Archives of Veterinary Science v. 9, n. 2, p. 73-79, 2004
Printed in Brazil
ISSN: 1517-784X

APPLICATION OF KJELDAHL AND DUMAS COMBUSTION METHODS FOR NITROGEN ANALYSIS

(Aplicação dos métodos de Kjeldahl e de Dumas para análise de nitrogênio)

SADER, A.P.O.[1]; OLIVEIRA, S.G.[2]; BERCHIELLI, T.T.[3]

[1]Química do Laboratório de Nutrição Animal, FCAV/Unesp - Jaboticabal, SP;
[2] Programa de Pós-graduação em Zootecnia, FCAV/Unesp - Jaboticabal, SP;
[3] Professora do Departamento de Zootecnia - FCAV/Unesp - Jaboticabal, SP, Pesquisadora do CNPq.

ABSTRACT – The Kjeldahl procedure has been replaced by Dumas combustion procedure for total N determination in feeds. These methods were compared using routine samples in the animal nutrition laboratory (concentrates, forages, excreta and duodenal content). NO_3-N covariate interfered on total N determination according to the procedure used. A significant correlation for crude protein (CP) content was observed between methods used for each group: grass silage component, Kjeldahl-N = 1.1661 + 0.9216 Dumas-N (R^2=0.99); energy feeds, Kjeldahl-N = 0.6376 + 0.9445 Dumas-N (R^2=0.72) and protein feeds, Kjeldahl-N = 6.5638 + 0.8615 Dumas-N (R^2=0.97). Means for total N contents from components, adjusted by covariance, were not different (P > 0.05) between both methods. Considering that the standard deviation for N content obtained by the two methods stayed within the intervals observed in the literature, corroborated by the fact that Dumas combustion procedure using the analyzer LECO-FP 528 LC is not harmful to the environment it was concluded, that this method can replace with advantages the Kjeldahl procedure in routine animal nutrition N analyzes.

Key words: Duma, Kjeldahl, Leco, Nitrogen.

RESUMO – O método padrão de Kjeldahl vem sendo substituído pelo método de combustão Dumas na determinação do nitrogênio total (NT) em alimentos. Estes procedimentos foram utilizados para avaliar amostras de rotina em laboratórios de nutrição animal (concentrados, forragens, excreta e conteúdo duodenal). A covariável nitrato (N-NO_3) interferiu na determinação do NT em função da metodologia empregada. Foi observada uma correlação positiva entre os métodos utilizados para cada grupo avaliado: ingredientes volumosos, Kjeldahl-N = 1.1661 + 0.9216 Dumas-N (R^2=0.99); alimentos energéticos, Kjeldahl-N = 0.6376 + 0.9445 Dumas-N (R^2=0.72) e alimentos protéicos, Kjeldahl-N = 6.5638 + 0.8615 Dumas-N (R^2=0.97). As médias para NT dos alimentos, ajustadas para a covariável, não diferiram entre os métodos (P > 0,05). O desvio padrão do teor de N dentro e entre os métodos utilizados estão dentro dos intervalos permitidos pela literatura, o que, associado ao fato da metodologia de Dumas consistir em uma técnica não poluente ao ambiente, indica ser esta metodologia capaz de substituir com vantagens o procedimento de Kjeldahl em análises de rotina em laboratórios de nutrição animal.

Palavras chave: Dumas, Kjeldahl, Leco, Nitrogênio.

Introduction

With the improvement in dry combustion nitrogen (N) analyzer technology and because of the expenses of disposing of hazardous laboratory waste chemicals, the Dumas method is replacing the traditional Kjeldahl method as the method of choice for N analysis. Consequently, comparison of the Dumas method with the Kjeldahl method is important (WATSON and GALLIHER, 2001). The Dumas method is rapid, simple (Van den NEUCKER *et al.*, 2002) and readily performed method permits doing many tests daily, using up a small amount of the sample and dispensing with the use of noxious chemicals (SEBECIC and

Correspondências para: Prof. Telma Teresinha Berchielli - Departamento de Zootecnia - Via de Acesso Prof. Paulo Donato Castelanne, s/n - Jaboticabal – SP - CEP 14884-900.

BALENOVIC, 2001; FRANCINI *et al.*, 2003).

These methods are to be based on an oxidative combustion and the samples are combusted with oxygen at approximately 900°-1200°C. The developing gas mixture is cleaned from disturbing substances and than the nitrogen oxides, containing in the gas mixture, are reduced to molecular nitrogen. Afterwards the content of nitrogen is measured with a heat conductivity detector (BUSCHMANN-FELLBACH and WESTPHAL, 2001; SAINT-DENIS and GOUPY, 2004). The Dumas method is well suitable for solids and liquids.

