Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Exploring Fourier transform mid-infra-red spectrometry to predict biochemical parameters in horse's blood

Andrea Minuti, Luigi Calamari, Giulia Ferronato, Annarita Ferrari, Olga Gachiuta, Paolo Bani & Erminio Trevisi

To cite this article: Andrea Minuti, Luigi Calamari, Giulia Ferronato, Annarita Ferrari, Olga Gachiuta, Paolo Bani & Erminio Trevisi (2019) Exploring Fourier transform mid-infra-red spectrometry to predict biochemical parameters in horse's blood, Italian Journal of Animal Science, 18:1, 1223-1230, DOI: 10.1080/1828051X.2019.1647121

To link to this article: https://doi.org/10.1080/1828051X.2019.1647121

Published online: 01 Aug 2019.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 1223–1230
https://doi.org/10.1080/1828051X.2019.1647121

Taylor & Francis
Taylor & Francis Group

PAPER

OPEN ACCESS Check for updates

Exploring Fourier transform mid-infra-red spectrometry to predict biochemical parameters in horse's blood

Andrea Minuti, Luigi Calamari, Giulia Ferronato, Annarita Ferrari, Olga Gachiuta, Paolo Bani and Erminio Trevisi

Dipartimento di Scienze Animali, degli Alimenti e della nutrizione - DIANA, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy

ABSTRACT

The aim of this study was to evaluate the use of Fourier transform mid-infra-red (FT-MIR) spectrometry to analyse blood biochemical parameters of the horse. For this purpose, mid infrared transmission spectra were acquired from plasma samples from 72 healthy horses. Each sample was also analysed using reference clinical chemical methods, and these results were used as calibrating values to develop predictive models by partial least squares method. The validation was carried out using external validation method. The coefficient of determination (R^2) and the ratio of prediction to deviation (RPD) showed high values for parameters regarding energy and protein metabolism. Among energy parameters, an excellent prediction model was developed for total cholesterol ($R^2 = 0.94$; RPD = 4.40) and triglycerides ($R^2 = 0.96$; RPD = 5.0) while fair results were obtained for cholesterol fractions (R^2 range: 0.75–0.80; RPD range: 2.0–2.3). Among protein metabolism parameters, excellent prediction models were developed for total protein, albumin, globulin (R^2 range: 0.96–0.99; RPD range: 5.40–9.30) and good prediction model for urea ($R^2 = 0.90$; RPD = 3.2), confirming previous results with the plasma of dairy cows. Our results highlight that FT-MIR spectrometry offers an accurate measurement of important plasma biomarkers for the evaluation of energy (cholesterol and triglycerides) and protein metabolism (urea), as well as for health status (albumin/globulin ratio). Our results may open an interesting perspective of a more cost-effective approach to monitoring the metabolic status and health conditions of the horse, with the future possibility to predict some blood biomarkers by the practitioner in field.

HIGHLIGHTS

- FT-MIR potential to measure blood parameters in horses was explored;
- Infra-red spectrometry can be used in horse's clinical chemistry;
- Fast and cost-effective metabolic status evaluation in horses;
- Accurate FT-MIR predictions for plasma protein and lipid fractions in horses.

ARTICLE HISTORY
Received 4 January 2019
Revised 24 June 2019
Accepted 18 July 2019

KEYWORDS
Horses; clinical chemistry; blood biochemical parameters; metabolic profile; FT-MIR spectroscopy

Introduction

Plasma or serum chemistry profiling can identify levels of several proteins, enzymes, electrolytes and other biochemical molecules that give information on general health status and metabolic condition as well as on individual organs' functionality. Therefore, plasma chemistry is used in diagnostic investigation to provide supportive evidence of a suspected diagnosis, as a prognostic indicator, to monitor metabolic changes that can be used to track the progress of disease in animals under treatment (Burlikowska et al. 2015) and to reveal health disorders already in a preclinical stage. Moreover, in sport horses, the routinary evaluation of a metabolic profile can provide important information in terms of nutritional status and performance evaluation in sport horses (Lindner 2000). The standard methodology to analyse those parameters is expensive and the time to get the results from the lab is often too long.

