

FOR DISCRIMINATION OF POULTRY MEAT AND PREDICTION OF CHOLESTEROL CONTENT

PRIMJENA KEMOMETRIKE I SPEKTROSKOPIJE U BLISKOM INFRACRVENOM PODRUČJU UZ REFLEKSIJU ZA RAZLIKOVANJE MESA PERADI I PREDVIĐANJE SADRŽAJA KOLESTEROLA

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SUMMARY

Four calibrations were made for cholesterol content in poultry meat (breasts and legs from chickens, cockerels, capons, and breasts and legs from geese). Standard uncertainties expressed as SECV (% , relative) for chickens, cockerels and capons were 9.2 for breasts and 7.8 for legs. These values for geese were 8.4 and 9.0, respectively. The discriminant method with the highest predictive ability, based on residuals RMSX Residuals, was used to classify the samples. Classification accuracy values were good and ranged, on average, from 96.8% to 98%. The NIRS calibrations on cholesterol content in the breast and leg meat of chickens, capons, cockerels, as well as in the breast and leg meat of geese, are suitable for rapid routine analyses to use in practice.

Key words: cholesterol, cross validation, uncertainty, NIRS

INTRODUCTION

Cholesterol is a very important constituent of the animal body and is required for normal functioning of human and animal organisms. It is used for synthesis of steroid hormones and bile acids, which play a major role in the digestion of fats. It is an extremely important component of nerve cells and determines their proper development. In addition, as a substrate necessary for the synthesis of vitamin D, it also affects the development of the skeletal system. However, it is well known that an excess of it is highly undesirable. For the purpose of determining the quality of dietary meat, it is important to determine the cholesterol content. The average cholesterol content (mg/g) in chicken breast ranges from 0.39 to 0.69, in chicken thigh from 0.58 to 0.84 (Pietrzak et al., 2013; Wang et al., 2006; Skřivan et al., 2002; Crespo and Esteve-Garcia, 2001; Konjufca et al., 1997), and in the meat of capons and cockerels from 54 mg/100g to 96 mg/100g (Calik et

al., 2017). These values are similar in the case of goose meat (0.50 – 0.83 mg/g; Buzala et al., 2014; Bielińska, 2012), which until recently was believed to be unhealthy due to higher fatness in comparison to the meat of other poultry species.

The most preferred parts of the poultry carcass are the breast and thigh, so the cholesterol concentration in them is important for many consumers. The cholesterol content can be higher in the thigh, possibly because of its higher fat content (Crespo and Esteve-Garcia, 2001). The crucial factor influencing the cholesterol content in meat is feeding. The concentration of this constituent is significantly influenced by the dietary fatty acid profile, especially in the thigh, in which tallow and olive oil diets produced higher values than sunflower and linseed oil diets (Crespo and Esteve-Garcia, 2001). In turn, Maraschiello et al. (1998) reported larger cholesterol concentrations in the breast of birds fed lard in comparison to those fed olive or sunflower oil.

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There are several methods for cholesterol content determination. One of the first was the colorimetric method for analysis of cholesterol in serum, using FeSO_4 with glacial acetic acid (Searcy and Berquist, 1960). The enzymatic methods are also commonly used for this purpose (Hanczakowski et al., 2004). In turn, the methods based on colorimetric reaction were easily applied to meat products analysis (Rhee et al., 1982). In other products e.g. milk, for total cholesterol determination a simplified enzymatic method was described (Kamelska et al., 2015). Recently, for raw materials and products of animal origin, the most common is a gas chromatography method with saponification of the sample and extraction with organic solvent. The relatively fast chromatographic method for direct – without any derivatization – cholesterol content determination in eggs was applied by Gašior and Pietras (2013). However, chromatographic methods are expensive and laborious, so less tiring and cheaper analytical methods for routine analyses are sought. The near-infrared reflectance spectroscopy could be such a method. What is more, an additional advantage is its speed, while maintaining sufficient accuracy. This feature is becoming increasingly important for recipients of the analysis results.

So, the aim of the study was to develop a NIRS calibration, and to evaluate the possibility of a rapid and accurate prediction of the cholesterol content in poultry meat.

