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Genotype x environmental interaction of growth traits to different levels of dietetic lysine for GIFT tilapia

N.R. Leite[a,*] namibiarleite@gmail.com, T.S. Campideli[a], M. Del. P. Rodriguez-Rodriguez[a], B. M. Pereira[a], T. A. Ferreira[c], L. R. A. Abreu[c], A.F.A. Fernandes[b], E. M. Turra[c], M.A. Silva[c], C.M. Bonafé[a]

[a]Departamento de Zootecnia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Rodovia MG 367, n. 5000 - Alto do Jacuba, Diamantina, Minas Gerais, Brazil

[b]Animal Science Department, University of Wisconsin - Madison, 470, Animal Science Building, 1675 Observatory Dr, Madison, WI 53706 USA

[c]Escola de Veterinária da Universidade Fedral de Minas Gerais, Avenida Antônio Carlos, n 6627 – caixa postal 567, Campus da UFMG, Belo Horizonte, Minas Gerais, Brazil

[*]Corresponding author.

Abstract

The effect of genotype x environment interaction for dietetic lysine on Nile tilapia of the GIFT line body weight, daily and total weight gains at 150 days of age was evaluated in this study. The experiment was composed of 700 fish from 26 full-sib families. Each fish was uniquely identified with a microchip tag and randomly distributed in 5 different levels of dietetic lysine (1.43, 1.53, 1.63, 1.73, 1.83%), each level had 7 replicates. Random regression models with homogeneity and heterogeneity (2, 3, and 4 classes) of residual variance were used for the estimation of the genetic parameters. Those models were evaluated via the BIC and model probability criterions. In order to access the sensitivity of the breeding values to the dietetic lysine levels, reaction norms, genetic and Spearman correlations were estimated. The results pointed out the need to consider heterogeneity of residual variance. Significant genotype x environment interaction between the levels of dietetic lysine was identified for daily weight gain. Thus, the results are suggestive that the predictions of the breeding values for the traits evaluated at 150 days of age for Nile tilapia of the GIFT line must consider the level of digestive lysine in the diet used in the production system.

Keywords: *Oreochromis niloticus*; Heterogeneity variance; Reaction Norms; Random regression models; genetic sensitivity.

1. INTRODUCTION

Fish nutritional requirements may change according to the species ability to use the given diet, and the food in suspension on the production system (Pezzato et al., 2004). According to Wilson Poe (1985), a correct balance between essential and non-essential amino acids is more important to fish than the ratio of crude protein in the diet. Thus, the principle of ideal protein can be defined as the exact balance between the amino acids in the diet that is necessary for the maintenance and growth. Following this principle, each essential amino acid is incorporated into the fish diet as a ratio of lysine as the reference amino acid (Furuya et al., 2005). The correct amino acid balance is important since a deficit of any essential amino acid can lead to nutritional disorder with a decrease of fish feed efficiency, weight gain and even disease resistance (Pezzato et al., 2004). Also, lysine is the most important amino acid to fish. This importance is because lysine is present at high levels in the fish body composition and it also is the first limiting amino acid in several protein sources. Therefore, it has been prioritized in fish nutrition (Furuya et al., 2004; Furuya et al., 2005; Rollin et al., 2003).

The identification of genotype x environment interaction (GEI) is of great importance to breeding programs, because in the presence of GEI the same genotype may have different breeding values across the environmental gradient. This may produce different animal performance and possible rank reordering (Kolmodin et al., 2002). Reaction norms are an interesting way to evaluate GEI since it allows the modeling of each animal breeding value across a continuum environmental variable. In those models, the environmental gradient is modeled as a regression covariable and thus, each individual has estimated random regression coefficients. This allows for estimation of genetic parameters and breeding values for any value of the environmental gradient that is in the studied range (Kolmodin et al., 2002; Santos et al., 2008; Rodrigues, 2012).

The objectives of this study were to: **1)** Evaluate the GEI for different levels of dietetic lysine on body weight (BW), daily weight gain (DWG) and total weight gain (TWG) of Nile tilapia from the GIFT line at 150 days of age; **2)** Assess the breeding values sensitivity to environmental levels via reaction norms.

2. MATERIAL AND METHODS

The experiment was conducted at the Aquaculture Laboratory of the Federal University of the Jequitinhonha and Mucuri Valleys (UFVJM), Campus JK, located in Diamantina, Minas Gerais - Brazil. The research was approved by the Committee on Ethics in Animal Use of the UFVJM (CEUA), under the protocol nº 057/2015. The experiment was held from November 2015 until May 2016. This period comprehends the breeding phase, family communal rearing and the grow out experiment until the fish reaches 150 days of age (60 days of the experiment).

To generate the full-sib families used in this experiment 26 females were mated to 20 males selected from the GIFT strain from the Aquaculture laboratory broodstock. These fish are descendants of the fish from the WorldFish Center that were introduced to Brazil in 2005 at the State University of Maringá. The females were selected based on their reproductive cycle. All the breeders were previously tagged with microchips (Passive Integrated Transponder - PIT tags). The selected breeders were moved to $1m^3$ tanks at a rate of 3 females and a male per breeding tank. The breeding units were on a recirculation aquaculture system (RAS) with constant aeration, controlled temperature ($\pm 25^oC$), mechanical, biological and ultraviolet filters. After a one-week period, the females that had fertilized eggs on their mouth were carefully captured and their eggs transferred to cylindrical incubators of 1.7 l. The remaining females were kept in the breeding tanks with the males for one more week. From the 20 males utilized to generate the families 1 mated with 3 females, generating 3 families of

half sibs and 4 males mated with 2 females generating 8 families of half-sib. The remaining of the males mated with only one female each. The date the eggs were collected was considered as birth date. Thus the age of the fish was measured as age post-spawning.

The incubators were connected to a RAS in which each incubator had constant water flow to individual aquariums of 3.4 l each (Figure 1). Therefore, after hatching the larvae of each family moved to the same aquarium. At the end of a period of approximately one week, the fish from the same family were transferred to a 70 l tank on another RAS where the temperature was maintained at 28 ºC. At this system, larvae were fed a powdered diet (45% crude protein), 4 times a day. In the next phase, fingerlings from the same family were transferred to a 140 l tanks on another RAS. At this system, the fish were fed a standard extruded diet (2-3 mm of diameter, 32% of crude protein) also 4 times a day. The fingerlings stayed in this system until they reach an average weight of 20g which was around the average age of 90 days. At this time, every fish was weighed and identified with a unique tag in the ventral area.

The tagging day was the first day of the growth experiment. After tagging and weighing, fish from the same family were randomly distributed to the experimental units, assuring at least one fish from each family at every experimental unit in order to promote connectivity. There was a total of 35 experimental tanks, each one of 1 m^3 of volume, and five treatments (dietetic lysine levels), thus 7 repetitions per treatment. Each experimental unit received a total of 20 individuals, therefore the experiment had a total of 700 fish (140/lysine level).

The tanks were maintained inside a greenhouse for control of environmental temperature. Natural illumination was the only light source. Also, all the tanks were connected to the same RAS that possessed ultraviolet light, mechanical and biological filters, aeration and electrical heaters (Figure 2). The water quality parameters were monitored

periodically and maintained at suitable levels for Nile tilapia and the tanks were cleaned and flushed weakly. Dissolved oxygen was always higher than 4 mg/l, ammonia below 0.5 mg/l, pH between 7 and 7.5 and temperature within 26 and 29 º C.

