the grower period (15-24 days of feed access), birds fed glucose in the DA group (17-26 day PH) had significantly higher ADG ($78.8 \pm 1.8 \text{ g day}^{-1}$) compared with birds fed glycerine in the IA group (15-24 day PH) ($68.0 \pm 4.4 \text{ g day}^{-1}$, Table 8). During the finisher period (25-35 day of feed access), the chickens fed glucose in the IA group (25-35 day PH) had higher ADG ($121.5 \pm 5.6 \text{ g day}^{-1}$) than glucose fed DA birds (27-37 day PH) ($110.2 \pm 9.5 \text{ g day}^{-1}$).

Daily feed consumption (DFC) was affected by interactions with the feeding program and dietary energy source. During the starter (1-14 day of feed access) and grower (15-24 day of feed access) periods, DFC did not differ among treatments. During the finisher period (25-35 day of feed access), the birds fed the control diet in the DA group (27-37 day PH) consumed more feed than birds fed glucose in the starter diet 215.2 vs. 191.6 g day^{-1} (Table 9). Throughout the experiment, male broilers consumed more feed than females (data not shown). Feed conversion ratio was not affected by the different feeding programs, gender and dietary energy sources and there were no interactions among these treatments (Table 10). As expected, older birds had a higher FCR than younger birds (data not shown).

Discussion

Newly hatched broiler chicks may be subjected to prolong periods without access feed and water under commercial conditions. This is because broiler chicks hatch over a 24 to 48 h hatch window (Uni and Ferket 2004; Willemsen et al. 2010; Buzala et al. 2015), and other hatchery practices, such as sexing, vaccination, and transportation can increase the period that early hatched chickens are deprived of feed and water for up to 72 hours (Willemsen et al. 2010). When studying effects of delay in access to feed on broiler growth performance, the broiler age

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is an important factor. There are two terms to describe chick age, biological age, which is defined as age in days in reference to the exact time of hatch, and chronological age, which is defined as the age in days in reference to the end of hatch of the whole flock (Willemssen et al. 2010). Early hatched birds often remain in the incubator until the incubation process was terminated. Early hatched chicks are subjected to a longer delay in access to feed than late hatched birds. Therefore, early hatched birds are more susceptible to the negative effects of a further delay in access to feed when based on birds chronological age (Careghi et al. 2005). In the current study, chronological age was used. If a nutritionally balanced diet is provided, Saki (2005) demonstrated that broilers can overcome the negative effects of an up to 24 h delay in access to feed and reach the same body weight on 35 day PH as birds fed immediately. In the current study, birds were not able to recover from a 36 h delay in access to feed by 35 days PH. This agrees with previous studies, where broiler growth were suppressed after experiencing a prolonged feed deprivation immediately after hatch.

During first days post hatch, newly hatched chicks can use glucose and sucrose efficiently (Batal and Parsons, 2002, 2004; Perry 2006), while dietary starch digestibility remains low (Noy and Sklan 1999a, 1999b; Sklan 2003). Young broilers also have a higher capacity to use dietary crude glycerine compared with older birds (Alvarenga et al. 2012; Lima et al. 2013; Henz et al. 2014). Lima et al. (2013) determined the average apparent metabolizable energy from three crude glycerine sources (70 – 80% glycerol) is 4461 kcal/ kg for 14 day old birds and 3715 kcal/ kg for 40 day old birds. This evidence suggests that, replacing dietary starches with crude glycerine, glucose or sucrose in young broiler (1-14 day PH) diets may enhance broiler growth. Previous studies focused on evaluating the effects of simple sugars on growth performance of young broilers were inconclusive. Simple sugars, such as, glucose, fructose, sucrose, maltose and

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dextrin were used either completely (Batal et al. 2004; Sulistiyanto et al. 1999) or partially (Longo et al. 2007; Wei et al. 1984) replacing starch in previous studies. Different dietary inclusion levels of simple sugars among studies also make it difficult to conduct cross study comparisons and estimated commercial applications. Wei et al. (1984) reported that providing newly hatched chicks with 5% sucrose or glucose for two weeks did not affect growth performance. In the current study, 8% glycerol, glucose and sucrose in the starter diets resulted in birds that were heavier than control birds. Modern broilers are undergoing intensive genetic selection for growth performance, including increased growth rate (Renema et al. 2007; Collins et al. 2014; Zuidhof et al. 2014). A recent study indicated that broiler 7 day body weight was not affected by ad libitum provision of an 8% glucose solution to newly hatched chicks (Jiang et al. 2008). This agrees with the current study, where broilers fed starter diets with glucose had similar body weights at 7 day of age (trial 1) and 7 day of feed access (trial 2). It is suggested that, heavier chicks at 7 day PH are heavier at market age (Saki, 2005). In the current study, crude glycerine produced heavier body weight at 35 day PH (trial 1) and 35d of feed access (trial 2) but did not affect body weight on 7 day PH or 7 days of feed access.

