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МАТЕМАТИЧЕСКОЕ МОДЕЛИРОВАНИЕ УРОВНЯ МАРГАНЦА В МЫШЕЧНОЙ ТКАНИ КРУПНОГО РОГАТОГО СКОТА

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Представлены результаты определения наиболее эффективной модели прогноза уровня марганца в мышечной ткани герефордского скота для прижизненной оценки элементного статуса животных малоинвазивными методами. Эксперимент проведен с помощью гематологического и биохимического исследования крови и атомно-абсорбционного анализа мышечной ткани крупного рогатого скота. Полученные данные использованы для подгонки регрессионной модели методом наименьших квадратов. Для анализа отобраны пробы скелетной мускулатуры массой 100 г с диафрагмальной мышцы от герефордского скота, разводимого в южной части Западной Сибири в условиях промышленного комплекса. Оценку концентрации марганца в тканях осуществляли методом атомно-абсорбционного анализа на спектрометре МГА-1000. Определение содержания эритроцитов, лейкоцитов и гемоглобина проводили на автоматическом гематологическом анализаторе PCE-90VET. Уровень протеина, альбуминов, глобулинов, мочевины, мочевой кислоты и холестерина определяли фотометрическими методами на полуавтоматическом биохимическом анализаторе Photometer-5010. Расчет эффектов регрессионных моделей осуществляли методом наименьших квадратов. Селекция лучшей модели по эффективности и точности оценки модели базировалась на комплексной оценке значений внутренних и внешних критериев качества. Между зависимой и независимыми переменными выявлены статистически значимые ассоциации ($p < 0{,}05$). Внутри пула предикторов отмечена скоррелированность ($p < 0{,}05$). В результате подгонки моделей получено оптимальное регрессионное уравнение, включающее два показателя (скорость оседания эритроцитов и уровень глобулинов), для прогноза уровня марганца в мышечной ткани крупного рогатого скота. Между главными эффектами модели отсутствуют признаки мультиколлинеарности, что подтверждает значения фактора инфляции дисперсии – 1,2. Полученная модель удовлетворяет необходимым допущениям в отношении остатков. Распределения остатков модели входят в доверительные интервалы кривой нормального распределения. Коэффициент автокорреляции был равен 0,039 ($p > 0{,}05$), что указывает на независимость остатков. Полученная модель может быть использована для прижизненной оценки концентрации марганца в мышечной ткани крупного рогатого скота.

Ключевые слова: марганец, крупный рогатый скот, герефордская порода, прогнозирование, регрессия

MATHEMATICAL MODELING OF THE MANGANESE LEVEL IN THE MUSCLE TISSUE OF CATTLE

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The results of determining the most effective model for predicting the level of manganese in the muscle tissue of Hereford cattle for in vivo assessment of the elemental status of animals by low invasive methods are presented. The experiment was carried out using hematological and biochemical blood tests and atomic absorption analysis of the muscle tissue of cattle. The data obtained are used to fit the regression model using the least square method. Skeletal muscle samples weighing 100 g from the diaphragm muscle of the Hereford cattle bred in the southern part of Western Siberia in the conditions of industrial complex were taken for analysis. Manganese concentration in tissues was assessed by atomic absorption analysis on an MGA-1000 spectrometer. The content of erythrocytes, leukocytes, and hemoglobin was determined on an automatic hematology analyzer

PCE-90VET. Protein, albumin, globulin, urea, uric acid, and cholesterol levels were determined by photometric methods on a Photometer-5010 semi-automatic biochemical analyzer. The effects of regression models were calculated using the least square method. Selection of the best model for efficiency and accuracy of model estimation was based on a comprehensive assessment of the values of internal and external quality criteria. Statistically significant associations ($p < 0.05$) were found between the dependent and independent variables. Within the pool of predictors, correlation ($p < 0.05$) was observed. As a result of model fitting, an optimal regression equation including two indicators (erythrocyte sedimentation rate and globulin level) for predicting manganese levels in bovine muscle tissue was obtained. There are no signs of multicollinearity between the main effects of the model, which confirms the values of the variance inflation factor - 1.2. The resulting model satisfies the necessary assumptions about the residuals. The distributions of the model residuals fall within the confidence intervals of the normal distribution curve. The autocorrelation coefficient was 0.039 ($p > 0.05$), indicating the independence of the residuals. The resulting model can be used for in vivo assessment of manganese concentration in bovine muscle tissue.

