

## Original Article

# Evaluation of Nutritional Value of Animal Feed by Near-Infrared Spectroscopy (NIR) and Multivariate Analysis

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## ABSTRACT

A vital component of maintaining livestock and other animals' health, productivity, and general well-being is high-quality animal feed. However, a lot of animal research programs frequently use feed compositions that lack thorough nutritional analyses or that use outdated composition techniques, which might result in diets that are not ideal. With the use of cutting-edge near-infrared spectrum (NIR) technology, which provides a rapid and precise assessment. This study intends to evaluate the nutritional value of frequently used animal feed used for such projects by comparing it to both internal and external standards and detect the accuracy of the near-infrared spectrum (NIR). For this purpose, five distinct samples of widely-used animal feed were collected from major farms. Each sample underwent five repetitions and was analyzed using NIR instrumentation and evaluated with multivariate analysis. The analysis covered moisture, protein, lipid, carbohydrate, ash, calcium, phosphate, fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent liquid (ADL), and starch content. The study revealed varying percentages of protein, fat, carbohydrates, minerals, calcium, phosphorus, and fiber in each feed sample. While these percentages differed between feeds, many samples generally met standard specifications for nutritional value. Addition to having a significant influence on animal performance and the quality of the animal output, feed's nutritional value is a major factor in raising animal productivity. Examined animal feeds are safe for use and can help meet the animals' nutritional demands.



## 1. Introduction

Feedstuffs, which include proteins (amino acids), carbohydrates, lipids, minerals, and vitamins, supply energy and nutrients necessary for development, reproduction, and overall health when combined with water. To provide a meal that contains all the important elements required for an animal's regular functioning, balanced diets include mixing substances in the proper quantities. The economic consequences of unbalanced diets may be seen in

terms of animal health, feed conversion efficiency, and ultimately the production of animal products (Gizzi & Givens, 2004, Hamasalim et al., 2021). Furthermore, the significant difference in the nutritional and food chemical compositions across studies due to the influence of several circumstances, including the year, the location of origin, the process used for manufacture or treatment, and the technique used to extract the oil (Anjum et al., 2012). Protein, vitamins, and minerals are the main

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nutrients needed by ruminant animals (NCR, 2001). While necessary, energy is not a chemical substance. Energy is a unit of labor that may be obtained from a variety of dietary elements, such as protein, fats, fiber, sugar, and starch. More prevalent forages, fodders, and agricultural wastes are composed of energy substrates, primarily fiber (Widodo, 2023). All required nutrients must be accessible for maximum usage, limiting production only to the animal's genetic capacity. The degree to which an animal's genetic potential is expressed depends on the nutrient that is first limiting. The selection of a feed supply is often based on its cost or relative availability (Hamasalim, 2009, Hassan & Hassan, 2010).

For maximum production, additional sources of nutrients must be used if the original source is unable to provide all the necessary ones (Coleman & Moore, 2003). The feed's nutritional value is determined by its content (protein, carbs, vitamins, and minerals), availability (digestibility), and effectiveness in using nutrients and energy. As a stand-in for digestible energy, digestible dry matter (also known as organic matter) is employed. The relative effectiveness of converting DE to metabolizable energy (ME) and the efficiency of converting ME to energy accessible for tissue accretion, milk production, or fiber synthesis are referred to as utilization (Hamasalim et al., 2016). Furthermore, variations in animal choices, physiological states, or genetic capacity for production, and consequently demand, lead to mistakes in fodder quality measures. This heterogeneity causes prediction models to contain mistakes and makes it challenging to accurately anticipate nutritive potential. Preciseness in the order in which distinct ecotypes are ranked may be more significant to plant breeders than total accuracy (Hassan & Hassan, 2009, Coleman & Moore, 2003). For livestock poultry, and fish, however, feedstuffs derived from animals are usually better than those derived from plants in terms of growth and health (Wu et al., 2018). This is partly because the former has higher quality (AA ratios and

digestibility) and more proteinogenic amino acids than the later. The nutritionally essential amino acids, which include leucine, lysine, methionine, threonine, and tryptophan, are proteins that are not produced by animal cells and have been the subject of much investigation in the past (Hou & Wu, 2018).