Glucose is directly absorbed in the small intestine without requiring enzymatic digestion (Moran et al. 2010). Glucose absorption is relatively low at hatch and then increases after consumption of feed (Sklan and Noy 2000). Providing a glucose based diet to newly hatched chicks has resulted in better feed efficiency in studies by others (Batal and Parsons 2002). In the current study, broiler feed efficiency was not affected by inclusion of 8% glucose (trial 1 and 2). Glucose based diet provides benefits to young chicks (1 – 7 day) as indicated by higher metabolic energy (Batal and Parsons 2002). Batal and Parsons (2002) also indicated that birds fed glucose based diets were lighter than those fed corn based diet after three weeks. A recent

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study indicated that completed replacement of corn starch with glucose resulted the increasing of ileal endogenous amino acids lost (Kong and Adeola 2013). The difference between glucose based diet and corn based diet fed birds observed in Batal and Parsons (2002)'s study may be result of increase endogenous amino acids loss. In the current study, male birds fed the 8% glucose starter diet were lighter than control birds after 35d of feed access. This is likely due to the fact that glucose has lower true metabolic energy than starch after 10 d PH (Sulistiyanto et al. 1999). These studies suggested that completely replacing starch with glucose after first week post hatch may not enhance broiler weight gain and final body weight. Current results demonstrated that partial replacement of starch with glucose or sucrose during first 14d PH enhanced broiler feed efficiency (1.62 vs. 1.70, Table 6) during 24-35 day PH. These results agreed with previous studies that partial replacement of starch with glucose or sucrose regarding feed conversion ratio (Wei et al. 1984; Batal and Parsons 2002; Jiang et al. 2008).

Broiler body weight did not differ between birds fed glucose and sucrose diets for 14 days. Batal and Parsons (2004) reported that broiler chicks fed a glucose-soybean meal based diet were heavier than those fed sucrose-soybean meal based diet after 21 days of feeding. Different glucose and sucrose inclusion levels (complete replacement of starch vs. 4 – 8% dietary inclusion level) and feeding times (21 days vs. 14 days) were used in both the current study and Batal and Parsons (2004). These differences may result in a difference in performance. Total amount of glucose supplement provided immediately after hatch is important for enhancing broiler performance (Noy and Pinchasov 1993). Broiler body weight at 21d was improved by providing a single dose 0.5ml 50% glucose solution immediately after hatch (Noy and Pinchasov 1993) but was not affected by 0.5ml 20% glucose solution (Takahashi and Akiba 2005). Therefore, it may be necessary to provide newly hatched birds with high glucose or sucrose

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inclusion ($\geq 8\%$) diets immediately after hatch to achieve maximum growth performance. Broiler body weight and weight gain were improved by supplying 9 – 16% sucrose in diets during the first 7 day PH (Longo et al. 2007). In the current study, inclusion of 8% glucose, sucrose or glycerine in the starter diet improved broiler body weight (Table 3), weight gain (Table 4) and FCR (Table 6) at market age. Feed cost represent the largest proportion of broiler production cost, minimizing feed cost will result in maximizing live birds profitability (Aviagen®, 2014). Older broilers consumed more feed compared with younger birds and had higher accumulated feed consumption (Aviagen®, 2016). Therefore, improving broiler FCR in later stages of growth may save overall feed cost and increase overall profitability.

