



## Estimate of body growth curve and feed intake of free-range chickens receiving different levels of digestible lysine

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### Abstract

**Aim of study:** To adjust nonlinear Gompertz model to describe the body growth and feed intake (FI) of free-range chickens of the CPK (Color Plumé) strain, receiving different levels of digestible lysine (dig-Lys), from 21 to 77 days of age.

**Area of study:** São Cristóvão, Sergipe, Brazil.

**Material and methods:** A total of 432 one-day-old unsexed chickens were used in the study. At 21 days of age, the birds were assigned to four treatments (0.85%; 0.97%; 1.09%; 1.21%) of dig-Lys, in a completely randomized design with four replicates, with 27 birds per experimental unit. The Gompertz model was used to estimate bird growth curves related to body weight (BW) and FI at each Lys level.

**Main results:** The tests of comparison between the parameters of the Gompertz model for each Lys level showed that parameter A varied, while parameters B (0.0329) and C (45.819) did not differ significantly. In parameter A, the dig-Lys level of 0.97% provided the highest BW estimate at maturity and maximum BW at the inflection point.

**Research highlights:** Use of non-linear models to predict nutritional requirements, helps farmers to optimize management decisions and, thus, maximize their profit. According to Gompertz model, it was possible to infer that the inclusion of 0.97% dig-Lys in the diet of mixed batches of free-range chickens of the CPK strain meets their nutritional requirements.

**Additional keywords:** amino acids; broilers; Color Plumé; nonlinear

**Abbreviations used:** BW (body weight); BWe (BW at the inflection point); CP (crude protein); CPK (Color Plumé); dig-Lys (digestible lysine); DF (degree of freedom); DFI (daily feed intake); DW (Durbin Watson statistics); DWG (daily weight gain); FC (feed conversion); FI (feed intake); FIe (FI estimated at the inflection point); FW (final weight); IW (initial weight); WG (weight gain); WGe (WG at the inflection point).

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## Introduction

Free-range chicken farming is an activity that represents an economic alternative for small and big entrepreneurs, considering that consumers seek products with different characteristics from those found in the conventional fast-growing chickens. However, free-range chickens have a slower growth rate and, consequently,

slaughtered at older ages, which in turn requires nutritional adjustments to improve their growth.

Nowadays, diets for chickens reared in free-range systems usually include corn and soybean meal as main energy and protein sources (Ferreira *et al.*, 2014); however, these ingredients may not be sufficient to meet the nutritional requirements of these animals, especially in terms of essential amino acids such as Lys and methionine.

Lysine-deficient diets might result in negative effects on muscle development and consequently affect growth performance, carcass characteristics and broilers body composition (Oliveira HG *et al.*, 2013; Oliveira RG *et al.*, 2016). Thus, establishing the dig-Lys requirements and its supplementation at different ages should be priority concerns in the production of these animals (Rosa *et al.*, 2014).

Body-growth estimates enable the adoption of management practices that maximize meat production, prioritizing the nutritional requirements of each growth phase. On this basis, estimating the nutritional requirements and adopting specific feeding programs based on the modeling of growth curves can optimize the management and efficiency of animal production (Köhn *et al.*, 2007). Moreover, these estimated data make possible to predict the optimal slaughter age as a function of the maximum growth rate (Gous, 2014).

Growth curves can be explained by several nonlinear mathematical models (*e.g.*, Robertson, Gompertz, Brody, Bertalanffy, and logistic) (Veloso *et al.*, 2015). Among them, the Gompertz-model equations have shown to be the most suitable for describing the growth of broilers (Neme *et al.*, 2006; Narinc *et al.*, 2010; Sakomura *et al.*, 2011; Grieser *et al.*, 2015).

The hypothesis proposed for the current study was whether dig-Lys is capable of altering the growth curve of free-range chickens. It is necessary to adjust different equations for each dietary level of dig-Lys. Besides that, this study proposes to adjust several nonlinear Gompertz equations to describe the body growth and feed intake (FI) of free-range chickens of the CPK (Color Plumé) strain fed diets with different levels of dig-Lys. Additionally, it aims to determine whether a single Gompertz equation and its parameters, considering them adequate to represent the four levels of dig-Lys and, therefore, define the best level of dig-Lys for free-range chicken at age of 22 to 77 days.

## Material and methods

This experiment was performed at the Federal University of Sergipe (UFS, Brazil). All procedures complied with the standards adopted by the Ethics Committee on Animal Experimentation of the UFS (approval no. 3/2012).

### Animals, experimental design and diets

A total of 432 one-day-old free-range chickens of the Color Plumé (CPK) strain, with an initial average body weight (BW) of  $39 \pm 1.3$  g, were used in the study. The birds were housed until 21 days of age in an experimental shed build of concrete floor and wood shavings, containing drinkers, feeders and heating source. After this period, and

with the BW average of  $470 \text{ g} \pm 1.7 \text{ g}$ ; 27 birds per cages (14 males and 13 females/cages); were distributed in a completely randomized design in four treatments with four replications. Each cage was considered as a single replica.

At 22 days of age, the birds were transferred to the free-range poultry sector in the semi-confinement system, with free access to paddocks during the day and collected at night. The sector is characterized by a warehouse with an internal area of 40 m<sup>2</sup>, covered with fiber cement tiles, with ceiling height of 2.90 m. The warehouse was divided into 16 boxes of 5.4 m<sup>2</sup> each. All boxes were equipped with a pendant water cooler and a tubular feeder of 20 kg capacity.

