Austin Journal of Analytical and Pharmaceutical Chemistry

Open Access

Austin Publishing Group

Research Article

Method Verification and Measurement of Uncertainty Estimation for the Proximate Analysis in Animal Feed- Single Laboratory Verification Protocol (Nordtest Approach)

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Received: June 27, 2022; **Accepted:** August 04, 2022; **Published:** August 11, 2022

Abstract

The purpose of this study was to verify the proximate analysis (DM percent, Ash percent, CP percent, EE percent, and CF percent) using AOAC approved methods at the Feed Quality Control Laboratory, DLS, Savar, Dhaka, and to reduce measurement uncertainty in each test in the laboratory. To verify the test procedures, a laboratory-made control sample (full-fat soybean), a certified control sample, and two Proficiency test feed samples (List A) were employed, together with various calibrate equipment (Table 1,2). This strategy was put to the test in terms of precision, accuracy, and consistency. Limits of Detection (LOD), Limits of Quantification (LOQ), Robustness, and Ruggedness, on the other hand, are irrelevant in these verification and uncertainty assessment techniques. Tests were carried out over a period of several months to verify this process and to assess the uncertainty more precisely. The Recovery percent was determined for the accuracy test, the Nordtest technique (Single Laboratory Verification Technique), and FAO. 2011 guidelines were used to assess the precision of the methods' long-term repeatability. The recovery% of the determination of DM%, Ash%, CF%, EE%, and CP% of the Laboratory manufactured Reference sample, FAPAS Proficiency test sample-1, and Sample 2 (Tables 3-7) were within 98-102%, while the precision was determined by long-term repeatability (SRw) was 0.87%, 0.13%, 0.29%, 0.25%, and 0.35%, respectively, which met the criterion (Table 2) of FAO guideline. The measurement uncertainty was computed using the Nordtest method (Single Laboratory Verification Technique) and FAO. 2011 criteria, based on the long-term repeatability of the proximal components in the acknowledged laboratory. According to the guideline, the Expanded Measurement Uncertainty for DM percent, Ash percent, CF percent, EE percent, and CP percent was 0.99, 0.89, 1.02, and 1.82 percent, respectively, which was quite satisfactory. So, in the respective laboratory-Feed Quality Control Laboratory, Department of Livestock Services, Savar, Dhaka, Bangladesh-this verification for proximate components analysis of the animal feed is validated.

Keywords: Proximate analysis; Method verification; Animal feed; Measurement uncertainty

Introduction

The concept "proximate composition" is commonly used in the feed/food industry to refer to the six components of Moisture, crude protein, ether extract, crude fiber, crude ash, and nitrogen-free extracts are all reported as a percentage of the total feed. The analysis of proximate components of animal feed and feedstuff has already been developed by multiple international bodies responsible for validating different test methods. This verification was done to check that the test procedure's validated methodologies (proximate analysis) were correct. This operation was carried out in the responsible test laboratory with the appropriate apparatus and chemicals, in line with the test protocol. The Nordtest method (Single Laboratory Verification Technique) and FAO. 2011 guidelines were used to verify the aforementioned test procedure (Table 1) and measurement Uncertainty.

Materials and Methods

Experimental place and date

From January 2021 to January 2022, the study was carried out at the Feed Quality Control Section, QC Laboratory, Savar, DLS, Dhaka, Bangladesh.

Preparation of laboratory made reference material

To ensure the accuracy of the method, a reference sample (working standard) with known and stable values should be run with each batch and analyzed and confirmed the recovery of analyses, according to Quality Assurance for Animal Feed, Analysis Laboratories, FAO Animal Production, and Health. Reference materials are usually pure substances, which are hard to come by in the case of feed. That's

Citation: Debnath M. Method Verification and Measurement of Uncertainty Estimation for the Proximate Analysis in Animal Feed- Single Laboratory Verification Protocol (Nordtest Approach). Austin J Anal Pharm Chem. 2022; 9(3): 1148.

why using a handmade feed reference sample (HRM) in the lab is so popular.

The following steps were considered to prepare a Homemade feed Reference Sample (HRM):

- From the overall volume of the sample, a 3kg representative sample (full fat soya bean) was taken.
- A 2 mm sieve grinder was used to ground the sample.
- Divided the sample into a small airtight container.
- Over the course of many days, six distinct runs with 18 identical samples were carried out utilizing varied equipment.
- Each test result was statistically examined (Tables 3-12).
- Every 18 tests in each parameter's mean value and standard deviation (SD not greater than 2) were calculated.

