THE EFFECT OF SUPPLEMENTAL DIETARY LIPASE AND COPPER ADDITION ON GROWTH PERFORMANCE AND FAT DIGESTIBILITY IN WEANING PIGS

by

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(Under the Direction of Michael J. Azain)

ABSTRACT

The current study conducted two experiments to determine if using lipase, copper or combination of lipase and copper supplement can improve the growth performance and nutrient digestibility of weaning pigs. The first experiment showed that the lipase supplementation cannot improve growth performance but did slightly enhanced fat, N and P digestibility in phase 1 and 3. The second experiment showed that the lipase supplementation showed a trend of improving growth performance, but it decreased fat and N digestibility in phase 1. The pigs treated with a combination of lipase and copper supplement had significantly better growth performance and N and P digestibility, but the fat digestibility was not improved with lipase or lipase and copper supplement. In the study, no consistent result showed that lipase to be beneficial to growth performance or fat digestibility. The effect of copper on improving lipase activity cannot be determined.

INDEX WORDS: Weaning pigs, Exogenous enzyme, Lipase, Copper, Fat digestibility, Growth

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CHAPTER 1

INTRODUCTION

The weaning stage is an important period for pigs. At the weaning stage, the pigs' diet changes from high-fat sow milk to high-carbohydrate dry pellet feed. In addition to the difference in diet nutrients, the weaning stress leads to a significant reduction in endogenous enzymes, which are important in nutrient digestion. Thus, the digestibility of feed nutrients dramatically decreases in the first two weeks after weaning. The fat digestibility of weaning pigs drops from its highest level of 96% (at the farrowing stage) to its lowest level of 65% (at the weaning stage). In order to provide the pigs a smoother transition process, researchers have tried different methods to help the weaning pigs go through these nutritional challenges. Using highly digestible fat for young pigs can improve the weaning pigs' fat digestibility, but opinions have varied on choosing fat sources. The debate on whether fat addition can help weaning pigs' growth performance is still ongoing. Additionally, feed is the largest cost in the pig industry. Production revenue is highly dependent on relative cost and production efficiency. Adding fat into the diet means increasing the feed cost. From an economic standpoint, this is not preferable unless growth performance is improved.

Enzyme supplementation can not only improve the feed's nutritional value and increase the pigs' nutrient digestibility, but also provide a safe and acceptable method for the consumer, as dietary enzymes are mostly extracted from plants or microorganisms. Phytase and carbohydratase are commonly used in animal feed and have become widely accepted in recent

years. To overcome the difference in gaps of fat content between sow milk and weaning diet and to increase the pigs' fat digestibility, lipase might be a good option to fulfill these requirements. However, the information related to lipase use in pig diets is very limited. Lipase has mostly been used to enhance other additive effects instead of being used alone to improve fat digestibility. The effect of using exogenous lipase on pig growth performance or fat digestibility has not yet been confirmed.

Copper is known as an essential trace mineral in animals' diet. The copper requirement is not very high (5-6 ppm) for pigs, but studies have shown that a high concentration of dietary copper can have a positive impact on pigs' growth performance, reproduction, and digestive enzyme activity. After the advantage of dietary copper addition was determined, the interest in studying copper increased. Studies have tested different levels of copper used in the diet and tested dietary copper from different sources. Some scholars have conducted deeper research on the effect of copper on enzyme and feed nutrients. The past research suggested that copper can enhance the pancreatic lipase activity and further improve fat digestibility. Dietary copper may also enhance the exogenous lipase activity. However, the effect of using dietary copper and exogenous lipase in the weaning diet has not been discussed before.

With the potential of weaning pig growth and the desires of markets and industries, the objective of this study was to determine the effect of adding exogenous lipase to the weaning pigs' diet (experiment 1) and if there was an interaction of lipase with growth promoting level of copper (experiment 2) on pigs' growth performance and fat digestibility.

CHAPTER 2

LITERATURE REVIEW

Weaning Stage of the Pigs

In swine production, the production stage is commonly separated into four stages: the gestation stage, the farrowing stage, the weaning (nursery) stage, and the grower/finisher stage. In the farrowing stage, the piglets remain with the sows after birth. The piglets get all of their nutrition needs and passive immunity from the high-fat colostrum and sow milk. For the piglet, this is the suckling period, and it lasts from day 0 after birth till day 21-28. The suckling pigs experience rapid growth in this period (Gill and Tomson,1956). After the suckling period, the piglets are separated from the sows on day 21-28 and begin their weaning adoption process. Piglets naturally wean around 70 days (d) of age (Whittemore and Green 2001), but in industrial pig production, piglets usually wean at 2-4 weeks (wk) of age in order to increase the litter turnover rate and reduce the cost of farrowing in the farms, as the farrowing room is the most expensive part of the swine farm and maximizing the number of litter produced is important for the financial success of the operation. (Dhuyvetter et al. 2014) Because of the early weaning, the pigs undergo weaning stress at the weaning stage (Brooks, 1984, Cera et al. 1988a, Dybkjær, 1992). They need to be separated from the sows, accept piglets from different litters and adapt to acute diet changes and environmental changes. According to Brooks's (1984) observations on water and feed intake post-weaning, some of the pigs did not start to drink water until the second day, and did not start to eat their first meal until 54 hours after weaning. Consequently, low water

consumption and underfeeding easily occur during the weaning stage. The diminishment of feed intake not only limits the growth potential of the piglets but also leads to the significant decrease in villi height in the small intestine and absorptive capacity (Owsley et al. 1986, Cera et al. 1988a, Clarke et al. 1971). At the same time, the activities of enzymes such as amylase, protease, lipase etc. drop dramatically at weaning (Cera et al. 1990, Jensen et al. 1997). All of these factors led to high ratio of diarrhea and increased mortality (NAHMS 2012). Fortunately, after the weaning pigs adapt to the diet and establish regular food intake and normal enzyme activity, pigs in the weaning period can use diets with different energy concentrations as efficiently as pigs in the grower/finisher stage. Thus, the weaning stage is not only a critical period for pigs to smoothly transit to the growth/finisher stage, but also a crucial point for obtaining an advantage in effective production in the pig industry. Effective weaning stage management and an appropriate nutrition program are important to maximize the growth potential during the weaning period and ensure a smoother and faster transition to the growth/finisher stage. (Held and Mendl 2001, Whittemore and Green 2001)

Enzymes Activities in Weaning Pigs

Enzymes play a vital role in the animal digestion process. The physiological development as measured by digestive enzyme activity of the gut is complete when the pigs reach 8 weeks of age (Hartman et al. 1961). This accounts for pigs naturally wean around 70 days of age. However, pigs in the swine industry are weaned at 21-35 days of age, leading to a dramatic drop in their endogenous enzyme activity. (Lindeman et al. 1986, Owsley et al. 1986) This is undoubtedly a big challenge to the swine industry, which requires efficient production and an effective feed conversion ratio.

In Cera et al. (1990) reported that, pancreatic lipase concentration (based on per gram of pancreas) decreased in both the pigs weaned on 21 and on 35 days of age (Figure 2-1). The body weights of the weaning pigs were lighter than those of suckling pigs of the same age. In relationship to age, the time of weaning and the pancreatic enzyme activity have been analyzed by Lindeman et al. (1986). The pigs in that study were weaned at 4 weeks of age. Enzyme determination was conducted at birth and at each week till week 6 of the experimental group. Before weaning, the total activities of chymotrypsin, trypsin, amylase and pancreatic lipase all increased with the increase of the body weight (BW) and pancreas weight. However, 1 week after weaning, the activities of enzymes such as amylase, chymotrypsin and lipase decreased. Even though this activity increased again at 6 and 7 weeks after weaning, some activity, like that of chymotrypsin and pancreatic lipase, increased at a lower rate. A decrease rate of recovery was also observed in the total activities of chymotrypsin, trypsin, amylase, and pancreatic lipase by Jensen et al. (1997). The total activity of amylase, chymotrypsin, carboxyl ester hydrolase and lipase, all experienced large drops at weaning. The enzyme concentration decrease is related to age and the increase of the pancreas size, but it is more highly associated with the sudden diet change and the decrease of fat content in weaner diets comparing to colostrum and milk. The piglets obtain nutrients from the sow milk, which contains 30-40% fat in dry matter basis during the suckling period (de Mann and Bowland 1963), but after weaning, typical commercial diets contain only 3-10% fat (Tokach et al. 1995)

Fat Digestibility in Weaning Pigs

During the suckling period, the apparent fat digestibility of pigs can be as high as 96%. When it comes to weaning, the digestibility is reduced to 65-80% (Cera et al. 1988a, Jensen et al.

1997). This acute change in digestibility is led by two factors. The first is the transition from a high-fat and highly digestible liquid diet of sow milk to the low-fat but high-carbohydrate nursery diet. The second is the low level of lipase activity in weaning pigs. (Cera et al. 1990) The inefficient use of fat strongly influences the growth performance of nursery pigs, including low feed intake, low BW gains and a high diarrhea rate. According to a NAHMS survey (2012), 16.1% of weaned pigs had problems with diarrhea and about 3.3% of weaned pigs died. Numerous research studies have been conducted to determine ways to minimize the effect brought about by low fat digestibility (Frobish et al. 1970, Lawrence and Maxwell 1983, Thaler et al. 1986, Cera et al. 1988b, Cera et al. 1989a, Tokach et al 1995, Jung et al. 2003).

In sow milk, the fat content on dry matter basis is about 30-40% (de Mann and Bowland 1963). When pigs are weaned, the fat content in the typical diet decreases to 3-10%. (Tokach et al. 1995) Dietary fat is crucial for weaning pigs because it is an important energy source for the animals and may reduce the weaning stress created by the acute change. Therefore, the addition of dietary fat is considered as a possible way to overcome the low fat digestibility problem and improve weaning pigs' growth performance. Thaler et al. (1986) investigate different levels of soy oil ranging from 0-5% added into weaning pigs' diets. The results showed that additional soy oil could significantly improve the weaning pigs' average daily gain, average daily feed intake and feed and gain ratio (F:G) at day 35. Adding 3% soy oil into the starter diet resulted in the most significantly improvement in average daily gain (ADG) and average daily feed intake (ADFI). In contrast, added fat in the diet did not benefit the growth performance of weaning pigs in another study (Tokach et al. 1995). An experiment by Lawrence and Maxwell (1983) examined that different levels of choice white grease were added into corn-soybean meal diets. The additional fat had a negative effect on the pigs' growth performance in the first 2 weeks of

the weaning period and no significant effect from 3 to 5 weeks. Adding fat to the nursery diet seems to produce inconsistent results on growth performance. As Lawrence and Maxwell (1983) note, "Fatty acid composition may influence the ability of the early weaned pig to utilize supplemental fat." Hypothetically, a fat source with similar fatty acid composition as the sow milk fat may help the growth performance of weaning pigs (Table 2-1), but since sow fat has relatively high C16:1, it is difficult to achieve this goal with a single source of animal fat or vegetable oil. Research has been conducted to test different kinds of fat sources, and the conclusions are varied. Frobish et al. (1970) tested the effect of using different fat sources on weaning pigs' diet in their study. In one of the experiments, butter, coconut oil, lard and soybean oil were used to substitute 10% of the starch in diets. The pigs on the control diet (without fat substitution) gained the most weight compared to those on other diets. Besides the control diet group, the pigs that were fed lard gained more weight than the pigs that were fed other fat sources, but the fat source effect on growth performance was not significantly different. Controversially, based on other studies' results, the pig growth performance with added lard or tallow was constantly inferior (Lawrence and Maxwell 1983, Cera et al. 1988b, Cera et al. 1989a).

Compared to animal fat, some research supports that vegetable oil, such as corn oil and soybean oil, can better improve pigs' growth performance. A study was conducted in Seoul, South Korea, in 2003 on weaning pigs' diets (Jung et al. 2003). In the study, 5% corn oil, soybean oil, tallow or fish oil were added in the test nursery diets, and growth performance, intestinal morphology and nutrient digestibility results were examined. Jung et al. (2003) found that using corn oil or soybean oil that contained a high proportion of unsaturated long-chain fatty acids can result in higher ADG and better F:G in the week 3 and 4 post-weaning compared to

other fat source. Regarding morphology, the corn oil resulted in higher villus height in the jejunum and ileum of weaned pigs than did soybean oil, tallow oil and fish oil. Additionally, in the metabolic trial result, the apparent digestibility of gross energy and crude fat in the corn oil diet group is significantly higher. Similar conclusions were found in Cera et al.'s study (1988b).

In the other experiment of Lawrence and Maxwell's study (1983), the effect of different sources of fat on neonatal pigs' growth performance was tested. The experiment used butter, corn oil, coconut oil and lard as fat sources in the diets, which contained 32% fat on a dry-matter basis. The results showed that the differences in fatty acid chain lengths between the different fat sources influenced the growth performance. The coconut oil diet group had the highest feed utilization rate, while the corn oil and lard groups' feed utilization rates were significantly lower. (Lawrence and Maxwell 1983) This finding is not limited to a single experiment. Several studies have reported that the rate of hydrolysis of medium-chain triglycerides (MCT) is faster than that of long-chain triglycerides (LCT) (Greenberger et al. 1966, Bach and Babayan 1982, Brady et al. 1982, Odle et al. 1989). Furthermore, medium-chain fatty acids can be utilized faster than long-chain fatty acids, as they are absorbed directly into the bloodstream. Therefore, high MCT might make the fat source more digestible, resulting in animals' better fat utilization. Coconut oil is a representative of good fat source, as it contains more than 60% medium-chain fatty acids. Pigs in the study that were fed the test diets containing coconut oil demonstrated the greatest ADG and ADFI, compared to the diets containing tallow or corn oil. The diets containing tallow and corn oil performed worse than the other two mixture diets. Coconut oil, corn oil, tallow, and mixtures of coconut:tallow, coconut:corn oil and corn oil:tallow (all combinations in 1:1 ratio) were added at rates of 8% to the basal diet to conduct this digestibility study (Cera et al. 1989a).

Despite several studies have demonstrated that fatty acid composition of diet does affect dietary fat utilization and creates a significant growth response in weaning pigs, more research is necessary to better predict the utilization of dietary fat based on fatty acid composition of the fat source and to provide a better fat source combination to use in pig diets.

Exogenous Enzymes Use in Swine Production

In animal digestive processes, the nutrients in the diet cannot be fully utilized due to the anti-nutritional factors in the diet and the shortage of endogenous enzymes that are essential for component break-down. The inefficient use of feed nutrients implies an enormous loss to the farm, because the animal feed is the major cost in the production system. Identifying a means to improve the efficiency of nutrient utilization is one of the biggest challenges to livestock industry. In past decades, feed enzymes have gained attention in the animal nutrition field because using exogenous enzymes can help to improve the efficiency of feed digestion, reduce anti-nutritional factors, improve the absorption of vitamins and minerals (Corring et al. 1978, Cera et al. 1988a, Maenz and Classen 1998, Barletta 2000, Bhat and Hazlewood 2001) and better maintain gut health (Dierick et al. 2002a, Park et al. 2017). The reasons for the rising interest in feed enzymes are not limited to these factors but are also driven by environmental and consumers' demands. "Since the animal better utilizes the feed, less is excreted. This results in manure volume being reduced by up to 20% and nitrogen excretion by up to 15% in pigs and 20% in poultry" (Sheppy 2001). Manure is considered a fertilizer that improves the quality of the soil, but once manure production exceeds the capacity of the environment, manure is also a source of environmental pollution (Sheppy 2001). Specifically, with the rapidly increasing demand for meat and animal production, manure becomes more likely a concern for the environment (Sheppy 2001). Nitrogen

is also an environmental concern, as it is a source of air and water pollution. Hence, decreasing animal waste using enzymes is an environmental-friendly approach. For customer demands and market requests, according to the United Nations report published in June 2017, the current world population of 7.6 billion is expected to reach 8.6 billion in 2030 (United Nations Department of Economic and Social Affairs/Population Division World Population Prospects, 2017). On the one hand, the growing population is pushing for greater efficiency in livestock production with lower production costs. On the other hand, consumers have a growing awareness of the health and well-being of livestock. Feed enzymes that are produced by microorganisms or plants are more acceptable to the consumers as feed additives.

Exploration of exogenous enzymes started in the 1970s. Phytases were used in aiding phytic acid breakdown; ß-glucanase and xylanases were used in fiber digestion; and protease and lipase were used to improve the digestibility of protein and lipid (Corring et al. 1978, Cera et al. 1988a, Maenz and Classen 1998, Barletta 2000, Bhat and Hazlewood 2001). Feed enzymes are widely used in both swin and poultry diets. The use of phytases began commercially in swine nutrition in the early 1990s. Many studies have confirmed that phytase can improve the utilization of P and decrease the need for P supplementation in the pig diet (Rapp et al. 2001, Augspurger et al. 2003, Adeola et al. 2004). However, the performance of phytases in animals is highly related to the source of the enzymes (Schwarz et al. 2015). Phytase from plant sources is sensitive to high temperatures, so the positive effect of phytase is significantly reduced after pelleting (Jongbloed and Kemme 1990, Schwarz et al. 2015). Moreover, different microbial phytases have different effects on pigs due to differences in their structure or kinetic characteristics (Augspurger et al. 2003). In addition to phytases, the response to using other exogenous enzymes in the diet also varies. Carbohydrases like ß-glucanase and xylanases are

commercially used in poultry diets but are not widely used in the pig feed industry, as the effect of carbohydrases has not been confirmed in pigs. Some studies showed improved growth performance while others showed no effect (Svihus 2000, Barrera et al. 2007, Woyengo et al. 2008, Adeola and Cowieson 2011). Xylanase addition to the growing pigs' diet did not have a significant effect on body weight gain (Woyengo et al. 2008). Conversely, Barrera et al.'s study (2007) showed a 15% improvement in body weight gain for weaning pigs using a xylanase supplement. Nevertheless, researchers still hold contrasting views about the results, which may be due, but are not limited, to the following: the animal growing stage, the type of grains in the feed (Adeola and Cowieson 2011), and the digestive system (Svihus 2000). The digesta retention time in pigs is much longer than for poultry (32-85h vs. 4-8h) (Freire et al. 2000, Svihus 2000, Partanen et al. 2007), and this difference may can help to maximize the enzymes' activity potential in pigs. However, the pH environment in the stomach and small intestine of pigs are not suitable for enzymes. Additionally, pigs are less sensitive to soluble fibers' anti-nutritional effect, so the addition of ß-glucanase and xylanases in swine diets may not provide meaningful improvement in pig growth performance (Svihus 2000). Carbohyrases and phytases occupy more than 90% of the enzyme market (Adeola and Cowieson 2011). However, protease and lipase are not widely used in the current feed industry. The research history on protease and lipase is not as long as that of phytase, and the research results are very limited. Protease treatment was used in the weaning pigs fed a soybean meal diet (Caine et al. 1997). There was no significant improvement in growth performance or on the ileal digestibility of DM, CP or amino acids. However, Park et al. (2017) concluded that, with the addition of a microbial protease enzyme, pigs had higher ADG, G:F, villus height and villus height to crypt depth ratio. The protease treatment group also had low frequency of diarrhea. For lipase, Marzooqi and Leeson(1999)

showed that exogenous lipase can help to improve poultry growth performance and fat digestibility. Dietary lipase for pigs is often combined with other enzymes or other feed additives. A combination of xylanase and phospholipase increased the ileal digestibility of different nutrients and energy in weaning pigs (Diebold et al. 2004). In a study by Prykhodko et al. (2016), pigs treated with a combination of microbial amylase, protease, and lipase resulting the pre-weaning period had 17% higher ADG and reached slaughter weight earlier relative to the control group. Some pigs were treated with the enzyme treatment for two weeks after weaning at 35 days of age. The enzyme supplement did not affect the growth performance of this group of pigs but did show a tendency to increase body weight gain at 18 weeks post-weaning. This study result agreed with a similar study from Kim et al (2005)., which provided bacteria that produced active dietary enzymes for pigs and promoted growth performance. Dierick et al. (2002a, 2002b, 2003, 2004) have a series of studies associated with lipase addition in weaning pigs' diet. A study in 2002 showed that weaning pigs with microbial lipase and medium-chain fatty acids in their diet had the best feed conversion ratio. The lipase and added fatty acids significantly reduced gastric and duodenal luminal flora and were considered as potential antibiotics (Dierick et al. 2002a). Another study showed similar results using ingredients high in medium-chain fatty acids, cuphea seeds and microbial lipase in the pigs' diets (Dierick et al. 2003). Interestingly, in another study in 2002, lipase addition increased the lipolysis in the feed during storage and created side effects in diet digestion in weaning pigs (Dierick and Decupere. 2002b). In a more recent study in 2004, exogenous lipase was tested with or without an emulsifier in the weaning pigs' diets to observe the effect on digestibility of fat or other nutrients. Unexpectedly, with the lipase addition, fat digestibility was not affected, but the apparent ileal digestibility (AID) of dry matter (DM) and energy and the apparent fecal (AFD) digestibility improved significantly

