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DETECTION OF HEIFERS WITH DYSTOCIA USING ARTIFICIAL NEURAL NETWORKS WITH REGARD TO *ERα-BGL*I, *ERα-SNA*BI AND *CYP19-PVU*II GENOTYPES

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Abstract. The aim of this study was to detect heifers with dystocia using artificial neural networks (ANN). A total of 531 calving records of Holstein-Friesian heifers of Black-and-White strain and 8 diagnostic variables were used. The output variable was the class of calving difficulty: difficult or easy. Perceptrons with one (MLP1) and two (MLP2) hidden layers and radial basis function (RBF) networks were investigated. The root mean square error and the structure of selected ANN (number of neurons in the input, hidden and output layers) were 0.22, 10-4-1; 0.25, 10-17-17-1 and 0.19, 10-25-1 for MLP1, MLP2 and RBF, respectively. The percentage of correctly recognized heifers with difficult and easy calvings and that of correctly diagnosed heifers from both categories for the training and validation sets were approx. 90%. The same values for the test set were 75-83%, 82–88% and 82–86%, respectively. In both cases, no significant differences in these proportions were found. The following variables contributed most to the detection of heifers with dystocia: gestation length, BCS index, *CYP19-Pvu*II and *ERα-Bgl*I genotypes and percentage of HF genes in heifer's genotype.

Keywords: artificial neural networks, dairy heifers, dystocia, genotypes

INTRODUCTION

In cattle, dystocia occurs most frequently compared to all other species of farm animals. This problem is particularly significant in heifers, in which dystocia is two to three times more frequent than in multiparous cows [Tyczka 1998, Mee 2004]. Holstein heifers have even 4.7 times higher probability of difficult calving than multiparous cows do [Johanson and Berger 2003]. Besides pelvis anatomy, many factors affect calving difficulty, including those of genetic nature. From among many genes with potential effect on reproductive traits, oestrogen receptor α gene (*ERα*) [Szreder and Zwierzchowski 2004, Szreder et al. 2007] and aromatase cytochrome P450 gene (*CYP19*) [Vanselow et al. 1999] are mentioned. The effect of oestrogens on the course of parturition is unquestionable and the aforementioned genes are involved in the synthesis of oestrogens and their action on tar-

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get tissues. *CYP19* encodes an enzyme (aromatase cytochrome P450) that is responsible for binding a substrate and catalysing reaction leading to the formation of phenolic ring A, characteristic of oestrogens [Kowalewska-Łuczak 2006]. On the other hand, *ERα* encodes oestrogen receptor *α* that regulates transcription of target genes [Szreder et al. 2007]. Hence, in the present study the following 3 polymorphisms had been selected for analysis: *ERα-Bgl*I, *ERα-Sna*BI and *CYP19-Pvu*II.

The occurrence of dystocia is associated with many adverse effects, including financial ones. Therefore, obtaining earlier information on the possible course of calving could be very useful for a breeder. Such a possibility is given by, among others, artificial neural networks (ANN) belonging to artificial intelligence methods. That being so, the aim of the present study was: 1) preparation and quality evaluation of the selected types of ANN used for the detection of dystocia in heifers based on the selected diagnostic variables (with particular consideration of polymorphisms), 2) performing of such detection, 3) finding which variables had the greatest influence on the occurrence of dystocia.

MATERIAL AND METHODS

The research material comprised data on 531 calvings of Polish Holstein-Friesian heifers of Black-and-White strain occurring between 2004 and 2009. Heifers were kept in a loose barn located in the West Pomerania Province with runouts accessible over the whole year. The animals were fed a total mix ration (TMR). Only heifers without any serious disorders before calving (uterine torsion, ventral hernia etc.) were included in the statistical analysis. The description of the performance indices and genotype frequencies is presented in Table 1.

The following 8 traits – diagnostic (input) variables were used for analysis: HF – percentage of HF genes in heifer's genotype; BGLI – *ERα-Bgl*I genotype; SNABI – *ERα--Sna*BI genotype; PVUII – *CYP19-Pvu*II genotype; GEST – gestation length (in days); BCSI – BCS index calculated as a difference between BCS_B before calving and BCS_C at calving (in scores); SEASON – calving season (1– from October to May, 2 – from June to September, according to Klassen et al. [1990]), AGE – heifer's age at calving (in months).