Quality reference material

List A:

1. FAPAS QC MATERIAL, T10169QC, and Dairy ration, received date: 28.4.21, Expiry date: 30.04.22.

2. FAPAS Proficiency test Sample- 1, ID-FCNC7-AFE20, Soybean meal, test no. 10177.

3. FAPAS Proficiency test Sample-2, Pig Ration, and ID- 10176.

Laboratory analysis

Determination of DM and moisture%: The determination of dry matter, or more specifically, moisture, is perhaps the most often conducted analysis in the QC lab. Because the concentration of other nutrients is frequently expressed on a dry matter basis, this is an important analysis (as a percentage of the dry matter). The dry matter of the collected sample was calculated gravimetrically as the residue left after drying for 3-4 hours at 103 2o C in a ventilated oven. All of the samples were examined in triplicate, and mean values were calculated.

Calculation:

% Dry Matter = (W3 – W1) x 100 / (W2 – W1)

Where,

W1= Weight of empty dish (g)

W2= Weight of dish and sample (g)

W3= Weight of dish and sample after drying (g)

Determination of crude ash: The residual after burning the sample (5 g sample) at 550 20 °C for 3 hours in a preheated muffle furnace and oxidizing all organic matter was quantified gravimetrically. All of the samples were examined in triplicate, and mean values were calculated.

Calculation:

% Ash = (W3 – W1) x 100 / (W2 – W1)

Where,

W1 = Weight of empty dish (g),

W2 = Weight of the dish and sample (g), and

W3 = Weight of dish and residue after incineration (g)

Result was calculated on Dry Matter basis.

Determination of Crude Fiber: The Weende system is built around crude fiber analysis. The analysis was originally intended to divide plant carbohydrates into less digestible (crude fiber) and readily digestible portions (nitrogen-free extract; NFE). 1g of material was placed in the fiber crucible, along with 0.5-1 g of celite. The material was then digested using a solution of 1.25 percent sulphuric acid and 1.25 percent potassium hydroxide. After drying, the weight of the ash sample was calculated.

Calculation:

% Fibre = (W3 – W1) x 100 / (W2 – W1)

Where,

W1= Sample weight

W2= Weight of Crucible and sample after drying

W3= Weigh of Crucible and sample after ash

Determination of Ether Extract/Crude Fat by Soxhlet Apparatus: The terms "lipid" and "fat" are sometimes used interchangeably to describe a wide range of substances that are insoluble in water but soluble to variable degrees in "fat solvents" or "organic solvents" such as ether (diethyl ether), chloroform, alcohol (methanol, ethanol, etc.), acetone, benzene, and "petroleum ether."

In the soxhlet sample thimble cup, a 5 g test piece of the sample was placed. The sample cup was then filled with 100 mL diethyl ether. The filtering took place at a boiling temperature of 60-680 C, and Ether Extract was collected beneath the sample cup as a result of the filtration and distillation. The residue was weighed after drying.

Calculation:

% Fat = (W3 – W1) x 100 / (W2 – W1)

Where,

W1= Sample weight

W2= Weight of empty extraction cup & sample

W3= Weigh of extraction cup with extract & dry sample.

Determination of Crude Protein (CP) by DUMAS Method: For the verification of crude protein analysis for animal feed, the Dumas method (total combustion method) was utilized. One of the most typical analyses done in the nutrition laboratory is this approach. The Dumas technique for determining nitrogen is based on quantitative combustion digestion of the sample at 1030 degrees Celsius in the presence of oxygen, where the nitrogen is transformed to Nitrogen Oxides (NOx) gas. In a thermal conductivity cell, NOx is converted to N2, which is then measured. In a tin cup, pour roughly 0.2-0.5 g EDTA to the nearest 0.1 mg (W) for crude protein determination. Close the tin cup carefully as if it were airtight and set it in Dumas' device. Silage was burned at a temperature of 10300°C for combustion and 650°C for reduction. The crude protein content was determined by multiplying the measured nitrogen quantity by the required factor

Table 1: Analytical Standard Method.

SL	Constituents		Method	Major instruments
1	Proximate components	Determination of Dry Matter % of Animal feeds and feeding stuff	AOAC 930.15.2000	Forced air Oven
2		Determination of Crude Ash % of Animal feeds and feeding stuff	AOAC 942.05.2000	Muffle furnace
3		Determination of Crude protein % of Animal feeds and feeding stuff, DUMAS method	AOAC 990.03	DUMAS
4		Determination of Crude Fiber % of Animal feeds and feeding stuff	AOAC 978.10	Velp Scientific –FIWE Advance Automatic Fiber Extractor
5		Determination of Ether Extract % of Animal feeds and feeding stuff	AOAC 920.39	SER 158, solvent extractor, Velp scientific.