Before the beginning of the experiment, the fish were fed a standard commercial diet, as stated before. After the beginning of the experiment, which started at the tagging day (experimental day 0), they were fed only with the respective formulated diet. The five different diets used in this experiment were formulated following the recommendation for growing tilapia (NRC, 1993), and the main difference between diets was the lysine levels (Table 1). Fish were fed 4 times a day, *ad libitum* and any excess of food were removed from the tanks, to preserve water quality. The animals were weighed again after 60 days of the experiment. At the end of the experimental period, the fish had approximately 150 days of age. Because there is a variation in animal age, the initial and final weight measures need to be adjusted. Therefore, fish body weights were adjusted to the standardized ages of 90 and 150 days of age. The body weight at the standardized age was estimated assuming a unique linear growth curve for each fish.

The general random regression model used in the analysis can be described in matrix notation as:

$$y = Xb + Z_1u + Z_2f + e \quad (1)$$

where, ***y*** is the vector of observations; ***b*** is the vector of fixed effects of sex; ***u*** and ***f*** are the vectors of the random regression coefficients for additive genetic and common family environment respectively; ***X***, ***Z1***, and ***Z2*** are the index matrix of the observations, modified by the Legendre polynomials referent to the levels of the random regression covariable at ***b, u*** and ***f*** respectively; ***e*** is the vector of residuals.

The residuals were evaluated regarding been homogeny or heterogenic distributed on the levels of the random regression covariable. The different classes of residual variance evaluated were:

1: Homogeneity of residual variance (1.43 to 1.83 % of dietetic lysine);

2: Heterogeneity with 2 classes (1.43 to 1.53 and 1.54 to 1.83);

3: Heterogeneity with 3 classes (1.43 to 1.53, 1.54 to 1.63 and 1.64 to1.83);

4: Heterogeneity with 3 classes (1.43 to 1.53, 1.54 to 1.63, 1.64 to 1.73 and 1.74 to 1.83);

Also the model had the following assumptions:

$$E(y) = \mathrm{Xb} \tag{2}$$

$$E(Z_1 u) = E(Z_2 f) = E(e) = 0 \tag{3}$$

$$G = \begin{bmatrix} \sigma^2_{b0} & \sigma_{b0,b1} \\ \sigma_{b1,b0} & \sigma^2_{b1} \end{bmatrix} \tag{4}$$

$$V(y) = Z_1(A \otimes \mathrm{G})\mathrm{Z'}_1 + Z_2(\mathrm{I}_m \otimes \mathrm{F})\mathrm{Z'}_2 + \mathrm{I_n}\sigma^2_{ei} \tag{5}$$

where, $\boldsymbol{\sigma^2_{b0}}$ and $\boldsymbol{\sigma^2_{b1}}$ are the variance components of the intercept ($\boldsymbol{b_0}$) and linear ($\boldsymbol{b_1}$) random regression coefficients for the additive genetic effect and $\boldsymbol{\sigma_{b0,b1}}$ is the covariance component for the same random regression coefficients; $\boldsymbol{\sigma^2_{ei}}$ is the residual variance component for the class $\boldsymbol{i}$ = 1, 2, 3 or 4 (1 for homogeneity, 2, 3 and 4 heterogeneity classes of residual variance); $\boldsymbol{A}$ is the Wright's numerator relationship matrix, $\boldsymbol{G}$ and $\boldsymbol{F}$ are the random regression coefficients matrix for the additive genetic and family effects, respectively; $\boldsymbol{I_m}$ and $\boldsymbol{I_n}$ are index matrices, for the $\boldsymbol{m}$ females with progeny and $\boldsymbol{n}$ is the number of fish with measured observations. To model the random regression effects for the reaction norms, Legendre polynomials of at most 2nd order were used.

The Bayesian Information Criterion (BIC) was used in order to rank the models and select the model that presented better fit. The decision to use the BIC was because it penalizes

for the number of parameters in the model in a way to select more parsimonious models (Breda et al., 2006). The evaluated models accounted for a polynomial order of the trajectory (linear or quadratic) and the classes of residual variance (homogeny, 2, 3 or 4 classes for heterogeneity). The BIC described by Schwarz (1978) can be defined as:

$$BIC = -2\,Log\,L + p\,Log_e\,(N - r) \quad (6)$$

where, $\boldsymbol{p}$ is the number of parameters in the model, $\boldsymbol{N}$ is the total of observations, $\boldsymbol{r}$ is the rank of the index matrix for the fixed effects, and $\boldsymbol{Log\ L}$ is the logarithmic of the likelihood function. Also, the BIC values were used to estimate the model probability, using the model with lower BIC as a reference (Burnham and Anderson, 2004; Neath and Cavanaugh, 2012).

The additive genetic covariances were estimated using the program WOMBAT (Meyer, 2006) that is based on restricted maximum likelihood (REML). Thus, the additive genetic covariance between the levels $\boldsymbol{i}$ and $\boldsymbol{j}$ of dietetic lysine was estimated as follow:

$$\sigma_{a_{ij}} = Z_i G Z_j' \quad (7)$$

where, $\boldsymbol{Z_i} = [\boldsymbol{\Phi_0}(\boldsymbol{i}) \quad \boldsymbol{\Phi_1}(\boldsymbol{i})]$ and $\boldsymbol{Z_j} = [\boldsymbol{\Phi_0}(\boldsymbol{j}) \quad \boldsymbol{\Phi_1}(\boldsymbol{j})]$, $\boldsymbol{\Phi_0}(\boldsymbol{i})$ and $\boldsymbol{\Phi_1}(\boldsymbol{i})$ are the intercept and linear Legendre polynomials for the $\boldsymbol{i}$ level of the regression covariable (dietetic lysine).

Therefore, the narrow sense heritability ($\boldsymbol{h^2}$) can be estimated as a function of the level of dietetic lysine in the diet by the following formula:

$$h^2 = \frac{Z_I G Z_i'}{Z_i G Z_i' + Z_i F Z_i' + \sigma_{ej}^2} \quad (8)$$

where $\boldsymbol{i}$ is the level of dietetic lysine and $\boldsymbol{j}$ is the class of the residual variance for the respective level of dietetic lysine.

The genetic sensitivity of the breeding values for each trait (BW, DWG, and TWG) to changes in the environmental gradient was accessed through reaction norms of 25 randomly selected fish. Also, Spearman correlations were estimated for the 6, 12 and 18% of the fish with highest breeding values for the levels of dietetic lysine evaluated. The breeding values used in this analysis were estimated via single trait model and the Spearman correlations were estimated using the CORR procedure (S.A.S, version 9.0).

3. RESULTS

The fish reached an average BW of 79 g at 150 days of age with increasing average BW as the lysine level increases (Table 2). Also, the same trend is observed for TWG and for DWG. The differences between treatments are highlighted by the coefficient of variation observed, ranging from 31.82% to 48.3 for BW and from 31.5 to 58.7 for both weight gains at 1.73% and 1.43% of dietetic lysine respectively.