In the period from 1 to 21 d of age, all the birds received a diet containing 22% crude protein (CP), 2,950 kcal of metabolizable energy/kg, 1.20% of digestible lysine (dig-Lys), 0.94% calcium, and 0.42% available phosphorus. From 22 to 77 d of age broilers received their respective experimental diets, based on corn and soybean meal formulated to meet poultry nutritional requirements, according to Rostagno *et al.* (2011), except for the concentration of dig-Lys. The experimental diets were formulated as to contain 0.85, 0.97, 1.09, and 1.21% of dig-Lys (Table 1). The dietary treatments with 0.85% (T1) and 1.21% (T4) dig-Lys were combined by the dilution technique to generate dietary treatments 2 (T2), containing 0.97% of dig-Lys (67% T1 + 33% T4), and dietary treatments 3 (T3) with 1.09% of dig-Lys (33% T1 + 67% T4). Throughout the trial period water and feed were provided for *ad libitum* consumption.

The diets were analyzed for dry matter (DM), CP, ether extract and minerals contents as described for AOAC (2012). Amino acid profile was based on SIBNAS® (2020).

### Growth performance

The bird's BW and FI were obtained weekly and used to determine daily weight gain (DWG, g); daily feed intake (DFI, g/bird), and feed conversion (FC, g/g) corrected for mortality.

### Statistical analyses

The BW and FI of the birds at each dig-Lys level was estimated by the Gompertz nonlinear regression model:

$$Y = A. e^{-e^{-B(t-C)}}$$

where  $Y$ =estimate of BW or FI at time  $t$  (g);  $A$ =estimate of BW or FI at maturity (g);  $B$ =relative growth rate at the inflection point (g/day per g);  $t$ =age (days);  $C$ =age at the inflection point (days) or maximum growth; and the constant  $e=2.718281828459$ .

**Table 1.** Composition of the experimental diets during the grower phase, from 22 to 77 days of age

Ingredient (g/kg)	dig-Lys	
	0.85%	1.21%
Corn	617.2	627.2
Soybean meal (45% CP <sup>a</sup> )	277.1	264.3
Maize gluten (21% CP <sup>a</sup> )	50.0	50.0
Vegetable oil	27.7	23.7
Calcitic limestone	9.2	9.2
Dicalcium phosphate	12.3	12.4
Salt	4.2	4.2
L-lysine HCl	-	3.9
DL-methionine	0.9	3.6
Vitamin supplement <sup>b</sup>	1.0	1.0
Mineral supplement <sup>c</sup>	0.5	0.5
Total	1000.00	1000.00
<b>Analysis</b>		
Crude protein,%	18.09	18.09
Metabolizable energy, kcal/kg	3.050	3.050
Fat, %	5.912	5.551
Crude fiber, %	3.348	3.283
NDF, %	10.781	10.701
Ash, %	2.871	2.659
Calcium, %	0.667	0.667
Available phosphorus, %	0.330	0.330
Digestible phosphorus, %	0.300	0.300
Digestible arginine, %	1.127	1.090
Digestible isoleucine, %	0.707	0.685
Digestible lysine <sup>d</sup> ,%	0.850	1.210
Digestible methionine <sup>d</sup> ,%	0.346	0.612
Digestible methionine + cysteine <sup>d</sup> ,%	0.610	0.870
Digestible threonine <sup>d</sup> ,%	0.631	0.631
Digestible tryptophan <sup>d</sup> ,%	0.197	0.190
Digestible valine <sup>d</sup> ,%	0.794	0.772
Potassium	0.754	0.733
Sodium	0.187	0.187

<sup>a</sup>CP: crude protein. <sup>b</sup>Vitamin supplement. Provides per kilogram of product: retinol (vitamin A), 0.3 g; cholecalciferol (vitamin D3), 0.05 g; tocopherol (vitamin E), 0.0201 g; thiamine (vitamin B1), 2.0 g; pyridoxine (vitamin B6), 4.0 g; pantothenic acid, 12.0 g; biotin, 0.10 g; menadione (vitamin K3), 3.0 g; folic acid, 1.0 g; nicotinic acid, 50.0 g; cobalamin (vitamin B12), 15000 mcg; selenium, 0.25 g; and excipient q.s., 1000 g. <sup>c</sup>Mineral supplement. Provides per kilogram of product: manganese, 16.0 g; iron, 100.0 g; zinc, 100.0 g; copper, 20.0 g; cobalto, 2.0 g; iodine, 2.0 g; and excipient q.s., 1000 g. <sup>d</sup>Amino acid profile based on SIBNAS<sup>®</sup>. Observations: formulations without inclusion of growth promoters.

The model parameters (A, B, C) for each Lys level were adjusted by using the PROC NLIN procedure of SAS (9.0), where Marquardt interaction algorithm was applied. The initial values of the parameters were obtained

from Michalczuk *et al.* (2016). After the A, B, C parameters were obtained for each Lys level, it was possible to determine the suitability of the model or even whether a set of curves is identical, an intercept is common please

use or some of the parameters are similar among models; *i.e.*, if a set of equations can be represented by a common equation (Regazzi & Silva, 2004). Assuming homoscedasticity of data, performed Barlett's test (Shukor, 2016) was performed considering the appropriate test of variances homogeneity.

