



Research Paper

Systematic Review on Comparing Calculated and Laboratory Determined Crude Protein Estimates for Animal Feedstuffs and Diets

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ABSTRACT: Feed is a major determinant of the profitability and sustainability of any animal enterprise. The feed must be nutritionally balanced and economically formulated to meet the purpose of production. Crude protein is a parameter that is often used in the assessment of the quality state of feed and feedstuff. The crude protein can either be calculated or chemically determined using various methods. Calculated crude protein is easier and quicker to carry out than laboratory assay of feed composition. Differences between the estimates of calculated crude protein and the chemically determined composition have been reported. A survey of agricultural, veterinary, biological and evolutionary literature yielded 107 animal feeding trial studies in which the author(s) reported crude protein estimates for calculated composition and/or chemically determined compositions. Using suitable statistical tools and reliability tests the study was conducted to provide a basis for use of calculated methods in animal feeding trials. It was determined from these studies that the calculated crude protein composition is a true reflection of the chemically determined estimate and hence be used where laboratory assay is not readily available.

KEYWORDS: calculated; chemical composition; comparison; crude protein; feeding trial; methods;

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I. INTRODUCTION

Over a century research by nutrition experts has been to define the nutrient required by animals. With the help of this information, rations can be formulated from feeds which are natural materials that either alone or suitably mixed (González-Martín *et al.*, 2006) meet the requirements for health and efficient production of animals. The goal of any feeding program is to achieve an appropriate balance among available feed ingredients where total ration nutrient composition meets daily nutritional needs of the animals (Van Saun, 2014). Feed can be defined as any material that an animal can ingest, digest and the products of this process absorbed and utilized for the normal physiological functions of the animal. Feed industry exist to produce several variety of animal feed using flexible technological processes large number of raw materials are incorporated with a wide range of dosing (Pavlova *et al.*, 2011). Tremendous variation exists in nutrient composition between different feeds. Even within a feedstuff, there is potential for significant variation in composition; hence, this calls for a consistent analysis of feed samples prior to use for poultry flocks (Bhatti *et al.*, 2002; Van Saun, 2014). It is likely that the same could apply for other species of animals.

Feed has been reported to play an important role in economics of animal production as it constitutes about 60 – 70 percent in cost of production of eggs and poultry meat (North and Bell, 1990). The ultimate goal of feed analysis is to predict the productive response of animals when they are fed rations of a given composition. Chemical analysis of formulated feeds is been used to obtain crude protein for feed (Gul and Safdar, 2009; Houndonoubo *et al.*, 2012). Other researchers have tended to use the calculated crude protein of

feed because either lack of possibility to determine the actual compositional data or there is insufficient time to obtain an analysis (Stanton and LeValley, 2010).

The main quality factors of feeds are the energy value, the amount of crude fibre (CF; being very important in regard to digestibility), crude protein (CP; important for the balance and digestibility of essential amino acids) and the ether extract (EE), together with the different additives that may be present (Pavlova *et al.*, 2011).

Crude protein percentage is used as a measure to determine the protein content of an animal feed. It measures the total nitrogen content of a feed or feedstuff. Crude protein measures both nitrogen from proteins as well as from non-protein nitrogen sources in the feedstuff such as creatinine and urea. Crude protein differs from true protein measurement that quantifies the actual protein content and excludes non-protein nitrogen (Annigan, 2011).

The methods available for crude protein determination includes Kjeldahl, Dumas, applications of Ultra-Violet (UV) visible spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Infrared (IR) techniques (McClements, 2003; Stanton and LeValley, 2010; Krotz *et al.*, 2014; Van Saun, 2014). Factors which determines what method of determination of crude protein to use includes the intended use of obtained information, the equipment available, ease of operation, the desired accuracy, whether or not the technique is non-destructive, the sample preparation, method characteristics (e.g. sensitivity and specificity), speed (time required per analysis) and the number of samples analysed per batch (McClements, 2003).

A peruse through studies using varying feed and different animals that involve both chemically analyzed and calculated values for crude protein showed differences in the components (Davis *et al.*, 1962; Ashraf, 1981; Iyeghe-Erakpotobor *et al.*, 2006; Ahmed *et al.*, 2013; Alikwe *et al.*, 2014). No study reports the justification for the use of calculated crude protein (CCP) composition in place of chemically determined crude protein (DCP). The present study was undertaken to establish similarities and correlation between calculated crude protein of feed and chemically analyzed crude protein of feed.

Hypothesis H₀: the calculated crude protein value is equal to chemically determined value and that similar and/or dissimilar crude corresponding CCP and DCP are not different

H_A:the calculated crude protein value is not equal to chemically determined value and that similar and/or dissimilar corresponding CCP and DCP are different.

