Detection of susceptibility of dairy cows to clinical *mastitis* by artificial neural networks based on selected genotypes and milk production records

Daniel Zaborski*, Katarzyna Wojdak-Maksymiec**, and Wilhelm Grzesiak*

Abstract

The aim of this study was to verify the applicability of artificial neural networks to the detection of dairy cows susceptible to clinical mastitis based on veterinary records, milk recording data and selected genotypes (lactoferrin, lysozyme, tumor necrosis factor alpha and combined defensin genotypes). Moreover, we wanted to determine the effects of complete and reduced sets of predictors (input variables) on the detection performance of the neural models. A total of 24712 test-day records from 990 Polish Holstein-Friesian Black-and-White cows were analyzed. Eight continuous and eight categorical predictors (including proportion of Holstein-Friesian genes, calving age, milk yield and composition, four genotypes, lactation number and stage, calving season) were used. Health state (mastitis vs. healthy) was an output variable. Multilayer perceptrons and radial basis function networks were trained and tested, yielding the percentages of correctly detected cows susceptible to clinical mastitis and resistant ones in the range of 57.8 to 63.3 % and 60.3 to 66.6 %, respectively. The most significant factors affecting mastitis occurrence were: lactation number and stage, calving age, the season of calving and mastitis diagnosis, tumor necrosis factor alpha and combined defensin genotypes. Also, Lactroferrin genotype was quite significant for two neural models, whereas lysozyme genotype had a much smaller effect on the health status of cows. After reducing the initial set of 16 predictors to only five, decreased performance of the networks was observed. It can be concluded that an indication of cows susceptible to clinical mastitis may facilitate the application of preventive measures and consequently reduce mastitis incidence.

Keywords: *inflammation, udder, diagnosis, prevention, sensitivity analysis, dairy cattle*

Zusammenfassung

Erkennen einer Mastitis-Anfälligkeit von Milchkühen mittels künstlicher neuraler Netze auf Basis ausgewählter Genotypen und Milchleistungsdaten

Ziel der Studie war, die Eignung künstlicher neuraler Netze zu prüfen, auf Basis tierärztlicher Diagnosen, Milchleistungsdaten und ausgewählter Genotypen (Lactoferrin, Lysozym, Tumornekrosefaktor-α und kombinierte Defensin-Genotypen) die Anfälligkeit von Milchkühen zur klinischen Mastitis zu erkennen. Zudem wurde der Einfluss der Prädikatorenzahlen auf die Detektionsfähigkeit der neuralen Modelle geprüft. Es wurden 24712 Versuchstagsdaten von 990 polnischen Kühen (Rasse: Holstein-Friesian schwarz weiß) analysiert. Verwendet wurden 8 stetige und 8 nominale Prädikatoren (u. a. Genanteil der Rasse Holstein-Friesian, Abkalbealter/-saison, Milchertrag/-zusammensetzung, vier Genotypen, Laktationsnummer/-phase). Der Gesundheitszustand (Mastitis/gesund) war Ausgangsvariable. Mehrlagige Perzeptronen und Netze mit radialen Basisfunktionen wurden generiert und getestet. Die Werte bei den Mastitis-anfälligen Kühen lagen bei 57,8 bis 63,3 % , bei den resistenten Kühen bei 60,3 bis 66,6 %. Die signifikantesten Faktoren für das Auftreten von Mastitis waren: Laktationsnummer/-phase, Abkalbungsalter/-saison, Zeit der Mastitis-Diagnose, Tumornekrosefaktor α und die kombinierten Defensin-Genotypen. Für zwei neurale Netze war der Genotyp des Lactoferrin ebenfalls sehr signifikant, obwohl der Lysozym-Genotyp die Gesundheit nur wenig beeinflusste. Nach Reduzierung der Prädikatoren von 16 auf 5 wurde eine verminderte Aussagekraft der neuralen Netze beobachtet. Es kann festgehalten werden, dass eine Indikation von Mastitis-anfälligen Kühen Präventivmaßnahmen erleichtern und folglich die Mastitis-Häufigkeit in Herden reduzieren kann.

Schlüsselworte: *Entzündung, Euter, Diagnose, Vorbeugung, Analyse der Anfälligkeit, Milchvieh*

* West Pomeranian University of Technology, Laboratory of Biostatistics, Department of Ruminants Science, Doktora Judyma 10, 71-466 Szczecin, Poland

** West Pomeranian University of Technology, Department of Genetics and Animal Breeding, Piastow 45, 70-311 Szczecin , Poland

Contact: daniel.zaborski@zut.edu.pl

1 Introduction

Mastitis is defined as an inflammation of the mammary gland usually caused by bacterial infections (dos Reis et al., 2013; Hillerton and Berry, 2005). It results in a considerable reduction in milk yield (even up to approx. 375 kg for a single mastitis case) (Cavero et al., 2007) and the change in the content of its constituents (an increase in somatic cell count – SCC, concentration of whey proteins, albumins, immunoglobulins, lactoferrin, sodium and chlorine ions as well as a decrease in the content of lactose, lactalbumins, fat, casein, concentration of calcium and potassium ions) (Bruckmaier et al., 2004; Harmon, 1994), which consequently leads to large economic losses estimated at about USD 2 billion annually in the USA, about GBP 300 million annually in the UK, GBP 14 million annually in Northern Ireland, approx. EUR 693 per cow annually in Ireland and EUR 164 to 235, on average, per cow in the Netherlands (Viguier et al., 2009). These losses also result from the costs of diagnosis and treatment, increased labor intensity, too early culling of cows, diseases of calves, and susceptibility to other disorders post-partum (Wawron, 2007). The current European Union legal regulations require the determination of SCC in raw milk and antibiotic residues are prohibited. Therefore, milk that does not meet the above criteria cannot be sold and this generates additional costs for the farmer. Because antibiotic treatment of cows with mastitis is expensive and requires waiting period for the milk, mastitis prevention is rather preferred (Pyörälä, 2002). There are many risk factors for mastitis such as: genetic factors (e. g. genetic selection for a maximum milk yield), environmental factors (inappropriately carried out mechanical milking, confinement management system, inappropriate bedding and nutrition) and physiological factors (Harmon, 1994; Sordillo, 2005). They lead to the impairment of immunological mechanisms of the mammary gland and, consequently, increase its susceptibility to microbial infections such as: *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli* and others (Hettinga et al., 2008), although sometimes an inflammatory condition may have a non-infectious etiology (Bradley, 2002). A frequently encountered problem in mastitis detection under field conditions is the impossibility of indicating explicitly disease-causing factors in a herd (Bradley, 2002).

Susceptibility to mastitis is also affected by gene polymorphisms such as: tumor necrosis factor alpha gene (*TNF-α*), lactoferrin gene (*LTF*), macrophage expressed lysozyme encoding-gene (*mLYZ*) or genes encoding defensins (*DEF*). TNF is responsible for the activation of the whole immune system, increasing the permeability of vascular endothelium and inducing chemotaxis, whereas LTF has broad antibacterial, antiviral, antifungal and antiparasitic properties (Wojdak-Maksymiec and Mikolajczyk, 2012). On the other hand, LYZ acts as an antibacterial agent against vegetative cells of many different microorganisms; however, Gram-positive bacteria differ considerably in their sensitivity to lysozyme depending on strain, species and specific conditions, while Gram-negative bacteria are, generally, less sensitive to lysozyme mainly due to the protection of their cell wall by an external membrane, although it has been shown that, e. g., bovine milk lysozymes are effective against five Gram-negative bacteria species that are unaffected by the lysozyme from hen egg white, which proves that lysozyme activity differs depending on its source (Davidson et al., 2010). Finally, defensins constitute a wide and varied group of peptide antibiotics acting against bacteria, viruses and fungi (Bals, 2000; Tunzi et al., 2000), which can be divided into three groups (α, β and θ), from which the second one, among others, is largely represented in cattle and defensins from this group were identified, e. g. in the tracheal, intestinal and tongue mucosa (Diamond et al., 1991; Schonwetter et al., 1995; Tarver et al., 1998).

Considering a significant role of mastitis in cattle farming, an early indication of susceptible cows is of utmost importance. Such detection can be performed by artificial neural networks, among others, especially in a situation, when a larger number of variables are problematic in a traditional statistical analysis (Verleysen and François, 2005).

An artificial neural network is a specific information processing system simulating a biological nervous system. It is composed of basic processing units called artificial neurons connected with each other between network layers (each neuron of a preceding layer with each neuron of the next layer). Weights, corresponding to synaptic potentials in biological neurons, are associated with these connections. They constitute network's knowledge representation and are appropriately modified during the learning process, whose aim is to prepare the network for performing specific tasks, e. g. classification or regression ones. The artificial neural network (ANN) types most frequently applied to supervised classification (e. g. mastitis detection) are multilayer perceptrons with one (MLP1) and two (MLP2) hidden layers and radial basis function (RBF) networks. Recent applications of ANN in cattle farming include: prediction of 305-day milk yield for the first lactation (Njubi et al., 2010), prediction of 305-day lactation yield from test milking records (Abbassi Daloii et al., 2010; Tahmoorespur et al., 2012), prediction of lifetime milk yield in cows (Gandhi et al., 2009a, 2009b, 2010), analysis and classification of lactation curves for the first one hundred days of lactation (Cárdenas Mansilla, 2008), decision support for mating animals in a herd (Njubi et al., 2009), preslaughter evaluation of marbling based on ultrasound analysis (Fukuda et al., 2012) and the modeling of fermentation processes (concentration of acetate, propionate and butyrate) in the rumen (Craninx et al., 2008).