(Dierick and Decuypere 2004). Lipase was used to aid nutrient digestibility and digestion tract health, but it did not prove to have any significant effect on pigs' growth performance. On the other hand, Liu et al. (2010) showed that the addition of lipase can improve weaning pigs' growth performance and fat digestibility using a method of adding gene-modified cell cultures that can produce recombinant porcine lipase in weaning pig diets. In this study, the porcine gene was introduced into Pichia pastoris (P. pastoris). The transformed P. pastoris cell cultures were added into test groups' diets with 5,000 and 10,000 U/kg of recombinant lipase. There was no difference in growth performance between the 5,000 and 10,000 U/kg groups, but the pigs' BW, ADG, ADFI in test groups were significantly higher than the control group's. In fat digestibility, the pigs with 10,000 U/kg supplementation had better fat digestibility than the others. After Liu et al study, there was no other study using lipase as the single feed additive in swine diets, and had a similar conclusion. The current research data on protease and lipase is very limited. Therefore, a meaningful conclusion cannot be reached at this current stage. The dramatic change of endogenous enzymes of weaning pigs is evident (Lindeman et al., 1986), so the addition of protease or lipase is still considered as a potential way to improve pigs' growth performance and development of the digestive system. In the current feed market, exogenous enzyme products are used in weaning pigs' diet to improve pigs' health and ensure the effective growth. With more advanced research, the potential value of protease and lipase additions may be discovered and exogenous enzymes will provide more benefits to weaning pigs.

Dietary Copper

Copper (Cu) is an essential trace mineral for pig growth, reproduction and development of the immune system. The NRC Cu requirement is relatively low, about 5-6ppm (NRC, 2011).

Because of the low Cu requirement for maintaining the biological function of animals, it is difficult to induce copper deficiency in pigs with balanced diets. However, dietary copper addition to swine diet is a common nutritional strategy to overcome the shortage of copper in corn and soybean meal diets, as well as to promote reproduction and pig growth (Bunch et al. 1965, Braude 1967, Lillie and Forbish 1978, Mahan 1990, Cromwell 1993, Davis 2002, Yen et al. 2005, Mei 2010, Bikker et al. 2016). Previous studies have shown that feeding a higher concentration of dietary Cu (60ppm) to sows can improve reproduction in many ways, including shortening the weaning-to-estrus interval, increasing the successful breeding rate and improving the piglets' birth and weaning weights (Lillie and Forbish 1978, Mahan 1990, Cromwell 1993, Yen et al. 2005). Moreover, studies have discovered that providing Cu supplementation to weaning pigs can improve gut health in a role similar to that of antibiotics (Stahly et al. 1981), promote growth (Bunch et al. 1965, Braude 1967, Davis 2002, Mei 2010, Bikker et al. 2016), and improve fat digestibility (Dove and Haydon 1992, Dove 1993, Dove 1995).

Although providing Cu supplements to pigs can improve their performance, concerns about potential toxicity caused by high levels of copper supplementation have been raised. Toxicity might affect absorption, transportation and tissue storage of other trace minerals. Thus, different levels of dietary copper supplementation in pigs' diet have been tested in previous studies (Bunch et al. 1965, Roof and Mahan 1982, Dove 1995). Roof and Mahan (1982) compared groups of weaning pigs with different levels of Cu (0, 125, 250, 375, 500 ppm) supplementation for 5 weeks. Pigs that were fed with 250 ppm Cu had significant improvements in daily gain and feed intake. However, the growth performance of the 375-ppm group remained the same as that of the control group (0 ppm), and the pigs that were fed the 500-ppm Cu supplementation had a decrease in daily gain and feed intake (1982). A similar result was

obtained in a study by Bunch et al. (1965). With regard to nutrient digestibility, compared to other levels of Cu supplementation, Dove (1995) showed that the addition of 250 ppm copper to the diet can better improve fat digestibility, with or without fat addition (5%). Mei et al. (2010) showed that supplying 175 or 250 ppm Cu to weaning pigs improved the digestibility of nutrients, including fat, crude protein, calcium and phosphorus, as well as reduced lactobacilli in cecum. In sows, copper supplementation levels of 15, 30 and 60 ppm were tested, and the 60 ppm Cu group had the greatest improvement in piglets' birth weight (Lillie and Forbish 1978). In a latter study (Cromwell et al. 1993), 250 ppm Cu was tested and found to have a positive effect on litter size and pig's birth weight and weaning weight. There was no negative effect on sows or piglets (Cromwell et al. 1993). Based on these study results, 250 ppm Cu can provide consistent benefit to pigs (Cromwell et al. 1998, Dove 195, Mei et al. 2010), but copper toxicity effect may occur when dietary levels exceed 375 ppm (Roof and Mahan 1982).

Different sources of copper have also been tested in previous studies. Early in the 1960s, pigs were tested with 250 ppm Cu supplementation from copper sulfate, copper carbonate and copper-methionine (Bunch et al. 1965). The study indicated that all three sources of Cu significantly improved the ADG and the G:F when compared to the control diet. Among the three sources, the copper-methionine and copper sulfate groups had better G:F than the copper carbonate group. Furthermore, Cromwell et al. (1989) showed that Cu from copper sulfate can provide a consistently positive effect on pigs' growth performance. Accordingly, Cu from copper sulfate was more regularly used in experiments than other copper sources. However, numerous tests were conducted later to test different sources of Cu on pigs' performance and compare them with the Cu from copper sulfate. Cromwell et al. (1998) compared the effect of supplementing Cu from tri-basic copper chloride (TBCC) and copper sulfate, discovering that TBCC and copper

sulfate had a similar effect on weanling pigs. Shelton et al. (2011) also discovered that Cu from TBCC and copper sulfate can both improve pigs' growth performance, but copper sulfate had a marginally better influence than TBCC. One of their experiments showed that copper sulfate improved the ADG, ADFI, G:F and final BW of the pigs, while TBCC only improved the G:F in overall performance. Furthermore, chelated Cu sources have also been tested in many studies, as chelation can improve animals' trace mineral utilization. Zoubek et al. (1975) indicated that EDTA chelated Cu or the addition of EDTA with Cu did not enhance the efficiency of Cu uptake in pigs, but these results might be explained by the random collection times of blood samples. The blood Cu concentration was varied either directly after meals or directly before meals (Zoubek et al. 1975). In the past decade, different sources of chelated Cu have been tested in numerous studies, including Cu-lysine complex (Cu-Lys), cupric citrate (Cu-Cit), Cu-methionine (Cu-Met), etc. (Stansbury et al. 1990, Coffey et al. 1994, Armstrong et al. 2004, Huang et al. 2010, Ma et al. 2015, Davin et al. 2016). Most of the results showed that the copper sulfate and chelated Cu had similar positive impacts on growth performance (Stansbury et al. 1990, Armstrong et al. 2004, Huang et al. 2010, Davin et al. 2016). Interestingly, a Cu-Met complex was compared with copper sulfate in the Davin et al. study (2016) and the Huang et al. study (2010). Besides the positive effect on growth performance, the Cu-Met complex can improve the P and Ca concentrations in bone (Davin et al. 2016) and lower Cu concentration in feces (Huang et al. 2010). These findings are not limited to these two studies. Armstrong et al. (2004) and Ma et al. (2014) also showed that chelated Cu decreased fecal Cu excretion. These studies suggest that chelated Cu can be better absorbed or retained by pigs and can substitute for copper sulfate as a dietary copper source.

Dietary Copper and Enzymes

Copper (Cu) is a vital component in many enzymes involved the oxidation reaction. Copper is not only an essential component of digestive enzymes but also a vital cofactor in stimulating or suppressing some enzymes in the digestion process.

Dove conducted a series of studies investigating Cu and dietary fat digestion. In his early studies, the addition of 250 ppm dietary copper and dietary fat was found to promote pigs' growth and fat digestion (Dove and Haydon 1992, Dove 1993). He hypothesized that the growth promoting effect was caused by the improved enzyme activity, so his later study in 1996 tested the effect of copper on the digestive enzymes. The results of the animal experiment indicated that supplementing 250 ppm Cu to weaning pigs can significantly improve the activities of the small intestine lipase and phospholipase A. The lipase and phospholipase are highly associated with nutrient digestion. Even though there was no significant increase in pancreatic enzyme activity in the animal experiment, the in vitro assay result in his study indicated that Cu can stimulate pancreatic lipase activity (Luo and Dove, 1996). Thus, dietary copper has a positive impact on enzyme activity, which further promotes nutrient utilization and improves growth performance. In contrast to Luo and Dove's result, pancreatic or intestinal enzyme activity was not affected by dietary Cu in a study by Hedemann et al. (2006). The concentrations of the Cu in that study were 0 ppm and 175 ppm. The lack of response might have been due to an insufficient amount of Cu supplementation (Hedemann et al. 2006). More investigations are needed to determine the effect of different levels of copper on pigs' enzyme activity.

Implications

From an economic standpoint, improving the growth performance of weaning pigs, as well as having a more cost-efficient nursery diet, is critical for the pig industry. Even though the methods to improve diet formulations or pigs' performance are varied, using exogenous enzymes from microorganisms or plants is widely accepted by consumers. To overcome the insufficient excretion of endogenous enzymes in weaning pigs, enzyme additives are efficient and feasible means to improve digestibility and growth performance. Interest in dietary enzyme research has increased, particularly for phytase and carbohydrases. Although there is no direct evidence showing that lipase can significantly improve weaning pigs' growth performance and fat digestibility, based on the previous lipase research in poultry and studies on combining lipase with other additives, there is a reason to be optimistic regarding the effect of lipase on weaning pigs. Copper is a more traditional additive in pigs' diets, but the effect of copper on exogenous enzymes and the effect of combining copper and enzymes on weaning pigs are not well understood. Therefore, research on weaning pigs combining dietary lipase and dietary copper in weaning pigs' diet is needed.

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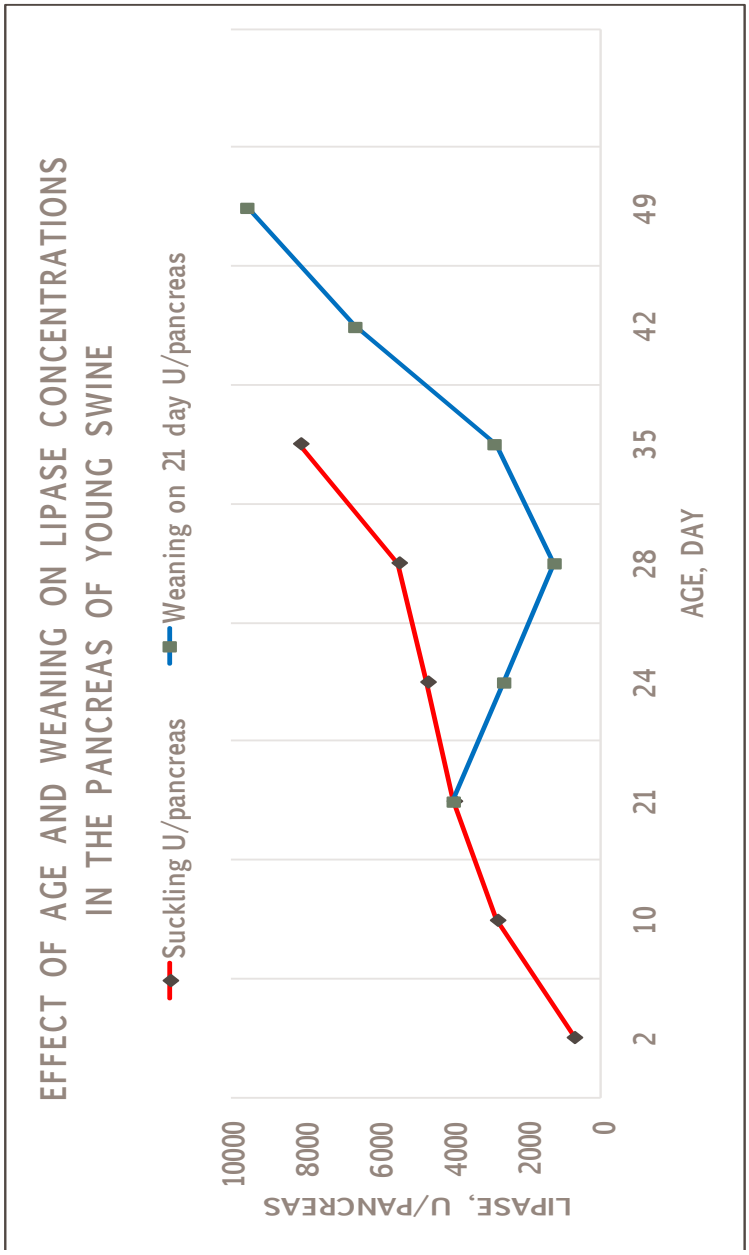

Adapted from Cera et al., 1990 data

Figure2-1. Effect of Age and Weaning on Lipase Concentration in the Pancreas of Young Swine

Table2-1. The Composition of Sow Milk Fat

	Sow Milk Fat	Fatty Acid (%)
Saturated	Caprinic (C10:0)	0.78
	Lauric (C12:0)	1.93
	Myrisitic (C14:0)	4.83
	Palmitic (C16:0)	30.85
	Stearic (C18:0)	5.26
	Arachidic (C20:0)	0.15
Unsaturated	Palmitoleic (C16:1)	8.98
	Oleic (C18:1)	33.15
	Linoleic (C18:2)	12.87
	Linolenic (C18:3)	1.21

Adapted from Mountzouris et al., 1999.

CHAPTER 3

THE EFFECT OF LIPASE ADDITION TO WEANING PIG DIET ON GROWTH PERFORMANCE AND FAT DIGESTIBILITY OF WEANING PIGS

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Abstract

An experiment was conducted to determine the effect of the addition of exogenous lipase on fat digestibility and growth performance in weaning pigs. A total of 100 pigs (initial weight = 6.00± 0.10 kg) were used in the experiment and fed with corn and soybean meal based diets. The pigs were randomly assigned to four treatments: positive control (PC), negative control (NC, lower fat and lower energy contents, no lipase), negative control+ 500 U/ kg lipase (NC+X), and negative control+ 1000 U/kg lipase (NC+2X). They were fed with phase 1 diet from day0-10 post-weaning and were switched to phase 2 diet (d7-21) and phase 3 diet (d21-35). During the experimental period, there was no effect of diet on pigs' body weight(BW), average daily gain(ADG), average daily feed intake(ADFI), or gain: feed ratio (G:F) ($P>0.05$) expect ADG in phase 3 and G:F in phase 1. However, NC+2X increased fat digestibility (61.50%) and phosphorous (P) digestibility (43.46%) in phase 1 and fat, nitrogen (N) and P digestibility in phase 3 (56.87%, 82.08% and 44.43%, respectively) compared to those of the NC group.

The results indicated that exogenous lipase addition might be able to increase digestibility of fat, nitrogen and phosphorus. However, there was no significant difference in growth performance between PC and NC, so the lipase effect on pigs' growth performance cannot be determined in this study. The effect of different level of lipase addition on growth performance and nutrient digestibility was not consistent, the optimal level of lipase added to the diet cannot be determined from this study.

Introduction

During the suckling period of the piglets, the piglets obtain most of the nutrients from the sow milk. The fat content in sow milk is high and the fat digestion of the piglets is very efficient.

However, the early weaning time in pig industries influences the natural process of adapting to the diet change, morphology change in gastrointestinal system and development of different digestive enzymes for piglets (Whittemore and Green 2001). As a result, the piglets have low feed intake and cannot efficiently use the fat in the weaning diet and they undergo different levels of diarrhea or growth depression. To avoid the loss caused by the inefficient fat digestion, different methods have been used to improve the health status of the piglets and maximize the potential of growth at the weaning stage, including but not limited to antibiotics use, dietary fat addition, substitution of different fat sources and exogenous enzyme addition. Studies show that supplementing fat to weaning pigs is not as effective as expected (Frobish et al. 1970, Leibbrandt et al. 1975, Lawrence and Maxwell 1983) and highly depends on the sources of fat (Lawrence and Maxwell, 1983, Jung et al. 2003), the chemicals structure of the fat (Bach and Babayan, 1982; Brady et al., 1982, Cera et al. 1989, Powles et al. 1994), the age of the pigs (Leibbrandt et al. 1975) and other factors. Therefore, fat addition to the pigs' diet is still under debate.

In past decades, exogenous enzymes have been widely used in swine diets in order to improve the digestibility of feed nutrients. For example, phytase had been used use in swine diet since the early 1990s. Numerous studies have proven that phytase can improve the utilization of phosphorus (P) and decrease the need of P supplementation in the pig diet. (Rapp et al. 2001, Augspurger et al. 2003, Adeola et al. 2004) When considering fat digestibility, lipase is the enzyme that helps pigs better utilize the fat in dry nursery feed, but the concentration and activity of lipase in weaning pigs decrease dramatically at weaning due to the acute diet change (Lindeman et al. 1986, Cera et al. 1989, Jensen et al. 1997). Supplementing exogenous lipase comes up as a possible solution. In previous studies, lipase has been used with other enzymes (Prykhodko et al. 2016) or other feed additives (emulsifier) as a possible substitute to antibiotics

(Dierick et al. 2002a), but it has rarely been used by itself to improve fat digestibility. In a study by Liu's et al. (2010), genetic transformed *P. pastoris* cell culture that can produce lipase inside the pig digestive tract was added into the weaning pigs diet. It demonstrated that lipase can help to improve the fat digestibility and promote growth. However, the information regarding the effect of lipase on the fat digestibility and growth performance of pig is still very limited. Therefore, this study was conducted to determine the effect of exogenous lipase supplementation in weaning diet on pigs' fat digestibility and growth performance.

