CORN PROTEIN CONTENT – A COMPARISON BETWEEN METHODS, ANALYSORS AND FARMERS EXPECTATIONS

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Abstract

In a process of problem solving and diet formulation the analysis offeed ingredients' proteins have to be done accurately, rapidly, cheep and if is possible at the farm level. As a general rule the accurate methods last too long and real time methods are not so exactly. The study is doing the comparison between the results of three methods 1. Classical chemistry (*Kjeldahl*method*)*, 2. Near infrared spectroscopy analysis (NIR) and 3. By FT-NIR spectrometer used for analyzing the corn's protein content. The "reference sample" was considered the result obtained from classical chemistry - Kjeldahl method and the comparison was between classical, NIR and FT-NIR methods, for corn's grains and corn's flour. Each measurement was performed twice and no significant difference was found between repetitions ($p<0.00$). The average protein content in corn for 39 samples was 7.82±0.16% by Kjeldahl method, by NIR equipment, 7.47±0.17% in corn's grains and 7.57±0.15% in corn's flour and by FT-NIR equipment, 8.40±0.29% in corn's grains and 7.80±0.12% in corn's flour.Comparing the results of NIR and FT-NIR measurements with Kjeldahl method results significant differences between first measurement (F=3.625 at p =0.007), second measurement (F=3.255 at p =0.013) and average measurements (F=3.486 at p =0.009). The smaller differences between reference results was in case of FT-NIR in corn's flour (0.02%) but were no significant differences by Turkey test comparison for any of NIR, FT-NIR, grains or flour. In conclusion, irrelevant to the method or equipment used for measurements it appears more feasible to run the samples at farm level.

Key words: Kjeldahl, NIR, FT-NIR, protein, corn
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The corn is the most used feed ingredient in swine and dairy industry even in pasture systems (Onan G., Huțu, I., 2016 Onan G., Huțu, I., Radunz, 2016). During several farm visits, in a process of problem solving the evaluation of diet and correction of diet weakness the evaluation and analysis of feed ingredients have to be done accurate, cheep and if is possible "in real time" at the farm level. On the practice of farm visit the extension service (Hutu I., 2004) have two variants: One is to take the sample, to send those to the Animal Nutrition Laboratory and to do the diet formulation in a high specialized software (*Brill*® family software) according with the result of laboratory analysis. Another one is to do the analysis in the frame of two hours during farm visit – in this case the ultra rapid analyses need it, regularly there are used near infrared spectroscopy analysis by portable NIR equipments.

All time between speedy and accuracy of results appears to be a contradictory relation. As a general rule the accurate methods take long time and speedy methods are not so exactly, and the time and all inexactitudes generate the additional costs for farm and decreases in gross margin for the production.

For all those reasons the extension service has to be sure that the result used is as close as possible with the reality of the farm (Matiuti, 2017). The study take into consideration the most expensive nutrient-the proteins form corn and the analysis of it. The study is performing the comparison between the results of three methods of protein content in corn: 1. classical (humid) chemistry – by *Kjeldahlmethod* and 2. by near infrared spectroscopy analysis with two types of equipments- one form *Foss InfraXact*® and the other form *Bruker Optics*®.

The specific objective of this report was to establish which method is feasible for "real time analysis" of protein content at the farm level – being the case of protein content of corn as a row material in two forms - grains and flour.

MATERIAL AND METHOD

Corn sample collection and preparation: 40 sample of 300 g corn grains (several variety such as Fundulea, KWS®, Pioneer ® and possible other varieties) was sampled from 85 partner farms of Extension unit were collected during farm visit of extension service during fall and winter season in West Romania. Each sample was divided in three: 1/3 for classical chemistry, 1/3 for near infrared

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spectroscopy analysis (NIR) as a grain and other one 1/3 was milled for using for NIR as flour. In case of classical chemistry, before milling the grain was dryedin a drying stove at 105°C for 5 to 8 hours, depending on humidity of row samples. For NIR and FTNIR methods, in order to keep the humidity and the proportion of chemical components the milling was done in *Foss Cyclotec™ 1093*grinding mill with same sieve.

