

DEFINITION OF NUTRITIONAL REQUIREMENTS FOR ZINC IN BROILERS USING BAYESIAN METHODOLOGY

Rick Anderson Freire MANGUEIRA¹
Taciana Villela SAVIAN¹
Roseli Aparecida LEANDRO¹

- **ABSTRACT:** Studies about nutritional requirements of animals are important, among other reasons, in order to have an adequate knowledge of the essential levels of minerals added in feed, and thus, to produce it efficiently, to avoid elimination of minerals through feces and urine and prevent intoxication of the animal due to excess of a certain nutrient. The data used for the study are related to Zinc (Zn) content, in ppm, in the broilers' tibia that received the same feed with nine different dosages of zinc. The quadratic segmented regression model with response plateau and the nonlinear segmented regression model with response plateau were fitted to data, using a bayesian approach and considering cases of homogeneity and heterogeneity of variances. The fitted models considering heterogeneity of variances were more appropriate for the data, according to the DIC fit criterion.
- **KEYWORDS:** Segmented regression; response plateau; zinc.

1 Introduction

Poultry production in Brazil has a fundamental importance in the lives of Brazilians, whether due to the economic aspect, generating employment and income; either by the nutritional aspect, since both the egg and the meat have proteins of high biological value. Thus, studies that aim to determine the ideal amount of nutrients to be applied in feed are essential to, at the same time, optimize animal production and reduce production costs which are around 70% of the final cost.

¹Universidade de São Paulo - USP, Escola Superior de Agricultura 'Luiz de Queiroz', Departamento de Ciências Exatas, CEP:13418-900, Piracicaba, SP, Brasil. E-mail: *rickanderson0310@yahoo.com.br*, *tusavian@usp.br*, *rleandr@usp.br*.

Minerals, both macrominerals and microminerals, are essential in animal nutrition and their deficiency can damage their performance, production and growth. Among the microminerals required in the diet in low concentrations there is zinc, which is related to the activation of about 300 enzymes in the animal organism (MCCALL; HUANG; FIERKE, 2000). Zinc deficiency causes, besides other disorders, reduction in weight gain, weaker bones, defects in shell eggs (BAIN *et al.*, 2006), low bone mineralization and dysfunction of the immune system (KIDD; FERKER; QURESHI, 1996; WANG *et al.*, 2002, FERREIRA, 2018).

Segmented regression models with response plateau are appropriate and frequently applied (SIQUEIRA *et al.*, 2009; EKLUND *et al.*, 2010; OZORES-HAMPTON *et al.*, 2012; GONÇALVES *et al.*, 2012; XIUFU, 2016; KIM *et al.*, 2018) in studies of nutritional requirements to establish the ideal level of a nutrient in the feed and may assist researchers in decision making regarding the diet of animals and also for animal or plant growth studies (SANTANA *et al.*, 2016). However, the parameters estimation of models is generally performed using the classical approach, which makes the process dependent on the sample size.

An alternative to classical methods is the bayesian approach that may be applied in cases of small samples. In this approach, the uncertainty related to the unknown quantities in the model (a prior distribution) are updated using information from the data (likelihood function), resulting in the updating of this uncertainty (a posterior distribution) (BOLSTAD, 2007; GELMAN *et al.*, 2014). Posterior distribution contains the necessary information for statistical inference and represents the existing knowledge about the problem.

The aim of this study was to fit two segmented regression models, one quadratic and one nonlinear, with response plateau using the bayesian approach to zinc deposition data in broilers' tibia, considering addition zinc dosages in the feed to the cases of homogeneity and heterogeneity of variances over the dosages.

2 Material and methods

The data used in this work refer to the Zinc (Zn) content, in ppm, deposited in the tibia of broilers considering different Zinc dosages, in ppm, added in the animal feed and are available in Rezende (2002). The experiment was a completely randomized design with eight repetitions and treatments were arranged in a factorial scheme (9x2) with nine doses of Zn, in ppm, in poultry feed (0, 15, 30, 45, 60, 75, 90, 105, 120 ppm) and the gender of the animals (males and females). The interaction gender vs dose was not significant just as in analysis performed by Rezende (2002), so, we chose to fit in this work disregarding the gender factor, in other words, there are 16 repetitions for each dose applied in the feed. The models considered were the quadratic segmented regression with response plateau (MQP) and the nonlinear segmented regression with response plateau (MNL), given by equations (1) and (2), respectively:

$$y_{ij} = \begin{cases} \alpha + \beta x_j + \gamma x_j^2 + \varepsilon_{ij}, & \text{for } x_j \leq x_0 \\ P + \varepsilon_{ij}, & \text{for } x_j > x_0 \end{cases} \quad (1)$$

$$y_{ij} = \begin{cases} \beta_1 + \exp(-\beta_3(x_j - \beta_2)^2) + \varepsilon_{ij}, & \text{for } x_j \leq x_0 \\ P + \varepsilon_{ij}, & \text{for } x_j > x_0 \end{cases} \quad (2)$$

so that: $j = 1, \dots, 9$; $i = 1, \dots, n$; y_{ij} is the Zn content, in ppm, deposited in the tibia of the i -th broiler that received the feed with j -th dose of Zn; x_j is the j -th dosage of Zn used in the feed, in ppm; α , β and γ are the parameters of the quadratic part of the model (1); β_1 , β_2 and β_3 are the parameters of the nonlinear part of the model (2); x_0 is the value of the Zn dosage added to the feed from which it is considered that there was stabilization in the deposition of the mineral in the animals' tibia (breakpoint); P is the response plateau, which can be interpreted as the maximum Zn content deposited in the tibia and ε_{ij} is the error associated with the fit of the model.

In the MQP model, the parameter x_0 called breakpoint is determined as a function of the parameters of the quadratic part of the model, i.e. $x_0 = -\beta/2\gamma$. In the MNL model, this parameter matches with parameter β_2 of the nonlinear part, as well as, parameter β_1 matches with response plateau P .

In this work, two structures were considered for the residual vector: with homogeneity and heterogeneity of variances over the dosages considered in the experiment.

The parameter estimation process was carried out using the bayesian methodology. The prior distributions considered to the parameters were normal distributions for the parameters α , β , γ , from MQP and β_1 and β_2 from MNL. For the parameter β_3 from MNL, a beta prior distribution was considered, $\beta_3 \sim \text{beta}(\alpha_{\beta_3}, \beta_{\beta_3})$, once the parameter is a rate with values between the interval $(0, 1)$. For the precision parameter τ in the MQP and MNL models, a gamma prior distribution was considered $\tau \sim \text{gamma}(a_\tau, b_\tau)$, with mean and variance being a_τ/b_τ and a_τ/b_τ^2 , respectively. The values of the hyperparameters μ_α , τ_α , μ_β , τ_β , μ_γ , τ_γ , μ_{β_1} , τ_{β_1} , μ_{β_2} , τ_{β_2} e α_{β_3} , β_{β_3} were assigned based on the estimates obtained by Rezende (2007), using the classical methodology. In addition, it was assumed that the errors follow a normal distribution.

These prior distributions combined with the likelihood function, which contains the data information, generated the posterior distributions, which were simulated samples from the parameters using Monte Carlo Markov Chains (MCMC). We used 10000 iterations in the MCMC process to fit the MQP and 200000 iterations to fit the MNL, with burn-in of 50% of first iterations in both models and thin of 10 values for the MQP and 200 values for the MNL, obtaining a posterior sample of 500 values for each parameter.

To check the evidence of convergence of the chains MCMC process, the Geweke test (GEWEKE, 1992) was used. The method consists of considering a function $g(\theta)$ and the simulated values in each iteration $g^i = g^1, g^2, \dots$ with $i = 1, 2, \dots, n$ sufficiently large. From this, the sample is divided into two sequences, with $n_A =$

$0.1n$ the first and $n_B = 0.5n$ the second and the means (\hat{g}_A and \hat{g}_B) and asymptotic variances (\hat{S}_g^A and \hat{S}_g^B) are calculated in each subsample. Then there is a Z-score:

$$Z - score = \frac{\hat{g}_A - \hat{g}_B}{\sqrt{\frac{\hat{S}_g^A}{n_A} + \frac{\hat{S}_g^B}{n_B}}} \sim N(0, 1).$$

If the standardized difference in means is large, there are signs of non-convergence in the chain.

The choice of the best model that fits the data was obtained using the deviance information criterion (DIC) proposed by Spiegelhalter *et al.* (2002), given by:

$$DIC = \hat{D} + 2p_D = \bar{D} + p_D$$

as $p_D = \bar{D} - \hat{D}$, being the posterior mean of the deviance and $\hat{D} = -2 \times \log(p(y|\hat{\theta}))$, being the best model the one that presents the least value of the criterion.