Table 2:

SL	Parameter	Target Limit
1	Standard Deviation	Not more than ±2
2	Recovery Limit%	98-101% for 100% concentration
3	Precision/ Repeatability%	1.3% for 100% concentration

(6.25) and represented as a percentage.

Calculation:

The Dumas apparatus, which is capable of performing the complete determination, calculates percent Nitrogen (percent N) automatically. The area of the peaks identified for the calibration standard (EDTA) and the samples are compared to calculate N content.

Calculation of crude protein (% CP) = % N x F

Where, F = 6.25

Verification Protocol

Quality Control

Statistical Analysis: This approach was tested for accuracy, precision, and trueness. Limits of Detection (LOD), Limits of quantification (LOQ), Robustness, and Ruggedness, on the other hand, are not relevant in these procedures for verifications and assessment of uncertainty. To verify this procedure, tests were carried out over a period of several months in order to calculate the uncertainty more precisely [1,2].

Result and Discussion

Accuracy and Precision

The DM, Ash, CF, EE, and CP Recovery % of the Laboratory made Reference sample, FAPAS Proficiency test sample-1 & Sample 2 (Table 3, 4, 5, 6 & 7) was between 98-102%, Whereas the precision was calculated by long-term repeatability (SRw) were 0.87%, 0.13%, 0.29%, 0.25% and 0.35% respectively that comply with the requirement (Table 2) of FAO guideline [1].

Measurement Uncertainty

To illustrate measurement uncertainty, the standard deviation of a state-of-knowledge probability distribution spanning the possible values attributable to a measured variable is widely utilized. Following the guideline [2], the measurement uncertainty was calculated from

Table 3:

Accuracy and Precision calculation for DM%					
Day	Sample	Observed Value	True Value	Accuracy	Precision
				% Recovery	% Long term Repeatability (SRw)
1	Reference sample	90.93	90.02	101.01	0.15
2	Reference sample	90.96	90.02	101.05	
3	Reference sample	90.93	90.02	101.01	
4	Reference sample	90.73	90.02	100.79	
5	Reference sample	90.58	90.02	100.62	
6	Reference sample	90.81	90.02	100.88	
7	Reference sample	90.64	90.02	100.68	
8	Reference sample	90.72	90.02	100.78	
9	Reference sample	90.85	90.02	100.93	
10	Reference sample	90.73	90.02	100.79	
11	Reference sample	90.54	90.02	100.58	
12	Reference sample	90.58	90.02	100.63	
13	FAPAS PT1	87.81	87.80	100.02	0.87
14	FAPAS PT1	89.04	87.80	101.41	
15	FAPAS PT2	91.32	90.44	100.97	0.07
16	FAPAS PT2	91.30	90.44	100.95	
17	FAPAS PT2	91.19	90.44	100.83	

Table 4:

Within laboratory Accuracy and Precision Ash%										
SL		Analytes/ Matrix	Day	no.of repeation	Sample Weight	Observed Value	True Value	Accuracy		Precision
					g			% Recovery	SD	% Long term Repeatability (SRw)
1	FAPAS QC sample Dairy ration	Ash%	1	6	1.5-2	7.92	8.03	98.57		0.01
			2	6	1.5-3	7.93	8.03	98.73		
			5	2	5	7.90	8.03	98.41		
					mean	7.92	8.03	98.57	0.01	
2	Laboratory made QC sample (382)	Ash%	1	2	2	4.93	4.86	101.44		0.13
			6	4	2	4.79	4.86	98.53		
			7	1	2	5.12	4.86	105.28		
			8	5	2	5.02	4.86	103.27		
			9	3	5	4.90	4.86	100.91		
			10	2	5	4.80	4.86	98.83		
			11	2	5	4.77	4.86	98.11	0.12	
					mean	4.90	4.86	100.91		

Table 5:

Within laboratory Accuracy and Precision CF%							Accuracy	Precision
SL		Analytes/Matrix	Day	no.of repeation	Observed Value	True value	% Recovery	% Long term Repeatability (SRw)
1	FAPAS QC sample dairy ration	CF%	1		9.71	9.41	103.21	0.29
			2		9.72	9.41	103.29	
			3		9.96	9.41	105.80	
			4		10.32	9.41	108.00	
2	Laboratory made QC sample (382)	CF%	1	2	12.00	12.20	98.36	0.17
			2	4	12.51	12.20	102.54	
			3	1	12.42	12.20	101.80	
			4	1	12.43	12.20	101.89	
			6	1	12.47	12.20	102.21	
			7	1	12.37	12.20	101.39	
			8	1	12.23	12.20	100.25	
			9	1	12.21	12.20	100.08	

the long-term repeatability of the proximate components in the respected laboratory.

