

## CASE STUDY

## Prediction models of iron level in beef muscle tissue toward ecological well-being

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## ABSTRACT

**BACKGROUND AND OBJECTIVES:** Elemental status is associated with the biochemical processes occurring in the body. Beef, consumed worldwide, is an excellent source of iron in terms of quantity and bioavailability, providing up to 18 percent of the daily requirement. The level of iron in muscle tissue affects beef quality. Current methods used to assess iron content in cattle muscles are laborious and complex. Accordingly, the current study aimed to develop a fast and simple method to assess the elemental status of animals in vivo and in a minimally invasive way based on an effective model for iron-level prediction by using blood-analysis results toward ecological well-being. This method can overcome the shortcomings of currently used approaches.

**METHODS:** Samples of diaphragmatic muscle weighing 100 grams, as well as blood samples, were obtained from Hereford cattle bred under typical conditions of an industrial complex in the south of Western Siberia, Russia. Elemental analysis was performed by atomic absorption method with electrothermal atomization. Regression analysis was conducted to estimate the relationships between iron level in the muscle tissue of Hereford cattle and independent values (blood parameters). An optimum model for predicting the iron level was established. The coefficients of regression models were calculated using the least squares method, and the values of the dependent variable corresponded with the Gaussian ones. A high correlation existed between independent variables.

**FINDINGS:** An optimum model for predicting the iron level in the muscle tissue of Hereford cattle was established. It contained three predictors, namely, number of erythrocytes, color index, and globulin, as a result of selection based on internal and external-quality criteria. The model meets the necessary assumptions: the residuals are normally distributed, no autocorrelations exist, and the observations are influential. Furthermore, no signs of multicollinearity exist between the main effects of the model (variance-inflation factor = 1.2–1.7).

**CONCLUSION:** The model can be used for the intravital analysis of iron level in the muscle tissue of cattle. In contrast to currently used methods, the approach proposed can be used for intravital analysis of the level of iron in muscle tissue, which is the most important advantage of the developed approach. The results can be used in ecology to assess ecological well-being and determine the allowable load of iron in animals. For veterinary medicine, the resulting model enables the evaluation of the iron level in the muscle tissue of Hereford cattle during their lifetime. Studying the effect of different factors on meat quality may allow to decrease or avoid useless measures used in farming, such as the excessive use of feed additives. In turn, these measures can decrease resource exploitation and increase farming productivity. Therefore, the results can guide the further development of sustainable farming.

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## INTRODUCTION

In the body of adult animals, the average concentration of iron (Fe) is 0.005 percent (%) –0.006% in natural humidity. Most iron is in the form of organic compounds that can be divided into two groups: porphyrin (heme iron) (70%–75%) and nonheme iron (25%–30%). Hemic iron is represented by hemoglobin, myoglobin, and heme-containing enzymes (cytochromes, cytochrome oxidase, catalase, and peroxidase). Nonheme iron comprises transferrin, ferritin, hemosiderin, and some iron proteinates, including ferroflavoprotein (Diniz *et al.*, 2019). This element involves many biochemical processes: oxygen transport, blood production, energy metabolism, immune functions, and many others (Diniz *et al.*, 2016; Kupczyński *et al.*, 2017). According to a meta-analysis (Institute of Medicine, 2001), the median dietary intake of iron is approximately 16–18 milligrams per day (mg/day) for men and 12 mg/day for women, whereas the tolerable upper intake level for adults is 45 mg/day. Excessive intake of iron has a harmful effect and causes symptoms of poisoning, despite its crucial role in living organisms (Institute of Medicine, 2001; Khaleghnia *et al.*, 2021; Garmyn *et al.*, 2011). Excessive iron has various adverse effects, including gastrointestinal and cardiovascular diseases caused by oxidative stress (Institute of Medicine, 2001; Geissler and Singh, 2011). Moreover, iron content in food usually does not exceed 5 milligrams per 100 grams (mg/100 g) (Table 1), so the problem

owing to excessive iron consumption with food is not relevant in most cases. Different types of meat are the main source of iron for human population. Thus, the content of iron (and other elements) in different types of meat, as well as other types of food, should be controlled to solve several tasks. These tasks include regulating human diet, estimating the effect of different factors on iron content in food, and avoiding excess of iron and other metals in food (Table 1).

### Iron content in beef

Beef, consumed worldwide, is an excellent source of iron in terms of quantity and bioavailability because iron content in beef exceeds that in other types of meat, providing up to 18% of the daily requirement (Valenzuela *et al.*, 2009; Duan *et al.*, 2012; Mateescu, 2014). Recent studies have shown that muscle iron content can affect beef-quality parameters. Iron content in meat including beef is associated with flavor and juiciness (Mateescu *et al.*, 2013a), meat structure (Kim *et al.*, 2010), palatability (Garmyn *et al.*, 2011), red color intensity, and lipid oxidation (Purohit *et al.*, 2015). Monounsaturated fatty acid levels are positively associated with semitendinosus iron content in crossbreeds of beef cattle, whereas cholesterol and polyunsaturated fatty acid levels are negatively correlated (Ahlberg *et al.*, 2014). Molecular mechanisms of the effect of iron and other mineral contents on different gene expression,

Table 1: Summary of the studies on iron content in different type of food

Subject of the study	Summary	Reference
Raw and cooked red	The effect of different factors on the analysis of iron accuracy is studied.	Lombardi-Boccia <i>et al.</i> , 2009
Beef meat and viscera	Bovine cuts of meat have a low variation in total Fe, and heme Fe comprises more than 60% of the total Fe.	Valenzuela <i>et al.</i> , 2009
Soil, pasture sward, and blood plasma of extensive reared bulls	No relationships exist among iron soil, forage, and blood concentration in beef cattle.	Pavlik <i>et al.</i> , 2013
Rice, wheat, or corn-containing products.	A novel method for iron extraction and determination is proposed.	Niedzielski <i>et al.</i> , 2014
Fish, shrimp, and prawn.	A modified heme-iron-extraction method has been proposed. It reveals the underestimation of previous analyses of iron content in seafood.	Wheal, <i>et al.</i> , 2016
Calf blood	The effects of feeding protein–iron complex on productive performance and indicators of calf metabolism are studied. Low doses of iron in diet positively affect calf metabolism parameters.	Kupczyński <i>et al.</i> , 2017
Paste from poultry and cattle bone	The potential role of bone paste as a source of minerals in the meat industry is demonstrated.	Kakimov <i>et al.</i> , 2021
Beef	Metal content (including iron) in beef samples from different locations is studied.	Sabow <i>et al.</i> , 2021
Reindeer meat	A meta-analysis of reindeer meat samples reveals significant differences in iron content among meat from different regions	Andronov <i>et al.</i> , 2022