Much attention has also been paid to the application of ANN to mastitis detection based on parameters such as: milk electrical conductivity, lactation and daily milk yield, fat and protein content in milk, milk flow rate, lactation length, number and stage, cow body temperature, and somatic cell count (Ankinakatte et al., 2013; Cavero et al., 2008; Hassan et al., 2009; López-Benavides et al., 2003; Nielen et al., 1995a,b; Wang and Samarasinghe, 2005; Yang et al., 1999, 2000).

Therefore, the aim of the present study was to verify the applicability of different ANN types to the detection of dairy cows susceptible to mastitis based on veterinary records (clinical mastitis cases), milk recording data, the mean values of natural logarithm of SCC for the sires of the studied cows and the above-mentioned genotypes. Moreover, we wanted to determine the effects of both complete and reduced sets of selected predictors (input variables) on the detection of predicted mastitis cases in cows.

Table 1
Means and standard deviations (in parentheses) for continuous predictors in the training (L) and test (T) data sets

Set	n	HF (%)	AGE (months)	MILK (kg)	FT (%)	PR (%)	LAE (%)	UR (mg/l)	SIRE ln(cells/ml)
L	18539	85.70 (13.97)	50.26 (20.11)	34.10 (9.54)	4.02 (0.80)	3.55 (0.42)	4.82 (0.25)	242.21 (80.16)	5.28 (0.56)
T	6173	85.92 (13.78)	50.87 (20.43)	34.16 (9.62)	4.01 (0.79)	3.55 (0.42)	4.81 (0.26)	239.86 (79.65)	5.28 (0.58)
Total	24712	85.75 (13.93)	50.41 (20.19)	34.12 (9.56)	4.02 (0.80)	3.55 (0.42)	4.82 (0.25)	241.62 (80.03)	5.28 (0.56)

Variable abbreviations are as given in material and methods section

2 Materials and methods

The study involved a total of 990 Polish Holstein-Friesian Black-and-White cows kept in one open barn throughout the year. They were not pastured in any season. The building in which the animals were housed consisted of two wings. The milking parlor and the room for cooling and storing milk were located between them. The building was a roof shelter with incomplete side walls (there was a clearance between the roof and the walls to enable the access of light and air circulation). The animals were not stall-tied, which facilitated their driving to the milking parlor. The stalls were bedded with straw. The cows were fed a normalized diet in the form of a total mixed ration (TMR) prepared from maize silage. The maize silage was mixed with seasonal ingredients (e. g. green forage, root crops). In addition, nutrition was supplemented with individually determined (from the current lactation yield records) portions of a concentrate and diet supplements. The feed was fed from a mixer-wagon directly to a trough. All cows were watered *ad libitum* from automatic drinkers. Milking was carried out twice daily in a herringbone milking parlor. The mean herd milk yield was 10,914 kg. Udders and teats were examined for clinical mastitis signs during each milking. The signs were easily visible without extra equipment or were confirmed using a thermometer or a strip cup (in order to provide high visibility of clots or flakes present in the milk). All alarming symptoms were reported to an experienced veterinarian employed on the farm, who ultimately diagnosed clinical mastitis cases. Cows were dried-off six weeks before an estimated calving date. Antibiotic protection was used if the symptoms of an inflammatory condition classified as mastitis were observed during the dry-off period. Otherwise, antibiotic therapy was avoided.

The present study included test milking records and data on mastitis occurrence collected between September 2003 and April 2008 from cows kept on the farm located in the northwestern region of our country. An initial set of 38794 information records was reduced to 24712 after editing for erroneous and incomplete data. The following predictors (input variables) were used: percentage of Holstein-Friesian genes (HF), calving age (AGE), daily milk yield (MILK), fat percentage in milk (FT), protein percentage in milk (PR), lactose percentage in milk (LAE), urea content in milk (UR), arithmetic mean of lnSCC for a cow's sire determined from the lnSCC of his daughters (SIRE), Lactroferrin genotype (LTF), tumor necrosis factor alpha genotype (TNF-α), lysozyme genotype (mLYZ), combined defensin genotypes (CDG), lactation number (LACT), test milking season (TESTS; autumn from September to November, winter from December to February, spring from March to May and summer from June to August), calving season (CALS; defined as previously described) and lactation stage (STAG; first stage from 0 to 30 days, second stage from 31 to 60 days, etc. until 315 days of lactation). Mean values of continuous predictors are given in Table 1 and the distributions of categorical predictors are presented in Table 2.

Genotypes were assayed with polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method based on the DNA isolated from peripheral blood. The details on genetic analyses are presented elsewhere (Wojdak-Maksymiec, 2009).

Mastitis class determined based on veterinarian's diagnosis (acute or chronic mastitis, drying-off with antibiotic protection) was an output variable with two categories: a cow with mastitis and a healthy cow. Acute mastitis was characterized by a sudden onset of local and systemic clinical symptoms. The diseased quarter was red, hot, edematous, painful, sensitive to the touch, and the milk quality was changed. It was more or less watery with the presence of serous or purulent secretion or blood. Systemic symptoms included an increase in internal body temperature, a higher pulse rate, weakness, a decrease in or a lack of appetite, reduced milk yield or no milk yield at all. Chronic mastitis, on the other hand, was characterized by mild, local clinical symptoms in the form of small changes in milk such as the presence of lumps, flakes, watery consistency and slight discoloration. The mastitic quarter was sometimes slightly swollen, red and sensitive to the touch. The whole data set (24712 records) was randomly divided into a training set (L; 18539 records) for network preparation and a test set (T; 6173 records) for the verification of mastitis detection performance (Table 1).

Table 2
Distributions of categorical predictors and output (dependent) variable in the training (L) and test (T) data sets

Set	L		T		Total	
Category	n	%	n	%	n	%
LTF						
AB	8403	45.33	2796	45.29	11199	45.32
AA	10136	54.67	3377	54.71	13513	54.68
TNF-α						
CC	6352	34.26	2138	34.63	8490	34.36
CT	8374	45.17	2829	45.83	11203	45.33
TT	3813	20.57	1206	19.54	5019	20.31
mLYZ						
CC	17628	95.09	5903	95.63	23531	95.22
CT	911	4.91	270	4.37	1181	4.78
CDG						
A1A2/B1B2/C1C2	13955	75.27	4616	74.78	18571	75.15
A1A2/B2/C1C2	428	2.31	157	2.54	585	2.37
A2/B1B2/C2	106	0.57	42	0.68	148	0.60
A1A2/B1B2/C1	763	4.12	253	4.10	1016	4.11
A2/B1B2/C1C2	518	2.79	178	2.88	696	2.82
A1A2/B1/C1C2	406	2.19	146	2.37	552	2.23
A1A2/B1B2/C2	464	2.50	151	2.45	615	2.49
A1/B1B2/C2	207	1.12	62	1.00	269	1.09
A1/B1B2/C1	169	0.91	60	0.97	229	0.93
A1/B1B2/C1C2	586	3.16	189	3.06	775	3.14
A1A2/B1B2/C2C2	82	0.44	25	0.40	107	0.43
A1A2/B2/C1	65	0.35	26	0.42	91	0.37
A2/B2/C1C2	131	0.71	40	0.65	171	0.69
A1/B1/C1C2	280	1.51	95	1.54	375	1.52
A1A2/B1/C1	123	0.66	49	0.79	172	0.70
A1/B1/C1	37	0.20	14	0.23	51	0.21
A1A2/B1B1/C1	21	0.11	8	0.13	29	0.12
A2/B2/C1	18	0.10	9	0.15	27	0.11
A2/B1B2/C1	62	0.33	23	0.37	85	0.34
A1A2/B1/C2	92	0.50	25	0.40	117	0.47
A1/B2/C1C2	16	0.09	2	0.03	18	0.07
A1A2/B2/C2	10	0.05	3	0.05	13	0.05
LACT						
I	4712	25.42	1516	24.56	6228	25.20
II	4947	26.68	1639	26.55	6586	26.65
III	3603	19.43	1224	19.83	4827	19.53
IV	2802	15.11	914	14.81	3716	15.04
V	1778	9.59	621	10.06	2399	9.71
VI	697	3.76	259	4.20	956	3.87
TESTS						
Autumn	5157	27.82	1723	27.91	6880	27.84
Winter	5315	28.67	1725	27.94	7040	28.49
Spring	5091	27.46	1728	27.99	6819	27.59
Summer	2976	16.05	997	16.15	3973	16.08
CALS						
Autumn	4355	23.49	1458	23.62	5813	23.52
Winter	5066	27.33	1627	26.36	6693	27.08
Spring	4395	23.71	1488	24.10	5883	23.81
Summer	4723	25.48	1600	25.92	6323	25.59

Set	L		T		Total	
Category	n	%	n	%	n	%
STAG						
I	1773	9.56	584	9.46	2357	9.54
II	1981	10.69	678	10.98	2659	10.76
III	1938	10.45	665	10.77	2603	10.53
IV	1964	10.59	660	10.69	2624	10.62
V	1912	10.31	644	10.43	2556	10.34
VI	1913	10.32	626	10.14	2539	10.27
VII	1883	10.16	618	10.01	2501	10.12
VIII	1785	9.63	607	9.83	2392	9.68
IX	1683	9.08	545	8.83	2228	9.02
X	1246	6.72	383	6.20	1629	6.59
XI	461	2.49	163	2.64	624	2.53
MAST – output variable						
Mastitis	1404	7.57	455	7.37	1859	7.52
Healthy	17135	92.43	5718	92.63	22853	92.48

Variable abbreviations are as given in material and methods section

The ratio of "mastitis" to "healthy" records in the training and test sets was approx. 1:10. A validation subset (6182 records) was also randomly created from part of the training records for the current monitoring of learning process and elimination of overtraining.