Infra-red, vibrational, spectroscopy deals with measurements of the electromagnetic spectrum that originates from transitions between quantised energy states due to vibrations of molecular bonds (Long 2005). Applications of infra-red spectroscopy as an

CONTACT Dr Andrea Minuti andrea.minuti@unicatt.it Dipartimento di Scienze Animali, degli Alimenti e della nutrizione – DIANA, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy

Supplemental data for this article can be accessed here.

analytical tool in different areas of animal production are receiving growing interest and attention. This technology has several advantages over other analytical techniques: rapidity of analysis, no use of chemicals, minimal or no sample preparation and easy applicability in different work environments (on/in/at-line applications) (Kruse-Jarres et al. 1990; Shaw et al. 1998). This, along with the availability of cost-effective micro infra-red instruments available in the field, offer the possibility to use the developed prediction models to measure directly in field an ever-wider set of parameters on different biological fluids. In particular, FT-MIR technology provides information on a levy large number of analytes because of the absorptions bands are sensitive to chemical composition of individual constituents (Karoui 2018). The application of infra-red spectroscopy in clinical chemistry showed interesting results for human (Shaw et al. 1998; Shaw and Mantsch 2006) and we have recently reported in dairy cows accurate FT-MIR prediction models for several plasma biomarkers to evaluation animals' metabolism and inflammatory status, including cholesterol and plasmatic protein fractions (Calamari et al. 2016). At the moment, to the best of our knowledge, no reports have investigated the possibility of using infra-red spectroscopy to analyse a set of biochemical parameters included in a metabolic profile in horses. The objective of the present research was to test the feasibility of developing prediction models of the main blood biochemical parameters for horses using Fourier transform mid-infra-red spectrometry (FT-MIR).

Materials and methods

Study design

The research protocol and the animal care were in accordance with the european directive 2010/63/EU on the protection of animals used for scientific purposes. The trial involved 72 horses raised in 5 stables located in North-West Italy. The blood samples were collected during routine check-up performed by the veterinary staff of the stables from spring 2015 to winter 2016. Every animal was sampled only one-time during sample collection. The horses were similarly distributed for sex with 33 males and 39 females. The age of the horses ranged from 3 to 24 years with 22 young horses (less than 10 years old), 33 adult horses (from 10 to 18 years old) and 18 old horses (greater than 18 years old). The most numerous breeds were: Arabian (33 horses), Spanish Anglo-Arabian (14 horses), Sella Italiano (9 horses), Nederlands horse (4 horses) and others (12 horses). The detailed information about race/breed, sex, age, physiological stage, working activities and diet of the horses is included in Supplementary Table S1.

Blood sampling and analyses with reference methods

Blood samples were collected before the morning meal, by venipuncture from the jugular vein, using 10-mL Li-heparin treated tubes (Vacuette, containing 18 U of Li-heparin/mL, Kremsmünster, Austria) and immediately cooled in an ice-water bath. The plasma obtained by centrifugation (3500×*g* for 16 min at 4 °C) was separated into two aliquots: the first aliquot was immediately used to collect the infra-red spectra; the second one was stocked at −20 °C until metabolites analysis and the results were to develop prediction models based on mid-infra-red spectra. Plasma metabolites used as calibrating values were analysed at 37 °C by an automated clinical analyser (ILAB 650, Instrumentation Laboratory, Lexington, MA, USA) using the methodology previously described by Calamari et al. (2016) and reported in Table 1. Commercial kits from Instrumentation Laboratory SpA (Werfen, Italy) were used to measure glucose, total cholesterol, urea, Ca, P, Mg, total protein, albumin, total bilirubin, and creatinine from Wako (Chemicals GmbH, Neuss, Germany) to measured non-esterified fatty acids (NEFA) and Zn. Electrolytes, Na, K and Cl were measured by the potentiometer method (ion-selective electrode connected to ILAB 650). Kinetic analysis was adopted to determine the activity of enzymes: alkaline phosphatase (AP; EC 3.1.3.1), aspartate aminotransferase (AST; EC 2.6.1.1), γ-glutamyltransferase (GGT; EC 2.3.2.2), lactate dehydrogenase (LDH; EC 1.1.1.27) using Instrumentation Laboratory kits (Instrumentation Laboratory SpA). Ceruloplasmin and haptoglobin were measured using the methodology described by Calamari et al. (2016). Triglycerides were measured using a kit of Instrumentation Laboratory. Triglycerides were measured using Trinder endpoint methodology (glycerol oxidase/peroxidase), after hydrolysis of triglycerides to glycerol. The high-density lipoproteins (HDL) and low-density lipoproteins (LDL) cholesterol were measured using a commercial kit (Randox Laboratories Limited, Crumlin, UK) using Trinder endpoint methodology [cholesterol oxidase (CHOD)/peroxidase (POD)] after a hydrolysis of cholesterol esters to free cholesterol. Paraoxonase (PON) activity was measured according to the methodology described by Bionaz et al. (2007). Myeloperoxidase (MPO) activity was measured according to the methodology

Table 1. Analytical reference methods (Calamari et al. 2016) and descriptive statistic of the variables measured in 72 horse plasma samples using the reference methods.