Body condition score was measured on a 5-point scale [Ferguson et al. 1994]. The obtained scores were transformed so that ANN properly interpreted the fact that both too high and too low body condition does not favour easy calvings. The optimum was set at 3.50 scores and higher values were subtracted from this optimum (e.g. if a heifer obtained 3.75 scores, it was recorded as 3.25 scores).

Polymorphic sites analysed using PCR-RFLP method are shown in Table 2. The applied primer sequences, melting temperatures and product lengths have been described by Szreder and Zwierzchowski [2004], Szreder et al. [2007] and Vanselow et al. [1999] for *ERα-Bgl*I, *ERα-Sna*BI and *CYP19-Pvu*II polymorphisms, respectively. PCR and electrophoresis conditions have been given by Jędrzejczak et al. [2011]. The variants of obtained genotypes and their frequencies are presented in Tables 1 and 2.

Table 1. Input (diagnostic) data
Tabela 1. Dane wejściowe (diagnostyczne)

Variable Zmienna		Calvings – Wycielenia: difficult „A" (n_A= 67) trudne		easy „B" (n_B= 464) łatwe		total (n = 531) razem	
		$\overline{x}$	s	$\overline{x}$	s	$\overline{x}$	s
HF, %		91.46	6.16	88.51	7.57	88.88	7.47
GEST, days – dni		282.39	4.64	277.10	1.79	277.77	2.93
BCSI, scores – pkt.		0.21	0.42	0.00	0.01	0.03	0.17
AGE, months – miesiące		24.96	2.58	26.64	3.63	26.43	3.55
		n	%	n	%	n	%
BGLI	*GG*	59	88.06	428	92.24	487	91.71
	AG	8	11.94	36	7.76	44	8.29
SNABI	*AA*	60	89.55	431	92.89	491	92.47
	AG	7	10.45	33	7.11	40	7.53
PVUII	*AB*	11	16.42	54	11.64	65	12.24
	AA	56	83.58	408	87.93	464	87.38
	BB	–	–	2	0.43	2	0.38
SEASON	1	15	22.39	162	34.91	177	33.33
	2	52	77.61	302	65.09	354	66.67

HF – percentage of HF genes in heifer's genotype – procent genów HF w genotypie jałówki; GEST – gestation length – długość ciąży; BCSI – body condition score index – indeks kondycji; AGE – age at calving – wiek przy wycieleniu; BGLI – *ERα-Bgl*I genotype – genotyp *ERα-Bgl*I; SNABI – *ERα-Sna*BI genotype – genotyp *ERα-Sna*BI; PVUII – *CYP19-Pvu*II genotype – genotyp *CYP19-Pvu*II; s – standard deviation – odchylenie standardowe; n – sample size – liczebność.

Table 2. Analyzed polymorphic sites and obtained genotypes
Tabela 2. Analizowane miejsca polimorficzne oraz otrzymane genotypy

Polymorphism Polimorfizm	Transition A/G at position Tranzycja A/G w pozycji	Genotype Genotyp	Restriction fragment length (bp) Długości fragmentów restrykcyjnych (pz)
*ERα-Bgl*I	151	*AG*	242, 182, 60
		GG	182, 60
*ERα-Sna*BI	–1213	*AA*	340
		AG	340, 225, 115
*CYP19-Pvu*II	1044	*AA*	405
		AB	405, 327, 78
		BB	327, 78

The output variable was the class of calving difficulty: A – difficult calving (dystocia) performed with great help of man and/or veterinarian, with possible complications, B – easy calving, that is, spontaneous one, or with little help of man. The number of difficult and easy calvings is presented in Table 3.

Table 3. Mean values of the analysed variables (standard deviations in parentheses) and distribution of calvings in the subsets
Tabela 3. Średnie wartości analizowanych zmiennych (odchylenia standardowe podano w nawiasach) oraz rozkład wycieleń w podzbiorach

Set Zbiór	n	HF, %	GEST, days – dni	BCSI scores – pkt.	AGE months – mies.	Calvings – Wycielenia	
						difficult „A” trudne	easy „B” łatwe
L+V	430	88.70 (7.78)	278 (2.88)	0.03 (0.18)	26.44 (3.76)	55 (12.79%)	375 (87.21%)
T	101	89.64 (5.94)	278 (3.13)	0.00 (0.02)	26.38 (2.49)	12 (11.88%)	89 (88.12%)
Total Razem	531	88.88 (7.47)	278 (2.93)	0.03 (0.17)	26.43 (3.55)	67 (12.62%)	464 (87.38%)

L – training – uczący; V – validation – walidacyjny; T – test – testowy; n – No. of records – liczba rekordów; HF – percentage of HF genes in heifer's genotype – procent genów HF w genotypie jałówki; GEST – gestation length – długość ciąży; BCSI – body condition score index – indeks kondycji; AGE – age at calving – wiek przy wycieleniu.

