Comparison of crystalline lysine and intact lysine used as a supplement in practical diets of Channel catfish (*Ictalurus Punctatus)* and Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Lysine is an essential amino acid commonly deficient in practical diets especially those contain high levels of plant protein ingredient, resulting in reduced growth and feed efficiency of aquatic animals. Supplementation of lysine in the formulated diets is essential to improve the nutritional values of feed. In general, there are two popular ways to improve the levels of essential amino acid in the diets, by feeding mixtures of complementary proteins and by supplementing deficient proteins with crystalline amino acids. Historically, crystalline lysine has been used commercially to improve the amino acid balance and consequently the nutritional profile of the diets. However, the use of crystalline lysine has been observed to cause reduced growth and feed efficiency of fish in some studies related to palatability, pH change, leaching and rapid uptake. The introduction of intact lysine via high lysine corn protein concentrate, which contains high concentrations of lysine and other essential amino acids, is a potential alternative to crystalline lysine. The study was conducted using channel catfish and tilapia to evaluate the production performance of these fish to increasing levels of lysine from crystalline and intact lysine supplemented in practical diets. A practical lysine deficient basal diet was developed using corn protein concentrate (Empyreal® 75) as a primary protein source. To this diet either graded levels of crystalline lysine or similar levels from a high lysine corn protein concentrate (Lysto™) were used, thus allowing the comparison of both source. The results of this study indicated that channel catfish and tilapia have positive performance with increasing lysine supplementation up to the requirement. Fish fed with the higher levels of lysine had the

highest weight gain and lowest feed conversion ratio. The regression analysis on the increased level of inclusion of crystalline lysine and intact lysine showed a general trend with parallel increases. Based on the growth data obtained from this study, it can be concluded that the effectiveness of using intact lysine via high lysine corn protein concentrate as a lysine supplement is not significantly different from crystalline lysine. Thus, the high lysine corn protein concentrate is a feasible ingredient for aquaculture feeds, which can be used to partially substitute soybean meal without addition of crystalline lysine.

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CHAPTER I

INTRODUCTION

Global fish production has expanded steadily in the last five decades with food fish aquaculture production increasing at an average annual rate of 6.2% in the period 2000–2012 (FAO, 2014). In fact, aquaculture has achieved national and international significance with total fish supplies accounted for 42.2% of total fish produced by capture fisheries and aquaculture in 2012 (FAO, 2014). Given the state of world's fisheries, expansion of aquaculture production with more intensive practices is necessary to meet the future demand for seafood and fisheries product.

Similar to livestock and poultry production, nutrition plays a very important role in semi-intensive and intensive aquaculture farming practices. According to FAO (2014), more than two thirds of aquaculture production was achieved through the use of artificial feeds. Although better understanding in aquatic animal nutrition and ingredient processing has recently created more benefit for aquaculture development, the continuously increasing price of feed ingredients, particularly for carnivorous species, seems to be a challenge for the industry.

Feed costs often account for 50% of total variable costs of production. Protein is considered the single most important component of aquatic feeds with its supplements generally constitute the highest cost ingredients especially for carnivorous and marine fish, which tend to have higher protein requirements (Wilson, 2002). Historically, fish meal has been the protein source of choice in commercial feed formulations of aquatic animals because its suitable digestibility, palatability, as well as the ability to provide all of the dietary essential amino acids required for growth of fish (Abdelghany, 2003). Aquaculture industry is estimated to have accounted for 73% of global fishmeal production and 71% of fish oil consumption by 2010

(International Fishmeal and Fish Oil Organization, 2013). However, according to FAO (2014), the price of fishmeal has increased continuously from about US$ 600 per metric ton to approximately US$ 1,900 per metric ton during the period 2003-2013. As a consequence, the use of other alternative protein ingredients from cheaper sources, such as byproducts from agriculture, fisheries or plant protein ingredients, has become more cost appealing to the aquaculture industry.

Plant-based proteins are considered the most likely candidates for fish meal replacement owing to its consistent quality, worldwide availability, sustainability and relatively low cost (Watanabe, 2002). Indeed recently, studies have shown that economical feeds can be formulated successfully with very high levels of plant-based protein ingredients as long as the feed formulation is meeting all the essential amino acid requirements of the animal (Kaushik et al., 1995; Lee et al., 2002, Barrow et al., 2007, Rodehutscord et al., 1995, Silva et al., 2009).

Channel catfish and tilapia are two primary food fish species cultured in the US. Similar to other species of growing animals, alternatives to high-cost protein supplements in formulated diets for channel catfish and tilapia are desirable to reduce feed costs and increase profits in modern farming operations. In recent years, manufacturers have been focusing on using plant proteins in least-cost diet formulations, which typically contain at least 90% plant products. Wilson (1994) and Francis (2001) suggested that it is very important to consider the appropriate level of carbohydrate for application as well as anti-nutrients in plants that may cause adverse effects on animal health. Gatlin (2007) also demonstrated that although the use of plant protein ingredients offers considerable potential with a content of comparable crude protein to fish meal, they may be less digestible and deficient in one or more of the ten essential amino acids.

Lysine is an essential amino acid that is commonly low in plant protein ingredients, notably cereal grain byproducts, such as corn gluten meal and wheat gluten (Hardy and Barrows, 2002; Gatlin et al., 2007). This is critical because lysine is the essential amino acid with the highest concentration in the carcasses of many fish species (Ahmed and Khan, 2004). Building upon the concept of the "Liebig barrel", when animals are given feeds deficient in lysine, the body cannot use other amino acids effectively. In addition to this, lysine is carnitine biosynthesis, which is required for normal lipid oxidation in the mitochondria (Harpaz, 2005). The deficiency of lysine in the feed will result in reduced growth and feed efficiency of fish (NRC, 2011).

Quite often lysine supplementation is required in plant-based diets for normal growth of aquatic species. Studies about lysine requirements in tilapia and channel catfish have been conducted with the use of dose–response experiments and utilized graded supplementation. According to Santiago and Lovell (1988), the dietary lysine requirement of 14.3 g/kg (5.1 g/100 g crude protein) was recommended for Nile tilapia fingerlings. The digestible lysine dose of 15.4 g/kg (5.5 g/100 g of digestible protein) was applied successfully for Nile tilapia fingerlings (Bomfim et al., 2010), which is close to the value previously found by Furuya et al. (2006), who estimated the requirement of digestible lysine of 14.3 g/kg (5.4 g/100 g of digestible protein) for the same fish species. In channel catfish, studies were conducted to assess the lysine requirement and evaluated the effects of feeding lysine-supplemented diets. Robison et al. (1980) indicated that the lysine requirement for fingerling channel catfish was approximately 1.5% of the dry diet or 5.0% of the dietary protein, which confirmed their previously reported lysine requirement for channel catfish at 5.1% of crude protein.

In aquafeeds, essential amino acids can be balanced by mixing protein sources with different amino acid profiles or using crystalline amino acids. Crystalline amino acids have been used commercially as cost-effective supplements to overcome the deficiencies of individual amino acids in terrestrial animals (Standing committee of Agriculture, 1987; NRC, 1994). Recently, the increased utilization of alternative protein sources with “imperfect” essential amino acid profiles due to continuously increased fish meal prices has resulted in the increased use of crystalline amino acid to meet the requirement of fish. However, the efficacy of crystalline amino acids in aquatic animal is still debatable. There are some disadvantages of using crystalline amino acids related to palatability, pH change, leaching and rapid uptake. Several studies have demonstrated that crystalline amino acids appear to be utilized with lower efficiency than essential amino acids supplied by intact protein due to faster absorption in the gastrointestinal tract compared to protein-bound amino acids (Deshimaru, 1976; Yamada et al., 1981a; Kaushik and Dabrowski, 1983; Murai et al., 1987; Cowey and Walton, 1988; Tantikitti and March, 1995; Zarate and Lovell, 1997; Zarate et al., 1999; Fox et al., 2006). In addition to this, the results from other experiments also indicated that the efficiency of using crystalline amino acids as supplements has been affected by pH with the confirmation from Nose (1974) and Murai (1981) that cyprinids and shrimp showed positive performance with the adjustment of pH when using feed with the high level of crystalline amino acids. Fox (2006) demonstrated that water stability of the diet and feeding behavior of the animal could also affect efficiency of crystalline amino acid utilization with the observation of poor ability to utilize crystalline amino acids in crustaceans.

In recent years, adaptation of new plant biotechnology has allowed the introduction of high lysine corn protein concentrate (Lysto^{TM}, Cargill) that will make the same contribution as feasible with intact lysine sources to substitute relatively expensive crystalline lysine in the practical diets of aquatic animal. This new product contains a high concentration of methionine, lysine and other essential amino acids and can improve the amino acid balance and, consequently, the nutritional profile of the diet. This ingredient's nutritional profile, on an as-is basis, contains about 75.8% protein, 5.3% lysine and 1.8% methionine compared to the conventional corn protein concentrate with about 76.2% protein, 1.1% lysine and 1.7% methionine. In addition to this, the prices of corn protein concentrate and high lysine corn protein concentrate are about US$ 740 per metric ton and US$ 817, respectively. The use of one metric ton of high lysine corn protein concentrate will provide 42 kg higher of lysine than the use of corn protein concentrate with an extra payment of US$ 77 (US$ 1.8 per kilogram lysine). Given the debate over the use of crystalline amino acids and the potential benefits of using high lysine corn protein concentrate, this research sought to evaluate the potential of this new high lysine ingredient as compared to traditional crystalline supplements.

Two specific objectives are summarized as follows:

1. Test the production performance (final weight, weight gain, survival, feed conversion ratio) and apparent net protein retention of channel catfish and tilapia fingerlings to increasing levels of lysine from crystalline and intact lysine supplemented in practical diets.
2. Compare the effectiveness of high lysine corn protein concentrate (Lysto™) as a lysine source replacing corn protein concentrate (Empyreal® 75) and compare its performance against purified lysine to maintain optimum growth in channel catfish and tilapia fingerlings.

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CHAPTER II

COMPARISON OF CRYSTALLINE LYSINE AND INTACT LYSINE USED AS A SUPPLEMENT IN PRACTICAL DIETS OF CHANNEL CATFISH (*Ictalurus punctatus)*

Abstract

A growth trial was conducted using channel catfish, *Ictalurus punctatus,* fingerlings to evaluate the relative performance of fish to increasing levels of lysine from crystalline and intact lysine supplemented in practical diets. A high lysine corn protein concentrate (Lysto^{TM}, Cargill) was used as the intact lysine source. The basal and test diets were designed to be isonitrogenous and isoenergetic containing 32% protein and 8% lipid using primarily plant-based protein sources. Nine dietary treatments with four replicates were assigned randomly in which the first five diets were designed to contain increasing levels of dietary lysine. Analyzed values of 1.21%, 1.34%, 1.45%, 1.56%, 1.72% using a crystalline lysine supplement were tested. The remaining diets were formulated using increasing levels of high lysine corn protein concentrate, which diets were analyzed to contain 1.40%, 1.57%, 1.77%, 1.91% lysine. A total of 780 fish were randomly stocked into 36 aquaria with 20 fish per/tank. Fish were fed based on percent body weight twice a day for a 10-week period. The effects of different levels of lysine on growth rate, feed conversion ratio and protein retention were studied. The results obtained after ten weeks indicated that channel catfish have positive performance with increasing lysine supplement. Based on the growth data obtained from this experiment, it can be concluded that the effectiveness of using high lysine corn protein concentrate as a lysine supplement is not significantly different from that of purified lysine.

1. Introduction

Farming of channel catfish *Ictalurus punctatus* is the most economically valuable aquaculture industry in the USA (Tucker 1996). Commercial production of channel catfish developed in the southern USA during the late 1950s and early 1960s. Mississippi, Alabama, Arkansas, and Texas are the top four channel catfish–producing states, contributing over 94% of U.S. total sales (NASS, 2015). The US farm-raised catfish industry is, however, severely threatened by external market competition from less expensive imported species. From 2005 to 2013, the percentages of channel catfish sales have declined by 60% of the market share for frozen catfish and catfish-like fillet products in the U.S while imported frozen catfish-like fillet products have increased from 20% to 80% (Hanson and Sites, 2014). Much of this decline relates to the higher domestic catfish fillet price and to catfish feed prices that increase the cost of producing domestic farm-raised catfish. U.S. farm-raised catfish producers have been struggling to reduce production costs maintaining an optimal nutritional profile of diets.

Given relatively low cost alternative feed ingredients, the utilization of plant protein as a partial replacement for fishmeal is considered a promising protein source. However, high level of plant protein inclusion in aquafeeds might cause severe essential amino acid deficiency in feed, resulting in poor growth and feed utilization rate of aquatic species (Abimorad et al., 2009). The study of Dias (1999) demonstrated that the use of maize gluten and soy protein concentrate significantly reduced the feed intake and growth in European seabass (*Dicentrarchus labrax* L.) compared to fish meal utilization. Burel (2000) experienced the same results for turbot fed plant protein sources as fish meal substitutes. The reduced growth was also observed on channel catfish fed 70% corn distiller's dried grains with solubles (CDDGS) (Webster, 1991) compared to those of the diets using 0% and 35% in the diets.