Analysis of protein content was done for each sample two times for each three methods/equipments:

The first method used was classical *Kjeldahl* (humid chemistry), by fully automated *Kjeldahl™8400 Analyzer Unit (FOSS Hillerød 3400, DK)*from Animal Nutrition Laboratory – *Horia Cernescu Research Unit (figure 1).*

The second method was near infrared Near-infrared reflectance spectroscopy(NIR) with equipment *Foss InfraXact®* (Hillerød 3400, DK). The *Foss InfraXact* specters were developed for the accurate and fast prediction by equipment producers' calibrations *(figure 2)*. The equipment was on electrical power independent laboratory (Auto laboratory of Animal Production).

The third equipment was a FT-NIRspectrometer (*BrukerMatrix I ®,* Boston, MA, SUA) with INGOT® calibrations(multiple calibration dairy package, swine and pet food).

NIR spectroscopy is an outstanding tool due to its simple operation, short analysis time, small sample requirement, low-cost and non-destructive measurement (Haughey et al. 2008; Spragg, 2017; Yang et al. 2013).

Theoretically, the differences between equipments are given by differences between NIR and FTNIR (signal Fourier-transformed): IR spectroscopy gets absorption of a monochromatic IR light at a time and draws the spectrum, whereas, in FTIR, multi-chromatic takes a summarized absorption of light and distributes it to create a spectrum using FTIR.

By specialized noncommercial sources (Burns & Ciurczak, E.W. 2011, *Hussain et all, 2019*) there are some points of difference between IR and FTIR such as:

- NIR takes a single spectrum -FTIR takes a number of scans.
- NIR used monochromatic light whereas FTIR used polychromatic light.
- FTIR scans up to 50 times in a minute and giving better resolution.
- FTIR is a fast technique than IR.
- Sample preparation in the traditional IR is time-consuming, while nowadays in FTIR uses Attenuated Total Reflection (ATR). Therefore, there is no need to prepare the sample.

So, according to several suppliers the FT-NIR spectrometers are more modern, given rapid analysis, high quality spectrum, reproducibility, ease of use and low maintenance.

Figure 1. ***Kjeldahl™8400 Analyzer Unit,*** **Animal Nutrition Laboratory**

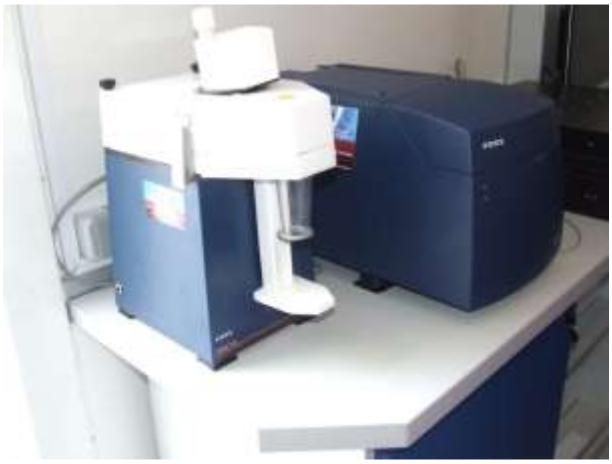

Figure 2. **NIR *Foss InfraXact®* in the Animal Production auto laboratory**

Statistical Analysis: Analysis of external results – protein values - were performed using one way *Analysis of Variance (ANOVA)*for the classical chemistry, NIR and FT-NIR equipments. For NIR and FT-NIR grain and also flour samples were used. The "reference sample" was considered the result obtained from classical chemistry - Kjeldahl method and the comparison was between classical and NIR and FT-NIR methods, for corn's grains and corn's flour. Also the comparison between first and second measurements was performing by *Student test* (paired samples).

RESULTS AND DISCUSSIONS

The measures were performed twice on each method – classical chemistry, NIR and FTNIR and a average between was also done.