which in turn affect meat quality, are considered in previous works (Diniz *et al.*, 2019; Mateescu *et al.*, 2013b). Iron may affect the expression of various genes through different mechanisms. Conversely, some genes may affect iron content in muscle tissues, which may be used to regulate iron content in meat in the future (Mateescu *et al.*, 2013b). Thus, iron content is one of the key characteristics affecting meat quality. The technique for assessing the interior by the elemental status of animals by blood parameters has yet to become widespread in agricultural production, even though the elemental status is associated with the biochemical processes occurring in the body (Kupczyński *et al.*, 2017; Diniz *et al.*, 2019). This technique is due to the high financial costs and laboriousness of studying the chemical composition of animal organs and tissues (Miroshnikov *et al.*, 2019; Miroshnikov *et al.*, 2020). Currently used approaches to determine metal (including iron) content in different types of meat are based on sample preparation involving stages of meat sample freezing, drying, and metal extraction with different reagents. Further analysis of the obtained extracts through atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry methods or other methods is also needed. This scheme is used to analyze element content in different types of food (Table 1), as well as animal hair (Miroshnikov *et al.*, 2019; Miroshnikov *et al.*, 2020). Currently, this approach may be considered as the main one for meat analysis (King *et al.*, 2023). Depending on the sample properties and the aim of the analysis, all stages of the analysis may be optimized, for example, more effective reagents for metal extraction may be used (King *et al.*, 2023). However, it does not significantly decrease the time and labor consumption to determine the elemental status of meat. Thus, the main shortcomings of currently used techniques for analyzing iron (and other elements) in meat is complexity, presence of several stages, and high costs. Notably, another disadvantage of currently used methods to determine iron content in meat is the impossibility of intravital assessment of iron content, which in turn does not allow correct iron content in cattle meat before slaughter. Thus, the development of fast and simple approaches, which may enable the determination of iron content in meat including that in beef, is an urgent task and investigation in this area. Animal blood

is an easily accessible, simple, and easy-to-select biological material. Furthermore, hematological and biochemical analyses do not require expensive laboratory equipment, are fast in sample preparation and execution, and do not require high financial costs. Therefore, this biomaterial is well suited for the role of an *in vivo* indicator of the content of heavy metals in the organs and tissues of animals. Furthermore, no data exist on robust relationships between blood-analysis results and iron content in meat, which does not allow using these data for iron-content estimation. The current study aimed to establish an optimum and effective model for predicting iron level in the muscle tissue of Hereford cattle, which enabled the assessment of the elemental status of animals *in vivo* and in a minimally invasive way toward food health consumption and ecological well-being. Notwithstanding the importance of other elements' contents in meat that determine food quality, It was focused on iron because it is the main element affecting the nutritional value of food, and beef is one the main sources of iron for the human population. This study was performed in in the Novosibirsk region of Russia in 2022–2023.

## MATERIALS AND METHODS

The study was approved by the expert commission of the Federal State State-Funded Educational Institution of Higher Education “Novosibirsk State Agricultural University.” Hereford cattle (n=30) bred in the south of Western Siberia (Russia) were studied. The animals were kept under standard conditions of an industrial complex in compliance with veterinary and zootechnical requirements following the law and under normal conditions for each species and breed. Feeding was performed with a typical complete feed, considering the age, live weight, and direction of animal productivity.

### Sample selection

The animals were slaughtered through the current requirements, technological instructions, and legal documents in force on the territory of the Russian Federation. Before slaughter, studies on their mucous membranes, skin, and derivatives were conducted. At the time of slaughter, the animals were clinically healthy. The animals were placed on a 12–18-hour preslaughter starvation diet. Organs and tissues were sampled immediately after the massacre; they

were frozen and stored at -18 degrees Celsius (°C) to -24 °C. Samples of skeletal muscles weighing 100 g were collected from the diaphragmatic muscle. Blood samples were collected from the jugular vein of the animals and stabilized with 5% sodium citrate. Blood samples were delivered to the laboratory within 6–12 hours, where hematological and biochemical analyses was performed.

#### Sample preparation for analysis

A portion of muscle tissue was crushed for atomic absorption analysis. From a homogeneous mass, a sample weighing 2–5 grams was selected and placed in a 50 milliliter (mL) quartz cup and poured with 25–50 mL of ethanol, covered with filter paper, and left at an ambient temperature of 10–25 °C for 24 hours. Then, the samples were dried at low heat. After cooling, a solution of nitric acid (HNO<sub>3</sub>) (1:1) was added in small portions, and the oxidation reaction was monitored, preventing the rapid evolution of foam. After the samples reacted with the HNO<sub>3</sub> nitric acid solution, their heating slowed to charring. Quartz cups with charred pieces were placed in a muffle furnace heated to 250 °C. They were tested, gradually raising the temperature to 510 °C, and kept for at least 4 hours until complete ignition. The samples were then treated with 5 mL of concentrated nitric acid HNO<sub>3</sub> and 1 mL of perchloric acid HClO<sub>4</sub>, the lids were covered, and the solution was heated strongly with fire until the answer became clear. After removing the caps, the samples were evaporated to a dry residue, which was treated with 5 mL of hydrochloric acid solution (1:1) and evaporated to wet salts. Then, the resulting sample was transferred into a container with 10 mL volume. The resulting working solution was analyzed on an MGA-1000 atomic absorption spectrometer (Lumeks LLC (Limited Liability Company)), Russia). The hematological parameters determined were the level of erythrocytes, leukocytes, and hemoglobin. An automatic hematology analyzer PCE-90VET (Hight Technology Inc, USA) was used. Biochemical parameters were determined using photometric methods on a Photometer-5010 semiautomatic biochemical analyzer (Robert Riele GmbH and Co KG, Germany) using reagents manufactured by Vector-Best CJSC (Closed Joint-Stock Company) and Olvex Diagnosticum LLC. Hemoglobin level was assessed at a wavelength of 540 nm by the Hemichromes method

using a Hemoglobin-Novo reagent kit manufactured by Vector-Best CJSC. Hemoglobin concentration was proportional to the hemichrome color intensity.

#### Statistical analysis

The original data were processed using the statistical programming language R. Model-fitting conditions were tested according to the exploratory data analysis protocol (Zuur *et al.*, 2010). Potential outliers were analyzed using the Grubbs test (Adikaram *et al.*, 2015). They determined whether the data distributions are Gaussian using the Shapiro–Wilk test (Razali *et al.*, 2011; da Silva Diniz *et al.*, 2020). The correlation coefficient between variables was calculated using the Spearman test (Xiao, 2019). Multicollinearity was assessed by calculating the variance-inflation coefficient for each parameter (Zuur *et al.*, 2010) and by using a graphical method with the scatterplot matrix of regression-model variables. The model coefficients were calculated by the least squares method. Multiple comparisons of influential observations were made by Bonferroni correction (Aickin and Gensler, 1996). The conditions for the independence of the model's residuals were tested using the Durbin–Watson test (Chen, 2016).

