Effects of long-term feeding of corn co-product-based diets on growth, fillet color, and fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*

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20 **Abstract**

21 We conducted a 24-week feeding trial with Nile tilapia *Oreochromis niloticus* to evaluate the
22 effects of long-term feeding of corn co-product–based diets on growth, fillet color, and fillet fatty
23 acid and amino acid composition. Five iso-nitrogenous diets were prepared (34% protein). The
24 control diet included 10% fishmeal. Fishmeal was eliminated from the other four diets, and 50%
25 of the dietary protein was supplied by one of four corn co-products, namely high-protein
26 distillers’ dried grains (HPDDG), distillers’ dried grains with solubles (DDGS), corn gluten meal
27 (CGM), and corn protein concentrate (CPC). Fish with an initial mean weight of 21 g were fed
28 one of the five diets twice a day to near satiety. Fish fed the control, HPDDG, or DDGS diet had
29 significantly higher ($P < 0.05$) mean weight gain, specific growth rates, mean feed intake, protein
30 efficiency ratio, and survival than those fed the other diets. Fish in these three treatments also
31 had the lowest food conversion ratio. The dietary treatments did not affect the lightness, redness,
32 yellowness, or crude protein and total amino acid content of fish fillets. Fillet lipid and ash
33 content were highest in the CGM group. Fillet fatty acid composition was greatly affected by the
34 dietary treatments. The CGM and CPC groups had significantly highest palmitic acid (16:0) and
35 total saturated fatty acid levels, whereas linoleic acid (18:2n-6) and total polyunsaturated fatty
36 acid levels were highest in the DDGS group. Similarly, the total n-6 level was highest in the
37 DDGS group, followed by HPDDG. The total n-3 levels and n-3: n-6 ratios in the fillets of the
38 control group were almost double those of the corn-based dietary groups. Our results suggest that
39 dietary inclusion of HPDDG or DDGS in non-fishmeal diets at up to 50% of dietary protein does
40 not negatively affect growth performance, feed utilization efficiency, or fillet color and amino
41 acid composition, but further improvement of n-3 fatty acid composition is necessary to ensure
42 human health benefits.

43 Keywords: amino acids, distillers' dried grains with solubles, fatty acids, fillet color, growth
44 performance, high-protein distillers' dried grains

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1. Introduction

In response to the rising demand for, and cost of, fishmeal, the use of dietary alternatives is becoming a common practice in aqua-feed formulations. Among the categories of alternatives, plant-based ingredients have long received great attention (Gatlin *et al.*, 2007, Hansen *et al.*, 2006, Olsen *et al.*, 2007, Vilhelmsson *et al.*, 2004). Corn co-products such as corn gluten meal (CGM), distillers' dried grains with solubles (DDGS), and high-protein distillers' dried grains (HPDDG) are among the plant-based ingredients rich in digestible proteins. Unlike conventional plant protein sources such as soybean meal, these co-products are free from anti-nutritional factors (Robinson and Li, 2008, Shiau *et al.*, 1987) and deficient in only a few amino acids (Cheng and Hardy, 2004). Yellow pigments inherent in the corn, however, limit the proportion of corn co-products that is acceptable for use in feeding food fishes. For instance, xanthophylls in corn-based ingredients could alter the fish fillet color (Gatlin *et al.*, 2007).

Fillet color is an important sensory attribute of food fish that directly determines the acceptability of the product (Dhanapal *et al.*, 2013, Gatlin *et al.*, 2007, Ross, 2000). It is also among the attributes of fish quality that could easily be altered by changes in the pigments in dietary ingredients (Gatlin *et al.*, 2007, Skonberg *et al.*, 1998). The effects of dietary pigments on fillet color have been reported in rainbow trout and some other food fish species, including salmon (Akhtar *et al.*, 1999, Buttle *et al.*, 2001, Li *et al.*, 2007, Skonberg *et al.*, 1998). Changes in flesh color caused by feed ingredients can have a negative effect on sale if the resulting product does not meet consumers' expectations (Park *et al.*, 1997, Skonberg *et al.*, 1998). Pigmentation or coloration is considered a desirable trait in certain food fishes such as trout and salmon (Li *et al.*, 2007), whereas the same attribute is highly undesirable in some white-fleshed fish such as channel catfish (Li *et al.*, 2011, Li *et al.*, 2007) and tilapia (Girao *et al.*, 2012).

Even though fillet color is among the attributes that could be strongly influenced by the pigments present in the protein source (Gatlin *et al.*, 2007), it has been among the criteria that have received the least attention during the evaluation of alternatives for fishmeal. A variety of corn-based ingredients have been tested in tilapia (Coyle *et al.*, 2004, Schaeffer *et al.*, 2010, Suprayudi *et al.*, 2015, Tidwell *et al.*, 2000, Wu *et al.*, 1997, Wu *et al.*, 1996, Wu *et al.*, 1994); almost all of the studies have focused primarily on evaluating the fishes' growth performance and feed utilization efficiencies. To our knowledge, not a single nutritional study has evaluated the effects of the pigments in corn-based ingredients on the fillet color of Nile tilapia.

