

Rapid protein quantification in chicken breast: A comparison of near infrared spectroscopy and the Kjeldahl method

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ABSTRACT

The study focuses on the importance of slow-growing native Italian poultry breeds, specifically the Bianca di Saluzzo, in biodiversity conservation and local product valorisation, highlighting the need for rapid, precise, and reliable measurements to ensure accurate breed evaluation and support sustainable breeding practices. These breeds, raised through traditional methods with attention to animal welfare, contribute to cultural and gastronomic identity while promoting sustainability. Accurate measurement of meat quality parameters like protein content is crucial for maintaining consumer trust and meeting food regulations. The research compares two protein determination methods: the traditional Kjeldahl method (Kj.m.) and the Near-Infrared Spectroscopy (NIRS). While the Kj.m. is accurate but labour-intensive and hazardous, NIRS offers a faster, non-destructive alternative. The study involved 192 Bianca di Saluzzo chickens, with protein content in breast meat analysed using both methods. Results showed a statistically significant difference between the methods, with NIRS slightly underestimating protein content compared to Kj.m. Despite this, NIRS demonstrated acceptable accuracy and significant advantages in speed and safety. The study suggests further calibration and refinement of NIRS to improve its precision for poultry meat from slow-growing chickens. Integrating NIRS technology can enhance farm efficiency, sustainability, and biodiversity conservation, providing reliable data on meat quality and nutritional composition. This technological advancement supports small farmers and improves consumer confidence in local poultry products.

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1. INTRODUCTION

Slow-growing native Italian poultry breeds, such as the Bianca di Saluzzo, play a fundamental role in the conservation of biodiversity and in the valorisation of typical local products [1]. The Bianca di Saluzzo is a prized breed originating from Piedmont, a region localized in the north-western part of Italy, characterized by slow growth and superior quality meat, renowned for its rich flavour and succulent consistency [2]. Raising local breeds require traditional methods and particular

attention to animal welfare, aspects that contribute to preserving local agricultural traditions and promoting sustainable production. These breeds offer added value both in terms of taste, sustainability and immune status, strengthening the cultural and gastronomic identity of the regions in which they are raised [3]. Native slow-growing poultry breeds exhibit greater tolerance and resilience to dietary variations, with a more diverse and stable gut microbiota compared to broilers, promoting better gut health and reducing the need for antimicrobial interventions [4].

In this context, precise measurements in poultry meat quality are essential to ensure products that are safe, nutritious and compliant with market standards. High accuracy in measuring parameters such as protein content, fat and moisture is crucial to maintain consumer confidence and meeting food regulations. Technological advancement, such as the use of near-infrared spectroscopy (NIRS), may support poultry biodiversity and smallholder farmers by providing tools for rapid and accurate analysis [5]. These technologies allow for more efficient and informed management of poultry genetic resources, improving product quality and offering added value that promotes the sustainability and competitiveness of small farmers in the global market [6].