The equations were compared using an asymptotic test based on the likelihood ratio to test the identity of the models, with approximation given by F statistics, using the PROC NLIN procedure of SAS (vers. 9.0). The NLIN procedure was executed in unrestricted mode termed full model ( $\Omega$ ), and with restrictions which were specified by  $\varphi_1$  or  $H_{01}$ ,  $\varphi_2$  or  $H_{02}$ ,  $\varphi_3$  or  $H_{03}$ ,  $\varphi_4$  or  $H_{04}$ ,  $\varphi_5$  or  $H_{05}$ ,  $\varphi_6$  or  $H_{06}$ , and  $\varphi_7$  or  $H_{07}$ , to obtain the estimates necessary to run the statistical test of the considered hypotheses (data not shown).

In order to estimate the values of the different parameters of the statistical model to maximize the likelihood of the observed data at 5% significance level, the likelihood ratio test was applied as suggested by Regazzi & Silva (2010). The  $H_0$  hypothesis of the reduced model was considered adequate when  $H_0$  is rejected; *i.e.*,  $F_0 \geq F\alpha$ . A test for lack of fit of the models was undertaken following the methodology of Regazzi & Silva (2010). The fit criteria used were the adjusted coefficient of determination ( $R^2$ ) and the Durbin Watson statistics (DW) (Neter *et al.*, 1985).

## Results

Bartlett's test showed that the hypothesis of homogeneity of variances (*i.e.*, equality of variances) was not rejected ( $p > 0.05\%$ ).

### Estimates of parameters of Gompertz equation

The parameters ( $A$ ,  $B$ ,  $C$ ) of the equations obtained for each dig-Lys level (Table 2) indicate that BW at maturity, represented by  $A$ , increased for the birds that received diets with 0.97% dig-Lys ( $A=5210.0$ ); however, as the age of the birds increases, gain rate decreases ( $B=0.0306$ ), causing an extension of the curve growth, since the age for maximum growth ( $C=48.0$ ) was higher in relation to the other levels analyzed. Associated with this result, the Gompertz equation derivation allowed to verify that the maximum  $BW_e$  of the birds related to the dietary treatment with 0.97% of dig-Lys was higher ( $BW_e=1916.6g$ ), but with  $WGe$  (58.6g) lower when compared to treatment with 0.85% of dig-Lys. For this level of inclusion of Lys,  $C$  parameter (age at the inflection point) values indicated that the CPK chickens attained their highest  $WGe$  at 44.5 days of age, on average.

Determination coefficients higher than 99% were observed and a high correlation (DW statistics) between observed and estimated data was obtained for BW at the dig-Lys level of 0.97% (Table 2). However, for FI, the dig-Lys level of 0.85% showed the best correlation between observed and estimated data.

### Determination of Gompertz equations parameters for each digestible lysine level

The parameters similarity was assessed based on formulated hypotheses (Table 3), aiming to verify whether a single equation for the four levels of dig-Lys can be considered adequate, or if certain parameters values in the model can be considered as similar. The hypotheses  $H_0$  (1),

**Table 2.** Estimates of the parameters of Gompertz equations adjusted for body weight and feed intake of CPKa broilers receiving different dig-Lys levels

	Lys%	Parameter			$BW_e(g)^b$	$WGe(g)^c$	Adjustment criterion	
		$A$	$B$	$C$			$R^2$	DW <sup>d</sup>
Body weight, g	0.85	4696.9	0.0343	44.5	1727.9	59.2	0.9998	2.089
	0.97	5210.0	0.0306	48.0	1916.6	58.6	0.9996	1.349
	1.09	4798.9	0.0328	45.9	1765.4	57.9	0.9999	1.901
	1.21	4632.4	0.0339	45.0	1704.2	57.7	0.9998	2.324
	Lys%	$A$	$B$	$C$	$FIe(g)^e$	$WGe(g)$	$R^2$	DW
Feed intake, g	0.85	17151.8	0.0277	62.1	6309.7	174.7	0.9999	1.303
	0.97	17550.6	0.0276	62.5	6456.2	178.2	0.9999	1.608
	1.09	17721.2	0.0278	62.5	6519.2	181.2	0.9999	2.068
	1.21	18464.2	0.0272	64.2	6792.6	184.7	0.9999	1.544

<sup>a</sup>CPK=Color Plumé strain. <sup>b</sup> $BW_e$ = body weight estimated at the inflection point, obtained by the  $A/e$  derivative. <sup>c</sup> $WGe$ = weight gain estimated at the inflection point, obtained by the  $A * B/e$  derivative. <sup>d</sup>DW= Durbin Watson statistics. <sup>e</sup> $FIe$ = feed intake estimated at the inflection point, obtained by the  $A/e$  derivative.

