# Comparison between feed microscopy and chemical methods for determining of crude protein and crude fiber content of commercial mixed feeds

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Abstract. The use of chemical methods in the determination of protein content in feed raw materials is time consuming and costly. The aim of this study to determine the amount of crude protein and crude fiber in mixed feeds using methods feed microscopy and chemical methods. Cattle feed, cattle milk feed, lamb raising feed, and meat chick feed have been used to study mixed feeds. By determining the results to indicate that feeds microscopy method approximately how much closer to chemical methods. The percentages of raw materials of crude protein and crude fiber in mixed feeds were determined with stereo microscopy and compared with obtained results of chemical methods. As a result there is no statistically difference in crude protein between feed microscopy method and chemical method. Feed microscopy estimating method can be used instead of chemical methods for crude fiber analysis, so that it is determined that feed microscopy method cannot be used instead of chemical methods. As a result protein analysis, so that it is determined that feed microscopy method cannot be used instead of chemical methods. As a result fiber analysis, so that it is determined that feed microscopy method cannot be used instead of chemical method. As a result, feed microscopy method can be suggested because crude protein content in raw materials of feed is more economical and shorter than chemical method.

Key words: Feed microscopy, chemical method, crude protein, crude fiber.

#### **INTRODUCTION**

Quality control of mixed feeds and raw materials is mostly done by chemical analysis methods. Although chemical analysis methods are extremely accurate, they take a long time and the process of deciding raw material purchase of feed factories is prolonged. Inspection and quality control in laboratories cause time loss and are seen as entrenched methods (Gines & Vohringer, 1998; Makowski, 1998).

Various studies are being carried out in order to prevent this loss or to reduce the most loss. One of the most important of these studies is the feed microscope (Hahn & Kruyskamp, 1997). Feed microscopy, the quality of mixed feed or feed ingredients can be decided more quickly (Klein & Marguard, 2005).

This decision is about whether it is more fraudulent, mold and pest status, physical properties such as foreign material presence. However, the ability to determine nutritional values such as crude protein and crude cellulose contained by feed microscopy can help us makes better decisions.

The feed to be subjected to the microscopic examination may consist of a single feed material or may be a mixed feed formed of two or more feed materials. Again, this subject is subject to forage crushing, crushing, grinding, baking etc. (Akyildiz, 1984; Pinotti et al., 2016), as well as in the case of preserving the natural structure.

However, in order to perform feed microscopic examination in pellet mixed feeds, the pellets must first be opened in water. During this wait, the colors of the raw materials change and they get the colors of each other. For this reason it is extremely difficult to work with feed microscopy in pellet feeds.

In order to gain experience in microscopic analysis, a programmed study and continuous practice are necessary. Getting information about the ingredients and qualities of raw materials can be done in 5–10 minutes by microscopy even though it takes hours by chemical analysis. Feed microscopy is an important and early part of an integrated quality control program developed for feed production (Khajarern & Khajarern, 2008).

In this study, the feasibility of crude protein and crude cellulose ratios in feeds was investigated more rapidly by feed microscopy instead of chemical methods and the results obtained were evaluated statistically.

### **MATERIALS AND METHODS**

The material of the study is composed of different mixed feeds. Cattle feed, cattle milk feed, lamb raising feed, and meat chick feed have been used to study mixed feeds that can vary in the content of various animals. Powder mixed feeds was provided in 5 kg packages from the Malkara Birlik Feed Plant. Analyzes of crude protein, raw cellulose and feed microscopy of mixed feeds were carried out in the Feed Analysis Laboratory of Tekirdağ Food Control Laboratory Directorate. The 500 g packages for six samples were formed from each of the 5 kg mixed feeds and a total of 24 samples were obtained. These samples were numbered 1 to 24 and numbered, which one was recorded at one time, the analyzes were finished and the evaluation stage was not looked up. For analysis of crude protein and crude cellulose, each sample of 4 samples was grinded at about 50 g to prepare an analytical sample. For the microscopic analysis, about 50 g was taken from the sieves with 1 mm and 0.5 mm mesh and separated into 3 fractions and placed in petri dishes and made ready to be examined in stereo microscope.

Incubation method (AOAC 2003) and Leco Nitrogen-Protein device were used in crude protein analysis. This method is applicable to mixed feed and mixed feed raw materials containing 0.2–20% nitrogen (N). The principle is to measure N high thermal conductivity (850–950 °C) with pure oxygen (99.9%), for example, with the help of thermal conductivity (thermal conductivity) resulting from the burning end result and multiply by the appropriate protein factor to be expressed as% protein. When the device is ready to run the sample, empty calibrations are given for empty calibrations when the temperature and pressures are reached, and empty calibrations are performed by selecting 3 of them from the results. Thus, N from the gas cylinders is reset. Before working on the sample, 0.15 g is weighed from the EDTA, which is indicated by the N value certificate, to calibrate the nitrogen to perform the nitrogen calibration, and several values are selected from the results and N calibrations are made. After the calibrations are made, the grinded sample is weighed at about 0.25 g. The protein turnover factor is

also entered into the device prior to sampling. This factor is 6.25 for mixed feeds. After the factor is entered, the device gives the results as% protein.