Statistica Neural Networks software (2000) was used for creation and training of ANN. The whole data set was divided into three parts (training, validation and test sets containing 330, 100 and 101 records, respectively). The detailed description of the ANN and learning algorithms applied has been presented in the study by Grzesiak et al. [2010]. The quality of ANN was determined with the root mean square error (RMS) for the training and validation sets. Perceptrons with one (MLP1) and two (MLP2) hidden layers and radial basis function networks (RBF) were investigated.

For the quality evaluation of the prepared ANN, sensitivity (*Se*), specificity (*Sp*) and accuracy (*Acc*) were applied. Assuming that *a* denotes the number of correctly recognized difficult calvings, *b* – number of incorrectly recognized easy calvings, *c* – number of incorrectly recognized difficult calvings and *d* – number of correctly recognized easy calvings, the probabilities were calculated according to the following formulae:

$$Se = \frac{a}{a+c},\ Sp = \frac{d}{b+d},\ \ Acc = \frac{a+d}{a+b+c+d}$$

The differences between probabilities were determined for the combined training and validation sets and for the test set using the test for proportions. In order to evaluate how well the model fitted the data, Akaike information criterion (AIC) was also used [Grzesiak et al. 2010]. The lower the AIC value the better the fit of the model.

After preparation and quality evaluation of ANN their usefulness for the detection of dystocia based on the test set was verified. The aforementioned probabilities, receiver operating characteristic (ROC) curves and area under curve (AUC) were used for this purpose. AUC = 1 indicates perfect discrimination and AUC = 0.5 no discrimination at all [Bradley 1997].

The last stage of the research was the indication of the input variables with the significant influence on the determination of calving difficulty class. The ratio index and rang were used for this purpose (the higher the ratio index and the lower the rang the greater the significance of a given variable [Grzesiak et al. 2010]).

RESULTS AND DISCUSSION

The following three ANN were selected for further analysis (one ANN with the lowest RMS for each type): MLP1 with the structure 10-4-1 (the number of neurons in the input, hidden and output layers, respectively, RMS = 0.22), MLP2 with the structure 10-17-17-1 (17 neurons in each hidden layer, RMS = 0.25) and RBF network with the structure 10-25-1 (RMS = 0.19).

Statistically significant differences between the analysed probabilities for individual types of ANN were not recorded (Table 4). Sensitivity and specificity were approx. 90%. Accuracy was also high and amounted to approx. 89%.

Table 4. Values of probabilities for artificial neural networks
Tabela 4. Wartości prawdopodobieństw dla sztucznych sieci neuronowych

Set Zbiór	n	Sensitivity Czułość	Specificity Specyficzność	Accuracy Trafność
MLP1 (RMS = 0.22)				
L	330	0.9048	0.8819	0.8848
V	100	0.8462	0.9425	0.9300
L+V	430	0.8909	0.8960	0.8953
T	101	0.8333	0.8202	0.8218
MLP2 (RMS = 0.25)				
L	330	0.9348	0.9085	0.9121
V	100	0.7778	0.8022	0.8000
L+V	430	0.9091	0.8827	0.8860
T	101	0.7500	0.8764	0.8614
RBF (RMS = 0.19)				
L	330	0.8780	0.8927	0.8909
V	100	1.0000	0.8721	0.8900
L+V	430	0.9091	0.8880	0.8907
T	101	0.7500	0.8539	0.8416

L – training – uczący; V – validation – walidacyjny; T – test – testowy; MLP1 – perceptron with one hidden layer – perceptron z jedną warstwą ukrytą; MLP2 – perceptron with two hidden layers – perceptron z dwoma warstwami ukrytymi; RBF – radial basis function network – sieć o radialnych funkcjach bazowych; RMS – root mean square error – pierwiastek błędu średniokwadratowego.

The calculated AIC values were 23.69, 24.78 and 24.23 for MLP1, MLP2 and RBF network, respectively.