II. MATERIALS AND METHODS

In carrying out feeding trials, animal scientists, biologists and veterinarians formulate diets usually at graded levels to determine the effect of such ingredients or feeds on certain selected growth, productive and reproductive parameters of animals. The ingredients and feed are calculated and analyzed to ensure that they meet the nutrient requirement of the animals that are to be tested. An internet (using Google Scholar and Yippy search engines) and manual (printed materials) search for animal experiments in which feed for animals were formulated was carried out (Table 1).

Table 1: Journal type and number of Calculated and laboratory determined estimates and the number selected from each category.

Source Journal	CCP	DCP	SEL
Agricultural	53	7	7
Veterinary	20	2	2
Biological	27	2	2
Evolutionary	7	-	-
Total	107	11	11

CP=calculated crude protein, DCP= determined crude protein. SEL= selected

A survey of agricultural, veterinary, biological and evolutionary literature yielded 107 animal feeding trial studies in which the author(s) reported crude protein estimates for calculated composition and/or chemically determined compositions. Out of 107 studies, eleven (11) were selected as they reported crude protein for both chemically analyzed and calculated compositions. From the 11 selected studies, a data set consisting of 62 pairs of calculated composition and chemically determined composition matrices was obtained. The final data encompass more than 10 years of research involving 5 different species of animals including rabbits, pigs, Japanese quails, broilers and laying hens (Table 2).

Table 2: Source of calculated and laboratory determined crude protein

Authors	CCP	DCP
Ani (2007)	16.75	15.65
	16.82	14.65
	16.90	15.65
	17.01	16.50
Onyimonyi and Okeke (2007)	17.90	18.92
Ari <i>et al.</i> (2011)	22.82	21.93
	21.30	20.56
	21.33	21.60
	19.69	22.32
Idiong <i>et al.</i> (2007)	19.55	21.67
	20.00	20.80
	20.02	20.25
	20.16	22.20
Ahmed <i>et al.</i> (2013)	20.20	19.80
	20.30	21.44
	20.40	20.60
	22.50	25.90
Ahmed <i>et al.</i> (2013)	22.00	25.40
	22.20	22.10
	22.20	21.00
	21.60	20.00
Akade <i>et al.</i> (2012)	20.30	21.00
	20.11	20.40
	20.28	20.18
	20.45	20.41
Oresanya (2005)	25.28	25.37
	30.11	30.44
Amaefule <i>et al.</i> (2011)	17.14	15.63
	17.44	15.40
	17.44	13.64
	18.04	13.65
Rashid <i>et al.</i> (2004)	18.34	17.23
	19.00	20.57
	15.29	16.09
	19.28	20.34
Sun (2007)	15.16	15.81
	22.00	19.62
	22.00	20.02
	22.00	20.79
	22.00	20.28

	20.00	19.31
	20.00	18.83
	20.00	18.94
	20.00	18.20
	17.50	14.88
	17.50	16.98
	17.50	15.32
	17.50	16.63
	16.50	15.12
	16.50	15.63
	16.50	14.58
	16.50	14.91
Alikwe <i>et al.</i> (2014)	21.37	23.35
	21.21	22.87
	20.18	21.70
	19.20	21.23
	18.13	21.06
	23.10	23.35
	23.25	22.87
	23.31	21.70
	23.30	21.23
	23.24	21.06

CCP=calculated crude protein, *DCP*= determined crude protein

III. DATA ANALYSIS

Any two crude protein values within the same row of a matrix from the original data set exhibit dependence (Waitt and Levin, 1998). Hence, randomization tests are useful because they require no prior assumptions regarding the distribution of the test statistic. A web based number tables generator (<http://tools.perceptus.ca/number-tables.php>) was used to randomize the estimates of the calculated crude protein and the chemically analyzed for the original dataset of this study. The original dataset was randomized for both values four times.

The values from the randomization process were then used to obtain disparity and the mean of the between corresponding estimates of calculated crude protein values and chemically determined crude protein values or subsets thereof to assess how close were the estimates of calculated crude protein and chemically analysed. The original and randomized data were subjected to statistical analysis using formulas of Cheverud (1988) and Roff (1998).

$$D_{CD} = \sum_{I=1}^{i=n} \frac{[CCP_i - DCP_i]}{N}$$

$$D_{CCP1-CCP2} = \sum_{I=1}^{i=n} \frac{[CCP_i - CCP_j]}{N}$$

$$D_{DCP1-DCP2} = \sum_{I=1}^{i=n} \frac{[DCP_i - DCP_j]}{N}$$

Where D_{CD} is the mean disparity between the calculated crude protein and the chemically determined crude protein obtained from the original data. N is the number of observations used in the study. CCP_i and DCP_i are the calculated crude protein and chemically determined crude protein from the original data respectively. CCP_1 and CCP_2 are the randomized data for four times and DCP_1 and DCP_2 are the corresponding randomized estimates for the chemically determined crude protein. The data were tested for normality of distribution using Kolmogorov-Smirnov test (Willis *et al.*, 1991; Waitt and Levin, 1998).

