



Comparison of Different Non-Linear Models for Describing Plasma Lysozyme Activity in Quail

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Abstract

Lysozyme activity is one of the nonspecific immunity parameters measured by changing the amount of adsorption at different times. The objective of the present study was to compare five non-linear models including Gompertz, Richards, Logistic, Lopez, and Weibull to describe the cumulative plasma lysozyme activity in quails. In total 1364 plasma samples (1004 females and 360 males) were collected and the cumulative lysozyme activity was calculated by turbidimetric method assay in *Micrococcus luteus*. The goodness-of-fit of models was compared according to different criteria of Maximum log-likelihood, Akaike information criterion, Mean square error, and Bayesian information criterion. The results showed that the Gompertz model was the best model for describing of decreasing cumulative pattern of lysozyme activity in female and male quails and provided satisfactory predictions of lysozyme activity at different times (30, 60, 90, 120, 150, 180, 210, 240, 270, 300 seconds). The parameters of all models were higher in females than males except for the k parameter which was greater in the males. Male quails had higher values for time and lysozyme activity than females at inflection points, whereas the absolute growth rate in 30, 150, and 300 seconds was predicted higher in female quails. In conclusion, the Gompertz model can be used accurately to evaluate cumulative lysozyme activity patterns in both sexes of quails.

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Introduction

Domestic poultry is often bred for egg or meat production separately, but some species of poultry have dual-purpose. Numerous studies have been conducted to evaluate the impact of genetic backgrounds of poultry on their growth performance; but the results showed that long-term selection to increase the growth rate results in the lower function of the immune system (Leshchinsky and Klasing, 2001). Therefore, improving the growth efficiency of poultry will lead to their susceptibility and vulnerability to pathogens. Many breeders use antibiotics and vaccines to solve this problem. It should be noted that immunization of the herd against all pathogens is not possible and the use of antibiotics not only increases the production costs and microbial

resistance but also, they have adverse effects on the quality of meat and eggs. Therefore, in terms of food safety, the European Union has banned the use of antibiotics in EU member states since 2006 (Garcia *et al.*, 2007).

The economic efficiency of the poultry industry mainly depends on the rate of deaths caused by diseases and stress factors. The stress factors may lead to hormonal changes, reduction of feed intake, metabolic and nutritional disorders, and immunosuppression in the birds. However, different adaptive capability has been observed in different strains to cope with environmental stresses and diseases (Goerlich *et al.*, 2012). The world poultry production system suffers from extensive losses due to different climatic conditions, production systems,

and management. The production of stocks that are resistant to a wide range of diseases offers the promising prospect to reduce the costs of treatments and vaccination programs. Creating genetic resistance has several benefits, especially, the increased immune response to vaccines, decreasing the expenditure on prophylactic and vaccination programs, and increasing survival and profitability (Gavora and Spencer, 1979; Pathak *et al.*, 2018).

In general, the immune system is divided into two categories: nonspecific (intrinsic) and specific (acquired) immunity (Kumari *et al.*, 2006). In intrinsic immunity, the body is the first line of defense against pathogens (Bao *et al.*, 2015), responding much faster than the specific immune system to infections (Saurabh and Sahoo., 2008). The lysozyme enzyme is one of the most important factors in the nonspecific immune system that was introduced by Alexander Fleming in 1922 (Jolles and Jolles, 1984). This enzyme is also known as muramidase and N-acetyl muramide-hydrolase, a polypeptide with a molecular weight of 14.3 kDa with antibacterial activity, which is present in a wide range of vertebrates (Huopalahti *et al.*, 2007). The white blood cell granules including neutrophils, monocytes, macrophages, and tissues rich in leukocytes produced the lysozyme enzyme (Holloway *et al.*, 1993).

The quail is the smallest animal species that became domesticated in the 11th century as a songbird (Wilkinson *et al.*, 2018). This bird is a dual-purpose bird and is bred for meat and egg production. In recent years, the quail used as an experimental animal model in scientific studies due to its remarkable properties (Vali *et al.*, 2005; Minvielle, 1998).