Lysine is generally considered to be the first limiting amino acid in diets of channel catfish with high level of plant protein inclusion. According to Li (2004), if feeds are formulated to meet a minimum lysine requirement, the requirements for all other amino acids are met. In fact, studies about the use of plant protein sources as fish meal replacement conducted on channel catfish showed better response of this species with lysine supplementation. Inclusion of 70% CDDGS supplemented with lysine (0.4%) increased growth performance and feed utilization efficiency of channel catfish relative to diets containing the same level of CDDGS without added lysine (Webster, 1991). Another study by Lim et al. (2009) suggested that, with lysine supplementation to the level equal to that of the control diet, 40% CDDGS can be included in channel catfish diet containing 8% fish meal without affecting growth and feed efficiency. In a pond production trial conducted in Mississippi, Robinson and Li (2008) reported that CDDGS could be used up to at least 30% when the diet was supplemented with lysine. Andrews and Page (1974) also confirmed that even though lysine is relatively abundant in soybean meal, the supplementation of soybean meal with crystalline lysine has been shown to enhance the growth and feed efficiency of channel catfish juveniles.

The utilization of crystalline amino acids has been shown to eliminate the adverse effects of using plant-based meal, as proven in Atlantic salmon (*Salmo slar*) (Anderson et al., 1993; Espe et al., 2007), red sea bream (*Pagrus major*) (Takagi et al., 2001), common carp (Nose, 1979), rainbow trout (*Oncorhynchus mykiss*) (Gaylord and Barrows, 2009). Wilson et al. (1977) and Robinson et al. (1980) also concluded that dietary crystalline lysine could be supplemented in purified form to eliminate the lysine deficiency in soybean meal. However, several results from other studies indicated that the efficiency of using crystalline lysine on growth performance of different fish species compared to intact lysine showed somewhat inconsistent results

(Thebault, 1985; Lumbard, 1997; Dabrowski et al., 2003; Peres and Oliva-Teles, 2005). Ambardekar (2007) described evidence that amino acids provided in purified crystalline form are not used as effectively as the amino acids obtained from intact proteins in the feed ingredients of channel catfish.

Recently, high lysine corn protein concentrate with about five times higher lysine content than conventional corn protein concentrate (5.3% versus 1.1%) was developed with the introduction of new plant biotechnology. It is possible that the combination of high lysine corn protein concentrate and soybean meal will attain the optimum lysine content in feed without crystalline lysine supplementation. Hence, the objectives of this study were to evaluate the relative performance of channel catfish to increasing levels of lysine from crystalline and intact lysine supplemented in practical diets. A high lysine corn protein concentrate (Lysto™, Cargill) was used as the intact lysine source.

2. Material and method

2.1 Experiment Diets

The basal diets for channel catfish were designed to contain 32% protein and 8% lipid using primarily plant-based protein sources. All diets across the substitution levels were free of marine protein sources except for menhaden fish oil, the only marine ingredient, to ensure palatability and provide long chain polyunsaturated fatty acids. The basal and test diets were formulated to meet the nutritional requirements of channel catfish in which soybean meal, cornstarch and menhaden fish oil were used to keep the isonitrogenous and isolipidic characteristics of the diets. The first five diets contained the corn protein concentrate, Empyreal® 75, at 12.0 percent, with an increasing inclusion of crystalline L-lysine (0.0, 0.15, 0.30, 0.45 and

0.60 percent of the diet) (Table 1). The total lysine was increased by the supplementation of crystalline lysine from 1.2 up to 1.7 percent of the diet (Table 3). Whereas, corn protein concentrate enriched with L-lysine (Lysto™) was used as a substitution of the corn protein concentrate (Empyreal® 75) in the last four diets at the following rates: 9:3, 6:6, 3:9, and 0:12 in percentage of the diet (Table 1). The lysine levels in these diets ranged from 1.4 to 1.9 percent of the diet (Table 3). The test diets were then prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 minutes. Hot water was then added to the mixture in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 4-mm diameter die in a meat grinder, dried at 70°C to a moisture less than 10%, and stored in the freezer at -20° C until used. A sample of each feed was collected and analyzed for proximate (Table 2) and amino acid compositions (Table 3) following AOAC (1995) procedures by the Experiment Station Chemical Laboratories, University of Missouri, Columbia.

2.2 Culture Methods

Channel catfish *Ictalurus punctatus* were obtained from E.W. Shell Fisheries Research Center, Auburn University, Auburn, AL. The trial utilized 9 dietary treatments with 4 replicates in each treatment. Juvenile catfish were reared two weeks in the nursery tanks before stocking into experimental aquaria. Juvenile channel catfish (initial weight 7.58 ± 0.05g) were randomly stocked into 36 aquaria, which are components of a 3,800-L indoor recirculation system, at 20 fish per aquarium. Samples of fish from the initial stocking were retained for later protein analysis and later used for protein retention determination.

Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation using air stones in each aquarium and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen and water temperature were measured twice a day using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH) while pH, TAN and nitrite-nitrogen (NO_2-N) were measured twice per week. Photoperiod was set at 14 h light and 10 h dark. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were maintained within acceptable ranges for channel catfish at 5.29±0.49 mg/L, 28.38±1.31°C, 2.20±0.49 ppt, 7.24±0.02, 0.10±0.08 mg/L, and 0.04±0.02 mg/L, respectively (Table 4).

Diets were offered to fish at 3-5% body weight daily at 08h00 and 16h00 according to fish size and divided into two equal feedings. Fish were weighed every two weeks. Daily feed rations were calculated based on % body weight and held constant for all treatments at a given time. The rations were adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine final weight, weight gain, survival and feed conversion ratio.

Crude protein in initial and final whole body samples were tested by the Kjeldahl method (Williams, 1984). Protein retention was calculated as follows:

Protein retention (%) = (final weight x final protein content) - (initial weight x initial protein content) x 100/protein intake.

2.3 Statistical Analysis

All data were subjected to a one-way analysis of variance to determine significant differences ($P < 0.05$) among the treatments, which was followed by Student-Neuman Keuls' multiple comparison test to distinguish significant differences among treatment means. The analysis of covariance ANCOVA was used to compare the two regression lines of channel catfish responses to the use of crystalline versus intact lysine. All the data were analyzed using SAS (V9.4. SAS Institute, Cary, NC, USA).

3. Results

Channel catfish in this study had better response for growth performance with higher lysine supplement. There were significant differences ($P<0.05$) among mean final weight (FW), weight gain (WG), feed conversion ratio (FCR), and apparent net protein retention (ANPR) of fish receiving various dietary treatments (Table 5). The fish fed diets with higher lysine supplement had higher mean FW, WG, ANPR and lower FCR. Fish fed diet 1 (1.21% lysine) without added lysine had the lowest mean FW, WG, ANPR and highest FCR. Mean FW, WG, and ANPR of fish in this treatment were significantly lower than those of diets 4, 5, 8, and 9, which contained high lysine levels at above 1.50 percent of the diets. There were no significant differences observed among mean FW, WG, ANPR of fish fed diet 1 and the two lowest inclusion levels of lysine in the crystalline and intact diets even. The same situation was found when analyzing the mean FCR, ranging from 1.67 down to 1.20 ($P<0.0001$; PSE=0.0468).

Analysis of covariance of mean FW, WG, FCR, and ANPR of channel catfish receiving various dietary treatments is presented in Table 6. No combined effects of lysine sources and lysine levels were observed on mean FW, WG, FCR of channel catfish fed two different lysine sources. The slope, P value and intercept P were not significantly different ($P>0.05$) among FW, WG (Figure 1), FCR (Figure 3) of fish fed crystalline lysine and intact lysine. From the above results, it can be concluded that high lysine corn protein concentrate can be used as effectively as crystalline lysine by channel catfish.

However, combined effects of lysine sources and lysine levels were observed on mean ANPR of channel catfish fed two different lysine sources. Channel catfish fed crystalline lysine showed better response for protein retention than that of fish fed intact lysine with the adjusted mean value were 46.5% and 43.1%, respectively. The Y intercepts of the two regression lines for crystalline lysine and protein-bound lysine were significantly different ($P<0.05$). The regression line for catfish fed intact lysine was $Y=30.7X-2.6$ ($R^2=0.82$), and the line for catfish fed crystalline lysine was $Y=37.5X-9.4$ ($R^2=0.72$) (Figure 2).

4. Discussion

The substitution of fish meal with plant-based protein sources has shown that it is necessary to pay greater attention to satisfying dietary amino acid requirements in the formulation of channel catfish diets (Wilson et al. 1981). Essential amino acid requirements have been established for a number of fishes and shrimps. In channel catfish, various studies have determined the essential amino acid requirements as percentage dry matter with the value of arginine (1.2%), methionine plus cysteine (0.6%), histidine (0.4%), isoleucine (0.6%), leucine (0.8%), valine (0.7%), phenylalanine and tyrosine (1.1%), threonine (0.5%), tryptophan (0.1%) (Robinson et al., 1981; Robinson et al., 1980b; Harding et al., 1977; Wilson et al., 1980, Robinson et al., 1980a, Wilson et al., 1978). The studies about lysine requirement and the effects of feeding lysine-supplemented diets were also conducted on channel catfish. Robison et al. (1980) indicated that the lysine requirement for fingerling channel catfish was approximately 1.5% of the dry diet or 5.0% of the dietary protein, which confirmed the previously reported lysine requirement for channel catfish at 5.1% of crude protein. The basal diet in this present study met the minimum requirements of channel catfish for all essential amino acids except lysine (Table 3).

Supplementation of lysine in the formulated diets with high levels of plant-based protein is essential to improve the growth performance of channel catfish. In the present study, growth performance of channel catfish responded to different levels of lysine supplementation from both intact and crystalline lysine sources, showing significant improvement compared to the control diet without added lysine. These results are in agreement with the findings of Robinson et al. (1980) who reported that channel catfish were able to use crystalline lysine effectively as a supplement in diets containing 30% crude protein, contrary to the prior studies of Andrews et al. (1977) who reported that channel catfish were unable to use crystalline amino acid. However, according to the present study results, the supplementation of increasing lysine levels up to 1.91 % of the diet did not result in any reduced growth or feed efficiency of channel catfish, which were observed before by Robinson (1980) at a lysine concentration of 1.87%. The results of that study demonstrated that fish fed diets with 1.87% lysine grew the same as those fed 1.21% but less than those fed 1.54% lysine which was consider the lysine requirement for channel catfish. However, Robinson (1980) indicated that this depression at the higher lysine level was unexpected since levels of lysine exceeding the requirement have not been shown to depress growth in catfish in prior studies. Although in the present study channel catfish fed diets with 1.91% lysine had highest percent weight gain, no significant differences among mean final weights of fish in this experiment were observed with fish fed 1.56%, and 1.77% lysine. Thus, it is possible that lysine level of 1.56% present in the diets was sufficient for growth. This result is close to the result observed by Robinson (1980) with the lysine requirement suggested at 1.51% of diet. The same patterns were observed on the feed conversion ratios, showing better responses of catfish to increasing lysine supplementation. Channel catfish fed diets containing lysine below the requirement level at 1.51% (Robinson, 1980) did not use the feed effectively. Channel catfish fed the control diet with 1.21% lysine experienced the highest mean feed conversion ratio at around 1.6. In contrast, the mean feed conversion ratios were much lower in the diets containing lysine level at or above the requirement. The results showed that no significant differences were found between the mean feed conversion ratios of channel catfish fed diet containing 1.56%, 1.72%, 1.77%, 191% even though fish fed the highest lysine level at 1.91% had the lowest mean feed conversion ratio.

Investigations into the efficacy of intact lysine, high lysine corn protein concentrate as a replacement of crystalline lysine on growth performance of channel catfish have been conducted. In the present study, effects of lysine supplementation from two sources were not significant different even though the adjusted mean for the weight gain of the fish fed crystalline lysine was higher at 437% compared to the fish fed intact lysine at 420%. Similarly, earlier studies with rainbow trout (*Oncorhynchus mykiss),* Atlantic salmon (*Salmo salar* L.), and Asian seabass (*Lates calcarifer* Bloch) showed that crystalline amino acids were utilized as efficiently as those of intact protein origin in meeting essential amino acid requirements of fish (Murai et al., 1987; Kim et al., 1991; Espe and Lied, 1994; Rollin, 1999; Williams et al., 2001; Rollin et al, 2003; Espe et al., 2006). Perez-Jimenez (2014) has recently demonstrated that up to 50% protein-bound amino acids can be replaced by crystalline amino acids in diets for Senegalese sole juveniles without negative effects in fish performance. Yu et al. (2012) also indicated that the efficiency of lysine enhanced corn protein concentrate, as a substitution to purified lysine, appears comparable with crystalline lysine for shrimp *L. vannamei*.