Pearson, Kendall-Tau and Spearman correlation coefficients (Akanno and Ibe, 2005; Visscher *et al.*, 2008) were determined using the bivariate correlation protocol of SPSS and Cohen's Kappa (Cohen, 1968; Landis and Koch, 1977; Viera and Garrett, 2005) was run to determine the relationship and level of agreement between the calculated crude protein and the chemically determined crude protein. The level of agreement was determined using the interpretation of Kappa (Landis and Koch, 1977; Table 3). Mantel (1967) test was used to test similarity and dissimilarity amongst the corresponding CCP and DCP matrix.

Table 3: Landis and Koch (1977) Interpretation of Kappa

Kappa	Agreement
< 0	Less than chance agreement
0.01–0.20	Slight agreement
0.21– 0.40	Fair agreement
0.41–0.60	Moderate agreement
0.61–0.80	Substantial agreement
0.81–0.99	Almost perfect agreement

Furthermore, the Wilcoxon signed-rank test (Akeeson *et al.*, 2008; McDonald, 2009) for matched pairs (nonparametric tests algorithms) and Friedman's test (Kerr *et al.*, 2002; Rebollo *et al.*, 2006) were used to test the hypothesis of the hypothesis of the study. IBM SPSS (2011) was used in running the analysis of data. The level of significance was set at $P < 0.05$.

IV. RESULTS

The result for descriptive statistic for the original data is shown in Table 4. The same values applied to the randomized data set (Table 4). The mean and the standard error for the calculated crude protein was $19.93\% \pm 0.34$ while the chemically determined crude protein content was $19.61\% \pm 0.43$. The DCP was more variable than the CCP and had coefficient of variation for DCP and CCP of 17.12 and 13.37, respectively. The scatter plot graph of calculated crude protein versus chemically determined crude protein is shown in Figure 1.

Table 4: Descriptive statistics of CCP and DCP from the original data set obtained from 11 studies from year 2004 to year 2014.

	N	Range	Min	Max	Mean	SEM	Var	CV
CCP	62	14.95	15.16	30.11	19.93	0.34	7.1	13.37
DCP	62	16.80	13.64	30.44	19.61	0.43	11.27	17.21

N= number of observation, *min*= minimum estimate, *max*= maximum estimate, *var*= variance, *CV*=coefficient of variation, *SEM*=standard error of the mean, *CCP*= calculated crude protein, *DCP*= determined crude protein

Table 5: Descriptive statistics of randomized CCP and DCP from the original data set obtained from 11 studies from year 2004 to year 2014

	N	Range	Min	Max	Mean	SEM	Var	CV
CCP1	62	14.95	15.16	30.11	19.93	0.34	7.1	13.37
CCP2	62	14.95	15.16	30.11	19.93	0.34	7.1	13.37
CCP3	62	14.95	15.16	30.11	19.93	0.34	7.1	13.37
CCP4	62	14.95	15.16	30.11	19.93	0.34	7.1	13.37
DCP1	62	16.80	13.64	30.44	19.61	0.43	11.27	17.21
DCP2	62	16.80	13.64	30.44	19.61	0.43	11.27	17.21
DCP3	62	16.80	13.64	30.44	19.61	0.43	11.27	17.21
DCP4	62	16.80	13.64	30.44	19.61	0.43	11.27	17.21

N= number of observation, *min*= minimum estimate, *max*= maximum estimate, *var*= variance, *CV*=coefficient of variation, *SEM*=standard error of the mean, *CCP*= calculated crude protein, *DCP*= determined crude protein

Differences between the CCP and DCP for the original as well as the randomized data are presented in Table 7. The mean value for all the disparity between calculated crude protein and their corresponding laboratory determined crude protein was 0.32. The variance amongst the disparity for the original data had the least

estimate when compared with the other randomized estimates. The result obtained for Kolmogrov-Smirnov test for normality indicated no significance and hence disparities for all the various subsets are normally distributed. Although the mean disparity for the original data subset obtained was 0.32 corresponds to that obtained for data of the randomization, the original data had the narrower estimate of variance, standard deviation and range of values. The analysis of variance of the disparity for both data subsets demonstrated no difference