MATERIAL AND METHODS

Experimental plan. A total of 360 poultry samples of breast meat (354 samples were included in the calibration) and 333 poultry samples of leg meat (327 samples were included in the calibration) were used in this study. Four calibrations were made: for breast meat from chickens, capons, cockerels (216 samples), and geese (138 samples), and for leg meat from chickens, capons, cockerels (186 samples), and geese (141 samples).

Chickens. The broiler chickens came from two experiments. All the birds were reared in 42 days on a deep litter under electronically controlled environmental conditions (temperature, lighting regime, air humidity). The chickens were fed commercial broiler diets, starter (1 – 21 days), grower (22 – 35 days), and finisher (36 – 42 days). In

the first experiment, the birds were fed ad libitum; in the second they were feed restricted from 08:00 to 14:00 h and had free access to feed during the remaining time. In this experiment the animals were divided into 4 groups; each of them received soybean oil (1), linseed oil (2), a mixture of soybean and linseed oil (3), or beef tallow (4). Water was available ad libitum for all the animals throughout the experiments.

Capons and cockerels. Castration was performed at 9 weeks of age under local anesthesia by a veterinary surgeon and complied with the requirements established by the Ethics Commission No. 953 of 10 July 2012. The capons and cockerels were kept under good environmental conditions (temperature of 18 – 20 °C, relative humidity of 60 – 75%) on litter, at a stocking density of 7 birds/m². The birds received feeds based on cereal-soybean meal, and were provided with free access to water. At 24 weeks of age they were slaughtered (12 hours prior to slaughter the birds received no feed, but had continuous access to water; Calik et al., 2017).

Geese. All the geese up to 13 weeks of age were reared and fed in the same way, in accordance with the fattening technology system of the National Research Institute of Animal Production-Experimental Plant in Koluda Wielka (Kuyavian-Pomeranian Voivodeship, in north-central Poland) (Bielińska et al., 2015). At that time they received complete feed mixtures containing, among others, maize, barley, wheat, and freshly cut grass. The birds also had the access to pasture. At the end of the fattening, the nutrition was diversified in four groups; each group of geese received only the oats, wheat, maize, or barley grains.

Gas chromatography analysis. The validated gas chromatography method, also used for analysis of eggs, was adapted as a reference for cholesterol content determination in meat (Gašior and Pietras, 2013). After the thawing (from a temperature of -18 °C) of the meat sample (without the skin), the cholesterol was determined after saponification and hexane extraction of the sample using an FID detector and column with 5% phenyl, 95% dimethylpolysiloxane phase. The reference analysis was carried out using derivatization with a silylation reagent as follows: after saponification and evaporation (40 °C, under the stream of nitrogen) 100 μl of SylonTM HTP (Sigma-Aldrich, USA) was added to the sample. Next, the derivatization was carried out (for 45 min at 80 °C), followed by chromatographic analysis.

Reagents and equipment. The following reagents (least pure for analysis grade) were used: n-hexane (Merck, Germany), KOH (POCH S.A., Poland), NaCl (POCH S.A., Poland), ethanol 96% (Chempur, Poland), cholesterol (5-Cholesten-3 β -ol, Sigma-Aldrich, USA), internal standard – 5 α -cholestane (>97%, Sigma-Aldrich, USA). The gas chromatograph GC 2010 (Shimadzu, Japan) with a flame-ionization detector and AOC-5000 autosampler was used. For the NIR calibration, the InfraAct™ 7500 analyzer (Foss, Denmark), working in the range 570 – 1850 nm, with ISIScan 4.6.10.14815 and WinISI 4.7.0.14943 software packages applied. The validation of NIRS calibration was performed based on ISO 12099:2010.

Standard uncertainty of NIRS and reference analysis. Standard Error of Cross Validation – SECV or SECV (%), relative) – as a measure of standard uncertainty for NIRS analysis was calculated. The standard uncertainty for the reference analysis comprised of within-laboratory reproducibility and other components not included in the reproducibility, such as recovery and chemical standard uncertainties (Gašior and Pietras, 2013). These uncertainty components were combined according to the law of propagation of uncertainty (Ellison et al., 2000).