## RESULTS AND DISCUSSION

#### Model fitting

According to the methodology, regression analysis began with the creation of a complete model containing all predictors by using Eq. 1 (Zuur *et al.*, 2010).

$$y \sim x_1 + x_2 + x_3 + x_4 + x_5 + x_6 + x_7 + x_8 + x_9 + x_{10} + x_{11} + x_{12} \quad (1)$$

Where, y is the response variable, and x<sub>1</sub>–x<sub>12</sub> are independent variables.

For convenience, the independent variables are renamed. According to Table 2, the dependent variable (y) is the muscles' level of iron in milligram per kilogram (mg/kg).

An essential step in exploratory analysis is the selection of regression models and the choice of predictors to assess multicollinearity. The models are unstable in the presence of multicollinearity in estimating coefficients. Consequently, analyzing individual factors' contribution to the response variable's variance is challenging. A paradox may arise

Table 2: Designation and interpretation of independent variables used to select regression models

Indicator	Unit of measure	Variable value in the model
Fe level in blood	millimoles per liter (mmol/L)	x1
Leukocytes	$\times 10^9$ pieces (pcs.)	x2
Erythrocytes	$\times 10^{12}$ pcs.	x3
Hemoglobin	gram per liter (g/L)	x4
Erythrocyte sedimentation rate	millimeters per hour (mm/h)	x5
Color indicator of blood		x6
Protein	g/L	x7
Albumin	g/L	x8
Globulin	g/L	x9
Urea	mmol/L	x10
Uric acid	micromole per liter ( $\mu$ mol/L)	x11
Cholesterol	mmol/L	x12

when the coefficients of the regression model are statistically insignificant. At the same time, the model as a whole is significant (the null hypothesis tested by F-statistics about the equality of all coefficients to zero is rejected). Accordingly, the values of the Spearman correlation coefficient were calculated and a correlation matrix and scatterplots were built to assess the linear relationship between variables (Figs. 1 and 2). The calculated correlation coefficients are in the lower triangle on a red background, and the significance levels for these coefficients are in the upper triangle. Analysis showed a relatively large number of relationships among variables, many of which are physiologically determined. For example, the color index of blood reflects the degree of saturation of red blood cells with hemoglobin. It represents the ratio of red blood cells and hemoglobin in blood. Likewise, the concentration of iron in blood is related to the number of red blood cells, and the total protein level is associated with the concentration of globulins, one of its main fractions. Naturally, these indicators have a high correlation. In other cases, the connection could be more precise. For example, a positive relationship between the level of iron in the blood and the concentration of uric acid may be due to the possibility of creating complexes from them, thereby increasing the antioxidant activity of the latter (Davies *et al.*, 1986).

Another complex way to assess the degree of

multicollinearity of a complex of predictors is to calculate the variance-inflation factor (VIF). A higher VIF for each predictor corresponds with a closer linear relationship with the rest of the independent variables. The VIF values of predictor dispersion were calculated for all candidate models (Table 3).

The example of the general model clearly shows the high multicollinearity of the model parameters, especially the protein and its constituent fractions of globulins and albumin. The correlation is less pronounced in the value of the color index of blood, calculated from the number of erythrocytes and hemoglobin. Including a complete set of predictors in the model leads to duplication of the influence of independent variables on the response value and creates excessive information noise. The rest of the indicators have a relatively low VIF weight, so the estimate of the remaining coefficients should be statistically significant.

#### Regression-model selection

Table 4 gives estimates of the coefficients of the complete model containing all independent variables. Consequently, a predictable situation is observed when, owing to the high multicollinearity, the estimates of all coefficients of the complete linear regression model turn out to be statistically insignificant according to the t-test. The F-test also indicates the statistical significance of the entire