	Analytical reference methods			Descriptive statistic				
Item	Methodology	Wavelength, nm	CV[a]	Mean	SD[b]	Min.[c]	Max.[d]	CV[e]
Minerals								
Ca, mmol/L	Endpoint	570	1.40	3.10	0.12	2.77	3.42	4
Na, mmol/L	ISE device		0.90	139.60	1.20	136.70	142.50	1
K, mmol/L	ISE device		1.30	3.83	0.51	2.14	5.12	13
Cl, mmol/L	ISE device		1.50	104.70	1.50	101.70	108.30	1
Zn, µmol/L	Endpoint	546		10.48	1.40	7.22	15.89	13
Mg, mmol/L	Rate	340	1.40	0.71	0.06	0.56	0.82	8
Inorganic P, mmol/L	Endpoint	340	2.00	1.17	0.20	0.75	1.66	17
Enzymes								
AST[f], U/L	Rate	340	2.10	304.60	103.70	201.30	755.00	34
LDH[g], U/L	Rate	340	1.00	451.90	138.70	229.90	928.20	31
AP[h], U/L	Rate	405	1.70	130.30	37.80	67.20	227.90	29
Paraoxonase, U/mL	Endpoint	405	6.77	103.10	19.60	64.80	147.20	19
GGT[i], U/L	Rate	405	3.72	27.97	7.33	9.90	48.51	26
Myeloperoxidase, U/L	Rate	450	5.13	492.70	84.00	272.30	665.10	17
Ceruloplasmin, µmol/L	Endpoint	546	3.48	2.82	0.68	1.58	4.98	24
Protein and energy markers								
NEFA[j], mmol/L	Endpoint	546	1.50	0.19	0.13	0.05	0.51	69
Glucose, mmol/L	Endpoint	510	1.50	5.35	0.38	4.50	6.17	7
LDL[k] cholesterol, mmol/L	Endpoint	600	3.30	0.63	0.23	0.20	1.31	37
HDL[l] cholesterol, mmol/L	Endpoint	600	3.10	1.36	0.20	0.93	1.97	14
Urea, mmol/L	Endpoint	340	1.20	5.27	0.93	3.38	7.63	18
Total cholesterol, mmol/L	Endpoint	510	2.10	2.42	0.36	1.59	3.42	15
Triglycerides, mmol/L	Endpoint	510	2.40	0.32	0.09	0.13	0.62	30
Albumin, g/L	Endpoint	600	1.80	35.09	1.70	30.90	39.32	5
Globulin, g/L				32.70	3.93	25.70	44.50	12
Total protein, g/L	Endpoint	546	1.20	67.80	3.81	60.00	79.00	6
Other parameters								
Creatinine, µmol/L	Endpoint	510	5.40	106.50	13.10	80.50	137.70	12
Haptoglobin, g/L	Endpoint	450	13.54	1.01	0.30	0.16	2.03	30
FRAP[m], µmol/L	Endpoint	600	7.70	164.60	32.10	73.50	246.00	20
Total bilirubin, µmol/L	Endpoint	546	6.70	25.19	8.54	10.54	55.06	34

[a]Coefficient of variation = calculated on the results obtained between runs according to the National Committee for Clinical Laboratory Standards (Document EP3-T: Guidelines for Manufacturers for Establishing Performance Claims for Clinical Chemistry Methods, Replication Experiment Evaluation," Villanova, PA, 1982.).
[b]Standard deviation.
[c]minimum.
[d]maximum.
[e]coefficient of variation of the plasma variables measured with reference method.
[f]aspartate aminotransferase.
[g]lactate dehydrogenase.
[h]alkaline phosphatase.
[i]γ-glutamyl transferase.
[j]non-esterified fatty acids.
[k]low-density lipoproteins.
[l]high-density lipoproteins.
[m]ferric reducing antioxidant power.

described by Bradley et al. (1982), and Ferric Reducing Antioxidant Power (FRAP) according to Benzie and Strain (1999).