**Table 3.** Estimates of the parameters of the full model ( $\Omega$ ) and reduced models ( $\varphi 1, \varphi 2, \varphi 3, \varphi 4, \varphi 5, \varphi 6, \varphi 7$ ) and respective residual sum of squares (RSS) and number of residual degrees of freedom (RDF) of the regression

	Estimate of model parameters							
	$\Omega$	$\varphi 1$	$\varphi 2$	$\varphi 3$	$\varphi 4$	$\varphi 5$	$\varphi 6$	$\varphi 7$
<i>A1</i>	4696.5		4842.8	4877	-	-	4870.3	-
<i>B1</i>	0.0343	0.0331	-	0.0327	-	0.0337	-	-
<i>C1</i>	44.515	45.446	45.33	-	45.4504	-	-	-
<i>A2</i>	5197.2	-	4916.9	4895.3	-	-	4894.9	-
<i>B2</i>	0.0306	0.0335	-	0.0329	-	0.0341	-	-
<i>C2</i>	47.992	45.398	46.038	-	45.3541	-	-	-
<i>A3</i>	4798.9	-	4799.3	4780.2	-	-	4784.3	-
<i>B3</i>	0.0328	0.0326	-	0.033	-	0.0328	-	-
<i>C3</i>	45.961	46.17	45.94	-	46.2312	-	-	-
<i>A4</i>	4632.4	-	4735.3	4739	-	-	4740	-
<i>B4</i>	0.0339	0.0321	-	0.0329	-	0.0322	-	-
<i>C4</i>	45.035	46.451	45.77	-	46.5589	-	-	-
<i>A</i>	-	4828.2	-	-	4833.2	4782.5	-	4821.3
<i>B</i>	-	-	0.0329	-	0.0328	-	0.0329	0.0329
<i>C</i>	-	-	-	45.823	-	45.5287	45.819	45.8116
RSS	474830	492513	485476	487457	511392	559752	488098	602491
RDF	132	135	135	135	138	138	138	141

H0 (2), H0 (3), H0 (4), H0 (5), H0 (6), H0 (7) represent, respectively, the reduced models ( $\varphi 1, \varphi 2, \varphi 3, \varphi 4, \varphi 5, \varphi 6, \varphi 7$ ).  $\varphi 1$  model reduced in parameter *A*;  $\varphi 2$  model reduced in parameter *B*;  $\varphi 3$  model reduced in parameter *C*;  $\varphi 4$  model reduced in parameters *A* and *B*;  $\varphi 5$  reduced model in parameters *A* and *C*;  $\varphi 6$  reduced model in parameters *B* and *C*;  $\varphi 7$  reduced model in parameters *A, B* and *C*.

In the test results for the seven formulated hypotheses (Table 4), only H0 (5) and H0 (7) were significant; *i.e.*, they did not allow for the use of reduced models in parameters *A* and *C* and a common equation for the four levels of dig-Lys, respectively. However, the other hypotheses H0 (1), H0 (2), H0 (3), H0 (4), and H0 (6) were not rejected ( $p > 0.05$ ), thus their use was accepted. Therefore, among the non-rejected hypotheses, H0 (6) was chosen, as it had the highest p-value.

For each dig-Lys level, models with different *A* parameters and equal *B* and *C* parameters were applied. The following adjusted equations were thus generated:

$$\text{For 0.850\% dig-Lys: } Y = 4870.3 \cdot e^{-e^{-0.0329(t-45.819)}}$$

$$\text{For 0.850\% dig-Lys: } Y = 4894.9 \cdot e^{-e^{-0.0329(t-45.819)}}$$

$$\text{For 1.090\% dig-Lys: } Y = 4784.3 \cdot e^{-e^{-0.0329(t-45.819)}}$$

$$\text{For 1.210\% dig-Lys: } Y = 4740 \cdot e^{-e^{-0.0329(t-45.819)}}$$

The test for the lack of fit of the models chosen with different *A* parameters and equal *B* and *C* parameters (Table 5) did not show significance ( $p > 0.05$ ), suggesting that the model is adequate for describing the data.

### Performance of CPK chickens based on the analysis of equation parameters

Considering that the model showed a different *A* parameter and equal *B* and *C* parameters for each dig-Lys level tested, a test was developed to determine whether parameter *A* or weight at maturity in the equation obtained with the dig-Lys level of 0.85% (*A1*) differed from the 0.97% (*A2*), 1.09% (*A3*), and 1.21% (*A4*) (their possible combinations are presented in Table 6). The dig-Lys level of 0.97% provided higher weight at maturity (*A*) than the levels of 0.85 and 1.21%. However, it did not differ ( $p < 0.089$ ) from the Lys level of 1.09% for this parameter. The other comparisons between dig-Lys levels for weight at maturity did not differ from each other (Table 7).

The derivations of Gompertz equation for the Lys levels (Figs. 1A and 1B) showed that the estimated BW

**Table 4.** Results of the tests of hypotheses H0(1), H0(2), H0(3), H0(4), H0(5), H0(6), H0(7) by the F statistics<sup>a</sup>

Hypothesis	Measured <i>F</i>	DF <sup>b</sup>	<i>p</i> -value
H0 (1): $A1=A2=A3=A4=A$	1.6386	3;135	0.1834
H0 (2): $B1=B2=B3=B4=B$	0.9865	3;135	0.4012
H0 (3): $C1=C2=C3=C4=C$	1.1701	3;135	0.1699
H0 (4): $A1=A2=A3=A4=A$ $B1=B2=B3=B4=B$	1.6940	6;138	0.1269
H0 (5): $A1=A2=A3=A4=A$ $C1=C2=C3=C4=C$	3.9346	6;138	$1.161 \times 10^{-3}$
H0 (6): $B1=B2=B3=B4=B$ $C1=C2=C3=C4=C$	0.6147	6;138	0.7182
H0 (7): $A1=A2=A3=A4=A$ $B1=B2=B3=B4=B$ $C1=C2=C3=C4=C$	3.9432	9;141	$1.684 \times 10^{-4}$

<sup>a</sup> $A1-A4$ ;  $B1-B4$ ;  $C1-C4$ = equation parameters for each dig-Lys level tested (0.85%, 0.97%, 1.09%, and 1.21%). <sup>b</sup>DF=degrees of freedom.