Training data were pre-processed before being fed to the network's input layer through the scaling of continuous predictors to an appropriate interval (using the mini-max method) and the conversion of categorical predictors to a numeric form (using the binary or one-of-N encoding). Network's output values were then converted to a class label during the post-processing stage (Bishop, 1995). A classical back-propagation algorithm proposed by Rumelhart et al. (1986) was used for the MLP network training. In this method, input signals were propagated forward through successive network layers to the output layer, where the network's response was compared against the real (desired) value of an output variable and thus an error of output neurons was calculated. Next, this error was back-propagated to preceding (hidden) layers and multiplied by the same weights that were used for passing the signal from input to output. This process was repeated iteratively until reaching the minimum of an error function. In the present study, the conjugate gradient algorithm was additionally used to fine-tune network's weights at the last stage of training. It consisted in an iterative determination of the search direction of an error function minimum, finding this minimum and then identification of a new search direction that was orthogonal to all previous directions (Haykin, 2009). The training of the RBF networks was performed in two stages: determination of the RBF centers (in the form of weights of the hidden layer neurons) and RBF radii (in the form of hidden neurons' bias values) and optimization of the output (linear) layer using pseudoinversion (StatSoft, 1998).

Network performance was assessed based on the root mean squared error (RMSE) on the validation set (Salehi et al., 1998). Statistica® Neural Networks software (v. 4.0F, StatSoft Inc., Tulsa, OK, USA) was used for building and training the MLP and RBF networks. It enabled the choice of an optimal network architecture, learning algorithm and network parameters (error function, activation functions, acceptance and rejection thresholds, learning epochs, learning rate and momentum, as well as the appropriate methods of the RBF center and radius determination for the RBF networks). The exhaustive search mode of the program was selected in order to maintain the trade-off between network's performance and complexity. Calculations were performed using Pentium 2.40GHz IBM-PC compatible machine and lasted for approx. 40 hours. The MLP and RBF networks with the lowest RMSE on the validation set were used for the detection of cows susceptible to mastitis.

Akaike information criterion (AIC), Bayesian information criterion (BIC) and the G-square statistic were applied to the comparison of the MLP and RBF networks quality (performance on the training and validation sets). They were calculated according to the following formulae (Dayton, 2003; Liddle, 2007; StatSoft, 2011):

$$AIC = N\ln(MSE) + 2k \quad (1), \text{ or}$$

$$AIC_c = N\ln(MSE) + 2k + \frac{2k(k+1)}{(N-k-1)} \text{ if } N/k \le 40 \quad (2),$$

$$BIC = N\ln(MSE) + k\ln(N) \quad (3),$$

$$G^2 = 2\sum_{i=1}^{2} O_i \ln\left(\frac{O_i}{E_i}\right) \quad (4),$$

where

MSE mean squared error,
k number of model parameters,
N number of training records,
O_i observed number of records,
E_i number of records predicted by the model.

The minimum values of these criteria were considered when choosing the best model. The detection performance of the MLP and RBF networks was assessed using sensitivity (Se), specificity (Sp) and accuracy (Acc). In addition, false positive P(FP) and false negative P(FN) rates, as well as the *a posteriori* probabilities of true positives P(PSTP) and true negatives P(PSTN) were calculated (Yang et al., 1999). Statistical significance of the differences between the probabilities was determined with the test for proportions. Moreover, the receiver operating characteristic (ROC) curves were plotted and the area under the curves (AUC) was estimated. The ROC curve shows the relationship between sensitivity and the false positive rate (1-specificty), summarizing, at the same time, the sensitivity and specificity values for different cut-off points. The standard error of AUC [SE(AUC)] was also computed according to the following formula (Greiner et al., 2000):

$$SE(AUC) = \sqrt{\frac{AUC(1-AUC)+(N_1-1)(Q_1-AUC^2)+(N_0-1)(Q_2-AUC^2)}{N_0 N_1}} \quad (5),$$

$$Q_1 = \frac{AUC}{2-AUC};\ Q_2 = \frac{2AUC^2}{1+AUC} \quad (6),$$

where

N_0 number of records without mastitis,

N_1 number of records with mastitis ($N_0 + N_1 = N$).

Apart from assessing the detection performance of the neural models based on the test set, the sequence of predictors (input variables) was also established according to their contribution to the determination of mastitis/health class (mastitis vs. no mastitis). The following criteria were used for this purpose:

1. Ratio of the network's error after removing a given predictor to the error for the full model (the greater the ratio, the more important the predictor);
2. RMSE after variable's removal;
3. Rank, ordering predictors according to their decreasing error (rank of 1 denotes the most significant variable, rank of 2 indicates slightly less significant variable, etc.).

Network's sensitivity analysis was performed on the training and validation sets.

Bearing in mind that smaller networks learn easier, better generalize acquired knowledge and are less costly in exploitation (require fewer input variables whose values need to be determined), we also aimed in the present study at checking (based on the error ratio) how the reduction of a predictor set affected the quality of the models and their detection performance. In order to do so, a two-stage procedure was applied, in which the number of predictors was reduced twice (first to the eight most significant variables out of 16 in the initial model and then to only five most important ones out of the eight previously used). After each stage, the networks were re-trained and re-evaluated. The same statistical tests as previously described were applied to difference significance testing.

3 Results

3.1 Model quality

Of the analyzed ANNs, three- and four-layer MLP networks were characterized by the RMSE values on the validation set equal to 0.2626 and 0.2627, respectively. They had the following architectures: 60-2-1 and 60-31-31-1 (the number of neurons in the input layer, hidden layer(s) and the output layer, respectively). The first one was trained for 50 epochs with the back-propagation algorithm and, additionally, for 14 epochs with the conjugate gradient method, whereas the second one was trained for 46 epochs with the back-propagation algorithm only. On the other hand, the RBF network (with a 60-51-1 architecture) was characterized by the RMSE value of 0.2656. The values of the quality measures for the MLPs with one and two hidden layers and the RBF networks calculated from the training set are shown in Table 3. As can be seen, significant differences in Se were recorded between MLPs and the RBF networks ($P \leq 0.05$). Moreover, significant differences in Sp and Acc were observed for each pair of the neural models ($P \leq 0.05$).

Table 3
Values of G^2, AIC, BIC, sensitivity (Se), specificity (Sp) and accuracy (Acc) for the training set (n = 18539)

Parameter	MLP1	MLP2	RBF
G^2	16013.80	15252.06	18238.64
AIC	-50247.52	-44107.54	-45395.82
BIC	-49269.07	-22378.15	-30092.92
Se (%)	65.53[a]	67.59[a]	61.89[b]
Sp (%)	64.88[a]	66.17[b]	61.09[c]
Acc (%)	64.93[a]	66.28[b]	61.15[c]

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network,
AIC – Akaike information criterion,
BIC – Bayesian information criterion,
a, b, c – values within rows with different superscript letters are significantly different ($P \leq 0.05$)

3.2. The most significant variables

The results of network sensitivity analysis (indicating input variables most significant to the model) are presented in Table 4. The most important predictors for the neural models included: STAG and LACT (for all models), AGE (especially for MLP1), CALS (for MLP2 and the RBF network), CDG (for MLPs) and TESTS (especially in the case of the RBF networks). Of the remaining genotypes investigated in the present study, also TNF-α and LTF had a relatively large effect on the output variable category (the former was ranked fifth by all network types and the latter was ranked sixth by MLP2 and the RBF network). Only the influence of mLYZ was much smaller. In general, it can be stated (based on the error ratio) that none of the analyzed input variables had a dominant influence on mastitis class in relation to other variables.

Table 4
Sensitivity analysis results for artificial neural networks

Item	Variable	MLP1			MLP2			RBF		
		Rank	Error	Ratio	Rank	Error	Ratio	Rank	Error	Ratio
1.	LTF	16	0.2529	1.00024	6	0.2495	1.00873	6	0.2575	1.00037
2.	TNF-α	5	0.2545	1.00649	5	0.2496	1.00927	5	0.2577	1.00114
3.	mLYZ	11	0.2531	1.00080	12	0.2479	1.00234	8	0.2575	1.00020
4.	CDG	4	0.2551	1.00896	4	0.2512	1.01580	12	0.2574	1.00001
5.	LACT	2	0.2559	1.01215	2	0.2517	1.01769	2	0.2580	1.00224
6.	TESTS	13	0.2531	1.00076	7	0.2491	1.00703	4	0.2577	1.00124
7.	CALS	7	0.2539	1.00418	3	0.2515	1.01695	3	0.2578	1.00167
8.	STAG	1	0.2566	1.01492	1	0.2547	1.02983	1	0.2598	1.00936
9.	MILK	8	0.2537	1.00350	14	0.2477	1.00170	9	0.2574	1.00011
10.	FT	9	0.2537	1.00348	11	0.2479	1.00251	14	0.2574	0.99997
11.	PR	12	0.2531	1.00077	16	0.2473	1.00000	15	0.2574	0.99994
12.	LAE	6	0.2543	1.00553	8	0.2487	1.00537	7	0.2575	1.00027
13.	UR	10	0.2536	1.00288	10	0.2480	1.00292	10	0.2574	1.00011
14.	HF	14	0.2530	1.00062	13	0.2478	1.00181	11	0.2574	1.00006
15.	AGE	3	0.2552	1.00925	9	0.2482	1.00343	16	0.2574	0.99990
16.	SIRE	15	0.2530	1.00055	15	0.2477	1.00155	13	0.2574	1.00000

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network, variable abbreviations are as given in material and methods section

3.3 Mastitis detection performance

Mastitis detection results for individual ANN types obtained from the test set (which was not used during the network training and shows its effectiveness during exploitation on new data) are presented in Table 5. Statistically significant differences in Sp, Acc and P(FP) were found for each pair of the analyzed networks ($P \leq 0.05$).