Contrary to the results of this present study, Zarate (1997) reported that free lysine L-lysine HCl were utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish. Zarate et al. (1999) indicated that free lysine was absorbed faster than protein-bound lysine by channel catfish. Evidence from fish other than channel catfish, such as trout and hybrid striped bass, also suggests that amino acids in intact protein are utilized more efficiently than those provided in crystalline form, possibly because crystalline amino acids are absorbed and catabolized more rapidly than amino acids from intact proteins (Yamada et al., 1981; Sveier et al., 2001; Liu et al., 2002; El Haroun and Bureau, 2006; Hauler et al., 2007; Dabrowski et al., 2010). However, according to Amogh (2009), efficiency with which crystalline amino acid supplements are utilized for protein synthesis could be affected by the rate at which intact proteins in a mixture of dietary ingredients are digested. The utilization efficiency of dietary crystalline amino acid might be reduced when slowly digested proteins compose a major portion of the diet. Likewise, Gahl et al. (1995) found that the lysine utilization efficiency for growth was 0.81 in pigs fed soybean meal diets but only 0.68 in pigs fed a mixture of soybean meal and corn gluten meal. Nang Thu et al. (2007) also demonstrated that the lysine utilization efficiency for growth of rainbow trout varied with the plant protein source. Thus, the difference in digestibility of protein sources and the way in which amino acids are incorporated in the diets might be the reason for the difference in the results of this present study and those prior studies.

Other added benefits of lysine supplementation include improved protein levels of the carcass. In this present study, dietary lysine supplementation significantly increased protein retention of whole channel catfish body. Numerous studies with terrestrial (Noblet et al., 1987; Revington et al., 1992; Moran and Bilgili, 1990; Friesen et al., 1994) and aquatic (Yang et al., 2010; Cheng et al., 2003; Mai et al., 2006; Rodehutscord et al., 2000) animals have shown that supplementation of lysine to lysine-deficient diets resulted in increased protein retention.

Similarly, studies with marketable size of channel catfish illustrated that increasing the lysine inclusion of lysine-deficient diets caused increased crude protein and reduced fat content of fillets (Robinson, 1991, Munsiri and Lovell, 1993). The results from this present study support previous findings that the use of crystalline lysine as a supplementation showed positive response for protein retention of channel catfish. Webster (1992) observed that the protein content of channel catfish fed a diet with 90% CDDGS with added purified lysine was significantly higher in protein as compared to those fed the 90% CDDGS diets without lysine supplementation. Li (2012) revealed that channel catfish fed diets containing 30 and 40% WDDGS without lysine supplementation had significantly lower protein content than that of the control fish while no significant differences were observed in fishes fed the same diets with added lysine. However, according to the results of this present study, channel catfish fed crystalline lysine had better response for protein retention than fish fed protein-bound lysine from high lysine corn protein concentrate. The poor performance of channel catfish fed high lysine corn protein concentrate in this present study is an unexpected result since fish showed the same response for weight gain with the utilization of intact lysine and crystalline lysine.

5. Conclusion

Regardless of differences in protein retention, high lysine corn protein concentrate was utilized as efficiently in meeting the essential amino acid requirements of the channel catfish as those of crystalline form. The corn protein concentrate with dried L-lysine fermentation product is a feasible ingredient for aquaculture feeds which can be used to partially substitute soybean meal without addition of crystalline lysine.

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Table 1. Ingredient compositions (g 100 g^{-1} as-is) of nine experimental diets with increasing levels of lysine supplement from crystalline lysine and intact lysine formulated to contain 32% protein, 8% lipid and offered to juvenile channel catfish

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Soybean meal[1]	25.28	25.28	25.28	25.28	25.28	25.28	25.28	25.28	25.28
DDGS[2]	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Menhaden Fish oil[3]	4.82	4.82	4.82	4.82	4.82	4.81	4.80	4.79	4.82
Whole wheat[4]	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Corn starch[5]	10.12	10.12	10.12	10.12	10.12	10.10	10.08	10.06	10.12
Trace mineral premix[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[7]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CP dibasic[5]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lecethin[9]	0.50	0.50	0.50	0.50`	0.50	0.50	0.50	0.50	0.50
CPC[10]	12.00	12.00	12.00	12.00	12.00	9.00	6.00	3.00	12.00
HLCPC[10]	0.00	0.00	0.00	0.00	0.00	3.03	6.06	9.09	0.00
Methionine-DL[11]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
L-Lysine[15]	0.00	0.15	0.30	0.45	0.60	0.00	0.00	0.00	0.00
Glutamic acid[16]	0.60	0.45	0.30	0.15	0.00	0.60	0.60	0.60	0.60

[1]De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[2] Poet Dakote Gold Inc., Sioux Falls, SD, USA.
[3] Omega Protein Inc., Reedville, VA, USA.

[4] MP Biomedicals Inc., Solon, Ohio, USA.
[5] MP Biochemicals Inc., Solon, OH, USA.
[6] Trace mineral: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[7] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B_{12}, Inositol, Vitamin A acetate, Vitamin D_3, Vitamin E, cellulose
[8] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[9] The Solae Company, St. Louis, MO, USA.
[10] Empyreal® 75, Lysto™ Cargill Corn Milling, Cargill, Inc., Blair, NE, USA
[11] Aldrich-Sigma, St. Louis, MO, USA.

Table 2. Analyzed proximate composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to channel catfish

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Protein	30.80	30.96	30.9	30.76	31.47	30.98	31.21	30.9	30.68
Moisture	9.36	9.34	10.12	10.29	8.99	9.25	8.39	10.11	9.93
Fat	7.96	7.83	7.86	7.98	7.82	7.81	7.73	7.62	7.73
Fiber	3.21	3.46	3.07	3.28	2.91	3.22	3.3	3.31	3.41
Ash	4.08	4.06	3.99	4.02	4.06	4.09	4.14	4.1	4.05

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 3. Analyzed amino acids composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to channel catfish

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Taurine	0.10	0.11	0.11	0.11	0.11	0.10	0.11	0.11	0.11
Hydroxyproline	0.08	0.02	0.10	0.07	0.00	0.07	0.08	0.07	0.05
Aspartic Acid	2.39	2.44	2.39	2.38	2.49	2.39	2.40	2.37	2.37
Threonine	1.08	1.08	1.05	1.05	1.10	1.07	1.06	1.04	1.04
Serine	1.36	1.34	1.29	1.30	1.37	1.34	1.32	1.31	1.29
Glutamic Acid	6.59	6.44	6.14	6.01	6.17	6.43	6.45	6.39	6.34
Proline	2.28	2.29	2.18	2.20	2.29	2.23	2.21	2.17	2.15
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	1.09	1.11	1.07	1.09	1.13	1.10	1.09	1.10	1.08
Alanine	1.88	1.90	1.78	1.81	1.90	1.82	1.80	1.76	1.75
Cysteine	0.49	0.49	0.49	0.48	0.50	0.48	0.49	0.47	0.48
Valine	1.46	1.48	1.42	1.42	1.47	1.43	1.42	1.41	1.39
Methionine	0.61	0.62	0.62	0.60	0.64	0.63	0.62	0.60	0.61
Isoleucine	1.34	1.34	1.31	1.30	1.35	1.29	1.31	1.29	1.27
Leucine	3.50	3.50	3.30	3.32	3.50	3.33	3.33	3.21	3.20
Tyrosine	1.29	1.28	1.25	1.23	1.28	1.20	1.24	1.19	1.21

Phenylalanine	1.68	1.68	1.61	1.61	1.69	1.63	1.63	1.59	1.58
Hydroxylysine	0.09	0.12	0.02	0.08	0.07	0.10	0.10	0.09	0.09
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	1.21	1.34	1.45	1.56	1.72	1.40	1.57	1.77	1.91
Histidine	0.72	0.73	0.71	0.71	0.74	0.72	0.72	0.72	0.71
Arginine	1.52	1.54	1.50	1.50	1.55	1.52	1.51	1.52	1.51
Tryptophan	0.33	0.36	0.35	0.34	0.36	0.34	0.36	0.36	0.35

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 4. Summary of mean water quality values observed over the ten-week growth trial for channel catfish reared in a semi-closed recirculation system. Values represent the mean, standard deviation, minimum, and maximum readings.

Parameter(C)	Mean	Standard deviation	Minimum	Maximum
Channel catfish (semi-closed recirculation system)				
Dissolved oxygen (mg L^{-1})	5.29	0.49	3.59	6.57
Temperature (˚C)	28.38	1.31	21.70	29.50
Salinity (ppt)	2.20	0.49	1.10	3.60
pH	7.24	0.02	6.49	7.84
Total ammonia-nitrogen (mg L^{-1})	0.10	0.08	0.00	0.24
Nitrite-N (mg L^{-1})	0.04	0.02	0.00	0.06

Table 5. Mean response of channel catfish fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over a ten-week growth period[1].

Diet	Lysine level	Final weight (g)	Weight gain (%)	FCR	Survival (%)	ANPR
1	1.21	33.39[c]	345.64[d]	1.67[a]	100.00	34.72[e]
2	1.34	36.10[c]	378.08[d]	1.56[ab]	100.00	40.45[ed]
3	1.45	38.73[bc]	416.53[cd]	1.44[bc]	100.00	46.68[bcd]
4	1.56	44.16[ab]	480.53[bc]	1.31[cd]	100.00	52.14[abc]
5	1.72	46.40[a]	506.61[ab]	1.34[cd]	100.00	52.28[abc]
6	1.4	36.44[c]	377.79[d]	1.58[ab]	100.00	39.85[ed]
7	1.57	38.21[bc]	399.80[d]	1.53[ab]	100.00	44.84[cd]
8	1.77	45.87[a]	493.73[abc]	1.24[d]	100.00	53.56[ab]
9	1.91	50.35[a]	571.09[a]	1.20[d]	100.00	54.93[a]
P-value		<0.0001	<0.0001	<0.0001	NS	<0.0001
PSE[2]		1.9760	23.6430	0.0468	0.0000	2.0071

[1]Means in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Student Newman-Keuls' multiple range test.
[2]Pooled standard error.

Table 6. Analysis of covariance output of channel catfish fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over a ten-week growth period

	Adjusted mean least-squares means			
Lysine source	Final weight (g)	Weight gain (%)	FCR	ANPR (%)
Crystalline lysine	40.7	437.1	1.44	46.5
Intact lysine	39.0	414.0	1.50	43.1
Slope P value	0.58	0.70	0.97	0.26
Intercept P value	0.17	0.12	0.07	0.01

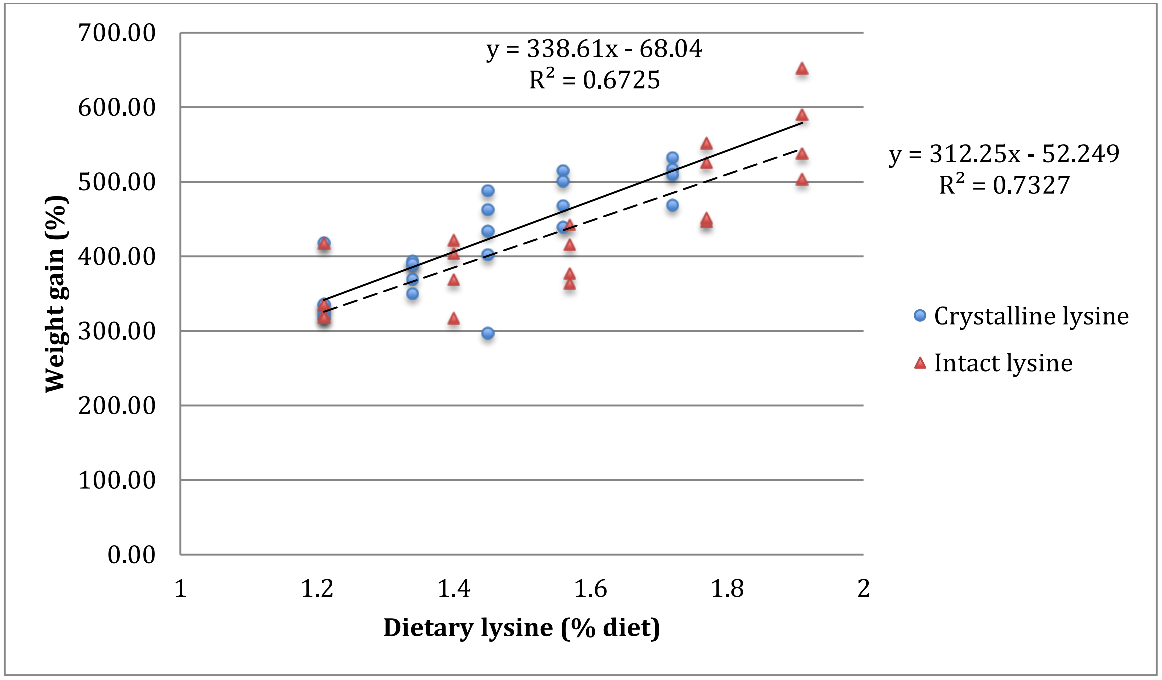

Figure 1. Regression of mean weight gain of channel catfish against dietary lysine levels. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