All analyses of this work were performed using the free software R (R CORE TEAM, 2021). In the fitting of the models to the data, the package R2OpenBUGS designed by Sturtz, Ligges e Gelman (2005) was used. To obtain a posterior summary of model parameters and chain convergence test, the coda package proposed by Plummer *et al.* (2006) was used.

3 Results and discussion

The means of the Zn content in the tibia of the animals for each dose of Zn applied in the feed are shown in Table 1 with the respective standard deviations. It can be seen that the standard deviation of the Zn content is quite different at each level of the dose factor, in other words, it may be said that there is heterogeneity of variances. Even so, the models with the response plateau were fitted considering both the homogeneity and heterogeneity of variance.

Table 1 - Means and standard deviations of each dose of Zn in poultry feed

Doses	Mean	Standard deviation
0	207.13	28.73
15	241.20	10.85
30	270.27	14.86
45	275.81	10.18
60	282.31	15.16
75	289.35	14.21
90	295.87	19.07
105	298.61	25.97
120	284.66	35.17

When verifying the evidence of convergence of the chains MCMC process for the model parameters, in Table 2, the result of the Geweke convergence test showed that for the MQP parameters and for the MNL parameters, the test presents Z-scores between the range of -1.96 and 1.96, i.e., there is evidence of convergence of the chains for all parameters. Furthermore, according to Kéry (2010) bayesian estimates are adequate, according to the MC error, when their estimates are small and less than 5% in relation to the SD of each parameter, which can be verified in this work for all parameters.

In Table 2, it can be seen that the fit presented an estimate for the MQP breakpoint with homogeneity of variance of $\hat{x}_0 = 66.89$ ppm, that represents the level of Zn in the feed which the deposition of this nutrient in the animals' tibia would be stabilized. For MNL considering homogeneity of variance, the breakpoint estimate was $\hat{x}_0 = 68.37$ ppm. These estimates for breakpoint are lower than those found by Rezend *et al.* (2007), in MQP, which were $\hat{x}_0 = 71.80$ ppm and similar to the MNL which was $\hat{x}_0 = 68.10$ ppm, fitting the same models with a response plateau for the mean levels of Zn in the tibia considering each dose using a classical approach. This is due to the fact that the authors used the mean of the Zn content in each of the dosages in the fit and not the use of all repetitions of the content as a function of the doses.

Table 2 - Posterior means, standard deviations (SD), a posterior Monte Carlo error (MC), 95% HPD credibility interval and Geweke convergence test (Z), of the parameters of the MQP and MNL models

Model	Parameter	Mean	SD	MC	L.I	L.S	Z
MQP (1 variance)	α	208.05	5.15	0.18	197.40	217.70	1.27
	β	2.51	0.43	0.01	1.75	3.40	-0.78
	γ	-0.0196	0.0062	0.0002	-0.0321	-0.0089	0.60
	x_0	66.89	9.72	0.29	48.99	86.77	0.20
	P	290.23	2.80	0.05	284.80	295.70	-0.82
MNL (1 variance)	$\beta_1 = P$	290.68	2.67	0.05	285.5	295.9	1.01
	$\beta_2 = x_0$	68.37	8.25	0.18	53.49	84.77	0.64
	β_3	0.00007	0.00002	0.00007	0.00004	0.00011	-0.46
MQP (9 variances)	α	211.88	5.71	0.33	200.30	222.80	-0.83
	β	2.19	0.39	0.02	1.46	2.96	0.68
	γ	-0.0160	0.0052	0.0003	-0.0259	-0.0071	-0.60
	x_0	73.26	10.43	0.54	52.99	93.46	-0.35
	P	290.12	3.28	0.11	284.00	296.70	0.45
MNL (9 variances)	$\beta_1 = P$	290.56	3.06	0.07	284.60	296.50	0.06
	$\beta_2 = x_0$	74.03	8.92	0.25	56.93	92.00	0.07
	β_3	0.000057	0.00002	0.00002	0.00003	0.0001	0.02

The Zn levels found in the fit of this study were also lower than those found by Gomes *et al.* (2009), who used quadratic regression models to estimate the adequate level of Zn in the feed of male and female chickens. The authors determined that

ideal Zn requirement for feed would be 82.20 ppm for males and 85.70 ppm for females.