Table 5
Results of mastitis detection in cows based on the test set (n = 6173)

Parameter (%)	MLP1	MLP2	RBF
Se	63.30	62.20	57.80
Sp	65.04[a]	66.58[b]	60.30[c]
Acc	64.91[a]	66.26[b]	60.12[c]
P(FP)	34.96[a]	33.42[b]	39.70[c]
P(FN)	36.70	37.80	42.20
P(PSTP)	12.59[a]	12.90	10.38[b]
P(PSTN)	95.70	95.68	94.73

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network,
a, b, c – values within rows with different superscript letters are significantly different ($P \leq 0.05$), parameter abbreviations are as given in material and methods section

The ROC curves and their corresponding AUC and SE(AUC) values calculated from the test set for the MLP networks with one and two hidden layers and the RBF networks are presented in Figure 1. The AUC values were higher for MLPs compared with those for the RBF networks [AUC ± SE(AUC) was 0.6863 ± 0.0142, 0.6857 ± 0.0142 and 0.6295 ± 0.0145 for the MLP1, MLP2 and RBF networks, respectively].

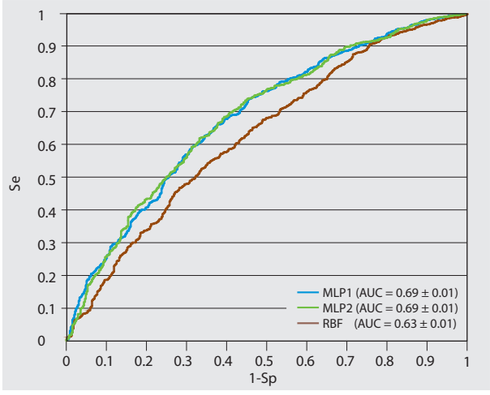

Figure 1
The ROC curves plotted for different ANN types based on the test set

3.4 Effect of reducing the number of input variables on the quality and detection performance of the models

Input variables included in individual ANN models after the first and the second stage of reduction are given in Table 6. In general, all networks selected the same set of predictors at both reduction stages, although their sequence of importance varied depending on the network type. The main differences in the first phase of reduction concerned AGE and MILK, which were retained only by MLP1 as well as mLYZ, which was selected only by the RBF network. At the second stage, AGE was still retrained only by MLP1, LAE by MLP2 and CALS and TESTS by the RBF network (Table 6).

Table 6
Input variables included in individual neural models after the first and the second stage of reduction

Rank	MLP1	MLP2	RBF
First stage of reduction			
1.	STAG	STAG	STAG
2.	LACT	LAE	LACT
3.	AGE	CDG	TESTS
4.	CDG	LACT	CALS
5.	TNF-α	TNF-α	TNF-α
6.	LAE	TESTS	mLYZ
7.	MILK	CALS	LTF
8.	CALS	LTF	LAE
Second stage of reduction			
1.	LACT	STAG	LACT
2.	STAG	CDG	STAG
3.	CDG	LACT	TNF-α
4.	AGE	TNF-α	CALS
5.	TNF-α	LAE	TESTS

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network, variable abbreviations are as given in material and methods section

The values of individual probabilities calculated from the training set for the ANNs with reduced numbers of input variables are presented in Table 7. The G^2 values increased with a decreasing number of input variables (which shows the quality deterioration of the neural models since smaller G^2 values indicate better models), except for MLP2, for which an initial increase (for the model with eight inputs) was followed by a decrease in G^2 (for the model with only five variables). In the case of AIC for MLP1, a reduction in the number of inputs resulted in its constant increase, whereas AIC for MLP2 and RBF initially decreased (for the model with eight inputs) and then increased (for the model with only five predictors). A similar trend in BIC was observed for all ANNs, for which the BIC value first fell (after the first stage of reduction) and then rose (after the second stage of reduction) (Table 7). A statistically significant difference in Se ($P \leq 0.05$) was present for the RBF network between the full model or the model with eight variables and the model with only five variables (Table 7). Also statistically significant differences in Sp and Acc ($P \leq 0.05$) were found for each pair of the MLP1 and RBF models, whereas significant differences in Sp and Acc ($P \leq 0.05$) for MLP2 exited between the full model and the model with eight variables or that with only five inputs (Table 7).

The next stage of the present study was the evaluation of the modified models in terms of their detection performance. The probability values (expressed as a percentage) for the ANNs with a reduced set of predictors calculated from the test set (n = 6173) are shown in Table 8. Specificity, accuracy and P(FP) differed significantly ($P \leq 0.05$) for all model types. Significant differences in Sp and P(FP) were found for MLP1 for each pair of compared models, whereas the respective differences for MLP2 were observed only between the full model and both reduced models. Finally, in the case of the RBF networks, differences in Sp and P(FP) existed between the full model or that with eight variables and the model with five inputs. The significant differences in Acc, on the other hand, were found between the full model or the one with eight variables and that with five predictors in the case of MLP1 and the RBF network, and between the full model and both reduced models for MLP2 (Table 8).

Table 7
Values of G^2, AIC, BIC, sensitivity (Se), specificity (Sp) and accuracy (Acc) for the training set (n = 18539) after the reduction of an initial set of input variables

Variables	Type	G^2	AIC	BIC	Se (%)	Sp (%)	Acc (%)
All	MLP1	16013.80	-50247.52	-49269.07	65.53	64.88^a	64.93^a
8	MLP1	16627.82	-50215.25	-49409.00	66.38	63.66^b	63.87^b
5	MLP1	17802.48	-49691.91	-47922.86	64.67	61.65^c	61.87^c
All	MLP2	15252.06	-44107.54	-22378.15	67.59	66.17^a	66.28^a
8	MLP2	15970.67	-49972.72	-48642.02	65.53	64.96^b	65.00^b
5	MLP2	15857.59	-43870.90	-22696.54	66.60	65.09^b	65.20^b
All	RBF	18238.64	-45395.82	-30092.92	61.89^a	61.09^a	61.15^a
8	RBF	19172.99	-49409.98	-47954.04	60.75^a	59.53^b	59.63^b
5	RBF	20923.86	-49041.05	-47483.35	56.70^b	56.89^c	56.87^c

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network,
AIC – Akaike information criterion,
BIC – Bayesian information criterion,
a, b, c – values within columns with different superscript letters are significantly different ($P \leq 0.05$)

Table 8
Results of mastitis detection in cows obtained on the test set (n = 6173) for the ANNs with a reduced set of predictors

Variables	Type	Se (%)	Sp (%)	Acc (%)	P(FP) (%)	P(FN) (%)	P(PSTP) (%)	P(PSTN) (%)
All	MLP1	63.30	65.04^a	64.91^a	34.96^a	36.70	12.59	95.70
8	MLP1	63.74	63.64^b	63.65^a	36.36^b	36.26	12.24	95.66
5	MLP1	61.10	61.09^c	61.09^b	38.91^c	38.90	11.11	95.18
All	MLP2	62.20	66.58^a	66.26^a	33.42^a	37.80	12.90	95.68
8	MLP2	64.40	64.39^b	64.39^b	35.61^b	35.60	12.58	95.79
5	MLP2	59.78	64.43^b	64.09^b	35.57^b	40.22	11.80	95.27
All	RBF	57.80	60.30^a	60.12^a	39.70^a	42.20	10.38	94.73
8	RBF	55.16	58.94^a	58.66^a	41.06^a	44.84	9.66	94.29
5	RBF	56.70	56.10^b	56.15^b	43.90^b	43.30	9.32	94.21

MLP1 – multilayer perceptron with one hidden layer,
MLP2 – multilayer perceptron with two hidden layers,
RBF – radial basis function network,
a, b, c – values within columns with different superscript letters are significantly different ($P \leq 0.05$), parameter abbreviations are as given in material and methods section

The ROC curves plotted based on the test set for individual ANN types after reducing the number of input variables and their corresponding AUC and SE(AUC) values are given in Figures 2 and 3. Similarly as in the case when all predictors were considered, the AUC values for multilayer perceptrons after retaining only the eight most significant input variables were higher than those for the RBF networks [AUC ± SE(AUC) values were 0.6861 ± 0.0142, 0.6908 ± 0.0142 and 0.6174 ± 0.0145 for the MLP1, MLP2 and RBF networks, respectively). Also, after retaining only the five most significant predictors, the same trend in the AUC values for individual ANN types was present (MLP2 had the highest AUC value of 0.6639 ± 0.0143, MLP1 had a slightly smaller AUC of 0.6472 ± 0.0144, whereas the RBF network had the smallest AUC of 0.5862 ± 0.0145).