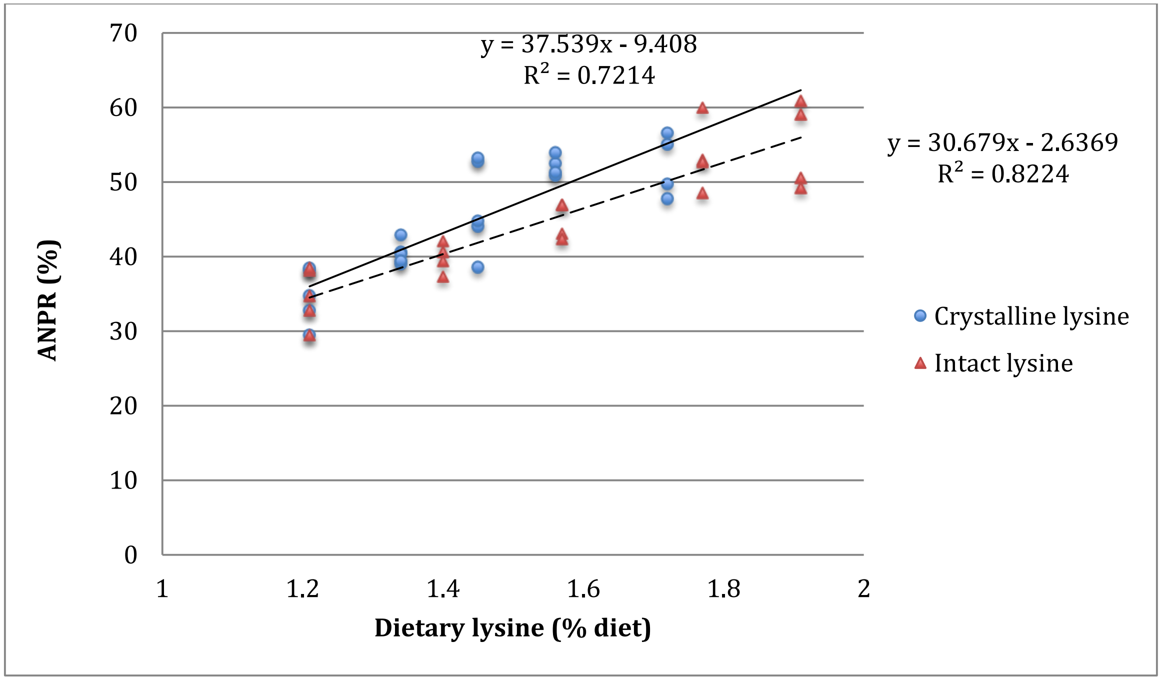

Figure 2. Regression of mean apparent net protein retention of channel catfish against dietary lysine levels. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

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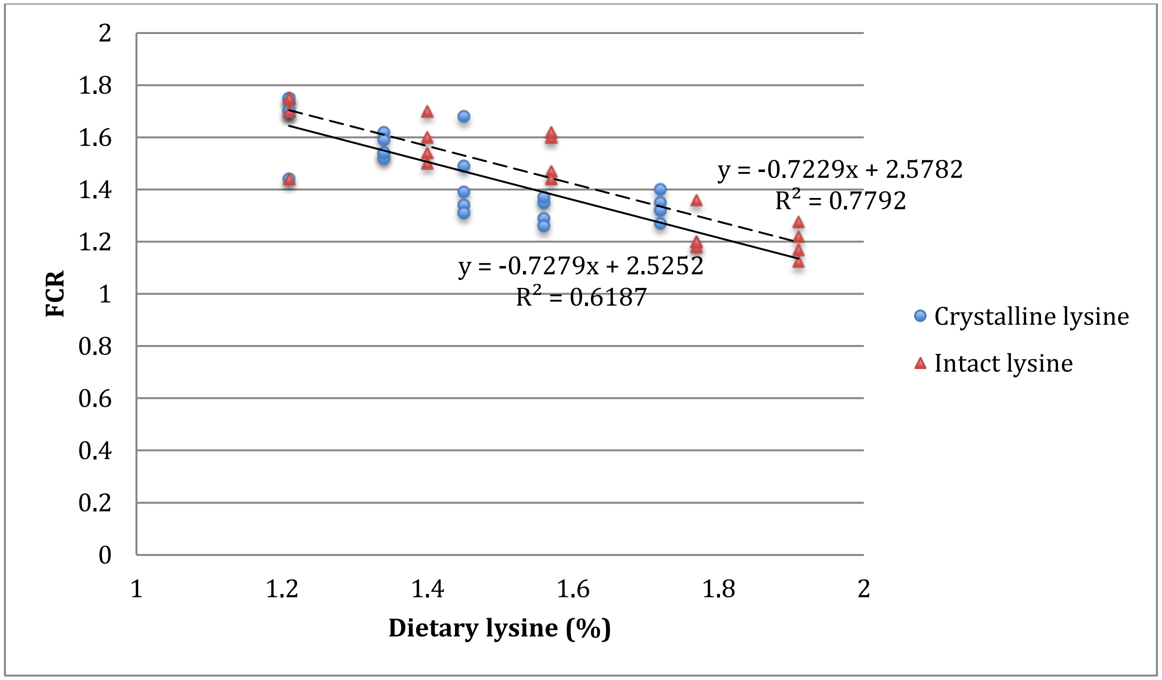

Figure 3. Regression of mean feed conversion ratio of channel catfish against dietary lysine levels. Solid line represents diets containing crystalline lysine, dashed line is diets with intact lysine.

CHAPTER III

COMPARISON OF CRYSTALLINE LYSINE AND INTACT LYSINE USED AS A SUPPLEMENT IN PRACTICAL DIETS OF NILE TILAPIA (*Oreochromis niloticus*)

Abstract

Two feeding trials were conducted using juvenile Nile tilapia, *Oreochromis niloticus*, to compare the efficiency of crystalline versus intact lysine in practical diets. A high lysine corn protein concentrate (Lysto^{TM}, Cargill) was used as the intact lysine source. The basal and test diets used in both experiments were designed to be isonitrogenous and isoenergetic containing 32% protein and 8% lipid using primarily plant-based protein sources. In the first growth trial, nine dietary treatments ranging in lysine levels from 1.15% (basal diet) to 1.88% lysine were offered to juvenile fish (6.41 g mean weight). The results obtained after ten weeks indicated that tilapia have positive performance with increasing lysine supplement from both crystalline and intact lysine source. A second series of diets with a broader range of inclusion level of lysine (0.92% to 2.02% diet) was formulated to confirm the results obtained in the first experiment. Lysine from two sources was added to 11 diets to produce analyzed lysine levels of 0.92%, 1.11%, 1.23%, 1.34%, 1.54% 1.65% in the first six diets using crystalline lysine and 1.13%, 1.38%, 1.61%, 1.81%, 2.02% in the last five diets using intact lysine. Juvenile Nile tilapia (6.40 g mean weight) in triplicate aquaria were randomly assigned to each of the experimental diets and fed for eight weeks. The dose–response to increasing dietary lysine levels was particularly clear in the second experiment. Broken-line regression analysis using thermal growth coefficient as a growth rate metric indicated that the lysine requirement of juvenile Nile tilapia was 1.49% while the estimate using saturation kinetics model was slightly lower at 1.42%. The analysis of covariance was conducted on all diets excluding diets 6, 10, 11, which contained an excess of the

estimated lysine requirement for tilapia. This analysis indicated there was no significant effect of the type of supplement indicating both are viable options. Overall, the study findings support the view of prior studies that high lysine corn protein concentrate can be used effectively to improve growth performance of tilapia to a level comparable to that obtained from crystalline lysine supplemented diet.

1. Introduction

The farming of tilapias, including Nile tilapia and some other cichlids species, is the most widespread type of aquaculture in the world. In 2013, world production of farmed tilapia is estimated to exceed 4,677,613 mt (Fitzsimmons et al., 2014) in which Nile tilapia is, by far, the predominant cultured tilapia, accounting for more than 70% of total tilapia production at 3,436,526 mt (FAO, 2014).

Global aquaculture industry including tilapia farming has recently been experiencing higher costs of production, in particular for feeds which usually represent 50% of expenses in aquaculture farms (FAO, 2014). Hence, the development of cost-effective feeds is critical in maximizing profitability. As protein is the most costly component of nutritionally complete feeds, the utilization of cheap and locally available plant protein sources would reduce feed costs. Quite often, these ingredients are limited in essential amino acid, especially lysine which is considered the first limiting amino acid of aquatic species (NRC, 2011).

Many studies conducted using Nile tilapia *Oreochromis niloticus* have demonstrated that adverse effects of plant protein could be eliminated by adjusting the amino acid profile thought the addition of purified limiting amino acids. Tacon et al. (1983) reported that supplementation of 0.8% D, C-methionine to a diet in which 75% of brown fish meal was replaced by soybean meal improved the growth performance of *O. niloticus* to a level comparable to that of a fish meal diet. The study of El-Saidy and Gaber (2002) observed significantly improved weight gain and feed efficiency of Nile tilapia fed a diet containing 55% soybean meal supplemented with 1% methionine and 0.5% lysine compared to that of fish fed the control diet containing 20% menhaden fish meal and 30% soybean meal. However, several studies have demonstrated that crystalline amino acid appears to be utilized with lower efficiency than essential amino acid supplied by intact protein due to faster absorption in the gastrointestinal compared to protein-bound amino acids (Cowey and Walton, 1988; Tantikitti and March, 1995; Zarate and Lovell, 1997; Zarate et al., 1999; Fox et al., 2006). Thus, there is an interest in both crystalline and intact protein sources to balance amino acid profiles.

The development of a high lysine corn protein concentrate makes for an interesting option for lysine supplementation. On an as-is basis, this product contains about 75.8% of protein, 5.3% of lysine and 1.8% of methionine. The incorporation of high lysine corn protein concentrate is expected to contribute as an alternative intact lysine source to substitute for crystalline lysine in the practical diets of aquatic animal. Hence, the objectives of this study were to evaluate the relative performance of tilapia to increasing levels of lysine from crystalline and intact lysine supplemented in practical diets. A high lysine corn protein concentrate (Lysto™, Cargill) was used as the intact lysine source.

2. Material and method

2.1 Experiment Diets

The basal and test diets used in both experiments were designed to be isonitrogenous and isoenergetic containing 32% protein and 8% lipid using primarily plant-based protein. The same formulation of experimental diets used for channel catfish was applied for tilapia in the first experiment. The first five diets contained the corn protein concentrate, Empyreal® 75, at 12.0 percent, with an increasing inclusion of crystalline lysine (0.0, 0.15, 0.30, 0.45 and 0.60 percent of the diet) whereas, corn protein concentrate enriched with L-lysine (Lysto™) was used as a substitution of the corn protein concentrate (Empyreal® 75) in the last four diets at the following rates: 9:3, 6:6, 3:9, and 0:12 in percentage of the diet (Table 7).

The second experiment was designed to confirm the efficiency of using high lysine corn protein concentrate as a substitution of crystalline lysine for juvenile Nile tilapia. In the second experiment, diets with higher inclusions level of corn protein concentrate at 20 percent were used to give a larger range of lysine levels. The basal diet contained reduced amounts of soybean meal and corn dried distiller's grains with solubles at 13.21 and 17.00 percent respectively compared to 25.28 and 20 percent on an 'as is' basis of feeds for channel catfish and tilapia in the first trial. The first six sets of diets contained the corn protein concentrate, Empyreal® 75, at 20.0 percent, with an increasing inclusion of crystalline L-lysine (0.2, 0.4, 0.6, 0.8 and 1.0 percent of the diet) (Table 8). The total lysine was increased by the supplementation of crystalline lysine from 0.9 up to 1.7 percent of the diet (Table 10). The last five sets of diets contained corn protein concentrate enriched with L-lysine (Lysto™) as a substitution of the corn protein concentrate (Empyreal® 75) at the following rates: 16:4, 12:8, 8:12, 4:16, 0:20 in percentage of the diet (Table 8). The lysine levels in these diets ranged from 1.1 to 2.0 percent of the diet (Table 10). The test diets were then prepared in Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 minutes. Hot water was then added to the mixture in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 4-mm-diameter die in a meat grinder, dried at 70° C to a moisture less than 10%, and stored in the freezer at -20° C until used. A sample of each feed was collected and analyzed for proximate and amino acid compositions (Table 9, Table 10, Table 11, Table 12) following AOAC (1995) procedures by the Experiment Station Chemical Laboratories, University of Missouri, Columbia.