For the response plateau (P) (Table 2), the fit of the MQP model, in the case in which homogeneity of variance was considered, resulted in an estimate of the mean Zn content found in the broilers' tibia of $\hat{P} = 290.23$ ppm and was higher to that found by Rezende *et al.* (2007), which was 281.1 ppm using the same model for the mean Zn content in each dose of the same experiment. This would be the maximum deposition of Zn in the broilers' tibia considering the ideal level of Zn in their feed. In MNL, the stabilization in mineral deposition was estimated at $\hat{P} = 290.68$ ppm, which was very close to that obtained by the fit of the MQP model.

In cases where heterogeneity of variances was considered (Table 2), the breakpoint showed a posterior mean of $\hat{x}_0 = 73.26$ ppm for MQP and $\hat{x}_0 = 74.03$ ppm when considering the MNL, in others word, very close estimates in both fitted models and higher those obtained in the fit of MQP and MNL considering one variance. For these obtained values of adequate dosage of zinc in the feed of the broilers, a deposition of zinc in the tibia of the animals of $\hat{P} = 290.12$ ppm and $\hat{P} = 290.60$ ppm was obtained, respectively, for the MQP and MNL models. In addition, with respect to the ideal dosage in the feed, the MNL showed a lower HPD credibility interval than in the MQP model with a 95% credibility level (Figure 1).

According to Rostagno *et al.* (2017), the level of Zn supplementation for the initial period of the broilers is 68.72 ppm in the feed, i.e., the fit here presented have estimates close to those proposed by Rostagno *et al.* (2017). Moreover, Trevisol (2019) estimated a supplementation of 66.36 ppm of Zn in the total broiler diet, a value also close to that estimated in this study. Regarding the concentration of Zn in the broilers' tibia, Trevisol (2019) found an average concentration of Zn of 357.57 ppm in broilers in the initial period, being higher than those estimated in the models fitted in this study.

Furthermore, comparing the fitted models using the deviance information criterion (DIC), the MQP, considering homogeneity of variance, was DIC=1240. The MNL also considering homogeneity of variance, obtained the same value DIC=1240. In the case in which heterogeneity of variances was considered, the MQP obtained DIC=1211 and the MNL DIC=1212, i.e., similar values. Thus, according to the DIC criterion, both models in which heterogeneity of variances was considered are more suitable for fitting to the zinc deposition data in the broiler's tibia, as they had lower DIC values. Rezende *et al.* (2007) concluded that MQP and MNL obtained satisfactory fits, but recommend MNL, because it has directly the breakpoint and the response plateau.

The DIC result corroborates with the residual graphs shown in Figure 2, i.e., the models in which heterogeneity of variances were considered had the best fit.

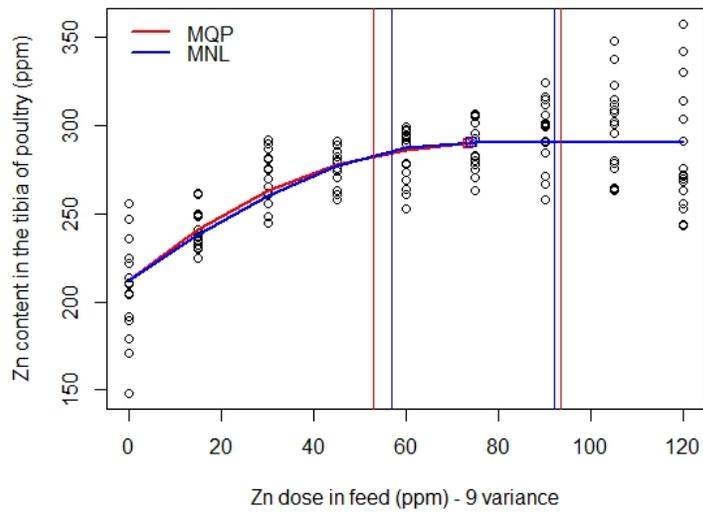
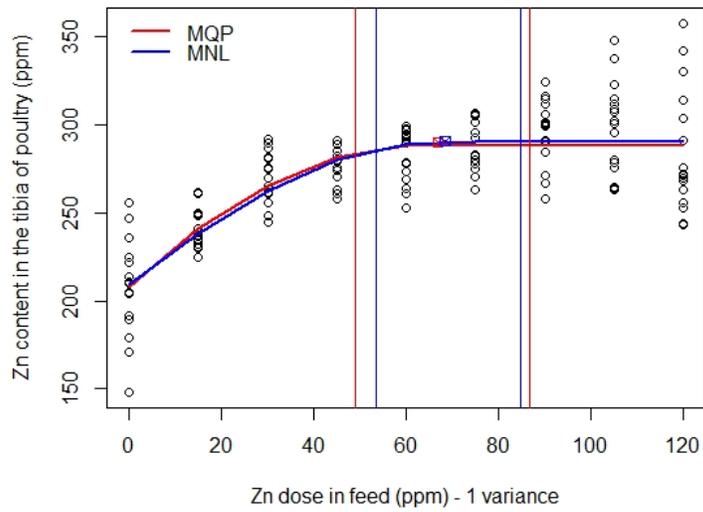


Figure 1 - Fits of MQP and MNL models, in which homogeneity and heterogeneity of variances were considered.