The quartz method was used for crude cellulose analysis (OJ (EU) Regulation 2009). In order to dissolve the organic substances outside the raw cellulose present in the method's principle feedstock; the feedstuff is boiled in successive concentrations with sulfuric acid and potassium hydroxide. Possible organic residues which remain after filtration are washed with dilute sulfuric acid, sodium hydroxide, water and acetone. The residue is dried, weighed and burned. The weight difference seen in the burning result gives the amount of crude cellulose. 1.0 g of feed sample is weighed into a 250 ml beaker. 100 ml of 1.25% sulfuric acid solution is added and heated. After boiling, add 2–3 drops of anti-foaming agent (silicone, amyl alcohol etc.) and boil for 30 minutes. To keep the volume constant at the time of boiling, the beaker is covered with a cooling system (such as a 500-ml drippy round balloon provided with cold water circulation) or clock glass. After the end of the period, 10 ml of 28% potassium hydroxide solution is added and boiled for a further 30 min. On the other side, the glass filter (gosch sieve) is filled with quartz sand to a height of 8–10 mm. Before filtering, the quartz sand is thoroughly moistened with hot distilled water and sucked with water tromp or vacuum pump to form a tight quartz sand layer. The welded specimen is filtered through the hot glass filter. Crude cellulose particles may become clogged during filtration. To prevent this, the vacuum is cut and the top of the quartz sand layer is lightly mixed with a glass baguette. To the filtration, the residue on the quartz sand layer is washed twice with hot distilled water, 10 ml of 1% sulfuric acid solution, again with hot distilled water, then with 10 ml of 1% sodium hydroxide solution, again with hot distilled water and 10 ml of 1% Sulfuric acid solution and then washed twice with hot distilled water again. Finally, washed again with acetone, vacuum must be cut in order to ensure that the raw cellulose residue is well moistened during different washing operations. After washing and filtration, the glass filter residues are dried in an automatic drying cabinet at 130 °C for 1 hour. After cooling in the desiccator, weigh the sample (I). Weighing II is obtained by weighing in a desiccator at a temperature of 550–600 °C for 30 minutes. The research was carried out with 2 methods and 4 randomly selected randomized Parsell Experiment Method and variance analysis was made with the SPSS statistical program and the significances were checked by Duncan test (Soysal, 2000).

#### RESULTS

Samples of 5 kg of powdered cattle feed, cattle milk feed, lamb raising feed and broiler chick feeds of 500 g for six sampled were obtained from the Malkara Birlik Feed Factory in Malkara district of Tekirdağ and these 24 samples were raw Protein and crude cellulose analyzes were performed with feed microscopy and chemical methods. The results obtained are summarized in Table 1. The results of the crude protein and crude fiber analyzes, which were made by two methods according to the randomized plot design, were evaluated statistically. There is no difference between the results of crude protein analysis made with feed microscopy and the results of protein analysis made with chemical method and feed microscopy can be used instead of chemical method in crude protein analysis. According to the average of the analysis results made with the two methods, the correlations r = 0.982 and  $r^2 = 0.964$  were found. In these figures, the prediction method with feed microscopy for crude protein analysis supports the usability

of chemical methods. According to Duncan test results, raw protein ratios of mixed feeds are different from each other. Thus, the availability of feed microscopy has been proven statistically in mixed feeds with different protein ratios and therefore different rations. In terms of raw cellulose, cattle fattening feed and cattle milk feed are similar, but other feeds are not similar. The similarity between cattle feed and cattle milk diets made it difficult to estimate the proportions of raw materials correctly.

METOT	Mixed (Powder) Feeds	Analyses	Recurrence (%)					
			1	2	3	4	5	6
Feed	Feed (Fattening	Crude protein	16.44	16.14	15.71	15.76	16.49	15.98
microscopy	bull)	Crude fiber	10.13	10.47	10.21	9.93	11.03	10.61
	Feed (dairy cow)	Crude protein	17.99	18.23	18.46	18.25	18.94	18.60
		Crude fiber	9.35	9.58	9.35	9.04	9.19	9.74
	Feed (lamb)	Crude protein	17.46	17.23	17.97	17.11	16.40	16.39
		Crude fiber	8.62	8.11	8.84	8.65	8.20	9.01
	Feed (broiler)	Crude protein	23.69	23.33	22.90	22.82	22.98	23.82
		Crude fiber	6.94	6.83	6.29	6.61	6.79	6.90
Chemical	Feed (Fattening	Crude protein	16.23	16.30	16.58	16.43	16.60	16.56
methods	bull)	Crude fiber	11.48	11.48	10.92	11.82	11.24	11.18
	Feed (dairy cow)	Crude protein	18.49	18.35	18.23	18.45	18.42	18.43
		Crude fiber	11.15	11.18	11.21	11.56	11.29	11.94
	Feed (lamb)	Crude protein	16.55	16.99	16.92	16.70	16.46	17.07
		Crude fiber	10.02	9.59	9.50	9.01	9.02	9.43
	Feed (broiler)	Crude protein	24.04	23.84	23.73	24.21	24.63	24.43
		Crude fiber	5.38	5.62	5.18	5.21	5.26	5.32