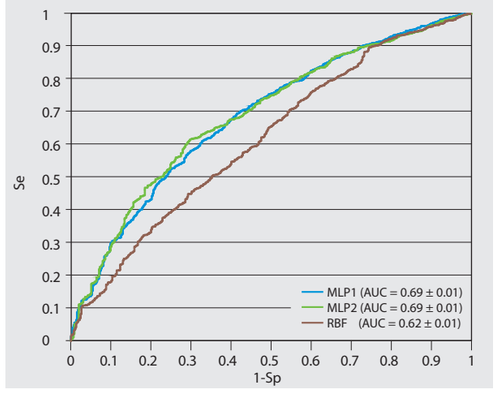

Figure 2
The ROC curves for individual ANN types plotted based on the test set for models including only the eight most significant input variables (Se – sensitivity, Sp – specificity, MLP1 – multilayer perceptron with one hidden layer, MLP2 – multilayer perceptron with two hidden layers, RBF – radial basis function network)

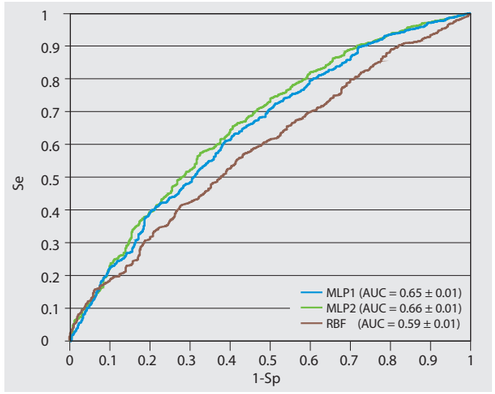

Figure 3
The ROC curves for individual ANN types plotted based on the test set for models including only the five most significant input variables (Se – sensitivity, Sp – specificity, MLP1 – multilayer perceptron with one hidden layer, MLP2 – multilayer perceptron with two hidden layers, RBF – radial basis function network)

4 Discussion

4. 1 Model quality

Considering the values of probabilities such as sensitivity (percentage of correctly diagnosed cows with mastitis), specificity (percentage of correctly identified healthy cows) and accuracy (percentage of correctly identified cows from both classes), calculated from the training set (Table 3), it can be stated that, in general, classification quality was good, which was also reflected in the low RMSE values (0.2626 to 0.2656) for individual ANNs. The RBF network was characterized by a worse quality compared with MLPs, which was further confirmed by the statistically significant differences in the calculated probabilities.

Montgomery et al. (1987), using discriminant function analysis for mastitis detection in cows based on predictors such as: previous mastitis occurrence, decreased body condition, changed milk organoleptic properties, udder swelling and raised body temperature, obtained a correct classification rate of 0.78, sensitivity ranging from 0.32 to 0.45 and specificity ranging between 0.91 and 0.97, whereas in the study by Nielen et al. (1995a), on mastitis detection using ANN (based on electrical conductivity, milk yield and its temperature during milking), sensitivity on a training set was approx. 0.75 and specificity was approx. 0.90. In a later study on mastitis detection (based only on milk electrical conductivity and quarter milk yield) Nielen et al. (1995b) obtained sensitivity of 0.92 and specificity of 1.0. The research on mastitis detection in cows (using ANNs and data on milk electrical conductivity, milk flow rate and the number of days in milk) was also carried out by Cavero et al. (2008), who recorded sensitivity in the range of 0.75 to 0.81 and 0.80 to 0.82 and specificity in the range of 0.60 to 0.66 and 0.77 to 0.79 depending on mastitis definition (SCC above 100,000 cells/ml or above 400,000 cells/ml). Finally, Krieter et al. (2007) assuming a minimum block sensitivity of 0.80, noted the specificity of 0.61 and 0.78 depending on mastitis definition, using the aforementioned input variables. As can be seen from the above review, sensitivity, specificity and accuracy in the present study were similar to or lower than those obtained by other authors, which resulted mainly from the use of weaker predictors in our work.

The values of G^2, AIC and BIC (Table 3) showing the goodness-of-fit and the trade-off between goodness-of-fit and model complexity, indicated that the best model was MLP2 according to G^2 and MLP1 according to AIC and BIC.

4.2 The most significant variables

As can be seen from Table 4, the most important mastitis predictors for ANNs were STAG, LACT, AGE, CALS, TESTS and the variables referring to genotypes, i. e. CDG, TNF-α and LTF.

Many studies have shown a significant effect of lactation stage (the STAG variable) on mastitis incidence, although results reported by different authors vary. In the study by Bunch et al. (1984) on cows in their first or second lactation, it was found that, on average, approx. two-fifths of clinical mastitis cases occurred before 30 days of lactation. Similar results were reported by Hammer et al. (2012), who observed the greatest risk of mastitis at a quarter level between 10 and 29 days in milk. On the other hand, an investigation carried out in the Scandinavian countries (Valde et al., 2004) showed that the highest risk of mastitis occurred from two days antepartum to 14 days post-partum. In Polish Holstein-Friesian Black-and-White cattle, Bogucki et al. (2014), who studied an effect of lactation stage on SCC in milk, found that the natural logarithm of SCC increased steadily with successive lactation stages, whereas Nałęcz-Tarwacka and Dembińska (2013) did not observe any significant effect of lactation stage on SCC.

As for primipara, most clinical mastitis cases occur at an early stage of lactation, until about 30 days in milk (Myllys and Rautala, 1995; Nyman et al., 2007; Svensson et al., 2006). Persson Waller et al. (2009) showed that two-thirds of all mastitis cases in primipara occurred directly before calving or in the first month of lactation – most frequently during the first week post-calving. Moreover, these authors also found significant differences in mastitis incidence depending on lactation stage between primi- and multipara. For the former, 65 % cases occurred in the period from seven days antepartum to 30 days postpartum, while 15 % and 20 % in the period from 31 to 120 days in milk and beyond 120 days in milk, respectively. Respective percentages for older cows were 36 %, 35 % and 28 %. In Polish Holstein-Friesian Black-and-White primipara, Gierdziewicz et al. (2009) stated that SCC was highest at the beginning and end of lactation, while its level at peak lactation (third and second month) was lowest. This result was further confirmed by Guliński et al. (2016), who found that SCC was lowest during the three middle months of lactation (sixth to eighth) and the natural logarithm of SCC was significantly higher in the ninth and tenth month of lactation compared with all the preceding ones. The authors explained this phenomenon by the "dilution" effect associated with a higher milk yield at peak lactation.

The next significant variables, i. e. lactation number or parity (LACT) and calving age (AGE) are usually discussed together. The general trend is towards an increased mastitis incidence with older age/higher parity (Olde Riekerink et al., 2007; Persson Waller et al., 2009; Wolf et al., 2010). Also Rupp and Boichard (2000) reported the highest mastitis incidence in the second and sixth lactation, respectively, whereas Valde et al. (2004) estimated the risk of mastitis during a 305-day lactation at 0.127 to 0.215 in primiparous cows and 0.204 to 0.358 in cows of second or higher parity. In addition, Hammer et al. (2012), who studied 2- to 9-year-old cows grazed on pasture, found the more frequent occurrence of mastitis with an increasing parity. According to Pantoja et al. (2009), the likelihood of mastitis in an udder quarter of a cow in her fifth or later lactation is 4.2 times higher than that in the udder quarters of cows in their second lactation. An effect of lactation number on the risk of clinical mastitis may also be attributed to the pathogen species responsible for this condition (Green et al., 2004). In the study on Polish Holstein-Friesian Black-and-White cows, it was found that the lowest SCC was characteristic of the cows in their first lactation compared with subsequent ones and that SCC rose with increasing parity/calving age (Gierdziewicz et al., 2009; Król et al., 2009; Nałęcz-Tarwacka and Dembińska, 2013; Otwinowska-Mindur et al., 2008). Similar observations in the same breed were made by Bogucki et al. (2014), who recoded the lowest natural logarithm of SCC in the second lactation, slightly higher one in the first lactation and the highest one in the third and subsequent lactations.

Important input variables indicated by the network sensitivity analysis (especially for the RBF network) included also calving season (CALS; third position) and mastitis occurrence season (TESTS; fourth position. In a study on 242 Polish Holstein-Friesian cows kept on a single farm, a higher incidence of mastitis was observed during summer months (June, July and August) compared with winter ones (Miciński et al., 2010), whereas in US Holsteins the highest mastitis occurrence was typical of the cooler months of the year (Abdel Azim et al., 2005). In the work by Olde Riekerink et al. (2007), a highly significant effect of season on mastitis incidence was found in Dutch Holstein-Friesian and Friesian cows. The authors observed a generally higher risk of clinical mastitis in late autumn (December) than in summer and a slightly increased frequency of mastitis cases was noticed in the second half of July in the herds with a high bulk tank SCC, which resulted from a wider spread of *E. coli* and *Staphylococcus aureus* pathogens in this period. The changes of mastitis frequency in different seasons were also reported for other microbial species. Finally, an interaction between season and management system was additionally recorded. Similar seasonal trends caused by a different spread of mastitis-causing pathogens were shown by Østerås et al. (2006), whereas Hristov et al. (2007) did not find any significant effect of season on mastitis incidence. Also, in the study on the effect of calving season on the content of SCC in the milk from Polish Holstein-Friesian Black-and-White cows, it was found that this effect was statistically insignificant (Nałęcz-Tarwacka and Dembińska, 2013). However, other works on the same breed showed that the differences in the SCC between seasons were statistically significant. For instance, Otwinowska-Mindur et al. (2008) reported that the somatic cell score was highest between April and June and lowest between October and December, whereas Guliński and Salamończyk (2007) found that SCC was highest from June to August and lowest from September to November.