Another important area that needs to be addressed in evaluating alternative ingredients in fish feeds is the nutritional profile of the resulting fish flesh. Fish is a good source of long-chain n-3, highly unsaturated fatty acids (HUFA), essential amino acids, and micronutrients such as vitamins and minerals, and this unique nutritional composition has led to a rising demand for fish and fish products worldwide (Dergal *et al.*, 2013, Vagner and Santigosa, 2011). Moreover, fish is a source of affordable, quality protein for people in developing countries, including the world's most populous countries (such as China, India, Indonesia, Pakistan, and Bangladesh), which are home to the bulk of the world's undernourished people (FAO, 2014). Compared to fishmeal, the plant-based ingredients used as protein sources for aqua-feeds contain relatively low levels of n-3 polyunsaturated fatty acids (PUFA) (Gatlin *et al.*, 2007, Ng *et al.*, 2013) and are poor in certain essential amino acids (Gatlin *et al.*, 2007, Olsen *et al.*, 2007).

Our objectives here, therefore, were to evaluate the effects of long-term feeding of zero-fishmeal diets containing different corn co-products on the growth performance, fillet color, and fillet fatty acid and amino acid composition of Nile tilapia, *Oreochromis niloticus*.

2. Materials and Methods

2.1 Growth trial

2.1.1 Experimental diets

Five iso-nitrogenous (34% protein) and iso-energetic experimental diets (fishmeal-based control diet and four corn co-product-based diets) were formulated to fulfill the known nutritional requirements of juvenile Nile tilapia. The proximate compositions of the main protein sources and the ingredient compositions of the diets are given in Table 1. Proximate compositions together with amino acid and fatty acid compositions of the diets are given in Tables 2 and 3, respectively. The control diet included 10% fishmeal. Fishmeal was eliminated from the other four diets, and 50% of the dietary protein was supplied by one of four corn co-products, namely high-protein distillers' dried grains (HPDDG), distillers' dried grains with solubles (DDGS), corn gluten meal (CGM), or corn protein concentrate (CPC). Crystalline amino acids (L-lysine, DL-methionine and L-tryptophan) were used to compensate for the essential amino acid deficiencies in the corn-based diets (Table 2). Before they were pelletized, the ingredients were ground to pass through a screen (0.5- μ m mesh) and then mixed by using a horizontal mixer. The mixture was then moistened by adding distilled water (~20%), pelletized and freeze-dried in a vacuum freeze-drier (RLE-206; Kyowa Vacuum Tec, Saitama, Japan). Diets were stored at 5 °C until use. The five diets were named according to the main protein ingredients, namely control (FM), HPDDG, DDGS, CGM, and CPC.

< Tables 1 – 3 >

2.1.2 Experimental fish and rearing conditions

Nile tilapia fingerlings were obtained from the Laboratory of Fish Culture, Tokyo University of Marine Science and Technology, Tokyo, Japan. Fish were acclimated to the experimental conditions for 2 weeks before the start of the experiment. During this acclimation period, fish were hand-fed the control diet to near satiety twice a day. After the acclimatization period, food was withheld for 24 h, after which 200 fish with similar initial body weights (21.0 to 22.0 g) were selected for the experiment. Groups of 18 fish were bulk weighed and stocked into 10 glass tanks (60 L). Five treatments in duplicate were randomly allocated to the 10 tanks. Each tank was connected to a freshwater recirculating system in which the water temperature was maintained at 25 ± 1 °C by using electric heaters. The water flow rate was maintained at 0.5 L min⁻¹ and aeration was provided continuously to each tank via submerged air stones. Approximately 50% of the water in the recirculating system was replaced with dechlorinated tap water once every 2 weeks. Fish were hand-fed to near satiety twice a day for 24 weeks and daily feed intakes were recorded.

2.2 Data collection and sampling

During the 24-week experiment, the fish in each tank were bulk-weighed at 4-week intervals. At the end of the experiment, fish were starved for 24 h and anaesthetized with 0.2% 2-phenoxyethanol before being handled. The weight and length of the fish in each tank were individually measured with an electric balance and a measuring board, respectively, to the nearest first decimal place.

Five fish from each tank were euthanized and used to calculate the hepatosomatic index (HSI), viscerosomatic index (VSI), intra-peritoneal fat (IPF) ratio, and fillet yield (FY). The fillet samples used for fillet yield determination were immediately frozen at -30 °C for final chemical

149 analysis. Frozen samples were minced in a centrifugal mill (Model ZM 1; Retsch, Haan,
150 Germany), freeze-dried in a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tec), and kept at –
151 30 °C until analysis.

152 Mean weight gain (MWG, g), percentage specific growth rate (SGR, %), mean feed
153 intake (MFI,g), food conversion ratio (FCR), percent survival (SR, %), protein efficiency ratio
154 (PER), HSI, VSI, IPF ratio, FY, and the coefficient of condition (K) were calculated by using the
155 following equations to compare fish growth, nutrient utilization efficiency, and body indices
156 among treatments.