The standard Kjeldahl method to determine total N has been used for a great number of applications. However, one of the limitations recognized for the standard Kjeldahl to analyze N is the inability to quantify NO_3-N (Nitrate-N) which can be a significant fraction of the total N in soil and plants samples (SILVERTOOTH and WESTERMAN, 1988). MCGEEHAN and NAYLOR (1988) confirmed that the Kjeldahl nitrogen values for materials containing nitrates were lower than values obtained using combustion techniques. In general, the combustion process determines slightly higher nitrogen values than wet oxidation techniques when analyzing plant material (SIMONNE *et al.*, 1995; ETHERIDGE *et al.*, 1998). JUNG *et al.* (2003) found that the Kjeldahl method gave slightly, but significantly, lower values than did the Dumas method, but the difference between the methods depended on the type of foodstuff (THOMPSON *et al.*, 2002). Otherwise, BLONDEL and VIAN (1993) working with human albumin and immunoglobulin at different concentrations, found no significant differences between methods. Nitrogen values obtained for soil by the Kjeldahl analysis also were lower than values obtained through the combustion process. WANG *et al.* (1993) concluded that the Kjeldahl procedure was more precise than the combustion technique on finely ground samples, recommending that more research data are needed on soils to determine which procedure is the most accurate.

COSGROVE *et al.* (1985) confirmed that nitrate-N can represent a large fraction of N in vegetables fertilized with NO_3 and NELSON and SOMMERS (1980) considered that some nitrates are not recovered due to the incomplete breakdown of N-O bonds during digestion, but nitrate-N recovery during automated combustion method is total and does not involve solvents. Therefore, many authors confirmed that the Dumas combustion procedure could replace the Kjeldahl method for crude protein analysis in plants. However, either method recovers different N fractions.

SIMONNE *et al.* (1994) and ETHERIDGE *et al.* (1998) concluded that results for N content by Dumas method were significantly higher than by Kjeldahl's. However, for Dumas-N procedure the coefficient of variation for standard AAFCO (The Association of American Feed Control Officials) cattle and broiler feeds, measured repeatedly over an 18-month period, were 2.23 (n= 90) and 2.12 g/100g (n=177), respectively (ETHERIDGE *et al.*, 1998). In conclusion, Dumas combustion procedure may replace Kjeldahl procedure for routine analysis in animal nutrition laboratory. However, ETHERIDGE *et al.* (1998) did not describe the group of cattle and broiler feeds studied.

Dumas combustion method has been adapted for several applications to determine N since the method does not cause environmental damage and gives accurate and faster results (SIMONE *et al.*, 1995; MATEJOVIC, 1997; ETHERIDGE *et al.*, 1998). However, little information is available on the influence of plant N status on Kjeldahl-N and Dumas-N determination.

The objective of this work was to compare analytical methodologies, Kjeldahl and Dumas methods, using routine samples in animal nutrition laboratory.

Materials and Methods

Samples analyzed: Conventional feeds samples from an animal nutrition laboratory used for these analyzes were dried at 65° C in a forced air oven, after which they were milled through a 20-mesh screen. Samples used in this experiment were previously divided into four different groups, based on Morrison's classification (MORRISON, 1966): Energy

feeds: corn grain, citrus pulp, manioc peel + pulp silage (MPSP), manioc scraps + pulp silage (MSSP) and wheat meal; protein feeds: fish meal, viscera meal, soybean meal, cottonseed meal and poultry litter + pulp; Grass silage: sugar cane, sugar cane + pulp silage (SCSP), coastcross hay (*Cynodon dactylon*), corn silage (CS), tifton hay (*Cynodon* spp.), jurema black (*Mimosa tenuiflora*), mororó *(Bauhinia cheilantha*) and sabiá (*Mimosa caesalpinifolia*); digest and excreta components: duodenum SCSP, feces SCSP, duodenal MPSP, feces MPSP, duodenum CS, feces CS, duodenal MSSP and feces MSSP.

Methodologies for crude protein analysis: Crude protein analysis (total N) was conducted by micro Kyeldahl (AOAC, 1990) and Dumas combustion methods. To perform analysis by combustion procedure, 0.1g sample feed was transferred to a tin container after which into a combustion chamber (850° C) utilizing an automated reading (FP 528 LC, Leco). The mixture of gases released during combustion in this method was catalytically converted to N_2 quantitative by passing the gas through a conductivity cell.