**Table 5.** Lack of fit for the chosen model

Causes of variation	DF <sup>a</sup>	SS <sup>b</sup>	MS <sup>c</sup>	Measured <i>F</i>	<i>p</i> -value
Treatment	9	57187.6	6354.18	1.511	0.1492
Residual (pure error)	144	605531.8	4205.08		
Regression residual	153	23696			

<sup>a</sup>DF=degree of freedom. <sup>b</sup>SS=sum of square. <sup>c</sup>MS=mean square.

**Table 6.** Results of the comparisons of the *A* parameters of the Gompertz equations for the different levels of dig-Lys

Hypothesis	Lys level	Measured <i>F</i>	DF <sup>a</sup>	<i>p</i> -value
H0(1): $A1=A2=A$	0.850 = 0.970	4.381	1;19	0.050
H0(2): $A1=A3=A$	0.850 = 1.090	0.479	1;19	0.497
H0(3): $A1=A4=A$	0.850 = 1.210	0.068	1;19	0.796
H0(4): $A2=A3=A$	0.970 = 1.090	3.208	1;19	0.089
H0(5): $A2=A4=A$	0.970 = 1.210	5.448	1;19	0.030
H0(6): $A3=A4=A$	1.090 = 1.210	0.757	1;19	0.395

<sup>a</sup>DF=degree of freedom.

(Fig. 1A) increased linearly, and the dig-Lys level, which resulted in the highest BW, was 0.97%. The same was observed for estimated DWG, in which the treatment containing 0.97% of dig-Lys was higher, generating the best WG among all levels. Overall, it can be observed that after the animal reaches maturity, at around 48 days of age, its DWG declines irrespective of the treatment, which is expected. Feed intake rose with the increasing concentrations of dig-Lys in the diets (Fig. 1C). The highest and lowest FI values were obtained in the treatments with 1.21% and 0.85% dig-Lys, respectively.

When evaluating the total production period (1 to 77 days of age), the performance information of the obser-

ved data demonstrated that birds of dietary treatment with 0.97% dig-Lys presented higher DWG and FI values, compared to other experimental treatments (Table 7). When analyzing the data from 22 to 77 days, it appears that there was an improvement in the FI of the birds of this treatment.

The comparison between the observed data (Table 7) and the estimated data (Table 8) allows us to infer that the Gompertz models demonstrated accuracy in estimating the information, since the estimated final weight (FW=3420 g) at 77 days was close to the information observed (FW=3480 g) in the dietary treatment of 0.97% dig-Lys.

**Table 7.** Observed growth performance of CPKa broilers in function of dig-Lys levels.

Period (days)	Variable	dig-Lys level (%)				SEMb	p-value
		0.85	0.97	1.09	1.21		
1 to 77	FW <sup>c</sup> (g)	3,418.0	3,480.0	3,363.0	3,315.0	38.16	0.0518
	WG <sup>d</sup> (g)	3,378.0xy	3,440.0x	3,323.0xy	3,275.0y	37.99	0.0471
	FI <sup>e</sup> (g)	8,700.0	8,960.0	9,066.0	9,195.0	132.92	0.1088
	FC <sup>f</sup>	2.57x	2.60xy	2.73xy	2.80y	0.054	0.0311
22 to 77	IW <sup>g</sup> (g)	470	468	468	467	0.756	0.1574
	WG (g)	2,948.0	3,012.0	2,895.0	2,848.0	37.89	0.0505
	FI (g)	7,976.0	8,236.0	8,342.0	8,472.0	132.2	0.1088
	FC	2.70x	2.73xy	2.88xy	2.97y	0.063	0.0331

<sup>a</sup>CPK=Color Plumé strain. <sup>b</sup>SEM= mean standard error. <sup>c</sup>FW=final weight. <sup>d</sup>WG=weight gain. <sup>e</sup>FI=feed intake. <sup>f</sup>FC=feed conversion. <sup>g</sup>IW=initial weight. x,y: significant differences between metabolizable energy levels.

Further, an estimated daily dig-Lys intake of 1.73 g was found in the animals supplemented with 0.97% dig-Lys at 62 days of age (Table 8), whereas at 77 days, the daily intake of the amino acid by the chickens was 1.61 g.

## Discussion

Based on the estimated parameters, it was found that supplementation with 0.97% of dig-Lys provided greater BW at maturity (parameter A) corroborating with the observed data, where the birds submitted to this treatment were heavier at 77 days. However, the growth curve prolongation estimated by parameter B, may indicate more time for these birds to reach their maximum body weight (BWe), between 48 days when supplemented with 0.97% of dig-Lys and on average 45 days for the others dietary treatments. After these ages, growth rates are reduced (Santos *et al.*, 2005), since the BWe on the growth curve represents the exact moment when the growth rate changes from increasing to decreasing (Mohammed, 2015).