NIRS measurements, calibration, validation parameters and discriminant analysis. For NIRS, the fresh meat samples without the skin were analyzed. For fortification the samples with cholesterol, a water-alcoholic (20%) mixture was added to a meat portion and then thoroughly mixed to get homogeneity. The samples were ground and scanned in triplicates using a 6.5 cm in diameter cuvette. For the calibration, the modified partial least square (MPLS) and mathematical data pre-treatment methods: SNV and Detrend, as well as 2.8.6.1, 3.5.5.1, and 1.4.4.1 derivatives, were applied. The cross validation was performed on 6 test sets, successively being excluded from the calibration set, so that the number of elements in each set was about 15% of the total number of samples. The parameters, such as minimal or maximal cholesterol content in the calibration set (MIN or MAX), average, standard deviation (SD), standard error of calibration (SEC), standard error of prediction corrected for bias (SEP), bias, slope, coefficient of determination (RSQ), and standard error of cross validation (SECV) were estimated. A discriminant analysis and classification were also performed.

RESULTS AND DISCUSSION

Calibrations and cross validation. Four calibrations were performed: for breast meat and leg meat from the chickens, capons, cockerels, and for breast meat and leg meat from the geese. The optimal derivative treatments were selected. For the chickens, capons and cockerels, spectra were transformed by the 2.8.6.1 derivative for breasts, and 3.5.5.1 for legs. For goose meat – both breasts and legs – the derivative 1.4.4.1 was applied. The cholesterol content values presented by other authors in chicken meat (Pietrzak et al., 2013; Wang et al., 2006; Skřivan et al., 2002; Crespo and Esteve-Garcia, 2001; Konjufca et al., 1997), and in capon and cockerel meat (Calik et al., 2017), are similar to the values found in this study. In turn, the cholesterol content in goose meat was only slightly higher compared to data from the literature (50 mg/100g – 83 mg/100g, Buzala et al., 2014; Bielińska, 2012), which can be explained by the fact that a newer analytical technique – gas chromatography – was applied for the reference analysis.

The method for chromatographic cholesterol determination in the meat (reference analysis) was validated in accordance with EN ISO/IEC 17025 and verified in inter-laboratory comparisons. The values of cholesterol content in the goose meat were within a narrow range (meat came from an experiment in which no differences were observed between the birds fed grain of oats, wheat, barley, and maize). This influenced the low values of the coefficient of determination RSQ (0.136 and 0.076 in breast and legs, respectively), in contrast to the meat of chickens, capons and cockerels (RSQ: 0.738 and 0.437 for breast and leg, respectively), for which this range was broader. Therefore, to ensure that the cholesterol was “well-read” by the NIRS technique, the meat samples were fortified with cholesterol. The fortification confirmed the effectiveness of the NIRS method for the prediction of cholesterol content, because the coefficients of determination were increased to the values that can be considered good (Li et al., 2016), while maintaining a low SEC and SECV values (Table 1). NIRS standard uncertainty, ranging between 7.8% and 9.2%, was very comparable to the within-laboratory standard uncertainty for the reference GC-FID method. As such, good NIRS calibrations were obtained, with low standard error of cross validation, as a measure of standard uncer-

tainty for NIRS prediction. The principles of uncertainty estimation for cholesterol determination were shown in the works by Gašior et al. (2013, 2005), and others (Arendarski, 2003; Dobecki, 2004).

The cross validation was performed. Validation charts for one of the six validation sets and validation characteristics for cholesterol content prediction in poultry meat are presented in Figures 1 – 4 and Table 2, respectively. The values of predicted cholesterol content (mg/g) ranged from 0.45 to 1.21, and the overall average was 0.84. The slope varied for the selected validation sets, but SEP and bias were relatively low, although the SEP values were slightly larger for the goose meat. The RSQ values for the chicken, capon and cockerel meat were higher than for the goose meat (Table 2).

Discriminant analysis and classification. Six discriminant methods were viewed (PLS2, Correlation, Maximum Distance, Mahalanobis Distance, RMSX Residuals, and MaximumX Residual). RMSX Residuals was selected, as it had the most predictive power. The discriminant analysis was performed at the following parameters: Scatter Correction, SNV_Detrend, Math Treatment 1,4,4,1, Wavelengths 578,1090,8; 1108,1842,8. The visualization in three-dimensional space shows a good differentiation of all four groups of poultry meat: 1/ breasts from chickens, capons, cockerels, 2/ legs from chickens, capons, cockerels, 3/ breasts from geese, and 4/ legs from geese (Fig. 5). The results for cross validation of the classification model based on five test sets, are presented in Table 3.