Lysozyme activity is measured by the change of its activity per time so a quantitative trait can be analyzed by non-linear regression models. Therefore, in the genetic evaluation of blood plasma lysozyme activity, the parameters of nonlinear models can be considered as the components of lysozyme activity instead of the mean change of lysozyme activity over time. Although, there are different nonlinear models for describing time-dependent changes, using the appropriate model to describe the response behavior is an important factor in prediction accuracy. Thus, the present study was conducted to compare the capability of five non-linear models for describing the cumulative plasma lysozyme activity in quails.

Materials and methods

Bird management

The present research was performed on the population of Japanese quails raised in the Research Center of Domestic Animals (RCDA) at the University of Zabol, Zabol, Iran. The study was

conducted following the general ethical guidelines of the Animal Care and Use Committee of the RCSDA.

A total of 1364 (1004 females and 360 males) Japanese quails data was collected from the RCSDA farm. A diet containing 250 g CP/kg and 2900 kcal J ME/kg was used to feed quails from d 1 to 45. Accessibility of the birds to the feed and water was *ad libitum*. The room temperature was 35 °C in the first week, then decreased gradually to 20–25 °C for the latter weeks. During the experiment, the lighting program was 23 h light and 1 h dark.

Lysozyme activity measurement

At d 45 of age, blood samples were collected from the wing vein of the birds in the tubes containing EDTA. Samples were instantly placed in the ice tank, and plasma was centrifuged at $2500 \times g$ for 10 min at 4 °C, and then stored at –80 °C until antibody analyses. Turbidimetric assay (Jiang *et al.*, 2001) was used to determine the activity of the plasma lysozyme enzyme. The used microorganism in the assay was *Micrococcus luteus* and the absorption rate was recorded at the wavelength (450 nanometers) in 5 minutes every 30 seconds. The difference between the records at different times was considered as the cumulative reduction of plasma lysozyme activity taking into account the amount of adsorption rate at time zero.

Non-linear models

Five non-linear models used for describing the cumulative reduction of plasma lysozyme activity are shown in Table 1. These models were fitted to the female and male datasets separately. After fitting the models, a comparison of fitted models and selection of the best model was performed by following goodness of fit criteria (Teleken *et al.*, 2017):

- 1) $AIC = n \ln(SSE/n) + 2k$.
- 2) $MSE = SSE/(n-k)$.
- 3) Log likelihood
- 4) $BIC = n \ln(SSE/n) + k \ln(n)$.

where AIC, MSE, and BIC, n, SSE, and k are the Akaike's Information Criteria, Mean square error, Bayesian Information Criterion, number of observations, sum square of errors, and the number of model parameters, respectively. The MSE, AIC, and BIC adjusted the residual sum of square (RSS) for the number of parameters in the model and can be used for comparison of models as goodness-fit criterion (Darmani Kuhl and France, 2019). The smaller values of MSE, AIC, and BIC are better while a larger value of log-likelihood represents the better fitness of the models. The statistical differences in cumulative lysozyme activity between females and males were determined by Student's t-test.

Table 1. Equation of non-linear models used in this study

Model	Equation*	t_i	w_i
Gompertz	$W = W_0 \exp\left\{[1 - \exp(-kt)] \ln\left(\frac{W_f}{W_0}\right)\right\}$	$\frac{1}{k} \left[\ln\left(\ln\left(\frac{W_f}{W_0}\right)\right) \right]$	$\frac{W_f}{e}$
Richards	$W = \frac{W_0 W_f}{[W_0^m + (W_f^m - W_0^m)e^{-kt}]^{1/m}}$	$\frac{1}{k} \times \ln\left(\frac{m}{(W_f^m - W_0^m)/W_0^m}\right)$	$\frac{W_f}{\sqrt[m]{m+1}}$
Logistic	$W = \frac{W_0 W_f}{[W_0 + (W_f - W_0)\exp(-kt)]}$	$\frac{1}{k} \ln\left(\frac{W_f - W_0}{W_0}\right)$	$\frac{W_f}{2}$
Lopez	$W = \frac{(W_0 b^k + W_f t^k)}{(b^k + t^k)}$	$b \left(\frac{k-1}{k+1}\right)^{1/2}$	$\frac{[(1 + \frac{1}{k})W_0 + (1 - \frac{1}{k})W_f]}{2}$
Weibull	$W = W_f - (W_f - W_0)\exp[-(kt)^m]$	$\frac{1}{k} \left(\frac{m-1}{m}\right)^{1/m}$	$W_f - (W_f - W_0)\exp\left(-\frac{m-1}{m}\right)$