Increasing production of biodiesel results in increased availability of crude glycerine for broiler feed. However, previous studies evaluating effects of crude glycerine on broiler performance were inconsistent. Dietary inclusion of crude glycerine (81% glycerol) up to 10% level can improve broiler feed utilization and weight gain between 7 and 28 days of age (McLea et al. 2011). In the current study, dietary inclusion of up to 8% crude glycerine (98% glycerol) in starter diet (1 – 14 day of feed access) did not negatively affect broiler growth performance. Current results agreed with Cerrate et al. (2006) where 10% dietary crude glycerine (80% glycerol) could be added without compromising broiler growth performance up to 14 days of age. More recent studies suggested that dietary inclusion of 10% crude glycerine (around 81% glycerol) can improve broiler weight gain (Henz et al. 2014; Ugrnani et al. 2014) and feed conversion ratio (Henz et al. 2014; Ugrnani et al. 2014; Mandalawi et al. 2014). In the current study, 8% crude glycerine (98% glycerol) was used. The total dietary inclusion level of glycerol is comparable with 10% crude glycerine (containing around 80% glycerol) used in previous experiments. Dietary inclusion of 15% crude glycerine (81% glycerol) did not compromise

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broiler growth performance but increased litter moisture (Henz et al. 2014). Increasing litter moisture is not desired under commercial production because it may lead to increased ammonia levels and disease susceptibility. Methanol is present at various levels (0.01 to 3.10%) in different crude glycerine products and no increase in litter quality was observed in previous studies (Jung and Batal 2011a; 2011b; Alvarenga et al. 2012). Consumption of methanol can result neurological disorders, blindness and even death in animals (Alvarenga et al. 2012). Therefore, it is necessary to consider methanol levels in different crude glycerine products during feed formulation. However, there are limited studies to evaluate negative effects of methanol associated with crude glycerine products in broiler growth performance (Alvarenga et al. 2012). A recent study indicated that, dietary inclusion of 5% crude glycerine (73.9% glycerol and 3.4% methanol) did not affect broiler growth performance during 1 – 14 day PH (Jung and Batal 2011b). The crude glycerine (98% glycerol) used in current study has a higher level of glycerol and lower methanol (trace) than crude glycerine products used in studies by Jung and Batal (2011b). The concern about methanol level was minimal in this study. Alvarenga et al. (2012) suggested that the optimal dietary inclusion level of crude glycerine is approximately 5%. The current study suggested that, a high quality crude glycerine (high glycerol and low methanol) can be included up to 8% in starter diets without negatively affecting broiler growth. Results from trial 1 in the current study further indicated that, dietary inclusion of crude glycerine in broiler starter diets enhance broiler body weight at 35 day PH (Table 3) and improve feed efficiency during 24-35 day PH (Table 7). Therefore, partial replacement of starch with high quality crude glycerine in broiler starter diets may enhance growth and reduce feed cost in later of production.

Delayed access to feed can result in reduced body weight, compromised growth performance, increased disease susceptibility and mortality (Willemssen et al. 2010; Abed et al.

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2011, Lamot et al. 2014). Newly hatched broiler chicks will lose 0.14g of BW/h if feed is unavailable (Sklan et al. 2000). Delayed access to feed also results in a reduction of satellite cell proliferation in newly hatched birds which will result in a reduction of muscle yield at market age (Berri et al. 2007). Juul – Madsen et al. (2004), Abed et al. (2011) and Rammouz et al. (2011) demonstrated that broiler chicks are able to overcome up to a 24 h (chronological age) delay in access to feed and reach the same final weight as birds with immediate feed access post hatch. Results of previous studies did not reflect individual bird growth performance when bird age was considered as chronological age. In the first trial, 36 h DA birds were lighter than immediate feed access birds at same chronological age. This result agreed with previous studies which indicated that 36 h of delayed access to feed resulted in lighter birds at same chronological age (Bhanja et al. 2010; Kornasio et al. 2011). Conversely, Cengiz et al. (2012) reported that, Ross 308 birds were able to reach the same final body weight after a 36 h delay in access to feed and water when organic acid was used as a feed supplement. The reason that broilers were able to recover from delayed feed access in Cengiz et al. (2012) study maybe because the results of organic acid enhanced GIT health and histological structures. In the second trial, DA birds lost around 2 g BW after 48 h DA. Previous studies indicated that, newly hatched birds can lose around 3 to 5 g BW if birds were not provided access to feed and water (Bigot et al. 2003; Bhanja et al. 2009). The weight loss occurred is because the newly hatched chicks utilized their body reserve and dehydration. In the current study, birds were placed in floor pens with wood shaving bedding and free access to water. The lower weight loss (2 g vs. 3 to 5 g BW) in the current study may suggest the DA birds were less dehydrated. A delay in access to feed may result in birds consuming wood shavings during first two days PH. Noy and Sklan (1999a) reported that, saw dust fed chicks lost less weight than unfed birds. In previous studies, 48 h DA birds were lighter

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than IA birds at the same chronological age (Juul – Madsen et al. 2004; Abed et al. 2011; Rammouz et al. 2011). The current study indicated that broilers were able to compensate for 36 h of feed deprivation at same chronological age and were able to compensate for 48 h of feed deprivation if they were able to access to feed for a similar period of time. Delayed feed access can reduce broiler chick immune responses (Shira et al. 2005) and health of small intestine (Simon et al. 2014). These factors were not measured in current study. In future studies, chicken immune response can be used to evaluate effects of delayed access to feed on broilers.