Materials and Methods

Animal Handling and Data Collection

The experimental protocols used in this study were approved by the Animal Care and Use Committee of the University of Georgia (UGA) (UGA Animal Care and Use, A2015 01-007-Y2-A0)

A total of 100 healthy crossbred pigs (PIC C 29 female x True Choice EBX or EB5 male) from the University of Georgia (UGA) Swine Center were randomly selected to use in 2 trials. Pigs in this experiment were 6 ± 0.1 kg at weaning, and the growth weight mostly meets the phases outlined in the 2012 NRC. The pigs were weaned at day 21 after birth and allotted into four diet treatment groups. They were housed in an environmentally controlled room with the temperature at 25-27 degrees Celsius and 24-hour daylight. In each trial, pigs were placed into 16 pens based on sex, weight and litter number. Each pen was 0.94m wide and 1.83m long and had 3-4 pigs; 4 pens were allotted per diet treatment. Four dietary treatments(Table3-1) were: 1) a positive control meeting all the NRC requirements for this age of pig, 2) a negative control diet with lower energy density, and two test diets with two levels of lipase addition in negative

control diets, 3) 500U/kg or 4) 1000U/kg given to the pigs. Pigs were fed ad libitum. The dietary treatments were formulated bases on the 3 phases outlined for weight and age in the 2012 NRC and met or exceeded all the 2012 NRC nutrient requirements, except that the negative controls were 100 kcal/kg below the stated energy requirements. Phase 1 diets were fed from day 0-10 post-weaning. On day 10 post-weaning, pigs were switched to phase 2 diets. On day 21, pigs were switched to feed phase 3 diets from day 21-35. At the end of study, the pigs returned to the UGA Swine Center. For fat digestibility analysis, 0.3% TiO2 was added to diets as digestibility marker to determine nutrient digestibility in pigs. Diets were produced at the UGA Poultry Science Department Feed Mill and were fed in pellet form. The composition of the diets is shown in Table 3-2.

Pigs were weighed on days 0, 10, 21 and 35 post-weaning. At the same time, feed intake was monitored at each weigh-in day. Diet samples were collected at each phase and stored at 4°C for further determination of nutrient profile. At the last 3 days of each phase, fecal samples from each pen were collected twice daily and stored at -20°C in the freezer. At the end of the study, the frozen fecal samples were dried in the Grieve Shelf oven SA-350 (The Grieve Cooperation, Round Lake, IL) at 60°C for a week. The dried fecal samples were ground into 2mm powder in the Wiley Mill (Thomas Scientific, Swedesboro, NJ) and then stored at room temperature for further analysis.

Sample Analysis

Feed samples were analyzed in triplicate for gross energy concentration (GE), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF), ether extract (EE), titanium and phosphorus content. Fecal samples were analyzed in duplicate for CP, ADF, NDF, EE, titanium and phosphorus content in the Animal Science Department lab. Gross energy

concentration in diets was examined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). The Leco FP-628 Nitrogen Analyzer was used to analyze crude protein in the diet and fecal samples (Leco Corporation, St. Joseph, MI). NDF and ADF were analyzed using Ankom A200 and A2000 fiber analyzers (Ankom Technology, Macedon, NY) with NDF Method Method 13 and ADF Method Method 12, respectively. Ether exact content was determined using an ANKOM XT15 Extractor (Labconco CO, Kansas City, MI) with AOCS Official Procedure Am 5-04.

For the mineral assay, diet and fecal samples were ashed for 6h at 550 °C in a Fisher Isotemp Muffle Furnace (Fisher Scientific, Suwanee, GA). After the sample cooled to room temperature, 10 ml sulfuric acid was added to each sample and heated for 1 h. Then, the solutions were diluted with distilled water into the total volume of 50 ml. After sitting for one night, the solutions were centrifuged and used for TiO2 (Short et al. 1996, Titgemeyer et al. 2001) and phosphorous (Herbert et al. 1971, Short et al. 1996) concentration assessment using a UV spectrophotometer, measuring the absorbance at 410 nm (TiO2) and 660 nm (Phosphorous).

The apparent total digestibility (ATTD) of nutrient was calculated with the following equation:

$$\mathbf{AATD} = \mathbf{1} - \frac{[\mathbf{Marker}]_{\mathbf{feed}}}{[\mathbf{Nutrient}]_{\mathbf{feed}}} \times \frac{[\mathbf{Nutrient}]_{\mathbf{feces}}}{[\mathbf{Marker}]_{\mathbf{feces}}}$$

Statistical Analysis

All data were analyzed using the PROC GLM procedure in SAS (SAS 9.4, SAS Institute, Inc., Cary, NC) to evaluate the effect of diet treatment (4 treatments) and trial (2 trials), diet treatment × trial interaction on body weight (BW), average daily gain (ADG), average daily feed intake (ADF) and gain and feed ratio (G:F) and digestibility of fat, nitrogen and phosphorous.

The pen was considered as the experimental unit. The results are presented as least squares means (LS Means) for the diet treatment effect, with significance at $P<0.05$ and trends at $P<0.20$. The differences between NC and lipase treatment groups were determined using a simple contrast.

Results

Diet Composition Analysis

The analyzed composition of diets for phase 1-3 is listed in Table 3-3 and Figure3-1.

Diets were formulated based on the requirements of NRC (NRC, 2012) and the special requirements that were needed to conduct the experiment. PC diets in all phases have higher energy concentration than other diets. Different from expectation, instead of having same energy concentration in phase1 and phase2 diet and lower energy concentration in phase 3 diet, the energy concentrations in phase 2 and 3 are similar while phase 1 diet energy concentration is about 2% higher than phase 2's, except for NC in phase 3. The energy difference between PC and NC diets was shown in the analyzed result, Other components such as crude fat, crude protein, fiber (NDF and ADF), etc., were similar to the expected values.

Growth Performance

The results of growth performances are shown in Table 3-4, Figure 3-2 and 3-3.

Generally, there were no significant main effects of the trial or the diet x trial. The diet with lipase in either level did not have any significant effect on BW(kg), ADG (PC, NC, NC+X, and NC+2X: 445, 443, 429 and 441g/day respectively), ADFI (PC, NC, NC+X, and NC+2X: 625, 644, 616 and 647 g/day respectively) or G:F (PC, NC, NC+X, and NC+2X: 0.69, 0.71, 0.69 and 0.70 respectively). The negative control in the experiment had similar or even better

performance than the positive control (BW: 20.65 vs. 21.48, ADG: 445 vs. 443, ADFI: 625 vs. 644, G: F: 0.71 vs. 0.69; P>0.20).

In Phase 1 (day0-10 post-weaning), there was no significant effect of diet on BW, ADG, ADFI, but the pigs in NC+ 2X diet had the highest BW(8.47kg), ADG(234g/day), and ADFI(255g/day). The growth performance of NC was better than PC but it was not significant.

In phase 2 (day11-21 post-weaning), there was no significant effect of diet on pigs' growth performance including BW, ADG, ADFI and G:F, but the pigs in the NC+ 2X diet had the highest BW (17.58kg), ADG (502g/day), and highest ADFI (595g/day) and the NC+1X diet had the highest G: F (0.87). The pigs in NC +2X had a slightly better growth performance than those in the PC and NC.

In phase 3 (day22-35), there was no significant effect of diet on pigs' BW, ADG, ADFI or G:F. The highest ADG (687 g/day), ADFI (1012g/day) and G: F (0.69) were all from the NC diet group. The NC+2X group had the highest BW, but it was just slightly higher than that of the NC group.

Nutrient Digestibility

The results of nutrient digestibility are shown in the Table 3-5, Figure 3-4, 3-5, 3-6 and 3-7.

In phase 1 (day0-10), the effect of diets on fat digestibility and the P digestibility was significant. The pigs in the positive control group had the highest fat digestibility (68.65% vs. 53.11, 59.09 and 61.50%), and NC+2X had the second-highest fat digestibility, at 61.50%, but it was not significantly different from the other two. For P digestibility, the PC and NC+2X had the highest digestibility (43.79 and 43.46%), while NC+X had significantly lower digestibility

(32.37%). When it comes to N digestibility, the NC+2X had the highest number (78.69 vs. 75.49,76.94 and 74.77%), but the result was not significant (P>0.2).

In phase 2 (day11-21), the effect of diets on fat digestibility (P=0.005) and N digestibility (P=0.002) were both significant. The highest fat digestibility was PC group (69.92%), and the second was the NC group (62.94%) and followed by the NC+X (61.98%). NC +2X (52.09) was significantly lower than other groups. The NC group also had the highest N digestibility, at 77.64%, but the PC group had the lowest N digestibility (71.88%). Different from the phase 1, the fat, N and P digestibility of the NC+X group (61.98, 77.29 and 35.21% respectively) were all better than those of the NC+2X group (52.09, 74.90 and 30.79% respectively).

In phase 3 (day22-35), the effect of diets on fat digestibility (P=0.001) N digestibility (P=0.04) and P digestibility (P<0.0001) were all significant. The fat and P digestibility in the PC group were significantly higher than in the other three groups (fat: 73.76 vs. 55.99, 45.37, and 56.87%, P: 54.22 vs. 32.76, 36.21, and 44.43%). For the N digestibility, the pigs on NC +2X diet had the highest N digestibility (82.08 vs. 80.85, 79.35, and 78.74%), but it was not significant.

Discussion

In contrast to the highly significant positive results in the study of Liu et al. (2010), the lipase addition did not bring any significant benefit to the growth performance of the weaning pigs in the current study (Table 3-4). When comparing the PC and NC groups, there was no significant (P<0.05) effect of the diets on growth performance. Based on the experimental design, 100 kcal/kg ME gap and fat content difference between the PC and NC group were expected to show a difference in growth performance of the weaning pigs and help further observe the effect of supplementing lipase to weaning pigs. As shown in figure 3-2, the pigs in NC group had

higher BW than the PC group in phase 1, 2 and 3 (8.21, 17.18, 21.48 vs. 7.88, 16.83, 20.65 kg), and higher ADG and G:F than PC group in phase 1 and 3 (ADG: 225, 687 vs. 196, 621 g/day, $0.05<P<0.1$; G:F: 0.80, 0.64 vs. 0.92, 0.68, $0.05<P<0.2$). In overall, ADG, ADFI and G:F result in PC group and NC group were almost the same. The lower energy content in the NC diets were confirmed in the diet analysis, but the pigs on the NC diet did not preform as expected. The hypothesis of this result is that with only 100kcal/kg ME gap between PC and NC group, the pigs in the NC group might be still getting enough energy to grow and even use the diet more efficient than the PC group. The energy levels setting of PC and NC in the current study affected the observation of effect of lipase on growth performance. However, aside from the unexpected results from PC and NC diet, when only comparing the growth performance of NC and NC+2X (figure 3-3), the NC +2X had slightly better BW in phase 1 to 3, ADG, ADFI in phase 1 and 2, G:F in phase 2. Therefore, the lipase supplement still has potential to improve the growth performance of the weaning pigs.

The inconsistent growth performances of NC+X and NC+2X, compared to NC were noticed. This might be due to the side effect of lipase supplementation on diets. Derrick and Decuypere conducted a study about endogenous lipolysis in feedstuffs in 2002 (Dierick and Decuypere 2002b). The study found that: with exogenous lipase, lipolysis in the diets was more pronounced than those without. Lipolysis leads to higher Free fatty acids (FFAs) in the diet and further affect the growth performance of the animals as FFAs are cytotoxic to intestinal cells. (Penn et al. 2014) When looking into the growth performance of NC+X group, NC+X group had a slightly lower BW, ADG and ADFI and G:F in phase1 and 3, compared to NC group. NC+X group also had lower nutrient digestibility than the NC group, except for the P digestibility in phase 3. The side effect from the exogenous lipase was shown on the pigs' growth performance

of the NC+X group. However, when the lipase addition level is high enough, the side effect might be neutralized by the stronger positive effect from increasing the nutrient digestibility. This might be an explanation for the marginally better growth performance and nutrient digestibility in NC+2X group. With the combination of the side effect and the positive effect of lipase, the lipase addition in this current study did not show a consistent impact on growth performance and the benefit on the growth performance of weaning pigs cannot be confirmed.

When it comes to nutrient digestibility, the PC diet had significantly higher fat digestibility than the other diet groups in phase 1 and 3($P<0.05$). Cera et al. (1990) and Jensen et al. (1997) indicated that fat content in diet affect the activities of the weaning pigs' endogenous lipase and further affect the fat digestibility in weaning pigs. Thus, the reason for the high fat digestibility in PC group might be the high fat content in the PC diet (Figure 3-1). The NC+2X had the second highest fat digestibility in phase 1 and 3 (The reason for the high fat digestibility of NC and NC+X in phase 2 was not clear.), even though it was not significantly different than that of the NC group. Based on this result, lipase supplementation has potential to increase fat digestibility, but the fat digestibility of the PC diet was too high, thus, the lower fat diet groups with the added lipase (NC+2X) cannot achieve the same fat digestibility as the PC diet group. Furthermore, in the study of Dierick and Decuypere. (2004), they pointed out that the potential effect of lipase on improving fat digestibility was weakened by the high unsaturated to saturated fatty acids ratio(U/S) and the rather high initial digestibility value of the fat source. Compared to other fat sources, soybean oil, a long-chain highly unsaturated fat source with 8564kcal/kg ME, is highly digestible to weaning pigs. Therefore, the soybean oil in the current study might weaken the potential lipase effect, and its high digestibility left very little room for the exogenous lipase to improve the fat digestibility.

Interestingly, aside from the phase 2 result, the N digestibility of the NC+2X group in phase 1 and phase 3 (P=0.04) was higher than that of the PC group (figure 3-4 and 3-6). This was not the first time finding out that lipase improved other nonfat nutrient digestibility. In the past studies, lipase addition also affected the apparent ileal digestibility (AID) of dry matter (DM), protein and energy (Bontempo et al. 1994, Dierick and Decuypere 2004) However, the reason for that is still undefined. For phase 2, the nutrient digestibility of NC+X and NC+2X were even lower than the NC group (figure 3-5). The reason for this was not clear. It might be the side effect from lipase that was mentioned, or lipase supplementation is counter-productive to the fat digestibility. Similar situations also occurred in past studies, the emulsify agent -- lecithin in Frobish et al. study (1969) and the exogenous lipase and emulsifier in Derrick and Decuypere's study (2004) both decreased the fat utilization in weaning pigs, as the fat source itself already had very high digestibility. The nutrient digestibility in current study was not consistent throughout the experiment period, the possible reasons above cannot be confirmed.

In Pykhodko's study, the enzyme treatment (amylase, protease and lipase) that weaning pigs were on did not have any effect on growth performance at 2wk but casued an increase in BW gain tendency in pigs at 18wk after weaning (Prykhodko et al. 2016). It might due to the improvement in gut maturation and the health of the digestive tract (Dierick et al. 2002a, Prykhodko et al. 2016). Therefore, exogenous enzymes might have no immediate effect on growth performance. In the present study, the NC+2X had marginal growth performance improvement in phase 1 and 2, the lipase supplementation slightly increased the fat digestibility in phase 1 and 3 compared to NC group, even though the highest digestibility were in the PC group. Optimistically, based on the reason found in Prykhodko et al. (2016) and the digestibility result from the present study, the positive effect from lipase supplementation may not have been

reflected in the pigs' growth performance as the present study ended at day35 post-weaning. With the positive influence of lipase on fat digestibility and digestive tract, the positive effect of lipase on growth performance may become significant if the experiment is longer.

Conclusion

In this study, the lipase did not effectively improve growth performance and nutrient digestibility. This might be due to the lipolysis side effect from the lipase and the high initial fat digestibility or the impropriate energy concentration gap design between PC and NC. However, with the marginal growth performance and fat digestibility improvement from the lipase, lipase addition still has potential to improve growth performance later when the pigs reach the grower/finisher stage, as the lipase enhanced nutrient digestibility and strengthened the digestive tract. In future studies, a new experimental design is needed to create a more suitable energy concentration gap between the positive control and the negative control, and a vitro test should be conducted to demonstrate the activities of exogenous lipase in the feed. Also, to better demonstrate the lipase effect on fat digestibility, using similar fat content in PC and NC diets or using a low-quantity fat source in the diets should be considered. More research is needed to confirm the significance of using exogenous lipase in weaning pigs' diet.

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Table 3-1. Diet Treatment Design

Treatment	Description	Details
1	Positive Control (PC)	NRC levels of energy and amino acids
2	Negative Control (NC)	100 kcal/kg below NRC on energy, meet requirement on amino acids and minerals
3	NC + X Lipase	
4	NC + 2X Lipase	

- X =1 portion of lipase = 500U/kg lipase

Table 3-2. Diet Composition in Each Phase

	Phase 1 Day (0-10)		**Phase 2 Day (11-21)**		**Phase 3 Day (21-35)**	
	Positive	Negative	Positive	Negative	Positive	Negative
Corn	29.44	31.72	43.89	46.15	55.76	58.02
Soybean Meal	16.15	15.99	31.26	31.1	33.55	33.38
Oats	10	10	5	5	5	5
Fat	**3.43**	**1.33**	**3.25**	**1.16**	**2.19**	**0.1**
Whey	27.5	27.5	10	10	0	0
Fish meal	5	5	3	3	0	0
Plasma Protein	5	5	0	0	0	0
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.12	0.11	0.11	0.11	0.07	0.07
Threonine	0.04	0.03	0.02	0.02	0.02	0.02
Limestone	0.86	0.88	0.68	0.69	0.85	0.85
Dicalcium Phosphate	0.18	0.16	1.08	1.07	1.52	1.51
Salt	0.2	0.2	0.25	0.25	0.35	0.35
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral Premix[b]	0.15	0.15	0.15	0.15	0.15	0.15
Zinc Oxide	0.38	0.38	0.25	0.25	0	0
Antibiotic	1	1	0.5	0.5	0	0
Nutrient Content						
Energy, kcal/kg ME	3400	**3300**	3400	**3300**	3350	3250
Lysine, SID %	1.5	1.5	1.35	1.35	1.23	1.23
Calcium, %	0.85	0.85	0.8	0.8	0.75	0.75
Phosphorus, STTD %	0.45	0.45	0.4	0.4	0.35	0.35

- Lipase (500 or 1000 U/kg) added to negative control diet in each phase.
- 0.3% TiO2 used as digestibility marker.
- a: the vitamin premix provided the following per kg of complete diet: 1100 IU vitamin A, 1376 IU vitamin D3, 44IU vitamin E, 4.4 mg vitamin K, 8.3 mg riboflavin, 50 mg niacin, 28mg pantothenic acid, 23 µg vitamin B12.
- b: the trace mineral premix provided the following per kg of complete diet: 165 mg Fe (FeSO4• H2O), 16.5 mg Cu (CuSO4• H2O), 36 mg Mn (MnSO4), 165 mg Zn (ZnO), 0.3 mg I (Ca(IO3)2) and 0.3 mg Se (Na2SeO3)

Table3-3. Analyzed Composition of Each Diet in Each Phase

Phase	Diet	Energy (kcal/kg GE)	Fat %	Protein %	Fiber % (NDF)	Fiber % (ADF)	Phosphorus %	Titanium %
Phase 1	PC	4147.22	6.41	20.11	6.42	1.59	0.629	0.29
	NC	4011.67	4.32	21.23	7.73	1.94	0.632	0.34
	NC+1x	4053.41	3.92	22.98	5.94	1.61	0.554	0.35
	NC+2x	4036.63	3.78	22.29	7.10	1.56	0.566	0.34
Phase 2	PC	4041.85	5.97	22.26	9.18	2.21	0.567	0.33
	NC	3990.64	3.93	21.72	8.98	2.21	0.608	0.33
	NC+1x	3994.12	4.36	22.95	9.22	2.02	0.608	0.29
	NC+2x	3969.67	4.13	23.12	8.42	2.21	0.620	0.36
Phase 3	PC	3996.95	5.59	20.37	10.63	1.97	0.635	0.25
	NC	3793.63	2.70	20.20	8.88	1.75	0.567	0.32
	NC+1x	3945.93	2.34	22.17	11.95	2.37	0.634	0.28
	NC+2x	3964.05	2.21	22.21	8.40	1.69	0.580	0.26