*W in all models is the cumulative lysozyme activity of a bird at different times t, W_0 , W_f , and k are initial and final cumulative lysozyme activity, and coefficient of relative growth or maturing index, respectively. The parameter b is the time at approximately half maximum cumulative lysozyme activity, m represents the shape parameter, and time (t_i), lysozyme activity (w_i) at inflection points.

Statistical analysis

Different models were fitted to explain the variation of cumulative lysozyme activity data in females and males separately by using *nlme* package in R software (Pinheiro et al., 2014). The model parameters and their different criteria of goodness of fit were estimated for each of the sex groups, separately. The time and lysozyme activity at the inflection point and absolute growth rate (AGR) in the 30, 150, and 300 seconds were estimated using the model parameters by the equations introduced by Moharrery and Mirzaei (2014). AFR can be derived from a general differential growth models, which means that the growth rate of a biological system is dependent on time and lysozyme activity (Moharrery and Mirzaei, 2014). Afterward, the correlation between the predicted and observed values of the cumulative

lysozyme activity was used to compare the accuracy of the models.

Results

Table 2 shows the mean (standard deviation) of the cumulative lysozyme activity measured as the mean of decreased lysozyme activity of plasma for male and female quails at different times. The high standard deviation of the means indicates the high variation of lysozyme activity in the studied quails. At the first 2 times, the mean of the cumulative lysozyme activity in male quails was slightly higher than in females, however, the males had higher cumulative lysozyme activity in other times except for the 300 seconds. In total differences in the cumulative lysozyme activity between males and females were not significant.

Table 2. Cumulative lysozyme activity at different ages for male and female quails

Time (sec)	Lysozyme activity	
	female	male
30	31.827 (38.206)	31.738 (33.947)
60	52.445 (49.398)	52.106 (45.553)
90	67.166 (56.785)	68.806 (52.950)
120	80.302 (63.313)	82.297 (58.804)
150	91.249 (69.039)	93.794 (61.934)
180	100.819 (73.843)	103.408 (65.538)
210	108.578 (76.984)	113.039 (68.188)
240	115.914 (80.420)	121.789 (70.917)
270	135.330 (86.229)	135.413 (72.078)
300	156.072 (96.215)	148.170 (75.701)

Data presented as the mean of decreased lysozyme activity and standard deviations are in parentheses. The average cumulative lysozyme activity at different ages had no significant difference ($P < 0.05$).

Figure 1 shows the cumulative lysozyme activity change trends for female and male quails. The difference in cumulative lysozyme activity between females and males at times of 30, 60, and 270 seconds were lower than at other times. After a time

of 60 seconds, males had a higher cumulative lysozyme activity than females, but finally, at the time of 300 seconds, females showed more cumulative lysozyme activity than males.