157
$$\text{MWG (g)} = [\text{final weight (FW, g)} - \text{initial weight (IW, g)}]$$

158
$$\text{SGR (\%)} = (\ln \text{FW} - \ln \text{IW})/(\text{time, days}) \times 100$$

159
$$\text{MFI(g)} = \text{total feed intake of fish (g)}/\text{number of fish}$$

160
$$\text{FCR} = \text{feed intake (g)}/\text{weight gain (g)}$$

161
$$\text{SR (\%)} = \text{number of fish at harvest}/\text{number of fish stocked} \times 100$$

162
$$\text{PER} = \text{body weight gain (g)}/\text{protein intake (g)}$$

163
$$\text{HSI} = 100 \times \text{liver weight (g)}/\text{bodyweight (g)}$$

164
$$\text{VSI} = 100 \times \text{visceral weight (g)}/\text{bodyweight (g)}$$

165
$$\text{IPF} = 100 \times \text{weight of peritoneal fat (g)}/\text{bodyweight (g)}$$

166
$$\text{Fillet yield} = 100 \times \text{fillet weight (g)}/\text{bodyweight (g)}$$

167
$$\text{K} = 100 \times \text{body weight (g)}/(\text{total length [cm]})^3$$

2.3 Colorimetric analysis of fillets

Fillet color was assessed instrumentally by using a tristimulus colorimeter (Chroma meter, model CR 13; Minolta, Osaka, Japan), which compares the reflectance of light from an object (fish fillet) with that of a standard calibration plate (number 12456). Lightness (L^* , negative for blackness and positive for whiteness), red–green chromaticity (a^* , negative for greenness and positive for redness), and yellow–blue chromaticity (b^* , negative for blueness and positive for yellowness) were measured for each fillet. Five fish per tank (right-side fillet of each fish) were used for the instrumental colorimetric analysis. The colorimetric values of the white muscle in five places (three dorsal, two ventral) in a single fillet were analyzed and the average value was used for calculations. Values were obtained from the same area of the fillet for all fish analyzed. Values for a^* and b^* , representing redness and yellowness respectively, were used to calculate the hue and chroma. Hue is the relationship between redness and yellowness, which is expressed as $H_{ab}^\circ = \tan^{-1}(b^*/a^*)$ (Hunt, 1977). Hue is an angular measurement of color where 0° and 90° denote red and yellow hues, respectively. Chroma is expressed as $C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$ and gives information about the clarity and intensity of the color. The total color difference between the control and corn-based treatments is given by ΔE , which is calculated as $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ where ΔL^* , Δa^* and Δb^* refer to the differences between the control and corn-based treatments.

2.4 Chemical analysis

Feed ingredients, diets, and muscle samples were analyzed in accordance with standard procedures for chemical analysis (AOAC, 2005). All samples were finely ground and analyzed in triplicate. Dry-matter content of each sample was calculated from the weight loss after drying

at 105 °C to a constant weight. Ash content was determined after the incineration of samples in a muffle furnace at 550 °C for 16 h. The Kjeldhal method was used for crude protein analysis (N content $\times$ 6.25). Total lipids in the samples were extracted after homogenization in a chloroform: methanol mixture (2:1, v:v) according to the method of [Folch *et al.* \(1957\)](#).

For fatty acid extraction, lipids were saponified with 1 mL 50% KOH in 15 mL 100% ethanol at 80 °C for 60 min. The saponifiable matter was then esterified with 7% boron trifluoride (BF₃) in methanol (Morita Chemical Industries Co., Ltd., Osaka, Japan) and heated for 20 min at 80 °C ([Morrison and Smith, 1964](#)). Fatty acid methyl ester (FAME) preparations were made up in hexane (20 mg/mL) and analyzed by using a gas chromatograph (GC) (GC-2025; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector and a silica capillary column (L $\times$ I.D., 30 m $\times$ 0.32 mm, df 0.25 μ m; Supelcowax 10 Fused Silica Capillary GC column; Supelco, Bellefonte, PA, USA) as described by [Kabeya *et al.* \(2014\)](#). Helium was used as the carrier gas and the thermal program was set to increase the column temperature from 170 to 260 °C at 2 °C min⁻¹. The temperature in both the injection port and the detector was adjusted to 260 °C. FAME peaks were identified by comparing the retention time of each with that of the appropriate FAME standard. Amino acid content was analyzed by using an automatic amino acid analyzer (JLC-500/v; JEOL, Tokyo, Japan) ([Boonyoung *et al.*, 2013](#)). For total amino acids, samples were digested at 110 °C for 22 h with 4 M methanosulphonic acid (Sigma-Aldrich, St. Louis, MO, USA); the digested solution was then passed through a 0.45- μ m membrane filter and injected into the automatic amino acid analyzer.

2.5 Statistical analysis

Data were initially checked for normality and equal variance by using Levene's test. The effects of different corn co-products on Nile tilapia growth performance, feed utilization efficiency, body indices, and fillet quality were compared in a one-way analysis of variance (ANOVA) at the 5% significance level ($P < 0.05$). When ANOVA was found to be significant, Tukey's multiple range test was used to detect differences among treatments. Statistical analysis of fillet fatty acid composition (area %) was performed on arcsine-transformed data (Zar, 1999). All statistical analyses were performed with SPSS version 16.0.

3. Results

3.1 Growth performance and feed utilization efficiencies

The experimental diets affected growth performance and feed utilization efficiencies of fish (Table 4). Compared with the control, CGM and CPC showed significantly lower MWG, % SGR, and MFI. These responses in the HPDDG and DDGS treatments were not different from those in the control. The significantly lowest percent survival was observed in CPC; there were no significant differences in survival between the other treatments. Feed utilization efficiencies in terms of FCR and PER were also affected by the diet treatments. Fish fed HPDDG and DDGS performed equally well in terms of FCR and PER compared with fish fed the control diet with fishmeal. The highest FCRs and lowest PERs were observed in the CGM and CPC groups.