Determination of nitrite and nitrate content as total nitrite: Total nitrite content determination of the samples was conducted in a manual spectrophotometer HACH, DR/ 2000 model, software 3.0, method no. 8151 for plants, considering 0.5g homogenate being mixed to 100 ml/min, deionizated water, after 25ml was used for reading NO_3-N, added of NitraVer® reagent (HACH Kit), reading at 500 nm using a specific software for NO_3-N, after 5 minutes.

Statistical analysis: Each feed group corresponded to an experiment which was randomly distributed, in a factorial scheme, feeds number (N) x methodologies for analysis of crude protein (2) with 3 replications. One analysis of covariance was performed to each experiment where total N content was used as covariate considering the Dumas method as capable of recovery reading of NO_3-N. Statistical analyzes were performed using PROC GLM and PROC REG (SAS, 1996). Regression analysis were performed to characterize the effect of Dumas method as a function of the Kjeldahl method for different sample components group, being: Kjeldahl-N = b_o + b_i Dumas-N where Kjeldahl-N = nitrogen determined by the Kjeldahl method, b_o = intercept, b_i and Dumas-N = nitrogen determined by the Dumas method.

Results and Discussion

The reproducibility of the Dumas method has been reported to be 0.1% N for concentrations below 0.7% N and 4% relative above 0.7% N (GISLUM *et al*., 2004). SAINT-DENIS and GOUPY (2004) found that the Dumas method for the quantitative determination of organic nitrogen was at least as precise as the Kjheldal method, but considerably faster, which makes it of great interest for research and industrial applications.

Crude protein contents, despite having some components displaying higher nitrate values, did not differ between methods employed for some components (TABLE 1), suggesting that NO_3-N was not recovered by the Kjeldahl method due to incomplete breakdown to N- N and N-O bonds during digestion, as well as for other components; NO_3-N could be almost all recovered, as previously mentioned by NELSON and SOMMERS (1980).

For all the feeds studied, regression analysis showed that NO_3-N content (covariate) interfered on crude protein content due to the method employed. After adjustment to NO_3-N there was no effect for methodology ($P>0.05$), as obtained by SIMONNE *et al.* (1994).

Differences were not found among the components used, except for wheat meal ($P<0.05$). SIMONNE *et al.* (1994) showed that N content determined by Kjeldahl and Dumas were affected by N present on plants (TABLE 2). Regression analysis of the CP content indicated a significant correlation for either method, adjusted for nitrate ($P<0.05$): $Y = 0.6376 + 0.9445X$ ($R^2 = 0.7187$).

TABLE 1 – NO_3-N AND CP CONTENTS OF COMPONENTS (FEEDS) FROM A ROUTINE ANIMAL NUTRITION LABORATORY AS DETERMINED BY THE KJELDAHL (CP_K) AND DUMAS COMBUSTION (CP_D) METHODS. (%).

Components	CP_K (±SD)	CP_D (±SD)	N-NO_3 (±SD)
Energy feeds			
Corn grain	8.51 ± 0.27	8.39 ± 0.11	0.11 ± 0.01
Citric pulp	6.73 ± 0.15	7.08 ± 0.14	0.38 ± 0.01
Silage (peel + pulp)	5.68 ± 0.10	6.25 ± 0.02	0.00 ± 0.00
Silage (scrap + pulp)	4.39 ± 0.05	5.04 ± 0.04	0.00 ± 0.00
Wheat meal	15.20 ± 0.01	15.49 ± 0.10	0.19 ± 0.01
Protein feeds			
Poultry litter + pulp	18.83 ± 0.64	18.70 ± 0.47	0.36 ± 0.04
Fish meal	60.00 ± 0.44	62.56 ± 1.09	0.27 ± 0.03
Viscera meal	52.20 ± 1.25	54.58 ± 0.72	1.15 ± 0.10
Soybean meal	44.38 ± 0.62	46.18 ± 0.44	0.14 ± 0.01
Cottonseed meal	46.77 ± 0.68	48.38 ± 0.15	0.34 ± 0.01
Roughage			
Sugar cane	1.70 ± 0.01	2.18 ± 0.01	0.11 ± 0.01
Corn silage	7.04 ± 0.15	7.97 ± 0.14	0.16 ± 0.03
Silage (sugar cane + pulp)	4.63 ± 0.09	6.03 ± 0.17	0.01 ± 0.00
Coastcross hay	6.40 ± 0.01	7.02 ± 0.09	0.22 ± 0.03
Tifton hay	14.87 ± 0.12	15.76 ± 0.29	0.25 ± 0.04
Jurema preta	15.87 ± 0.29	17.06 ± 0.12	0.44 ± 0.01
Mororó	16.87 ± 0.35	18.13 ± 0.13	0.44 ± 0.01
Sabiá	19.73 ± 0.15	20.38 ± 0.13	0.51 ± 0.02
Digest and excreta			
Silage (sugar cane +pulp) duodenal	16.55 ± 0.19	17.66 ± 0.18	0.01 ± 0.00
Silage (sugar cane + pulp) feces	9.79 ± 0.17	10.57 ± 0.21	0.14 ± 0.00
Silage (sugar cane +pulp) duodenal	16.42 ± 0.24	16.70 ± 0.10	0.03 ± 0.01
Silage (sugar cane + pulp) feces	10.03 ± 0.25	10.67 ± 0.14	0.15 ± 0.01
Corn silage (duodenal)	20.83 ± 0.06	21.37 ± 0.05	0.00 ± 0.00
Corn silage (feces)	11.56 ± 0.27	12.53 ± 0.54	0.13 ± 0.01
Silage (scrap + pulp) duodenal	20.17 ± 0.24	20.55 ± 0.57	0.01 ± 0.01
Silage (scrap + pulp) feces	10.62 ± 0.27	12.15 ± 0.04	0.11 ± 0.01