Conclusion:

Partial replacement of starch-rich cereal with glucose, sucrose or crude glycerine (98% glycerol) in starter diets can be used to improve broiler performance during later stages of growth, without negative effects on broiler growth performance. Crude glycerine, a biodiesel by-product, is a potential dietary energy source for young broilers and can be safely used in broiler starter diet up to 8% level (8% glycerol dietary inclusion). Effects of easily utilize dietary energy sources on broiler immune system development under delayed feed access condition should be evaluated in the future.

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1 Table 1. Diet formulations of glucose, sucrose or glycerine starter diets in both trials, as fed basis.

Feed	Control	Glucose		Sucrose		Glycerine	
		4%	8%	4%	8%	4%	8%
Ingredient %							
Corn	44.072	39.722	40.468	39.722	40.468	39.791	40.607
Soybean meal	38.73	38.903	34.59	38.903	34.591	38.503	33.79
Wheat	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Tallow-grease blend	3.050	3.226	2.792	3.226	2.792	3.557	3.455
Glucose	0.000	4.001	8.002	0	0	0	0
Sucrose	0.000	0	0	4.001	8.001	0	0
Glycerol	0.000	0	0	0	0	4.001	8.002
Limestone	1.659	1.659	1.647	1.659	1.647	1.658	1.644
Mono-Dicalcium phosphate	0.859	0.859	0.884	0.859	0.884	0.861	0.889
Methionine Premix ^a	0.641	0.641	0.645	0.641	0.645	0.642	0.646
Mineral & Vitamin Premix ^b	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Iodized salt	0.432	0.432	0.434	0.432	0.434	0.431	0.433
Lysine-HCL (76% lysine)	0.040	0.040	0.021	0.040	0.021	0.039	0.017
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	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Calculated nutrient composition							
AMEn (kcal/kg)	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
Total phosphorus (%)	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Lysine (%)	1.43	1.43	1.43	1.43	1.43	1.43	1.43
Methionine (%)	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Methionine + cysteine (%)	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Histidine (%)	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Arginine (%)	1.66	1.66	1.66	1.66	1.66	1.66	1.66

2 **Note:** ^a Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

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^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; D1 Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7 mg; 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 Methylene Disalicylate, Alpharma, Inc., Fort Lee, NJ, USA (providing 4.4 mg tonne-1 mixed feed)

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33 Table 2. The diet formulations for chicks receiving common grower and finisher diet in both trials, as fed basis.

Feed Ingredients %	Grower	Finisher
Corn	49.700	55.908
Soybean meal	31.396	26.018
Wheat	10.002	10.000
Tallow-grease blend	4.607	3.988
Limestone	1.427	1.393
Mono-Di calcium Phosphate	0.715	0.641
Methionine premix ^a	0.586	0.529
Mineral and Vitamin Premix ^b	0.500	0.500
Iodized salt	0.402	0.400
Lysine-HCL (76% lysine)	0.111	0.123
Amprolium ^c	0.050	0.000
BMD ^d	0.004	0.000
Pel-stik ^e	0.500	0.500
Total	100.000	100.000
Calculated nutrient composition		
AMEn (kcal/kg)	3150	3200
Crude Protein (%)	20.00	18.00
Calcium (%)	0.92	0.85
Total phosphorus (%)	0.55	0.52
Lysine (%)	1.24	1.09
Methionine (%)	0.45	0.41
Methionine + cysteine (%)	0.95	0.86
Histidine (%)	0.53	0.47
Arginine (%)	1.40	1.23

34 **Note:** ^a Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

35 ^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;
36 riboflavin, 7.6 mg; D1 Ca-pantothenate, 13.5 mg; vitamin B12, 0.023 mg; niacin, 29.7; folic acid, 4.0 mg, choline, 801 mg; biotin, 0.3
37 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
38 mg.

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^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)

^e Pel – stik -- Pellet Binder – Uniscope Inc., Johnstown, CO, USA.

