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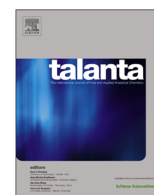

Multicomponent blood lipid analysis by means of near infrared spectroscopy, in geese

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ABSTRACT

This study provides accurate near infrared (NIR) spectroscopic models on some laboratory determined clinicochemical parameters (i.e. total lipid (5.57 ± 1.95 g/l), triglyceride (2.59 ± 1.36 mmol/l), total cholesterol (3.81 ± 0.68 mmol/l), high density lipoprotein (HDL) cholesterol (2.45 ± 0.58 mmol/l)) of blood serum samples of fattened geese. To increase the performance of multivariate chemometrics, samples significantly deviating from the regression models implying laboratory error were excluded from the final calibration datasets. Reference data of excluded samples having outlier spectra in principal component analysis were not marked as false. Samples deviating from the regression models but having non outlier spectra in PCA were identified as having false reference constituent values. Based on the NIR selection methods, 5% of the reference measurement data were rated as doubtful. The achieved models reached R^2 of 0.864, 0.966, 0.850, 0.793, and RMSE of 0.639 g/l, 0.232 mmol/l, 0.210 mmol/l, 0.241 mmol/l for total lipid, triglyceride, total cholesterol and HDL cholesterol, respectively, during independent validation. Classical analytical techniques focus on single constituents and often require chemicals, time-consuming measurements, and experienced technicians. NIR technique provides a quick, cost effective, non-hazardous alternative method for analysis of several constituents based on one single spectrum of each sample, and it also offers the possibility for looking at the laboratory reference data critically. Evaluation of reference data to identify and exclude falsely analyzed samples can provide warning feedback to the reference laboratory, especially in the case of analyses where laboratory methods are not perfectly suited to the subjected material and there is an increased chance of laboratory error.

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1. Introduction

Foie gras as a luxury food product is made of specially fattened duck or goose liver. Total foie gras production of the European Union was around 25,000 t in 2014 covering world's 90% [1]. The fatty goose liver production of Hungary is nearly 2000 t per annum, being a world leader, and the country has old traditions of fattening [2]. During gavage the birds are strongly overfed with high-fat diets that provides, besides 8–10-fold liver mass increase, severe dyslipidemia [3]. Because of the force-feeding procedure, gavage-based foie gras production is controversial and production or retailed products are denied in several countries, and there is an expressed need for avoiding gavage in foie gras production. Evaluating the effects of gavage or gavage-free feeding alternatives, and production performances of different breeds requires biochemical analytical background [4]. Thus, quick and accurate analysis of body fluids, primarily blood plasma or serum is essential to evaluate health or nutritional status of animals. The most characteristic alteration of serum blood biochemical constituents during hepatic steatosis concerns the blood lipids: total lipid, triglyceride and the lipoproteins, i.e. total and high density lipoprotein (HDL) cholesterol.

In the routine clinicochemical laboratory analysis the current practice is applying automated equipment utilizing enzymatic and direct photometric methods for the metabolite and enzyme measurements, while most ion concentrations are measured with

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ion-selective electrodes [5]. Because of hyperlipidemia, it is often problematic to get accurate results for blood parameters of fattened geese using automats widely applied in human diagnostics. Moreover, some of the analyses are loaded with uncertainty derived from the improper standard substrate used for calibration [6].

The widespread application of near infrared (NIR) spectroscopy for the quick estimation of biochemical components in divergent biological matrices is reasoned by multiple factors, such as the short analysis time, reagent-less work, lack of waste production, and the parallel estimation possibility of multiple components. These advantages provoked the expansion of the NIR technique in clinical chemistry. Possibly one of the earliest approaches was performed by Hall & Pollard [7] describing the estimation of serum proteins, triglycerides and glucose, with the very important warning that purified chemical standard solutions cannot be applied as a basis for quantitative analysis. Instead, to develop calibrations, a real primary sample set with known clinicochemical values is needed. This methodology was utilized by Heise et al. [8] reporting the successful assay of total protein, glucose, total cholesterol, triglyceride, and urea in EDTA-treated human plasma, recommending the use of wide wavelength regions. Domján et al. [9] and Turza et al. [10] made important steps towards simplification of the sample application, namely authors analyzed also whole (anticoagulant treated) blood, Turza et al. [10] even directly in sampling tubes instead of cuvettes. From a physiological aspect it is worth mentioning that the NIR spectroscopy based estimations are not limited to chemically clearly defined molecules (e.g. glucose [10] or urea [7]), but it is also accurate for heterogeneous compounds, like total protein (albumin and globulins [8]), and for highly complex particles, like lipoproteins [9,11]. Petter et al. [11] described NIR method useful as a potential alternative or even supplementary clinical method for the quick determination of low density lipoprotein (LDL) and HDL in human serum.