As before, for the detection of dystocia on the basis of the test set, no statistically significant differences in the probability values for the individual types of ANN were found (Table 4). Sensitivity for MLP2 and RBF network was somewhat lower than that for MLP1. Besides, the values of the remaining probabilities were high and exceeded 82%.

AUC for the test set (Fig. 1) was the greatest for the RBF network and the smallest for MLP2.

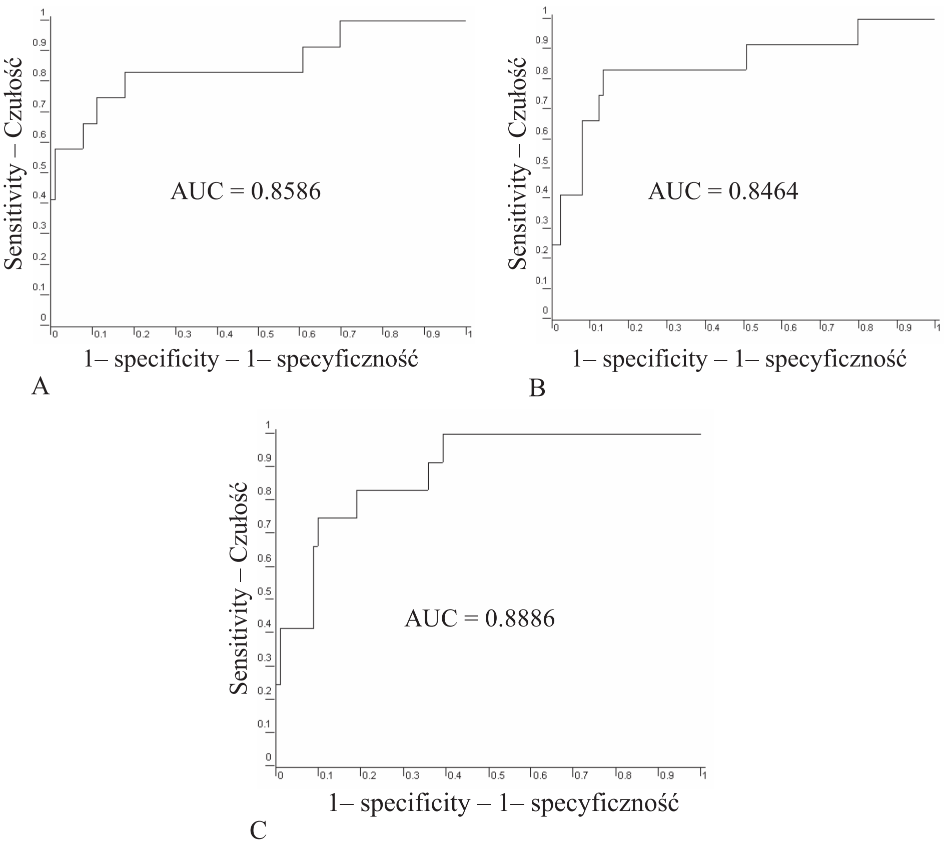

Fig. 1. Receiver operating characteristic (ROC) curves for detection of dystocia in heifers: A – using perceptron with one hidden layer (MLP1), B – using perceptron with two hidden layers (MLP2), C – using radial basis function (RBF) networks

Rys. 1. Krzywe charakterystyki roboczej odbiorcy (ROC) dla detekcji trudnych wycieleń u jałówek: A – za pomocą perceptronu z jedną warstwą ukrytą (MLP1), B – za pomocą perceptronu z dwoma warstwami ukrytymi (MLP2), C – za pomocą sieci o radialnych funkcjach bazowych (RBF)

Gestation length had the greatest influence on the determination of calving difficulty class for all types of networks (Table 5). For MLP1 and MLP2, the major effect of body condition score index was also observed, and for RBF network, the effect of calving season was noted. Moreover, the variables denoting the *ERα-Bgl*I and *CYP19-Pvu*II genotypes and percentage of HF genes had somewhat less influence.

The RMS value for the individual ANN indicated their good quality. Sensitivity and specificity calculated based on the training and validation sets (Table 4) obtained in the present research were similar to those obtained by other authors in similar studies. For example, Krieter et al. [2006], in the study on oestrus detection in cows using ANN, recorded comparable values of sensitivity (0.79) and specificity (1.00). On the other hand, Cavero et al. [2008] obtained sensitivity in the range 0.60–0.66 and 0.77–0.79 depending on the way of defining the occurrence of mastitis (the criterion was the somatic cell count: above 100,000 · ml^{-1} or above 400,000 · ml^{-1}).