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59 Table 3. The effects of dietary energy sources (glucose, sucrose and glycerine), inclusion levels (0, 4 and 8%) and feed access time (36
60 hrs delayed vs. immediate feed access) on broiler body weight (g), trial 1.

		Age (day)			
		7	14	24	35
Effects					
Energy sources (Source)	Glycerine	136.6±15.6	376.9±31.3	1142.3±65.9a	2254.8±132.8a
	Glucose	133.2±17.6	357.9±32.1	1089.7±68.2b	2216.0±124.5b
	Sucrose	131.4±16.6	357.7±30.0	1075.5±70.0b	2215.5±55.9b
Inclusion level (Level)	0%	132.6±16.3	354.9±30.2	1106.5±74.2	2198.2±93.9
	4%	132.1±14.1	362.3±28.0	1082.3±88.5	2229.1±122.5
	8%	136.5±13.4	375.4±29.0	1118.7±82.3	2259.0±128.7
Feed access (Program)	Immediate	146.1±7.7a	389.7±14.8a	1159.1±56.7a	2309.9±99.6a
	Delayed	121.4±7.5b	338.7±16.0b	1045.9±62.3b	2147.6±66.7b
ANOVA P value					
Source		P=0.95	P=0.26	P<0.01	P<0.01
Level	linear	P=0.07	P<0.01	P=0.40	P<0.01
	Quadratic	P=0.17	P=0.20	P=0.02	P=0.98
Program		P=0.03	P<0.01	P<0.01	P<0.01
Source * Level		P=0.99	P=0.74	P=0.64	P=0.28
Source * Program		P=0.42	P=0.60	P=0.70	P=0.40
Level * Program		P=0.42	P=0.50	P=0.83	P=0.24
Source * level * Program		P=0.90	P=0.09	P=0.85	P=0.76

61 **Note:** Mean ± SEM on each sampling day with different letters (a-b) are significantly different (P<0.05).

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Table 4. The effects of dietary energy sources (glucose, sucrose and glycerine), inclusion levels (0, 4 and 8%) and feed access time (36 hrs delayed vs. immediate feed access) on broiler daily feed consumption (g day⁻¹), trial 1.

		Growth stage (day)			
		0-7	8-14	15-24	25-35
Effect					
Energy sources (Source)	Glycerine	15.2±2.5	50.6±4.0	104.4±24.1	191.3±11.1
	Glucose	15.6±2.8	47.5±3.2	104.3±14.9	186.0±9.7
	Sucrose	15.0±2.3	47.4±5.2	103.4±16.9	183.6±10.0
Inclusion level (Level)	0%	14.9±2.5	46.1±3.0	101.9±11.1	185.1±9.7
	4%	15.4±2.2	48.0±3.9	105.1±6.9	185.9±9.6
	8%	15.5±2.6	51.4±3.9	105.1±8.3	189.9±12.1
Feed access (Program)	Immediate	17.55±1.09a	50.78±4.3a	109.57±4.81a	191.33±12.54a
	Delayed	12.96±0.66b	46.25±4.29b	98.52±7.65b	182.60±9.36b
ANOVA P value					
Source		P=0.95	P=0.14	P=0.85	P=0.07
Level	Linear	P=0.02	P<0.01	P=0.14	P=0.09
	Quadratic	P=0.33	P=0.37	P=0.38	P=0.49
Program		P<0.01	P<0.01	P<0.01	P<0.01
Source * Level		P=0.97	P=0.69	P=0.45	P=0.16
Source * Program		P=0.28	P=0.19	P=0.11	P=0.98
Level * Program		P=0.08	P=0.87	P=0.59	P=0.65
Source * level * Program		P=0.47	P=0.54	P=0.55	P=0.64

Note: Mean ± SEM on each sampling day with different letters (a-b) are significantly different (P<0.05).

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71 Table 5. The effects of dietary energy sources (glucose, sucrose and glycerine), inclusion levels (0, 4 and 8%) and feed access time (36
72 hrs delayed vs. immediate feed access) on birds' feed conversion ratio (FCR), trial 1.