Although the results indicate late growth, diets with 0.97% of dig-Lys showed high BWe when compared to the observed performance data, it appears that this dietary treatment provided the best results for WG and FC, in relation to the other levels evaluated Lys.

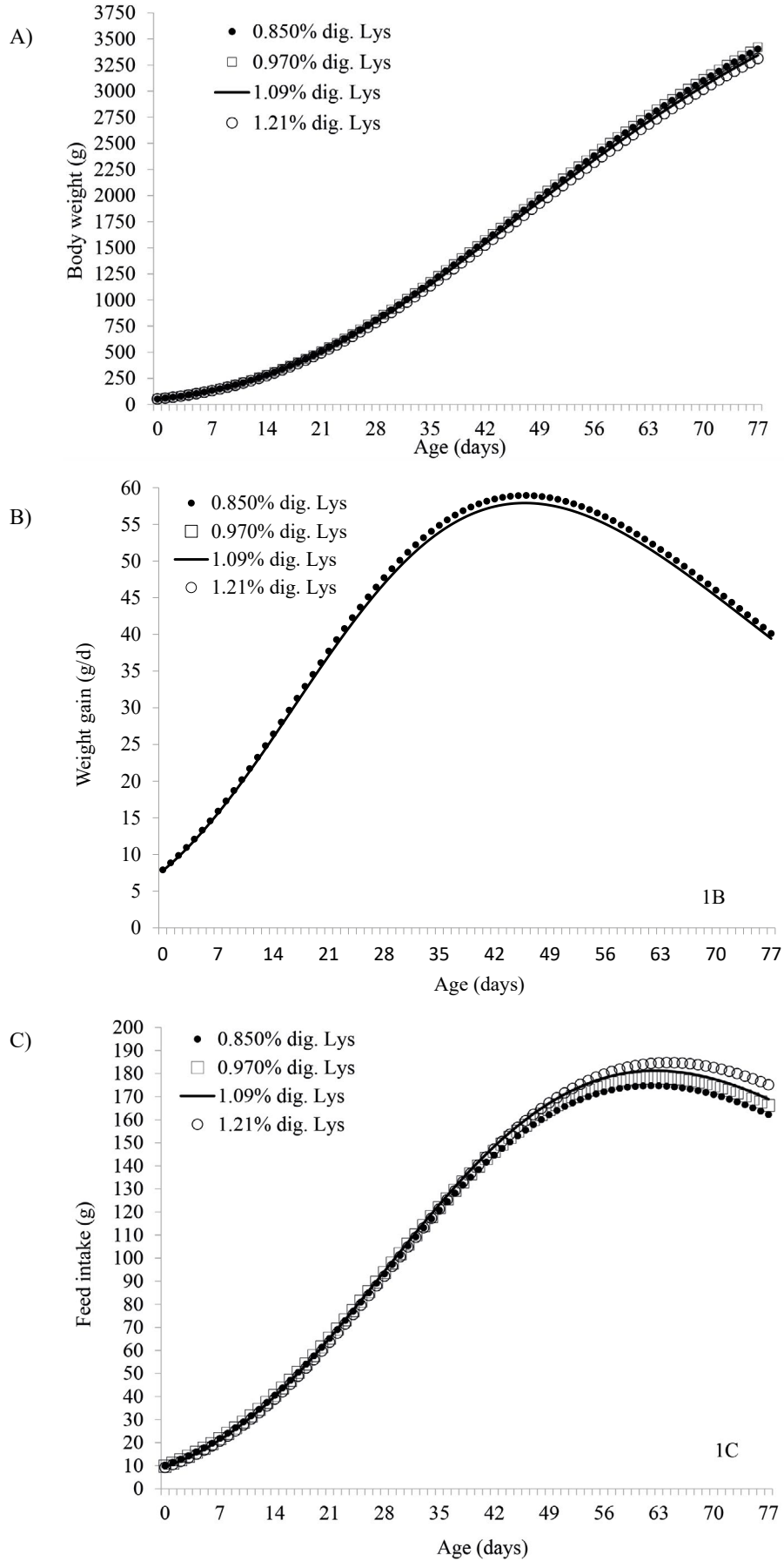
In this experiment, the parameters of Gompertz equation were similar to those found by Michalczuk *et al.* (2016), who evaluated the growth potential of free-range chickens of the Greenleg Partridge (CCGP) strain crossed with a commercial fast-growing strain and obtained 5921.7 for parameter A, 0.03 for parameter B, and 46.82 for parameter C.

The 45.85 days of age found in the present experiment for the C parameter for the CPK chickens were similar those obtained by Santos *et al.* (2005) in free-range Paraiso Pedrês chickens, which achieved their highest WG at around 46 days of age. In comparison with chickens of the

Isa Label strain, the CPK chickens showed to be earlier, since the former strain had their maximum weight gain at 52.5 days of age. Veloso *et al.* (2015), on the other hand, used the Gompertz model in Redbro (CPK) chickens and observed that they did not reach their maximum weight at the end of the experiment, but were still in the period of growth and muscle deposition.