- All analysis conducted in the Animal and Dairy Science Department lab of University of Georgia(UGA)
- PC= Positive control, NC= Negative control diet, NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase
- All were the means of triplicate analysis, means in a same phase group with different superscripts differ $P<0.05$

Table 3-4. Pig Growth Performance with Each Diet Treatment in Each Phase

Diet	Positive Control	Negative Control	NC+X	NC+2X	SEM	P-Value
Body Weight (kg)						
Phase 1 (day1-10)	7.88	8.21	7.92	8.47	0.58	NS
Phase 2 (day10-21)	12.80	12.80	12.41	13.29	0.81	NS
Phase 3 (day21-35)	20.65	21.48	20.12	21.57	1.11	NS
Daily Gain (kg/day)						
Phase 1 (day1-10)	196	225	189	234	20	NS
Phase 2 (day10-21)	490	480	476	502	29	NS
Phase 3 (day21-35)	621	687	572	643	30	0.1
Overall (day1-35)	445	443	429	441	26	NS
Daily Feed Intake (kg/day)						
Phase 1 (day1-10)	236	244	226	255	19	NS
Phase 2 (day10-21)	584	594	550	595	35	NS
Phase 3 (day21-35)	975	1012	988	1010	39	NS
Overall (day0-35)	625	644	616	647	28	NS
Gain: Feed						
Phase 1 (day1-10)	0.8	0.92	0.82	0.91	0.043	0.2
Phase 2 (day10-21)	0.84	0.82	0.87	0.85	0.025	NS
Phase 3 (day21-35)	0.64	0.69	0.59	0.65	0.034	NS
Overall (day1-35)	0.69	0.71	0.69	0.7	0.027	NS

- Result are LS Means for 8 pens per diet treatment in each phase, NS = Not Significant (P > 0.2)
- NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase
- NS = Not Significant (P > 0.2)

Table 3-5. Nutrient Digestibility of Each Diet Treatment in Each Phase

Diet	Positive Control	Negative Control	NC+X	NC+2X	SEM	P-Value
Phase 1 (day1-10)						
Fat Digestibility, %	68.65[b]	53.11[a]	59.09[a]	61.50[a]	3.23	0.02
N Digestibility, %	75.49[ab]	76.94[ab]	74.77[a]	78.69[b]	1.20	NS
P Digestibility, %	43.79[b]	40.14[b]	32.37[a]	43.46[b]	2.25	0.004
Phase 2 (day10-21)						
Fat Digestibility, %	69.92[b]	62.94[b]	61.98[b]	52.09[a]	2.94	0.005
N Digestibility, %	71.88[a]	77.64[b]	77.29[b]	74.90[b]	1.03	0.002
P Digestibility, %	26.39	35.31	35.21	30.79	3.07	0.1
Phase 3 (day21-35)						
Fat Digestibility, %	73.76[c]	55.99[b]	45.37[a]	56.87[b]	1.31	0.001
N Digestibility, %	80.85[ab]	79.35[a]	78.74[a]	82.08[b]	0.84	0.04
P Digestibility, %	54.22[c]	32.76[a]	36.21[a]	44.43[b]	2.47	<0.0001

- Result are LS Means for 8 pens per diet treatment in each phase, digestibility was determined using TiO2 as the marker.
- NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase
- Means within a row lacking a common superscript letter ($P < 0.05$)
- NS = Not Significant ($P > 0.2$)

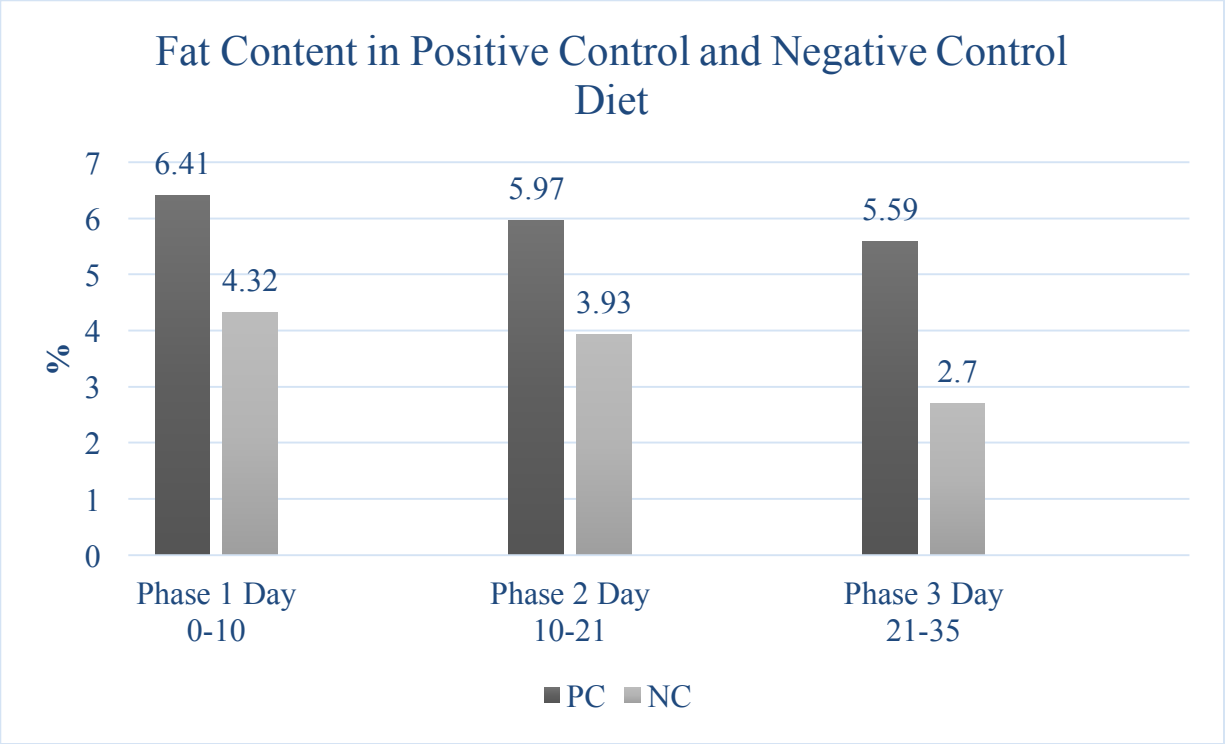

Figure3-1. The Fat Content in Positive Control and Negative Control Diets in Each Phase

- PC= Positive control, NC= Negative control diet

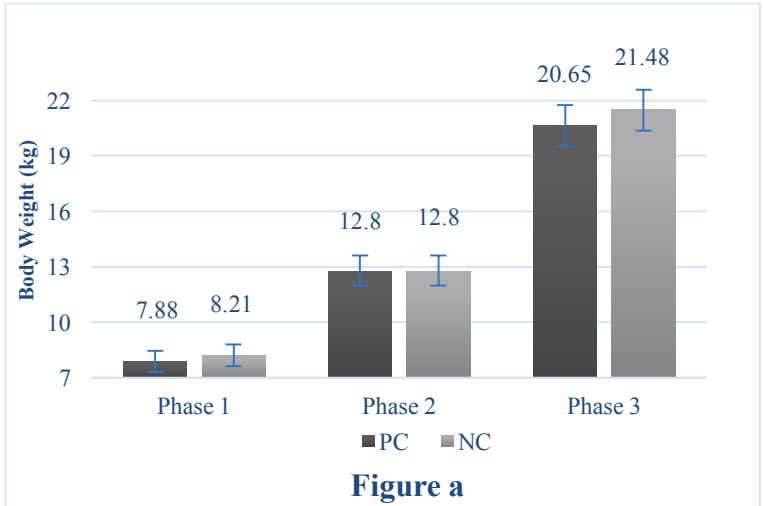

Figure a

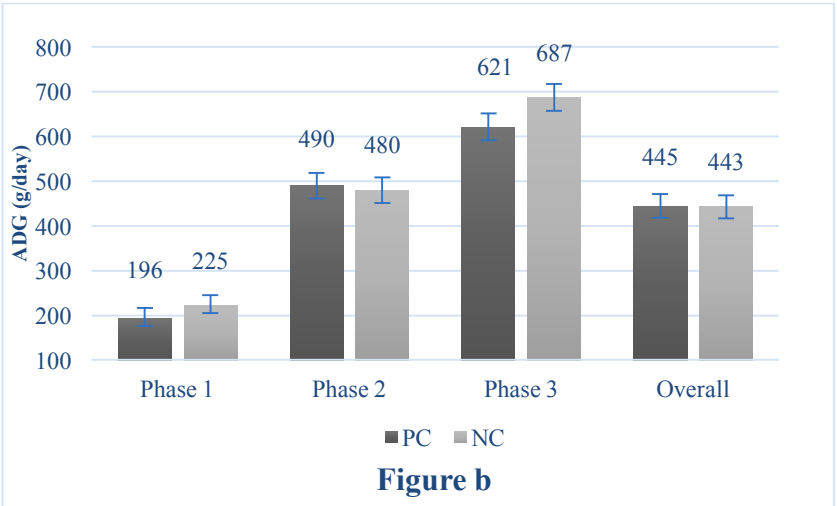

Figure b

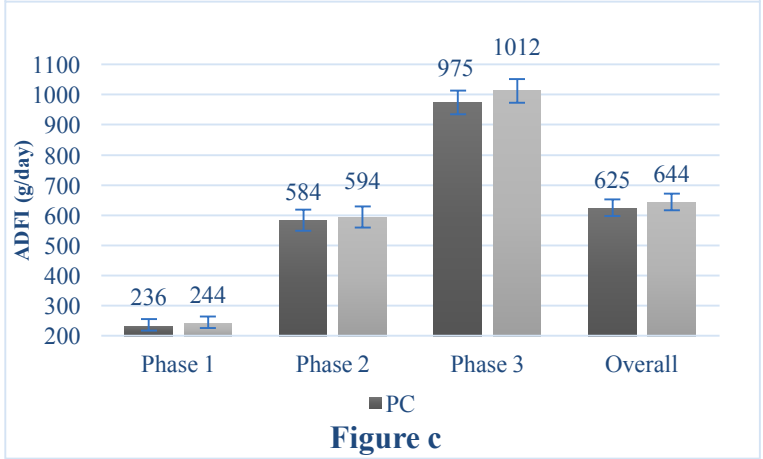

Figure c

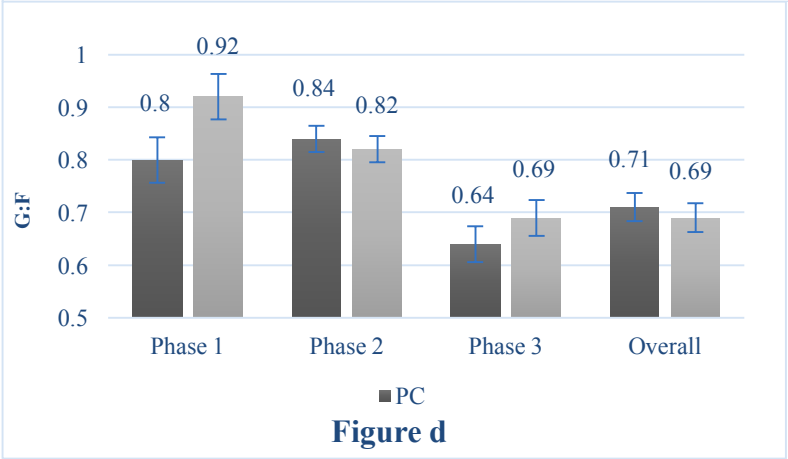

Figure d

Figure3-2. The Growth Performance of Pigs on PC and NC Diets in Each Phase (P>0.05)

- Figure a: body weight gain on PC and NC diet; figure b: average daily gain on PC and NC diet; figure c: average daily feed intake on PC and NC diet; figure d: gain and feed ratio on PC and NC diet.
- PC= Positive control, NC= Negative control diet

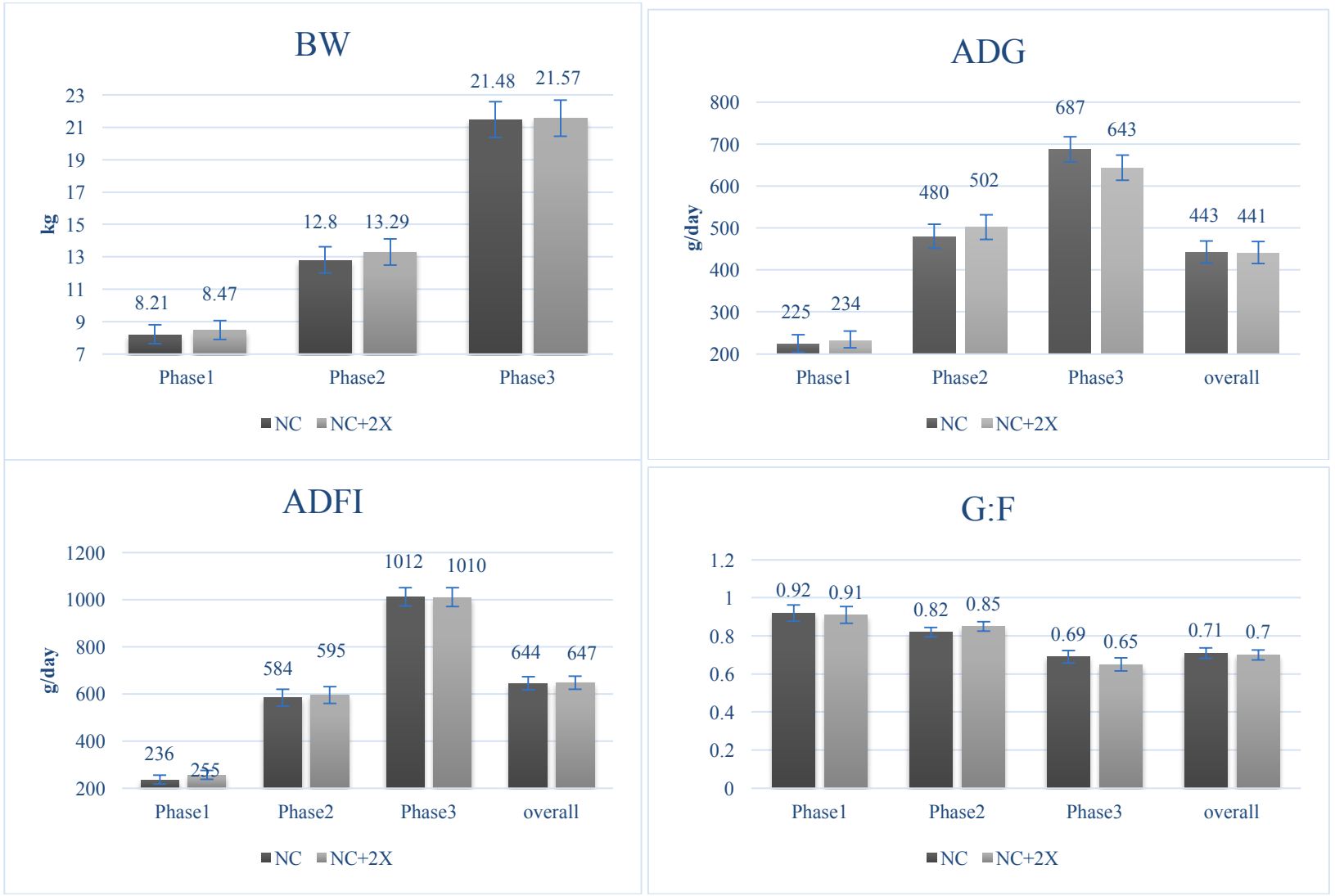

Figure 3-3: The Growth Performance of Pigs on NC and NC+2X Diets in Each Phase (P>0.05)

- Figure BW: body weight gain on NC and NC+2X diet; figure ADG: average daily gain on NC and NC+2X diet; figure ADFI: average daily feed intake on NC and NC+2X diet; figure G:F: gain and feed ratio on NC and NC+2X diet.
- NC= Negative control diet, NC+2X = Negative Control +1000U/kg lipase

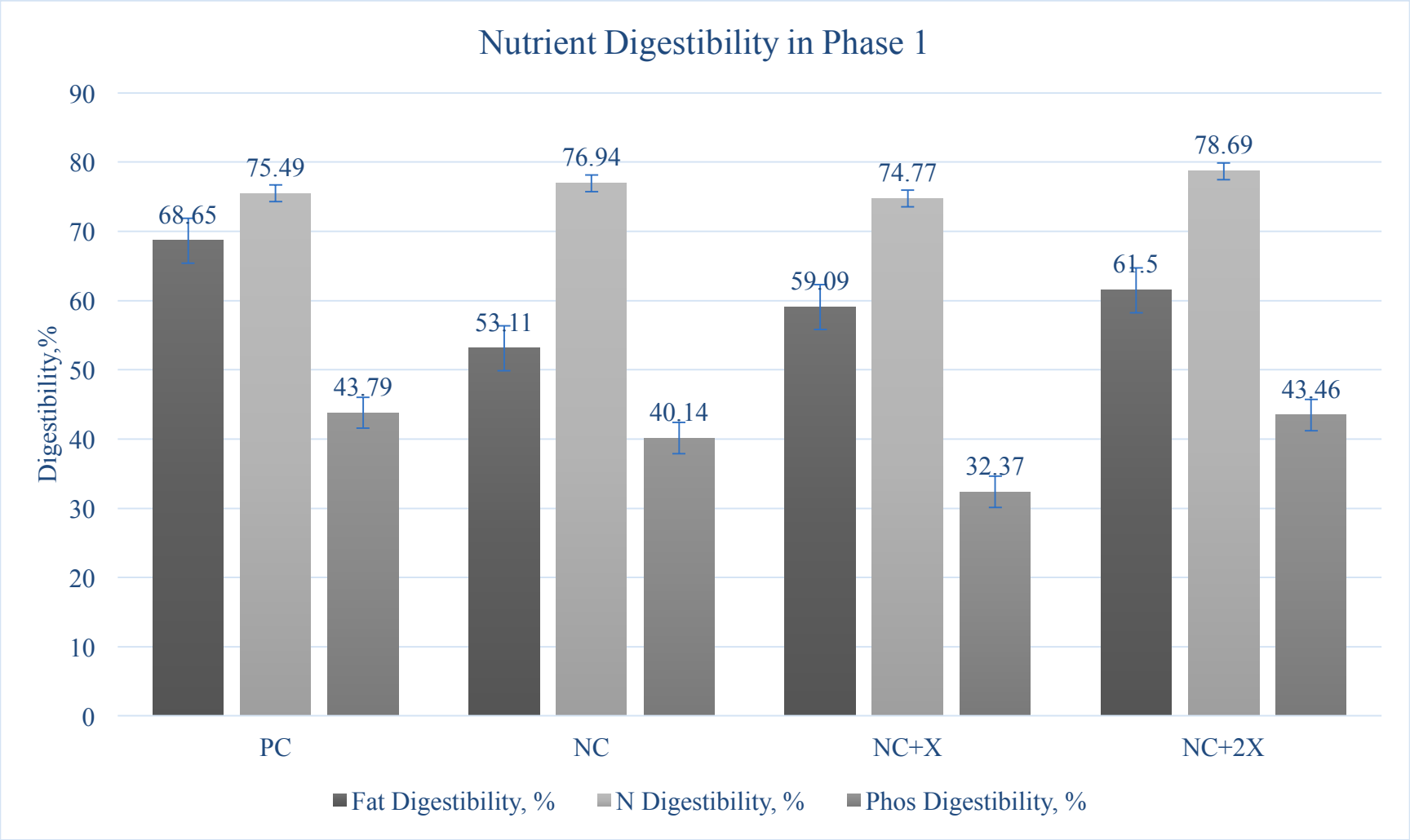

Figure 3-4. Nutrient Digestibility in Phase 1

- Fat, nitrogen, phosphorous digestibility on PC diet, NC diet, NC+X and NC+2X diets.
- PC= Positive control, NC= Negative control diet, NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase

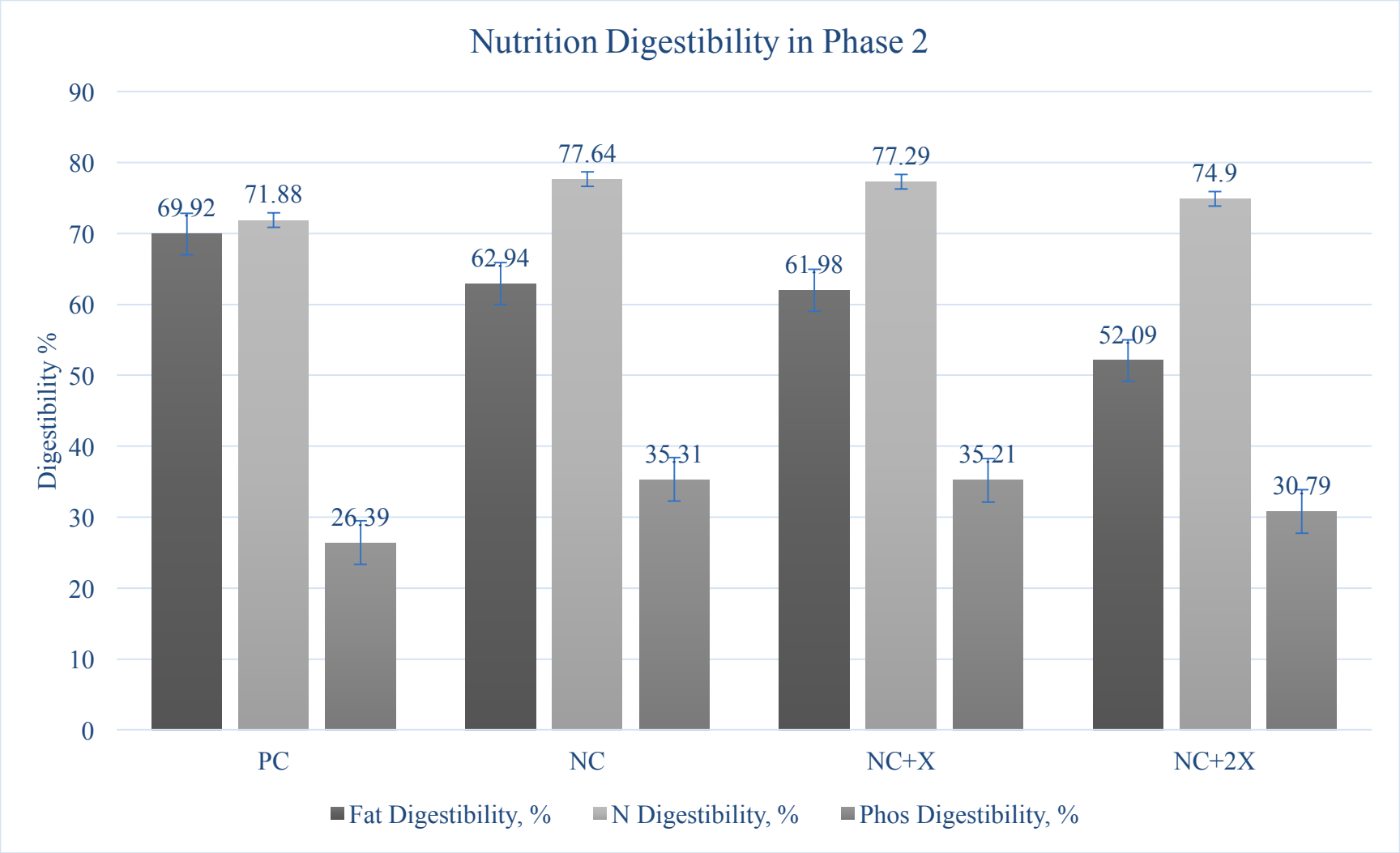

Figure 3-5. Nutrient Digestibility in Phase 2

- Fat, nitrogen, phosphorous digestibility on PC diet, NC diet, NC+X and NC+2X diets.
- PC= Positive control, NC= Negative control diet, NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase

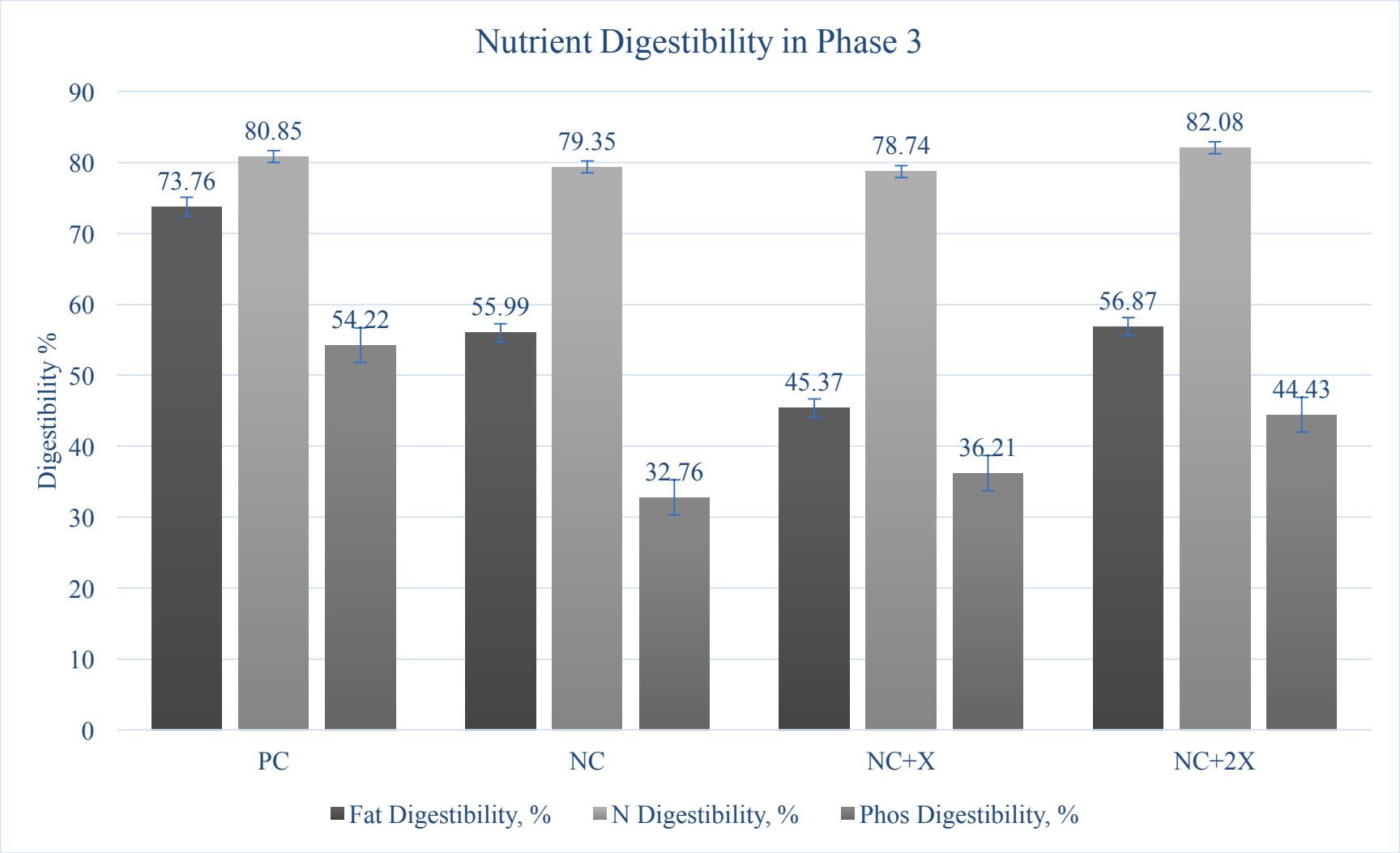

Figure 3-6. Nutrient Digestibility in Phase 3

- Fat, nitrogen, phosphorous digestibility on PC diet, NC diet, NC+X and NC+2X diets.
- PC= Positive control, NC= Negative control diet, NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase

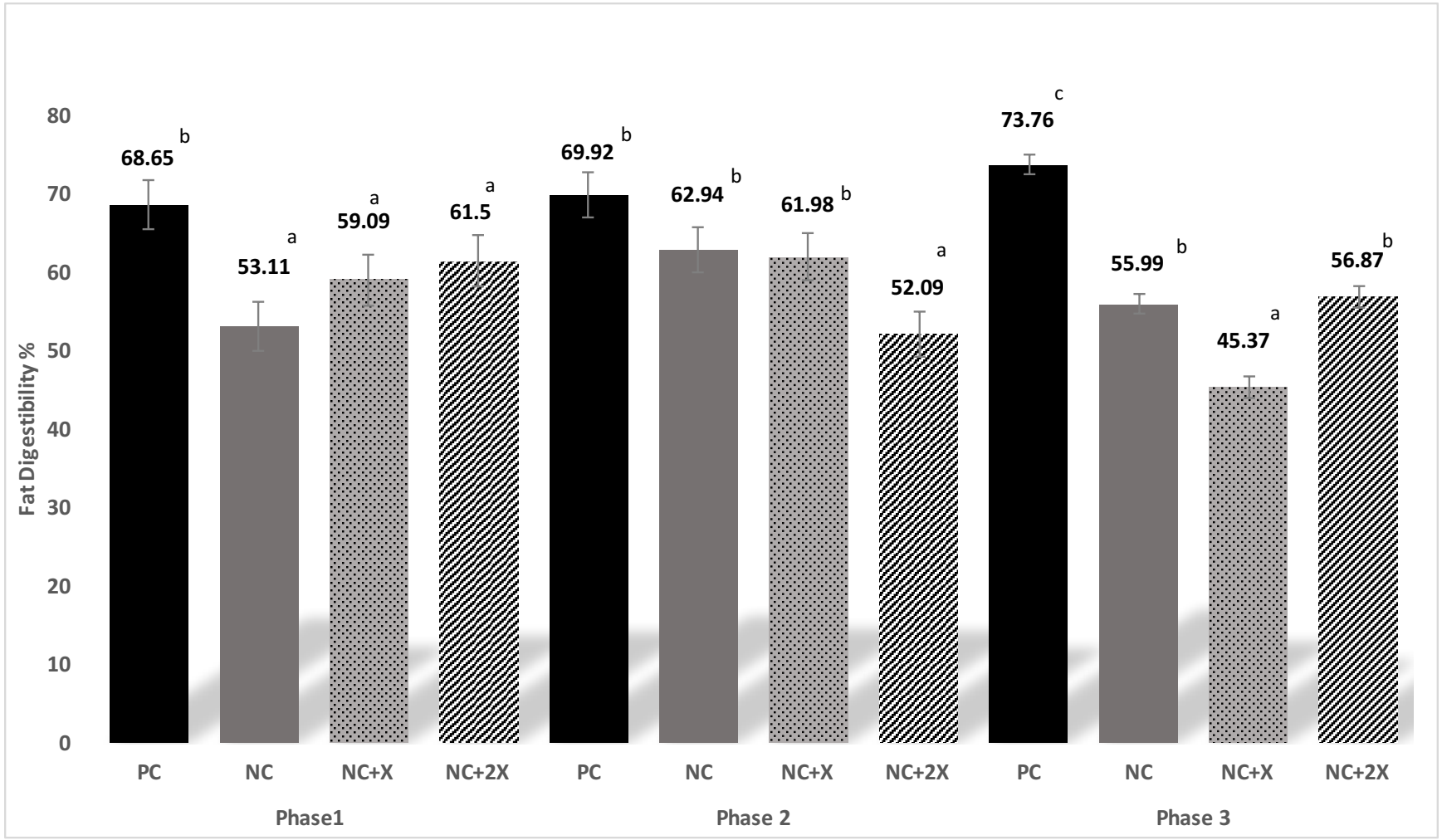

Figure 3-7 Fat Digestibility of Different Diet Treatment on Each Phase

- Fat, nitrogen, phosphorous digestibility on PC diet, NC diet, NC+X and NC+2X diets.
- Means in a same phase group with different superscripts differ $P<0.05$
- PC= Positive control, NC= Negative control diet, NC + X = Negative Control +500U/kg lipase, NC+2X = Negative Control +1000U/kg lipase

CHAPTER 4

THE EFFECT OF LIPASE AND COPPER SUPPLEMENTATION ON GROWTH PERFORMANCE AND FAT DIGESTIBILITY IN WEANING PIGS

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Abstract

An experiment was conducted to determine the effect of lipase and copper supplementation on weaning pigs' growth performance and fat digestibility. A total of 192 pigs (initial weight = 5.94±0.03 kg) were used in the experiment and fed with corn and soybean meal-based diets. The pigs were randomly assigned to four treatments: control diet (C), control + 1000 U/kg lipase (CL), control + 250 ppm copper (CCu), and control + 1000 U/kg lipase + 250 ppm copper (CLCu). They were fed with phase 1 diet for 14 days (day 0-14 post-weaning) and were then switched to phase 2 diet (day 14-28 post-weaning). During the experimental period, copper had a significant effect on overall bodyweight (BW), average daily gain (ADG), average daily feed intake (ADFI) and gain:feed ratio (G:F), as well as fat, N, fiber and phosphorus digestibility ($P<0.05$). Lipase tended to improve ADG and G:F ($0.05<P<0.2$), and it improved the N and phosphorous digestibility in phase 2. However, the effect of lipase on nutrient digestibility was not consistent. The CL group had a significantly lower fat digestibility (65.37%) in phase 1, but significantly higher fat digestibility (73.31%) in phase 2, compared to C group (71.94, 68.82%). There was no lipase and copper interaction effect on growth performance. The interaction effect improved the N digestibility, but it was not consistent on fat, fiber or phosphorous digestibility.

The results indicated that copper has a positive impact on the weaning pigs and can be used as a growth promoter when added into the diet. Exogenous lipase might improve the growth performance of weaning pigs in the long run, but it had an unclear effect on fat digestibility. This might be due to the side effect of endogenous lipolysis in the feed. In addition, the diet with lipase and copper significantly increased the growth performance of the treatment group compared to the control group, but the lipase and copper interaction effect was not significant,

and the effect on nutrient digestibility was inconsistent. The effect of the addition of copper on exogenous lipase activity could not be determined.

Introduction

The growth performance and nutrient digestibility of weaning pigs are always a concern in the pig industry. At the weaning stage, the piglets are separated from the sows and moved to a new environment, and they need to adapt to the diet change from high-fat sow milk to high-carbohydrate dry food (de Mann and Bowland 1963, Tokach et al. 1995). Additionally, during this period, the activity of lipase in the weaning pigs decreases dramatically, and their fat digestibility drops from 96% to 60%-80% (Cera et al. 1990 and 1988a, Jensen et al. 1997). The low fat content in the diet and the inefficient fat utilization lead to growth depression in weaning pigs. Therefore, different methods have been used to improve pigs' growth performance and fat digestibility (Thaler et al. 1986, Jung et al 2013). Exogenous enzymes have been tested in different animals' diets and are widely used as supplements in animal feed (Dierick and Decuypere 2004, Liu et al. 2010, Prykhodko et al. 2016). However, the effect of exogenous lipase on pigs is not well understood. Research data associated with exogenous lipase in pigs are very limited. In the previous experiment, which added lipase to the weaning pigs' diet, the lipase supplementation did not cause any improvement to the growth performance, but it partially increased the fat, N and phosphorus digestibility compared to the positive group.

Copper (Cu) is one of the essential trace minerals for animal nutrition. High-concentration (250ppm) copper supplementation is commonly used as a growth promoter in weaning pigs' diets (Roof and Mahan 1982, Cromwell et al 1998, Mei et al. 2010). A study showed that 250 ppm Cu supplementation can also stimulate pancreatic lipase activity (Luo and Dove 1996).

Therefore, copper might have the potential to stimulate exogenous lipase activity. Based on the previous experiment result and the potential effect of copper, the exogenous lipase effect on pigs may be strengthened with copper addition, and the exogenous lipase and copper supplementation might further minimize the negative impact of weaning. In this experiment, dietary copper and exogenous lipase were added into nursery diets to determine the effect on growth performance and fat digestibility of the weaning pigs.

Materials and Methods

Animal Handling and Data Collection

The experimental protocols used in this study were approved by the Animal Care and Use Committee of the University of Georgia (UGA) (UGA Animal Care and Use, A2015 01-007-Y2-A0)

A total of 192 healthy crossbred pigs (PIC C 29 female x True Choice EBX or EB5 male) from the University of Georgia (UGA) Swine Center were randomly selected to use in 2 trials. Pigs at this experiment were 5.94 ± 0.03 kg at weaning, and the growing weight mostly meets the criteria of the phases outlined in the 2012 NRC. In each trial, the pigs were weaned at day 21 of age and were placed into 24 pens based on sex, weight, litter number and genetic breeding lines. Each pen was 0.94 wide and 1.83m long and had 4 pigs, and there were 6 pens per diet treatment. The four dietary treatments (Table 4-1) were: 1) control diet (C), 2) control diet with lipase (CL), 3) control diet with 250 ppm copper (CCu), and 4) control diet with lipase and 250 ppm copper (CLCu). The dietary treatments were formulated to meet or exceed all the 2012 NRC nutrient requirements. Copper supplementation was provided at 250 ppm in the CCu and CLCu, as most of the studies showed that 250 ppm copper concentration can improve pig

performance and enzyme activity better than other concentrations (Roof and Mahan 1982, Cromwell et al 1993, Mei et al. 2010). Phase 1 diets were fed from day 0-14 post-weaning. On day 15 post-weaning, pigs were switched into phase 2 diets, which were fed from day 15-28 post-weaning. At the end of the study, the pigs were returned to the UGA Swine Center. For nutrient digestibility analysis, 0.3% TiO2 was added to the diets as a digestibility marker to determine the nutrient digestibility in pigs. Diets were manufactured at the UGA Poultry Science Department Feed Mill and were fed in pellet form. The composition of the diets is shown in Table 4-2 and 4-3.