Accurate protein determination is crucial in the food industry for several key reasons, including nutritional labelling, quality control, and process optimization. Nutritional labelling ensures that consumers are well-informed about the protein content in food products, which is vital for dietary planning and managing specific health conditions. Quality control involves monitoring the consistency and safety of food products, ensuring that they meet regulatory standards and consumer expectations. Process optimization helps manufacturers improve production efficiency and product quality [7]. Traditionally, the Kjeldahl method (Kj.m.) has been widely used for protein quantification due to its reliability in measuring nitrogen content, which is then converted to protein content using a conversion factor. The Kj.m. is well-established and provides a comprehensive measure of total nitrogen in a sample, which has made it a standard technique in the industry for many years. However, this method has several limitations. It is time-consuming, involving multiple steps such as digestion, distillation, and titration, each requiring careful handling and precise conditions. The method also requires the use of hazardous chemicals, including concentrated sulfuric acid and strong bases, which pose safety and environmental risks. Additionally, the accuracy of the Kj.m. can be compromised by the presence of non-protein nitrogenous substances in food samples. These substances, such as urea, nucleic acids, and certain amino acids, can contribute to the total nitrogen measured by the Kj.m., leading to an overestimation of the actual protein content. This potential for inaccuracy necessitates careful consideration and correction to ensure that the protein content reported on nutritional labels is precise and reliable [8]. Due to these limitations, there is an ongoing interest in developing alternative methods for protein determination that are faster, safer, and more specific. Techniques such as the Dumas method [9], which also measures nitrogen content but uses combustion and gas chromatography, and advanced spectroscopic methods like NIRS, are being explored for their potential to provide accurate and efficient protein quantification. This method can offer significant advantages in terms of speed, safety, and specificity, contributing to improved quality control and process optimization in the food industry [10],[11]. In particular, NIRS is emerging as a faster and more convenient alternative, offering rapid, non-destructive analysis with high sensitivity [12]. Studies have shown that NIRS, when combined with chemometric techniques like partial least squares regression (PLSR), can effectively predict protein content in various food matrices, including maize, and plant feed materials, with satisfactory accuracy [13]-[15]. Furthermore, the use of NIRS technology to evaluate lesions and meat quality in chicken breasts represents an important innovation in the poultry sector. With its ability to penetrate the surface layers of meat and detect changes in tissue, NIRS can provide detailed information on the structure and

composition of chicken meat. This allows industry operators to identify any lesions or defects in the meat, evaluate the freshness and quality of the product, and make informed decisions to ensure consumer safety and satisfaction. The adoption of this technology can therefore contribute to improving the quality control and management of poultry production, guaranteeing high standards of the finished product.

Despite its advantages, NIRS can be affected by the presence of other macronutrients, such as fat, which can lead to overestimation of protein content if not properly calibrated [16]. In comparison, the Kj.m., while accurate, is labour-intensive and susceptible to tampering through the addition of non-protein nitrogenous substances. Therefore, while the Kj.m. remains a gold standard, NIRS presents a promising alternative for rapid and efficient protein determination in chicken breast samples, provided that appropriate calibration and validation are performed to account for potential interferences. The objective of this study is to assess the protein content in samples of breast meat from a local, slow-growing breed. This entails a comparison between the traditional Kj.m. and the innovative NIRS method. Through this comparative analysis, the study seeks to distinguish the effectiveness and precision of each technique in quantifying protein levels. By juxtaposing these conventional and advanced methodologies, the research aims to illuminate their unique strengths and limitations, thereby enhancing and progressing protein analysis methodologies in agricultural research and practice.

2. MATERIALS AND METHODS

2.1 Birds and husbandry

The trial was conducted at the poultry facility of the University of Turin, located in north-west Italy, with approval from the University's Bioethical Committee (No. 814715). To support the use and conservation of local genetic resources, the animals used in the trial were hatched from eggs collected at the Avian Conservation Centre for Local Genetic Resources, which is also affiliated with the University of Turin. This centre plays a crucial role in preserving and studying native avian species, thereby contributing to the maintenance of poultry biodiversity and enhancing the understanding of their unique genetic traits.

In total, 192 Bianca di Saluzzo chickens were divided into 24 pens, each housing 8 animals [17]. Each pen measured 2.0×3.2 meters, with the floor covered in rice hulls as bedding. This low stocking density helps prevent overcrowding, reduces the risk of disease and decreases stress among animals, all of which help improve the quality of the meat produced. Extensive free-range breeding allows chickens to benefit from a varied diet and greater physical activity, elements which positively reflect on the nutritional and organoleptic profile of the meat.