<Table 4>

Long-term feeding with corn co-product-based diets did not affect certain body indices such as the IPF ratio, VSI, and FY (Table 5). There were significant differences in the HSI among treatments. The highest and lowest HSIs were observed in the CGM and DDGS treatments, respectively (Table 5). However the HSI in the control group was not statistically

different from that in either HPDDG or CPC. Significantly highest condition factor (K) was in the control group (Table 5).

<Table 5>

3.2 Fillet color

The chromatic parameters of fillets from Nile tilapia were not affected by the dietary treatments (Table 6). The lightness (L*) value ranged from 41.5 to 48.0. Even though redness (a*) and yellowness (b*) were numerically highest in the CPC and control groups, respectively, there were no observed statistical differences among treatments.

<Table 6>

3.3 Nutritional quality of fillets

The nutritional quality of fillets was determined on the basis of proximate composition, total fatty acid composition, and amino acid composition. Proximate fillet compositions are given in Table 7.

<Table 7>

There were no statistical differences in dry matter or crude protein contents of fillets from fish in the different dietary groups (Table 7). CGM treatment gave the highest lipid content ($P < 0.05$) compared with the control. The other three treatments were not significantly different from either CGM or the control in terms of crude lipid content of fillets (Table 7). The highest ash content was also obtained with CGM; the other treatments gave statistically similar ash contents (Table 7).

Nineteen different fatty acids were identified in fish fillets (Table 8). Linoleic acid (LA, 18:2n-6), oleic acid (18:1n-9), and palmitic acid (16:0) occurred in highest proportions in all treatments; these three fatty acids collectively accounted for two-thirds of the total fatty acid pool. With the exception of two fatty acids (oleic acid [18:1n-9], and α -linolenic acid [LNA, 18:3n-3]), the fatty acid profile of the Nile tilapia fillets was significantly affected by the treatments. Among five dietary groups, linoleic acid was significantly highest in the DDGS group, followed by the HPDDG. The highest and the lowest fillet percentages of stearic acid (18:0) were observed in CPC and DDGS, respectively. Among C-20 and C-22 PUFA, the level of arachidonic acid (ARA, 20:4n-6) was significantly higher with all of the corn-based treatments than with the control. Fillet levels of adrenic acid (22:4n-6) and decosapentaenoic acid (22:5n-6) were highest in DDGS, whereas the fillet level of adrenic acid was lowest in CGM. Fillets of both the control and CGM groups had the lowest levels of decosapentaenoic acid (22:5n-6). As expected, fillet of fishmeal based control group had the significantly higher levels of eicosapentaenoic acid (EPA, 20:5n-3), decosapentaenoic acid (DPA, 22:5n-3), and decosahexaenoic acid (DHA, 22:6n-3) than that of corn based treatments. The proportion of individual saturated fatty acids (SFA) was significantly highest in the fillets of the CGM and CPC groups, whereas the proportion of PUFA was highest in the DDGS-group fillets. The ratio of n-3 PUFA to n-6 PUFA in the fillets of the control group given fishmeal was more than double that of the fillets from the corn-based treatments. The calculated PUFA to SFA ratio was above 0.83 in all treatments.

<Table 8>

There were no significant differences in total amino acid composition of Nile tilapia fillets among different dietary groups. Among the total amino acids, glutamic acid, aspartic acid,

and lysine were found at relatively high percentages in tilapia fillets, whereas tryptophan was the least available amino acid (Fig. 1).

<Fig. 1>

4. Discussion

The growth rates of fish were affected by the dietary treatments. Growth is a function of feed intake and the capacity of the fish to utilize ingested food (Carter *et al.*, 2001). We clearly observed this relationship: the control, HPDDG, and DDGS groups showed feed intake levels higher than those of the CGM and CPC groups, and the effects of lower feed intake in the latter treatments were clearly noticed in the lower specific growth rates. The observed variations in growth rates are therefore likely explained by the differences in treatments.

Dietary availability of essential nutrients such as amino acids is an important determinant of feed intake in fish (De la Higuera, 2001). Dietary amino acid profiles in this study were not equal among treatments, and the differences in feed intake observed here can be explained by the differences in dietary amino acids. Even if the diets were formulated to fulfill the essential amino acid requirements (Santiago and Lovell, 1988) of Nile tilapia, the analytical data showed that CGM and CPC were deficient in dietary arginine and histidine. Feed intake is known to decrease when dietary essential nutrients are deficient (De la Higuera, 2001, Gómez-Requeni *et al.*, 2004). Therefore, differences in amino acid composition among diets are, plausibly, responsible for the observed variation in feed intake and thereby for differences in growth performance.

In addition, HPDDG and DDGS, which yielded growth performances similar to the control, are co-products of a corn dry-milling process, whereas CGM and CPC are co-products of corn wet-milling. Because all four corn co-products were made from the same ingredient, the

observed differences in feed intake and growth are likely attributable to difference in the corn processing method. Unlike wet-milling, dry-milling processes the whole corn kernel, which is subjected to subsequent saccharification and fermentation before the co-products are processed (Weigel *et al.*, 2005, Weigel *et al.*, 1997, Wu *et al.*, 1997). Dry-milling co-products such as HPDDG and DDGS usually contain fermentation residues, including yeast cells (*Saccharomyces cerevisiae*) (Belyea *et al.*, 2004, Han and Liu, 2010, Weigel *et al.*, 1997) and other unidentified nutrients formed during the fermentation–distillation process (Weigel *et al.*, 1997). Corn wet-milling uses steeping and fractionation technology to separate the corn kernel into protein, starch, oil, and fiber components, and the protein portion is not subjected to fermentation (Weigel *et al.*, 1997, Wu *et al.*, 1997). Therefore, the greater feed intake and growth performance observed in the HPDDG and DDGS groups than in the CGM and CPC groups could be associated with the availability of yeast and other unidentified fermentation residues remaining in the dry-milled co-products. This hypothesis is supported by the work of Abdel-Tawwab *et al.* (2008), who reported improved growth performance of Nile tilapia juveniles fed diets supplemented with commercial live yeast (*S. cerevisiae*); they noted that the yeast enhanced appetite and consequently improved feed intake. Lim *et al.* (2007) also reported improved feed consumption and growth of Nile tilapia fed diets containing DDGS.