The regression models that had the best fit were the ones that accounted for heterogeneity of residual variance with 2 classes (Table 3). But, while the best regression models for BW and TWG accounted for Legendre polynomials of quadratic order, the model for DWG accounted only for a linear trajectory. The model probability in the way it was formulated (Table 3) shows how the other models differ from the model that had better fit. It is worth notice how distant are the model probability for the quadratic trajectory in relation to the linear trajectory for DWG. For each trait, the selected model was used for the analysis that followed.

The random regression coefficients for the selected models are present in Table 4. For the 3 traits evaluated the regression coefficients referent to the intercept (b_0) captured most of the variance, may it be for additive genetic or family effects, if compared to the linear coefficient (b_1) or their covariance (b_0b_1). The correlation (r_{b0b1}) between the intercept and the

linear coefficients for the additive genetic effect was low and negative for DWG (-0.077) and positive for both BW (0.588) and TWG (0.135). But for the family effect, the opposite is observed, the r_{b0b1} for DWG was positive (0.14) and negative for BW (-0.99) and TWG (-0.338). The estimated additive genetic correlations between the different levels of dietetic lysine were high and positive for every trait evaluated (Figure 3). The lowest estimates were approximately 0.75 for DWG. Moreover, those lower estimates of genetic correlations were between the combinations of dietetic lysine that are more distant (1.43 and 1.83%).

Changes in the estimates of h^2, additive genetic family and residual variances across the environmental gradient can be observed for the studied traits (Figure 4). The estimates of h^2 for BW, DWG and TWG presented similar trends with lower values at 1.43% of dietetic lysine and increasing as the percentage of lysine increase. The lowest estimated h^2 value was of 0.15 for BW at 1.43% of lysine and increased as the lysine level increases. The increase in heritability from 1.43 to the other levels of lysine is also observed for DWG and TWG. However, the curve for DWG practically lose the increasing behavior for values higher than 1.53% of lysine while the curve for TWD presents an increasing trend, but less step than the presented by h^2 for BW (Figure 4). The family effect presented low importance for DWG across the whole environmental gradient (0.010 to 0.012). On the other hand, for BW and TWG the family effect variance presented higher estimates at 1.43% of dietetic lysine (152.07 and 71.45 for BW and TWG respectively) with decreasing importance as the percentage of lysine increase (Figure 4).

In order to evaluate if there is a reordering of the EBVs for the studied traits, the genetic sensitivity, and Spearman correlations were estimated. The genetic sensitivity of the EBVs was evaluated via reaction norms for 25 randomly selected fish (Figure 5). In the reaction norms for BW, there is an increased dispersion of the EBVs as the percentage of dietetic lysine increases with discrete reordering for some individuals. On the order hand, the

reaction norms for DWG presented stronger reordering of the animals, with decreasing dispersion of the EBVs as the percentage of lysine increase. The reaction norms for TWG presented some reordering but the magnitude of the EBVs was more constant across the levels of the environmental covariable. Those results are in agreement with the genetic correlations presented in Figure 3 since the lower values of genetic correlation estimated for DWG were of 0.75 for 1.43 and 1.83% of dietetic lysine. Also, the results presented in this study are in accordance with Robertson (1959) claims that genetic correlations below 0.8 are indicative of GEI with reordering of the estimated breeding values. Therefore, GEI was observed for all 3 traits evaluated, may it be only due to a change in dispersion of the EBVs, as for BW, or reordering of the rank, as for DWG.

As a final assessment of the effects of GEI, it is important to evaluate how it affects the selection of fish to be breeders for the next generation. Thus, the Spearman correlation between the top (6, 12 and 18%) selected individuals for 3 levels of dietetic lysine (1.43, 1.63 and 1.83%) were estimated (Table 5). Following the finds of genetic correlations (Figure 3), the estimates of Spearman correlation for BW and TWG were high (0.92 to 0.99) for any combination of lysine levels and percentage of selected animals. This result is also in accordance with the reaction norms (figure 5) indicating that there is a low reordering of the EBVs for those traits across the levels of dietetic lysine. On the other hand, Spearman correlations for DWG, and consecutively the percentage of similarity between selected fish were lower. The lower Spearman correlation (0.09) being between 1.43 and 1.83% of dietetic lysine for the top 12% of fish selected resulting in only 55% of similarity between the selected fish. The results of Spearman correlation for DWG is in accordance with Crews Jr. & Franke (1998) claims that correlation values lower than 0.7 can result in a reordering of the classification, compromising the selection for divergent environments.

4. DISCUSSION

The descriptive statistic results are evidence of high divergence of phenotypic variance for the evaluated traits, with higher CV for TWG and DWG (Table 2). Previous studies with tilapia of the GIFT strain presented a coefficient of variation for BW similar to the present work, ranging from 32 to 50% (Bentsen et al., 2012; Khaw et al., 2012; Santos, 2009). The observed differences in the estimated averages and the reduction of CV at higher levels of dietetic lysine (most pronounced at 1.73%) are indicative of possible heterogeneity of the phenotypic variance partition. Thus, not unexpectedly all selected models accounted for heterogeneity of residual variance with 2 residual levels (Table 4). Regarding the residual variance, great change is observed between the estimated variance for the 2 classes fitted (the first class for dietetic lysine levels between 1.43 to 1.53 and the second from 1.54 to 1.83). The estimated residual variance was approximately 1.6 times higher for the first class (Figure 4). This difference is evidence of the actual heterogeneity of residual variance for Nile tilapia of the GIFT line across the gradient of dietetic lysine. However, this still is not sufficient information for the evaluation of GEI.

One way to evaluate GEI is via interpretation of the correlation between the random regression coefficients for the additive genetic effect. In the literature, negative additive genetic correlations between the random regression coefficients are indicative of reordering of the estimated breeding values (EBV) (Su et al., 2006). On the other hand, positive correlations are indicative of a joint increase of both variables as the gradient of the regression covariable increase. These correlations between the coefficients will reflect on the slope that describes the sensitivity of the fish to changes in the environmental gradient (Felipe et al., 2012). Another measure of GEI is the additive genetic correlation between the same trait across the environmental levels. Genetic correlations below 0.8 may be indicative of GEI (Robertson, 1959) and possible reordering of the animals' rank. While higher values of genetic correlation

indicate stability of the estimated breeding values, thus animals rank may not be affected across the environments (Yoshida et al., 2013). However, a better approach is to use the break-even genetic correlation, which is the threshold value that determines the need to break a breeding program into smaller programs for each environment. In order to estimate the actual break-even genetic correlation, the selection intensity that can be applied in each different environment is needed. Also, Sae-Lim et al., (2015) comment on the importance of taking other factors into account as the relative importance of each environment. Thus, in order to decide if it is necessary to consider selection for different environments the cost of developing alternative selection programs and the sib test should be weighed against its benefits. But, overall the break-even genetic correlation for fish will be between 0.7 to 0.8 (Sae-Lim et al., 2015).