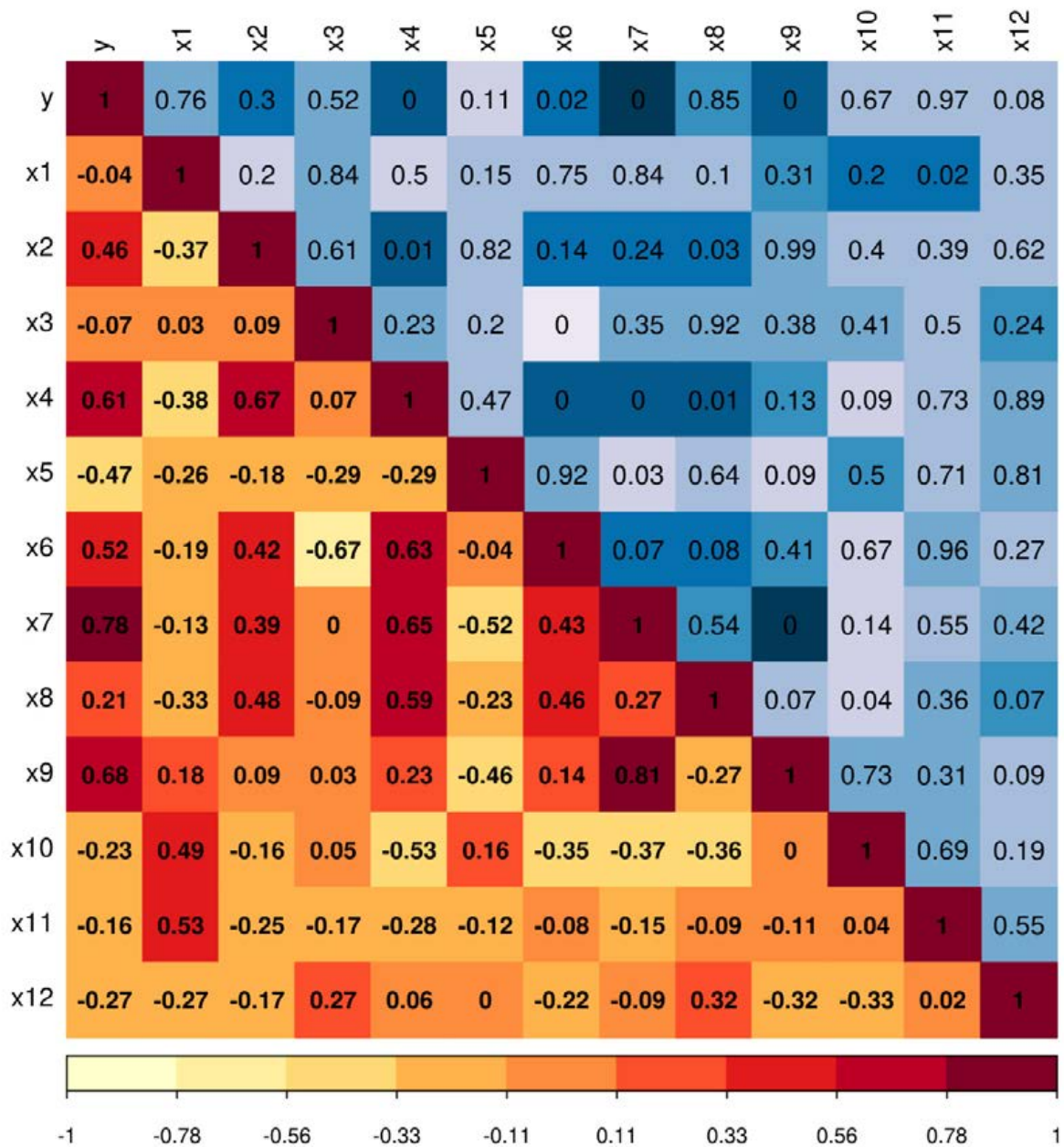


Fig. 1: Correlation matrix of regression-model variables

model. One of the most effective ways to reduce multicollinearity is to select an informative set of predictors.

The optimum structure model with tuned parameters should provide the “best” value of a

particular quality criterion. However, with many variables, achieving the optimum value for all quality criteria is almost impossible, so several suboptimal candidate models are created for the subsequent selection of the working model. Stepwise regression



Fig. 2: Matrix of scatterplots of regression-model variables

Table 3: Dispersion of inflation-factor values for regression models' coefficients to assess iron level in muscle tissue

Predictor	Complete model	$y \sim x_3 + x_4 + x_6 + x_7 + x_{12}$	$y \sim x_3 + x_4 + x_6 + x_8 + x_9 + x_{12}$	$y \sim x_3 + x_6 + x_9$
x1	2.1	—	—	—
x2	4.1	—	—	—
x3	27.2	13.8	15.5	1.7
x4	34.2	14.5	18.1	—
x5	2.3	—	—	—
x6	37.9	21	22.4	1.7
x7	631.5	1.8	—	—
x8	156.6	—	2.8	—
x9	697.5	—	2.1	1.2
x10	3.4	—	—	—
x11	1.8	—	—	—
x12	1.9	1.2	1.5	—

Table 4: Parameters for estimating the coefficients of the complete model to predict the iron level in muscle tissue from blood parameters

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	$P_t^*$
Int.	-25.655	25.605	-1.002	0.338
x1	-2.483	45.661	-0.054	0.958
x2	-0.181	0.345	-0.525	0.61
x3	3.166	2.865	1.105	0.293
x4	-0.094	0.179	-0.523	0.611
x5	0.523	2.608	0.2	0.845
x6	17.562	15.91	1.104	0.293
x7	-0.573	1.308	-0.438	0.67
x8	0.888	1.265	0.702	0.497
x9	0.823	1.288	0.639	0.536
x10	0.54	0.903	0.598	0.562
x11	-0.001	0.015	-0.09	0.93
x12	-1.856	1.66	-1.118	0.288

RSE<sup>2</sup> – 4.95; F-statistic – 3.98, P = 0.015.

Here and below: <sup>1</sup>Int. Is the free term of the equation; RSE<sup>2</sup> is the estimate of the standard deviation of residuals (residual standard error); \*  $P_t$  – significance level of t-statistics; SE (standard Error).

Table 5: Parameters for estimating the coefficients of the candidate model selected by the combined algorithm

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	$P_t$
Int.	-21.170	14.601	-1.450	0.165
x3	3.555	1.830	1.943	0.069
x4	-0.140	0.110	-1.267	0.222
x6	21.055	10.373	2.030	0.058
x8	0.217	0.144	1.509	0.150
x9	0.231	0.059	3.899	0.001
x12	-1.735	1.233	-1.407	0.180

RSE – 4.20; F-statistic – 10.78, P < 0.001.

analysis is performed using a combined algorithm “stepwise forward and backward selection”. In the first step, the best one, according to the Akaike information criterion, is selected from all predictors. Then, the following variable with the optimum solution with the first coefficient of the model is set. The algorithm stops when the extremum of the criterion value is reached. Afterwards, the exclusion stage replaces the location of the inclusion of variables. In this case, all combinations of variables are sorted out. Then, a less informative predictor is excluded from the model by the value of the specified quality criterion, and so on, until the criterion extremum is reached. The model

obtained by this method is presented in Table 5. Compared with the entire model, the estimate of the standard deviation of the residuals and the F-statistic is significantly lower, showing the superiority of this model over the general one.

A more illustrative method for selecting the optimum regression model is to sequentially build all possible regression models with an assessment of the quality of each of them. The method’s main disadvantage is the need to use significant computing power. In this case, when using 12 independent variables, 4096 regression models were built. Consequently, the best models were ranked according to the main internal-



Table 6: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) according to the value of the Akaike information criterion

Model equation	df	p	SSE	MSE	R <sup>2</sup>	R <sup>2</sup> <sub>adj</sub>	AIC	BIC
$y \sim 1+x_3+x_4+x_6+x_7+x_{12}$	18	5	310.281	17.238	0.785	0.725	143.535	159.946
$y \sim 1+x_3+x_6+x_9$	20	3	372.917	18.646	0.741	0.702	143.948	158.003
$y \sim 1+x_3+x_6+x_9+x_{12}$	19	4	348.338	18.334	0.758	0.707	144.312	159.545
$y \sim 1+x_3+x_6+x_7+x_{12}$	19	4	350.013	18.422	0.757	0.706	144.427	159.66
$y \sim 1+x_2+x_3+x_6+x_7+x_{10}+x_{12}$	17	6	296.436	17.437	0.794	0.722	144.44	162.028

Here and below: df is degrees of freedom, p is the number of model coefficients, SSE is the sum of squared errors, and MSE is the mean-squared error.