The particular significance of feedstuffs derived from animals in enhancing the feed efficiency, development, and health of animals (including fish and companion animals) is demonstrated by the composition of proteins (amino acids) and associated nitrogenous nutrients in feedstuffs for animal diets (Li & Wu, 2020). Three often-confused steps for feed evaluation were proposed by (Murray, 1996) Making sure a feed material is what it claims to be is the first stage (qualitative analysis). Quantitative analysis, the second phase, involves determining its ranking among similar streams. Nutritional analysis, the third phase, involves estimating the probable animal performance that an intended animal will exhibit after consuming the item. Each of the three processes holds significance and can be implemented for certain feeds (FAO, 2010). The purpose of this research is to evaluate the nutritional content of animal feed used in animal projects, compare it to national and international standards, and assess the nutritional content of animals using a sophisticated and trustworthy tool—the near infrared spectrum.

## 2. Epidemiology and pathophysiology

### 2.1 Animal feed collection

The animal feed for this study was sourced from multiple livestock field in Sulaimani governorate. Five distinct samples of animal feed were collected (A, B, C, D and E), and they have been used across all large farms producing animal products. Five repetitions were gathered from each of these various samples. Samples were placed in sterile bags and sent to the laboratory for NIR testing. They were then kept at 25°C for two days until analysis. Moisture, protein, lipid, carbohydrate, ash, calcium, phosphate, fiber, neutral detergent fiber (NDF), acid

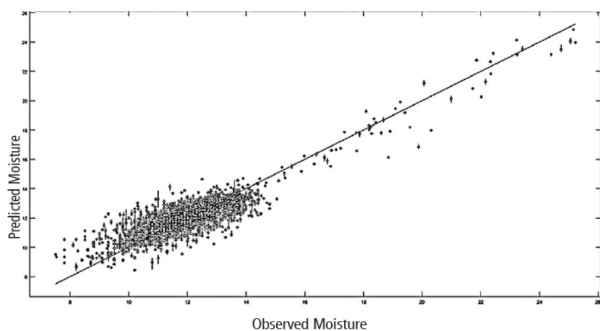
detergent fiber (ADF), acid detergent liquid (ADL), and starch were all assessed in the samples.

### 2.2 Near infrared spectrum (NIR)

In this study, feed samples from feed producer enterprises in the governorate of Sulaimani were used. These samples included various feed kinds for sheep, horses, dairy, and beef cattle. Spectral data were obtained for every sample utilizing an open-faced big sample dish and numerous DA 7250 devices. Certain samples were examined without any further sample preparations, such as grinding. Using the PerkinElmer Hongis Regression, HR, Artificial Neural Network Regression, ANN, Partial Least Squares Regression, and PLS algorithms, calibrations were created to predict the associations between the gathered NIR spectra and reference chemical data.

### 2.3 Calibration and Prediction

In final analysis, it can be said that the DA 7250 can precisely test samples of ruminant feed in just under six seconds. There is no need to grind samples before measurement and calibration because the calibrations may be utilized on any DA 7250 equipment for a wide range of product kinds and formulations. NIR vs. Reference the Figures (1-3) below show the moisture, protein, and fat model calibration plots.