In Brazil, free-range chicken strains are commercially divided into two categories: i) the super heavy for which a greater weight gain potential is expected, consisting of birds originating from crosses between earlier-growing strains; and ii) the heavy, which are hardy and late in weight gain (Lemos *et al.*, 2018), so the growth curve is slow. Paraiso Pedrês and Color Plumé (CPK), for instance, belong to the super heavy category, while ISA Label is part of the heavy category. Several studies have detected differences in the growth of free-range chicken genotypes (Santos *et al.*, 2005; Rizzi *et al.*, 2013; Eleroğlu *et al.*, 2014; Tavares *et al.*, 2015), demonstrating that knowing the differences of the growth speed between broiler rustic lines, allows the adoption of management techniques influencing the feeding behavior of birds, the breeding system and to select which genetic strain to use (Veloso *et al.*, 2015) aiming to reduce the age at slaughter and, therefore, decrease production costs.

In general, broiler lines have different growth potentials and, in order for birds to express this potential, it is essential that the requirements mainly of Lys are met, since this is the reference amino acid to estimate the requirements for other amino acids, in addition this is directly related to protein deposition in chicken carcasses (Santos *et al.*, 2014; Cardoso *et al.*, 2020). Based on the data from the present study, it is reinforced that the Lys needs to be adjusted to each growth phase, as observed by Oliveira *et al.* (2016), who recommend 0.85% of dig-Lys for free-range Redbro chickens (CPK) aged 57 to 70 days. With this recommendation, the authors obtained 0.624 g/bird WG, values lower than those found in the present



**Figure 1.** Body weight (A), weight gain (B) and feed intake (C) of CPK broilers adjusted by the Gompertz equation in the period from 1 to 77 days of age.



**Table 8.** Estimated growth performance<sup>a</sup> of CPK<sup>b</sup> chickens, in the period of 1 to 77 days of age, from a mixed lot by deriving the Gompertz equation.

Age (days)	BW <sup>c</sup> (g)	DWG <sup>d</sup> (g/day)	DFI <sup>e</sup> (g/day)	CFI <sup>f</sup> (g)	FC <sup>g</sup> (g/g)	Lys intake <sup>h</sup> (g/day)
0	54	-	-	-	-	-
1	62	8.0	11.1	11	1.40	0.11
2	71	8.9	12.58	24	1.41	0.12
3	82	9.9	14.15	38	1.43	0.14
4	93	11.0	15.85	54	1.44	0.15
5	106	12.2	17.68	71	1.45	0.17
6	120	13.4	19.65	91	1.47	0.19
7	136	14.7	21.77	113	1.48	0.21
8	152	16.0	24.02	137	1.50	0.23
9	170	17.4	26.42	163	1.52	0.26
10	190	18.8	28.96	192	1.54	0.28
11	211	20.3	31.65	224	1.56	0.31
12	234	21.8	34.47	258	1.58	0.33
13	258	23.4	37.43	296	1.60	0.36
14	283	25.0	40.52	336	1.62	0.39
15	311	26.6	43.74	380	1.65	0.42
16	340	28.2	47.08	427	1.67	0.46
17	370	29.8	50.54	478	1.69	0.49
18	403	31.5	54.1	532	1.72	0.52
19	437	33.1	57.77	590	1.75	0.56
20	472	34.7	61.52	651	1.77	0.6
21	509	36.3	65.37	716	1.80	0.63
22	548	37.9	69.28	786	1.83	0.67
23	588	39.5	73.26	859	1.86	0.71
24	630	41.0	77.29	936	1.88	0.75
25	673	42.5	81.36	1018	1.91	0.79
26	718	43.9	85.46	1103	1.95	0.83
27	764	45.3	89.59	1193	1.98	0.87
28	811	46.7	93.72	1286	2.01	0.91
29	860	48.0	97.84	1384	2.04	0.95
30	910	49.2	101.96	1486	2.07	0.99
31	960	50.4	106.04	1592	2.11	1.03
32	1012	51.5	110.09	1702	2.14	1.07
33	1065	52.5	114.1	1816	2.17	1.11
34	1119	53.4	118.04	1934	2.21	1.14
35	1174	54.3	121.92	2056	2.24	1.18
36	1230	55.1	125.71	2182	2.28	1.22
37	1286	55.9	129.42	2312	2.32	1.26
38	1343	56.5	133.04	2445	2.35	1.29
39	1400	57.1	136.55	2581	2.39	1.32
40	1458	57.6	139.95	2721	2.43	1.36
41	1516	58.1	143.23	2864	2.47	1.39
42	1575	58.5	146.38	3011	2.50	1.42

<sup>a</sup>Experimental diets 0.97% dig-Lys. <sup>b</sup>CPK=Color Plumé strain. <sup>c</sup>BW=average body weight. <sup>d</sup>DWG=daily weight gain. <sup>e</sup>DFI=daily feed intake. <sup>f</sup>CFI=cumulative feed intake. <sup>g</sup>FC=feed conversion. <sup>h</sup>Lys intake=daily lysine intake.

**Table 8 (Cont.).** Estimated growth performance of CPK<sub>b</sub> chickens, in the period of 1 to 77 days of age, from a mixed lot by deriving the Gompertz equation.