Table 7: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) according to the value of the Bayesian information criterion

Model equation	df	p	SSE	MSE	R <sup>2</sup>	R <sup>2</sup> <sub>adj</sub>	AIC	BIC
$y \sim 1+x_3+x_6+x_9$	20	3	372.92	18.65	0.74	0.7	143.95	158
$y \sim 1+x_7+x_{12}$	21	2	436.75	20.8	0.7	0.67	145.74	158.62
$y \sim 1+x_4+x_9$	21	2	439.64	20.94	0.7	0.67	145.9	158.78
$y \sim 1+x_7$	22	1	509.75	23.17	0.65	0.63	147.45	159.15
$y \sim 1+x_4+x_7+x_{12}$	20	3	392.96	19.65	0.73	0.69	145.21	159.26

Table 8: Ranking of the best regression models for predicting the level of iron in muscle tissue (mg/kg) by the value of the adjusted coefficient of determination

Model equation	df	p <sup>*</sup>	SSE	MSE	R <sup>2</sup>	R <sup>2</sup> <sub>adj</sub>	AIC	BIC
$y \sim 1+x_3+x_4+x_6+x_7+x_{12}$	18	5	310.28	17.24	0.79	0.73	143.54	159.95
$y \sim 1+x_2+x_3+x_6+x_7+x_{10}+x_{12}$	17	6	296.44	17.44	0.79	0.72	144.44	162.03
$y \sim 1+x_3+x_4+x_6+x_7+x_8+x_9+x_{12}$	16	7	281.01	17.56	0.81	0.72	145.16	163.92
$y \sim 1+x_3+x_4+x_6+x_8+x_9+x_{12}$	17	6	299.91	17.64	0.79	0.72	144.72	162.31
$y \sim 1+x_3+x_4+x_6+x_7+x_{10}+x_{12}$	17	6	303.93	17.88	0.79	0.72	145.04	162.63

quality criteria, namely, Akaike information criterion (AIC) (Table 6), (Bayesian information criterion (BIC) (Table 7), and R<sup>2</sup> adjusted (adj) (Table 8).

The adjusted coefficient of determination is the greediest; that is, a model with many parameters will often be preferred, even though each criterion considers the number of predictors in the model. For example, the best model for R<sup>2</sup> adj includes five predictors. A more balanced approach is implemented using the information criteria AIC and BIC, imposing a “penalty” for adding new parameters. Their main difference is that the BIC is more sensitive to adding new parameters and prefers the most compact models. This criterion is found when analyzing Tables 3 and 4. According to the AIC, the best model contains five coefficients, and the following has 3. Moreover, according to the BIC, the best model includes three

independent variables. Although the value of the standard deviation of the residuals is slightly higher than that of the previous model, the value of the F-statistic is higher (Table 9).

Thus, for further analysis, three models can be selected (the best according to internal-quality criteria), and the whole model with all coefficients can be left for comparison. The most compact models with minimal multicollinearity are worth giving preference. Therefore, if it is considered the selected candidate models based on the VIF (Table 2), multicollinearity would be absent only in the model with three predictors (the best according to the BIC). When using the Mallows test (Fig. 3b), the best model has the same variables as the Bayesian information test (Fig. 3a).

The above estimates of the quality of fit of the

Table 9: Parameters for estimating the coefficients of the candidate model to predict the iron level in muscle tissue (mg/kg) from blood parameters

Coefficient notation	Odds estimates	Coefficient SE	t-statistic	P <sub>t</sub> *
Int.	-9.583	6.925	-1.384	0.182
x3	1.551	0.631	2.456	0.023
x6	11.503	2.966	3.878	0.001
x9	0.212	0.046	4.570	<0.001

RSE<sup>2</sup> – 4.318; F-statistic – 19.1, P < 0.001.

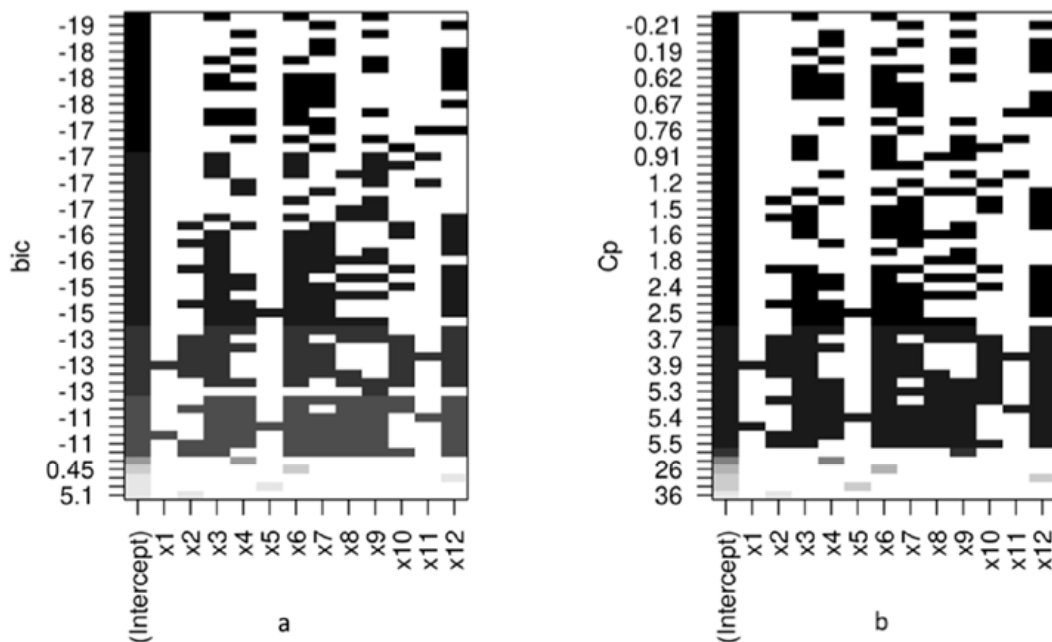


Fig. 3: Ranking models for predicting iron level in muscle tissue by using the Bayesian information criterion (a) and the Mallow criterion (b)

regression model refer to “internal” criteria because their calculations are based on the same data used to calculate the model. Therefore, estimates often provide biased measures of the actual function of the process, which are based on sampled empirical values of small samples. Unbiased forecasts can be obtained only by applying external-quality criteria. They are also effective against model overcomplication and allow model selection with an optimum number of parameters. The most informative external criterion is cross-validation (CV). The prediction error of the response variable is estimated during the course of multiple random splitting of the initial sample into training and testing. Some of them are based

on leave-one-out CV:  $n$  (sets) of regression models are fitted on  $(n-1)$  sample values, and the excluded observation is used each time to calculate the prediction error. Visualization of the best candidate models, divided into three blocks ( $k=3$ ) by the CV method, shows that the regression lines of the model for BIC and Mallows’s  $C_p$  ( $C_p$ ) (Fig. 4a) provide a more accurate forecast relative to the others (Fig. 4b and c), and the complete model (Fig. 4d) is the worst fit to predict the level of iron in muscle tissue.