Of the variables referring to the genotypes of lactoferrin (LTF), tumor necrosis factor alpha (TNF-α), lysozyme (mLYZ) and defensins (CDG), three predictors, i. e. TNF-α, CDG, and LTF were ranked relatively high by all ANN types. As described earlier, TNF-α is a pro-inflammatory cytokine, which activates the whole immune system and increases the vascular endothelial permeability, facilitating diapedesis and, as a chemoattractant, inducing chemotaxis, that is, the migration of leucocytes towards the focus of inflammation (Wojdak-Maksymiec and Mikołajczyk, 2012). Moreover, this cytokine raises the secretion of many other cytokines, being itself secreted at higher amounts by phagocytic cells as a result of bacterial lipopolysaccharide action (Wojdak-Maksymiec et al., 2013). These authors also found a significant effect of an interaction between a TNF-α genotype and lactation number on mastitis incidence and the *T* allele was associated with a lower number of mastitis cases in earlier lactations. On the other hand, the action of defensins consists in damaging the cell membrane of microorganisms and its perforation, which results in the loss of intracellular content and the penetration of the molecules that could not enter the cell initially. At the same time, the synthesis of nucleic acids and proteins as well as the respiration process are inhibited (Mak, 1994; Risso, 2000). Due to their broad antibiotic spectrum, defensins are also involved in combating pathogens that most often cause mastitis in dairy cows (Roosen et al., 2004; Ryniewicz et al., 2003).

Finally, LTF acts against many microbial species including Gram-negative and Gram-positive bacteria, enveloped and non-enveloped viruses, fungi and other parasites. It is capable of binding iron ions as well as phosphorus and zinc, which are necessary for growth promotion. Therefore, the action of LTF limits the availability of these agents to potential pathogens (Wojdak-Maksymiec et al., 2013). LTF is present in many secretory fluids of mammals and secondary granules of neutrophils being an important mediator in host defense against different environmental factors (Zimecki et al., 2004). In cattle, bovine LTF present in milk is involved in the innate response of the mammary gland against bacterial infections. It was shown that its concentration depended on the SCC level in Polish Holstein-Friesian cows, along with the concentrations of interleukin-1β and tumor necrosis factor alpha, which makes these inflammatory mediators potentially useful indicators of mastitis occurrence (Sobczuk-Szul et al., 2014). At the molecular level, it was also found that the polymorphism within intron 6 of the *LTF* gene identified with PCR-RFLP in the same breed using the *Eco*RI restriction enzyme was associated with the level of SCC (the *BB* genotype was related to a significantly higher SCC level compared with *AA* and *AB*) (Sender et al., 2006).

The last investigated genotype (lysozyme) had a less significant effect on mastitis status, similarly to other input variables included in the neural models (variables referring to milk yield and composition, proportion of Holstein-Friesian genes and the mean lnSCC for a sire).

4.3 Detection performance

After training the MLP and RBF networks, the best one from each category was selected and used on the test set in order to verify its effectiveness of diagnosing cows susceptible to mastitis. These records were not used for the network training or its monitoring. Considering calculated probabilities and sensitivity in particular (percentage of correctly detected cows with mastitis), it can be stated the ability of the models to detect this condition was good (approx. 62 %) (StatSoft, 1998). Statistically significant differences in Sp, Acc and P(FP) were present between all neural model types, however, the lowest values were observed for the RBF networks, which proves their somewhat worse detection properties (Table 5). In the study by Montgomery et al. (1987) on mastitis detection using discriminant function analysis, sensitivity, specificity and accuracy on a test set were 0.39, 0.91 and 0.75, respectively, whereas in the aforementioned work by Nielen et al. (1995b) the respective values were 0.33 to 0.77; 0.69 to 1.00 and 0.67 to 0.77, depending on the manner of test records selection. Also, Yang et al. (1999) and Yang et al. (2000) obtained quite different values of discussed probabilities depending on the way, in which test records were selected and the proportion of mastitis and non-mastitis cases (sensitivity, specificity and accuracy in the range of 0.24 to 0.75, 0.67 to 1.0 and 0.55 to 0.99, respectively). On the other hand, sensitivity and specificity in the study by Cavero et al. (2008) were 0.63 to 0.93 and 0.38 to 0.87 depending on mastitis definition, whereas Krieter et al. (2007) obtained specificity of 0.51 to 0.75, assuming the minimum sensitivity of 0.80. These data show that the values reported by different authors are similar to those in the present study, although

Sun et al. (2010), using ANNs for the same purpose, reported Se, Sp and Acc in the range of 0.79 to 0.87, 0.91 to 0.92 and 0.87 to 0.91, respectively, which were relatively high and higher than those in the present work. Finally, it should be mentioned that ANNs were also applied to mastitis detection, in which more than two categories of an output variable were distinguished (Hassan et al., 2009).

It is also important for mastitis detection to avoid false positive results (i. e. healthy cows diagnosed as ill) and, especially, false negative ones (cows suffering from mastitis recognized as healthy by the model), which in the latter case result in much more serious consequences. The rate of the so-called false alarms was similar for both MLP types (approx. 35 %) and only the RBF network was characterized by their higher percentage (Table 5). Also P(FN) values were at a similar level for all ANN types and comparable with the false positive rate. In the available literature on mastitis detection using ANN, these values vary considerably in the range of 0.0 to 0.76 (Cavero et al., 2008; Nielen et al., 1995b; Wang and Samarasinghe, 2005; Yang et al., 1999).

Additional probabilities calculated from the classification (confusion) matrix included P(PSTP) and P(PSTN), which indicate the proportions of cows assigned to the "healthy" or "mastitis" class by the model that really belonged to these categories. The P(PSTP) values obtained in the present study (Table 5) were definitely too low for all ANN types. An opposite situation occurred in the case of P(PSTN), which proves high reliability of predicting healthy cases by ANNs. The P(PSTP) and P(PSTN) values reported in the available literature are in a relatively broad range from 0.02 to 1.00 (Nielen et al., 1995b; Yang et al., 1999)

The last stage of the detection performance assessment was the plotting of the ROC curves (Figure 1) for the test set records and the calculation of AUC. AUC assumes the values in the range of 0.5 (no discrimination) to 1.0 (perfect discrimination), constituting, at the same time, the probability that a measure or predicted risk is higher for a case belonging to a given class than for a case that does not belong to this class (Cook, 2007). In the present study, the optimum cut-off value minimizing the number of misclassifications to both distinguished classes, was 0.93 (assuming equal misclassification costs), while the highest AUC value (indicating the best discrimination power of the neural model) was found for MLP1 (Figure 1). For comparison, Yang et al. (1999) reported the AUC values in the range of 0.77 to 0.87 depending on the proportion of mastitis to non-mastitis records and the optimal cut-off value.

4.4 An effect of a reduced number of input variables on the quality and detection performance of the models

A reduction in the number of input variables for ANN may lead to obtaining a model of higher quality, learning more easily, having better generalization abilities and being less costly during its exploitation. The final set of five variables was almost the same for all ANN types, except for AGE, LAE and the season of calving and test day, which were selected only by MLP1, MLP2 and the RBF network, respectively. Unfortunately, this final set for MLPs included also CDG, whose practical use can be problematic due to the necessity of determining polymorphic variants of defensins, which is rather costly and requires laboratory tests. A similar study on an effect of different combinations of input variables on the quality of the neural models used for mastitis detection was preformed by Yang et al. (2000), who analyzed models trained only on the basis of milk recording data, conformation traits data or both data sets combined. The most significant production variables were lactation stage, milk yield and SCC, which were similar to those in the present study (except for SCC), whereas the influence of conformation traits was much smaller. The same authors in their previous study (Yang et al., 1999) investigated MLPs trained on a data set of only traditional mastitis predictors (such as cow age, lactation stage and SCC) and on a combined data set of traditional and additional predictors (e. g. calving season, milk composition, conformation class) showing that the effect of additional variables was negligible. In other studies on the use of ANN for mastitis detection, different possible sets of predictors were also analyzed, although on a much smaller scale. For instance, Heald et al. (2000) reduced an initial set of 23 predictors to only 13, retaining in the model variables such as: mean SCC for lactation, test-day SCC, 305-day milk yield, lactation number, days in milk, the ratio of monthly milk yields for two subsequent months of lactation, the number of test days with somatic cell score (SCS) above 4.9 and the contribution of cow's individual SCC to bulk tank SCC. As can be seen, the final set of selected predictors determined from the sensitivity analysis was similar to the set of five input variables in the present study. Also, Sun et al. (2010) investigated four different sets of predictors such as: running mean of the normalized quarter milk yield and the normalized electrical conductivity, deviation from the minimum of the second aforementioned variable, as well as the first three principal components, concluding that the best prediction properties (sensitivity, specificity and accuracy) were characteristic of MLPs trained on principal components. On the other hand, Ankinakatte et al. (2013) considered five different sets of input variables such as: days in milk, SCS, concentration of lactate dehydrogenase, electrical conductivity and daily milk yield, finding that the exclusion of the second and the last above-mentioned variables significantly deteriorated ANN detection performance.