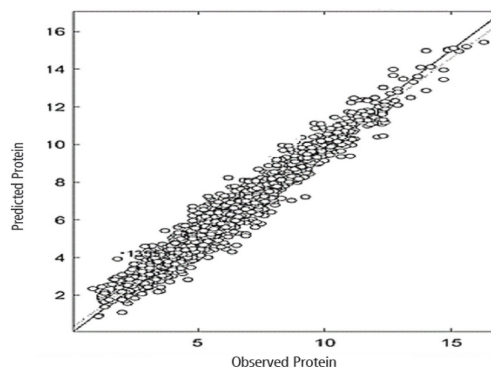


**Figure 1.** Moisture: The DA 7250 calibrations can be used to determine moisture when monitoring the cooling/drying.

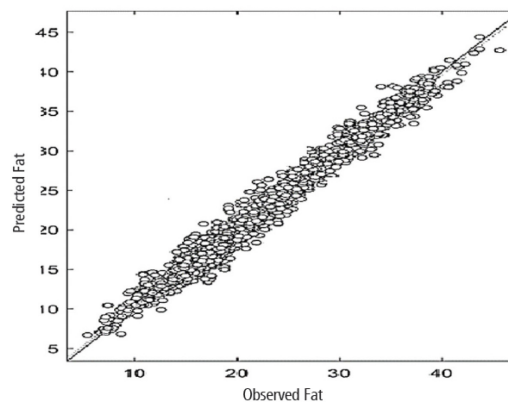
### 2.4 Methods of data analysis

The outcomes of the experiment were statistically assessed using Microsoft Excel Office 2019, XLSTAT 2019.3.2.64, and OriginPro 2020. The

significant correlation difference test indicated a probability value of 0.05 as significant, and a one-way ANOVA was employed. Duncan's test was used to compare the means of the animal feed (Vidal et al., 2020).



**Figure 2.** Protein: Results are very consistent from low to high Protein samples, with very small differences between reference analysis and NIR predictions.



**Figure 3.** Fat: The calibration covers a very wide range, and accurately measures high fat samples. This means that all types of products can be analyzed with the same calibration.

## 3. Results and discussion

The nutritional content of the examined animal feed, including moisture, dry matter, protein, fat, ash, calcium, and phosphorus, is shown in Table 1. At a significance level of ( $P < 0.05$ ), unique statistics were identified for every nutritional component. All of the animal feed types had different moisture contents; feed type D (11.524%) had the greatest moisture content, while feed type B (8.846%) had the lowest. All five varieties of animal feed, however, complied with the accepted standards. Similarly, all animal

**Table 1:** The nutritional value of some animal feed determined by NIR.

Components%	Feed A	Feed B	Feed C	Feed D	Feed E	Mean	Acceptable limit	Observations	Std. deviation	Standard
Moisture	9.408 <sup>b</sup>	8.846 <sup>d</sup>	9.436 <sup>b</sup>	11.524 <sup>a</sup>	10.028 <sup>c</sup>	9.848	Max. 12	25	0.937	IQS1616/1990: ISO 6496: ISO 5983-1
Dry matter	90.278 <sup>b</sup>	91.050 <sup>a</sup>	90.264 <sup>b</sup>	89.152 <sup>d</sup>	89.410 <sup>c</sup>	90.031	Min. 88	25	0.697	IQS1616/1990: ISO 6496: ISO 5983-1
Protein	12.450 <sup>e</sup>	17.954 <sup>a</sup>	13.580 <sup>d</sup>	17.684 <sup>b</sup>	17.188 <sup>c</sup>	15.771	Min. 12	25	2.339	IQS1616/1990: ISO 6496: ISO 5983-1
Fat	1.358 <sup>e</sup>	3.656 <sup>c</sup>	4.434 <sup>a</sup>	1.894 <sup>d</sup>	4.060 <sup>b</sup>	3.08	Min. 2	25	1.25	IQS1616/1990: ISO 6496: ISO 5983-1
Ash	1.726 <sup>d</sup>	6.566 <sup>a</sup>	6.478 <sup>b</sup>	3.886 <sup>c</sup>	0.956 <sup>e</sup>	3.922	Max.8	25	2.378	IQS1616/1990: ISO 6496: ISO 5983-1
Calcium	0.544 <sup>d</sup>	1.858 <sup>a</sup>	1.754 <sup>b</sup>	1.622 <sup>c</sup>	0.524 <sup>e</sup>	1.26	Min. 0.5	25	0.61	IQS1616/1990: ISO 6496: ISO 5983-1
Phosphorus	0.446 <sup>d</sup>	0.612 <sup>c</sup>	0.856 <sup>a</sup>	0.690 <sup>b</sup>	0.356 <sup>e</sup>	0.592	Min. 0.3	25	0.181	IQS1616/1990: ISO 6496: ISO 5983-1

\*a, b, c, d, e indicates statistical differences between the groups feed (P<0.05) (4).