FT-MIR spectroscopy

FT-MIR measurements were performed with a MilkoScan FT (Foss Electric, Hillerød, Denmark). The instrument was equipped with a flow cell with a heating system and a homogeniser to standardise the samples conditions. The spectra were collected in the range of 5012–925 cm^{-1} with a spectral resolution of 3.8 cm^{-1}. Every collected spectrum was composed by 1060 points as percentage transmittance. The absorbance was then calculated for each spectrum, and only informative areas between 3200 and 900 cm^{-1} were retained (Foss Electric 2002). Ten millilitres of plasma were required to acquire 10 interferograms that were averaged to produce a single spectrum.

Data analysis

The infra-red spectra of the analysed samples were processed by the OPUS© (Bruker Optik GmbH, Ettlingen, Germany) software to develop calibrations curves for each parameter by using partial least square (PLS) fit method. The Mahalanobis distances were

used for the detection of outliers. According to Shaw and Mantsch (2006), for each parameter two independent datasets were created: a calibration dataset with 54 samples (75% of the total samples) and a validation dataset constituted by 18 samples (25% of the total samples). The calibration and the validation datasets were automatically created by the software for each parameter in order to be similar in terms of mean, range and standard deviation. The spectra data from calibration dataset were preprocessed by maths functions that included: (a) vector normalisation (SNV; it normalises a spectrum by first calculating the average intensity value and subsequent subtraction of this value from the spectrum. Then the sum of the squared intensities is calculated, and the spectrum is divided by the square root of this sum); (b) First derivative (it calculates the first derivative of spectrum. This method emphasises steep edges of a peak. It is used to emphasise pronounced but small features over a broad background); and (c) First derivative + SNV. The developed calibration model was internal validated using cross-validation (live-one-out) procedure. To assess the accuracy of the prediction models, the following statistics were used: (i) the coefficients of determination R^2 for calibration; (ii) the root mean square error of estimation (RMSEE) for calibration and (iii) the number of extracted factors. The prediction models with the highest R^2 and lowest RMSEE and number of extracted factors were retained.

The prediction models were applied to the validation dataset containing the samples previously excluded from the calibration. The accuracy of the model was evaluated using R^2 for validation, root mean square error of prediction (RMSEP) and RPD according to the criteria suggested by Urbano Cuadrado et al. (2005) and Williams (2014). Moreover, the bias between predicted and reference values in the validation dataset was estimated according to Bland and Altman (1986) using SAS software (SAS Institute Inc., Cary, NC, USA; release 8.0). A *t*-test was applied to establish if the mean value of differences was significantly different from zero ($p < .05$), that would indicate the existence of a fixed bias.

Results

Descriptive statistics are displayed in Table 1. The horses selected for this study were all clinically healthy with the concentrations of the blood parameters within the reference ranges reported for horses without clinical signs of disease (Calamari et al. 1990, 2010; Abeni et al. 2013). The blood parameters with the lowest variability (CV <10%; Table 1), in ascending order, were Na, Cl, Ca, albumin, total protein, glucose and Mg. The greatest variability (CV ≥30%; Table 1), in ascending order, was observed for triglycerides, haptoglobin, LDH, AST, total bilirubin, LDL cholesterol and NEFA.

Table 2 shows the summary of PLS prediction models. The number of extracted factors with the PLS procedure for each prediction model ranged from 1 to 8. A low number (≤4) of extracted factors was observed for Ca, Na, K, Zn, AST, LDH, AP, paraoxonase, myeloperoxidase, ceruloplasmin, albumin, globulin, total protein, creatinine, haptoglobin, FRAP, total bilirubin, whereas a high number (≥6) was found for Mg, inorganic P, GGT, NEFA, glucose, LDL cholesterol, HDL cholesterol, urea, total cholesterol, triglycerides and albumin globulin ratio.

The R^2 in validation ranged from 0.01 to 0.99. Values of R^2 below 0.66 were observed, in increasing order, for AST, Ca, LDH, creatinine, Na, K, haptoglobin, Cl, AP, NEFA, paraoxonase, glucose, FRAP, GGT, Zn, myeloperoxidase, total bilirubin, Mg and ceruloplasmin. The R^2 in validation ranged from 0.66 to 0.81 for the prediction model of LDL cholesterol and HDL cholesterol. Values of R^2 in validation greater than 0.82 were observed, in increasing order, for the prediction models of inorganic P, urea, total cholesterol, triglycerides, albumin, albumin globulin ratio, globulin and total protein.