Although we found here that the % SGRs were lower in the CPC and CGM treatments than in the other treatments, our observations are consistent with the reported literature for Nile tilapia of similar size (Fontainhas-Fernandes *et al.*, 1999, Likongwe *et al.*, 1996). Our highest PERs were in the control, HPDDG, and DDGS groups, which showed higher growth rates compared to other two treatments, indicating that these groups had the most efficient feed utilization for growth. There were no significant differences in fillet yields of tilapia in the

different dietary treatments; this shows that Nile tilapia are capable of converting corn co-products and other ingredients into edible muscle mass at rates similar to those when the fish are fed a fishmeal-based diet.

The lightness of Nile tilapia fillets, as measured instrumentally, has been reported as 55.2 and 52.7 by [Girao *et al.* \(2012\)](#) and [Lima *et al.* \(2015\)](#), respectively, whereas we obtained values here of 41.5 to 48.0. In contrast, the redness measured in these two earlier studies was comparable to our values. Yellowness was reported as 5.5 by [Lima *et al.* \(2015\)](#) and 9.7 by [Girao *et al.* \(2012\)](#), whereas our values ranged from 1.3 to 3.2. This indicates that dietary inclusion of corn-based ingredients does not enhance the generally observed yellowness in tilapia fillets. Xanthophyll retention in fish is a function of a wide array of factors such as uptake, absorption, transport, metabolism and excretion ([Torrissen *et al.*, 1989](#)). Many fish species have the ability to convert one pigment into another ([Kaisuyama and Matsuno, 1988](#), [Matsuno, 1991](#), [Yamashita *et al.*, 1996](#)) and this ability varies considerably between species. The most important metabolic products of carotenoids in some animals are the retinoids; [Matsuno \(1991\)](#) reported the direct bioconversion of xanthophylls astaxanthin, zeaxanthin, lutein, and tunaxanthin into 3-dehydroretinol in Nile tilapia. The instrumentally measured fillet color values in our study clearly indicate that the yellow pigments in corn-based ingredients are not deposited in the fillet to the extent that the fillets exhibit clear visual differences in color. Further studies are required to confirm this observation.

After 24 weeks of growth trials, the fatty acid composition of Nile tilapia fillets mirrored that of their respective diets. This matches similar previous observations in Nile tilapia and red hybrid tilapia ([Justi *et al.*, 2003](#), [Ng *et al.*, 2013](#), [Tadesse *et al.*, 2003](#), [Teoh *et al.*, 2011](#)). In general, HUFA such as 20:4n-6, 22:5n-3 and 22:6n-3 show selective deposition and retention in

fish fillets, as their concentrations in fillets are always higher than those in the diet. We saw a opposite selective utilization of 18:2n-6 and 18:3n-3 in which the dietary concentration was always higher than that of fillet. This result also suggests that 18:2n-6 and 18:3n-3 is readily utilized by fish when present at high concentrations. The dietary long-chain polyunsaturated fatty acids (LC-PUFA) made up less than 0.25% of the four corn-based diets; the LC-PUFA concentration in the fishmeal-based control diet was 10 times greater. However, at the end of 24 weeks, the LC-PUFA level in fillets from the corn-based treatments was almost two-thirds that of the control. This indicates the effectiveness of fatty acid desaturase and elongase enzymes in the herbivorous Nile tilapia.

The individual fatty acid compositions of fillets from fish in the corn-based treatments in this study, however, further indicated that the activity of fatty acid desaturase and elongase in the bio-conversion of 18:3n-3 to its long-chain homologs was insufficient to maintain the proportions of EPA and DHA in fish fed non-fishmeal diets at the same levels as in fish fed the fishmeal-based control diet. We did not use fish oil here: fishmeal was the only marine-derived feedstuff used and it was present only in the control. This result might therefore expose the possible effects of using fishmeal- and fish oil-free diets in commercial tilapia culture on the fatty acid composition of the fish produced. However, the PUFA:SFA ratio in all treatments ranged between 0.83 and 1.39 which is well above the minimum value of 0.45 recommended by the Department of Health of the UK ([HMSO, 1994](#)) and confirms that fish fed corn co-product are suitable for human consumption.

5. Conclusions

Among tested ingredients, HPDDG and DDGS were the best corn co-products to formulate a zero-fishmeal diet for Nile tilapia. This study provide convincing evidence for the dietary inclusion of higher percentage of corn co-products did not negatively affect on fillet quality in terms of amino acid profile and the fillet color. However, improvement of n-3 highly unsaturated fatty acids is necessary in HPDDG and DDGS diet to ensure human health benefits. Application of fish oil-based finishing diet could be a possible solution to overcome this problem, and further studies are recommended in this regard.