**Table 2:** The value of carbohydrates and their types in animal feed determined by NIR.

Tests	Feed A	Feed B	Feed C	Feed D	Feed E	Mean	Acceptable limit	Observations	Std. deviation	Standard
Fiber	5.187 <sup>c</sup>	12.283 <sup>b</sup>	17.767 <sup>a</sup>	4.153 <sup>d</sup>	2.223 <sup>e</sup>	8.323	15-17	25	2.023	IQS1616/1990: ISO 6496: ISO 5983-1
Starch	53.613 <sup>b</sup>	13.450 <sup>c</sup>	19.633 <sup>d</sup>	45.133 <sup>c</sup>	55.560 <sup>a</sup>	37.478	Min. 10	25	1.176	ISO 6496: ISO 5983-1
NDF	13.910 <sup>e</sup>	28.967 <sup>b</sup>	39.473 <sup>a</sup>	8.827 <sup>c</sup>	8.910 <sup>d</sup>	20.017	Min. 33	25	1.631	ISO 6496: ISO 5983-1
ADF	2.560 <sup>d</sup>	21.957 <sup>b</sup>	24.553 <sup>a</sup>	3.843 <sup>e</sup>	2.023 <sup>c</sup>	10.987	Min. 21	25	1.421	ISO 6496: ISO 5983-1
ADL	1.867 <sup>c</sup>	7.967 <sup>a</sup>	6.913 <sup>b</sup>	1.663 <sup>d</sup>	0.760 <sup>e</sup>	3.834	Max. 43	25	1.091	ISO 6496: ISO 5983-1

\*a, b, c, d, e indicates statistical differences between the groups feed (P<0.05).

**Table 3:** Pearson linear correlation coefficient matrix for tested parameters.

	Moisture	Dry matter	Protein	Fat	Ash	Calcium	Phosphorus	Fiber	Starch	NDF	ADF	ADL
Moisture	1											
Dry matter	-0.90105	1										
Protein	0.34602	-0.21404	1									
Fat	-0.38963	0.21882	0.22302	1								
Ash	-0.31158	0.5833	0.11958	0.41367	1							
Calcium	-0.00491	0.33773	0.32851	0.29933	0.94562	1						
Phosphorus	0.07043	0.17651	-0.09703	0.25175	0.84593	0.86184	1					
Fiber	-0.54567	0.65277	-0.27374	0.54553	0.88121	0.70477	0.78844	1				
Starch	0.50171	-0.7138	-0.13256	-0.53153	-0.97024	-0.86255	-0.71184	-0.89353	1			
NDF	-0.62615	0.69606	-0.28573	0.5943	0.84517	0.64317	0.71579	0.99357	-0.88418	1		
ADF	-0.57294	0.70419	-0.03604	0.63086	0.92959	0.77447	0.72338	0.96429	-0.97116	0.96555	1	
ADL	-0.63769	0.81353	0.00876	0.5195	0.92874	0.77042	0.64134	0.914	-0.98471	0.91841	0.97618	1

feed varieties met the regulatory criteria for dry matter levels, which were within the acceptable range (IQS, 1999). According to (Saiful et al., 2015) moisture is a crucial component of raw materials used to produce animal feed. The weather in Sulaimani governorate determines how much feed is produced during the cultivation season, but in certain seasons, storage issues and weather can make handling high-moisture feed difficult for both farmers and feed-producing corporations (2).

Based on statistical analysis, the protein levels in the tested animal diets differed. We compared the means using the Duncan test, and feed type B (17.954%) had the greatest protein level and feed type A (12.450%) had the lowest. All varieties of feed samples, however, had protein levels that were

within the acceptable range. The main component of diet that promotes growth is protein. Comparatively speaking to the current study, the mean value of crude protein in a study spanning 15 nations was determined to be 8.01% (Thomas, 2002). Furthermore, crude protein in diets fulfills two purposes for ruminant animals. The rumen bacteria require nitrogen (N) for their growth, while the small intestine needs amino acids for absorption and use by the ruminant animal in question. There are two sources of amino acid supply: microbial protein (MP), which is obtained by assimilation of ruminal NH<sub>3</sub>, and feed protein that avoids microbial breakdown (Broderick, 1994). Additionally, (Karr-Lilienthal et al., 2006): discovered that soybean meal's crude protein levels ranged from 45.1% to 52.6%. Despite the fact that Crude Protein Content was observed to vary from 31.6 to 41.7% by (Spragg & Rod, 2007).