Table 1 Calibrations characteristic for cholesterol content in poultry meat (breasts, legs, fortified with cholesterol) and a comparison of standard uncertainties for NIRS and reference analysis

Tablica 1. Značajke kalibracija za sadržaj kolesterola u mesu peradi (prsna, bataci pojačani kolesterolom) i usporedba standardnih kolebljivosti za BIRS i referentna analiza

	Chickens, capons, cockerels – Pilići, kopuni, pijetlovi		Geese - Guske	
	Breast - prsa	Leg - noga	Breast - prsa	Leg - noga
N	216	186	138	141
MIN (mg/g)	0.452	0.723	0.452	0.605
MAX (mg/g)	2.520	2.631	2.479	2.380
Average (mg/g)	1.097	1.126	1.245	1.095
SD (mg/g)	0.638	0.464	0.598	0.456
SEC	0.060	0.072	0.081	0.076
RSQ	0.9066	0.8750	0.8869	0.8820
SECV (mg/g)	0.058	0.071	0.081	0.078
Uncertainty - Nesigurnost				
Reference analysis – u_{ref} (% , relative)	9.9			
NIRS – as SECV (% , relative)	9.2	7.8	8.4	9.0
$SECV/u_{ref}$	0.93	0.79	0.85	0.91

N – number of samples in the calibration set – broj uzoraka u kalibracijskom setu
 MIN/MAX – minimal/maximal cholesterol content in the calibration set – minimalni/maksimalni sadržaj kolesterola u kalibracijskom setu
 Average – average cholesterol content in the calibration set – prosječan sadržaj kolesterola u kalibracijskom setu
 SD – standard deviation of the cholesterol content in the calibration range – standardna devijacija u kalibracijskom setu
 SEC – standard error of calibration – standardna greška u kalibraciji
 RSQ – coefficient of determination (correlation coefficient squared) – koeficijent determinacije (kvadratni korelacijski koeficijent)
 SECV – standard uncertainty of NIR analysis as standard error of cross validation - standardna nesigurnost NIR analize kao standardna pogreška prijelazne valjanosti
 u_{ref} – estimated in the laboratory standard uncertainty of the reference analysis, as relative% - procijenjena laboratorijska standardna nesigurnost referentne analize, relativno %

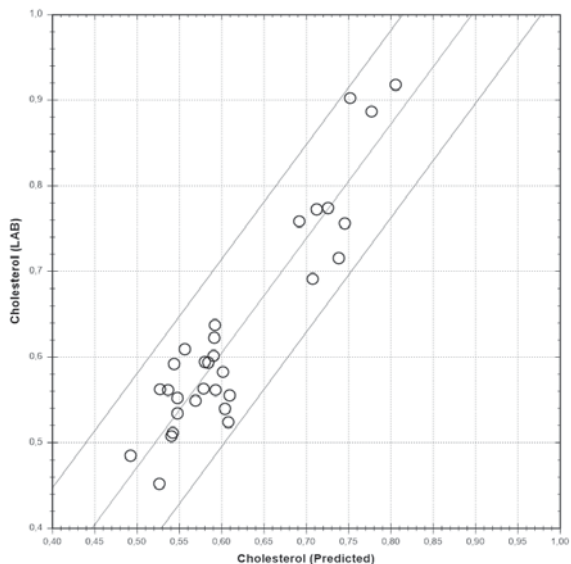


Fig. 1 Validation chart for one of the six validation sets, for cholesterol content prediction (mg/g) in the breast meat of chickens, capons, cockerels. Abscissa: NIR prediction values, ordinate: reference values (LAB).

Slika 1. Tablica procjene za jednu od šest skupina procjene za predviđanje sadržaja kolesterola (mg/g) u mesu prsa pilića, kopuna i pijetlića

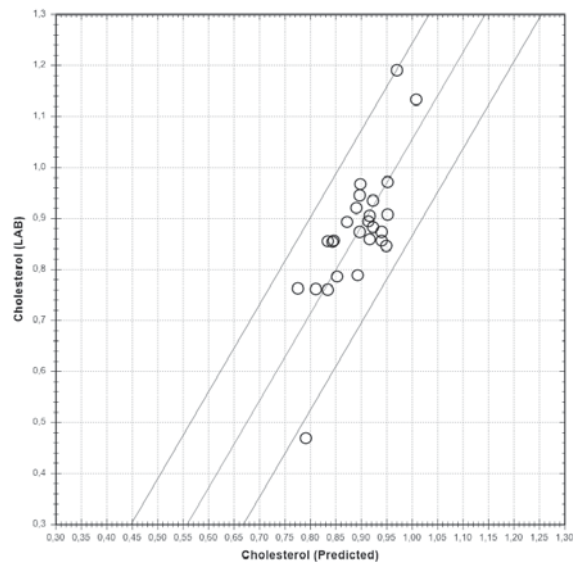


Fig. 2 Validation chart for one of the six validation sets, for cholesterol content prediction (mg/g) in the leg meat of chickens, capons, cockerels. Abscissa: NIR prediction values, ordinate: reference values (LAB).