In the current work, the values of genetic correlation were typically high. The only estimate below 0.8 was for DWG between 1.43 and 1.83 % of lysine (Figure 3). Even though these values of genetic correlation are indicative of negligible GEI there were two modes of interaction observed in the reaction norms (Figure 5). One is an increase in dispersion, observed for BW as the level of dietetic lysine increases, the second is reordering of ranks that are observed for DWG. Also, the reorder is more accentuated if comparing 1.43 and 1.83 % of lysine. These results are in agreement with the correlation estimated between the random regression coefficients for the additive genetic effect. As a final assessment of the impact of GEI Spearman correlations between selected fish under different lysine levels was estimated (Table 5). The results of Spearman correlation under different selection intensities presented indicate that selection for BW and TWG can be performed at any level of dietetic lysine without prejudice if aiming for selection or production at any of the lysine levels evaluated (Table 5). Thus, in order to evaluate the possible genetic gain over the generations for a given trait, the h^2 estimates can be used. Regarding h^2 for BW, Rutten et al. (2005) reported h^2

estimates of 0.24 for Nile tilapia BW at 151 days of age while Turra et al. (2016) reported h^2 estimates of 0.62 for Nile tilapia at 168 days. Both works were conducted in RAS, as the present one. However, neither work studied GEI for dietetic lysine. The ranging values for h^2 in the current work (0.15 to 0.44) are a reflex of the behavior of the GEI for BW and lysine levels. Thus, the higher values of h^2 estimated at the higher levels of dietetic lysine may be used to define selection strategies since higher h^2 estimates for a given trait of a population are related to higher realized genetic gain with selection (Mota, et al., 2015).

The results of Spearman correlation for DWG show a strong indication that GEI for dietetic lysine can be a major concern if the selection is focused on DWG, mostly between 1.43 and 1.83% of dietetic lysine. Also, the descriptive statistics show an increase in BW, TWG, and DWG as the level of dietetic lysine rise. This suggests that the 1.43% preconized for Nile tilapia (NRC, 1993) may not be the optimum level for improved tilapia strains. In fact, recent works evaluating the response of Nile tilapia to different levels of dietetic lysine are in agreement with our findings. Nguyen and Davis (2016) working with fish of initial BW of 6.4g and final BW of 40g showed a linear improvement of BW and feed conversion ratio as lysine levels increase from 1.13 to 2.02%. In another study with Nile tilapia of initial BW of 275g and final BW of 550g, both the final BW and fillet yield followed a quadratic regression on lysine levels with optimum around 1.5% (Michelato et al., 2016). These findings from the literature and the results presented here are indicative that Nile tilapia presents a different response to different levels of lysine and that GEI is an important factor to these differences.

Therefore, the estimates of the variance components and h^2 for BW, DWG, and TWG are indicative that the use of higher levels of lysine on the diet may be advantageous for selection of tilapia of the GIFT strain with a final weight of 80g of body weight. It is important to highlight that the results presented here may not be the same for older fish. In

fact, the genetic correlation between fish BW will decrease as the differences between the ages evaluated increase (He et al., 2017; Rutten et al., 2005; Turra et al., 2012). Also, the results from the present work suggest that this decrease can be more drastic if there are not only different ages but also different levels of dietetic lysine. Thus, it would be important for a breeding program to use the same levels of dietetic lysine as used in commercial diets at grow out farms in order to mitigate the effects of GEI. However, the decision of which level of dietetic lysine to use, or even if the split of the growth phase in more different levels involves an economic analysis that will be specific to different situations and markets.

5. CONCLUSIONS

Residual variance presented heterogenic behavior for the growth traits evaluated across the gradient of dietetic lysine. This is indicative of changes in the partition of the total phenotypic variance with possible changes on the importance of the additive genetic component at different levels of the gradient of the random regression covariable. Thus, these result demonstrates the importance to take into account the possibility of heterogeneity of variance in studies of GEI via reaction norms.

The estimated genetic correlations, reaction norms and Spearman correlations for BW and TWG of Nile tilapia of the GIFT line at 150 days of age presented similar trends and were divergent from the results obtained for DWG. The findings of the present study points for GEI with reordering of the EBVs for DWG across the levels of dietetic lysine in the diet.

Therefore, if the focus of selection in the GIFT tilapia population evaluated is on BW or TWG at 150 days of age it would be interesting to promote selection using a diet that has 1.83% of dietetic lysine instead of 1.43%. Since this will account for faster genetic progress because of the higher heritability estimates for 1.83% of dietetic lysine. However, it is important to note that, for DWG differences between the dietetic lysine environment for the

selection and target/production fish groups may compromise the realized genetic gains. Thus, estimated breeding values for DWG at 150 days of age for lower levels of dietetic lysine should not be used for selection at higher levels, and vice-versa. Ultimately, studies of the economic viability of using higher levels of dietetic lysine should be conducted in order to identify the best level.

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REFERENCES

Bentsen, H.B., Gjerde, B., Nguyen, H.N., Rye,M., Ponzoni, R.W., De Vera,M.S.P., Bolivar, H.L., Velasco, R.R., Danting, J.C., Dionisio, E.E., Longalong, F.M., Reyes, R.A., Abella, T.A., Tayamen, M.M., Eknath, A.E., 2012. Genetic improvement of farmed tilapias: genetic parameters for body weight at harvest in Nile tilapia (Oreochromis niloticus) during five generations of testing in multiple environments. Aquaculture 338-341, 56–65. DOI:10.1016/j.aquaculture.2012.01.027

Breda, F. C., Albuquerque, L. G., Yamaki, M., Filho, J. C. R., Sarmento, J. L. R., Lopes, P. S., Rodrigues, M. T., 2006. Estimação de parâmetros genéticos para produção de leite de cabras da raça Alpina. Revista Brasileira de Zootecnia 35, 396-404. DOI:10.1590/S1516-35982006000200009

Burnham, K.P., Anderson, D.R., 2004. Multimodel inference: Understanding AIC and BIC in model selection. Sociological methods & research 33, 261-304. DOI:10.1177/0049124104268644

Crews JR., D. H., Franke, D. E., 1998. Heterogeneity of variances for carcass traits by percentage brahman inheritance. Journal of Animal Science 76, 1803-1809. DOI:10.2527/1998.7671803x

Felipe, V. P. S., Freitas, L. S, Santos, G. G., Valente, B. D., Wenceslau, R. R., Silva, M. A., Corrêa, G. S., Corrêa, A. B., 2012. Utilização de modelos de norma de reação com variância residual heterogênea para estudo de valores genéticos de peso de codornas de corte em função de níveis de proteína bruta na dieta. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 64, 991-1000. DOI:10.1590/S0102-09352012000400028

Furuya, W. M., Botaro, D., Neves, P. R., Silva, L. C. R., Hayashi, C., 2004. Exigência de lisina pela tilápia do Nilo (*Oreochromis niloticus*), na terminação. Ciência Rural 34, 1571-1577.