Keywords: manganese, cattle, Hereford breed, prediction, regression

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INTRODUCTION

Manganese is an essential micronutrient, with a requirement of 20 mg of manganese/kg of feed for beef cattle [1–3]. Deficiency of this element results in disturbances in carbohydrate and lipid metabolism, growth retardation, dermatitis, impaired synthesis of bone tissue and the reproductive system [4, 5]. It has been established that the key enzymes sensitive to manganese deficiency in the diet are glycosyltransferases and xylosyltransferases (enzymes activated by manganese and involved in the synthesis of proteoglycans and, consequently, in bone formation), as well as arginase and mitochondrial superoxide dismutase (manganese metalloenzymes) [6–9]. Decreased fertility due to manganese deficiency is a result of impaired synthesis of cholesterol and related compounds (ergosterol, ergosteryl sitosterol, stigmasterol, etc.) necessary for the synthesis of sex hormones and other steroids [10–12]. Replenishing manganese deficiency in ruminant animals can be achieved through the use of multicomponent mineral feed supplements [13, 14]. As a preventive measure, manganese-containing additives are used when there is only unconfirmed suspicion of micronutrient deficiency [15].

The elemental status is associated with biochemical processes occurring in the organism [16–18]. Identifying these patterns will allow for the development of the methods for assessing the levels of micronutrients in animal tissues during their lifetime. Hematological and biochemical blood parameters can serve as predictors and biomarkers of manganese levels.

The purpose of this study was to identify the most effective predictive model for manganese levels in the muscle tissue of the Hereford cattle, which would allow for non-invasive assessment of the animals' elemental status during their lifetime.

The research objectives were achieved through hematological and biochemical analysis of blood samples and atomic absorption analysis of muscle tissue in beef cattle. The obtained data were used to fit the regression models using the least squares method.

MATERIAL AND METHODS

Samples of skeletal muscle (*n* = 22) were taken from the diaphragm muscle of the Hereford cattle bred in the southern part of Western Siberia. The animals were kept under standard conditions of an industrial complex, complying with veterinary and zootechnical requirements (GOST 32855-2014, GOST 26090-84, GOST R 52254-2004). At the time of slaughter, the animals were clinically healthy. Blood samples were taken from the jugular vein of the animals and stabilized with 5% sodium citrate. Analysis of the muscle tissue samples was performed according to GOST R 55484-2013 using an electrothermal atomic absorption spectrometer MGA-1000. Hemoglobin level, red blood cell count, and leukocyte count were determined using a PCE-90VET hematological analyzer. Biochemical analysis of serum was conducted using a Photometer-5010 biochemical analyzer.

Statistical analysis of the raw data was performed in the R environment. Model fitting was done using the least squares method according to the exploratory data analysis protocol [19]. The Shapiro-Wilk test was used to assess the normality of residual distributions. Spearman's rank correlation coefficient was calculated to assess the correlation between model variables. The presence of multicollinearity in the parameters of the candidate models was assessed based on the variance inflation factor values and visual inspection of the scatterplot matrix. Influential observations in the model residuals were identified using the Grubbs' test. Multiple comparisons of influential observations in the model residuals were conducted using Bonferroni correction. The Durbin-Watson test was used to assess the independence of the model residuals.

For convenience of analysis and description, the names of the original variables have been changed (see Table 1).

Табл. 1. Обозначение и расшифровка для комплекса независимых переменных, используемых для селекции регрессионных моделей

Table 1. Designation and interpretation for the complex of independent variables used for the selection of regression models

Indicator	Unit of measurement	Variable in the model
Fe level in the blood	mmol/L	*x*1
Leukocytes	$\times 10^9$ units	*x*2
Erythrocytes	$\times 10^{12}$ units	*x*3
Hemoglobin	g/l	*x*4
ESR	мм/ч	*x*5
Blood color index	Ratio	*x*6
Protein	g/l	*x*7
Albumin	g/l	*x*8
Globulin	g/l	*x*9
Urea	mmol/L	*x*10
Uric acid	μmol/L	*x*11
Cholesterol	mmol/L	*x*12

RESULTS AND DISCUSSION

In order to fit the regression models using the least squares method, it is necessary to assess the correlation between the predictors. When multicollinearity is detected among the model parameters, the coefficient values will be unstable, and the analysis of the contribution of each effect to the variance of the dependent variable will be difficult. Therefore, Spearman correlation coefficients were calculated to assess the associations between the variables (see Figure 1), and scatter plots were constructed (see Figure 2).

Four statistically significant correlations were found between the dependent and independent variables: hemoglobin, blood color index, albumin, and erythrocyte sedimentation rate (ESR). Hemoglobin concentration is associated with albumin and blood color index values, while ESR level is not related to these variables. In the selection of the models, it is necessary to choose a set of predictors that will allow for the calculation of the most effective and compact model, where the parameters do not duplicate each other's influence on the variance of the dependent variable.