Age (days)	BW <sup>c</sup> (g)	DWG <sup>d</sup> (g/day)	DFI <sup>e</sup> (g/day)	CFI <sup>f</sup> (g)	FC <sup>g</sup> (g/g)	Lys intake <sup>h</sup> (g/day)
43	1634	58.7	149.4	3160	2.54	1.45
44	1693	59.0	152.29	3312	2.58	1.48
45	1752	59.1	155.03	3467	2.62	1.5
46	1811	59.2	157.63	3625	2.66	1.53
47	1870	59.2	160.08	3785	2.70	1.55
48	1929	59.2	162.38	3947	2.74	1.58
49	1988	59.1	164.52	4112	2.78	1.6
50	2047	58.9	166.51	4278	2.83	1.62
51	2106	58.7	168.33	4447	2.87	1.63
52	2164	58.4	170.00	4617	2.91	1.65
53	2222	58.1	171.52	4788	2.95	1.66
54	2280	57.7	172.87	4961	2.99	1.68
55	2337	57.3	174.06	5135	3.04	1.69
56	2393	56.8	175.1	5310	3.08	1.7
57	2449	56.3	175.99	5486	3.12	1.71
58	2505	55.8	176.72	5663	3.17	1.71
59	2560	55.2	177.3	5840	3.21	1.72
60	2614	54.6	177.74	6018	3.26	1.72
61	2668	53.9	178.03	6196	3.30	1.73
62	2720	53.3	178.18	6374	3.35	1.73
63	2773	52.6	178.19	6553	3.39	1.73
64	2824	51.8	178.06	6731	3.44	1.73
65	2875	51.1	177.81	6908	3.48	1.72
66	2925	50.3	177.43	7086	3.53	1.72
67	2974	49.5	176.93	7263	3.57	1.72
68	3022	48.7	176.31	7439	3.62	1.71
69	3070	47.9	175.58	7615	3.66	1.70
70	3116	47.1	174.74	7789	3.71	1.69
71	3162	46.3	173.79	7963	3.76	1.69
72	3207	45.4	172.75	8136	3.80	1.68
73	3251	44.6	171.61	8308	3.85	1.66
74	3295	43.7	170.38	8478	3.90	1.65
75	3337	42.9	169.07	8647	3.94	1.64
76	3379	42.0	167.68	8815	3.99	1.63
77	3420	41.2	166.21	8981	4.04	1.61

<sup>a</sup>Experimental diets 0.97% dig-Lys. <sup>b</sup>CPK=Color Plumé strain. <sup>c</sup>BW=average body weight. <sup>d</sup>DWG=daily weight gain. <sup>e</sup>DFI=daily feed intake. <sup>f</sup>CFI=cumulative feed intake. <sup>g</sup>FC=feed conversion. <sup>h</sup>Lys intake=daily lysine intake.

study, which estimated 0.728 g/bird WG when offering 0.97% of dig-Lys for the same period (Table 8).

The present study evaluated whether different levels of dig-Lys can alter the growth curves of free-range chickens. Therefore, the parameter equality test identified that an equation is required for each level of dig-Lys, with different A parameters, and equal B and C parameters. Morais *et al.* (2015) formulated several hypotheses to

check the equality of nonlinear-model parameters to describe the growth curve of four free-range chicken strains. Those authors found that, for males, parameters A, B, and D should be considered equal for all strains, and to describe the growth curve, only parameter C of the quadratic logarithmic model is different for the four strains.

A comparison between dig-Lys levels for parameter A showed that the intermediate level of 0.97% provided

the highest body weight at maturity in the mixed lot of CPK chickens. Conflicting results were found by Tavares *et al.* (2015), who observed an elevated weight at maturity in Redbro (CPK) animals fed diets with 0.108% of dig-Lys. Santos *et al.* (2014) recommended, based in several mathematical models, that for CPK broilers the dietary supply of Lys should be 1.13% and 1.09% in the phases of 29-56 days and 56-84 days of age, respectively.

Overall, the performance variables showed that the birds receiving the treatment with the lowest inclusion of dig-Lys (0.85%) had the lowest FI, which is associated with the best feed conversion (FC) throughout the experimental period. Similar findings were reported by Rosa *et al.* (2014), who found that chickens of the slow growth strain supplemented with 0.90% of dig-Lys had lower FI and FC.

The results regarding the observed performance variables were similar to the data estimated by the Gompertz model, demonstrating its accuracy in describing the growth curve of birds receiving different levels of dig-Lys. The FI, FC, WG and BW values in this study, for the period 1 to 77 days of age, were higher than those found by Tavares *et al.* (2015), who investigated the growth development in free-range chicken strain Redbro (CPK) from 1 to 84 days of age, finding that FI, FC, DWG and final weight values were 5,703g, 2.36 g/kg, 28.71 g/bird/day and 2,497 g, respectively.

The Gompertz equation was efficient in describing the body growth and FI behavior of free-range CPK chickens, considering that the observed and estimated data were highly correlated. Equations with different A parameters and equal B and C parameters were established for each dig-Lys level. It is recommended for 0.97% of dig-Lys the equation  $Y = 4894.9 \cdot e^{-e^{-0.0329(t-45.819)}}$ .

The highest estimated BW at maturity (parameter A) and BWe was observed at the dig-Lys level of 0.97%, which differed significantly from the other levels.

In conclusion, the parameters of the nonlinear Gompertz model accurately described the body growth and FI behavior of free-range chickens of the CPK strain. Based on the obtained results, we recommend the dig-Lys level of 0.97% in the diet of free-range CPK chickens of mixed lot in the period of 22 to 77 days of age. To increase weight gain, we recommend splitting the supplementation of dig-Lys for CPK chickens of both sexes into two periods: 21 to 49 days and 50 to 77 days.

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