The average protein content in corn for 39 samples was 7.82±0.16% by classical chemistry *Kjeldahl*, 7.47±0.17% by NIR in corn's grains, 7.57±0.15% by NIR in corn's flour, 8.40±0.29%

by FT-NIR in corn's grains and 7.80±0.12% by FT-NIR in corn's flour.

The results of the study show significant correlations between measurements but no significant difference between repetitions. All correlations between the first and the second measurements was significant ($p<0,001$): in generally, the correlations was higher in classical chemistry (r = +0.990, at p =0.00) and for milled samples (r = +0.990, at p =0.00 for NIR and r = +0.944, at p =0.00 for FTNIR method). The differences between the first and the second measurements was higher in corn grains, but not significant statistically (0.082±0.06% at p=0.205 for NIR and 0.127±0.11% at p=0.273 for FTNIR method).

As a first observation the variability of measurement is smaller in classical chemistry (in terms of standard deviation, SD = 0.147%) and milled sample (corn's flour) both NIR (SD=0.147%) and FTNIR methods (SD=0.264%).

Comparing the results of NIR and FT-NIR, for grain and flour measurements with Kjeldahl method results, the study found the significant differences between the first measure (F=3.625 at p =0.007), the second measure (F=3.255 at p =0.013) and the average of both measurements (F=3.486 at p =0.009).

Comparison of classical Kjeldahl with NIR and FTNIR by *Student* test, the differences between averages of measurements are significantly in case of NIR for corn's grains (*paired difference* = 0.35±0.10, t=3.526 at p =0.001) and corn's flour (*paired difference* =0.25±0.05, t=5.178 at p =0.000)and FTNIR for corn's grains (*paired difference* = -0.57±0.22, t=-2.555 at p =0.015). The study statistics underline the accuracy of FTNIR for corn's flour with classical results (*paired difference* =0.23±0.07, t=0.302 at p =0.764). In our study the tendency of NIR method was to overestimate the protein percent and underestimate the protein percent for FTNIR form corn's grain. The best estimation was obtained after milling the sample in FTNIR method.

Regarding the time, the most time consuming is classical chemistry: draying 5-8 hours, milling, weighting (1/4 hour), mineralization (6 hours), cooling, distillation and titration (1/2 hour) that means 2-3 working days in the lab. In case of using NIR and FTNIR equipment just 5 minutes, the time for spectrums collection, are needed. If the flour is use as a material the time for milling increases with maximum 10 minutes.

Conventional methods of analysis for quality control involve time consumption, a lot of labour, expensive procedures and chemical reagents (Cozzolino, 2012, Karoui et al., 2010, Weeranantanaphan et al., 2011).

In conclusion, by farmer expectations, time consumption, preparation of sample (grains or flour) and speed of obtained results, the use of any instruments (NIR and FTNIR) at the farm level is better than taken sample for analysis in laboratory.

By the accuracy of results the milled samples and FTNIR equipment is better but NIR equipment is robust and easy to work in the farm conditions. Even the differences between estimations of corns' flour are significant (average NIR estimation = 7.57±0.16%, average FTNIR estimation = 7.80±0.12, *paired difference* = -0.22±0.06, t=-3.682 at p =0.001) practically, the value of analysis can be applicable at farm level. Like in other study, the results indicated that corn nutritive values could be fast and accurately predicted by NIRS (Yang et al., 2011). In conclusion, in extension service, any portable NIR or FTIR instruments with adequate calibrations are preferable for analyzing quality and safety of cereals (Hussain *et al.* 2019) to time consuming classical chemistry.

CONCLUSIONS

The results for protein content in corn have a limited variability given by type of raw material (corn's grain or corn's flour) and type of the used method (Kjeldahl, NIR or FT-NIR).

By theoretically point of view the differences are significant but by practical point of view in extension services, the time to obtain the results is more important than accuracy of the results.

In extension service conducted in animal farms, any portable NIR or FTIR equipment with adequate calibrations is preferable to time consuming classical chemistry.

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