Selection of the candidate models was based

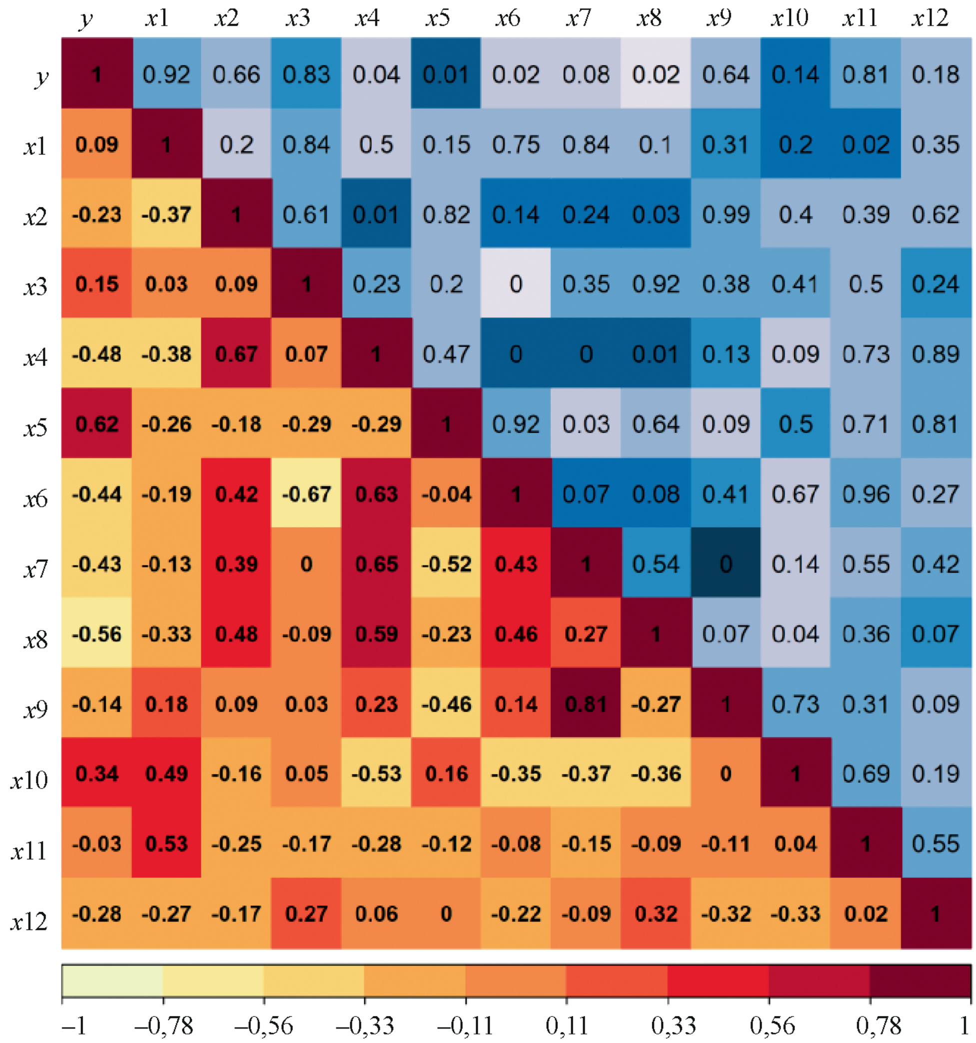

Рис. 1. Корреляционная матрица (на красном фоне значения коэффициентов корреляции, на синем – их уровней значимости)

Fig. 1. Correlation matrix (on the red background are the values of the correlation coefficients, on the blue background are their significance levels)

on the assessments of internal quality criteria, including the Akaike information criterion (AIC), adjusted coefficient of determination (R2 adj), Mallow's criterion (Cp), and Bayesian information criterion (BIC). Based on the evaluations of the first two quality criteria, a model with four predictors was constructed (see Table 2). By considering the latter two criteria, a more compact model with two predictors was obtained (see Figure 3).

The evaluation of the obtained models and their effects is presented in Tables 3 and 4. Although the estimation of the residual standard deviation was slightly lower for the model with four predictors, only one coefficient (x9) was statistically significant, even though the value of the F-statistic was below the critical value, allowing us to reject the hypothesis of all coefficients being equal to zero (see Table 3). On the other hand, the model with two independent variables had a higher F-statistic value, leading to an improvement in the level of significance by a factor of two compared to the previous model (see Table 4).

Calculation of the variance inflation factor for the full model and candidate models is presented in Table 5. It is evident that the candidate models do not exhibit multicollinearity among the predictors, unlike the full model, where more than half of the coefficients are highly correlated.