As for the quality criteria calculated from the training set for ANNs with reduced sets of predictors (Table 7), a general upward trend for G^2 was visible, which indicates a lower ANN quality (except for MLP2, for which a decrease in G^2 was observed for the model with five variables relative to the one with eight predictors). A different situation was, however, observed for AIC and BIC, whose values changed differently depending on the network type. In the case of MLP1, AIC constantly increased, whereas for MLP2 and RBF, the values first decreased and then increased. The same trend was observed in BIC for all classifiers and consisted in an initial fall of the criterion value and then its rise. This result shows that the initial reduction in the number of input variables led to

less complex models that still fit the training data sufficiently well. However, further reduction in the number of predictors (and a model complexity) resulted in their poorer goodness-of-fit, which was reflected in the higher values of the information criteria applied. Also, the significantly lower Se, Sp and Acc values were observed for some ANN types after removing some input variables. The elimination of predictors had a similar effect on mastitis detection on the test set (Table 8). Sp, Acc, and P(FP) differed significantly for all network types, while the remaining probabilities remained at a similar level irrespective of the number of predictors. An additional verification of the effect of input variables reduction on the network detection performance was performed by plotting the ROC curves and calculating the AUC values (on the test set). The downward trend was present for MLP1 and the RBF network, while MLP2 was characterized by the different values of this area depending on the number of predictors included.

To sum up, it should be stated that the reduction in the number of input variables may be advantageous in their application to mastitis detection on a farm (especially with regard to genotype determination) but an effect of this procedure on the quality of ANNs can be observed. In the case of MLPs, a combined defensin genotype was also included in the final set of five variables, and the necessity of its determination may be quite costly.

5 Conclusions

The results of mastitis detection in dairy cows obtained in the present study from the relatively easily available data on milk recording and the four selected genotypes, using three types of ANNs, showed satisfactory effectiveness. MLPs were slightly better in this respect, although sensitivity, specificity and accuracy were similar for all ANNs. The role of mastitis risk factors described in the literature (lactation stage, parity, calving age, season) was confirmed in our study, whereas all remaining factors (daily milk yield, its composition, proportion of Holstein-Friesian genes or the mean lnSCC for a sire) had a small influence on the udder health status. Of the genotypes included in the neural models, tumor necrosis factor alpha, lactoferrin and combined defensin genotypes were relatively important, whereas the last considered genotype, i. e. lysozyme did not significantly improve the networks' detection performance.

It is possible to reduce the original set of 16 input variables to five, but a lower detection performance of the RBF network and the necessity of combined defensin genotype assay should be taken into account. An indication of cows more susceptible to mastitis facilitates the proper application of preventive measures, which may ultimately limit the number of cows suffering from mastitis in a herd.

References

Abbassi Daloii T, Tahmoorespur M, Nassiri MR (2010) Prediction of 305 days milk production using artificial neural network in Iranian dairy cattle. In: Book of abstracts of the 61st Annual Meeting of the European Association for Animal Production, Heraklion - Crete Island, Greece, 23-27 August 2010. Wageningen : Wageningen Acad Publ, p 177

Abdel-Azim GA, Freeman AE, Kehrli ME, Kelm SC, Burton JL, Kuck AL, Schnell S (2005) Genetic basis and risk factors for infectious and noninfectious diseases in US Holsteins : I. Estimation of genetic parameters for single diseases and general health. J Dairy Sci 88(3):1199–1207

Ankinakatte S, Norberg E, Løvendahl P, Edwards D, Højsgaard S (2013) Predicting mastitis in dairy cows using neural networks and generalized additive models : a comparison. Comput Electron Agric 99(11):1–6

Bals R (2000) Antimikrobielle Peptide und Peptidantibiotika. Med Klin 95(9):496–502

Bishop CM (1995) Neural networks for pattern recognition. Oxford : Clarendon Pr, 482 p

Bogucki M, Sawa A, Neja W (2014) Effect of changing the cow's milking system on daily yield and cytological quality of milk. Acta Sci Pol Zootech 13(4):17–26

Bradley AJ (2002) Bovine mastitis : an evolving disease. Vet J 164(2):116–128

Bruckmaier RM, Ontsouka CE, Blum JW (2004) Fractionized milk composition in dairy cows with subclinical mastitis. Vet Med (Praha) 49(8):283–290

Bunch KJ, Heneghan DJS, Hibbitt KG, Rowlands GJ (1984) Genetic influences on clinical mastitis and its relationship with milk yield, season and stage of lactation. Livest Prod Sci 11(1):91–104

Cárdenas Mansilla CS (2008) Recognition of lactation pattern curves through neural networks and discriminate analysis, during the first third of lactation in dairy cows from the IX Region. Agro Sur 36(1):43–48

Cavero D, Tölle K-H, Rave G, Buxadé C, Krieter J (2007) Analysing serial data for mastitis detection by means of local regression. Livest Sci 110(1):101–110

Cavero D, Tölle K-H, Henze C, Buxadé C, Krieter J (2008) Mastitis detection in dairy cows by application of neural networks. Livest Sci 114(2):280–286

Cook NR (2007) Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 115(7):928–935

Craninx M, Fievez V, Vlaeminck B, De Baets B (2008) Artificial neural network models of the rumen fermentation pattern in dairy cattle. Comput Electron Agric 60(2):226–238

Davidson PM, Sofos JN, Branen AL (2010) Antimicrobials in food. Boca Raton : CRC Pr, 720 p

Dayton CM (2003) Model comparisons using information measures. J Mod Appl Stat Meth 2(2):2

Diamond G, Zasloff M, Eck H, Brasseur M, Maloy WL, Bevins CL (1991) Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa : peptide isolation and cloning of a cDNA. Proc Nat Acad Sci USA 88(9):3952–3956

dos Reis CBM, Barreiro JR, Mestieri L, de Felício Porcionato MA, dos Santos MV (2013) Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. BMC Vet Res 9(1):67

Fukuda O, Nabeoka N, Miyajima T (2012) Estimation of marbling score in live cattle based on dynamic ultrasound image using a neural network. In: Proceedings of the 19th International Conference on Systems, Signals and Image Processing (IWSSIP), 2012 held in Vienna, Austria, 11-13 April 2012. Piscataway : IEEE, pp 276-279

Gandhi RS, Raja TV, Ruhil AP, Kumar A (2009a) Evolving prediction equations for lifetime milk production using artificial neural network methodology in Sahiwal cattle. In: Dairying for livelihood and growth! : 37th Dairy Industry Conference, Goa, India, 7-9 February 2009. New Delhi : Indian Dairy Ass, p 7

Gandhi RS, Raja TV, Ruhil AP, Kumar A (2009b) Prediction of lifetime milk production using artificial neural network in Sahiwal cattle. Indian J Anim Sci 79(10):1038–1040

Gandhi RS, Raja TV, Ruhil AP, Kumar A (2010) Artificial neural network versus multiple regression analysis for prediction of lifetime milk production in Sahiwal cattle. J Appl Anim Res 38(2):233–237

Gierdziewicz M, Otwinowska-Mindur A, Ptak E (2009) Effect of herd size and daily milk yield on somatic cell score in Polish Holstein-Friesian cows Black-and- White variety. Sci Ann Pol Soc Anim Prod 5(2):65-72