The CV error for the model has decreased by more than 110 times relative to the overall model selected based on the BIC. This error is also 26% better than the closest model selected based on the adjusted

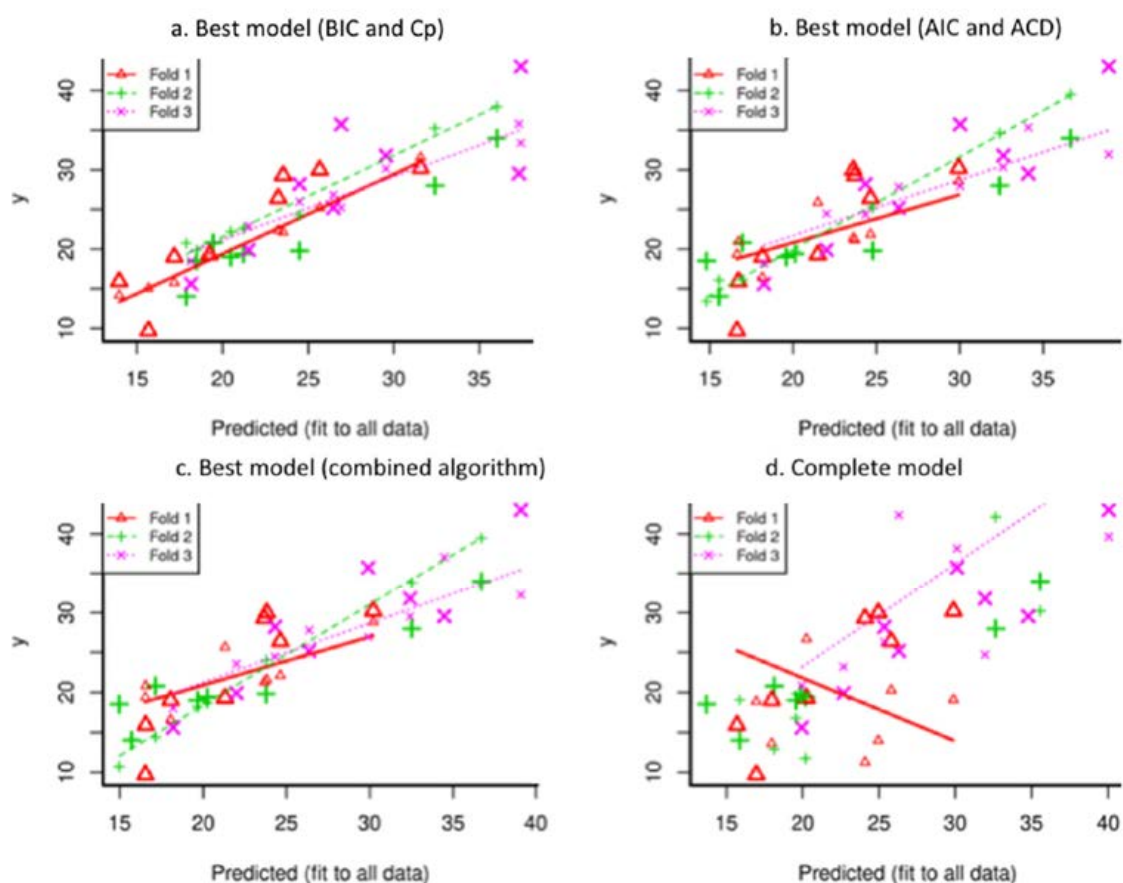


Fig. 4: Visualization of candidate models for assessing the level of iron in muscles by the cross-validation method: BIC and Cp (a), AIC and ACD (b), combined algorithm (c), and complete model (d)

Table 10: Estimated error in the cross-validation of regression models for predicting the level of iron in muscle tissue (mg/kg)

Model Formula	SS	df	MS
$y \sim 1+x_3+x_6+x_9$	561	24	23
$y \sim 1+x_3+x_4+x_6+x_7+x_{12}$	756	24	31
$y \sim 1+x_3+x_4+x_6+x_7+x_8+x_9+x_{12}$	2360	24	98
$y \sim 1+x_2+x_3+x_4+x_5+x_6+x_7+x_8+x_9+x_{10}+x_{11}+x_{12}$	62126	24	2589

Note: SS is the sum of squares, MS is the mean square.

determination coefficient and the Akaike information criterion (Table 10).

Thus, the optimum model includes three predictors ( $x_3$ ,  $x_6$ ,  $x_9$ ).

#### Verifying assumptions about model residuals

Verifying the assumptions regarding the model's

residuals is necessary to determine the adequacy of applying the least squares method. Using graphical methods, one can evaluate the distribution normality of residuals and the dependence on predicted values. (Figs. 5 and 6). Thus, the probability density curve of the residual distribution practically repeats the Gaussian distribution's confidence region (Fig. 5). The quantile plot of the standardized residuals

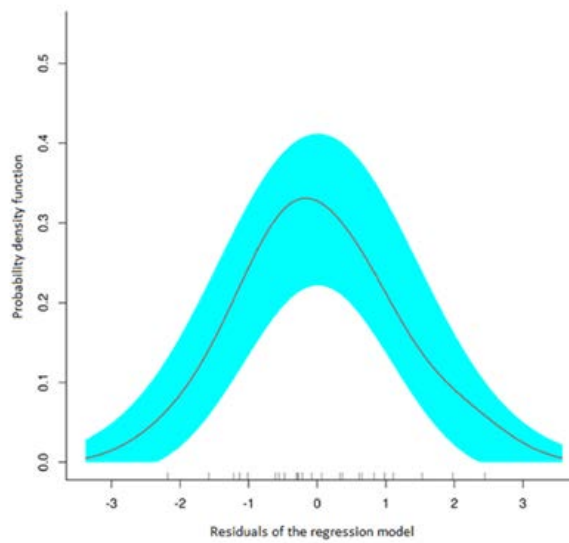


Fig. 5: Distribution of residuals of the regression model to assess the level of iron (mg/kg) in muscle tissue