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Table 1. Proximate composition of main ingredients¹ and formulation of experimental diets¹ for Nile tilapia, *Oreochromis niloticus* (g kg⁻¹ dry matter, *n* = 3)

Component	Fishmeal	HPDDG	DDGS	CGM	CPC
Dry matter	918	904	896	933	901
Crude protein	682	461	314	658	798
Crude lipid	100	37.7	77.1	103	95.8
Ash	113	38	66	41	16
Ingredient	Control	HPDDG	DDGS	CGM	CPC
Fishmeal	100	0	0	0	0
Corn co-product	0	332	524	235	194
Soybean meal	278	188	211	168	166
Poultry by-product meal	104	52	71	52	53
Wheat flour	308	225	56	302	329
Alpha starch	152	121	86	175	176
Vitamin mix ²	10	10	10	10	10
Mineral mix ³	16	16	16	16	16
Soy oil	41	46	18	32	44
L-lysine	0	5	4	6	7
DL-methionine	0	4	5	3	4
L-tryptophan	0	1	1	1	1

¹HPDDG, high-protein distillers' dried grain/HPDDG-based diet; DDGS, distillers' dried grains with solubles/DDGS-based diet; CGM, corn gluten meal/CGM-based diet; CPC, corn protein concentrate/CPC-based diet

²Vitamin mixture composition (unit kg⁻¹): Vitamin D3, 2,420,000 IU; Vitamin K3, 6050 mg; thiamine, 3025 mg; riboflavin, 3630 mg; pyridoxine, 2420 mg; cyanocobalamin, 6 mg; L-ascorbic acid, 368,900 mg; nicotinic acid, 24,200 mg; D-pantothenic acid, 6050 mg; inositol, 121,000 mg; d-biotin, 363 mg; folic acid, 908 mg; para-aminobenzoic acid, 3025 mg

³Mineral mixture composition (g kg⁻¹): Sodium chloride, 50; magnesium sulfate, 745; iron(III) citrate n-hydrate, 125; cellulose, 30; trace-element mixture, 50. The trace-element mixture contained (g kg⁻¹) zinc sulfate heptahydrate, 353; manganese sulfate, 162; copper (II) sulfate pentahydrate, 31; aluminum chloride hexahydrate, 10; cobalt chloride, 3; potassium iodate, 1; cellulose, 440.

Table 2. Proximate composition (g kg⁻¹ dry matter, *n* = 3) and essential amino acid composition (as a percentage of dietary protein, *n*= 3) of experimental diets¹ for Nile tilapia, *Oreochromis niloticus*

Component	Control	HPDDG	DDGS	CGM	CPC	
Dry matter	947.3	976.5	949.0	967.4	965.3	
Crude protein	342.9	348.7	350.9	336.4	343.2	
Crude lipid	78.3	66.8	69.6	93.8	87.8	
Ash	68.6	34.4	38.7	55.6	45.2	
Gross energy (MJ kg ⁻¹ diet) ²	20.0	20.4	20.4	20.5	20.6	
EAA ³						Requirement ⁴
Arginine	4.42	4.72	4.87	4.15	3.90	4.20
Histidine	1.80	1.80	1.85	1.65	1.63	1.72
Isoleucine	3.42	3.50	3.22	3.22	3.21	3.11
Leucine	5.91	7.80	7.69	10.06	8.74	3.39
Lysine	5.26	5.50	5.32	5.14	5.65	5.12
Methionine	3.23	2.80	2.69	2.75	2.69	2.68
Phenylalanine	3.82	4.45	4.39	5.21	4.74	3.75
Threonine	3.91	3.93	3.79	3.96	3.81	3.75
Tryptophan	1.01	1.10	1.06	1.11	1.04	1.00
Valine	3.40	2.98	2.94	3.63	3.15	2.80
NEAA ⁵						
Alanine	3.38	6.18	5.77	6.57	6.11	
Aspartic acid	9.24	9.10	7.93	7.24	7.01	
Cysteine	0.58	1.05	0.99	0.88	0.83	
Glutamic acid	19.53	20.97	19.13	22.46	21.46	
Glycine	5.46	5.24	4.59	4.01	3.92	
Serine	2.47	5.08	4.41	4.49	4.42	
Tyrosine	2.60	3.77	3.45	4.04	3.62	

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet;

CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

²Gross energy content was calculated assuming 23.7, 39.5 and 17.2 MJ per kg of protein, lipid and carbohydrates (nitrogen free extracts) respectively as described by Einen and Roem (1997)

³EAA, essential amino acids

⁴Essential amino acid requirement of Nile tilapia as described by Santiago and Lovell (1988)

⁵NEAA, non essential amino acids

Table 3. Fatty-acid composition of experimental diets¹ (area %, *n* = 3)

Fatty acid ²	Control	HPDDG	DDGS	CGM	CPC
14:0	1.13	0.15	0.15	0.16	0.18
16:0	16.58	15.08	15.41	16.79	14.67
18:0	3.85	3.34	2.74	2.99	3.33
18:1n-9	23.05	23.91	24.97	22.71	23.77
18:2n-6	42.06	49.72	50.60	49.80	49.16
18:3n-3	3.74	4.08	2.61	3.45	3.90
20:1n-9	0.02	0.12	0.02	0.22	0.03
20:4n-6	0.29	0.05	0.11	0.09	0.10
20:5n-3	1.19	ND ³	ND	0.11	0.03
22:6n-3	0.98	0.07	0.08	ND	0.04
Σ saturates	21.56	18.57	18.30	19.94	18.18
Σ monoenes	27.11	26.31	27.03	25.20	26.34
Σ PUFA ⁴	48.66	53.91	53.41	53.49	53.28
n-3:n-6	0.15	0.08	0.05	0.07	0.08