The pigs were housed in an environmentally controlled room with the temperature at 25-27 degrees Celsius and 24-hour daylight. They were weighed on days 0, 7, 14, 21 and 28 post-weaning. At the same time, feed intake was monitored at each weight day. Diet samples were collected at each phase and stored at 4 degrees Celsius in the refrigeration for further determination of the nutrient profile. For fecal collection, in trial 1, fecal samples from each pen were collected twice daily for the last 3 days of each phase. In trial 2, fecal samples were collected twice daily on the last two days of each week. All samples were stored at -20 degrees Celsius in the freezer. At the end of the study, the frozen fecal samples were dried in the Grieve Shelf Oven SA-350 (The Grieve Cooperation, Round Lake, IL) for one week. The dried fecal samples were ground into 2mm powder in the Wiley Mill (Thomas Scientific, Swedesboro, NJ) and then stored at room temperature for further analysis.

Sample Analysis

Feed samples were sent to the Feed and Environmental Water Laboratory for proximate analysis and mineral analysis (University of Georgia Cooperative Extension, Athens, GA). At the same time, feed samples were analyzed in triplicate for gross energy concentration (GE),

crude protein (CP), ADF, NDF, titanium and phosphorus content in the Animal and Dairy Science Department lab, and fecal samples were analyzed in duplicate for crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF), ether extract, titanium, and phosphorus content. Gross energy concentration in diets was examined by bomb calorimeter (Parr 1261, Parr Instrument Co., Moline, IL). A Leco FP-628 Nitrogen Analyzer was used to analyze crude protein in the diet and fecal samples (Leco Corporation, St. Joseph, MI). NDF and ADF were analyzed by an Ankom A200 and A2000 fiber analyzer (Ankom Technology, Macedon, NY) with NDF Method (Method 13) and ADF Method (Method 12), respectively. The fecal ether exact content was determined using an ANKOM XT15 Extractor (Labconco CO, Kansas City, MI) with AOCS Official Procedure Am 5-04.

For the mineral assay, the diet and fecal samples were ashed for 6 hours at 550 °C in a Fisher Isotemp Muffle Furnace (Fisher Scientific, Suwanee, GA). After the sample cooled to room temperature, 10 ml sulfuric acid was added to each sample and heated for 1 h. Then, the solutions were diluted with distilled water to the total volume of 50 ml. After sitting for one night, the solutions were centrifuged and used for TiO2 (Short et al. 1996, Titgemeyer et al. 2001) and phosphorous (Herbert et al. 1971, Short et al.1996) concentration assessment using a UV spectrophotometer, measuring the absorbance at 410 nm (TiO2) and 660 nm (Phosphorous).

The apparent total digestibility (ATTD) of nutrients were calculated with the following equation:

$$\mathbf{AATD} = \mathbf{1} - \frac{[\mathbf{Marker}]_{\mathbf{feed}}}{[\mathbf{Nutrient}]_{\mathbf{feed}}} \times \frac{[\mathbf{Nutrient}]_{\mathbf{feces}}}{[\mathbf{Marker}]_{\mathbf{feces}}}$$

Statistical Analysis

All data were analyzed using the PROC GLM procedure in SAS (SAS 9.4, SAS Institute, Inc., Cary, NC) to evaluate the effect of lipase supplementation, copper supplementation, lipase

× copper interaction, trial (2 trials) on body weight (BW), average daily gain (ADG), average daily feed intake (ADF) and gain:feed ratio (G:F) and digestibility of fat, N and phosphorous. The pen was considered as the experimental unit. The results are presented as least squares means (LS Means) for the lipase supplementation, copper supplementation, and lipase × copper interaction effect, with significance at $P<0.05$ and trends at $P<0.20$.

Results

The analyzed composition of diets for phase 1 and 2 is listed in Table 4-4 and Table 4-5

Diets were formulated based on the requirements of NRC (NRC, 2012) and all the analyzed nutrient content values were similar to the expected values. There was no significant difference in the nutrient contents among the four diet treatments. The copper levels in the diets (Table 4-5) were also similar to the expected levels. Diet CCu and CLCu had about 250ppm Copper both in phase 1 and 2.

Growth Performance

The results of growth performance are shown in Table 4-6 and Table 4-7 and Figure 4-1, 4-2 and 4-3.

Generally, no significant Cu × Lipase interactions or trial effects were observed in the growth performance responses in this study. There was no effect of Cu or lipase on BW from day 0-7. In addition, the effect of lipase on growth performance was mostly nonsignificant, but it did tend to improve ADG and G:F. However, Cu supplementation had a significant effect on most of the growth performance (table 4-7).

For lipase supplementation, comparing the BW, ADG, ADFI and G:F in the C and CCu groups to the CL and CLCu groups, there was no significant difference between them in general

(P>0.05), but the lipase groups had slightly better growth performance than the no lipase group (Figure 4-2). In addition, lipase supplementation had a trend of improving ADG and G:F in phase 1 and overall (Figure 4-7) (ADG: 237 vs. 254, 394 vs. 411 g/day; G:F: 0.88 vs. 0.91, 0.77 vs. 0.79; 0.05<P<0.2).

With regard to copper supplementation, the diet with copper (CCu and CLCu) significantly improved growth performance when compared to the C and CL groups (Figure 4-2). The day 14, 21, 28 BW, ADG, ADFI and G:F of the 250ppm Cu groups were significantly higher than those in the NRC Cu groups (Table 4-7)

When it comes to the lipase and Cu combination supplementation, the CLCu group had the highest BW, ADG, ADFI and G:F in phase 1, phase 2 and overall, compared to other diet groups (Figure 4-1). The differences between the C group and the CLCu group in BW from day14-28, ADG (phase 1: 201 vs. 280, phase 2: 516 vs. 592, overall: 364 vs. 438 g/day), ADFI (phase 1: 249 vs. 300, overall: 493 vs. 541g/day) and G:F (phase 1: 0.82 vs. 0.96, phase 2: 0.71 vs. 0.76, overall: 0.74 vs. 0.82) were significant (P<0.05), except BW from day 0-14 and ADFI in phase 2 (730 vs. 784g/day, P>0.05) (Figure 4-3).

Nutrients Digestibility

The results of nutrient digestibility are shown in Table 4-8 and Figure 4-4, 4-5, 4-6, and 4-7.

The lipase had a significant positive effect on the N (80.49, 83.35, 83.88 and 84.51%) and phosphorous digestibility (46.85, 55.88, 55.07 and 56.21%) in phase 2 (P<0.05), but it did not have any significant effect on the fiber digestibility in both phases (P>0.05). Moreover, the lipase supplementation had a trend of decreasing fat digestibility (71.94, 65.37, 70.57 and 72.81%) and phosphorus digestibility (51.38, 47.58, 57.97 and 57.10%) in phase 1 (0.05<P<0.2).

The fat digestibility and N digestibility of the CL group were significantly lower than those in the C group in phase 1 (71.94 vs. 65.37, 80.31 vs. 77.72%) (Figure 4-5), but the fat digestibility, N digestibility, fiber digestibility and phosphorous digestibility of the CL group in phase 2 were significantly higher (68.82 vs. 73.31, 80.49 vs. 83.35, 52.33 vs. 63.45, 46.85 vs. 55.88%).

The copper had a significant impact on N and phosphorous digestibility in both phases. It had no significant effect on fiber digestibility in phase 1 and only tended to improve fiber digestibility in phase 2 (52.33, 63.45, 58.12, 51.80%). When it comes to fat digestibility, the copper had a significant effect ($P=0.05$) or trend ($0.05<P<0.2$) on fat digestibility. However, the CCu group values in phase 1 were lower than those of the C and CLCu group (71.94, 70.57, 72.81%), while the CCu group value was higher than the CLCu value in phase 2 (72.48 vs. 67.07) (Figure 4-4). The copper effect on fat digestibility among the diets was not consistent.

Comparing the C and CLCu groups (Figure 4-7), the CLCu group had significantly higher N digestibility and phosphorous digestibility in both phases (Phase 1: 80.31 vs. 83.51, 51.38 vs. 57.10, Phase 2: 80.49 vs. 84.51, 46.85 vs. 56.21) ($P<0.05$). The copper and lipase effect or copper and lipase interaction effect were not consistent on fat digestibility and fiber digestibility. Although the differences were not significant ($P>0.05$), in phase 1, the fat and fiber digestibility in the CLCu group were slightly lower than those in the C group (71.94 vs. 72.81, 57.02 vs. 64.19), while in phase 2, the fat and fiber digestibility in CLCu group were lower than those in the C group (68.82 vs. 67.07, 52.33 vs. 51.81).

Discussion

Based on the current study's results of the growth performance in the pigs, the 250 ppm Cu from the copper sulfate generally provided a positive influence on the growth performance as

expected. The results matched the conclusion in the Cromwell et al. (1989) study, which indicated that 250 ppm Cu can provide a steadily positive effect on pigs' growth performance. For nutrient digestibility, in the current study, the Cu supplementation significantly improved the N and phosphorous digestibility. Previous studies have shown that Cu supplementation can improve nutrient digestibility. In Coble et al. (2016), copper addition to the pigs' diets improved the digestibility of dry matter and gross energy. Dove (1995) showed that the addition of dietary copper in the diet can improve fat digestibility. However, different from the results of that study, the fat digestibility did not show any consistent improvement with the Cu supplementation in the current study. The CCu diet contained 250 ppm Cu. In phase 1, the CCu group had fat digestibility similar to the C group's, while the CCu group had higher fat digestibility than the C group's in phase 2 (Figure 4-4). Previous studies have shown that Cu supplementation could not improve nutrient digestibility, Engle and Spears indicated that Cu supplementation had no effect on growth performance or lipid and cholesterol metabolism (2001), which Chowdhury et al.'s study (2004) showed that Cu supplementation did not enhance the fat digestibility of broiler chickens. Therefore, the effect of copper on fat digestibility is not consistent.

When it comes to the lipase effect, there was no significant lipase effect on growth performance, but the lipase groups (CL and CLCu) still had slightly better performance than the C and CCu groups ($P>0.05$) (Table 4-7). One explanation could be the sample size, the sample size of current animal experiment was 192, if we can increase the sample size, the effect might become significant. Another explanation could be that the improvement of pigs' growth performance might not be immediately detectable. In the Prykhodko et al. (2016) combination enzyme study, the weaning pigs treated with the enzyme treatment (amylase, protease and lipase) at 35 days of age for two weeks did not show any improvement in growth performance during

the two weeks, but tended to show body weight gain improvement at 18 weeks post-weaning. Lipase might improve the gut maturation and the health of the digestive tract. (Dierick et al. 2002a, Prykhodko et al. 2016). In the current study, significant improvement was not seen, but a trend of ADG and G:F overall improvement was seen in the results. Therefore, there might be a possibility that the growth performance of the pigs in the lipase supplementation group (CL, CLCu) would be significantly improved if the study could be extended. In additional, beside the two explanations above, another explanation might be that the lipase supplementation levels might not be high enough to promote pig growth at the weaning stage. Different levels of enzyme supplementation were used in the previous studies, and the results were varied. In the Liu et al. study (2010), two levels of recombinant lipase were added into the weaning pigs' diets, 5,000 and 10,000 U/kg, respectively. The growth performance between these two diet groups was similar but significantly better than the control group. A similar result was also found in the Bikker et al. study (2016). Consequently, there is a possibility that the level of lipase supplementation in the current study was not sufficient to increase the pigs' growth significantly.

In the study by Dierick and Decuypere (2004), the authors indicated that, with lipase addition, fat digestibility was not affected, but the apparent ileal digestibility (AID) of dry matter (DM) and energy and the apparent fecal (AFD) digestibility were improved. A similar result was shown in the current study. The lipase supplementation significantly promoted the N digestibility and phosphorous digestibility in phase 2. The main purpose of adding lipase to the diet was to improve fat digestibility, but the lipase did not promote the fat digestibility or fiber digestibility in the current study; moreover, the lipase effect on fat digestibility was not consistent. Although the lipase effect was not significant on fat digestibility in general, when only comparing the C and CL groups, the lipase supplementation significantly decreased the fat digestibility of the CL

group in phase 1; in contrast, it significantly increased the fat digestibility of the CL group in phase 2. This inconsistent result was unexpected. Dierick and Decuypere (2002b) pointed out that lipolysis in the diets with exogenous lipase was greater and faster than in the diets supplemented without exogenous lipase. Lipolysis increases the free fatty acids in the feeds, which might lead to a decrease in fat digestibility. The unexpected result might be due to the lipolysis caused by the exogenous lipase.

Based on the result of current study, even though the lipase and Cu interaction effect on nutrient digestibility was significant, the effects on different nutrients' digestibility or in different phases were varied. For example, the fat digestibility (Figure 4-4) in phase 1 for CL is 65.37%, but the rates of fat digestibility for C and CLCu were 71.94% and 72.81%. There was no interaction effect between C and CLCu; the significant effect was caused by the drastically low fat digestibility of the CL group. However, in phase 2, the fat digestibility of CLCu was 67.07%, but the rates of fat digestibility of CL and CCu were 73.31% and 72.48%. The significant effect was caused by the major differences in fat digestibility between CLCu and these two groups (CL and CCu). With regard to the interaction effect on growth performance, there was no significant lipase and Cu interaction effect ($P>0.05$). Comparing the growth performance of C and CLCu, there was a significant difference in BW from day 14-28, ADG, ADFI in phase 1 and overall, and G:F (Figure 4-3). This indicated that Cu and lipase addition in the diet can improve pigs' growth performance. However, the improvement might simply be caused by the Cu effect and the lipase effect. The improvement cannot prove that the combination of lipase and Cu had an interaction effect. The study's results did not show that the addition of copper improved the activity of exogenous lipase.

Conclusion

In this study, although the effect of copper on fat digestion is inconsistent, the copper supplementation had a positive impact on growth performance and N and phosphorus digestibility. Copper supplementation can be used in weaning pigs' diet as a growth promoter. The lipase supplement in the current study showed a trend of improving growth performance, to some extent, but it did not effectively improve the nutrient digestibility, which might be due to the side effect of lipolysis caused by the lipase. With better lipolysis prevention treatment, the lipase addition still has the potential to improve growth performance and fat digestibility. More research is needed to determine the effect of lipase on the performance of weaning pigs. The diet with lipase and copper supplements had significantly better growth performance results than the control group. However, the copper and lipase interaction effect was not significant. In addition, due to the inconsistent result of nutrient digestibility on different diets, the interaction effect of copper and lipase on nutrient digestibility was meaningless. In future study, a larger sample size of animal experiment should be conducted to better determine the lipase supplementation effect on growth performance of the weaning pigs. Also, a vitro experiment on different levels of copper with exogenous lipase should be conducted in order to determine if the copper addition can alter the exogenous lipase activity.

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Table 4-1. Diet Treatment Design

Diet	Cu	Lipase
1 (C)	No	No
2 (CL)	No	Yes
3 (CCu)	250ppm	No
4 (CLCu)	250ppm	Yes

- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Lipase supplementation concentrations in CL and CLCu were 1000U/kg
- The C and CL diet only have the copper from swine trace mineral premix, which were about 16.5ppm.

Table 4-2. Diet Composition in Phase 1

	Phase 1 Diet, Day 0 - 14			
Diet:	C	CL	CCu	CLCu
	%	%	%	%
Corn	27.38	27.38	27.28	27.28
Soybean Meal	8.91	8.91	8.91	8.91
Oats	10	10	10	10
Hamlet Protein	10	10	10	10
Soybean Oil	2.39	2.39	2.39	2.39
Whey	27.5	27.5	27.5	27.5
Fishmeal	5	5	5	5
Spray Dried Plasma	5	5	5	5
Salt	0.2	0.2	0.2	0.2
Limestone	0.92	0.92	0.92	0.92
Dicalcium Phosphate	0.04	0.04	0.04	0.04
Swine Vitamins[a]	0.25	0.25	0.25	0.25
Swine Trace Mineral[b]	0.15	0.15	0.15	0.15
Copper Sulfate	**-**	**-**	**0.1**	**0.1**
L-Lysine	0.2	0.2	0.2	0.2
DL-Methionine	0.06	0.06	0.06	0.06
Antibiotic (Mecadox)	1	1	1	1
Digestibility Marker[c]	0.3	0.3	0.3	0.3
Nutrient Content				
Energy, ME, kcal/kg	3400	3400	3400	3400
SID Lysine, %	1.5	1.5	1.5	1.5
Calcium, %	0.85	0.85	0.85	0.85
Phosphorus, STTD %	0.45	0.45	0.45	0.45

- Lipase (1000U/kg) added to diet CL and CLCu.
- a: the swine vitamins provided the following per kg of complete diet: 1100 IU vitamin A, 1376 IU vitamin D3, 44IU vitamin E, 4.4 mg vitamin K, 8.3 mg riboflavin, 50 mg niacin, 28mg pantothenic acid, 23 µg vitamin B12.
- b: the swine trace mineral provided the following per kg of complete diet: 165 mg Fe (FeSO4• H2O), 16.5 mg Cu (CuSO4• H2O), 36 mg Mn (MnSO4), 165 mg Zn (ZnO), 0.3 mg I (Ca(IO3)2) and 0.3 mg Se (Na2SeO3)
- c: digestibility marker is TiO2

Table 4-3 Diet Composition in Phase 2

	Phase 2 Diet, Day 14-28			
Diet:	C	CL	CCu	CLCu
	%	%	%	%
Corn	42.37	42.37	42..27	42.27
Soybean Meal	28.42	28.42	28.42	28.42
Oats	5	5	5	5
Hamlet Protein	5	5	5	5
Soybean Oil	2.59	2.59	2.59	2.59
Whey	10	10	10	10
Fishmeal	3	3	3	3
Salt	0.25	0.25	0.25	0.25
Limestone	0.9	0.9	9	0.9
Dicalsium Phosphate	0.68	0.68	0.68	0.68
Swine Vitamins[a]	0.25	0.25	0.25	0.25
Swine Trace Mineral[b]	0.15	0.15	0.15	0.15
Copper Sulfate	-	-	**0.1**	**0.1**
L-Lysine	0.2	0.2	0.2	0.2
DL-Methionine	0.07	0.07	0.07	0.07
L-Threonine	0.01	0.01	0.01	0.01
Antibiotic (Mecadox)	0.5	0.5	0.5	0.5
Digestibility Marker[c]	0.3	0.3	0.3	0.3
Nutrient Content				
Energy, ME, kcal/kg	3350	3350	3350	3350
SID Lysine, %	1.23	1.23	1.23	1.23
Calcium, %	0.7	0.7	0.7	0.7
Phosphorus, STTD %	0.33	0.33	0.33	0.33

- Lipase (1000U/kg) added to diet CL and CLCu.
- a: the swine vitamins provided the following per kg of complete diet: 1100 IU vitamin A, 1376 IU vitamin D3, 44IU vitamin E, 4.4 mg vitamin K, 8.3 mg riboflavin, 50 mg niacin, 28mg pantothenic acid, 23 µg vitamin B12.
- b: the swine trace mineral provided the following per kg of complete diet: 165 mg Fe (FeSO4• H2O), 16.5 mg Cu (CuSO4• H2O), 36 mg Mn (MnSO4), 165 mg Zn (ZnO), 0.3 mg I (Ca(IO3)2) and 0.3 mg Se (Na2SeO3)
- c: digestibility marker is TiO2

Table 4-4. Analyzed Composition of Each Diet in Each Phase

Phase	Diet	Energy (kcal/kg GE)	Dry Matter %	Moisture %	Ash %	Fat %	Protein %	Fiber % (NDF)	Fiber % (ADF)	Phosphorus %	Titanium %
Phase 1	C	4001.21	88.64	11.36	6.31	4.54	22.05	7.62	1.89	0.585	0.23
	CL	3989.19	88.12	11.88	6.69	4.14	22.85	7.94	1.54	0.632	0.27
	CCu	3996.52	88.34	11.66	6.66	4.11	23.27	7.99	1.82	0.554	0.29
	CLCu	4014.30	88.28	11.72	6.28	4.34	23.52	7.55	1.74	0.566	0.28
Phase 2	C	4062.84	86.87	13.13	5.91	4.58	23.46	7.66	2.74	0.567	0.27
	CL	4055.80	87.27	12.73	6.10	4.65	23.8	8.26	2.85	0.608	0.26
	CCu	4043.41	87.12	12.88	5.89	4.48	23.74	7.96	2.57	0.608	0.27
	CLCu	4082.99	86.73	13.27	5.75	4.01	24.77	7.89	2.89	0.620	0.27

- Analysis were conducted in Animal Science Department Laboratory and Feed and Environmental Water Laboratory of University of Georgia(UGA).
- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Energy, protein, fiber (NDF &ADF), phosphorous and titanium content results were from Animal Science Department Laboratory. Dry matter, moisture, ash and fat content (as-fed) results were from Feed and Environmental Water Laboratory.
- All were the means of triplicate analysis

Table 4-5. Analyzed Mineral Content of Each Diet in Each Phase

Phase	Diet	Phosphorus %	Potassium %	Calcium %	Magnesium %	Manganese ppm	Iron ppm	Copper ppm	Zinc ppm	Sodium ppm	Ca:P
Phase 1	C	0.58	0.9	0.98	0.13	101	175	**14**	137	2857	1.69
	CL	0.65	1.1	1.02	0.14	96	200	**18**	150	3374	1.59
	CCu	0.67	1.15	1.16	0.15	94	221	**209**	165	3755	1.73
	CLCu	0.62	1.09	1.04	0.15	98	207	**247**	149	3509	1.66
Phase 2	C	0.67	1.1	0.96	0.18	63	272	**23**	138	1742	1.45
	CL	0.64	1.15	0.99	0.18	58	256	**23**	149	1784	1.55
	CCu	0.66	1.08	1.1	0.17	65	328	**330**	150	1953	1.67
	CLCu	0.66	1.17	0.99	0.19	55	323	**260**	137	1704	1.51

- Analysis were conducted in Feed and Environmental Water Laboratory of University of Georgia(UGA).
- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Results were reported on an as sampled basis.