Table 5. Sequence of variables according to their influence on the determination of calving class
Tabela 5. Uszeregowanie zmiennych według ich wpływu na determinację klasy wycielenia

	HF	BGLI	SNABI	PVUII	GEST	BCSI	SEASON	AGE
				MLP1				
R	7	3	5	4	1	2	8	6
R I	1.0255	1.0868	1.0717	1.0794	1.5007	1.2882	1.0077	1.0602
				MLP2				
R	3	4	8	7	1	2	6	5
RI	1.0108	1.0107	0.9941	0.9982	1.3265	1.1172	1.0017	1.0046
				RBF				
R	5	7	8	4	1	3	2	6
RI	1.0149	0.9991	0.9988	1.0379	1.2595	1.1080	1.1226	1.0009

HF – percentage of HF genes in heifer's genotype – procent genów HF w genotypie jałówki; BGLI – *ERα-Bgl*I genotype – genotyp *ERα-Bgl*I; SNABI – *ERα-Sna*BI genotype – genotyp *ERα-Sna*BI; PVUII – *CYP19-Pvu*II genotype – genotyp *CYP19-Pvu*II; GEST – gestation length – długość ciąży; BCSI – body condition score index – indeks kondycji; AGE – age at calving – wiek przy wycieleniu; R – rang – ranga; RI – ratio index – wskaźnik ilorazu; MLP1 – perceptron with one hidden layer – perceptron z jedną warstwą ukrytą – MLP2 – perceptron with two hidden layers – perceptron z dwoma warstwami ukrytymi; RBF – radial basis function network – sieć o radialnych funkcjach bazowych.

The overall number of the correctly recognized calvings from both classes in the present study was also high.

The AIC value, applied to compare the models, indicated that MLP1 was characterised by the best fit to experimental data.

It seems obvious that the correct detection of heifer with dystocia (sensitivity) is much more important for a breeder than prediction of the occurrence of easy calving (specificity) and the results obtained in the present study should be discussed in this context. In the study by Hassan et al. [2009] concerning mastitis detection but with distinguishing three classes (no infection, infection caused by minor pathogens and infection caused by major pathogens) sensitivity for individual classes was in a broader range than that in the present research and amounted to 0.82–0.98, 0.44–0.86 and 0.20–0.40, respectively, depending on the proportion of records with and without infection. However, the value of sensitivity similar to that in the present study was obtained by Krieter et al. [2006]. The percentage of correctly recognized cows in oestrus was 78%. Even higher sensitivity was recorded by Sun [2010] and Pastell and Kujala [2007]. The percentage of correctly diagnosed cows with mastitis and lameness was 91% and 100%, respectively.

Also the values of specificity cited in the literature vary. Perfect specificity (equal to 1.0) was obtained by Krieter et al. [2006], whereas Hassan et al. [2009] using division into 3 categories reported specificity for individual classes amounting to 0.52–0.88, 0.75–0.97 and 0.99–1.00, respectively. In the study by Sun [2010], specificity was very similar to that in the present study (0.87), whereas in the work by Pastell and Kujala [2007] the percentage of correctly indicated cows without locomotion problems was 58%.

The accuracy obtained in the present study can be regarded as relatively high (Table 4). Somewhat lower level of correct classification was obtained by Heald et al. [2000] in the research on mastitis detection (0.57–0.71) and Gardner et al. [1999] in detection of ketosis in cows based on the parameters of exhaled air (0.76). However, Sun et al. [2010] and Pastell and Kujala [2007] reported higher accuracy. The percentage of correctly diagnosed healthy and ill cows (with mastitis or lameness) was 91% and 96%, respectively.

The relationship between sensitivity and the level of so-called false alarms (1-specificity) is depicted using ROC curves. The most favourable shape of this curve was characteristic of the RBF network, which is also indicated by the AUC value of 0.89 (Fig. 1). For comparison, in the study by Pastell and Kujala [2007] this value equalled 0.86.