		Growth stage (day)			
		0-7	8-14	15-24	25-35
Effect					
Energy sources (Source)	Glycerine	1.19±0.13	1.47±0.10	1.47±0.10	1.58±0.08b
	Glucose	1.23±0.08	1.48±0.08	1.46±0.20	1.63±0.12a
	Sucrose	1.23±0.14	1.44±0.07	1.42±0.09	1.63±0.12a
Inclusion level (Level)	0%	1.22±0.10	1.46±0.06	1.39±0.08	1.70±0.10
	4%	1.22±0.08	1.46±0.07	1.48±0.12	1.62±0.06
	8%	1.19±0.13	1.48±0.05	1.42±0.05	1.67±0.17
Feed access (Program)	Immediate	1.23±0.11	1.48±0.08	1.44±0.15	1.67±0.14
	Delayed	1.20±0.12	1.46±0.08	1.42±0.12	1.67±0.11
ANOVA P value					
Source		P=0.30	P=0.46	P=0.04	P<0.01
Level	Linear	P=0.45	P=0.19	P=0.32	P=0.36
	Quadratic	P=0.75	P=0.70	P=0.02	P=0.02
Program		P=0.30	P=0.51	P=0.43	P=0.88
Source* Level		P=0.32	P=0.74	P=0.06	P=0.25
Source * Program		P=0.70	P=0.38	P=0.11	P=0.46
Level * Program		P=0.19	P=0.85	P<0.01	P=0.82
Source * level * Program		P=0.13	P=0.87	P=0.22	P=0.21

73 **Note:** During grower (14-24d) and finisher (25-35d), FCR were affected by main effect energy source, however, Tukey – Kramer test
74 did not differentiate the means (P>0.05).
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78 Table 6. The effects of feeding programs and different levels of energy source interactions in starter diet on feed conversion ratio
 79 (FCR) during the 35 day post hatch, trial 1.

Feeding program		Immediately access			36 hours delayed access			ANOVA (p-value)
Inclusion (%)	Level	0	4	8	0	4	8	
Time								
0-7		1.26±0.09	1.19±0.07	1.16±0.14	1.20±0.02	1.25±0.02	1.24±0.02	P=0.19
8-14		1.47±0.07	1.48±0.09	1.49±0.09	1.44±0.07	1.45±0.08	1.49±0.10	P=0.85
15-24		1.32±0.08b	1.47±0.16a	1.44±0.16ab	1.44±0.12ab	1.47±0.13a	1.39±0.12b	P<0.01
25-35		1.69±0.13	1.63±0.07	1.67±0.19	1.72±0.09	1.61±0.09	1.68±0.14	P=0.82

80 **Note:** The differences of FCR during grower stage were identified from the ANOVA, but the Tukey – Kramer test did not
 81 differentiate the means (P>0.05).

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90 Table 7. The broiler body weights (g) on day 7, 14, 24 and 35 of feed access, trial 2.

		Growth stage (day of feed access)			
		0-7	8-14	15-24	25-35
Effect					
Male	Control	121.3±9.9	400.2±26.2	1153.9±46.3ab	2382.5±60.9a
	Glycerine	153.3±7.1	424.4±22.3	1186.7±82.0ab	2459.0±73.4a
	Glucose	153.4±8.8	413.8±21.5	1209.5±73.5a	2266.4±135.5b
Female	Control	145.6±7.2	388.2±24.7	1108.6±44.5bc	2260.4±95.9b
	Glycerine	144.5±10.8	408.4±24.8	1090.3±44.3c	2252.9±89.3b
	Glucose	149.0±8.2	410.2±32.0	1120.2±56.7c	2182.6±83.1b
Immediate access	Male	145.5±6.2	405.3±27.2	1130.4±48.7b	2388.5±73.5a
	Female	140.9±7.3	394.0±28.0	1074.9±41.2b	2197.7±99.7b
Delayed access	Male	139.8±12.3	420.3±18.5	1236.4±41.4a	2350.1±74.9a
	Female	151.9±7.6	410.3±19.1	1137.8±37.0b	2266.3±76.9b
Immediate access	Control	140.4±5.3	385.6±32.0	1089.8±39.9c	2276.0±13.3bc
	Glycerine	143.7±10.3	410.5±33.3	1097.2±66.8b	2268.3±180.3bc
	Glucose	145.4±4.2	403.0±32.4	1120.9±48.9b	2335.0±82.3ab
Delayed access	Control	126.5±11.9	402.8±12.7	1165.4±34.5ab	2359.4±75.4ab
	Glycerine	154.1±8.1	422.0±13.0	1187.2±66.0ab	2373.2±139.6a
	Glucose	157.0±8.6	421.0±14.4	1208.9±76.3a	2191.8±89.5c
ANOVA P value					
Gender		P=0.77	P=0.41	P<0.01	P<0.01
Energy Source ^a		P=0.47	P=0.33	P=0.08	P<0.01
Program ^b		P=0.86	P=0.29	P<0.01	P=0.58
Gender * Energy Source		P=0.73	P=0.65	P<0.01	P<0.01
Gender * Program		P=0.92	P=0.59	P<0.01	P<0.01
Energy Source * Program		P=0.80	P=0.64	P<0.01	P<0.01
Gender * Energy Source * Program		P=0.96	P=0.86	P=0.75	P=0.61

91 **Note:** Mean ± SEM on day 7, 14, 24 and 35 of feed access, with different letters (a-b) are significantly different (P<0.05).