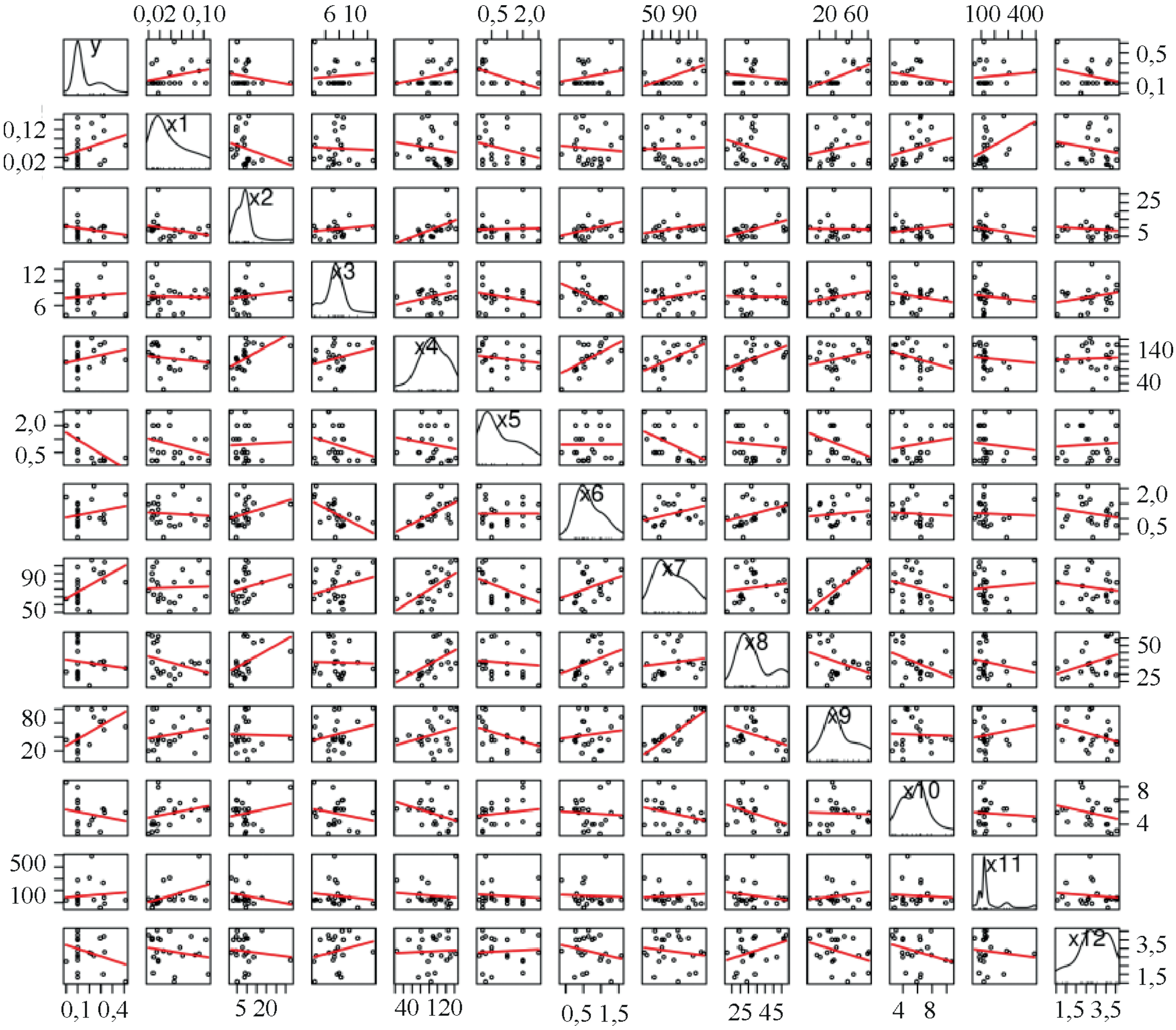

Рис. 2. Матрица диаграмм рассеяния параметров регрессионных моделей

Fig. 2. Matrix of scatterplots of regression models parameters

Табл. 2. Внутренние критерии оценки качества моделей-кандидатов прогноза уровня марганца в мышечной ткани, мг/кг

Table 2. Internal criteria for assessing the quality of candidate models for predicting the level of manganese in muscle tissue, mg/kg

Model formula	*df*	*p*	SSE	MSE	R^2	R^2_{adj}	AIC	BIC
$y \sim 1 + x1 + x3 + x9 + x10$	17	4	0,079	0,005	0,573	0,472	–49,358	–37,242
$y \sim 1 + x5 + x9$	19	2	0,097	0,005	0,478	0,423	–48,932	–38,998

Henceforward: SSE – square sum error; MSE – mean square error; R^2 – coefficient of determination; R^2_{adj} – adjusted coefficient of determination (ACD); AIC – Akaike information criterion; BIC – Bayesian information criterion.