The RPD for validation dataset in our study ranged from 2 to 3 for the prediction models of LDL cholesterol, HDL cholesterol and inorganic P. An excellent prediction ability was obtained for parameters related to energy and protein metabolism. Among energy parameters, calibration curves developed for total cholesterol and triglycerides had RPD values greater than 4. Among the parameters of protein, urea, albumin and albumin globulin ratio showed a RPD greater than 3 and total protein and globulin showed an RPD greater than 8 (Table 2). Moreover, the Bland–Altman plots (Supplementary Figure S1) showed for inorganic P, urea, albumin, globulin and total protein that there was a good concordance between the results obtained by FT-MIR and the reference. Also, the *t*-test indicated the absence of a fixed bias between predicted and reference values ($p > .05$).

Discussion

The accuracy of infra-red analysis is affected by the quality of the reference assays used (Barbano and Clark 1989). Precision has to do with how much

Table 2. Summary of partial least squares prediction models obtained with the calibration dataset ($n = 54$ samples) and validation dataset ($n = 18$ samples) from horse plasma samples.

	Calibration model			Validation model			
Parameters	Number of extracted factors	R^{2a}	RMSEE[b]	R^{2a}	RPD[c]	RMSEP[d]	Bias
Minerals							
Ca, mmol/L	1	0.070	0.110	0.030	1.020	0.150	−0.016
Na, mmol/L	1	0.150	1.070	0.190	1.140	1.280	−0.279
K, mmol/L	4	0.360	0.390	0.210	1.190	0.520	−0.173
Cl, mmol/L	5	0.520	1.030	0.260	1.180	1.430	−0.199
Zn, µmol/L	3	0.310	1.220	0.530	1.490	0.920	−0.189
Mg, mmol/L	7	0.590	0.040	0.560	1.540	0.040	0.008
Inorganic P, mmol/L	8	0.910	0.060	0.880	2.920	0.080	−0.002
Enzymes							
AST[e], U/L	1	0.110	93.900	0.010	1.000	115.000	24.200
LDH[f], U/L	1	0.130	117.000	0.150	1.100	158.000	39.400
AP[g], U/L	1	0.190	31.800	0.260	1.200	37.800	4.350
Paraoxonase, U/mL	2	0.320	15.900	0.340	1.200	17.400	−1.870
GGT[h], U/L	6	0.640	4.140	0.390	1.310	7.020	1.660
Myeloperoxidase, U/L	2	0.350	67.200	0.530	1.500	61.800	8.640
Ceruloplasmin, µmol/L	3	0.530	0.440	0.580	1.540	0.540	0.040
Protein and energy markers							
NEFA[i], mmol/L	6	0.570	0.090	0.330	1.230	0.100	0.011
Glucose, mmol/L	7	0.610	0.240	0.340	1.310	0.370	−0.133
LDL[j] cholesterol, mmol/L	7	0.810	0.100	0.750	2.020	0.130	0.018
HDL[k] cholesterol, mmol/L	7	0.790	0.090	0.800	2.260	0.090	0.024
Urea, mmol/L	7	0.860	0.320	0.900	3.180	0.390	−0.077
Total cholesterol, mmol/L	7	0.950	0.090	0.940	4.370	0.100	0.040
Triglycerides, mmol/L	7	0.920	0.030	0.960	4.960	0.020	0.002
Albumin, g/L	3	0.960	0.340	0.960	5.370	0.370	0.090
Albumin globulin ratio	7	0.980	0.020	0.970	5.920	0.030	0.006
Globulin, g/L	1	0.960	0.770	0.980	8.400	0.590	−0.216
Total protein, g/L	2	0.980	0.530	0.990	9.330	0.480	0.174
Other parameters							
Creatinine, µmol/L	1	0.200	11.400	0.150	1.100	13.200	2.380
Haptoglobin, g/L	1	0.150	0.280	0.240	1.150	0.280	−0.028
FRAP[l], µmol/L	4	0.420	22.400	0.370	1.400	27.100	11.300
Total bilirubin, µmol/L	4	0.360	6.870	0.530	1.660	6.110	2.920

[a]Coefficient of determination.
[b]root mean square error of estimation.
[c]residual prediction deviation.
[d]root mean square error of prediction.
[e]aspartate amino transferase.
[f]lactate dehydrogenase.
[g]alkaline phosphatase.
[h]γ-glutamyl transferase.
[i]non-esterified fatty acids.
[j]low-density lipoproteins.
[k]high-density lipoproteins.
[l]ferric reducing antioxidant power.