Table 4-6. Pigs Growth Performance with Each Diet Treatment in Each Phase

Diet	Control Diet (C)	C+Lipase (CL)	C+ Cu (CCu)	C+ Lipase+Cu (CLCu)	SEM	P-Value		
						Lipase	Cu	Lipase x Cu
Body Wt. (kg)								
Day 0	5.91	5.96	5.94	5.96	0.27	NS	NS	NS
Day7	6.65	6.81	7.03	7.10	0.30	NS	NS	NS
Day14	8.77 ^{a}	9.09 ^{ab}	9.72 ^{ab}	9.96 ^{b}	0.39	NS	0.03	NS
Day21	11.63^{a}	12.08^{ab}	13.03^{ab}	13.33^{b}	0.50	NS	0.01	NS
Day28	16.05^{a}	16.70^{ab}	17.84^{b}	18.27^{b}	0.61	NS	0.01	NS
Daily Gain (g/day)								
Day 0-7	106^{a}	122^{ab}	159^{bc}	163^{c}	13	NS	0.0008	NS
Day7-14	302^{a}	326^{ab}	385^{ab}	406^{b}	32	NS	0.02	NS
Day 14-21	408^{a}	426^{ab}	477^{b}	481^{b}	21	NS	0.006	NS
Day 21-28	631	660	688	705	27	NS	0.1	NS
Day 0-14 (Phase 1)	205^{a}	223^{a}	270^{b}	284^{b}	14	0.2	<.0001	NS
Day 14-28 (Phase 2)	519^{a}	544^{ab}	581^{bc}	593^{c}	24	NS	0.001	NS
Day 0-28 (Overall)	362^{a}	384^{a}	425^{b}	439^{b}	10	0.2	0.0002	NS
Daily Feed Intake (g/day)								
Day 0-7	148^{a}	167^{ab}	179^{b}	182^{b}	8	0.2	0.005	NS
Day7-14	350	347	390	404	20	NS	0.05	NS
Day 14-21	571	599	633	626	31	NS	0.1	NS
Day 21-28	885	907	927	935	32	NS	NS	NS
Day 0-14 (Phase 1)	249^{a}	257^{a}	284^{ab}	300^{b}	11	NS	0.006	NS
Day 14-28 (Phase 2)	728	753	780	780	29	NS	0.1	NS
Day 0-28 (Overall)	489^{a}	505^{a}	532^{a}	538^{b}	24	NS	0.02	NS
Gain: Feed								
Day 0-7	0.68^{a}	0.72^{a}	0.89^{b}	0.89^{b}	0.06	NS	0.004	NS
Day7-14	0.84^{a}	0.92^{ab}	0.97^{b}	1.00^{b}	0.04	0.2	0.01	NS
Day 14-21	0.72^{ab}	0.71^{a}	0.76^{ab}	0.77^{b}	0.02	NS	0.01	NS
Day 21-28	0.71^{a}	0.73^{ab}	0.75^{ab}	0.75^{b}	0.02	NS	0.1	NS
Day 0-14 (Phase 1)	0.82^{a}	0.87^{a}	0.95^{b}	0.96^{b}	0.02	0.1	<.0001	NS
Day 14-28 (Phase 2)	0.71^{a}	0.72^{a}	0.75^{ab}	0.76^{b}	0.01	NS	0.01	NS
Day 0-28 (Overall)	0.74^{a}	0.76^{a}	0.80^{b}	0.82^{b}	0.01	0.2	<.0001	NS

- Result are LS Means for 12 pens per diet treatment, 4 pigs per pen in each phase
- NS = Not Significant (P > 0.2). Means in a row with different superscripts differ P<0.05

Table 4-7. Pig Growth Performance with Lipase or Copper Supplementation in Each Phase

Diet	No Lipase C CCu	1000U/kg Lipase CL CLCu	NRC Cu C CL	250ppm Cu CCu CLCu	SEM	P-Value Lipase	P-Value Cu
Body Wt. (kg)							
Day 0	5.92	5.96	5.94	5.94	0.19	NS	NS
Day7	6.84	6.95	6.73	7.06	0.21	NS	NS
Day14	9.25	9.53	8.93	9.84	0.27	NS	0.03
Day21	12.33	12.71	11.85	13.18	0.35	NS	0.01
Day28	16.95	17.49	16.37	18.06	0.43	NS	0.008
Daily Gain (g/day)							
Day 0-14 (Phase 1)	237	254	214	278	10.4	0.2	<.0001
Day 14-28 (Phase 2)	550	569	532	587	14.3	NS	0.0089
Day 0-28 (Overall)	394	411	373	432	10.3	0.2	0.0002
Daily Feed Intake (g/day)							
Day 0-14 (Phase 1)	267	276	253	290	9.1	NS	0.006
Day 14-28 (Phase 2)	754	767	741	780	17.8	NS	0.1
Day 0-28 (Overall)	511	521	497	535	11.5	NS	0.02
Gain: Feed							
Day 0-14 (Phase 1)	0.88	0.91	0.84	0.95	0.02	0.1	<.0001
Day 14-28 (Phase 2)	0.73	0.74	0.72	0.75	0.01	NS	0.0101
Day 0-28 (Overall)	0.77	0.79	0.75	0.81	0.01	0.2	<.0001

- Result are LS Means for 12 pens per diet treatment, 4 pigs per pen in each phase
- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- No Lipase = the diet treatments without lipase supplementation (C and CCu), 1000U/kg Lipase = the diet treatments with 1000U/kg lipase supplementation (CL and CLCu), NRC Cu = the diet treatments without copper supplementation (C and CL), 250ppm Cu = the diet treatments with 250ppm copper supplementation (CCu and CLCu)
- NS = Not Significant ($P > 0.2$).

Table 4-8. Pig Nutrients Digestibility of Each Diet Treatment in Each Phase

Diet	Control Diet (C)	C+ Lipase (CL)	C+ Copper (CCu)	C+ Lipase+ Copper (CLCu)	SEM	P-Value		
						Lipase	Cu	Lipase*Cu
Fat digestibility								
Phase 1 (day 0-14)	71.94[b]	65.37[a]	70.57[b]	72.81[b]	1.45	0.2	0.05	0.007
Phase 2 (day 14-28)	68.82[a]	73.31[b]	72.48[b]	67.07[a]	1.00	NS	0.2	<.0001
Nitrogen digestibility	80.31[b]	77.72[a]	82.45[c]	83.51[c]	0.67	NS	<.0001	0.014
Phase 1 (day 0-14)	80.49[a]	83.35[b]	83.88[b]	84.51[b]	0.53	0.004	0.0004	0.05
Phase 2(day14-28)								
Fiber digestibility								
Phase 1 (day 0-14)	57.02	62.49	62.79	64.19	3.53	NS	NS	NS
Phase 2 (day 14-28)	52.33[a]	63.45[b]	58.51[b]	51.81[a]	1.90	NS	0.2	0.0001
Phosphorous digestibility								
Phase 1 (day 0-14)	51.38[a]	47.58[a]	57.97[b]	57.10[b]	1.50	0.1	<.0001	NS
Phase 2 (day 14-28)	46.85[a]	55.88[b]	55.07[b]	56.21[b]	0.95	<.0001	0.0002	0.0005

- The results are LS Means for 12 pens per diet treatment in each phase, 4 pigs per pen in each phase.
- Digestibility was determined by using TiO2 as the marker.
- NS = Not Significant ($P > 0.2$). Means in a row with different superscripts differ $P<0.05$

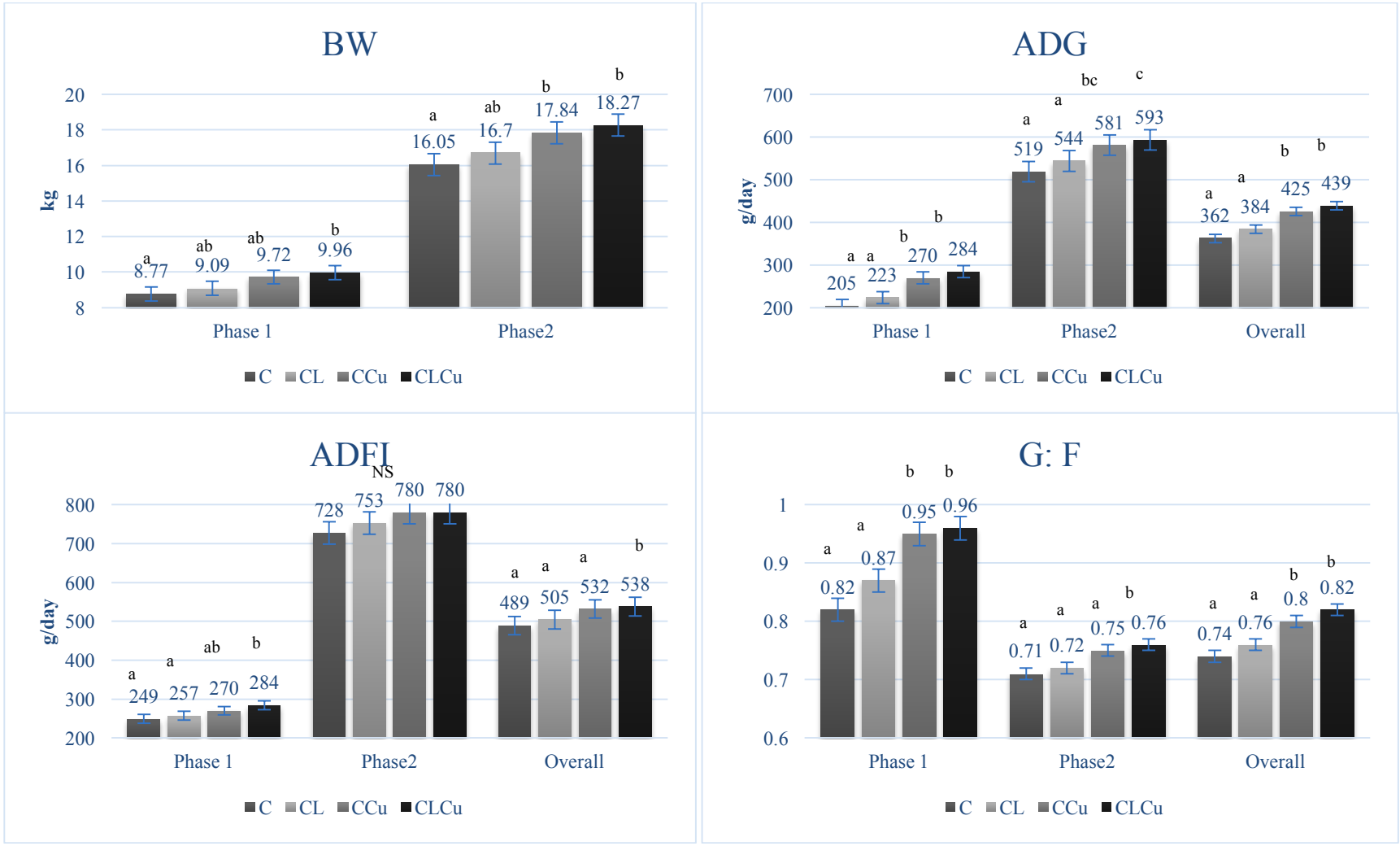

Figure 4-1. The Growth Performance of Pigs on Each Diet in Each Phase

- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Means in a phase group with different superscripts differ P<0.05

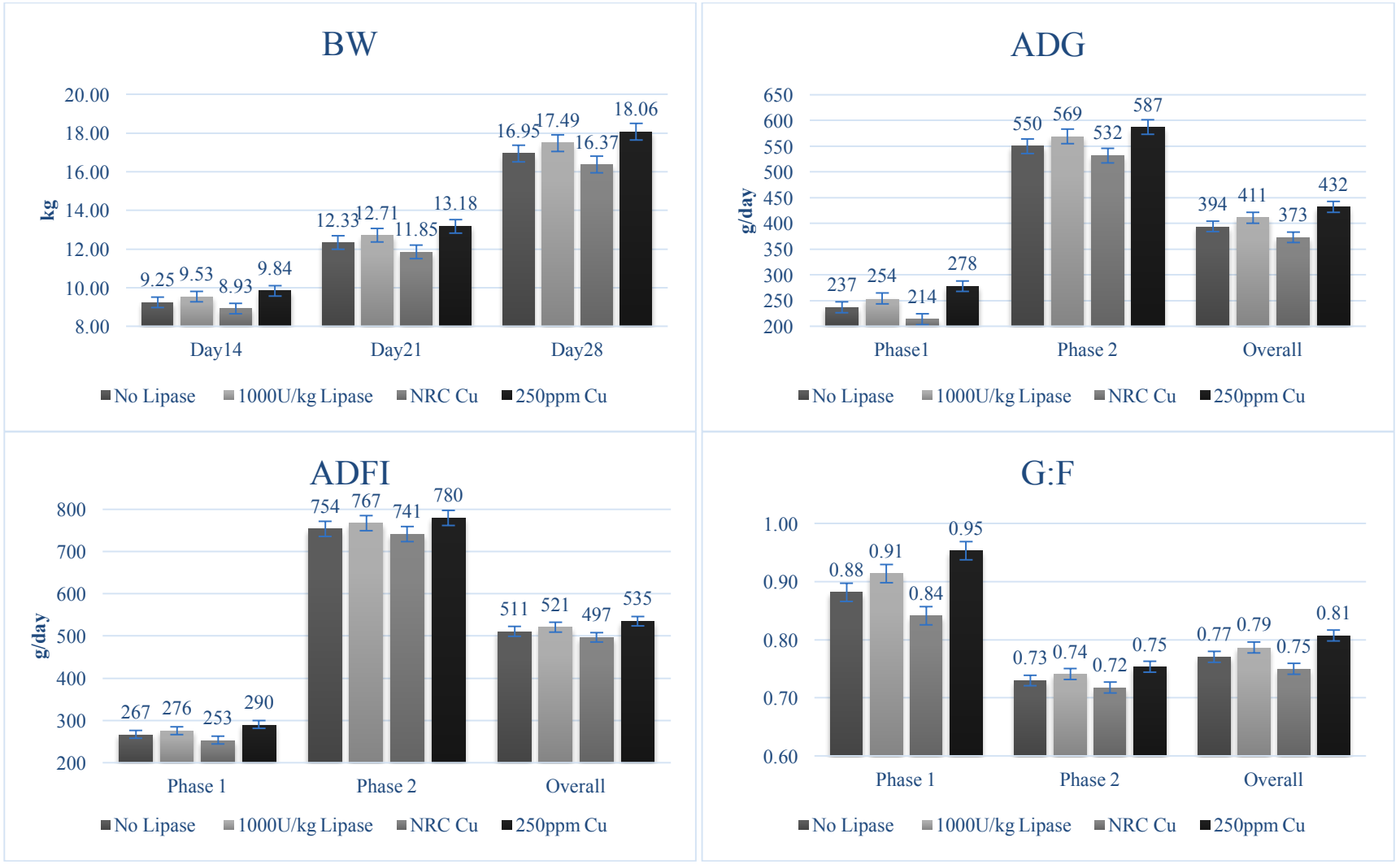

Figure 4-2. The Growth Performance of Pigs on Lipase or Copper Supplementation in Each Phase

- No Lipase = the diet treatments without lipase supplementation (C and CCu), 1000U/kg Lipase = the diet treatments with 1000U/kg lipase supplementation (CL and CLCu), NRC Cu = the diet treatments without copper supplementation (C and CL), 250ppm Cu = the diet treatments with 250ppm copper supplementation (CCu and CLCu)
- Means in a phase group with different superscripts differ $P<0.05$