Table 1. Crude protein and fiber analysis results of mixed feeds

The results obtained by feed microscopy for crude fiber analysis differ from those found by chemical methods. In the t-test between the averages, it was found to be important at the level of P < 0.05 for crude fiber and this difference was confirmed. However, the correlations for the results of raw fiber analysis with two methods are r = 0.931 and  $r^2 = 0.867$  (Table 2). This shows that there is a strong and linear relationship between the results obtained by the two methods for crude fiber analysis.

Table 2. Correlation results of feed microscopy and chemical methods

Analysis	Correlation coefficient (r)	Determination coefficient $(r^2)$
Crude protein	0.982**	0.964
Crude fiber	0.931**	0.867

Huss (1976) have found that very well-recognized and highly predictable multi-cell multiplier products in microscopic view, as well as coarse-grained or grossly lightweight components (rough bran, Barley products, sunflower seeds) will be overestimated, while some of the lesser-bodied materials (such as finely ground soy products) will generally be estimated below their value.

The difference between the two methods is significant in crude protein analysis, which is important in crude fiber analysis, can create a contradiction. However, if the ration contents are carefully examined, it will be seen that the crude fiber analysis results obtained by the feed microscopy in the rations where the soybean pulp is in excess are much higher than the chemical method and even in the crude protein analysis. The reason is that the soya is in two forms as whole soybean and soya bean casserole. However, in the feed microscope, these two forms cannot be distinguished from each other and are evaluated as soybean pulp. This results in illusion in estimation. The hit rate in his estimation is undoubtedly proportional to experience. The more the feed microcopies work with various rations, the more experience he will have. For this reason, it is necessary to practice continuously and create a good collection of raw materials.

## CONCLUSION

It has been determined that the difference between the results of the crude protein analyzes made by the two methods is statistically insignificant, i.e., the results are similar to each other, and therefore the prediction method can be used instead of the chemical methods by feed microscopy in crude protein analysis. The method used for crude cellulose analysis was significant at P < 0.01. That is, the results obtained by feed microscopy for raw cellulose analysis differed from the results obtained by chemical method, and this difference was confirmed by the fact that the difference between the averages in the t test was significant compared to P < 0.05. The most important reason for not developing the feed microscope is the lack of trained staff.

Because a feed microscope grows only in a few years, the Ministry of Food, Agriculture and Livestock and the universities should produce joint projects; organize courses and seminars for the identification and development of the feed microscope. For the spread of the feed microscope, which is expected to take place in the coming years, the students should be provided with a practical undergraduate course in the universities so that students learn the mixed feed and feed raw materials under the microscope so that they can graduate with preliminary knowledge about feed microscopy.

#### REFERENCES

- Akyildiz, A.R. 1984. *Feeds Information Laboratory Guide*. Ankara University Agric. Fac. Publication No: 895.
- AOAC 2003. AOAC Official Method 900.03 Protein (crude) in animal Feed Combustion Method.
- Gies, P. & Vöhringer, H. 1998. Feed microscopy-its special relevance for veterinary nutrition. *Journal of Animal Physiology and Animal Nutrition* 80, 207–212.
- Hahn, H., Kruyskamp, K.1997. Estimation of animal constituents in compounded feeds: comparison between feed microscopy and ELISA. *Agribiological Research* **50**(1), 54–63.
- Huss, W. 1976. *Microscopy and quality control in the manufacture of animal feeds* (Futtermittelmikroskopie zur Qualitaetskontrolle). Roche Information Service. (In German).
- Khajarern, J. & Khajarern, S. 2008. *Feed Microscopy and Quality Control Manual*. Khon Kaen University Faculty of Agriculture Kaen 40002, Tailand.

- Klein, H., Marquard, R. 2005. *Feed microscopy:atlas for the microscopic examination of feed containing vegetable and animal products*. Book: Feed microscopy: atlas for the microscopic examination of feed containing vegetable and animal products, 309 pp.
- Makowski, J.V. 1998. Feed microscopy. An undervalued quality assurance technique. 1998. International News on Fats, Oils and Related Materials. Vol. 9 No. 11, 1036–1037; 1040–1042.
- OJ (EU) Regulation. 2009. Official Journal of the European Union Ec. No: 152/2009 L54 26/2/2009 p 40–42.
- Pinotti, L., Ottoboni, M., Caprarulo, V., Giromini, C., Gottardo, D., Cheli, F., Fearn, T., Baldi, A. 2016. Microscopy in combination with image analysis for characterization of fishmeal material in aquafeed. *Animal Feed Science and Technology* (215), 156–164.
- Soysal, M.I. 2000. Biyometrinin Prensibleri. Trakya Univ. Ziraat Fak. Yayın No: 95. (In Turkish)