variability there is about the actual value measured when the assay is replicated. When an assay is run repeatedly on the same sample and the results obtained have little variability, the assay is said to have high precision (Analytical reference methods in Table 1). In our study, the reference assays used to analyse some blood parameters were characterised by sub-optimal repeatability between runs, with a CV greater than 10% for haptoglobin, and between 3 and 10%, in ascending order, for HDL cholesterol, LDL cholesterol, ceruloplasmin, GGT, myeloperoxidase, creatinine, total bilirubin, paraoxonase and FRAP. For almost all these blood parameters, the prediction models were characterised by a RPD lower than 2, which is considered to be the minimal threshold for acceptable approximate quantitative predictions (Saeys et al. 2005). Among these blood variables without optimal repeatability in the reference chemistry, only the prediction models of HDL and LDL cholesterol could be considered near to acceptability. The reason for this is likely due to two causes: (i) while for the peroxidase, myeloperoxidase and GGT the reference method in the plasma measured the enzyme activity, the HDL and LDL cholesterol fractions are measured as concentrations of the metabolites; and (ii) the plasma concentration of cholesterol fractions is high compared with concentration of ceruloplasmin and FRAP. Therefore, an improvement in the reference

assays for these blood parameters without optimal repeatability could enhance the predictive ability of the prediction model based on FT-MIR spectroscopy.

Overall, the prediction models of the enzyme's activities measured in this study had poor predictive ability. The inadequate estimation was at least in part due to the low proportion of high values in this dataset. For improving the predictive ability of FT-MIR spectroscopy in the assessment of enzyme activities, it could be necessary to obtain a more uniform distribution of data with more samples with high values. Moreover, the reference methods asses the enzymes activity but their absolute concentrations are not quantified. It is therefore possible that discrepancies exist between the amount and activity of the enzymes.

The current study still confirms the difficulty in predicting mineral content in plasma with FT-MIR spectroscopy (apart from inorganic P), in particular for minerals that are not included in organic compounds. Similar results were observed in our previous study on plasma dairy cow (Calamari et al. 2016) and in studies in cow milk, mainly for electrolytes. In particular, Soyeurt et al. (2009) showed poor prediction ability in FT-MIR spectroscopy for Mg, Na and K in milk. Conversely, Soyeurt et al. (2009) and Toffanin et al. (2015) have suggested the potential of FT-MIR spectroscopy to predict Ca and P content in cow milk. The difficulty observed in our study to predict mineral content in blood is likely due to two main reasons. First, among the minerals measured in the blood, a large proportion is in ionised form and not included in organic compounds. Approximately, 50% of total Ca in plasma is in the ionised form, and approximately 45% is linked to protein, whereas approximately 70% of Mg is ionised (Rosol and Capen 1997). Furthermore, in the present study, as we previously observed in dairy cow plasma (Calamari et al. 2016), the variability observed for the minerals in our dataset was lower than for other blood parameters. As before stated, the development of better prediction models requires a dataset containing samples with a greater variability, which allows for an improvement in the predictive ability of FT-MIR spectroscopy. To increase the variability of these parameters, it would be useful to include some animals with clinical diseases or with different physiological conditions that alter the minerals' blood concentration.

The RPD obtained with the validation of the prediction models ranged from 1.02 to 9.33. According to Williams (2014), six levels of prediction accuracy can be established based on RPD values for forages, feeds, soils, functionality factors, etc. For RPD values below 2.5, the calibration is considered poor and valuable only for a rough screening; values between 2.5 and 2.9 are considered fair and valuable for screening; between 3.0 and 3.4 are good and can be used for quality control; between 3.5 and 4.0 are very good and allow approximate quantitative predictions whereas above 4.0 the prediction is considered excellent and can be used for any application. The ranking of prediction accuracy from RPD is confirmed by the values of R^2. Karoui et al. (2006) propose that values for R^2 between 0.50 and 0.65 allow to be used only for discrimination between high and low concentrations, values for R^2 between 0.66 and 0.81 indicate approximate quantitative predictions, whereas R^2 values between 0.82 and 0.90 reveal good prediction. Calibration models with coefficient of determination above 0.91 are considered to be excellent. Thus, based on that interpretation of RPD and R^2, the prediction models developed for total cholesterol, triglycerides, albumin, albumin globulin ratio, globulin and total protein can be considered as excellent. These results confirm our previous study on plasma dairy cows (Calamari et al. 2016). Interesting are in particular the results obtained with FT-MIR for protein and protein fractions, considering that the Fourier transform infrared spectroscopy technique is a widely used tool in many different fields even to evaluate secondary and tertiary protein structure (Qi et al. 2004; Jing et al. 2016), differentiation of plasmin and plasminogen in milk (Ozen et al. 2003), secondary and tertiary structural changes in bovine plasminogen (Hayes et al. 2003) and casein (Curley et al. 1998) and casein fractions in goat's milk (Díaz-Carrillo et al. 1993). The ability of FT-MIR to predict protein fractions (i.e. albumin and globulin) offers the opportunity for more extensive evaluations than just protein metabolism. In fact, the hypoalbuminaemia may be caused, for example, by defective albumin synthesis during an inflammatory event (Minuti et al. 2013, 2014) that at the same time causes the rise of globulin fractions. The combined effect of these changes is a decrease in the albumin globulin ratio that is of greater clinical significance than the total protein concentration. Then, FT-MIR offers the possibility, through the prediction of protein fractions, also to obtain information concerning aspects of health status and welfare of animals, in diagnosing many different problems, including liver, kidney, gastrointestinal tract disease and inflammatory conditions (Trevisi and Bertoni 2009; Trevisi and Minuti 2018).