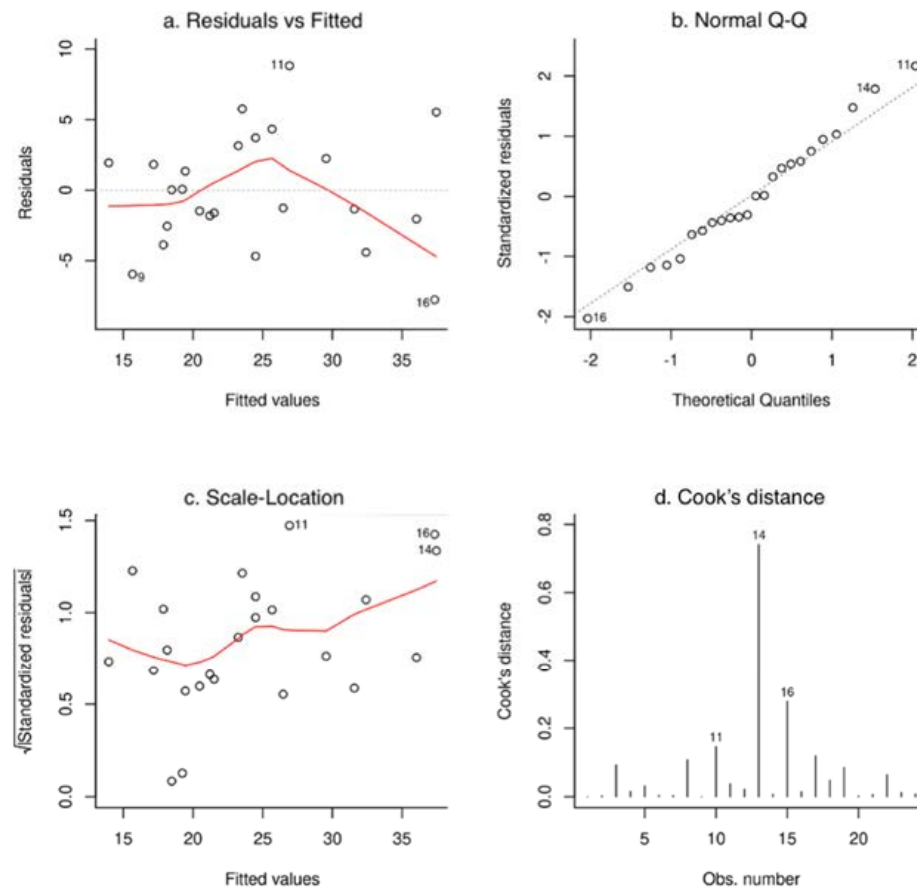


Fig. 6: Residuals versus response (a), quantile plot (b), square root of standardized residuals versus response (c), and Cook's distances (d)

and theoretically expected quantiles show that the values are distributed relatively normally (Fig. 6, top left field). This plot is also confirmed by the formal Shapiro–Wilk test ( $W = 1$ ;  $P = 1$ ).

The model's dispersion of residuals versus predicted values is shown in Fig. 6a, indicating the homogeneity of the variance. A smoothing line is plotted in red, which facilitates the analysis. It is close to a horizontal line, so the condition for uniform dispersion of residuals is satisfied. Fig. 6c also indicates the variance homogeneity of the residuals, the y-axis shows the square root of the standardized residuals, which are standardized by dividing each residual by its standard deviation. The standardization procedure improves the heterogeneity detection of their variance. The smoothing line, in this case, is also close to horizontal. Fig. 6d is built to identify “influential” observations. The visualized Cook's distance values show that three comments (highlighted by ordinal values) require careful consideration. First, student-t residuals are compared with the theoretically expected values of the t-distribution to ensure that they are not outliers. The significance level is calculated considering the

Bonferroni correction for the observation with maximum deviation. In the selected model, the deal with the maximum deviation is 2.42. Its adjusted level of significance ( $P$ ) is 0.62. This value is similar to the theoretically expected one, so none of the potentially influential observations is an outlier. The values of the criterion  $d=1.92$  are obtained, and they correspond with an autocorrelation coefficient of 0.025 ( $p=0.67$ ) owing to the Durbin–Watson test. Such a high significance level of the statistical error of the first kind indicates the absence of autocorrelation. Therefore, the condition for the independence of the model's residuals is satisfied. As a result of the selection and evaluation of the quality of models, the best predictive model, taking into account internal and external-quality criteria, contains three predictors: the number of erythrocytes, color index, and globulin. To predict the level of iron in the muscle tissue of cattle, it is proposed to use Eq. 2 which is made by the author based on data shown in Table 9.

$$y = -9,583 + 1,551 \times RC + 11,503 \times CCR + 0,212 \times G \quad (2)$$

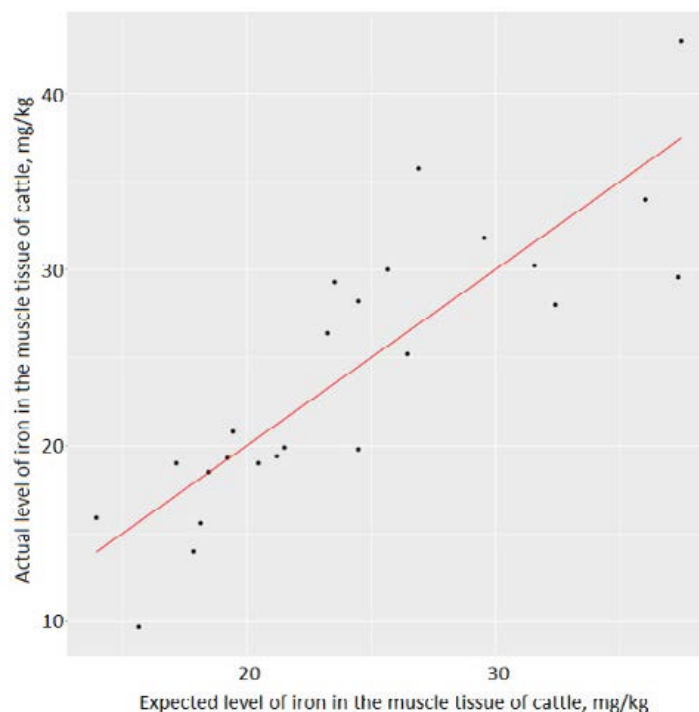


Fig. 7: Model of the expected and actual level of iron in the muscle tissue of cattle



Table 11: Summary of the studies on meat quality using novel techniques

Subject of the study	Method used and summary	Reference
Chicken breast and beef chops	Optomagnetic methods can be used to evaluate the spoilage of fresh beef and chicken meat when stored in a refrigerator.	<a href="#">Mileusnić et al., 2017</a>
Beef	The predictability of a detailed mineral profile of beef using different portable near-infrared spectrometers is studied. The methods can predict Fe and P contents.	<a href="#">Patel et al., 2020</a>
Different types of food	The work summarizes the results of studies on different nondestructive spectroscopic and imaging techniques for food quality. The authors conclude that these techniques may be used for food analysis, but the commercialization of these techniques is prevented by the high-cost equipment and generation of large data sets.	<a href="#">Edwards et al., 2021</a>
Different types of meat	The work summarizes results of studies on different techniques for meat quality (electronic nose, computer vision, spectroscopy, hyperspectral imaging (HSI), and multispectral imaging technologies). The authors conclude that future studies are required to enhance the accuracy, scalability, robustness, and simplicity of these technologies.	<a href="#">Khaled et al., 2021</a>
Pork and beef	The review concluded that HSI and visible/near-Infrared spectroscopy are the leading techniques for monitoring pork and beef quality and safety.	<a href="#">Sanchez et al., 2022</a>
Different types of meat	The work shows that different nondestructive spectroscopic and imaging techniques are promising for analyzing the quality of different types of meat.	<a href="#">Wu et al., 2022</a>

Where,  $y$  is the concentration of iron in muscle tissue (mg/kg), RC is the red count, CCR is the cell–color ratio, and G is globulin.

Visualization of the resulting model is displayed as a scatterplot of predicted and observed values ([Fig. 7](#)). The results obtained show a sufficient level of approximation and the absence of outliers.