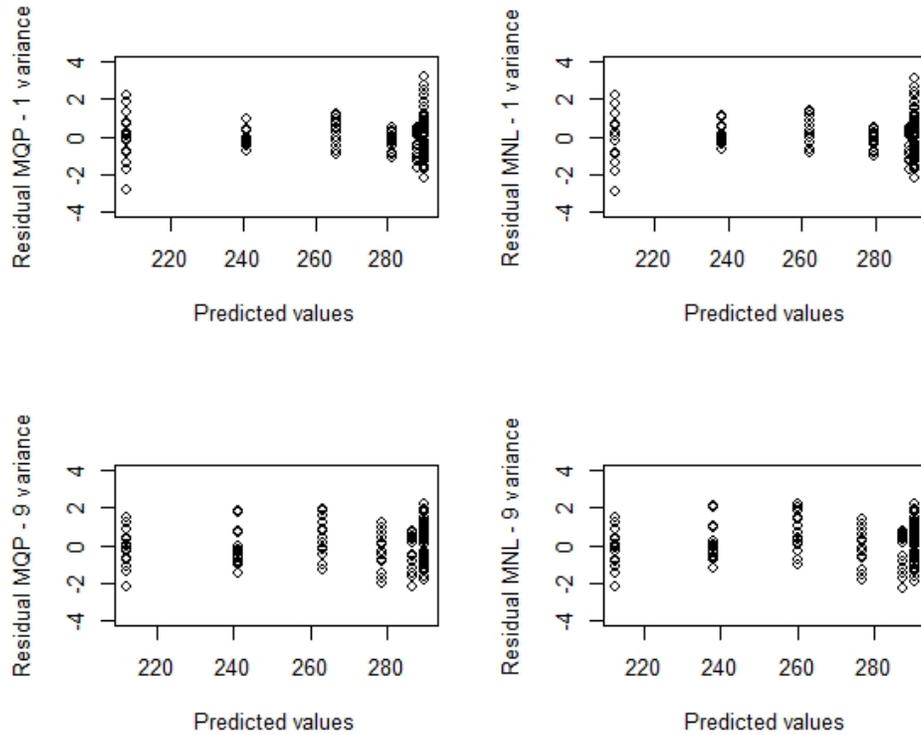


Figure 2 - Standardized residuals x predicted values by the MQP and MNL models.

Conclusions

The bayesian methodology proved to be appropriate for the fit of the quadratic segmented regression model with response plateau and the nonlinear segmented regression model with response plateau to the zinc content data in the broilers' tibia, considering homogeneity and heterogeneity of residual variances over dose factor levels.

Considering the heterogeneity of variances showed a better fit of the models, consequently, better estimates to parameters. For this case, the fit suggests dosages of 73.26 ppm and 74.03 ppm for the MQP and MNL models, respectively.

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- **RESUMO:** Os estudos de exigências nutricionais de animais são importantes, dentre outros motivos, para se ter o conhecimento adequado dos níveis essenciais de minerais adicionados em rações, e assim, produzi-la de forma eficiente, evitar eliminação de minerais pelas fezes e urina e impedir a intoxicação do animal por excesso de determinado nutriente. Os dados utilizados para o estudo são referentes ao teor de Zinco (Zn), em ppm, na tibia dos frangos de corte que receberam uma mesma ração com nove dosagens diferentes de zinco. Foram ajustados os modelos de regressão segmentada quadrática com platô de resposta e o modelo de regressão segmentada não linear com platô de resposta, utilizando uma abordagem bayesiana e considerando casos de homogeneidade e heterogeneidade de variâncias. Os modelos ajustados considerando heterogeneidade de variâncias foram mais adequados para os dados, de acordo com o critério de ajuste DIC.
- **PALAVRAS-CHAVE:** Modelos segmentados; platô de resposta; zinco.

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