Furuya, W.M., Botaro D., Macedo, R. M. G., Santos, V. G., Silva, L. C. R., Silva, T. C., Furuya, V. R. B., Sales, P. J. P., 2005. Aplicação do conceito de proteína ideal para redução dos níveis de proteína em dietas para tilápias do Nilo (*Oreochromis niloticus*). Revista Brasileira de Zootecnia 34, 1433-1441. DOI:10.1590/S1516-35982005000500002

He, J., Zhao, Y., Zhao, J., Gao, J., Han, D., Xu, P., Yang, R., 2017. Multivariate random regression analysis for body weight and main morphological traits in genetically improved farmed tilapia (Oreochromis niloticus). Genet. Sel. Evol. 49, 80. https://doi.org/10.1186/s12711-017-0357-7

Khaw, H.L., Ponzoni, R.W., Hamzah, A., Abu-Bakar, K.R., Bijma, P., 2012. Genotype by production environment interaction in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). Aquaculture 326–329, 53-60. DOI:10.1016/j.aquaculture.2011.11.016

Kolmodin, R., Strandberg, E., Madsen, P., Jensen, J., Jorjani, H., 2002. Genotype by Environment Interaction in Nordic Dairy Cattle Studied Using Reaction Norms. Acta Agriculturae Scandinavica, Section A — Animal Science 52, 11–24. DOI:10.1080/09064700252806380

Meyer, K., 2006. Wombat: A Program for Mixed Model Analyses by Restricted Maximum Likelihood (REML). University of New England, Armidale, NSW, Australia.

Michelato, M., de Oliveira Vidal, L.V., Xavier, T.O., de Moura, L.B., de Almeida, F.L.A., Pedrosa, V.B., Furuya, V.R.B., Furuya, W.M., 2016. Dietary lysine requirement to enhance muscle development and fillet yield of finishing Nile tilapia. Aquaculture 457, 124–130. https://doi.org/10.1016/j.aquaculture.2016.02.022

Mota, L.F., Abreu, L.R., Silva, M.A., Pires, A.V., Lima, H.J., Bonafé, C.M., Costa, L.S., Souza, K.A.R., Martins, P.G., 2015. Genotype × dietary (methionine+ cystine): Lysine ratio interaction for body weight of meat-type quails using reaction norm models. Livestock Science 182, 137-144. DOI:10.1016/j.livsci.2015.11.006

Neath A. A., Cavanaugh J. E., 2012. The Bayesian information criterion: background, derivation, and applications. Wiley Interdiscip. Reviews Computational Statistics 4, 199–203. DOI: 10.1002/wics.199

Nguyen, L., Davis, D.A., 2016. Comparison of crystalline lysine and intact lysine used as a supplement in practical diets of channel catfish (Ictalurus punctatus) and Nile tilapia (Oreochromis niloticus). Aquaculture 464, 331–339. https://doi.org/10.1016/j.aquaculture.2016.07.005

NRC., 1993. National Research Council. Nutrient Requirements of warm water fishes and shellfishes. Washington: National Academy 102.

Pezzato, L.E., Miranda, E. C., Fracalossi, D. M., Cyrino, J. E. C., 2004. Nutrição de peixes. In: CYRINO, J.E.C.; URBINATI, E.C.; FRACALOSSI, D.M.; CASTAGNOLLI, N. (Ed.) Tópicos especiais em piscicultura de água doce tropical intensiva. São Paulo: tecArt, 75-169.

Robertson, A., 1959. The sampling variance of genetic correlation coefficient. Biomethic 15, 469-485. DOI:10.2307/2527670

Rodrigues, D.T., 2012. Interação genótipos ambientes em animais via modelos de normas de reação. 86p. Dissertação (Mestrado em Estatística Aplicada e Biometria) – Universidade Federal de Viçosa, Viçosa-MG.

Rollin, X., Mambrini, M., Abboudi, T., Larondelle, Y., Kaushik, S. J., 2003. The optimum dietary indispensable amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry. British Journal of Nutrition 90, 865-876. DOI: 10.1079/BJN2003973

Rutten, M. J. M., Komen, H., Bovenhuis, H., 2005. Longitudinal genetic analysis of Nile tilapia (Oreochromis niloticus L.) body weight using a random regression model. Aquaculture 246, 101–113. DOI:10.1016/j.aquaculture.2004.12.020

Sae-Lim, P., Gjerde, B., Nielsen, H.M., Mulder, H., Kause, A., 2015. A review of genotype-by-environment interaction and micro-environmental sensitivity in aquaculture species. Rev. Aquac. 1–25. https://doi.org/10.1111/raq.12098

Santos, G. G., Corrêa, G S. S., Valente, B. D., Silva, M. A., Corrêa, A. B., Felipe, V. P. S., Wenceslau, R. R., 2008. Sensibilidade de valores genéticos de codornas de corte em crescimento às modificações de níveis de proteína das dietas. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 60, 1188-1196. DOI:10.1590/S0102-09352008000500022

Santos, A. I., 2009. Interação genótipo - ambiente e estimativas de parâmetros genéticos em tilápias. 97p. Tese (Doutorado em Zootecnia) - Universidade Estadual de Maringá, Maringá-PR.

SAS., 2002. STATISTICAL ANALYSIS SYSTEM. User's guide: Statistics. Version 9.0, NC; SAS Institute.

Schwarz, G., 1978. Estimating the dimension of a model. The annals of statistics 6, 461-464. DOI:10.1214/aos/1176344136

Su, G., Madsen, P., Lund, M.S., Sorensen, D., Korsgaard, I.R., Jensen, J., 2006. Bayesian analysis of the linear reaction norm model with unknown covariates. Journal of Animal Science 84, 1651-1657. DOI:10.2527/jas.2005-517

Turra, E.M., de Oliveira, D.A.A., Valente, B.D., Teixeira, E. de A., Prado, S. de A., de Melo, D.C., Fernandes, A.F.A., de Alvarenga, É.R., e Silva, M. de A., 2012. Estimation of genetic parameters for body weights of Nile tilapia Oreochromis niloticus using random regression models. Aquaculture 354, 31–37. https://doi.org/10.1016/j.aquaculture.2012.04.035

Turra, E.M., Toral, F.L.B., de Alvarenga, E.R., Raidan, F.S.S., Fernandes, A.F.A., Oliveira Alves, G.F., Sales, S.C.M., Teixeira, E. A., Manduca, L.G., Brito, T.S., Silva, M.A., Silva Junior, A.F., Almeida, L.F.C., Santos, C.R., Silva, M.A., 2016. Genotype × environment interaction for growth traits of Nile tilapia in biofloc technology, recirculating water and Cage systems. Aquaculture 460, 98-104. DOI:10.1016/j.aquaculture.2016.04.020

Wilson, R.P.; Poe, W.E., 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, Ictalurus punctatus. Comparative Biochemistry and Physiology 80B, 385-388. DOI: 10.1016/0305-0491(85)90224-X

Yoshida, G.M., de Oliveira, C.A.L., Kunita, N.M., de Oliveira, S.N., Alexandre Filho, L., Resende, E.K., Lopera-Barrero, N.M., Ribeiro, R.P., 2013. Resposta à seleção de características de desempenho e morfométricas de tilápia-do-nilo ao longo do período de cultivo. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65, 1815-1822. DOI:10.1590/S0102-09352013000600032

Table 1. Composition of the experimental diets formulated for tilapia in growth stage used for each of the five groups evaluated.