#### *Advantages of the proposed approach*

A fast, simple, and reliable method of predicting iron content in cattle meat was proposed. The method is based on blood analyses according to commonly used methods and further use of the results obtained to calculate iron content in cattle meat. Notably, many studies have focused on the development of methods for meat-quality analysis because currently used methods are characterized by high consumption of time and labor ([King et al., 2023](#)). Additionally, in literature, no data are available on the use of blood analysis to predict meat quality. Most studies have focused on the use of different spectroscopic, biochemical, and optical methods that allow fast and nondestructive meat analysis to estimate main quality parameters without tedious sample preparation ([Table 11](#)).

Despite the various methods proposed, they cannot solve the main disadvantage of currently used methods, i.e., they do not allow the prediction of elemental status of meat before slaughter.

Moreover, they have certain advantages on currently used methods as they do not require long-term preparation. The method developed in this work avoids long-term preparation and application of complex and expansive equipment and can be used to predict iron content in meat before slaughter. In turn, it enables the correction of the final iron content in meat by optimizing feed livestock and obtaining meat with required quality. Notably, iron-content control in meat (particularly beef) is one of the elements of so-called sustainable beef production ([Purchas and Busboom, 2005; Broom, 2021; Hubbart et al., 2023](#)) because iron content is one of the key meat-quality indicators. Thus, controlling the intravital iron content may be one the most effective tools to correct beef quality by understanding the effect of different factors on the final product quality, which may enable the development of approaches to obtain the meat of required quality through targeted methods of influence on certain meat-quality indicators. This in turn will allow only required methods to control food production and decrease the possible environmental impact of beef production, thereby avoiding the use of extensive methods to improve the quality and amount of meat produced, which can contribute to ecological well-being.

Using the method proposed, the conditions of cattle keeping may be regulated to improve the goal parameter (iron content) through a minimally invasive

approach (blood sampling and analysis), which is currently used routinely in veterinary practice. Thus, applying the proposed method will not include any special ethical implications.

#### *Limitations of this study and further research directions*

This study presents initial results on the development of the proposed method. Indeed, the model accuracy and the effect of different factors should be further studied in detail by using a large sample. In the present work, a comparatively small sample was used. Based on the results, it cannot unambiguously evaluate these factors affecting the model performance and the limitation of its application. Nevertheless, the results obtained clearly suggest that the proposed method is promising for future research and may be improved for further commercialization. Further development of the method proposed also requires study of the mechanisms affecting relationships between blood parameters and iron content in muscle tissues of cattle, which in turn may improve the proposed model and avoid its shortcomings owing to factors that decrease model accuracy. The results showed only empirical patterns observed in this work but cannot provide an explanation for them. The iron amount in the diet of calves affects key blood parameters (Kupczyński *et al.*, 2017). Some mutual effects probably determine blood parameters and iron content in livestock feed and muscle tissues, but their mechanisms should be studied. In addition to direct contributions to the increase in beef quality by regulating one of the key parameters of meat quality, further studies in the area of the study may be used as element of sustainable beef production. Given that the prediction of iron content cannot be used to directly solve most ecological problems of livestock farming (for example, gas emission), studying the effect of different factors on meat quality may allow to decrease or avoid useless measures used in farming, such as the excessive use of feed additives. Consequently, resource exploitation can decrease and farming productivity can increase.

#### **CONCLUSION**

A fast and simple method of assessing the elemental status of animals in vivo and in a minimally

invasive way is developed. The method is based on an effective model for predicting the iron level in the muscle tissue of Hereford cattle by using blood-analysis results. The coefficients of regression models using the least squares method and the values of the dependent variable corresponding with the Gaussian are calculated. A high correlation between independent variables is revealed. An optimum model for predicting the iron level in the muscle tissue of Hereford cattle was identified, containing three predictors: the number of erythrocytes, color index, and globulin as a result of selection based on internal and external-quality criteria. The model meets the necessary assumptions: the residuals are normally distributed, no autocorrelations exist, and the observations are influential. Furthermore, no signs of multicollinearity exist between the main effects of the model ( $VIF = 1.2-1.7$ ). The resulting model can be used for the intravital analysis of iron level in the muscle tissue of cattle, which is an important advantage of the developed approach to currently used methods. In the future, verifying this model on a test sample, increasing the accuracy of the forecast, and continuing to train the model are necessary. The results can be used to assess ecological well-being and determine the allowable load of iron on animals and its transfer to human. For veterinary medicine, the resulting model enables the evaluation of the iron level in the muscle tissue of Hereford cattle during their lifetime. Moreover, similar studies must be conducted on large populations and mixed linear models considering random effects must be established. It is believed that the method proposed may be used for cattle and for other types of meat animals. Analysis of literature reveals that the article possesses scientific novelty as it has not been found work based on similar approaches. The method is proposed may also be the simplest and fastest technique among currently available methods of predicting the content of one the key elements in meat. Therefore, the method has practical significance for commercialization.

#### **AUTHOR CONTRIBUTIONS**

K. Narozhnykh, as the study single author performed the study conceptualization and design, data collection, analysis and interpretation of results, and manuscript writing up.

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**CONFLICT OF INTEREST**

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

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**ABBREVIATIONS**

$\mu\text{mol/L}$	Micromole per liter	<i>adj</i>	Adjusted
%	Percent	<i>AIC</i>	Akaike information criterion
°C	Degree Celsius	<i>BIC</i>	Bayesian information criterion
AAS	Atomic absorption spectroscopy	<i>CCR</i>	Cell-color ratio
ACD	Adjusted coefficient of determination	<i>CJSC</i>	Closed Joint-Stock Company
		<i>Cp</i>	Mallows's <i>Cp</i>
		<i>CV</i>	Cross-validation
		<i>df</i>	Degrees of freedom
		<i>Eq.</i>	Equation
		<i>Fe</i>	Iron
		<i>G</i>	Globulin
		<i>g/L</i>	Gram per liter
		<i>HClO<sub>4</sub></i>	Perchloric acid
		<i>HNO<sub>3</sub></i>	Nitric acid
		<i>ICP-MS</i>	Inductively coupled plasma mass spectrometry
		<i>LLC</i>	Limited Liability Company
		<i>mg/100 g</i>	Milligram per 100 grams
		<i>mg/day</i>	Milligram per day
		<i>mg/kg</i>	Milligram per kilogram
		<i>mL</i>	Milliliter
		<i>mm/h</i>	Millimeters per hour
		<i>mmol/L</i>	Millimoles per liter
		<i>MS</i>	Mean square
		<i>MSE</i>	Mean squared error
		<i>nm</i>	Nanometer
		<i>pcs</i>	Pieces
		<i>RC</i>	Red count
		<i>RSE</i>	Residual standard error
		<i>SE</i>	Standard error
		<i>SS</i>	Sum of squares
		<i>SSE</i>	Sum of squared errors
		<i>VIF</i>	Variance inflation factor

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