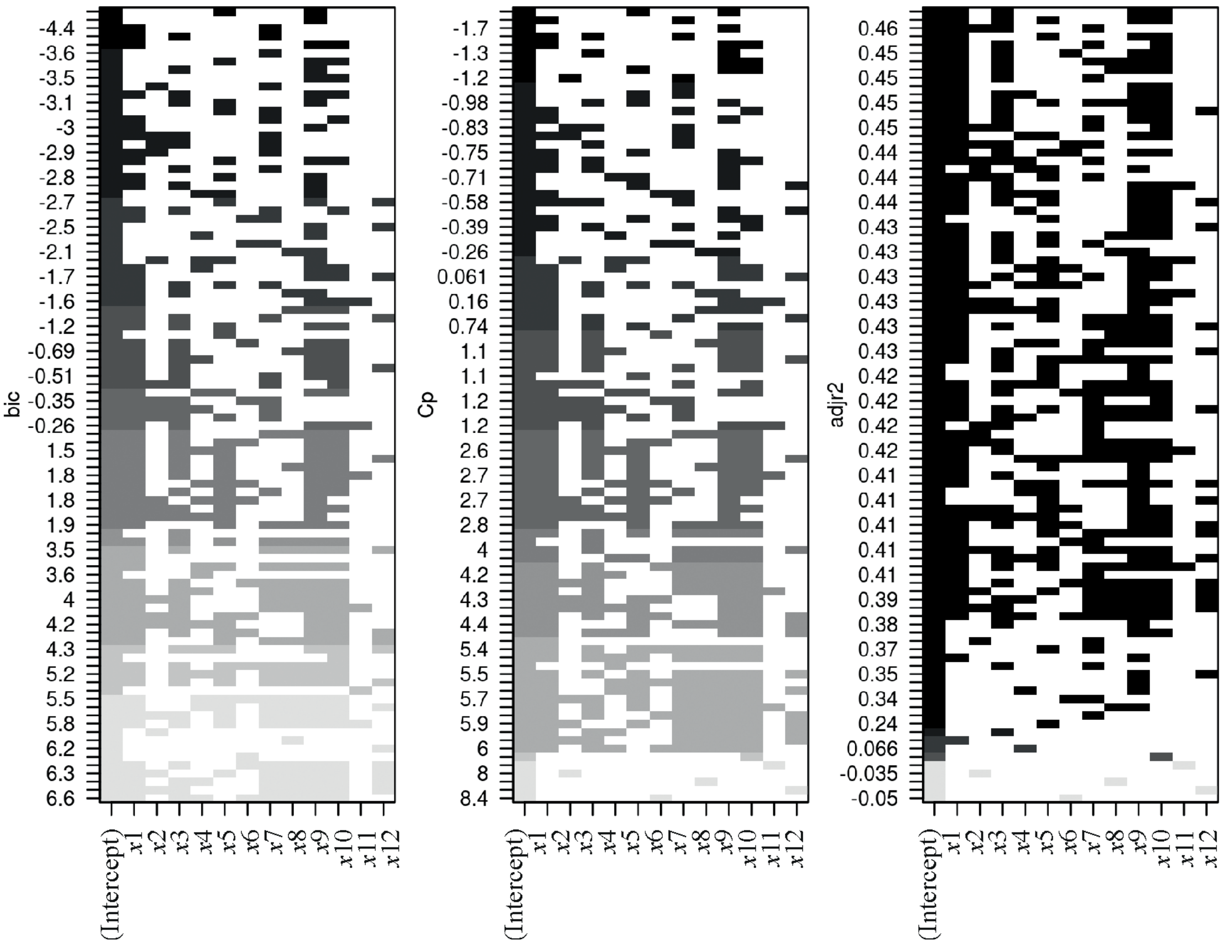

Рис. 3. Ранжирование моделей (слева направо) по BIC, критерию Мэллоу и R^2_{adj}

Fig. 3. Ranking of models (from left to right) according to the BIC, the Mallow criterion and R^2_{adj}

The choice of the best model was based on an external quality criterion for regression models – cross-validation. The visualization of cross-validation with the original dataset divided into 3 blocks is presented in Figure 4. The left graph shows that one of the regression lines deviates significantly from the overall trend, unlike the model with two predictors (on the right).