Ingredients (%)	Dietetic lysine levels (%)				
	1.43	1.53	1.63	1.73	1.83
Soybean meal 45%	46.69	46.69	46.69	46.69	46.69
Broken rice	22.66	22.66	22.66	22.66	22.66
Corn grain meal	9.40	9.40	9.40	9.40	9.40
Gluten 60%	10.00	10.00	10.00	10.00	10.00
Soybean oil	1.00	1.00	1.00	1.00	1.00
Calcite limestone	3.00	3.00	3.00	3.00	3.00
Bicalcium phosphate	3.00	3.00	3.00	3.00	3.00
Common Salt	0.50	0.50	0.50	0.50	0.50
Inert (Kaolin)	0.617	0.777	0.892	1.025	1.128
Vitamin & mineral Premix[(1)]	0.5	0.50	0.50	0.50	0.50
Vitamin C	0.05	0.05	0.05	0.05	0.05
L-lysine HCl	0.052	0.181	0.310	0.439	0.568
DL-methionine	0.306	0.362	0.426	0.487	0.548
L-threonine	0.265	0.500	0.542	0.649	0.756
L-glutamic acid	1.760	1.180	0.830	0.400	-
BHT	0.20	0.20	0.20	0.20	0.20
Estimated composition					
Dry matter (%)	84.58	84.92	85.23	85.54	85.83
Dietetic energy (kcal/kg)	3190.0	3190.0	3190.0	3190.0	3190.0
Crude protein (%)	31.42	31.42	31.42	31.42	31.42
Dietetic protein (%)	29.09	29.09	29.09	29.09	29.09
Crude fiber (%)	3.15	3.15	3.15	3.15	3.15
Ether extract (%)	2.53	2.53	2.53	2.53	2.53
Total phosphorus (%)	0.93	0.93	0.93	0.93	0.93
Available phosphorus (%)	0.68	0.68	0.68	0.68	0.68
Total calcium (%)	1.93	1.93	1.93	1.93	1.93
Total lysine (%)	1.53	1.63	1.73	1.83	1.93
Dietetic lysine (%)	1.43	1.53	1.63	1.73	1.83
Total Met + Cist (%)	1.26	1.31	1.37	1.44	1.50
Dietetic Met + Cist (%)	0.87	0.92	0.98	1.04	1.10
Total threonine (%)	1.45	1.56	1.67	1.78	1.89
Dietetic threonine (%)	1.10	1.18	1.25	1.33	1.41
Total arginine (%)	1.91	1.91	1.91	1.91	1.91
Dietetic arginine (%)	1.66	1.66	1.66	1.66	1.66
Total leucine (%)	2.98	2.98	2.98	2.98	2.98
Dietetic leucine (%)	2.95	2.95	2.95	2.95	2.95
Total tryptophan (%)	0.36	0.36	0.36	0.36	0.36
Dietetic tryptophan (%)	0.29	0.29	0.29	0.29	0.29
Starch (%)	30.08	0.08	30.08	30.08	30.08
Linoleic acid (%)	1.27	1.27	1.27	1.27	1.27

[(1)]Composition per Kg: Premix composition per Kg: Vitamin A - 1,200,000 UI; Vitamin D3 - 200,000 UI; Vitamin E - 1,200 mg; Vitamin K3 - 2,400 mg; Vitamin B1 - 4,800 mg; Vitamin B2 - 4,800 mg; Vitamin B6 - 4,800 mg; Vitamin B12 - 4,800 mg; Vitamin C - 48 g; Folic acid - 1,200 mg; Acid pantothenic 12,000 mg; Biotin - 48 mg; Cholin - 108 g; Niacin - 24,000 mg; Fe - 50,000 mg; Cu - 3,000 mg; Mn - 20,000 mg; Zn - 30,000 mg; I - 100 mg; Co - 10 mg; Se - 100 mg.

Table 2. Phenotypic mean and coefficient of variation (CV in percentage) at different levels of dietetic lysine for body weight (BW), total weight gain (TWG), and daily weight gain (DWG) of GIFT tilapia at 150 days of age.

	Lysine level (%)									
	1.43		**1.53**		**1.63**		**1.73**		**1.83**	
Trait	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
BW (g)	71.35	48.30	82.14	37.46	77.31	36.82	82.84	31.82	85.64	37.81
TWG (g)	54.43	58.74	64.89	43.27	60.27	43.65	65.75	35.55	66.42	41.77
DWG (g)	0.91	58.70	1.08	43.29	1.00	43.63	1.10	35.57	1.14	41.77

Table 3. Comparison between random regression models assuming different classes of residual variance, and linear or quadratic average trajectory for body weight (BW), daily weight gain (DWG) and total weight gain (TWG) of GIFT tilapia at 150 days of age.

Trait	**Average trajectory**	**Residual classes**	**N[1]**	**P[2]**	**Log L[3]**	**BIC[4]**	**MP[5]**
BW	Linear	1 class	676	7	-2570.603	5161.02	6.33
		2 classes	676	8	-2568.944	5160.53	4.96
		3 classes	676	9	-2568.82	5163.11	18.03
		4 classes	676	10	-2568.551	5165,4	56.71
	Quadratic	1 class	676	7	-2569.176	5158.16	1.52
		2 classes	**676**	**8**	**-2567.343**	**5157.33**	**1.00**
		3 classes	676	9	-2567.263	5160.00	3.80
		4 classes	676	10	-2566.933	5162.17	11.25
DWG	**Linear**	1 class	675	7	249.389	-478.973	1.93
		2 classes	**675**	**8**	**251.461**	**-480.288**	**1.00**
		3 classes	675	9	251.687	-477.910	3.28
		4 classes	675	10	251.69	-475.087	13.47
	Quadratic	1 class	675	7	246.65	-473.495	29.85
		2 classes	675	8	248.916	-475.198	12.74
		3 classes	675	9	249.081	-472.698	44.46
		4 classes	675	10	249.092	-469.891	180.96
TWG	Linear	1 class	675	7	-2498.056	5015.917	9.57
		2 classes	675	8	-2495.951	5014.536	4.80
		3 classes	675	9	-2495.715	5016.894	15.59
		4 classes	675	10	-2495.712	5019.717	63.96
	Quadratic	1 class	675	7	-2496.693	5013.191	2.45
		2 classes	**675**	**8**	**-2494.383**	**5011.400**	**1.00**
		3 classes	675	9	-2494.218	5013.900	3.49
		4 classes	675	10	-2494.208	5016.709	14.22

[1]N: Number of observations; [2]P: Number of parameters; [3]Log L: Logarithmic of the maximum likelihood function; [4]BIC: Bayesian information criterion.

Table 4. Estimates of covariance components for the intercept ($\mathbf{b_0}$) and linear ($\mathbf{b_1}$) random regression coefficients and correlation ($\mathbf{r_{b0b1}}$) for the additive genetic and family effect, and the total variance for each class of the residual variance for GIFT tilapia at 150 days of age.

Trait	**Trajectory**	**Additive genetic effect**

		b_0	b_0b_1	b_1	r_{b1b0}
BW[1]	Quadratic	504.5590	61.552800	21.679600	0.588500
DWG[2]	Linear	0.142798	-0.0025659	0.0077077	-0.0773
TWG[3]	Quadratic	485.618	9.62013	10.4378	0.1351
Trait	**Trajectory**	**Family effect**			
		b0	**b0b1**	**b1**	r_{b1b0}
BW[1]	Quadratic	116.029000	-41.310400	15.002000	-0.990200
DWG[2]	Linear	0.0210894	0.00041049	0.0003944	0.1423
TWG[3]	Quadratic	87.8798	-8.96128	7.99366	-0.3381
Trait	**Trajectory**	**Residual variance**			
		1 Class	**2 Classes**	**3 Classes**	**4 Classes**
BW[1]	Quadratic	827.4050	494.5160	-	-
DWG[2]	Linear	0.179576	0.10443	-	-
TWG[3]	Quadratic	683.266	383.144	-	-

[1]Body weight; [2]Daily weight gain; [3]Total weight gain.