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet;

CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

²Some fatty acids are not shown (16:1n-7, 18:1n-7, 18:4n-3, 20:1n-11, 20:4n-3, 22:1n-11, 22:1n-13, 22:4n-9, 22:5n-3)

³ND, not detectable

⁴PUFA, polyunsaturated fatty acids

Table 4. Growth performance and feed utilization efficiencies of Nile tilapia fed experimental diets¹ for 24 weeks (*n* = 2)

Parameter ²	Control	HPDDG	DDGS	CGM	CPC	<i>P</i> -value
MWG (g)	162.2 ± 16.8 ^a	160.7 ± 6.4 ^a	161.4 ± 10.0 ^a	88.3 ± 20.9 ^b	74.9 ± 5.2 ^b	0.005
%SGR	1.27 ± 0.04 ^a	1.26 ± 1.11 ^a	1.27 ± 0.03 ^a	0.96 ± 0.12 ^b	0.90 ± 0.03 ^b	0.009
MFI (g fish ⁻¹)	216.2 ± 21.1 ^a	222.2 ± 3.5 ^a	225.5 ± 1.2 ^a	148.8 ± 26.5 ^b	124.1 ± 9.4 ^b	0.008
FCR	1.33 ± 0.01 ^b	1.38 ± 0.07 ^b	1.4 ± 0.08 ^b	1.72 ± 0.15 ^a	1.66 ± 0.03 ^a	0.017
PER	2.31 ± 0.01 ^a	2.12 ± 0.11 ^a	2.30 ± 0.12 ^a	1.69 ± 0.15 ^b	1.68 ± 0.01 ^b	0.004
% Survival	97.2 ± 3.9 ^a	97.2 ± 3.9 ^a	97.2 ± 3.9 ^a	91.7 ± 11.8 ^a	52.7 ± 11.8 ^b	0.010

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P* > 0.05) by the Tukey's test

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet; CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

²MWG, mean weight gain; % SGR, specific growth rate (%); MFI, mean feed intake; FCR, food conversion ratio; PER, protein efficiency ratio

Table 5. Body indices of Nile tilapia fed experimental diets¹ for 24 weeks (*n* = 10)

Parameter ²	Control	HPDDG	DDGS	CGM	CPC	<i>P</i> -value
IPF ratio	1.88 ± 0.09	2.22 ± 0.64	1.50 ± 0.44	2.02 ± 1.05	1.34 ± 0.35	0.612
HSI	2.70 ± 0.32 ^b	2.70 ± 0.15 ^b	1.93 ± 0.30 ^c	3.45 ± 0.21 ^a	2.30 ± 0.26 ^{bc}	0.014
VSI	9.33 ± 0.92	10.92 ± 1.27	9.44 ± 0.35	11.62 ± 1.06	11.50 ± 0.30	0.116
Fillet yield	28.16 ± 0.87	27.52 ± 0.05	27.34 ± 0.68	27.14 ± 0.18	26.37 ± 0.82	0.080
K	2.01 ± 0.01 ^a	1.83 ± 0.00 ^c	1.89 ± 0.02 ^{bc}	1.94 ± 0.05 ^b	1.87 ± 0.01 ^{bc}	0.006

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P* > 0.05) by the Tukey's test

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet; CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

²IPF, intraperitoneal fat; HSI, hepatosomatic index; VSI, viscerosomatic index; K, condition factor

Table 6. Tristimulus color parameters of fillets from Nile tilapia fed experimental diets¹ for 24 weeks (*n* = 10)

Parameter ²	Control	HPDDG	DDGS	CGM	CPC	<i>P</i> -value
L*	47.8 ± 3.7	48.0 ± 0.7	47.8 ± 0.6	41.5 ± 2.4	41.8 ± 0.9	0.061
a*	1.3 ± 0.3	0.7 ± 0.3	1.2 ± 0.2	1.8 ± 0.5	2.3 ± 0.5	0.450
b*	3.2 ± 0.6	2.3 ± 0.2	2.3 ± 1.2	1.3 ± 0.7	2.2 ± 0.5	0.516
Chroma	3.5 ± 0.3	2.4 ± 0.2	2.7 ± 0.9	1.9 ± 1.2	3.3 ± 0.7	0.574
Hue (°)	67.5 ± 7.6	74.0 ± 5.6	54.5 ± 18.7	53.6 ± 13.1	43.3 ± 12.1	0.501
ΔE	0	1.11	0.97	6.61	6.22	

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P* > 0.05) by the Tukey's test

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet; CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

²L*, lightness; a*, redness; b*, yellowness; chroma, intensity of color; hue angle = 0° for redness and 90° for yellowness; ΔE, total color difference compared with control

Table 7. Proximate compositions of fillets from Nile tilapia fed experimental diets¹ for 24 weeks (% wet basis, $n = 3$)