The most significant variable (on the basis of ratio index and rang) for the determination of calving class (Table 5) appeared to be gestation length, which is in accordance with results reported by other authors. In general, both too short (below 265 days) and too long (above 285 days) gestation may adversely affect calving ease in heifers through the death of foetus, occurrence of twin pregnancies and premature calving in the first case and too large size of a foetus in the second case [Mee 2008]. In the analysis concerning Holstein primiparous cows in the USA, López de Maturana et al. [2008] found the non-linear relationship between gestation length and the occurrence of dystocia. Percentage of difficult calvings was the lowest at gestation length of approx. 273–275 days. Relatively constant level of calving difficulties remained at gestation length of 266 – 275 days and increased systematically after exceeding this value. On the other hand, in the study on Holstein primiparous cows in Denmark [Hansen et al. 2004], weak or medium genetic correlation between gestation length and calving difficulty was found.

The second most important variable was the difference in the body condition score of heifers (BCSI) before calving and at calving (Table 5). It is known that both too low and too high body condition is a factor favouring more difficult calvings [Meijering 1984, Hoffman et al. 1996]. According to Mee [2008], currently recommended BCS in Ireland

(6-point scale) at calving of heifers is 2.75–3.00 scores. In the study on Ayrshire and Holstein primiparous cows [Bastin et al. 2010], mainly positive genetic correlation between body condition score and calving ease determined by dam's genotype and negative correlation between BCS and calving ease for direct effect were found, whereas phenotypic correlations were close to zero.

The sequence of remaining variables differed depending on the type of ANN (Table 5). For RBF network, calving season appeared to be significant variable. For perceptrons, the percentage of genes of Holstein-Friesian breed and *ERα-Bgl*I genotype were quite important. Contrary to our expectations, the effect of the two remaining polymorphisms was less significant. In the case of *CYP19-Pvu*II polymorphism, the value of the ratio index proved its contribution to the determination of calving class, whereas this value for the *ERα-Sna*BI exceeded unity only for MLP1.

CONCLUSIONS

Summarizing the present research, it can be concluded that: 1) different types of ANN applied for the classification of dystocia in heifers were characterized by high and similar ability to classify difficult and easy calvings, 2) in particular MLP1 with four neurons in a hidden layer showed the best results in detecting dystocia (the highest sensitivity on the test set) 3) factors most influencing the course of parturition were: gestation length, BCSI, and to a lesser degree, percentage of HF genes in heifer's genotype and *CYP19-Pvu*II and *ERα-Bgl*I genotypes, 4) since the difference between body condition score before and during calving was an important variable affecting calving class, breeders may be suggested to assess BCS in order to obtain more precise information on the possible course of calving, 5) rather high level of the correct detection of dystocia in heifers obtained by means of ANN using a small number of explanatory variables indicates that ANN may be a useful indirect tool for the prevention of dystocia in heifers.

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DETEKCJA JAŁÓWEK Z TRUDNYMI PORODAMI ZA POMOCĄ SZTUCZNYCH SIECI NEURONOWYCH Z UWZGLĘDNIENIEM GENOTYPÓW *ERα-BGL*I, *ERα-SNA*BI I *CYP19-PVU*II

Streszczenie. Celem niniejszej pracy była detekcja jałówek z trudnymi porodami przy użyciu sztucznych sieci neuronowych (SSN). Wykorzystano w tym celu dane o 531 wycieleniach jałówek rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej oraz 8 zmiennych diagnostycznych. Zmienną wyjściową była klasa trudności porodu: trudny lub łatwy. Analizowano perceptrony z jedną (MLP1) i dwoma (MLP2) warstwami ukrytymi oraz sieci o radialnych funkcjach bazowych (RBF). Pierwiastek błędu średniokwadratowego oraz struktura wybranych SSN (liczba neuronów w warstwach wejściowej, ukrytej i wyjściowej) były następujące: 0,22, 10-4-1; 0,25, 10-17-17-1 i 0,19, 10-25-1 odpowiednio dla MLP1, MLP2 i RBF. Odsetek prawidłowo rozpoznanych jałówek z trudnymi i łatwymi porodami oraz odsetek prawidłowo zdiagnozowanych jałówek z obu kategorii dla zbioru uczącego i walidacyjnego wynosiły ok. 90%. Wartości te dla zbioru testowego wynosiły odpowiednio: 75–83%, 82–88% i 82–86%. W obu przypadkach nie stwierdzono istotnych statystycznie różnic między tymi proporcjami. Następujące zmienne miały największy wkład w detekcję jałówek z trudnymi porodami: długość ciąży, indeks BCS, genotypy *CYP19-Pvu*II i *ERα-Bgl*I oraz procentowy udział genów hf w genotypie jałówki.

Słowa kluczowe: genotypy, jałówki mleczne, sztuczne sieci neuronowe, trudny poród

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