Table 5. Spearman correlations and similarity (percentage of selected animals) between three levels of dietetic lysine (1.43,1.63, and 1.83%) if selected the 18, 12 or 6% of fish with the highest estimated breeding values at 150 days of age.

Trait	Selection	1.43 x 1.63	Similarity (%)	1.43 x 1.83	Similarity (%)	1.63 x 1.83	Similarity (%)
BW	18%	0.96	93.4	0.92	87.7	0.98	94.3
	12%	0.97	92.6	0.95	88.9	0.99	96.3
	6%	0.97	95.1	0.93	92.7	0.98	95.1
DWG	18%	0.83	85.0	0.33	65.0	0.59	80.0
	12%	0.83	85.0	0.09	55.0	0.52	70.0
	6%	0.86	82.5	0.10	55.0	0.16	72.5
TWG	18%	0.98	96.7	0.94	94.2	0.98	96.7
	12%	0.98	95.1	0.96	91.4	0.98	96.3
	6%	0.99	97.5	0.98	92.5	0.99	95.0

Figure 1. Incubation system (left) and detail of an incubator with larvae recently spawned (right)

Figure 2. Greenhouse with recirculation aquaculture system and tanks of 1 m^3 of volume

Figure 3. Additive genetic correlations for growth traits of Nile tilapia of the GIFT line across the gradient of dietetic lysine

Figure 4. Estimates of heritability, additive genetic and family variances across the gradient of dietetic lysine for body weight, daily and total weight gains at 150 days of age in tilapias of the GIFT line

Figure 5. Reaction norms of the estimated breeding values across the gradient of dietetic lysine for body weight, daily and total weight gains at 150 days of age of 25 randomly selected tilapias of the GIFT line

Highlights

- Lysine is the first limiting amino acid in tilapia nutrition and differences in it level may lead to changes in growth rate.
- Identification of genetic sensitivity to dietetic lysine for GIFT tilapia is of importance for the aquaculture production chain.
- Changes in estimated genetic components and breeding values with reordering of the evaluated animals.

Figure 1

Figure 2

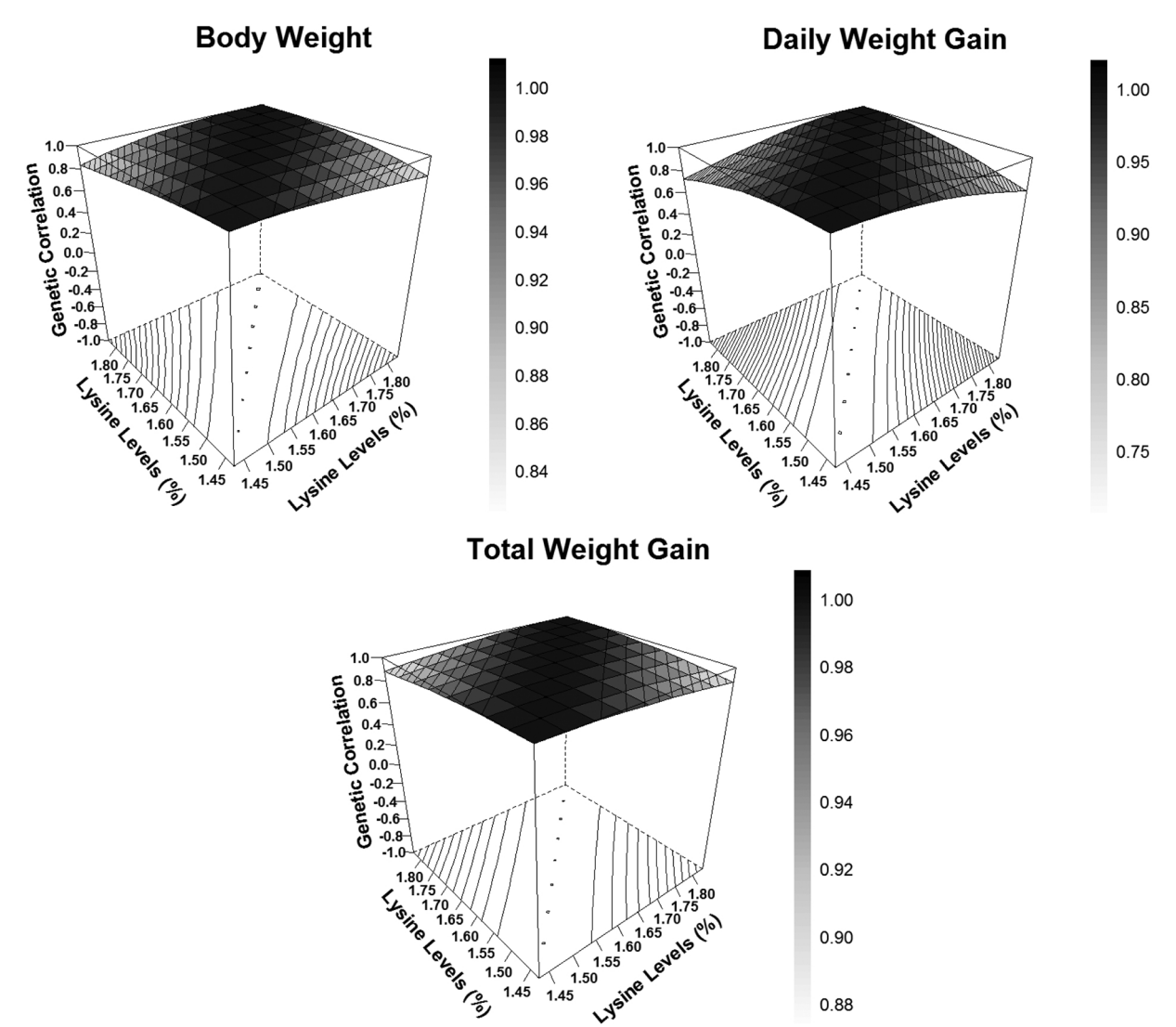

Body Weight
Genetic Correlation
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Lysine Levels (%)
1.80
1.75
1.70
1.65
1.60
1.55
1.50
1.45
Lysine Levels (%)
1.45
1.50
1.55
1.60
1.65
1.70
1.75
1.80
1.00
0.98
0.96
0.94
0.92
0.90
0.88
0.86
0.84
Daily Weight Gain
Genetic Correlation
Lysine Levels (%)
Lysine Levels (%)
1.00
0.95
0.90
0.85
0.80
0.75
Total Weight Gain
Genetic Correlation
Lysine Levels (%)
Lysine Levels (%)
1.00
0.98
0.96
0.94
0.92
0.90
0.88