Figure1. Scatter plot and regression line of calculated crude protein (CCP) on chemically determined crude protein (DCP)

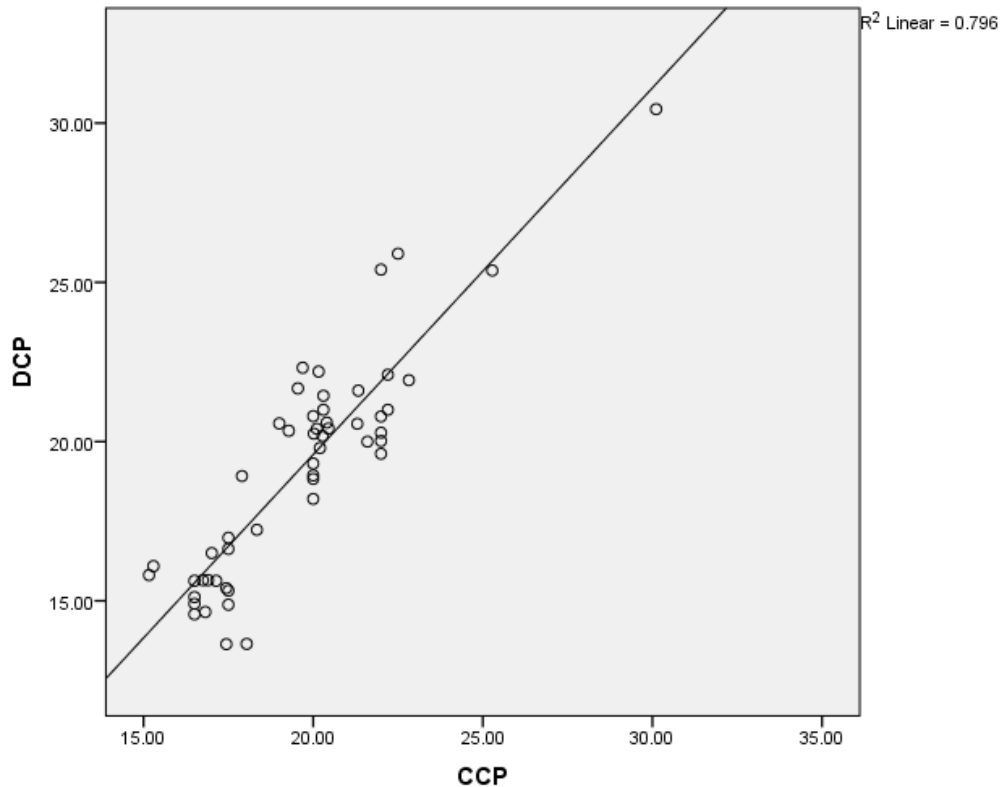


Table 7: Disparity between CCP and DCP from original and randomized dataset obtained from 11 studies from year 2004 to year 2014

	N	Range	Min	Max	Mean	SEM	Var
CCP-DCP	62	7.79	-3.40	4.39	0.32	0.21	2.80
CCP1-DCP1	62	19.95	-11.44	8.51	0.32	0.51	16.37
CCP2-DCP2	62	22.33	-10.42	11.91	0.32	0.56	19.44
CCP3-DCP3	62	23.16	-10.28	12.88	0.32	0.52	16.45
CCP4-DCP4	62	22.85	-13.30	9.55	0.32	0.56	19.28

CCP= calculated estimate of crude protein, DCP= chemically determined estimate of crude protein, Min= minimum, Max= maximum, SEM= standard error of the mean, Var= variance.

The correlation between the calculated crude protein and the chemically analyzed crude protein for the original data showed that the Pearson, Kendall-Tau and Spearman correlation indicated highly significant agreement ($P < 0.01$) estimated at 87%, 60.6% and 80%, respectively. The result obtained from running Cohen's kappa (κ) was -0.004. This is the proportion of agreement over and above chance agreement. Based on the guidelines from Landis & Koch (1977) Table 3, a kappa (κ) of -0.004 represents a less than chance agreement. Furthermore, since $p = .600$, our kappa (κ) coefficient is statistically non-significantly different.

The nonparametric test for two related samples using the original data resulted in non-significant a Wilcoxon ranked test with value of $P = 0.092$. The test using k-related samples comparing the original data set and the randomized data utilizing the Friedman test gave Monte Carlo value of $P = 0.961$ being non-significant.