2.2 Culture Methods

Two feeding trials were conducted at the E.W. Shell Fisheries Center, Auburn, AL. Both trials used Nile tilapia fry spawned at this center, which were stocked in the nursery tank until the beginning of each trial. The first trial was conducted in a flow-through system consisting of 39 aquaria. The aquaria for all the study groups were randomly assigned to treatments. Each of the nine treatments was replicated four times. Fish were kept in 132-L aquaria in a recirculation system with a flow rate of 0.45 L/min. The inlet water was pumped through a UV sterilizer (model ALQ60IL, Aqua Logic, San Diego, CA, U.S.A) before it was finally distributed back into each aquarium. In the second trial, juvenile tilapia fish were randomly stocked into 50-L aquaria of a 3,800-L indoor recirculation system. There were three replicates per treatment. Samples of fish from the initial stocking were retained for later protein retention analysis. Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA) and dissolved oxygen was maintained near saturation using air stones. Dissolved oxygen and water temperature were measured twice a day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH) while pH, TAN and nitrite-nitrogen were measured twice per week. Photoperiod was set at 14 h light and 10 h dark. The mean water quality variables during the first trial were as follows: dissolved oxygen, 5.98±0.62mg/L; water temperature, 27.51±0.96°C; TAN, 0.13±0.07 mg/L; nitrite-N, 0.03±0.12 mg/L; and pH, 7.08±0.35 for the first trial and dissolved oxygen, 5.40±0.39 mg/L; water temperature, 26.64±0.71 C; TAN, 0.78±0.42 mg/L; nitrite-N, 0.35±0.38 mg/L; and pH, 8.0 6 0.2 for the second trial (Table 13). These values were within optimal ranges for normal growth and health of juvenile Nile tilapia (El Gamal 1988; Wangead et al. 1988; Watanabe et al. 1993; El-Shafai et al. 2004).

Diets were offered to fish at 3-5% body weight daily at 08h00 and 16h00 according to fish size and divided into two equal feedings each day. Fish were weighed every two weeks. Daily feed rations were calculated based on % body weight and held constant for all treatments at a given time. The rations were adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine weight gain, survival and feed conversion ratio.

Crude protein in initial and final whole body samples were tested by the Kjeldahl method (Williams, 1984). Protein retention were calculated as follows:

Protein retention (%) = (final weight x final protein content) - (initial weight x initial protein content) x 100/protein intake.

2.3 Statistical Analysis

Data from both experiments were analyzed using one-way ANOVA to determine if there were significant differences ($P<0.05$) in mean FW, WG, FCR, ANPR. Student Newman Keuls' multiple comparison test was used to determine differences among treatment means. The analysis of covariance ANCOVA was used to compare two regression lines of tilapia responses to the use of crystalline versus intact lysine. In the second trial, one-slope straight broken-line and saturation kinetics model were used to determine estimates of requirements. All the data analyses were performed using SAS (V9.4. SAS Institute, Cary, NC, USA).

3. Results

Tilapia in the two growth trials showed improved growth performance with higher lysine supplement. There were significant differences ($P<0.05$) among mean final weight (FW), weight gain (WG), feed conversion ratio (FCR), apparent net protein retention (ANPR) of fish receiving various dietary treatments (Table 14 and Table 16). The fish fed diets with higher lysine supplement had higher mean FW, WG, ANPR and lower FCR. There were no significant differences in mean survival rate among treatments.

In the first trial, fish fed diet 1 (1.15%) without added lysine had the lowest mean FW, WG, ANPR and highest FCR. Mean FW, WG, and ANPR of fish in this treatment were significantly lower than those of the other diets. While fish fed diet 9 (1.88%) had significantly higher ($P<0.05$) mean WG than those reported for fish fed diets 2 and diet 6 with lysine inclusion at 1.32 and 1.35%, respectively, there were no significant differences observed among FW, WG of groups fed diets 3, 4, 5, 7, 8, 9. The apparent net protein retention ranged from 44% in the lowest inclusion level of lysine to 57% in the highest inclusion level (P-value <0.05). When compared to the three inclusion levels of lysine below 1.5% among themselves and the five inclusion levels of lysine above 1.5% among themselves, in both the crystalline and intact diets, there were no significant statistical differences. Although fish fed diet 9 produced the highest apparent net protein retention, no significant differences were observed among fish fed this diet and other groups with lysine inclusion above 1.5%. Fish fed diets 8 and 9 had significantly lower mean FCR ($P<0.05$) compared to those of the control and other diets; whereas, no significant differences among mean FCR of fish fed diet 1-7 were observed. Survival of Nile tilapia for all treatments ranged from 96.25% to 98.75%. Analysis of covariance of FW, WG, FCR, and ANPR of tilapia in the first trial is presented in Table 15. No combined effects of lysine sources and lysine levels were observed on FW, WG, FCR, and ANPR of tilapia fed the two different lysine sources. The slope P value and intercept P were not significantly different ($P>0.05$) among FW, WG (Figure 4), FCR (Figure 6), ANPR (Figure 5) of fish fed crystalline lysine and intact lysine.

In the second trial, fish fed diets with lysine supplement above 1.5% had significantly higher (P<0.05) mean WG than values reported for the other diets with added lysine below 1.5%. Even though fish fed diet 11 (2.02%) had the highest mean FW, WG and lowest FCR, no significant differences among fish in this group and groups fed diets with lysine supplemented above 1.5% were observed. The fish fed the control diet (0.92%) had significantly lower mean weight gain and ANPR compared to those of other groups (P<0.05). The same pattern was found when analyzing the mean FCR, ranging from 1.44 down to 1.12 (P<0.0001; PSE=0.0468). Broken-line regression analysis of mean weight gain indicated that the lysine requirement of juvenile Nile tilapia was 1.49% (1.44% - 1.54%) of the diet while saturation kinetics model indicated that lysine requirement of juvenile Nile tilapia was 1.42% (1.36% - 1.52%) of the diet (Figure 7).

Analysis of covariance of mean FW, WG, FCR, and ANPR of tilapia in the second trial are presented in Table 17. There were no significant differences among mean FW, WG, FCR, and ANPR of tilapia fed two different lysine sources. The slope P value and intercept P were not significantly different (P>0.05) among mean FW, WG (Figure 8), FCR (Figure 10), ANPR (Figure 9) of fish fed crystalline lysine and intact lysine. From the above results, it can be concluded that high lysine corn protein concentrate can be used as effectively as crystalline lysine by tilapia.

4. Discussion

As the cost of fish meal becomes relatively more expensive, locally available plant-derived proteins with consistent quality and sustainability are increasingly being used to produce cost-effective feed formulations. Like other aquatic species, many studies have been conducted to evaluate the efficiency of using plant protein sources as the replacement of fish meal in practical diets for tilapia (Novoa et al., 1997; Fasakin et al., 1999; El-Saidy and Gaber, 2003; El-Saidy and Gaber, 2004; Gaber, 2006; Borgeson, 2006; Thompson et al., 2012; Krome et al., 2014). Quite often, crystalline amino acids are used to meet the essential amino acid requirements of fish in diets with unbalanced amino acid profiles. According to Santiago and Lovell (1988), the essential amino acid requirements for growth of tilapia are arginine (1.2%), methionine plus cysteine (0.8%), histidine (1.0%), isoleucine (1.8%), leucine (1.9%), valine (1.6%), phenylalanine and tyrosine (1.6%), threonine (1.1%), tryptophan (0.28%). Santiago and Lovell (1988) also suggested the lysine requirement for Nile tilapia is 1.43 % of dry diet or 5.1 % of crude protein, while the digestible lysine utilization efficiencies of 0.72% for gain of juvenile tilapia were observed by He et al. (2013). The basal diet in this present study met the minimum requirements of tilapia for all essential amino acids except lysine (Table 7 and Table 8).

Supplementation of lysine in the formulated diets with high level of plant-based protein is often required as lysine levels are low. The results from both trials indicated that growth performance of tilapia showed better response with the supplementation of lysine from both intact and crystalline lysine sources. The dose–response to graded level of dietary lysine was particularly clear in the second trial which significant differences observed in terms of weight gain among treatments as dietary lysine levels increased from 0.92 to 2.02 % of the diet. Similarly, Lim et al. (2007) attributed the poor performance of Nile tilapia fed a diet containing 40% WDDGS to lysine deficiency, with lysine supplementation, however, at least 40% of WDDGS can be included in diets of juvenile tilapia as a replacement of a combination of soybean meal and corn meal. According to Teshima et al (1986), weight gain of *O. niloticus* was improved when a 35% protein diet formulated from casein was supplemented with either arginine and lysine or tryptophan and methionine. Furuya et al. (2004) also demonstrated that with the supplementation of lysine monohydrochloride at 2.14% and 0.2%, respectively, Jatropha platyphylla kernel meal and soy bean meal can be used to replace 62.5% fish meal protein without causing adverse effects on the growth performance of *O. niloticus*. The same patterns were observed on the feed conversion ratios, showing better responses of tilapia to graded level of dietary lysine. While particularly clear results obtained in tilapia of the second trial, tilapia in the first trial responded mildly to increased lysine inclusion in which no significant differences were observed among fish fed diets 1 to diet 7 with lysine inclusion ranging from 1.15% to 1.64%. Regardless of apparent net protein retention which showed better response of fish fed diet containing lysine inclusion above 1.5%, lysine level at 1.38% could be sufficient for growth of tilapia. Furuya (2012) observed similar results in his study which showed different lysine requirement of tilapia to obtain highest weight gain and highest rate of body protein deposition with the value of 15.96 g/kg lysine and 15.21 g/kg of lysine, respectively.

One-slope straight broken-line analysis of second trial indicated that the breakpoint for thermal growth coefficient occurred at 1.49% of the diet, while the estimate using saturation kinetics model was slightly lower at 1.42% of the diet. The results from this study are close to the value previously found by Bomfim et al. (2010) and Furuya et al. (2006) who estimated the requirement of 15.4 g/kg of digestible lysine (5.5 g/100 g of digestible protein) and 14.3 g/kg of digestible lysine (5.4 g/100 g of digestible protein), respectively, for the same fish species. Recently, Furuya et al (2012) also demonstrated that in diets balanced for the arginine:lysine ratio, the digestible lysine requirement of Nile tilapia fingerlings is 15.21 g/kg (5.41 g/100 g of digestible protein). These findings are a bit lower compared to the value proposed by NRC (2011) with the lysine requirement of 1.6% for tilapia *Oreochromis spp*. The higher lysine requirement for *O. niloticus* fingerlings of 7.12 g/100 g protein was though observed by Ovie

(2013). However, Bureau (2006) found that differences in experimental design and condition of research, such as fish size, culture protocols, models used and basal diets composition resulted in the variations in amino acid estimates. According to results of this present study, when lysine requirement has been met increasing levels of lysine supplement did not result in any reduced growth. Similarly, Bomfim et al. (2010) observed linear increase of lysine levels on the weight gain of Nile tilapia fingerlings fed diets containing 9.5, 11.0, 12.5, 14.0, 15.5 or 17.0 g/kg of lysine. These findings are contrary to the findings of Santiago and Lovell (1988) who reported that increasing dietary lysine beyond the requirement of tilapia at 1.43% would not provide significant additional growth. Furuya (2012) found that the excessive levels of lysine above 15.96 g/kg would result in reduced growth performance of Nile tilapia.

Investigations into the efficacy of intact lysine, high lysine corn protein concentrate as a replacement of crystalline lysine on growth performance of tilapia have been conducted. In the present study, effects of lysine supplementation from two sources were not significantly different with the adjusted means for the weight gain of the fish fed crystalline lysine and fish fed intact lysine were 1123% and 1112% for the first trial and 487% and 482% for the second trial. These findings are in agreement with Yu et al. (2012) who indicated that the efficiency of lysine enhanced corn protein concentrate, as a substitution to purified lysine, appeared comparable with crystalline lysine for shrimp *L. vannamei*. However, according to Nguyen et al. (2007) the efficacy of using crystalline amino acids in practical diets of fish is dependent on several things, such as the ability of species to absorb and use such amino acids for protein synthesis, source of protein used as well as for other physiological functions. William et al. (2010) observed that the efficiency of the utilization of crystalline amino acids was dependent on dietary protein level. He at al. (2012) found that the digestible protein, energy and amino acid utilization efficiencies decreased significantly with the increment of fish size in which the digestible lysine utilization efficiencies for gain of *O. niloticus* were 0.72 and 0.52 for juvenile (20.7) and adult fish (165 g), respectively. The differences in efficiency could be attributed to a difference of plant protein source between diets (Amogh, 2009). Yu and Zhang (2012) indicated that dietary microencapsulated lysine and methionine supplements improved the growth performance of juvenile tilapia compared to crystalline lysine.