At 147 and 174 days of age, two animals from each pen were selected based on average weight to ensure representativeness of the samples. This growth time interval, longer than intensive farming methods, allows the animals to develop naturally, with an overall improvement in the quality of the meat. The selected animals were slaughtered in compliance with EU regulations on animal welfare, which provide specific techniques to minimize pain and stress during slaughter. These regulations not only ensure ethical treatment of animals, but are also crucial to obtaining high-quality meat, as reduced pre-slaughter stress helps maintain the tenderness and flavour of the final product. Chicken breast samples were collected from the 96 slaughtered birds of Bianca di Saluzzo.

2.2 Breast fillet protein evaluation

The assessment of protein content was carried out through the utilization of two distinct methodologies: a conventional technique known as the Kj.m. and a cutting-edge approach termed NIRS. The analysis of the amount of protein in a sample of chicken breast with the Kj.m. is a standardized and widely used process in the chemical field for the determination of protein content.

After collecting chicken breast samples, it was critical to properly prepare them for analysis. To ensure the purity of the sample and remove any unwanted residues such as bones or skin, the breasts were carefully cleaned and subsequently cut into small homogeneous pieces. This preparation step was crucial to ensure that the samples were representative and that any contaminants did not influence the analysis results. Once prepared, the samples were frozen at -18°C to preserve their integrity until analysis.

The method (AOAC 988.05) is based on measuring the nitrogen present in proteins, which is then converted into a total protein value. Initially, the chicken breast sample is carefully weighed and placed in a digestion flask along with a specific amount of concentrated sulfuric acid (H₂SO₄) and a catalyst (typically copper or selenium sulphate). The mixture is heated until complete digestion, transforming the organic nitrogen present in the proteins into ammonium sulphate ((NH₄)₂SO₄), a step that takes a few hours and produces a clear solution. Subsequently, the solution is neutralized by adding sodium hydroxide (NaOH), releasing ammonia (NH₃). The solution is then distilled, and the ammonia is collected in a solution of boric acid (H₃BO₃), forming ammonium borate. The quantity of ammonia (and therefore nitrogen) is determined by titrating the boric acid solution containing ammonium borate with a standard solution of hydrochloric acid (HCl) or sulfuric acid (H₂SO₄). The titration endpoint is detected using a pH indicator or an automatic endpoint detection system. The total nitrogen content is calculated from the amount of acid used in the titration. Since the protein content is estimated by multiplying the total nitrogen by a specific factor (generally 6.25, since protein is assumed to contain approximately 16 % nitrogen), this gives the total amount of protein present in the chicken breast sample. This method, although complex and requiring careful execution, is very precise and reliable for determining protein content. The Kj.m. is universally recognized and widely used in the food, environmental and pharmaceutical fields for its accuracy in protein quantification.

Subsequently, the NIRS instrument used for the analysis was calibrated on broiler chicken breast samples. This was required due to the small availability of data on slow growing autochthonous chickens. This calibration was performed using reference samples known for their protein composition, ensuring that the instrument was carefully adjusted to obtain precise and reliable results. Broiler-specific calibration was essential to ensure that the unique characteristics of this type of chicken were adequately considered during the analysis.

Table 1. Statistical Analysis (GAM = Generalized Additive Model) performed to assess the Crude Protein (CP) content of breast fillet of an autochthonous slow growing chicken breed analysed with the Kjeldahl method (Kj.m.) and Near Infrared Spectroscopy (NIRS) method.

	Kj.m.	NIRS	Paired t-test (p-value)	GAM (R ²)
CP (%)	23.7 ± 0.75	25.1 ± 1.04	< 0.001	0.42

Protein analysis in the chicken breast samples was then performed using NIRS. Prior to analysis, samples were allowed to thaw at room temperature to avoid any freezing effects, ensuring that the sample structure was not compromised. For each sample, NIRS analysis was performed following a series of standardized procedures. The sample was placed in the NIRS device and infrared light was emitted onto its surface, recording the NIRS reflection spectrum.