The samples of animal feed had different fat contents. Feeds A (1.358%) and D (1.894%) had fat percentages below the allowable limit, but feeds B (3.656%), C (4.434%), and E (4.060%) had fat percentages within the allowable range. All contemporary computerized models for lactation ration formulation now incorporate fat. In order to produce herd milk, more fat will be needed (Palmquist et al., 2017). The goal of dairy research has been to enhance the amount of "beneficial fatty acids"

or "improve the healthfulness" of the milk fatty acid profile for a long time (Palmquist et al., 2006). According to some studies, the ether extract level in soybean meal varied from 4.1% to 9.0%, whereas (Hossain et al., 2012), stated that it varied from 1.0 to 18.0%. (Karr-Lilienthal et al., 2006) also discovered this variation. Furthermore, ethyl ether extract concentrations were found to vary from 8.5 to 17.0% by (Spragg & Rod, 2007)

Based on statistical analysis, we compared the quantities of ash in the feed animal samples using the Duncan test. Feed C had the greatest level (6.478%), whereas feed A had the lowest level (1.726%). All of the animal feed samples, however, had ash levels that were within the curriculum's acceptable limit (ISO, 1999). Crude ash is a type of empirical approach that is used to estimate total organic matter indirectly and is crucial to the nutritional interpretation of animal diets (Quirino et al., 2023). The inorganic residue left over after all biological matter has completely burned off is referred to as mineral matter or crude ash (Ismail, 2017; Liu, 2019). Because it makes it possible to estimate total OM—which includes all possible molecules that might provide energy—indirectly, crude ash is crucial to the nutritional interpretation of animal diets (Souz et al., 2017).

The Duncan test revealed that the calcium levels in the animal samples varied, but overall, they were within the acceptable range when compared to the standard (ISO, 2005). One significant element that influences the effectiveness of reproduction is calcium (Ca). Nutrient metabolism and circadian rhythms interact (Gao, et al., 2019). Notably, milk Ca and other mineral element concentrations are similarly influenced by the amount of calcium in the diet (Miller et al., 1994).

The same is true for the phosphorus level, where there were significant differences (0.05) across the animal feed samples. By standard, every animal feed sample was still below the allowable level. Because P in plant feedstuffs is bonded to phytate and non-ruminants lack the phytases necessary to break down phytate, P cannot be used by (Woyengo & Nyachoti, 2013). Less growth and production, as well as poor

bone formation and mineralization, might arise from an inadequate P supply in comparison to the requirements at a particular physiological stage of the animal (Misiura et al., 2020). When luminal P availability is low in comparison to the body's requirements, active transport of P is the major method of absorption; conversely, passive transport predominates when luminal P availability is high in comparison to the body's requirements (Yee et al., 2021; Hill & Vorland, 2021). Additionally, because bone-derived calcium phosphates are typically byproducts of the synthesis of gelatin from bones, they may be less expensive than calcium phosphates obtained from rock phosphate.

Table 2 lists the types and values of carbohydrates for the animal meals that have been examined. On the basis of a 0.05 significance threshold, statistical analysis has been carried out and compared using the Duncan test (Duncan, 1955).

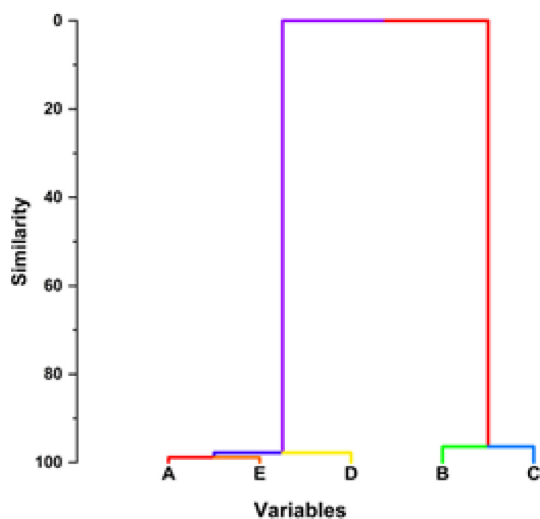
Variability was seen among the animal feed samples when the fiber levels were assessed. Feed type C had the highest level of fiber (17.767%), whereas feed type E had the lowest level (2.223%). A diet rich in complex carbohydrates, dietary fiber is characterized as "non-digestible polysaccharides." "A substrate that is selectively utilized by host microorganisms conferring a health benefit" is how the International Scientific Association for Probiotics and Prebiotics (ISAPP) defines a prebiotic (Hill et al., 2014). Animal production performance and palatability are negatively impacted by fiber, which is thought to be an antinutritional component in feed (Buxton & Redfearn, 1997). On the other hand, a number of studies have consistently shown that feeding animals more fiber can enhance their performance (Lai et al., 2015; Jiménez-Moreno et al., 2019). All animal feed samples, however, were within the standard's permitted range. Recent research has frequently highlighted the intricate function of fiber in animal feed. Diets rich in fiber and low in fat can improve intestinal health and lower the risk of cardiovascular disease (Simpson & Campbell, 2015).