Slika 2. Krivulja procjene za jednu od šest skupina procjene za predviđanje sadržaja kolesterola (mg/g) u mesu batka pilića, kopuna i pijetlića

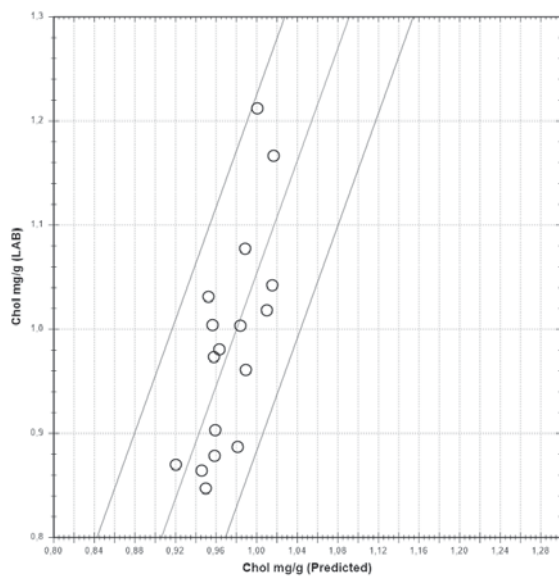


Fig. 3 Validation chart for one of the six validation sets, for cholesterol content prediction (mg/g) in the breast meat of geese. Abscissa: NIR prediction values, ordinate: reference values (LAB).

Slika 3. Krivulja procjene za jednu od šest skupina procjene za predviđanje sadržaja kolesterola u mesu prsa gusaka

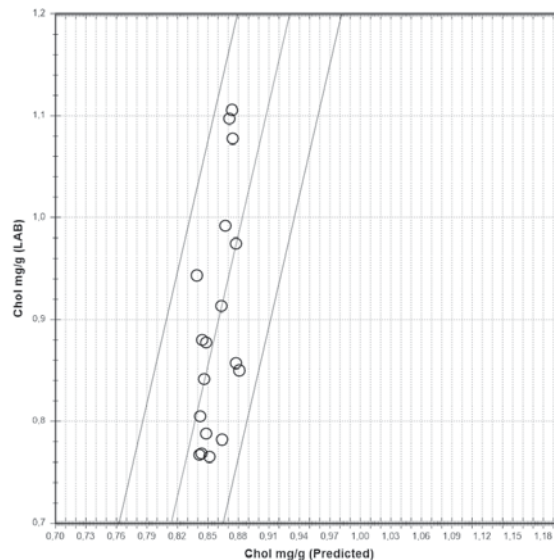


Fig. 4 Validation chart for one of the six validation sets, for cholesterol content prediction (mg/g) in the leg meat of geese. Abscissa: NIR prediction values, ordinate: reference values (LAB).

Slika 4. Krivulja procjene za jednu od šest skupina procjene predviđanja sadržaja kolesterola u mesu batka guske

The values for classification accuracies (total, for geese, and for chickens, capons, cockerels) were more than 96%, and show a large predictive power for the model constructed. Thus, the discri-

minant analysis allows assigning the sample to a specific meat group, and in case of doubt, it facilitates the selection of the calibration for cholesterol prediction.