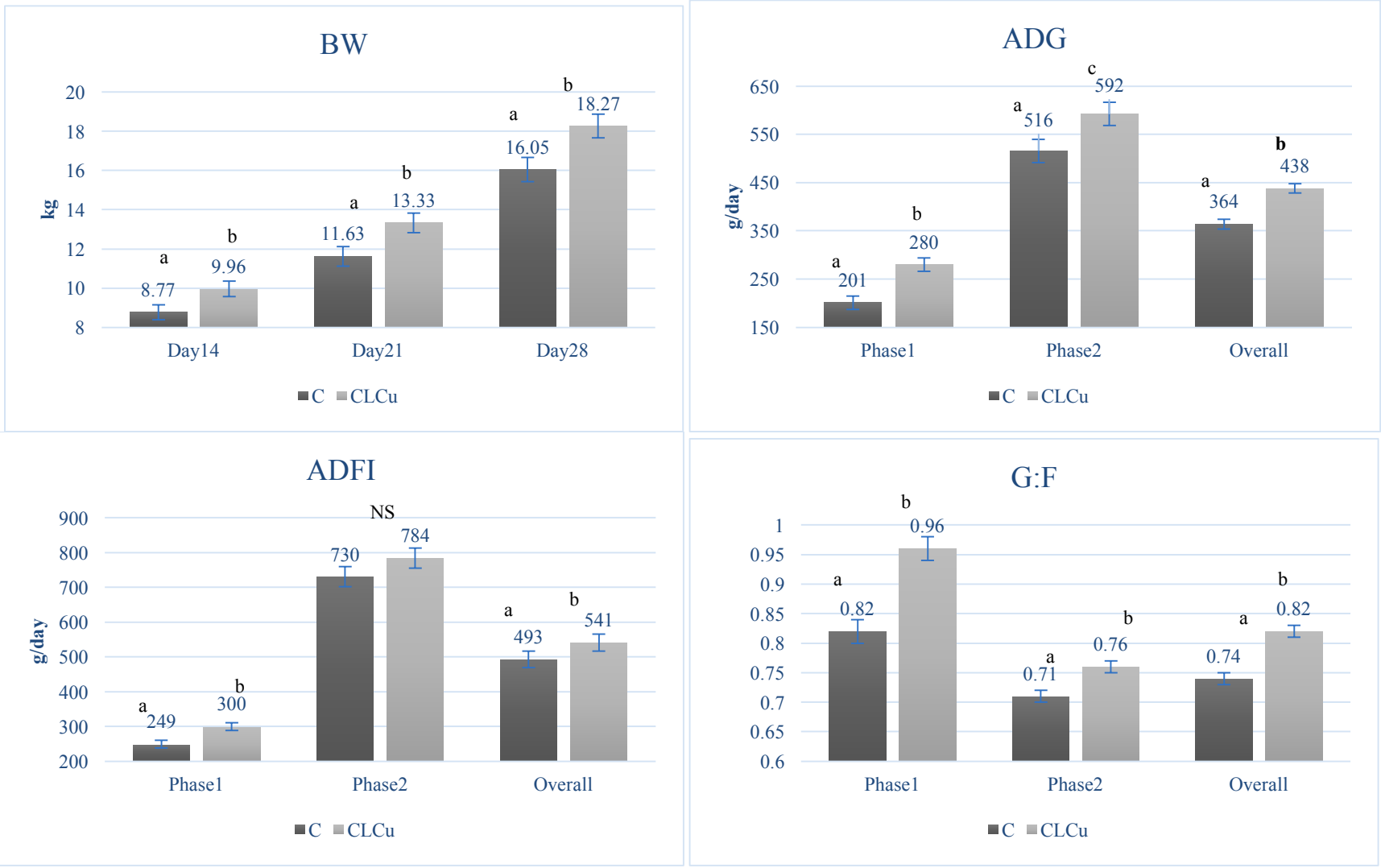

Figure 4-3. The Growth Performance of Pigs on C and CLCu Diets in Each Phase

- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Means in a phase group with different superscripts differ $P<0.05$

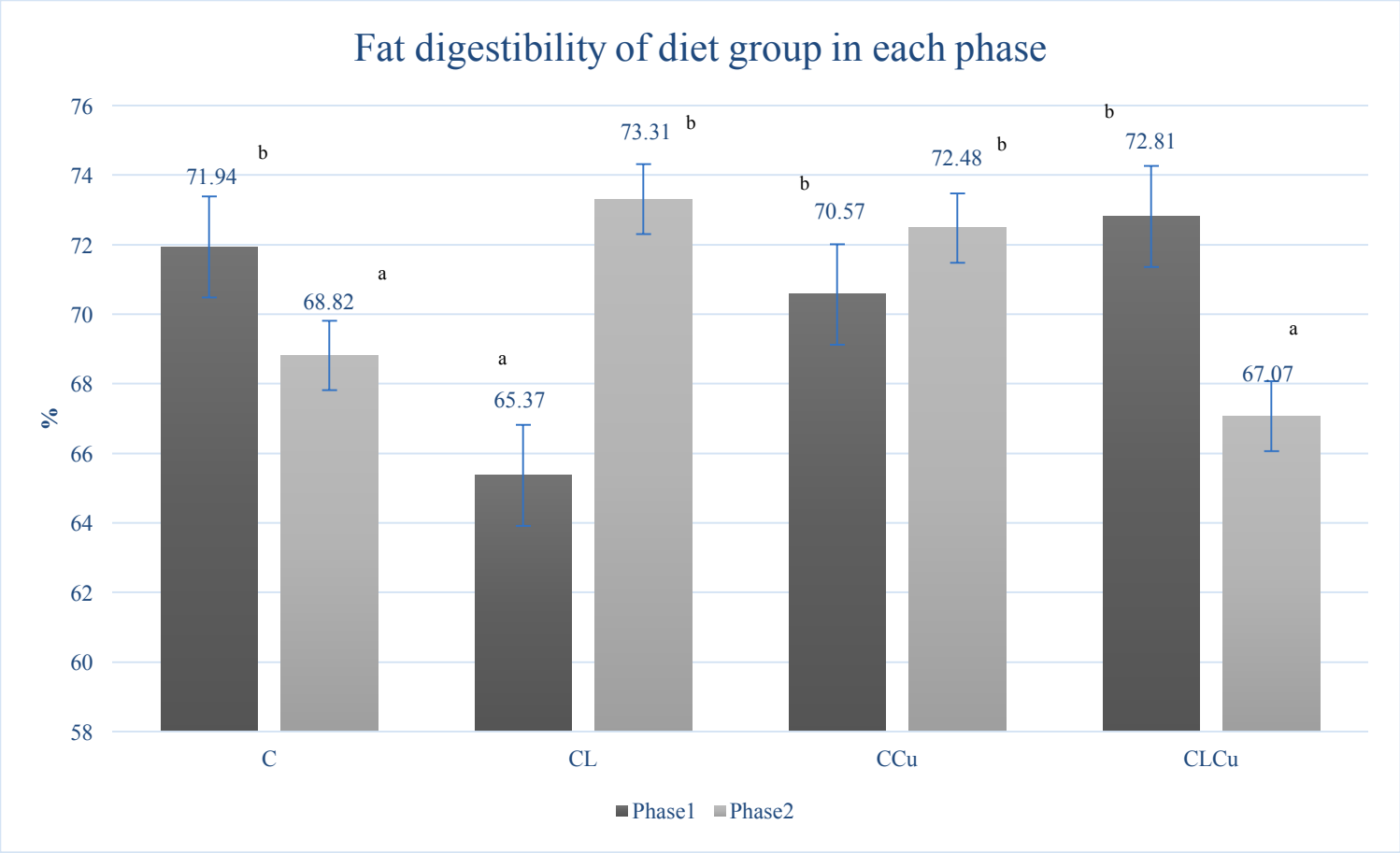

Figure 4-4. Fat Digestibility of Different Diet Treatment on Each Phase

- C= Control diet, CL= Control diet +1000U/kg Lipase, CCu= Control diet +250ppm Cu, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Means in a same phase group with different superscripts differ $P<0.05$

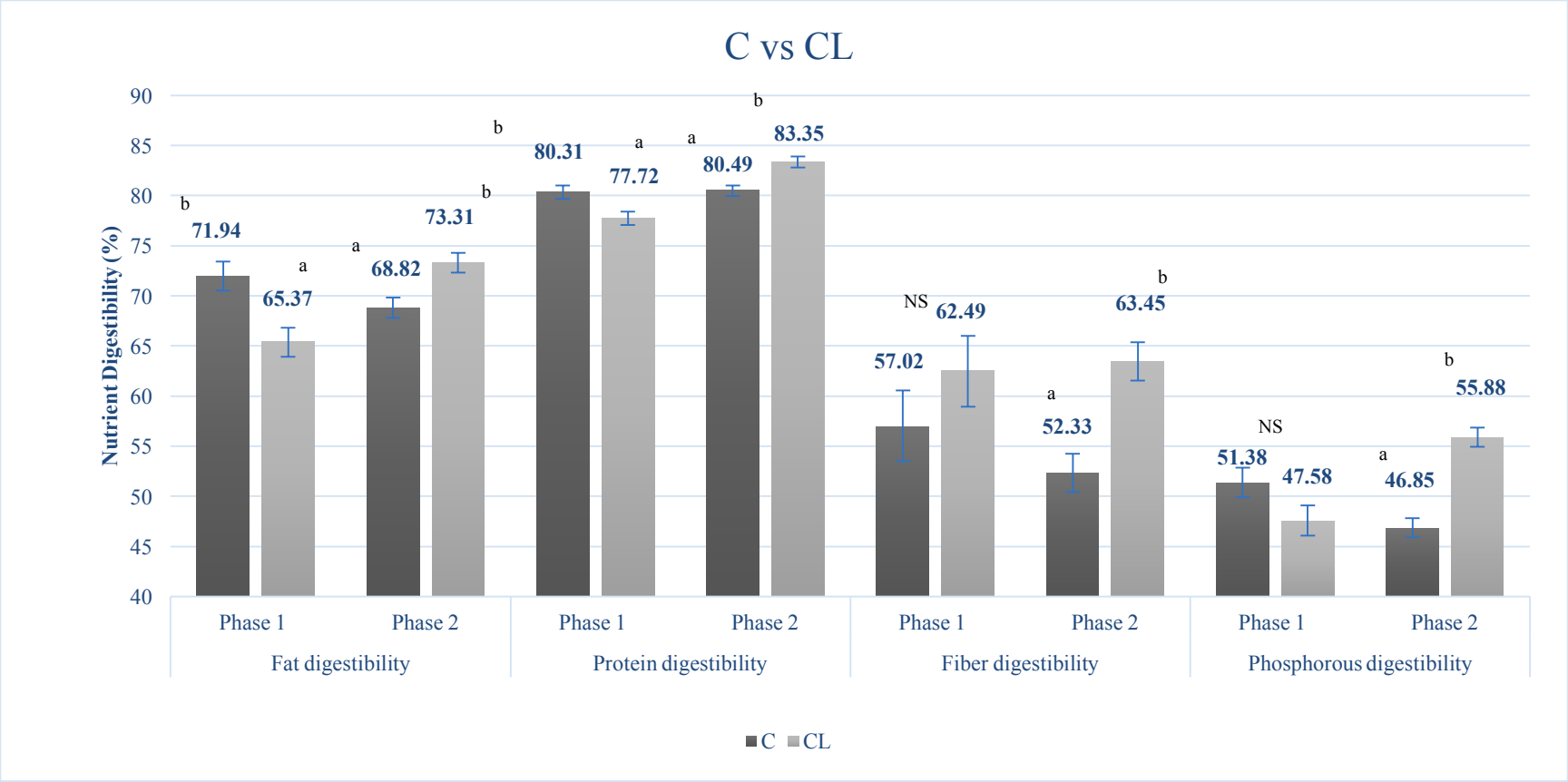

Figure 4-5. Nutrient Digestibility of C and CL Diets in Each Phase

- C= Control diet, CL= Control diet +1000U/kg Lipase
- Means in a same phase group with different superscripts differ P<0.05

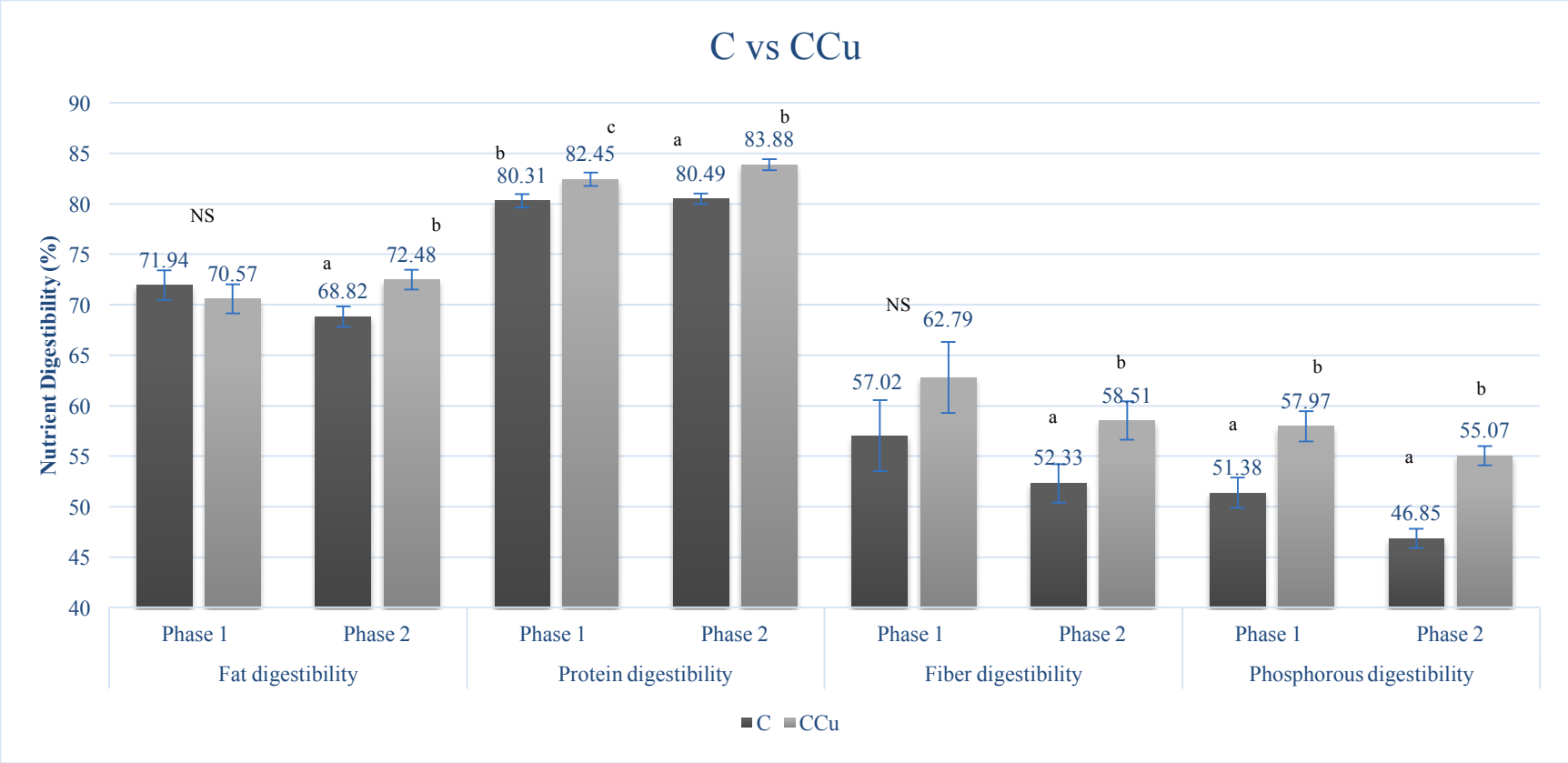

Figure 4-6. Nutrient Digestibility of C and CCu Diets in Each Phase

- C= Control diet, CCu= Control diet +250ppm Cu
- Means in a same phase group with different superscripts differ P<0.05

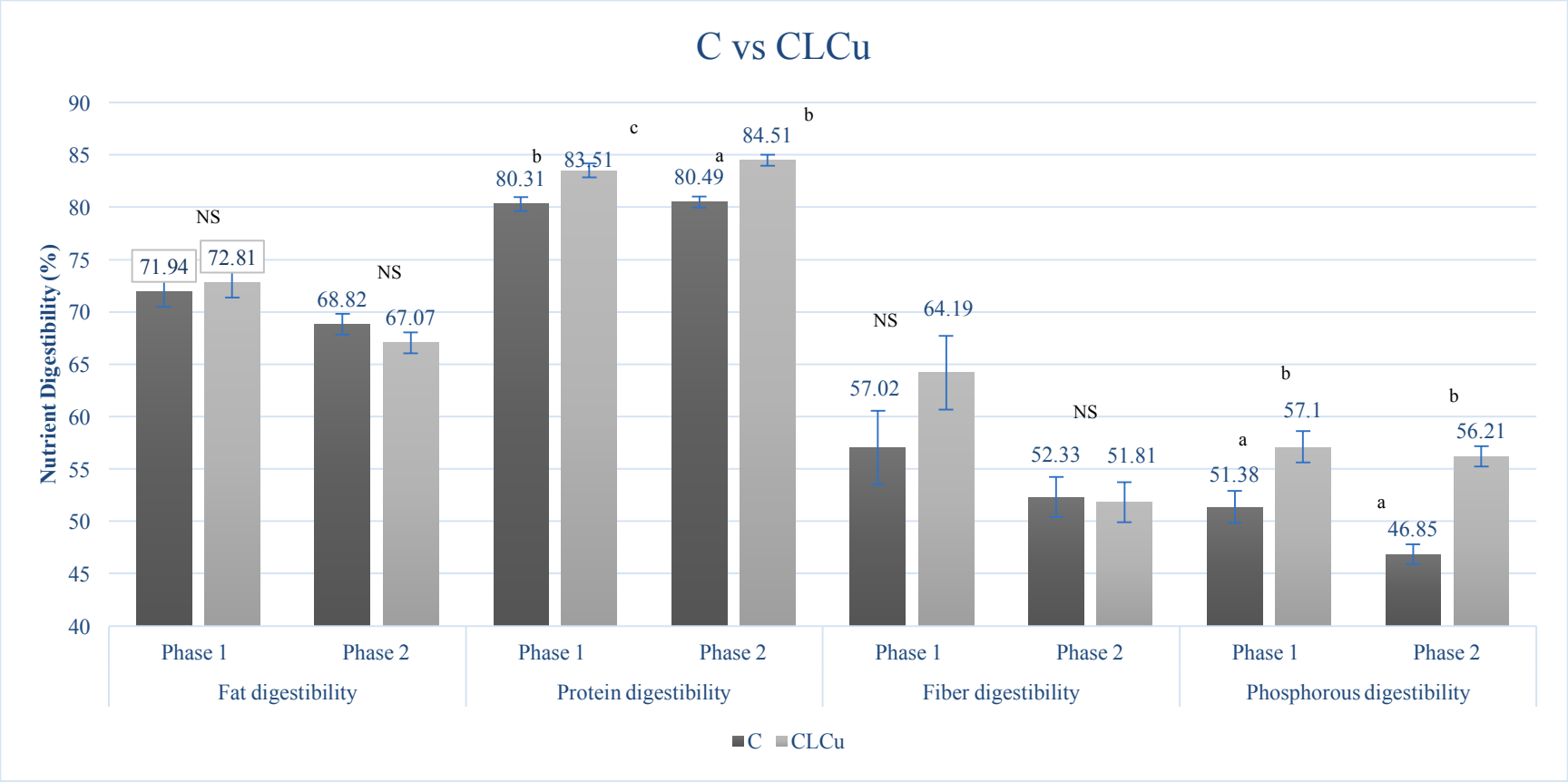

Figure 4-7. Nutrient Digestibility of C and CLCu Diets in Each Phase

- C= Control diet, CLCu= Control diet +1000U/kg Lipase +250ppm Cu
- Means in a same phase group with different superscripts differ P<0.05

CHAPTER 5

CONCLUSIONS

Exogenous enzymes have been widely used by the feed industry, as they can improve animals' feed utilization and further decrease the feed cost. Lipase is the important enzyme in fat digestion and has been shown to have a positive effect on growth performance in a few studies. In the present study, there were no significant results on growth performance or nutrient digestion. However, the lipase supplement had potential to improve growth performance in experiment 2. The negative effect on the growth performance in phase 1 and the fat digestibility in phase 2 might be due to the lipolysis effect or the diet designs. Therefore, the energy concentration difference between positive and negative control diets should be greater and more lipase concentration levels in the diets should be tested. Also, larger sample size of animal experiment should be conducted in the future. Copper has been shown to have a positive effect on improving endogenous enzyme activity. In the present study, although copper showed an effect on growth performance and N and phosphorous digestibility, copper's effect on improving exogenous lipase activity was not determined. Vitro studies on testing lipase bioactivities in the diets and the effect different levels of copper on exogenous lipase are needed in the future study.