The forecast quality of the candidate models can be assessed using the unbiased value of the coefficient of determination and the mean square for each of the candidate models (see Table 6). The obtained data indicate a significant superiority of the compact model, as it provides a significantly more accurate forecast and explains the variance of manganese levels in muscle tissue nearly three times better than the closest competing model.

Thus, as a result of the selection, it can be concluded that the best model for predicting the level of manganese in cattle muscle tissue contains two predictors (x5 and x9).

To consider the selected model sufficiently effective for forecasting, it is necessary to check the assumptions regarding its residuals. Formal tests, such as the Anderson-Darling test ($A = 0.5$; $p = 0.2$) and the Shapiro-Wilk test ($W = 0.9$; $p = 0.2$), indicate the conformity of the residual distribution to normality. The visualization of the probability density values of the residuals falls within the confidence interval for the normal distribution curve (see Figure 5).

The plot of standardized residual quantiles and theoretically expected quantiles also shows that their values are normally distributed (see Figure 6, top-left plot).

The top-left and bottom plots indicate the relative homogeneity of residual variances (see Figure 6). The detection of the potentially influential observations using Cook's distance is

Табл. 3. Параметры оценки коэффициентов модели-претендента прогноза уровня марганца в мышечной ткани от показателей крови, мг/кг

Table 3. Parameters for estimating the coefficients of the candidate model for predicting the level of manganese in the muscle tissue from blood parameters, mg/kg

Coefficient designation	Coefficient estimates	Standard errors of the coefficients	*t*-statistics	p_t
Integer[1]	0,090	0,084	1,115	0,280
*x*1	0,889	0,463	1,919	0,072
*x*3	0,011	0,009	1,323	0,203
*x*9	0,002	0,001	2,759	0,013
*x*10	–0,013	0,007	–1,738	0,100

Note. RSE = 0,068, *F*-statistic = 5,7, *p* = 0,004.

[1]Absolute term of an equation.

Табл. 4. Параметры оценки коэффициентов модели-претендента прогноза уровня марганца в мышечной ткани от показателей крови, мг/кг

Table 4. Parameters for estimating the coefficients of the candidate model for predicting the level of manganese in the muscle tissue from blood parameters, mg/kg

Coefficient designation	Coefficient estimates	Standard errors of the coefficients	*t*- statistics	p_t
Integer[1]	0,200	0,050	3,983	0,001
*x*5	–0,052	0,028	–1,873	0,077
*x*9	0,002	0,001	2,711	0,014

Note. RSE = 0,71, *F*-statistic = 8,7, *p* = 0,002.

[1]Absolute term of an equation.

Табл. 5. Значения фактора инфляции дисперсии для коэффициентов регрессионных моделей оценки уровня марганца в мышечной ткани

Table 5. Values of the dispersion inflation factor for the coefficients of regression models for estimating the level of manganese in the muscle tissue

Predictor	Full model	$y \sim x1 + x3 + x9 + x10$	$y \sim x3 + x5$
*x*1	2,1	1,1	–
*x*2	4,2	–	–
*x*3	24	1,1	1,2
*x*4	33,8	–	–
*x*5	2,3	–	1,2
*x*6	33,3	–	–
*x*7	595,9	–	–
*x*8	162,6	–	–
*x*9	658,6	1,2	–
*x*10	3,3	1,1	–
*x*11	1,8	–	–
*x*12	1,8	–	–

shown in the bottom-right plot of Figure 6. The ordinal numbers of three observations with high influence potential are displayed. However, visually, these instances align with the model on the previous graphs of Figure 6. A formal check of the most influential observation's conformity to the overall population was conducted with Bonferroni correction. In the selected model, the maximum value of the studentized residual was 2.52 and corresponded to an adjusted significance level of 0.47. The obtained results indicate that this maximum value does not differ from the other observations in the population. To test the hypothesis of independence of the residuals, the autocorrelation coefficient was calculated as 0.039 ($p = 0.32$), confirming the null hypothesis of independence of the residuals in the model.

Thus, to forecast the level of manganese in Hereford cattle muscle tissue, it is necessary to determine the globulin concentration and erythrocyte sedimentation rate and build a regression equation based on the obtained data:

$$y = 0.2 - 0.052 \times \mathrm{ESR} + 0.002 \times \mathrm{G},$$

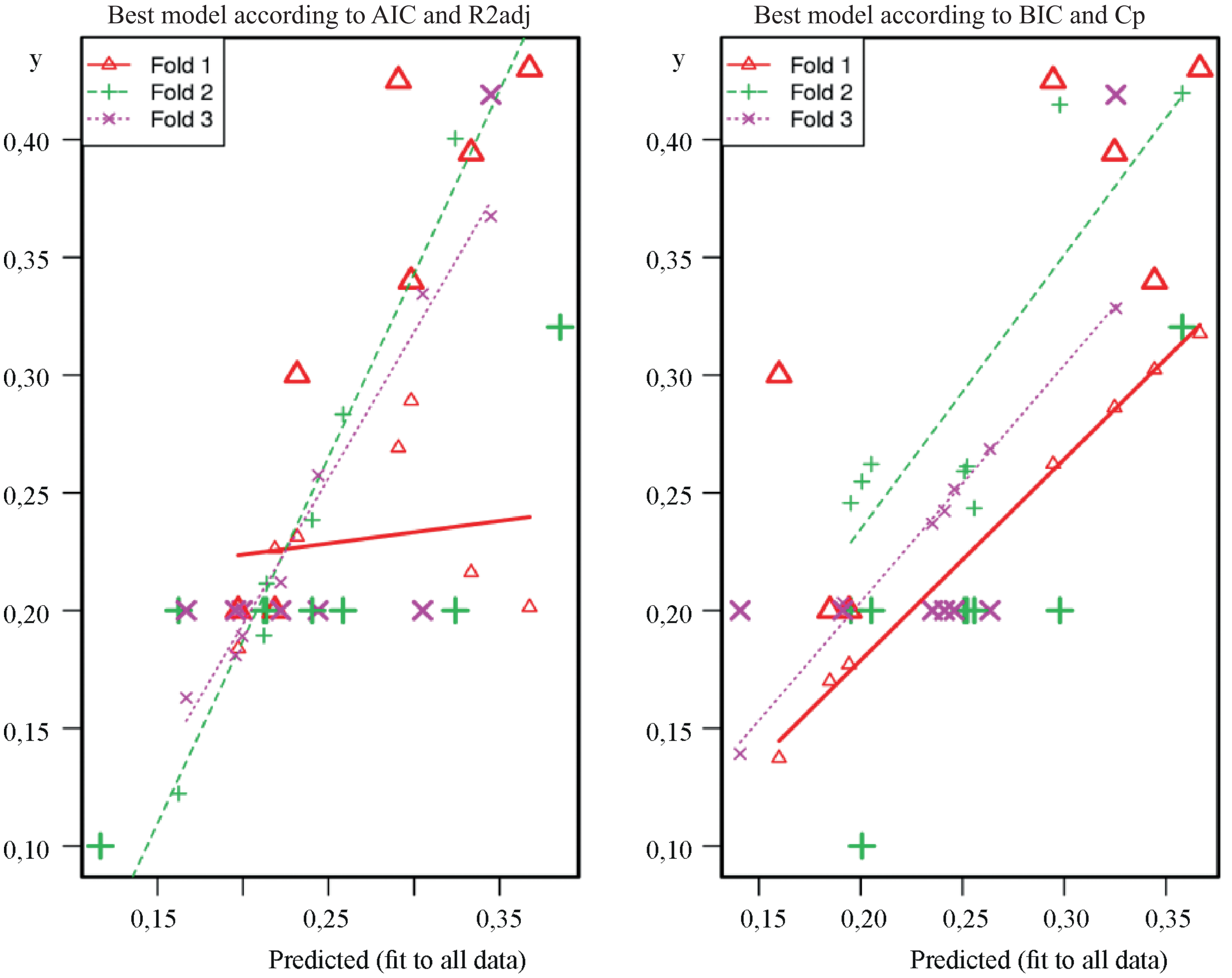

Рис. 4. Визуализация моделей-претендентов методом кросс-валидации с разбитием на 3 блока

Fig. 4. Visualization of candidate models for assessing by the cross-validation method divided into 3 blocks

Табл. 6. Кросс-валидация оценок регрессионных моделей прогноза уровня марганца в мышечной ткани

Table 6. Cross-validation of estimates of regression models for predicting the level of manganese in the muscle tissue

Model formula	SSE	*df*	MS	R^2	Cross-validation R^2
$y \sim 1 + x5 + x9$	0,2200	22	0,00996	0,478	0,227
$y \sim 1 + x1 + x3 + x9 + x10$	0,2242	22	0,01019	0,573	0,0826

where *y* is the manganese concentration in muscle tissue (mg/kg), ESR is the erythrocyte sedimentation rate (mm/h), and G is the globulin level (g/L).

Although the obtained results have demonstrated a sufficient level of statistical significance for the model and its coefficients, as well as the absence of outliers, this model requires training on new data as the precision of the forecast for manganese levels in muscle tissue will have wide confidence intervals with the available data. Therefore, additional data is needed to improve the model.