Table 2 Validation characteristics for cholesterol content prediction in poultry meat (breast, legs)

Tablica 2. Značajke procjene predviđanja sadržaja kolesterola u mesu peradi (prsna, bataci)

	Chickens, capons, cockerels - Pilići, kopuni, pijetlovi		Geese - Guske	
	Breast - prsna	Leg - noga	Breast - prsna	Leg - noga
N	31	26	17	18
MIN (mg/g)	0.452	0.469	0.878	0.765
MAX (mg/g)	0.918	1.190	1.212	1.105
Average (mg/g)	0.628	0.875	0.983	0.893
SEP	0.055	0.094	0.087	0.107
Bias	0.011	-0.02	0.01	0.035
Slope	1.16	1.68	1.08	3.47
RSQ	0.861	0.569	0.501	0.310

N – number of samples in the validation set - broj uzoraka u kalibracijskom setu

MIN/MAX – minimal/maximal cholesterol content in the validation set - minimalni/maksimalni sadržaj kolesterola u kalibracijskom setu

Average – average cholesterol content in the validation set - prosječan sadržaj kolesterola u kalibracijskom setu

SEP – standard error of prediction corrected for bias - standardna pogreška predviđanja ispravljena za pristranost

Bias – difference between the average of the predicted values and reference value - razlika između prosjeka predviđenih vrijednosti i referentne vrijednosti

RSQ – coefficient of determination (correlation coefficient squared) – koeficijent determinacije (kvadratni korelacijski koeficijent)

Table 3 Validation parameters of the classification predictive power for the RMSX Residuals method

Tablica 3. Parametri procjene klasifikacije mogućnosti predviđanja za metodu RMSX Residents

No. of the test set – broj testnog seta	Total accuracy, poultry – Ukupna točnost, perad	Classification accuracy – točnost klasifikacije (%)	
		Geese - Guske	Chickens, capons, cockerels – Pilići, kopuni, pijetlovi
1	97.5	97.5	97.5
2	98.8	100.0	97.5
3	95.0	97.5	97.5
4	96.3	97.5	97.5
5	96.3	97.5	95.0
		Average - Prosjek(%)	
	96.8	97.0	98.0

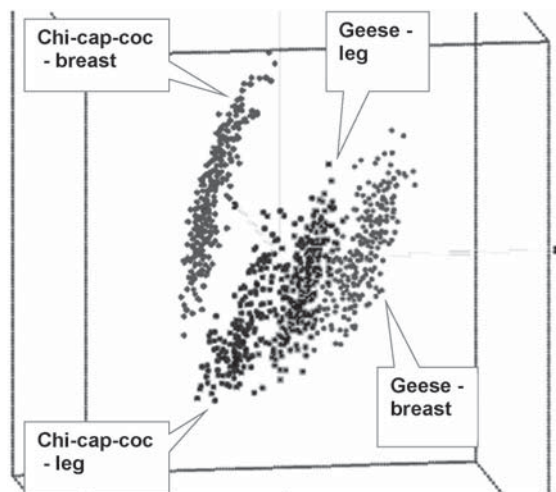


Fig. 5 3D-visualization for the 4 groups of poultry meat (Chi-cap-coc mean: chickens, capons, cockerels). Scatter correction SNV_Detrend, Math Treatment 1,4,4,1, Wavelengths 578,1090,8; 1108,1842,8.

Slika 5. 3-D slika četiriju skupina mesa peradi (pilića, kopuna i pijetlića)

CONCLUSIONS

The results obtained in this work show that NIRS can be used as a routine analysis method for the rapid determination of cholesterol content in the breast and leg meat of chickens, capons, cockerels, and also in the breast and leg meat of geese. The discriminant method with the highest predictive ability, based on RMSX residuals, was used to classify the samples. Classification accuracy values were very correct and ranged, on average, from 96.8% to 98%.

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SAŽETAK

Provedene su četiri kalibracije za sadržaj kolesterola u mesu peradi (prsna i bataci pilića, pijetlića, kopuna i gusaka). Standardne kolebljivosti izražene kao SECV (Standardna pogreška rotirajuće procjene) (%), relativno) za piliće, pijetliće i kopune bile su 9,2 za prsna i 7,8 za batak. Te su vrijednosti za guske iznosile 8,4 odnosno 9,0. Metoda diskriminacije s najvećom mogućnošću predviđanja na temelju RMSX Residents korištena je za klasificiranje uzoraka. Vrijednosti točnosti klasifikacije bile su dobre i kretale su se, uglavnom, od 96,8% do 98%. Kalibracije NIRS za sadržaj kolesterola u mesu prsna i bataka pilića, kopuna, pijetlića kao i mesu prsna i bataka gusaka dobre su za brzu rutinsku analizu u praksi.

Ključne riječi: kolesterol, rotirajuća procjena, kolebljivost