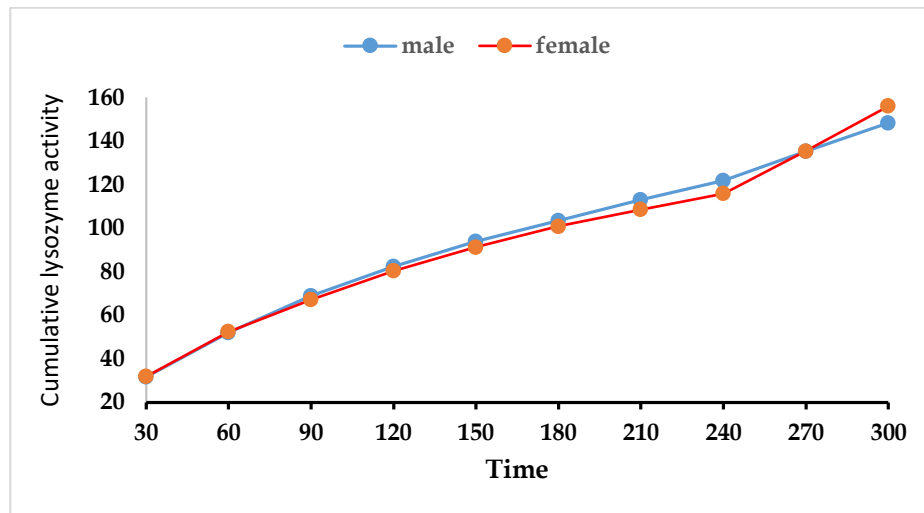


Figure 1. Cumulative lysozyme activity change trend in females and males

The estimated parameters of different models for the male and female quails are shown in Table 3. The initial cumulative lysozyme activity (w_0) parameter was smaller in the Richard model than in other models in both sexes and the highest value of w_0 belonged to the logistic model in females and males. The estimated values of w_f , m , and b parameters in females were higher than in males, whereas the males had a higher value of the k parameter. In Gompertz and Logistic models, the lowest estimated k parameter in both sexes was associated with the highest value of w_f parameter. The females had a higher value of the m parameter than males, indicating that female quails reached sooner to maximum cumulative lysozyme activity than males.

Table 4 shows the time and the cumulative plasma lysozyme activity at the inflection point estimated by different fitted models in each sex. The maximum and minimum time and lysozyme activity were estimated by logistic and Richards models in both females and

males, respectively. In all models, the t_i and w_i parameters were higher in females than males. The estimated absolute growth rate (AGR) in 30, 150, and 300 seconds is shown in Table 4. The higher value for AGR was estimated by the Lopez model (except for AGR₃₀ in females). In all models, the AGR₁₅₀ value was higher than in other epochs, except for the Richards model. However, the lowest value of AGR was observed in 300 seconds for all models which indicates lysozyme activity increased up to 150 seconds and then it decreased after 150 seconds. In the present study, the reduction of plasma lysozyme activity has been considered cumulatively. This reduction is higher in early times than the later times so the reduction of plasma lysozyme activity sometimes reaches zero at the time of 300 seconds. The reason for the low AGR₃₀₀ compared to AGR₃₀ and AGR₁₅₀ is also due to this subject. Generally, lysozyme activity was measured over 5 minutes and lysozyme activity remained constant in the later times.

Table 3. Estimated parameters of different models for cumulative lysozyme activity

Sex*	Model	$W_0 \pm SE$	$W_f \pm SE$	$k \pm SE$	$m \pm SE$	$b \pm SE$
Female	Gompertz	29.248±1.956	215.552±20.750	0.005±0.0006	-	-
	Logistic	33.231±1.652	183.350±11.410	0.009±0.0007	-	-
	Richards	23.619±4.382	180.000±33.563	0.006±0.003	-0.227±0.739	-
	Lopez	30.448±4.308	178.000±20.809	-	1.798±0.309	179.674±26.636
	Weibull	29.332±4.116	140.000±7.167	0.006±0.0004	1.620±0.209	-
Male	Gompertz	25.079±2.873	173.021±12.620	0.008±0.001	-	-
	Logistic	29.733±2.407	156.731±7.740	0.012±0.001	-	-
	Richards	20.557±6.638	160.000±22.715	0.008±0.005	-0.188±0.895	-
	Lopez	27.020±7.344	178.000±28.912	-	1.707±0.438	167.810±34.424
	Weibull	26.786±6.523	140.000±9.009	0.006±0.0006	1.576±0.289	-

W_0 , W_f , k , m and b are the initial and final cumulative lysozyme activity, coefficient of relative growth or maturing index, the shape parameter, and age at approximately half maximum decreased lysozyme activity, respectively.