There are several factors affecting the performance of NIR calibration models, such as sample preparation and presentation to scanning, geometry and operation of the spectrometer, environmental effects, disabilities of mathematical modelling, or the inaccuracy of the reference data. There are tests in use to evaluate most of these effects, but in many cases reference data are being considered as accurate, however this is not always checked.

Due to the multi collinearity of NIR spectral data, and the complexity caused by the overlapping combination and overtone bands, multivariate statistical methods are required for developing quantitative and qualitative models. Evaluation of the multivariate models according to chemometrics reveals the background of calibration and classification procedures, describes the relationship between the spectral data, the chemical composition, and the physical parameters [12]. Thus, through detailed chemometric analysis of NIR data, latent variables, and regression coefficient vectors it is possible to describe the chemical reason why the developed models work. Since model performance may decrease in any case when unreliable reference or spectral data are observed, evaluation of models can provide information on the input data.

Chemical constituents provide complex absorption signals in various parts of the NIR spectrum. Assuming a well-operated benchtop spectrometer, NIR spectra describe the samples very accurately, and the performance of the developed calibration model may be limited mostly by the slight inaccuracies of the reference data [13,14]. In this sense, NIR technique is useful in testing and evaluating the reference data, as errors of NIR models may refer to the errors of the laboratory reference data [15].

The objective of the present study was to develop NIR calibration models for further feeding and housing experiments which can predict geese's blood clinicochemical parameters, using different spectral regions, and spectra of different measurement modes – i.e. transmission and transflectance. Furthermore, our aim was to develop an automatable objective method to evaluate the outlier samples in order to increase model accuracy and give feedback to reference analytical laboratory in highly doubtful cases. The biological sample in focus was a highly characteristic, postprandial, high-fat avian blood serum, providing generally large variation (broad range) in the population investigated.

2. Materials and methods

2.1. Animals and blood sampling

Grey Landes type ganders were examined at 11th, 17th and 20th days within the *a priori* planned and legislated force-feeding protocol, performed at the experimental farm in a closed stable of the Kaposvár University by a professional animal caretaker. The force-feeding diet contained 4.91 g ether extract /100 g dry matter (DM), 12.78 g crude protein /100 g DM, 2.33 g crude ash /100 g DM and 12.4 MJ / kg metabolizable energy. The force-feeding was performed three times per day, until satiation, being a routine protocol. The investigation period was chosen due to the high blood concentration of the analytes in study. The same thirty geese were tested on day 11 and 17, while additional 15 geese were involved on day 20 ($n=30+30+45$). Blood samples were drawn from the *v. brachialis* after cutaneous local analgesia into 13 ml centrifuge tubes with 22 G sterile needles. Blood was left to clot at 35 °C, centrifuged on 1500 rpm for 10 min, before serum separation. Serum was siphoned and stored in 5 ml Eppendorf tubes at −70 °C until analysis. Animals after the force-feeding were sold for a professional goose abattoir and were processed industrially.

The study was carried out in strict accordance with the animal welfare protocol for experiments at Kaposvár University, and all efforts were made to minimize suffering. The protocol was approved by the Somogy County Animal Health and Food Chain Safety Directorate under the allowance number: XV-I-31/446-10/2012, in accordance with the concerning EU legislations.

2.2. Clinicochemical analysis

Clinicochemical analysis was performed on an automated equipment (Hitachi 917) in a single analytical run. Triglyceride (TG) concentration was measured on the basis of the Trinder reaction [16], total cholesterol (TCh) by the enzymatic method of Röschlau et al. [17], and high-density lipoprotein cholesterol (HDL-Ch) by the phosphotungstic acid/$MgCl_2$ precipitation method. Reagent kits for TG, TCh, and HDL-Ch were purchased from Human Diagnostics Ltd. (Wiesbaden, Germany). The total lipid (TL) content of serum was determined spectrophotometrically (Shimadzu UV 160 A equipment), with the sulfo-phospho-vanillin method and measured in 10 mm path length optical glass cuvettes [6]. There was no replicate measurement of reference clinicochemical data at the single blood serum samples.

2.3. Near infrared spectrum acquisition

Each geese blood serum sample was scanned using a FOSS NIRSystems 6500 spectrometer (FOSS NIRSystems, Inc., Laurel, MD, USA). Absorbance spectra (log1/*T* for transmission mode, and log1/*R* for transflectance mode, further on referred to as transmission spectra and transflectance spectra, respectively) were recorded with WinISI II, version 1.5 software (InfraSoft International LLC, State College, PA, USA). Spectra were recorded with a spectral increment of 2 nm in the 400–2498 nm interval. Acquisition of transmission spectra was performed with a 1 mm open-top

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