92 ^a Energy source has three levels: control, 8% glycerine and 8% glucose

93 ^b Program has two levels: immediate vs. 48 hours delayed feed access.

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95 Table 8. The average daily weight gain (g day^{-1}) during 1-7, 8-14, 15-24 and 25-35d of feed access, trial 2.

		Growth stage (day of feed access)			
		0-7	8-14	15-24	25-35
Effect					
Male	Control	20.4±0.9	39.8±4.6	75.4±4.1ab	122.9±5.9ab
	Glycerine	21.9±1.0	38.7±3.2	76.2±9.4ab	121.7±7.6ab
	Glucose	21.9±1.3	37.2±2.3	79.6±5.4a	110.8±11.6bc
Female	Control	20.8±1.0	34.7±3.3	72.0±2.7ab	114.5±6.8ab
	Glycerine	20.5±1.5	37.7±3.2	71.0±6.0b	109.2±6.1bc
	Glucose	21.3±1.2	37.3±4.2	68.2±4.1b	114.0±8.2c
Immediate access	Male	20.9±0.9	37.1±5.4	72.5±5.8b	122.2±7.2a
	Female	19.9±1.1	40.1±3.8	81.6±9.0a	114.8±141.1ab
Delayed access	Male	20.2±1.0	36.2±5.2	68.1±4.0b	112.2±7.7b
	Female	21.6±1.1	36.9±1.2	72.8±2.6b	113.0±7.0b
Immediate access	Control	20.3±8.0	35.1±3.9	71.2±3.8ab	117.8±9.3ab
	Glycerine	20.6±1.5	38.1±4.5	68.0±4.4b	112.3±9.5b
	Glucose	20.9±0.6	36.8±4.4	71.8±7.2ab	121.5±5.6a
Delayed access	Control	17.9±3.8	39.4±2.2	76.2±3.9ab	119.6±5.6ab
	Glycerine	21.8±1.2	38.2±1.3	76.4±6.3ab	118.5±8.4ab
	Glucose	22.4±1.2	37.7±1.7	78.8±5.7a	110.2±9.5b
ANOVA P value					
Gender		P=0.72	P=0.12	P<0.01	P<0.01
Energy Source ^a		P=0.23	P=0.79	P=0.16	P<0.01
Program ^b		P=0.88	P=0.16	P<0.01	P=0.03
Gender * Energy Source		P=0.38	P=0.31	P<0.01	P<0.01
Gender * Program		P=0.82	P=0.19	P<0.01	P<0.01
Energy Source * Program		P=0.49	P=0.47	P<0.01	P<0.01
Gender * Energy Source * Program		P=0.70	P=0.46	P=0.58	P=0.34

96 **Note:** Mean ± SEM on day 7, 14, 24 and 35 of feed access, with different letters (a-b) are significantly different ($P<0.05$).97 ^a Energy source has three levels: control, 8% glycerine and 8% glucose98 ^b Program has two levels: immediate vs. 48 hours delayed feed access.

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99 Table 9. The average daily feed consumption (g day⁻¹) during 1-7, 8-14, 15-24 and 25-35d of feed access, trial 2.