The goodness of fit criteria of fitted models in female and male quails is shown in table 5. All models fit well the cumulative plasma lysozyme activity data in females and males. The MSE, AIC,

and BIC values were smaller in the Gompertz model in both sex, and the Gompertz model had the best log-likelihood value. Thus, the Gompertz can be the best model for describing the trend of cumulative

plasma lysozyme activity in female and male quails. Based on four criteria, the Weibull model was the worst model for describing the trend of cumulative

plasma lysozyme activity compared to the other models in both sexes.

Table 4. Estimated time (t_i), lysozyme activity (w_i) at inflection points and absolute growth rate (AGR) at 30, 150, and 300 seconds using different non-linear models

Sex	Model	t_i (sec)	w_i	AGR (30)	AGR (150)	AGR (300)
Female	Gompertz	138.37	79.30	0.326	0.391	0.298
	Logistic	167.55	91.67	0.290	0.412	0.274
	Richards	81.136	57.90	0.420	0.403	0.219
	Lopez	89.42	63.19	0.410	0.895	0.763
	Weibull	92.12	64.52	0.349	0.434	0.116
Male	Gompertz	82.28	63.65	0.466	0.475	0.207
	Logistic	120.99	78.37	0.354	0.456	0.174
	Richards	66.514	52.85	0.491	0.418	0.167
	Lopez	76.43	58.29	0.509	0.916	0.738
	Weibull	88.00	61.44	0.373	0.432	0.120

Table 5. The goodness of fit criteria of fitted models for cumulative lysozyme activity in different models

Sex	Model	MSE [†]	AIC [‡]	BIC [#]	Log. Lik
Female	Gompertz	4778.7	104869.6	104898.1	-52430.8
	Logistic	4785.6	104882.8	104911.4	-52437.4
	Richards	4782.2	104875.4	104911.1	-52432.7
	Lopez	4791.54	104895.4	104931.0	-52442.7
	Weibull	4802.7	104916.9	104952.5	-52453.4
Male	Gompertz	3705.7	37162.6	37187.0	-18577.3
	Logistic	3712.5	37168.7	37193.2	-18580.4
	Richards	3708	37165.6	37196.2	-18577.8
	Lopez	3710.2	37167.6	37198.2	-18578.8
	Weibull	3719.2	37175.8	37206.4	-18582.9

[†] Mean square error (RSME); [‡]Bayesian information criterion (BIC), [#]Akaike information criterion (AIC), and log-likelihood (Log. Lik).

The correlation between observed and predicted values of the cumulative plasma lysozyme activity is presented in Table 6. In females and males, the correlation values were higher than 0.95, suggesting that all models accurately predicted the cumulative

plasma lysozyme activity. In female and male quails, the lowest correlation belonged to the Weibull model, the worst model for describing the cumulative plasma lysozyme activity.

Table 6. Correlation between observed and predicted values of cumulative lysozyme activity by different models

Sex	Model	Correlation	Standard error
Female	Gomperts	0.988	0.037
	Logistic	0.985	0.041
	Richards	0.985	0.041
	Lopez	0.979	0.048
	Weibull	0.973	0.054
Male	Gomperts	0.995	0.024
	Logistic	0.992	0.030
	Richards	0.993	0.027
	Lopez	0.992	0.029
	Weibull	0.988	0.036

Discussion

The standard deviation of cumulative plasma lysozyme activity had an incremental trend with times of measurement (Table 2). Similar results were obtained for body weight traits in quails (Aggrey, 2002; Nahashon *et al.*, 2006; Faraji-Arough *et al.*, 2018), which was a common phenomenon of time

series data. Raji *et al.* (2014) reported that the increase in standard deviation with time is expected for the time series data. Differences in the cumulative lysozyme activity between males and females were not significant. Sivaraman *et al.* (2005) reported that males of the broiler dam line tend to show lower serum lysozyme but the differences were not

significant. In the present study, the female quails had a higher cumulative plasma lysozyme activity than males most of the time. Differences in results can be due to the different species studied by Sivaraman *et al.* (2005). Also, it was reported that female quails were significantly heavier than male quails (Sezer and Tarhan, 2005), and considering the negative genetic correlation (Sivaraman *et al.*, 2005) between lysozyme activity and body weight, it is expected that females have lower lysozyme activity.