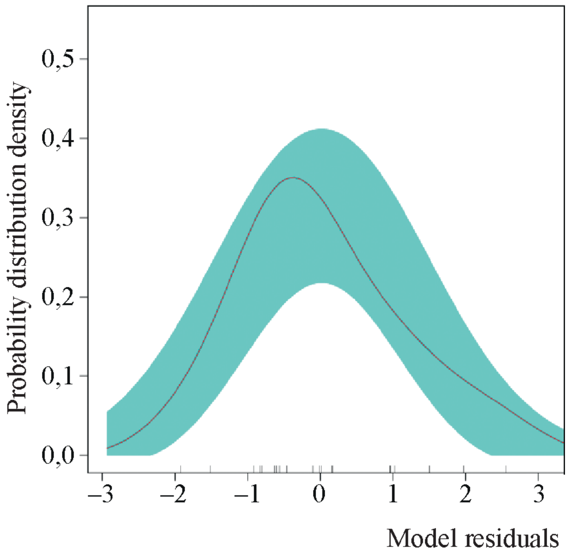

Рис. 5. Распределение остатков регрессионной модели оценки уровня марганца в мышечной ткани, мг/кг

Fig. 5. Residual distribution of the regression model for estimating the level of manganese in the muscle tissue, mg/kg

CONCLUSION

The obtained model can be used for the in vivo assessment of manganese levels in the Hereford cattle muscle tissue. The data obtained can be utilized for the purpose of ecological monitoring of elemental load on animals. The application of this method will enable the timely detection of Mn imbalances in muscle tissue and, through changes in diets, reduce or increase the concentration of this metal. Further training of the model is necessary to find a broader set of predictors to enhance the accuracy of the dependent variable estimates.

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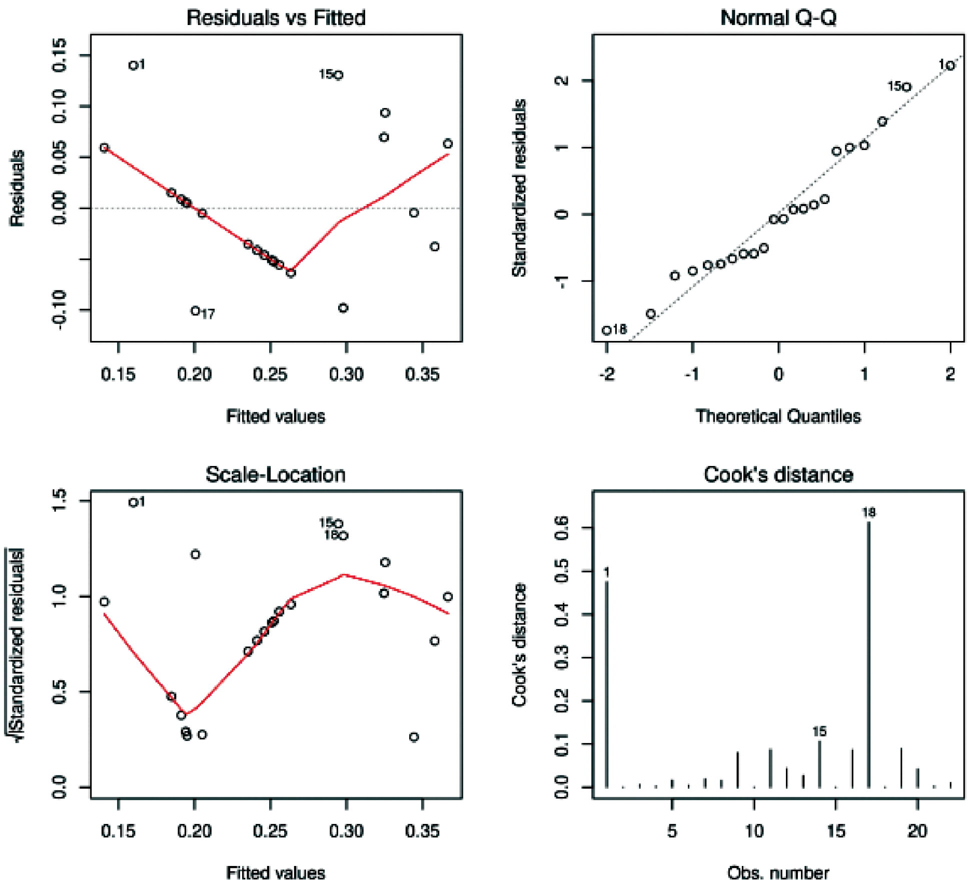

Рис. 6. Слева направо: остатки в зависимости от отклика, график квантилей, квадратный корень стандартизированных остатков в зависимости от отклика и дистанций Кука

Fig. 6. From left to right: residuals versus response, quantile plot, square root of standardized residuals versus response, and Cook's distances

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