2.3 Statistical analysis

To compare the amount of protein detected using the Kj.m. and the NIRS method we used a Wilcoxon signed-ranks test. The normality of distribution was assessed through the Shapiro–Wilk’s test. A Generalized Additive Model (GAM) was performed to analyze the relationship between the two data sets. It is a generalization of linear regression and allows to combine the contribution of each variable through non-linear functions. Statistical analysis was carried out using RStudio software (V.4.1.1).

3. RESULTS AND DISCUSSION

In this study, the protein content was determined using two different methods: the Kj.m. and the NIRS.

The NIRS method, which uses near-infrared reflectance to estimate protein content, produced results statistically different to those obtained with the Kj.m. ($t = -3.84$; p -value < 0.001) (Table 1). A non-linear relationship between the two datasets was identified by the GAM, with a coefficient of determination of 49 % (Table 1). While a relationship exists between the two data sets, the NIRS method is not perfectly aligned with the Kj.m. However, the results of the NIRS method showed a slight underestimation of the protein content, reporting a value of 23.7 % compared to 25.1 % obtained with the Kj.m. (Figure 1, Table 1).

This discrepancy could be attributed to various factors, including variation in sample composition due to differences in sample collection points. The chemical and physical composition of the sample can vary significantly depending on the sampling point, thus influencing the results obtained with different methods. Other causes could be related to the specificity of the

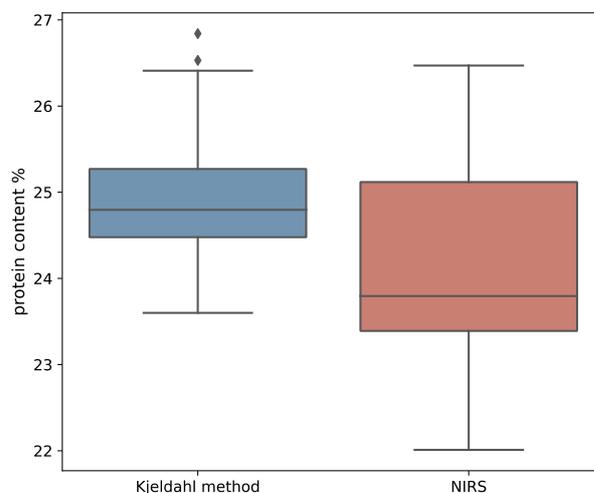


Figure 1. Crude Protein (CP) content of breast fillet of an autochthonous slow growing chicken breed analysed with the Kjeldahl method and Near Infrared Spectroscopy (NIRS) method.

NIRS method for certain matrices, to instrumental limitations or to the operating conditions during the analysis.

Despite this slight discrepancy, the NIRS method demonstrated acceptable outcomes in estimating protein content. This is particularly relevant considering the advantages of the NIRS method, such as the speed of analysis, non-destructiveness and the possibility of analyzing a large number of samples in a short time without the use of chemical reagents.

To further improve the agreement between the results of the NIRS method and those of the Kj.m., it would be appropriate to proceed with further refinement and calibration of the NIRS model. This could include using a larger and more diverse set of samples for calibration, optimizing operating conditions, and implementing corrections to account for variations in sample composition. By addressing factors contributing to the discrepancy, such as sample heterogeneity, calibration adjustments, and method optimization, the NIRS method could be further improved to provide even more accurate protein content measurements.

One of the limitations of this study is in fact due to the calibration performed on broiler meat which has different properties compared to the meat of slow-growing native breeds. In this context, poultry biodiversity is fundamental for the sustainability and resilience of agricultural ecosystems. Maintaining a wide range of poultry breeds contributes to food security, conservation of genetic resources and adaptability to different environmental and climatic conditions. In this context, the use of NIRS technology can offer significant help to small farmers. Thanks to this technology, it is possible to obtain rapid and reliable data on the nutritional composition of poultry feed and meat, essential elements for guaranteeing the quality and safety of poultry products.