In the study of growth curves in broiler chickens (Masoudi and Azarfar, 2017) and Khazak native chickens (Faraji-Arough *et al.*, 2019), the highest and lowest values of W_0 parameter were estimated by the use of Logistic and Richards models, respectively, that it was in agreement with our results. Also, the inverse association between W_f and k parameters in other studies (Adenaik *et al.*, 2017; Faraji-Arough *et al.*, 2019) proves our finding for Gompertz and Logistic models. The higher maturing index value for males and females was reported using the Logistic model (Aggrey, 2002, Narinc *et al.*, 2010; Faraji-Arough *et al.*, 2019), which is parallel to our findings.

The estimated values for time and cumulative plasma lysozyme activity at the inflection point were higher for the female models than for male models (Table 4). In other studies of growth curves in different poultry species, higher values of age and weight at inflection points were observed for males than females (Cooper, 2005; Narinc *et al.*, 2010; Goto *et al.*, 2010; Faraji-Arough *et al.*, 2019), which were in contrast to our results. The reason for the difference can be due to the difference in the studied species and traits. Similarly, age and weight at the inflection point for female quails were higher than for male quails in the study by Sezer and Tarhan (2005). The growth rate at 30 and 150 seconds was higher in male than female quails, whereas, the growth rate at 300 epochs for females was higher than male quails. Thus, it could be concluded that male quails reached sooner (Table 4) the inflection point than females, therefore, resulting in a shorter time for decreasing lysozyme activity in males than in females.

The Gompertz model showed a high correlation between observed and predicted cumulative plasma activity at different times. Models that show the difference between predicted and observed values at short interval are preferred over other models with deviations at a longer interval time (Adenaik *et al.*, 2017). According to the overall goodness of fit criteria, the Gompertz model was the best model for describing the cumulative plasma lysozyme activity in both sexes. The growth models are often used to describe the growth in various animal species such as quail (Alkan *et al.*, 2009; Narinc *et al.*, 2010; Raji *et*

al., 2014; Faraji-Arough *et al.*, 2018), broilers, and laying hens (Zhao *et al.*, 2015; Manjula *et al.*, 2016; Adenaik *et al.*, 2017; Faraji-Arough *et al.*, 2019). Usually, the Gompertz and Richard models were reported as the best models

The growth models are nonlinear sigmoid models that have an asymptote and an inflection point. However, the accuracy of the estimated model parameters depends on the data structure (Moharrery and Mirzaei, 2014). It is recommended that researchers should choose the flexible model with the least complexity among the available models. For example, the three-parameter models (Gompertz, Logistic) are simple and fit well short time data such as growth traits, but Richards models, that has an additional parameter, are more complex than three-parameter models and it is appropriate for a long time data series (Karkach, 2006; Darmani Kuhl *et al.*, 2010).

Interpretation and the understanding of the phenomenon can be facilitated with mathematical models due to having parameters with biological meaning (Fitzhugh, 1976). Furthermore, using mathematical models for biological events could be effective in identifying problems and solving solutions without making the cost of experimentation and animal manipulation. On the other hand, the required time to find a solution for each production system is reduced (Bindya *et al.*, 2010). Although the use of growth models was reported to describe the growth changes, in egg and milk production, there is no published paper on the use of the classical growth model to describe cumulative plasma lysozyme activity.

Conclusion

The present results showed that the Gompertz model was the best model for describing of decreasing cumulative pattern of lysozyme activity in both sexes and would obtain the most accurate estimations using the Gompertz model. All model parameters were higher in females than males except the k parameter. However, the k parameter, time (t_i) and lysozyme activity (w_i) at inflection points were high in male quails. Thus, the present study can be considered the first report to study lysozyme activity and this trait could be studied as an interpretive parameter by the use of an appropriate growth model instead of using the average activity over time.

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