Figure 3

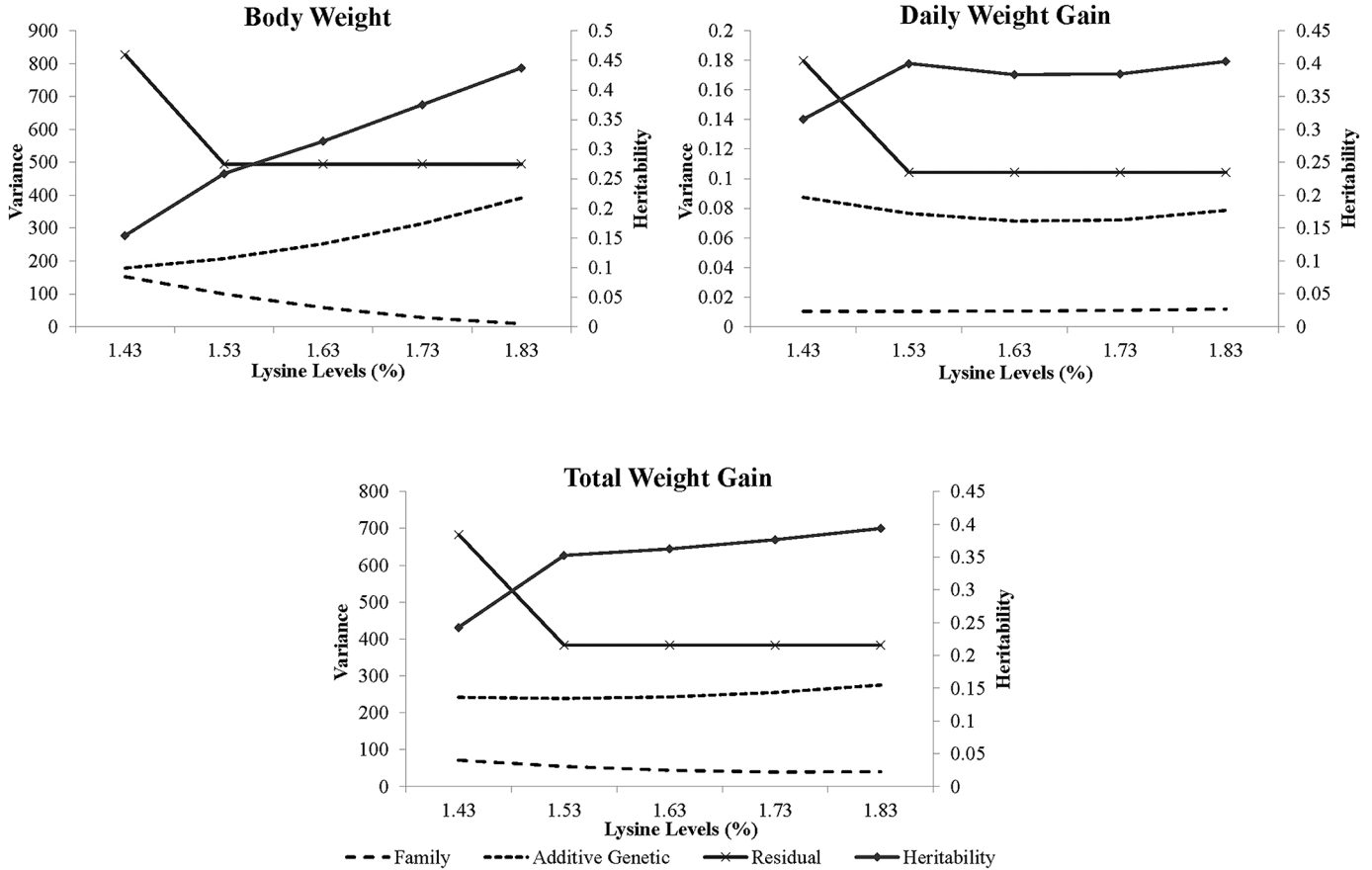
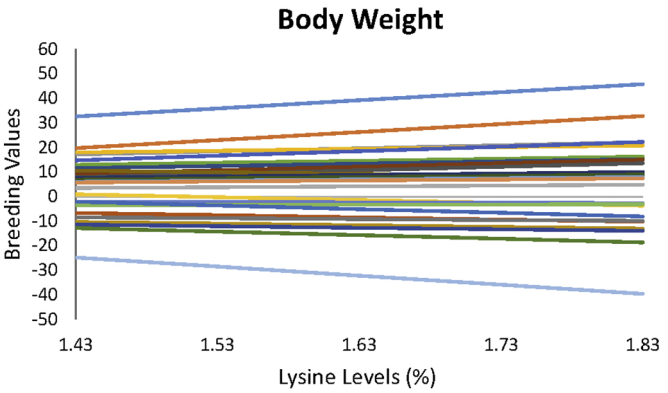
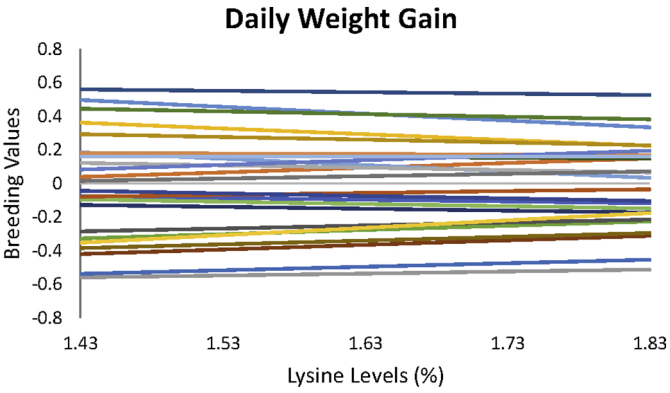

Body Weight
Variance
Heritability
Lysine Levels (%)
1.43
1.53
1.63
1.73
1.83
Daily Weight Gain
Variance
Heritability
Lysine Levels (%)
Total Weight Gain
Variance
Heritability
Lysine Levels (%)
Family
Additive Genetic
Residual
Heritability

Figure 4


Body Weight
Breeding Values
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
1.43
1.53
1.63
1.73
1.83
Lysine Levels (%)


Daily Weight Gain
Breeding Values
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
1.43
1.53
1.63
1.73
1.83
Lysine Levels (%)

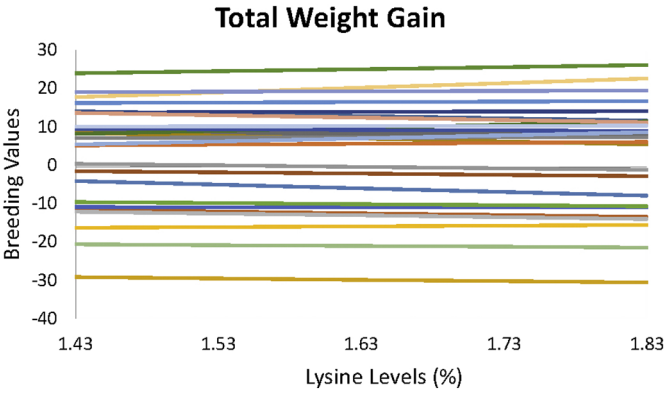

Total Weight Gain
Breeding Values
30
20
10
0
-10
-20
-30
-40
1.43
1.53
1.63
1.73
1.83
Lysine Levels (%)

Figure 5