V. DISCUSSION

Feed manufacturing involves the processing of mixtures of feedstuffs and feed additives into a usable form to increase profits of animal production by maximizing the nutritional value of a feedstuff or a mixture of feedstuffs. Nutrient requirements as established by research conducted at various agencies are continually being used as the basis for feed formulation for animals. Nutrient requirement data are updated frequently to ensure current data are available for formulating least cost feeds. Nutrient profiles of feedstuffs sometimes supplied by different suppliers are continually updated based on actual assays conducted over a number of years. When formulating diet for animals, a safety margin is used to account for variations in the nutrient content of feed ingredients (Robinson and Li, 1996).

A variety of biologic, chemical, enzymatic, and other sophisticated analytical and computational methods are used to evaluate nutrient content of feeds. Chemical methods can directly measure quantities of compounds associated with an essential nutrient; however, they tell us nothing about digestibility and absorbability. Biologic, enzymatic, and other sophisticated methods provide a more nutritional perspective to feed analysis; thus helping to better understand just how the animal will interact with its diet (Van Saun, 2014).

The problem with using this method is that feeds vary in their composition and the organic constituents (e.g., crude protein, ether extract, crude fibre, acid detergent fibre and neutral detergent fibre) can vary as much as 15 percent, the mineral constituents as much as 30 percent, and the energy values at least 10 percent from published and commonly used tables values (NRC, 1994; Aduku, 2004; Stanton and LeValley, 2010).

Most balanced diet formulations are currently based on proximate nutrient values. Increasing evidence suggests that nutrient values of dietary ingredients are also affected by active components such as enzyme inhibitors (Hall *et al.*, 2009; Alu, 2012; Dashe, 2015). Variations from commonly used table values in the levels of crude protein contents would be explained in terms of processing methods, geographical condition of the areas in which cereals and legumes are cultivated and to formulation in compound feeds (Bhatti *et al.*, 2002). Method of storage also influences the crude protein content due to certain metabolic activities during storage, composition of the feed (e.g. fibrous plant components are retained other dry matter lost). A slight amount of crude protein is lost during storage. However, the protein is lost at a slower rate than carbohydrates. Thus, due to drying off of the feedstuffs, crude protein concentration increases slightly during storage (Buckmaster *et al.*, 1989; Hall *et al.*, 2009).

The correlation for the original data set for CCP and DCP shows a strong and positive correlation (87%). A large positive matrix correlation indicates that correlations vary in similar directions, not that the magnitudes of individual correlations are identical (Waite and Levin, 1998). Furthermore, with a very low and non-significant estimate of Cohen's-Kappa (which shows that the agreement between the CCP and the laboratory determined crude estimates are largely due to chance), we can hypothesize that the corresponding estimates are similar. The sample size in this study was 62 and with a large sample size, the results will change as *P* values and confidence intervals are sensitive to sample size, and with a large enough sample size, the result can become statistically significant (Viera and Garrett, 2005).

Again, variables from two similar sources can be expressed in the form of dissimilarity matrices ("distance apart" for sample composition), leading to a consistent analytic framework that will allow answer the question without requiring the data to conform to particular distributions or assumptions (Goslee and Urban, 2007). The simple Mantel statistic is effectively the correlation between two dissimilarity matrices. This is a normalized version of the original Mantel statistic (Mantel, 1967). The hypothesis of a Mantel test is that the degree of dissimilarity in one dataset corresponds to the degree of dissimilarity in another independently-derived dataset (Goslee and Urban, 2007). Since the Monte Carlo value obtained for the test gave a non-significant value, it holds true that the similarities and dissimilarities between CCP and DCP are the same.

Both, the related samples sign test and the related samples Wilcoxon signed rank test resulted in a non-significant estimates of 0.162 and 0.092 respectively, the null hypothesis and that the Mantel test for dissimilarity between corresponding CCP and DCP are non-significant, it is save to conclude that although the corresponding CCP and DCP are different in terms of value, they are the same. Furthermore, the related samples Friedman's Two Way Analysis of Variance by Ranks and the Kendall's coefficient of concordance gave also non-significant values of 0.128 and 0.128 respectively; the null hypothesis that the corresponding CCP and DCP are different was rejected.

VI. CONCLUSION

Feed manufacturing involves the processing of mixtures of feedstuffs and feed additives into a usable form to increase profits of animal production by maximizing the nutritional value of a feedstuff or a mixture of feedstuffs. Nutrient requirements as established by research conducted at various agencies are continually being used as the basis for feed formulations. This study has shown that the estimates of calculated crude protein composition as being used currently are similar to chemically determined composition of crude protein.

Therefore, where running a chemical analysis of feedstuffs or diet is not possible in required time-frame, the calculated composition is a good alternative.

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