The added benefit of lysine supplementation was also observed in the body protein content of tilapia. In the present study, fish fed the control diet without added lysine experienced significantly lower protein content compared to other groups. The possible reason for protein deposition increased was that balanced amino acid profile promoted protein synthesis rates in muscle (Furuya et al., 2004). Similarly, Lim et al. (2007) found that fish fed the 40% CDDGS diet without added lysine had significantly lower whole-body protein content than the group fed the SBM control diet. That study also explained that decrease in protein content may be related to smaller size fish, which had less flesh and/or the imbalance of dietary essential amino acids, such as deficiency of lysine, which may contribute to reduced protein synthesis, as the protein content of fish fed diets containing the same level of CDDGS supplemented with lysine increased to a level that was statistically similar to that of the control fish. Studies conducted by Zhou et al. (2010a; 2011b) also demonstrated that protein deposition in dorsal muscle of juvenile black sea bream was increased markedly by supplementation of coated lysine (5%) and methionine (3.4%), even slightly higher than those of diets containing fish meal (Lu, 2014). Nwanna et al. (2012) reported that the protein content of the common carp (*Cyprinus carpio* L.) fed the DL-Methionine supplemented diet had significantly higher protein level in muscle than those of fish fed the Methionine-deficient diet. There is evidence that the source of dietary lysine (protein bound vs. free lysine) (Zarate and Lovell, 1997), the level of protein in the diet (Rodehutscord et al., 2000), and diet digestible energy content and lysine levels, affect lysine utilization for protein deposition in rainbow trout (Encarnacao, 2004).

The results of this study also demonstrated the relationship between temperature, density and amino acid requirement of animals. The tilapia in the first experiment showed better response for growth performance, protein retention and lower lysine requirement compared to the second experiment. While the tilapia in both experiment had the same initial mean weight, the average temperature of the first treatment was slightly higher than the second experiment with values at 27.51^0C and 26.64^0C respectively. According to Lupatsch et al. (2010) and He et al. (2013), maintenance needs were generally thought to be highly dependent on body size and temperature, thus were proportional to the metabolic body weight, while the needs for growth were governed by the amount and composition of the added body weight gain. Costas (2008) also suggested that crowding stress may affect amino acid requirements. The higher usage of free amino acids from the high stocking density group may be due to the higher demand for energy production in order to cope with stressful rearing conditions, higher rate of protein synthesis or due to synthesis of other important metabolites related to stress response. While in the second experiment fish experienced higher stocking density at 20 fish/50 L, the fish in the first experiment were kept in larger aquaria at 20fish/132 L.

5. Conclusion

High lysine corn protein concentrate was utilized as efficiently in meeting the essential amino acid requirements of the tilapia as those of crystalline form. The corn protein concentrate with dried L-lysine fermentation product is a feasible ingredient for aquaculture feeds, which can be used to partially substitute soybean meal without addition of crystalline lysine.

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Table 7. Ingredient compositions (g 100 g^{-1} as-is) of nine experimental diets with increasing levels of lysine supplement from crystalline lysine and intact lysine formulated to contain 32% protein, 8% lipid and offered to juvenile Nile tilapia in the first trial

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Soybean meal[1]	25.28	25.28	25.28	25.28	25.28	25.28	25.28	25.28	25.28
DDGS[2]	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Menhaden Fish oil[3]	4.82	4.82	4.82	4.82	4.82	4.81	4.80	4.79	4.82
Whole wheat[4]	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Corn starch[5]	10.12	10.12	10.12	10.12	10.12	10.10	10.08	10.06	10.12
Trace mineral premix[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[7]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C[8]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CP dibasic[5]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lecethin[9]	0.50	0.50	0.50	0.50`	0.50	0.50	0.50	0.50	0.50
CPC[10]	12.00	12.00	12.00	12.00	12.00	9.00	6.00	3.00	12.00
HLCPC[10]	0.00	0.00	0.00	0.00	0.00	3.03	6.06	9.09	0.00
Methionine-DL[11]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
L-Lysine[15]	0.00	0.15	0.30	0.45	0.60	0.00	0.00	0.00	0.00
Glutamic acid[16]	0.60	0.45	0.30	0.15	0.00	0.60	0.60	0.60	0.60

[1]De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[2] Poet Dakote Gold Inc., Sioux Falls, SD, USA.
[3] Omega Protein Inc., Reedville, VA, USA.

[4] MP Biomedicals Inc., Solon, Ohio, USA.
[5] MP Biochemicals Inc., Solon, OH, USA.
[6] Trace mineral: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[7] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B_{12}, Inositol, Vitamin A acetate, Vitamin D_3, Vitamin E, cellulose
[8] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[9] The Solae Company, St. Louis, MO, USA.
[10] Empyreal® 75, Lysto™ Cargill Corn Milling, Cargill, Inc., Blair, NE, USA
[11] Aldrich-Sigma, St. Louis, MO, USA.

Table 8. Ingredient compositions (g 100 g^{-1} as-is) of eleven experimental diets with increasing levels of lysine supplement from crystalline lysine and intact lysine formulated to contain 32% protein, 8% lipid and offered to juvenile Nile tilapia in the second trial

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Soybean meal	13.21	13.21	13.21	13.21	13.21	13.21	13.21	13.21	13.21	13.21	13.21
DDGS	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Fish oil	5.08	5.08	5.08	5.08	5.08	5.08	5.07	5.06	5.05	5.04	5.03
Whole wheat	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Corn starch	15.55	15.55	15.55	15.55	15.55	15.55	15.52	15.48	15.46	15.43	15.40
TMP	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CP dibasic	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lecethin	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
CPC	20.00	20.00	20.00	20.00	20.00	20.00	16.14	12.29	8.42	4.61	0.71
HLCPC	0.00	0.00	0.00	0.00	0.00	0.00	3.90	7.80	11.70	15.55	19.49
Methionine-DL	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.08	0.08	0.09	0.10
L-Lysine	0.00	0.20	0.40	0.60	0.80	1.00	0.00	0.00	0.00	0.00	0.00
Glutamic acid	1.00	0.80	0.60	0.40	0.20	0.00	0.99	0.98	0.98	0.97	0.96

[1]De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[2] Poet Dakote Gold Inc., Sioux Falls, SD, USA.
[3] Omega Protein Inc., Reedville, VA, USA.

[4] MP Biomedicals Inc., Solon, Ohio, USA.
[5] MP Biochemicals Inc., Solon, OH, USA.
[6] Trace mineral: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[7] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B_{12}, Inositol, Vitamin A acetate, Vitamin D_3, Vitamin E, cellulose
[8] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[9] The Solae Company, St. Louis, MO, USA.
[10] Empyreal® 75, Lysto™ Cargill Corn Milling, Cargill, Inc., Blair, NE, USA
[11] Aldrich-Sigma, St. Louis, MO, USA.

Table 9. Analyzed proximate composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to Nile tilapia in the first trial

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Protein	30.43	31.19	30.89	31.80	31.22	31.26	31.43	31.80	31.43
Moisture	11.68	7.64	11.36	7.95	9.54	9.27	8.53	8.43	8.29
Fat	6.73	7.22	6.47	6.86	6.67	6.79	6.76	6.87	7.33
Fiber	3.19	3.48	3.45	3.33	3.60	3.52	3.42	3.59	3.74
Ash	4.03	4.20	4.03	4.20	4.11	4.09	4.89	4.19	4.18

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 10. Analyzed proximate composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to Nile tilapia in the second trial

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Protein	31.84	32.95	32.00	31.24	32.06	32.20	32.23	31.90	32.54	31.28	31.92
Moisture	9.06	5.56	7.95	11.10	8.84	9.68	6.75	7.78	6.96	9.38	8.55
Fat	8.89	8.47	8.58	8.50	8.16	8.81	8.19	7.86	8.14	8.85	8.09
Fiber	2.82	3.15	2.96	2.81	2.57	2.40	2.92	3.01	3.03	2.86	2.74
Ash	4.17	4.39	4.20	4.12	4.21	4.17	4.32	4.32	4.36	4.25	4.25

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 11. Analyzed amino acids composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to Nile tilapia in the first trial

Dept #	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Taurine	0.09	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10
Hydroxyproline	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.06
Aspartic Acid	2.28	2.40	2.28	2.41	2.36	2.32	2.36	2.33	2.33
Threonine	1.04	1.10	1.05	1.10	1.08	1.07	1.08	1.07	1.06
Serine	1.27	1.36	1.30	1.40	1.35	1.36	1.33	1.35	1.34
Glutamic Acid	6.31	6.52	6.07	6.28	5.97	6.47	6.50	6.48	6.43
Proline	2.24	2.39	2.25	2.39	2.30	2.28	2.28	2.30	2.25
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	1.11	1.16	1.10	1.18	1.13	1.12	1.15	1.13	1.13
Alanine	1.79	1.91	1.79	1.93	1.85	1.82	1.82	1.82	1.79
Cysteine	0.48	0.50	0.47	0.50	0.49	0.48	0.48	0.49	0.47
Valine	1.39	1.46	1.39	1.46	1.41	1.39	1.39	1.40	1.36
Methionine	0.64	0.66	0.62	0.65	0.64	0.63	0.63	0.62	0.61
Isoleucine	1.23	1.31	1.23	1.29	1.26	1.24	1.25	1.25	1.22
Leucine	3.23	3.50	3.26	3.48	3.37	3.30	3.28	3.31	3.22

Tyrosine	1.17	1.24	1.16	1.22	1.20	1.18	1.19	1.18	1.18
Phenylalanine	1.58	1.69	1.59	1.68	1.64	1.61	1.61	1.62	1.59
Hydroxylysine	0.10	0.11	0.09	0.11	0.09	0.08	0.11	0.08	0.08
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	1.15	1.32	1.38	1.57	1.64	1.35	1.55	1.71	1.88
Histidine	0.70	0.73	0.70	0.74	0.71	0.71	0.72	0.72	0.71
Arginine	1.53	1.59	1.52	1.60	1.56	1.56	1.58	1.57	1.56
Tryptophan	0.32	0.35	0.37	0.35	0.32	0.34	0.33	0.33	0.33

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 12. Analyzed amino acids composition of the experimental diets[1] (g 100 g^{-1} as-is) fed to Nile tilapia in the second trial

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
Taurine	0.11	0.10	0.10	0.10	0.10	0.10	0.11	0.11	0.10	0.10	0.11
Hydroxyproline	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Aspartic Acid	2.17	2.20	2.15	2.08	2.21	2.13	2.17	2.20	2.16	2.11	2.07
Threonine	1.07	1.09	1.07	1.02	1.08	1.06	1.08	1.08	1.07	1.04	1.02
Serine	1.38	1.40	1.43	1.32	1.42	1.36	1.39	1.38	1.36	1.35	1.29
Glutamic Acid	7.18	7.07	6.83	6.33	6.52	6.17	7.24	7.22	7.13	6.84	6.76
Proline	2.55	2.58	2.57	2.42	2.57	2.54	2.58	2.57	2.52	2.43	2.41
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine	1.03	1.04	1.03	1.00	1.04	1.02	1.03	1.06	1.05	1.03	1.00
Alanine	2.12	2.11	2.13	2.02	2.14	2.10	2.13	2.15	2.09	2.00	1.96
Cysteine	0.55	0.56	0.55	0.53	0.56	0.53	0.55	0.55	0.55	0.53	0.53
Valine	1.40	1.40	1.40	1.34	1.40	1.38	1.40	1.41	1.38	1.32	1.29
Methionine	0.71	0.73	0.71	0.67	0.71	0.69	0.72	0.71	0.72	0.70	0.71
Isoleucine	1.22	1.24	1.24	1.18	1.24	1.21	1.23	1.23	1.20	1.16	1.15
Leucine	3.99	4.02	4.05	3.78	4.04	3.95	4.05	4.00	3.88	3.69	3.65
Tyrosine	1.25	1.30	1.28	1.22	1.29	1.26	1.26	1.27	1.26	1.22	1.17
Phenylalanine	1.70	1.81	1.79	1.69	1.80	1.76	1.72	1.70	1.75	1.67	1.57
Hydroxylysine	0.06	0.07	0.07	0.07	0.06	0.05	0.05	0.07	0.08	0.08	0.06
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02

Lysine	0.92	1.11	1.23	1.34	1.54	1.65	1.13	1.38	1.61	1.81	2.02
Histidine	0.69	0.69	0.69	0.67	0.69	0.68	0.70	0.70	0.69	0.67	0.67
Arginine	1.29	1.32	1.29	1.26	1.31	1.28	1.31	1.32	1.31	1.27	1.26
Tryptophan	0.30	0.31	0.26	0.29	0.31	0.30	0.31	0.33	0.28	0.29	0.28

[1] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri

Table 13. Summary of water quality values observed over the growth trials for Nile tilapia reared in a semi-closed recirculation system. Values represent the mean, standard deviation, minimum, and maximum readings

Parameter(C)	Mean	Standard deviation	Minimum	Maximum
Tilapia – first trial				
Dissolved oxygen (mg L^{-1})	5.98	0.62	4.50	7.17
Temperature (˚C)	27.51	0.96	24.50	29.50
Salinity (ppt)	1.86	0.74	0.50	2.80
pH	7.08	0.35	6.30	7.68
Total ammonia-nitrogen (mg L^{-1})[b]	0.13	0.07	0.03	0.20
Nitrite-N (mg L^{-1})	0.30	0.12	0.07	0.41
Tilapia – second trial				
Dissolved oxygen (mg L^{-1})	5.40	0.39	4.50	6.12
Temperature (˚C)	26.64	0.71	25.10	27.70
Salinity (ppt)	1.29	0.79	0.20	3.50
pH	6.98	0.26	6.40	7.25
Total ammonia-nitrogen (mg L^{-1})	0.78	0.42	0.07	1.65
Nitrite-N (mg L^{-1})	0.35	0.38	0.03	1.26

Table 14. Mean response of Nile tilapia fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over a ten-week growth period in the first trial.