According to the table 8-12, the Expand MU of DM%, Ash%, CF%, EE% and CP% were 0.99, 0.89, 0.89, 1.02 & 1.82% respectively.

Where,

sbias = Standard deviation of the bias estimates obtained and n is the number of bias estimates obtained.

u(Cref) = Assuming standard uncertainty of reference value.

$$RMS_{bias} = \sqrt{\frac{\sum (bias_2)^2}{n}}$$

$$u(bias) = \sqrt{RMS_{bias}{}^2 + s_{bias}{}^2 / n + u(Cref)^2}$$

Combined Uncertainty

$$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$$

Conclusion

The research also included a mechanism for verifying the Proximate Analysis of Animal Feed determination. Accuracy, Precision, Standard deviation, Expand Measurement Uncertainty, and other estimated parameters in the verification protocol were determined to match the required performance criteria, and the technique was verified for the intended application. However, according to the Nordtest technique, other statistical parameters such as -Limit of detection, internal repeatability, reproducibility, recovery, and linearity of the operating concentration range were not taken into account (Single Laboratory Verification Technique).

Recommendation

It is suggested that a long time further study with a reference certified sample be carried out in order to improve the Measurement

Table 6:

Within laboratory Accuracy and Precision EE%									
SL		Analytes/ Matrix	Day	no.of repeation	Observed value	True value	Accuracy		Precision
							% Recovery	SD	% Long term Repeatability (SRw)
1	FAPAS QC sample Dairy ration	EE%	1	1	4.46	4.53	98.45		0.03
			2	1	4.48	4.53	98.83		
			3	1	4.53	4.53	99.90		
					4.49	4.53	99.06	0.03	
2	Laboratory made QC sample (382)	EE%	1	4	18.83	18.58	101.35		0.25
			2	1	18.48	18.58	99.47		
			3	2	18.68	18.58	100.27		
			4	2	18.64	18.58	100.33		
			5	5	18.66	18.58	100.41		
			6	1	18.71	18.58	100.7		
			7	1	18.61	18.84	98.82		
			8	3	18.57	18.58	99.97		
			9	3	18.40	18.58	99.05		
			10	1	18.85	18.58	101.43		
			11	4	18.48	18.58	99.47		
			13	4	18.93	18.58	101.9		
			14	6	19.14	18.58	102.99		
			15	6	19.12	18.58	102.93		
			16	3	19.18	18.58	103.24		

Table 7:

Within laboratory Accuracy and Precision CP% DUMAS Method								
SL		Analytes/Matrix	Day	Observed Value	True Value	Accuracy		Precision
						% Recovery	Average	% Long term Repeatability (SRw)
1	FAPAS QC sample (445) Dairy ration	CP%	1	17.50	17.20	101.74	102.35	0.24
			2	17.84	17.20	103.69		
			3	17.36	17.20	100.93		
			5	17.60	17.20	102.33		
			6	17.38	17.20	101.07		
			7	17.95	17.20	104.37		
2	FAPAS known sample Soyabean meal, 1495		8	44.56	44.20	100.82	101.30	0.35
			9	45.06	44.20	101.95		
			10	44.55	44.20	100.80		
			11	44.27	44.20	100.15		
			12	45.11	44.20	102.06		
			13	44.69	44.20	101.12		
			14	45.19	44.20	102.25		

Table 8:

Calculation of measurement of Uncertainty for the evaluation of DM% in Laboratory made QC sample (Full fat Soyabean)									
Day	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
DM%	92.746	92.569	92.818	92.824	92.821	92.808	92.747	92.338	92.072
Mean	92.64								
SD/u(Rw)	0.27								
%DM control	92.66	%							
Standard deviation	0.39	%							
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**
bias from reference	0.08	-0.09	0.16	0.16	0.16	0.15	0.08	-0.32	0.00
RMSbias =	0.16	RMSbias2	0.03						
sbias =	0.10		sblas2/n	0.00		$RMS_{bias} = \sqrt{\frac{\sum (bias_2)^2}{n}}$			
u(Cref)	0.39		u(Cref)2	0.15					
u(bias) =	0.42	sbias is the standard deviation of the bias estimates obtained and n is the number of bias estimated obtained							
u(Rw) =	0.27			$u(bias) = \sqrt{RMS_{bias}{}^2 + s_{bias}{}^2 / n + u(Cref)^2}$					
uc=	0.50	%							
Expanded uncertaint	0.99	%	k=2	$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$					

Table 9:

Measurement uncertainity Laboratory made QC sample for Ash%											
Date	**day 1**	**day 2**	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 11**
Ash%	4.505	4.927	4.341	4.677	4.499	4.305	4.462	4.402	4.789	4.957	4.915
Mean	4.62										
SD/u(Rw)	0.25										
% Ash in control sample	**4.86**										
Standard deviation of QC	**0.17**										
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**
bias from reference	**-0.35**	**0.07**	**-0.52**	**-0.18**	**-0.36**	**-0.55**	**-0.40**	**-0.46**	**-0.07**	**0.10**	**0.06**
RMSbias =	**0.32**		**RMSbias2**	**0.10**							
sbias =	**0.25**		**sbias2/2**	**0.01**							
u(Cref) =	**0.17**		**u(Cref)2**	**0.03**							
u(bias) =	**0.37**										
u(Rw) =	**0.25**										
uc =	**0.45**	%									
Expnaded uncertainty U	**0.89**	%	**k=2**								

Table 10:

Measurement of Uncertainity calculation of Laboratory made QC sample for CF%								
Date	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
CF%	12.7	12.47	12.42	12.43	12.47	12.37	12.23	12.21
Mean	12.41							
SD/u(Rw)	0.15							
%CF in control sample =	12.22	12.22	%					
Standard deviation of QC sample =	0.34	0.34	%					
blas from reference value =	0.48	0.25	0.20	0.21	0.25	0.15	0.01	-0.01
RMSbias =		0.24		RMSbias2	0.06	$RMS_{bias} = \sqrt{\frac{\sum (bias_2)^2}{n}}$		
sbias =		0.11		sbias2/n	0.00			
u(Cref) =		0.34		u(Cref)2	0.11			
u(bias) =		0.42		$u(bias) = \sqrt{RMS_{bias}{}^2 + s_{bias}{}^2 / n + u(Cref)^2}$				
u(R) =		0.15		$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$				
uc =		0.44	%					
Expanded uncertainity U =		0.89	%	k=2				

Table 11:

Calucation of measurement of Uncertainity of Laboratory made QC sample (EE%)																
Date	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**	**Day 15**	**Day 16**
EE%	18.83	18.48	18.63	18.64	18.66	18.71	18.84	18.57	18.40	18.85	18.48	18.13	18.93	19.14	19.12	19.18
Mean	18.72															
SD/u(Rw)	0.29															
%EE in control sample	18.58	%														
Standard deviation of QC sample =	0.27	%														
bias from reference	0.25	-0.1	0.05	0.06	0.07	0.13	0.26	-0.01	-0.18	0.26	-0.10	-0.46	0.35	0.55	0.54	0.60
RMSbias =	0.31		RMSbias2	0.1												
sbias =	0.29		sbias2/n	0.01												
u(Cref) =	0.27		u(Cref)2	0.07												
u(bias) =	0.42															
u(Rw) =	0.29															
uc =	0.51	%														
Expanded uncertainity U =	1.02	%	k=2													

Table 12:

Measurement of Uncertainity of DUMAS Method - Reference Sample (soyabean meal) FAPAS							
Date	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
CP%	44.56	45.06	44.55	44.27	45.11	44.69	45.19
Mean	44.78						
SD/u(Rw)	0.35						
%CP in control sample (09.10.21-07.02.22) =	44.2	%					
Standard deviation of QC	0.50	%					
bias from reference value =	0.36	0.86	0.35	0.07	0.91	0.49	0.99
RMSbias =	0.66		RMSblas2	0.44			
sbias =	0.35		Sblas2/n	0.02			
u(Cref) =	0.50		u(Cref)2	0.25			
u(bias) =	0.84		$RMS_{bias} = \sqrt{\frac{\sum (bias_2)^2}{n}}$				
u(Rw) =	0.35						
			$u(bias) = \sqrt{RMS_{bias}{}^2 + s_{bias}{}^2 / n + u(Cref)^2}$				
uc =	0.91	%					
Expanded uncertainity U =	1.82	%	k=2				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7

Uncertainty of this test process.

Acknowledgments

The author wishes to express his gratitude to the Department of Livestock Services' Quality Control Laboratory for Livestock, Production Inputs, and Food Products for giving finances and chances to conduct this research at the QC Laboratory, DLS, Savar, Dhaka. Finally, a special thanks go out to all of the Feed Quality Control Section's employees for their helpfulness.

Author Responsibilities

Debnath Manika is a laboratory analyst, data collector, statistician, and information generator for the researcher. Manika was also in charge of drafting and quality control for the text. She provides a considerable contribution to the manuscript's data analysis. Abrham Ayele follows the publisher's rules when preparing the paper.

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