The integration of NIRS technology into breeding processes provides added value to the final product, improving the transparency and quality of information for consumers. This not only increases consumer confidence in local and traditional poultry products, but also the competitiveness of farmers in the market. The ability to monitor and optimize the nutritional composition of poultry feed and meat in real time allows farmers to quickly adapt to market needs and environmental conditions, thus improving the sustainability and resilience of their operations.

While the NIRS method has demonstrated good correlation with the Kj.m. and highlighted considerable potential as a rapid, non-destructive analysis technique, it is important to recognize that there is still room for improvement that can be explored to further optimize performance and accuracy of protein content measurements. This could involve a number of strategies, including updating calibrations, optimizing operating procedures, and implementing fixes for possible sources of error.

The adoption of innovative technologies such as NIRS spectroscopy can bring significant benefits to poultry farming on several fronts. First, the ability to perform rapid, non-invasive analysis can improve the operational efficiency of farms, allowing operators to quickly monitor the nutritional composition of poultry feed and meat. This can help optimize animals' diets, improving their overall health and well-being.

Furthermore, the use of NIRS can promote the sustainability of poultry farms. The possibility of analyzing samples quickly and non-destructively allows us to reduce the consumption of chemical reagents and limit the use of invasive processes, thus contributing to reducing the environmental impact of breeding activities.

In addition to the impacts on farm efficiency and sustainability, the adoption of NIRS technology can play a crucial role in the conservation of poultry biodiversity. Maintaining a diverse range of poultry breeds is essential to maintaining genetic diversity within farm bird populations. NIRS can help farmers evaluate and monitor the genetic composition of their herds, thus contributing to the conservation of genetic resources and biological diversity.

Moreover, NIRS has emerged as a promising non-destructive technique for assessing poultry meat quality abnormalities, which have become increasingly prevalent due to intensive genetic selection for rapid growth and high breast yield in broilers. These abnormalities, including white striping (WS), wooden breast (WB), and spaghetti meat (SM), significantly impact the sensory, nutritional, and technological properties of poultry meat, leading to substantial economic losses in the industry [18]-[20]. The histopathological characteristics of these conditions, such as muscle fibre degeneration and connective tissue defects, are linked to excessive muscle fibre hypertrophy and reduced capillary density, which compromise meat quality [21], [22]. Traditional methods for assessing meat quality are often destructive, time-consuming, and costly, necessitating the development of rapid, reliable, and non-destructive techniques like NIRS [23]. NIRS can effectively predict meat tenderness and other quality attributes by analysing the spectral data, thus offering a more efficient alternative to conventional methods [24]. Additionally, other advanced techniques like Fourier transform infrared spectroscopy (FT-IR) and multispectral imaging (MSI) have shown efficacy in predicting microbiological quality and classifying meat samples based on spoilage levels, further supporting the utility of non-destructive methods in meat quality assessment [25]. The increasing incidence of these abnormalities, exacerbated by environmental stressors and intensive production practices, underscores the need for improved monitoring and mitigation strategies to maintain meat quality and industry sustainability [26]. Therefore, integrating NIRS and other non-destructive techniques into routine quality control processes could significantly enhance the detection and management of poultry meat abnormalities, ensuring better product quality and consumer satisfaction.

Finally, the use of NIRS technology can play an important role in monitoring the quality of poultry products. By providing accurate and reliable data on the nutritional composition of poultry feed and meat, NIRS spectroscopy can improve transparency and traceability along the food supply chain, providing consumers with crucial information to make informed decisions about their food purchases.

4. CONCLUSIONS

The Kj.m., long regarded as the reference standard for protein determination, is known for its accuracy but is also time-consuming and labour-intensive. In contrast, the NIRS method provides a rapid, non-destructive alternative that is ideal for high-throughput analysis and offers potential for inline process monitoring. This study highlights the need to consider analytical requirements, sample volume, and desired accuracy when choosing between the Kj.m. and NIRS methods for protein analysis in chicken breast samples. By evaluating these factors, researchers and practitioners can make informed decisions about the most appropriate method for their specific needs and constraints.

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