Diet	Lysine level	Final weight (g)	Weight gain (%)	FCR	Survival (%)	ANPR
1	1.15	72.10^{d}	1021.82^{c}	1.04^{a}	98.75	43.93^{d}
2	1.32	76.01^{c}	1097.31^{b}	1.02^{ab}	96.25	47.58^{c}
3	1.38	77.94^{bc}	1130.24^{ab}	1.00^{abc}	96.25	50.21^{bc}
4	1.57	80.05^{ab}	1145.22^{ab}	1.00^{abc}	96.25	53.33^{ab}
5	1.64	81.39^{ab}	1152.13^{ab}	1.00^{abc}	98.75	56.22^{a}
6	1.35	77.51^{bc}	1107.32^{b}	1.00^{abc}	98.00	50.52^{bc}
7	1.55	79.14^{abc}	1137.82^{ab}	0.99^{abc}	98.75	54.48^{ab}
8	1.71	81.12^{ab}	1162.89^{ab}	0.98^{bc}	97.00	56.55^{a}
9	1.88	82.57^{a}	1187.49^{a}	0.96^{c}	96.25	57.31^{a}
P-value		<0.0001	<0.0001	<0.0001	0.7956	<0.0001
PSE		0.9725	16.5235	0.0121	1.5845	1.3202

[1]Means in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Student Newman-Keuls' multiple range test.
[2]Pooled standard error.

Table 15. Analysis of covariance output of Nile tilapia fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over a ten-week growth period in the first trial.

	Adjusted mean (least-squares mean)				
Lysine source	Final weight	Weight gain[1] (%)	FCR[2]	Survival (%)	ANPR[3] (%)
Crystalline lysine	78.4	1123.0	1.01	97.1	51.6
Intact lysine	77.7	1112.1	1.00	97.9	51.8
Slope P value	0.16	0.51	0.84	0.57	0.32
Intercept P value	0.36	0.37	0.58	0.44	0.81

Table 16. Mean response of Nile tilapia fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over an eight-week growth period in the second trial. Means in the same column with different superscripts are significantly different at P < 0.05

Diet	Lysine level	Final weight (g)	Weight gain (%)	TGC	FCR	Survival (%)	ANPR
1	0.92	31.98^{f}	401.98^{f}	0.0886^{e}	1.44^{a}	96.25	31.63^{d}
2	1.11	35.47^{e}	452.67^{e}	0.0958^{d}	1.31^{b}	96.25	34.10^{d}
3	1.23	37.97^{d}	489.52^{d}	0.1007^{c}	1.27^{b}	93.75	38.41^{c}
4	1.34	40.43^{c}	530.22^{c}	0.1057^{b}	1.22^{c}	97.50	42.38^{b}
5	1.54	42.55^{ab}	561.33^{b}	0.1095^{a}	1.19^{cd}	100.00	43.67^{ab}
6	1.65	43.18^{ab}	573.26^{ab}	0.1108^{a}	1.15^{de}	100.00	46.30^{a}
7	1.13	36.23^{e}	469.56^{e}	0.0977^{d}	1.30^{b}	98.75	34.36^{d}
8	1.38	39.92^{c}	525.37^{c}	0.1049^{b}	1.20^{cd}	98.33	41.10^{b}
9	1.61	42.09^{b}	561.75^{b}	0.1091^{a}	1.17^{cd}	100.00	43.83^{ab}
10	1.81	42.90^{ab}	568.37^{ab}	0.1102^{a}	1.16^{de}	100.00	46.09^{a}
11	2.02	43.79^{a}	590.98^{a}	0.1124^{a}	1.15^{e}	100.00	46.93^{a}
P-value		<0.0001	<0.0001	<0.0001	<0.0001	0.1770	<0.0001
PSE		0.4221	6.3714	0.0008	0.0142	1.6522	0.8468

[1]Means in the same column with different superscripts are significantly different at P < 0.05 based upon analysis of variance followed by Student Newman-Keuls' multiple range test.
[2]Pooled standard error.

Table 17. Analysis of covariance output of Nile tilapia fed diets with increasing levels of lysine supplement from crystalline lysine and intact lysine over an eight-week growth period in the second trial

	Adjusted mean (least-squares mean)				
Lysine source	Final weight (g)	Weight gain[1] (%)	FCR[2]	Survival (%)	ANPR[3] (%)
Crystalline lysine	37.7	487.0	1.29	96.7	38.0
Intact lysine	37.1	482.2	1.29	98.2	37.1
Slope P value	0.07	0.12	0.95	0.81	0.42
Intercept P value	0.11	0.37	0.85	0.24	0.22

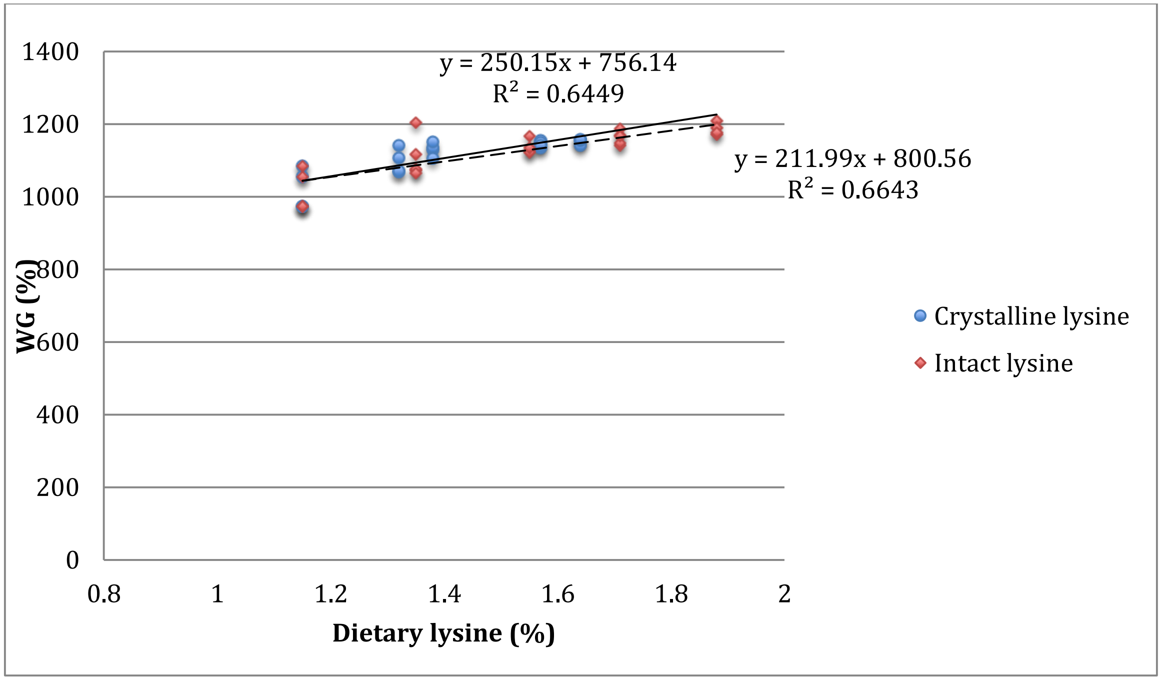

Figure 4. Regression of mean weight gain of Nile tilapia against dietary lysine levels in the first trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

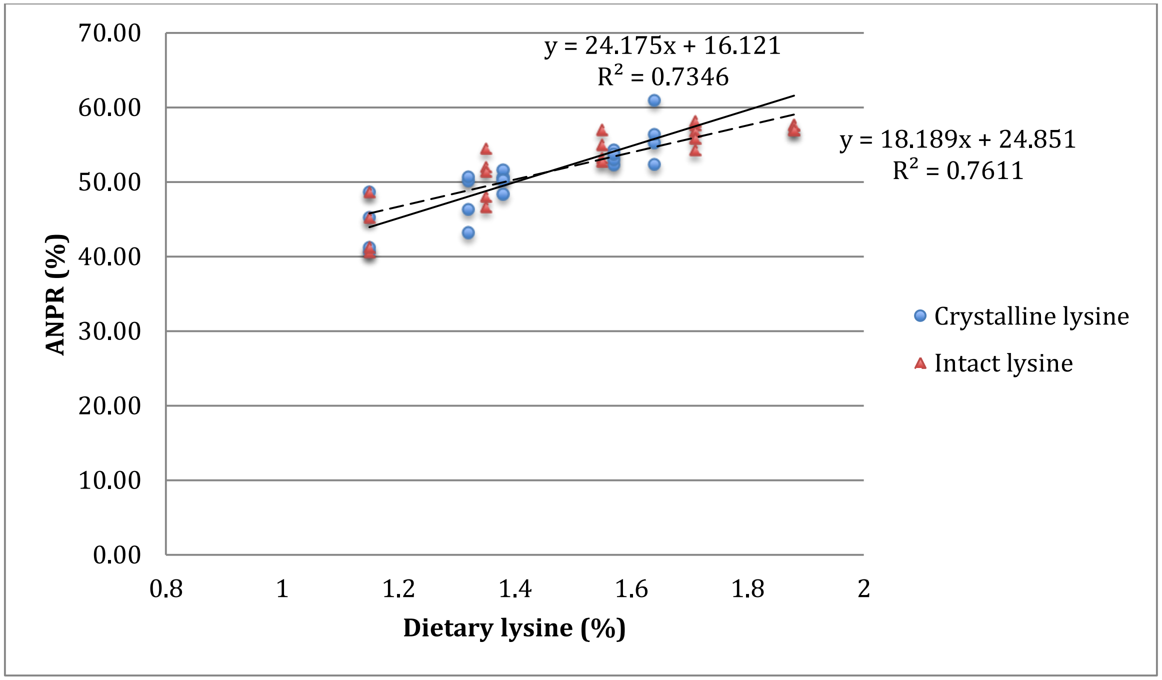

Figure 5. Regression of apparent net protein retention of Nile tilapia against dietary lysine levels in the first trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

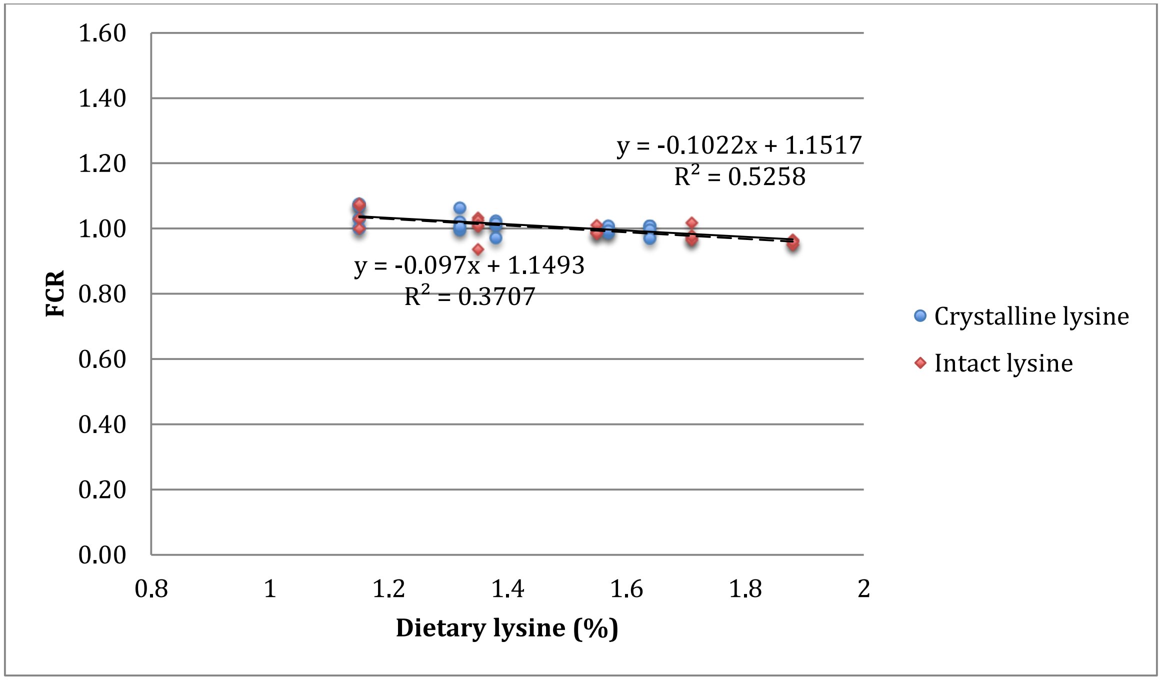

Figure 6. Regression of mean feed conversion ratio of Nile tilapia against dietary lysine levels in the first trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