Conclusions

The results obtained in this study are very promising and may open an interesting perspective for an easier and more cost-effective approach to monitoring biochemical parameters in horse's blood and more generally of animals. Our results highlight that FT-MIR spectroscopy offers accurate measurement of some plasma biomarkers of great importance for the evaluation of the energy and protein metabolism of horses. Moreover, the very good ability to predict total protein and some information concerning the alteration in protein fractions (albumin and globulin) offers the possibility to obtain information on health status and conditions implying alteration of albumin globulin ratio. Our results also suggest that some other plasma biomarkers related to mineral metabolism, inflammatory conditions, as well as enzyme activities are more difficult to predict. For these plasma biomarkers, further studies are needed involving a larger population and including horses in different health conditions to increase the variability within each plasma biomarker. Finally, along with the availability of cost-effective micro infra-red instruments that can be used in the field, together with the possibility of transferring calibration curves from one instrument to another, the future possibility to predict some blood biomarkers by the practitioner at horse-side seems feasible.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This manuscript is based on research supported by the "Romeo and Enrica Invernizzi foundation" Milan, Italy.

ORCID

Andrea Minuti http://orcid.org/0000-0002-0617-6571
Luigi Calamari http://orcid.org/0000-0002-1632-9762
Giulia Ferronato http://orcid.org/0000-0001-9153-8540
Annarita Ferrari http://orcid.org/0000-0001-5945-8540
Paolo Bani http://orcid.org/0000-0002-5334-1015
Erminio Trevisi http://orcid.org/0000-0003-1644-1911

References

Abeni F, Dal Prà A, Bertin G, Calamari L. 2013. Serum protein fraction in mature horses and relationship with metabolic and hematological parameters. J Equine Vet Sci. 33:905–911.

Barbano DM, Clark JL. 1989. Infrared milk analysis — challenges for the future. J Dairy Sci. 72:1627–1636.

Benzie IF, Strain JJ. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299:15–27.

Bionaz M, Trevisi E, Calamari L, Librandi F, Ferrari A, Bertoni G. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J Dairy Sci. 90:1740–1750.

Bland JM, Altman DG. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1:307–310.

Bradley PP, Priebat DA, Christensen RD, Rothstein G. 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 78:206–209.

Burlikowska K, Boguslawska-Tryk M, Szymeczko R, Piotrowska A. 2015. Haematological and biochemical blood parameters in horses used for sport and recreation. J Cent Eur Agric. 16:370–382.

Calamari L, Abeni F, Bertin G. 2010. Metabolic and hematological profiles in mature horses supplemented with different selenium sources and doses. J Anim Sci. 88: 650–659.

Calamari L, Cappa V, Parmeggiani F, Galizzi Vecchiotti G. 1990. Nota sul profilo metabolico di cavalli trottatori. Ippologia. 1:67–70.

Calamari L, Ferrari A, Minuti A, Trevisi E. 2016. Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on Fourier transform mid-infrared spectroscopy: preliminary results. BMC Vet Res. 12:4.

Curley DM, Kumosinski TF, Unruh JJ, Farrell HM. 1998. Changes in the secondary structure of bovine casein by Fourier transform infrared spectroscopy: effects of calcium and temperature. J Dairy Sci. 81:3154–3162.