		Growth stage (day of feed access)			
		0-7	8-14	15-24	25-35
Effect					
Male	Control	21.3±2.2	47.8±5.0	109.5±6.1	206.7±21.2
	Glycerine	22.9±2.4	51.4±2.4	112.1±6.0	207.1±12.9
	Glucose	24.2±2.7	51.4±3.2	108.8±6.3	199.8±11.7
Female	Control	20.9±2.0	49.5±3.0	104.2±2.3	203.7±11.2
	Glycerine	21.9±2.9	48.3±3.7	101.9±6.3	185.9±10.0
	Glucose	22.9±1.0	50.6±1.6	104.7±4.6	185.9±13.7
Immediate access	Male	23.3±2.3	51.7±2.4	108.8±4.5	203.0±10.7
	Female	22.4±2.8	48.7±3.9	111.5±4.6	206.1±13.1
Delayed access	Male	21.8±2.0	49.5±2.7	101.2±6.6	185.4±12.3
	Female	22.0±2.3	49.4±4.4	106.9±3.4	198.9±18.1
Immediate access	Control	22.3±2.3	49.5±2.8	105.4±6.4	195.1±12.7b
	Glycerine	22.1±2.2	51.2±3.1	104.2±8.7	192.3±18.4b
	Glucose	23.2±2.2	51.1±2.5	105.3±5.5	191.6±13.4b
Delayed access	Control	19.8±3.3	47.8±5.4	108.3±2.2	215.2±13.0a
	Glycerine	22.9±2.9	48.5±3.4	108.2±5.2	201.3±10.9ab
	Glucose	23.9±1.8	50.8±3.5	109.8±6.1	191.0±12.6b
ANOVA P value					
Gender		P=0.34	P=0.61	P<0.01	P<0.01
Energy Source ^a		P=0.10	P=0.30	P=0.93	P<0.01
Program ^b		P=0.88	P=0.16	P<0.01	P=0.03
Gender * Energy Source		P=0.31	P=0.42	P=0.29	P=0.16
Gender * Program		P=0.67	P=0.48	P=0.40	P=0.16
Energy Source* Program		P=0.28	P=0.61	P=0.65	P=0.03
Gender * Energy Source * Program		P=0.48	P=0.41	P=0.47	P=0.20

100 **Note:** Mean ± SEM on day 7, 14, 24 and 35 of feed access, with different letters (a-b) are significantly different (P<0.05).

101 ^a Energy source has three levels: control, 8% glycerine and 8% glucose

102 ^b Program has two levels: immediate vs. 48 hours delayed feed access.

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103 Table 10. The feed conversion ratio (FCR) during 1-7, 8-14, 15-24 and 25-35d of feed access, trial 2.

		Growth stage (day of feed access)			
		0-7	8-14	15-24	25-35
Effect					
Male	Control	1.11±0.02	1.30±0.14	1.46±0.08	1.63±0.12
	Glycerine	1.07±0.10	1.39±0.14	1.48±0.05	1.68±0.20
	Glucose	1.09±0.04	1.38±0.09	1.38±0.11	1.79±0.30
Female	Control	1.04±0.07	1.44±0.17	1.44±0.10	1.72±0.15
	Glycerine	1.14±0.04	1.30±0.14	1.50±0.05	1.70±0.08
	Glucose	1.08±0.12	1.36±0.14	1.48±0.08	1.64±0.11
Immediate access	Male	1.11±0.10	1.37±0.11	1.43±0.08	1.65±0.16
	Female	1.07±0.04	1.29±0.10	1.37±0.10	1.75±0.14
Delayed access	Male	1.10±0.07	1.37±0.15	1.49±0.10	1.67±0.10
	Female	1.08±0.08	1.36±0.14	1.46±0.05	1.71±0.12
Immediate access	Control	1.10±0.08	1.41±0.12	1.48±0.06	1.68±0.11
	Glycerine	1.12±0.10	1.41±0.17	1.53±0.07	1.71±0.15
	Glucose	1.10±0.10	1.39±0.12	1.47±0.07	1.58±0.11
Delayed access	Control	1.05±0.02	1.33±0.19	1.43±0.11	1.68±0.17
	Glycerine	1.10±0.08	1.35±0.10	1.44±0.07	1.67±0.14
	Glucose	1.08±0.05	1.28±0.11	1.38±0.12	1.84±0.25
ANOVA P value					
Gender		P=0.96	P=0.79	P=0.26	P=0.82
Energy Source ^a		P=0.79	P=0.81	P=0.29	P=0.71
Program ^b		P=0.57	P=0.04	P=0.05	P=0.07
Gender * Energy Source		P=0.75	P=0.21	P=0.37	P=0.15
Gender * Program		P=0.98	P=0.36	P=0.22	P=0.28
Energy Source* Program		P=0.94	P=0.09	P=0.06	P=0.27
Gender * Energy Source * Program		P=0.96	P=0.24	P=0.24	P=0.20

104 **Note:** ^a Energy source has three levels: control, 8% glycerine and 8% glucose105 ^b Program has two levels: immediate vs. 48 hours delayed feed access.

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