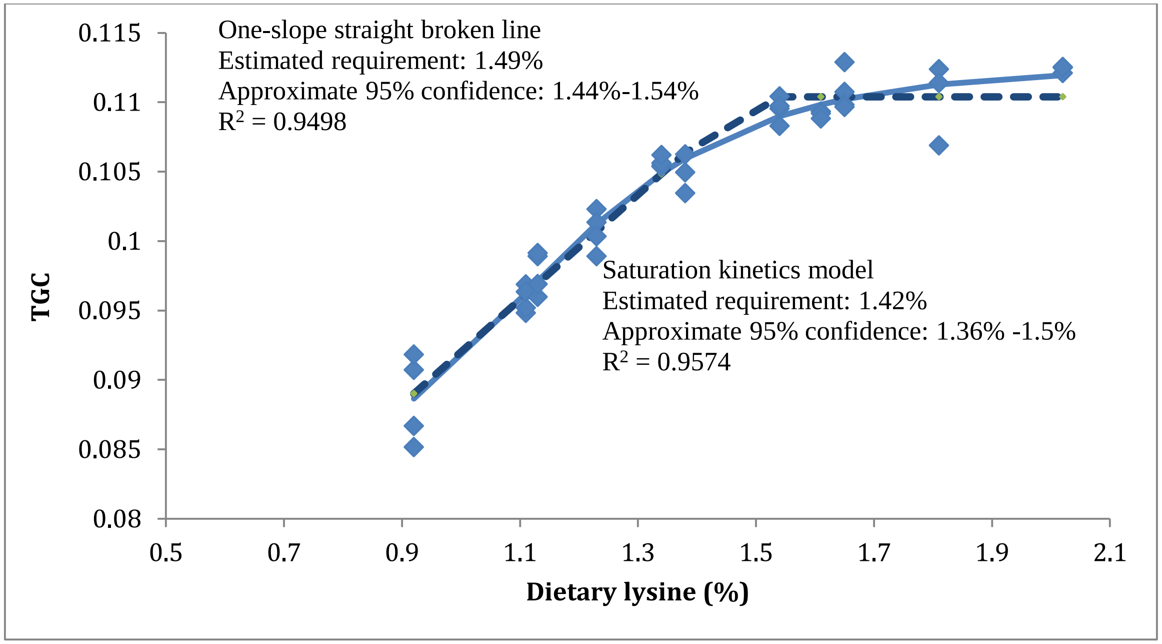

Figure 7. Regression of TGC vs. graded levels of dietary lysine in the second trial. The curve represents the predicted TGC based on the saturation kinetics model, fitted using the least-square method. The dashed line represents the predicted TGC based on the one-slope straight broken-line analysis, fitted using the least-squared method.

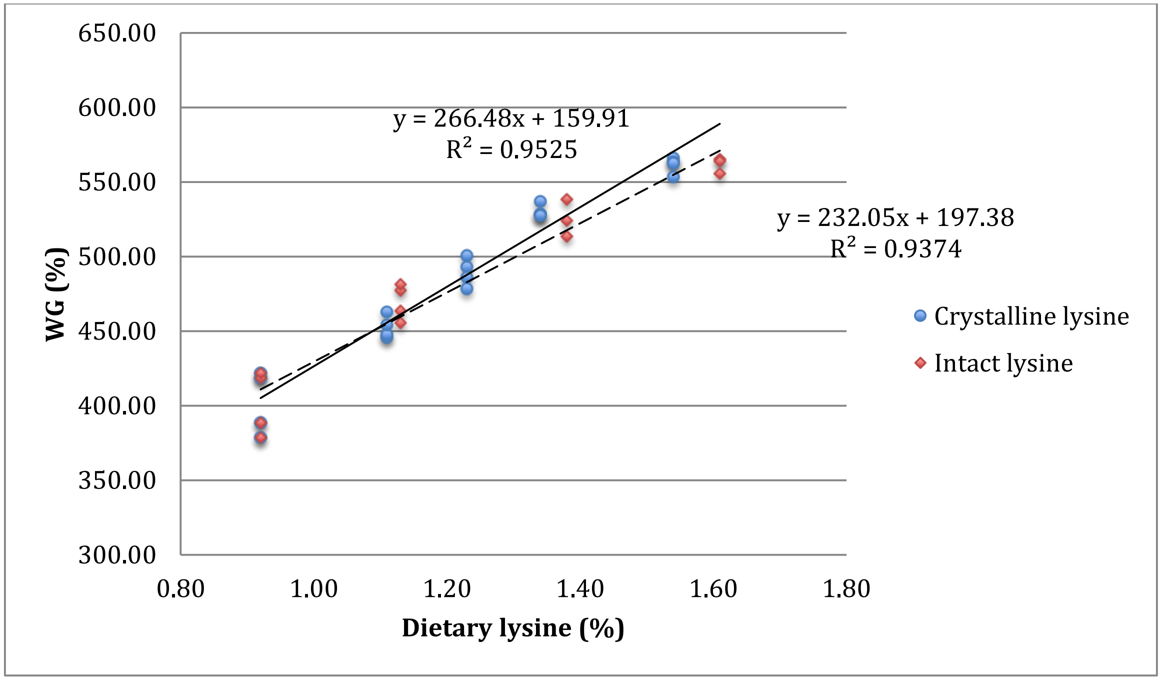

Figure 8. Regression of mean weight gain of Nile tilapia against dietary lysine levels in the second trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

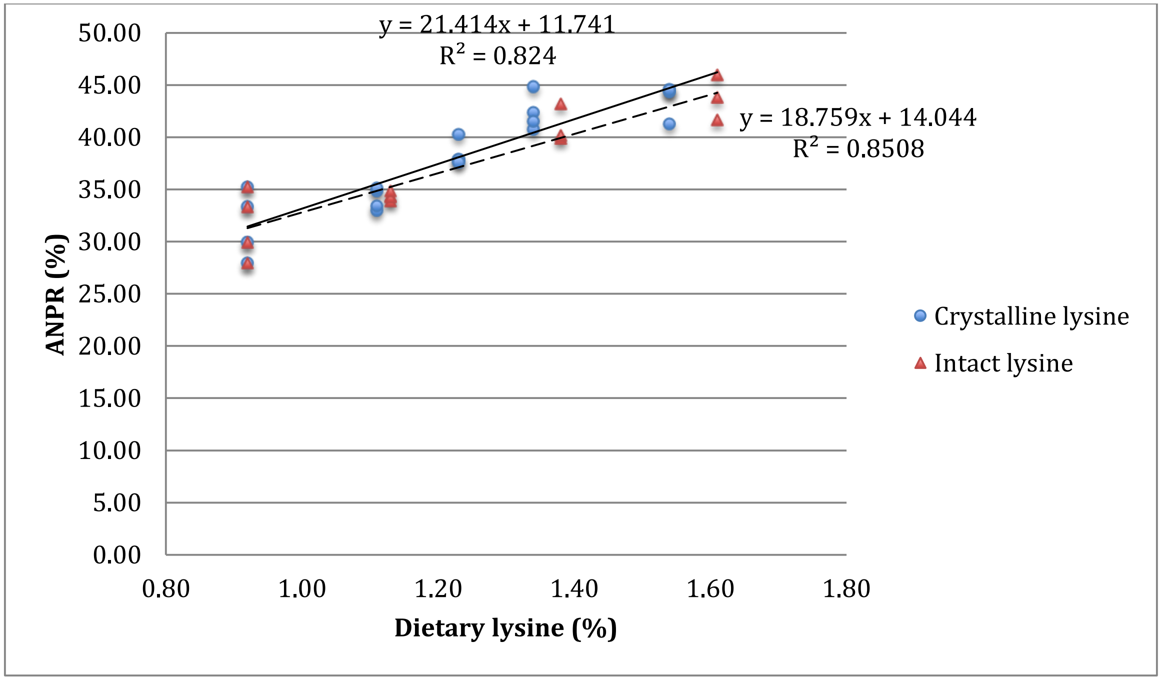

Figure 9. Regression of mean apparent net protein retention of Nile tilapia against dietary lysine levels in the second trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

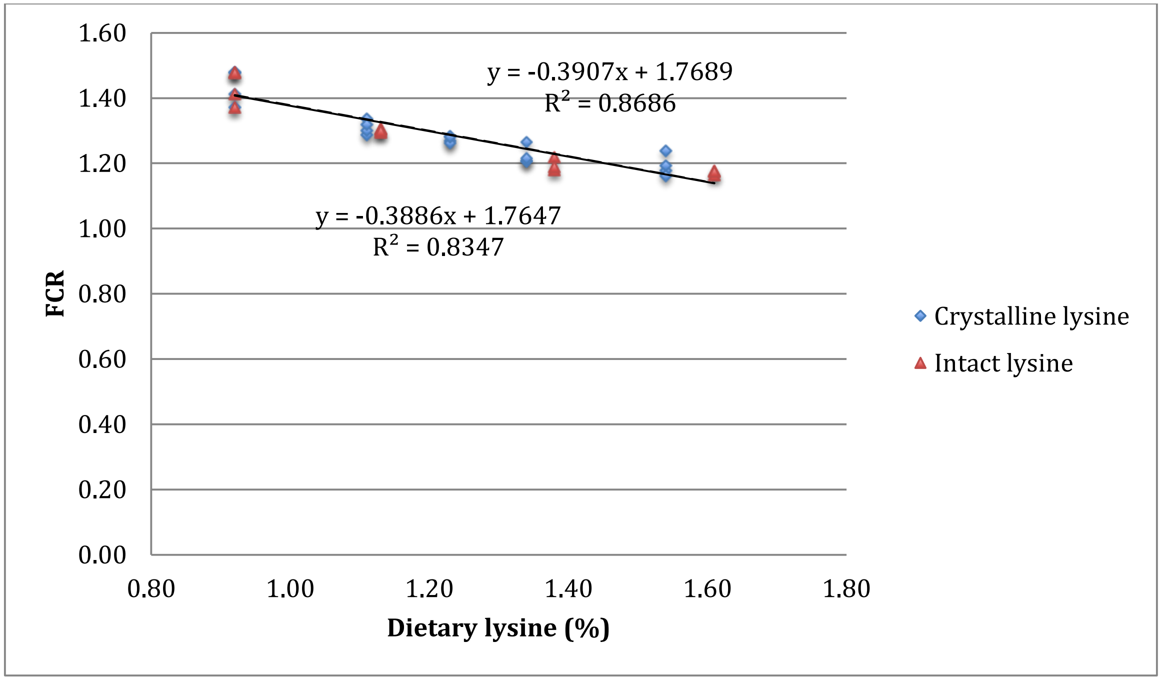

Figure 10. Regression of mean feed conversion ratio of Nile tilapia against dietary lysine levels in the second trial. Solid line represents diets containing crystalline lysine; dashed line is diets with intact lysine.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The use of plant-based protein ingredients as fish meal substitutes has become more cost appealing to aquaculture industry. However, high levels of plant protein inclusion in aquafeeds might cause severe essential amino acid deficiencies in feed, particularly lysine, resulting in poor growth and feed utilization rate of aquatic species. Quite often lysine supplementation is required in plant-based diets for normal growth of aquatic species, and crystalline lysine has been used commercially as a cost-effective supplement to overcome the deficiencies of individual amino acid in terrestrial animals. However, the use of crystalline lysine has been observed to cause reduced growth and feed efficiency of fish in some studies related to palatability, pH change, leaching and rapid uptake. High lysine corn protein concentrate with about 5 times higher lysine content than conventional corn protein concentrates (6.2% versus 1.1%) was developed with the introduction of new plant biotechnology would make the same contribution as feasible intact lysine source to crystalline lysine in the practical diets of aquatic animal. Given the debate over the use of crystalline amino acid, this research sought to evaluate the potential of this new high lysine ingredient as compared to traditional crystalline supplements using channel catfish and tilapia.

The results of this present study indicated that channel catfish and tilapia have positive performance with increasing lysine supplementation. Fish fed with the higher levels of lysine had the highest mean weight gain and lowest mean feed conversion ratio. Based on the growth data obtained from this study, it can be concluded that the effectiveness of using intact lysine as a lysine supplement is not significantly different from crystalline lysine. The regression analysis on the increase level of inclusion of crystalline lysine and intact lysine showed a general trend with parallel increases.

In conclusion, high lysine corn protein concentrate was utilized as efficiently in meeting the essential amino acid requirements of the tilapia and channel catfish as those of crystalline form. The corn protein concentrate with dried L-lysine fermentation product is a feasible ingredient for aquaculture feeds, which can be used to partially substitute soybean meal without addition of crystalline lysine.

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