Among the animal samples, there were significant differences in the amounts of starch at the 0.05

significance level. There were differences in the starch content of animal feed; the range was 13.45 to 55.56. Animal health and carbohydrates from food sources are strongly correlated; depending on the source, fiber can be classified as either pure prebiotic or composite dietary fiber (Prasad et al., 2019). For every animal feed sample, the amounts of NDF, ADF, and ADL were measured. With the exception of feed type C (39.47%), where the level was within the appropriate range, the levels were often lower than those that are allowed for the majority of meals. The samples of animal feed included varying amounts of neutral detergent fiber (NDF). The NDF levels were below the allowable limit in feed types A (13.910%), B (28.967%), D (8.827%), and E (8.910%), but within the allowable range in feed type C (39.473%). Additionally, the Duncan test revealed that the levels of acid detergent fiber (ADF) differed across the animal samples; in feed types A, D, and E, ADF was below the allowable threshold. The quantities of acid-detergent lignin (ADL) in all animal feed samples were within the allowable limit in accordance with the standard curriculum, even though feeds B and C were within the acceptable range.

Multivariate analysis for finding the correlation and similarity between variables has also been done, as shown in Table 3 and Figure 4. Table 3 reveals the Pearson linear correlation coefficient matrix for all parameters. In practice, correlation matrices are widely used to understand the relationship between several pairs of variables. The correlation coefficient's distance from zero indicates the strength of the correlation between the two variables. It may be used to evaluate statistical hypotheses, such as whether two variables have a significant correlation. The findings show that there is some strong direct correlation between parameters, which is colored blue in the table. Also, some highly significant reverse correlations were present and colored red. Hierarchical cluster analysis was also used to find the similarity between the variable feeds, as shown in figure 4. HCA was performed in this work based on Ward's method as an agglomeration method with Euclidean distance for measuring similarity. HCA is a

cluster analysis technique that aims at developing a hierarchical cluster pattern. It is a data analysis approach that sorts data points into clusters, or groupings, based on a set of common features. According to the finding results, three main clusters have been established as presented in the dendrogram, and the most similar cluster is between feeds A and E (cluster 1), and the second most similar cluster is between cluster 1 and feed D (cluster 2). Feed B and feed C (cluster 3) have similarities between them based on all parameters that have been measured in this study. The most dissimilarity is between cluster 3 and clusters 1 and 2.



**Figure 4.** HCA dendrogram for variables depending on all detected parameters.

#### 4. Conclusions

In conclusion, addition to having a significant influence on animal performance and the quality of the animal output, feed's nutritional value is a major factor in raising animal productivity. The near infrared spectrum (NIR) may be used to quickly and accurately determine the nutritional content of feed while also producing reliable findings. This study shows that while few of the nutritional content of the animal feed on the biggest farms in Sulaimani governorate is outside of acceptable bounds, it is not to the point where it is deemed dangerous. Overall, there are differences in the nutritional composition of the examined animal feed samples, but all of them

satisfy the recommended levels of moisture, protein, fat, ash, calcium, phosphorus, different types of carbohydrates, and fiber. This shows that the examined animal feeds are safe for use and can help meet the animals' nutritional demands.

#### Conflict of interest

The authors declare that there is no conflict of interest

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