Díaz-Carrillo E, Muñoz-Serrano A, Alonso-Moraga A, Serradilla-Manrique J. 1993. Near infrared calibrations for goat's milk components: protein, total casein, αs-, β- and κ-caseins, fat and lactose. J Near Infrared Spectrosc. 1:141.

Foss Electric. 2002. MilkoScan FT120. Type 71200. Reference manual. Issue 9 GB.

Hayes KD, Ozen BF, Nielsen SS, Mauer LJ. 2003. FTIR determination of ligand-induced secondary and tertiary structural changes in bovine plasminogen. J Dairy Res. 70:461–466.

Jing X, Yang C, Zhang L. 2016. Characterization and analysis of protein structures in oat bran. J Food Sci. 81: C2337–C2343.

Karoui R. 2018. Spectroscopic technique: mid-infrared (mir) and Fourier transform mid-infrared (FT-MIR) spectroscopies. In: Da-Wen Sun editor. Modern techniques for food authentication. Cambridge, (MA): Academic Press; p. 23–50.

Karoui R, Mouazen AM, Dufour É, Pillonel L, Picque D, Bosset J-O, De Baerdemaeker J. 2006. Mid-infrared spectrometry: a tool for the determination of chemical parameters in Emmental cheeses produced during winter. Lait. 86:83–97.

Kruse-Jarres JD, Janatsch G, Gless U, Marbach R, Heise HM. 1990. Glucose and other constituents of blood determined by ATR-FTIR-spectroscopy. Clin Chem. 36:401–402.

Lindner A. 2000. Use of blood biochemistry for positive performance diagnosis of sport horses in practice. Rev Méd Vét. 151:611–618.

Long DA. 2005. Handbook of vibrational spectroscopy, volumes 1–5, edited by J. M. Chalmers and P. R. Griffiths. John Wiley & Sons, Chichester, 2002, p. 3862. J. Raman Spectrosc. 36:271.

Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Bani P. 2013. Assessment of gastrointestinal permeability by lactulose test in sheep after repeated indomethacin treatment. J Anim Sci. 91:5646–5653.

Minuti A, Ahmed S, Trevisi E, Piccioli-Cappelli F, Bertoni G, Jahan N, Bani P. 2014. Experimental acute rumen acidosis in sheep: consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. J Anim Sci. 92:3966–3977.

Ozen BF, Hayes KD, Mauer LJ. 2003. Measurement of plasminogen concentration and differentiation of plasmin and plasminogen using Fourier-transform infrared spectroscopy. Int Dairy J. 13:441–446.

Qi PX, Wickham ED, Farrell HM. 2004. Thermal and alkaline denaturation of bovine beta-casein. Protein J. 23:389–402.

Rosol T, Capen C. 1997. Calcium-regulating hormones and diseases of abnormal mineral (calcium, phosphorus, magnesium) metabolism. In: Kaneko J, Harvey J, Bruss M, editors. Clinical biochemistry of domestic animals. 5th ed. San Diego (CA): Academic Press; p. 619.

Saeys W, Mouazen AM, Ramon H. 2005. Potential for onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst Eng. 91:393–402.

Shaw RA, Kotowich S, Leroux M, Mantsch HH. 1998. Multianalyte serum analysis using mid-infrared spectroscopy. Ann Clin Biochem. 35:624–632.

Shaw RA, Mantsch HH. 2006. Infrared spectroscopy in clinical and diagnostic analysis. Encycl Anal Chem.

Soyeurt H, Bruwier D, Romnee J-M, Gengler N, Bertozzi C, Veselko D, Dardenne P. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J Dairy Sci. 92:2444–2454.

Toffanin V, De Marchi M, Lopez-Villalobos N, Cassandro M. 2015. Effectiveness of mid-infrared spectroscopy for prediction of the contents of calcium and phosphorus, and titratable acidity of milk and their relationship with milk quality and coagulation properties. Int Dairy J. 41:68–73.

Trevisi E, Bertoni G. 2009. Some physiological and biochemical methods for acute and chronic stress evaluation in dairy cows. Ital J Anim Sci. 8:265–286.

Trevisi E, Minuti A. 2018. Assessment of the innate immune response in the periparturient cow. Res Vet Sci. 116: 47–54.

Urbano Cuadrado M, Luque de Castro MD, Pérez Juan PM, Gómez-Nieto MA. 2005. Comparison and joint use of near infrared spectroscopy and Fourier transform mid infrared spectroscopy for the determination of wine parameters. Talanta. 66:218–224.

Williams P. 2014. The RPD statistic: a tutorial note. NIR News. 25:22–26.