Component	Control	HPDDG	DDGS	CGM	CPC	<i>P</i> -value
Moisture	77.85 ± 0.21	77.60 ± 0.14	77.35 ± 0.21	77.30 ± 0.14	77.70 ± 0.14	0.096
Crude protein	19.60 ± 0.28	19.60 ± 0.00	19.65 ± 0.35	19.40 ± 0.14	19.35 ± 0.21	0.637
Crude lipid	1.80 ± 0.00 ^b	2.05 ± 0.07 ^{ab}	2.20 ± 0.00 ^{ab}	2.35 ± 0.07 ^a	2.05 ± 0.21 ^{ab}	0.024
Ash	1.30 ± 0.05 ^b	1.30 ± 0.00 ^b	1.26 ± 0.06 ^b	1.60 ± 0.08 ^a	1.50 ± 0.14 ^{ab}	0.013

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different ($P > 0.05$) by the Tukey's test

¹HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet; CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

Table 8. Fatty-acid¹ compositions of fillets of Nile tilapia fed experimental diets² for 24 weeks (area %, *n* = 3)

Fatty acid	Control	HPDDG	DDGS	CGM	CPC	<i>P</i>-value
14:0	2.02 ± 0.09 ^a	1.94 ± 0.05 ^a	1.40 ± 0.02 ^b	2.29 ± 0.19 ^a	2.30 ± 0.08 ^a	0.002
16:0	21.95 ± 0.40 ^{bc}	22.61 ± 0.29 ^b	20.19 ± 0.08 ^c	26.65 ± 1.01 ^a	25.74 ± 0.13 ^a	0.000
18:0	6.82 ± 0.02 ^b	6.35 ± 0.05 ^b	5.61 ± 0.02 ^c	6.66 ± 0.32 ^b	7.51 ± 0.00 ^a	0.000
18:1n-9	22.13 ± 0.04	22.01 ± 0.03	21.29 ± 0.84	23.04 ± 1.04	21.38 ± 0.19	0.067
18:2n-6	23.33 ± 0.07 ^c	25.41 ± 0.12 ^b	29.93 ± 0.00 ^a	21.17 ± 1.00 ^d	20.93 ± 0.05 ^d	0.000
18:3n-3	1.35 ± 0.17	1.34 ± 0.11	1.09 ± 0.09	0.91 ± 0.04	0.96 ± 0.08	0.084
20:4n-6	2.37 ± 0.09 ^b	2.92 ± 0.05 ^a	3.31 ± 0.07 ^a	3.12 ± 0.20 ^a	3.09 ± 0.03 ^a	0.000
20:5n-3	0.27 ± 0.01 ^a	0.06 ± 0.00 ^d	0.08 ± 0.00 ^{cd}	0.16 ± 0.00 ^b	0.11 ± 0.01 ^{bc}	0.000
22:4n-6	0.73 ± 0.01 ^b	0.77 ± 0.07 ^{ab}	0.92 ± 0.05 ^a	0.48 ± 0.05 ^c	0.71 ± 0.02 ^b	0.000
22:5n-6	1.18 ± 0.03 ^d	2.36 ± 0.13 ^b	2.70 ± 0.11 ^a	1.25 ± 0.12 ^d	2.01 ± 0.08 ^c	0.001
22:5n-3	0.96 ± 0.03 ^a	0.29 ± 0.03 ^b	0.30 ± 0.02 ^b	0.19 ± 0.00 ^c	0.34 ± 0.01 ^b	0.000
22:6n-3	3.61 ± 0.13 ^a	1.33 ± 0.09 ^c	1.30 ± 0.04 ^{cd}	1.02 ± 0.02 ^d	1.65 ± 0.06 ^b	0.000
Σ saturates	30.80 ± 0.47 ^b	30.90 ± 0.29 ^b	27.20 ± 0.08 ^c	35.60 ± 0.88 ^a	35.55 ± 0.04 ^a	0.000
Σ monoenes	28.99 ± 0.15	27.74 ± 0.07	26.14 ± 0.02	30.06 ± 0.94	28.05 ± 0.60	0.057
Σ PUFA	33.86 ± 0.23 ^b	34.04 ± 0.35 ^b	37.73 ± 0.06 ^a	29.70 ± 1.06 ^c	30.70 ± 0.32 ^c	0.008
n-3	6.25 ± 0.16 ^a	2.98 ± 0.08 ^b	2.76 ± 0.05 ^b	2.28 ± 0.06 ^c	3.07 ± 0.08 ^b	0.000
n-6	27.61 ± 0.07 ^c	31.06 ± 0.18 ^b	34.96 ± 0.11 ^a	27.42 ± 1.00 ^c	27.64 ± 0.24 ^c	0.000
n-3:n-6	0.23	0.10	0.08	0.08	0.11	
PUFA:SFA	1.10	1.10	1.39	0.83	0.86	

Values are Mean ± SEM. Means with the same superscript in each row are not significantly different (*P* > 0.05) by the Tukey's test

Standard errors below 0.01 are not given. ND, not detectable; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids

¹Some fatty acids are not shown (16:1n-7, 18:1n-7, 18:4n-3, 20:1n-9, 20:1n-11, 20:4n-3, 22:1n-11)

²HPDDG, high-protein distillers' dried grain-based diet; DDGS, distillers' dried grains with solubles-based diet; CGM, corn gluten meal-based diet; CPC, corn protein concentrate-based diet

Figure 1

Fig. 1: Total amino acid compositions of Nile tilapia fillets ($n = 3$). Bars represents the standard error of the mean and values were not significantly different among dietary groups ($P > 0.05$), Tukey's test