Green MJ, Green LE, Schukken YH, Bradley AJ, Peeler EJ, Barkema HW, de Haas Y, Collis VJ, Medley GF (2004) Somatic cell count distributions during lactation predict clinical mastitis. J Dairy Sci 87(5):1256–1264
Greiner M, Pfeiffer D, Smith R (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45(1–2):23–41
Guliński P, Salamończyk E (2007) Estimation of chosen milk performance traits, length of lactation and udder health in high-yielding Polish Holstein-Friesian cows of Black-and-White variety. Sci Ann Pol Soc Anim Prod 3(1):29–36
Guliński P, Wyszomierski K, Salamończyk E (2016) Relationship between somatic cell count and milk performance of Polish Holstein-Friesian cows. Sci Ann Pol Soc Anim Prod 12(1):17–23
Hammer JF, Morton JM, Kerrisk KL (2012) Quarter-milking-, quarter-, udder-and lactation-level risk factors and indicators for clinical mastitis during lactation in pasture-fed dairy cows managed in an automatic milking system. Aust Vet J 90(5):167–174
Harmon RJ (1994) Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci 77(7):2103–2112
Hassan KJ, Samarasinghe S, Lopez-Benavides MG (2009) Use of neural networks to detect minor and major pathogens that cause bovine mastitis. J Dairy Sci 92(4):1493–1499
Haykin SS (2009) Neural networks and learning machines. New York : Pearson, 906 p
Heald CW, Kim T, Sischo WM, Cooper JB, Wolfgang DR (2000) A computerized mastitis decision aid using farm-based records: An artificial neural network approach. J Dairy Sci 83(4):711-720
Hettinga KA, Van Valenberg HJF, Lam T, Van Hooijdonk ACM (2008) Detection of mastitis pathogens by analysis of volatile bacterial metabolites. J Dairy Sci 91(10):3834–3839
Hillerton JE, Berry EA (2005) Treating mastitis in the cow : a tradition or an archaism. J Appl Microbiol 98(6):1250–1255
Hristov S, Joksimović-Todorović M, Relić R, Stojanović B, Stanković B, Vuković D, Davidović V (2007) The influence of udder disinfections, period of lactation and season on cow mastitis occurrence. Savrem poljopr 56(1-2):138–143
Krieter J, Cavero D, Henze C (2007) Mastitis detection in dairy cows using neural networks. In: Böttinger S, Theuvsen L, Rank S, Morgenstern M (eds) Agrarinformatik im Spannungsfeld zwischen Regionalisierung und globalen Wertschöpfungsketten : Referate der 27. GIL Jahrestagung, 05.-07. März 2007, Stuttgart, Germany, pp 123-126
Król J, Litwinczuk A, Brodziak A, Topyla B (2009) Milk quality of three cow breeds during the successive years of performance. Sci Ann Pol Soc Anim Prod 5(4):181–188
Liddle AR (2007) Information criteria for astrophysical model selection. MNRAS Letters 377(1):L74–L78
López-Benavides MG, Samarasinghe S, Hickford JG (2003) The use of artificial neural networks to diagnose mastitis in dairy cattle. In: Proceedings of the International Joint Conference on Neural Networks 2003, Portland, Oregon 20-24 July 2003 : vol 1. Piscataway : IEEE, pp 582-585
Mak P (1994) Antibacterial proteins and peptides of human neutrophils : the search for new antibiotics. Adv Cell Biol Suppl 3:157–173
Miciński J, Zwierzchowski G, Ropelewska K (2010) Milking process management and the quantity and quality of milk produced in two milking systems. Sbornik Naucznych Trudow 1(1):289-299
Montgomery ME, White ME, Martin SW (1987) A comparison of discriminant analysis and logistic regression for the prediction of coliform mastitis in dairy cows. Can J Vet Res 51(4):495
Myllys V, Rautala H (1995) Characterization of clinical mastitis in primiparous heifers. J Dairy Sci 78(3):538–545
Nałęcz-Tarwacka T, Dembińska B (2013) The influence of some factors on somatic cell count in milk of high yielding cows. Prz Hod 81(4):3-5
Nielen M, Schukken YH, Brand A, Haring S, Ferwerda-van Zonneveld RT (1995a) Comparison of analysis techniques for on-line detection of clinical mastitis. J Dairy Sci 78(5):1050–1061
Nielen M, Spigt MH, Schukken YH, Deluyker HA, Maatje K, Brand A (1995b) Application of a neural network to analyse on-line milking parlour data for the detection of clinical mastitis in dairy cows. Prev Vet Med 22(1):15–28
Njubi DM, Wakhungu J, Badamana MS (2009) Mating decision support system using computer neural network model in Kenyan Holstein-Friesian dairy cattle [online]. To be found at <http://www.lrrd.org/lrrd21/4/njub21045.htm> [quoted 21.08.2016]
Njubi DM, Wakhungu JW, Badamana MS (2010) Use of test-day records to predict first lactation 305-day milk yield using artificial neural network in Kenyan Holstein–Friesian dairy cows. Trop Anim Health Prod 42(4):639–644
Nyman A-K, Ekman T, Emanuelson U, Gustafsson AH, Holtenius K, Waller KP, Sandgren CH (2007) Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. Prev Vet Med 78(2):142–160
Olde Riekerink RGM, Barkema HW, Stryhn H (2007) The effect of season on somatic cell count and the incidence of clinical mastitis. J Dairy Sci 90(4):1704–1715
Østerås O, Sølverød L, Reksen O (2006) Milk culture results in a large Norwegian survey : effects of season, parity, days in milk, resistance, and clustering. J Dairy Sci 89(3):1010–1023
Otwinowska-Mindur A, Gierdziewicz M, Ptak E (2008) Effect of year, season and age of calving on somatic cell score in Polish Holstein-Friesian cows of Black-and-White variety. Sci Ann Pol Soc Anim Prod 4(2):29–36
Pantoja JCF, Hulland C, Ruegg PL (2009) Somatic cell count status across the dry period as a risk factor for the development of clinical mastitis in the subsequent lactation. J Dairy Sci 92(1):139–148
Persson Waller K, Bengtsson B, Lindberg A, Nyman A, Ericsson Unnerstad H (2009) Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows : influence of breed and stage of lactation. Vet Microbiol 134(1):89–94
Pyörälä S (2002) New strategies to prevent mastitis. Reprod Dom Anim 37(4):211–216
Risso A (2000) Leukocyte antimicrobial peptides : multifunctional effector molecules of innate immunity. J Leukocyte Biol 68(6):785–792
Roosen S, Exner K, Paul S (2004) Bovine beta-defensins : identification and characterization of novel bovine beta-defensin genes and their expression in mammary gland tissue. Mamm Genome 15:834–842
Rumelhart DE, Hintont GE, Williams RJ (1986) Learning representations by back-propagating errors. Nature 323(6088):533–536
Rupp R, Boichard D (2000) Relationship of early first lactation somatic cell count with risk of subsequent first clinical mastitis. Livest Prod Sci 62(2):169–180
Ryniewicz Z, Zwierzchowski L, Bagnicka E, Flisikowski K, Maj A, Krzyżewski J, Strzałkowska N (2003) Association of the polymorphism at defensin gene loci with dairy production traits and milk somatic cell count in Black-and-White cows. Anim Sci Pap Rep 21:209–222
Salehi F, Lacroix R, Wade KM (1998) Improving dairy yield predictions through combined record classifiers and specialized artificial neural networks. Comput Electron Agric 20(3):199–213
Schonwetter BS, Stolzenberg ED, Zasloff MA (1995) Epithelial antibiotics induced at sites of inflammation. Science 267(5204):1645–1648
Sender G, Korwin-Kossakowska A, Hameid KGA, Prusak B (2006) Association of the polymorphism of some genes with the occurrence of mastitis in cattle. Med Weter 62(5):563–565
Sobczuk-Szul M, Wielgosz-Groth Z, Nogalski Z, Pogorzelska-Przybyłek P (2014) Changes in the content of whey proteins during lactation in cow's milk with a different somatic cells count. Vet Zootech 45(87):79–84
Sordillo LM (2005) Factors affecting mammary gland immunity and mastitis susceptibility. Livest Prod Sci 98(1):89–99
StatSoft (1998) Statistica neural networks user guide. Tulsa OK : Statsoft, 315 p
StatSoft (2011) Statistica 10 user guide. Tulsa OK : Statsoft
Sun Z, Samarasinghe S, Jago J (2010) Detection of mastitis and its stage of progression by automatic milking systems using artificial neural networks. J Dairy Res 77(02):168–175
Svensson C, Nyman A-K, Waller KP, Emanuelson U (2006) Effects of housing, management, and health of dairy heifers on first-lactation udder health in southwest Sweden. J Dairy Sci 89(6):1990–1999
Tahmoorespur M, Hosseinnia P, Teimurian M, Aslaminejad AA (2012) Predictions of 305-day milk yield in Iranian dairy cattle using test-day records by artificial neural network. Indian J Anim Sci 82(5):63-68

Tarver AP, Clark DP, Diamond G, Russell JP, Erdjument-Bromage H, Tempst P, Cohen KS, Jones DE, Sweeney RW, Wines M, Hwang S, Bevins CL (1998) Enteric beta-defensin : molecular cloning and characterization of a gene with inducible intestinal epithelial cell expression associated with *Cryptosporidium parvum* infection. Infect Immun 66(3):1045–1056
Tunzi CR, Harper PA, Bar-Oz B, Valore EV, Semple JL, Watson-MacDonell J, Ganz T, Ito S (2000) Beta-defensin expression in human mammary gland epithelia. Pediatr Res 48(1):30–35
Valde JP, Lawson LG, Lindberg A, Agger JF, Saloniemi H, Osteras O (2004) Cumulative risk of bovine mastitis treatments in Denmark, Finland, Norway and Sweden. Acta Vet Scand 45(3-4):201–210
Verleysen M, François D (2005) The curse of dimensionality in data mining and time series prediction. In: Cabestany J, Prieto A, Sandoval F (eds) Computational intelligence and bioinspired systems : 8th International Work-Conference on Artificial Neural Networks, IWANN 2005, Vilanova i la Geltru, Barcelona, Spain, June 8-10, 2005 ; proceedings. Berlin : Springer, pp 758-770
Viguier C, Arora S, Gilmartin N, Welbeck K, O'Kennedy R (2009) Mastitis detection : current trends and future perspectives. Trends Biotechnol 27(8):486–493
Wang E, Samarasinghe S (2005) On-line detection of mastitis in dairy herds using artificial neural networks [online]. To be found at <http://www.mssanz.org.au/modsim05/papers/wang_e.pdf> [quoted 22.08.2016]
Wawron W (2007) Drying-off with non-antibiotic preparation protection. Biul Lub Izby Lek-Weter 2007(3):11–17
Wojdak-Maksymiec K (2009) Poszukiwanie związków pomiędzy wybranymi genami nieswoistej odpowiedzi immunologicznej a liczbą komórek somatycznych i zawartością niektórych składników w mleku krów. Szczecin : ZUT, 126 p
Wojdak-Maksymiec K, Mikolajczyk K (2012) Interactions between *TNF-α, LTF* and *mLYZ* gene variants in determining somatic cell count in Jersey cows. Pak Vet J 32(4):477–482
Wojdak-Maksymiec K, Szyda J, Strabel T (2013) Parity-dependent association between *TNF-alpha* and *LTF* gene polymorphisms and clinical mastitis in dairy cattle. BMC Vet Res 9(1):114
Wolf J, Wolfová M, Štípková M (2010) A model for the genetic evaluation of number of clinical mastitis cases per lactation in Czech Holstein cows. J Dairy Sci 93(3):1193–1204
Yang XZ, Lacroix R, Wade KM (1999) Neural detection of mastitis from dairy herd improvement records. Trans ASAE 42(4):1063–1071
Yang XZ, Lacroix R, Wade KM (2000) Investigation into the production and conformation traits associated with clinical mastitis using artificial neural networks. Can J Anim Sci 80(3):415–426
Zimecki M, Artym J, Chodaczek G, Kocieba M, Kruzel ML (2004). Protective effects of lactoferrin in Escherichia coli-induced bacteremia in mice : relationship to reduced serum TNF alpha level and increased turnover of neutrophils. Inflamm Res 53(7):292-296