TABLE 2 – CRUDE PROTEIN CONTENT OF ENERGY FEEDS AS DETERMINED BY THE KJELDAHL (CP_K) AND DUMAS COMBUSTION (CP_D) METHODS. (%).

Components	CPK (± SD)	CPD (± SD)
Corn grain	7.95 ± 0.27	8.52 ± 0.11
Citric pulp	6.70 ± 0.15	7.88 ± 0.13
Silage (peel + pulp)	8.13 ± 0.09	7.47 ± 0.02
Silage (scrap + pulp)	7.69 ± 0.05	7.03 ± 0.04
Wheat meal	10.43* ± 0.01	10.92* ± 0.09

Means adjusted by NO_3-N.
Means in the same row followed by * differ ($P < 0.05$).

Crude protein values for protein feeds obtained for either Kjeldahl or Dumas procedures, adjusted by covariate NO_3 -N are presented in TABLE 3. Covariance analysis showed that NO_3-N interfered on CP content on protein feeds group. After adjustment for this covariate, were not verified effects of methodologies and interaction effect on methods among feeds (P>0.05). Otherwise, a high correlation for CP content was observed between methods analyzed (P<0.01): Y=6.538 + 0.815X (R^2 =0.9699).

TABLE 3 – CRUDE PROTEIN CONTENTS OF PROTEIN FEEDS AS DETERMINED BY THE KJELDAHL (CP_K) AND DUMAS COMBUSTION (CP_D) METHODS, ADJUSTED FOR NO_3-N. (%).

Components	CPK (±SD)	CP D (±SD)
Poultry litter + pulp	35.37 ± 0.64	33.88 ± 0.47
Cottonseed meal	46.33 ± 0.68	46.65 ± 0.15
Fish meal	51.52 ± 0.44	53.07 ± 1.09
Soybean meal	45.39 ± 0.62	45.42 ± 0.44
Viscera meal	48.46 ± 1.25	46.47 ± 0.72

Means adjusted by NO_3-N covariate.

Also, in roughage feeds (TABLE 4), NO_3-N covariate interfered on results due to the methods used to analyze CP, annulling the effect of methods after adjustment for covariate. The results obtained for interaction among feeds, only CP content for silage (poultry litter + pulp) for Kjeldahl-CP different Dumas-CP after adjustment by covariate. Regression analysis showed high correlation between Kjeldahl and Dumas crude protein content: Y = 1.1661 + 0.9216X, R^2 = 0.9883 (P<0.01).

TABLE 4 – CRUDE PROTEIN CONTENTS OF ROUGHAGE COMPONENTS AS DETERMINED BY KJELDAHL (CP_K) AND DUMAS COMBUSTION (CP_D) METHODS, ADJUSTED FOR NO_3-N. (%).

Components	CPK (± SD)	CPD (± SD)
Sugar cane	5.35 ± 0.01	± 0.01
Coastcross hay	8.34 ± 0.01	8.46 ± 0.09
Jurema Preta)	14.36 ± 0.29	14.54 ± 0.12
Mororó	14.99 ± 0.35	15.25 ± 0.13
Sabiá	16.82 ± 0.15	16.29 ± 0.13
Silage (poultry litter + pulp)	7.22* ± 0.09	8.59* ± 0.17
Corn silage	8.75 ± 0.15	9.32 ± 0.14
Tifton hay	13.72 ± 0.12	14.04 ± 0.29

Means adjusted by NO_3-N covariate.
Means in the same row followed by * differ (P < 0.05).

Analyzing the use of these methods for measurement of total N from digest it has been observed that although there was an effect of covariate (P<0.01), the methods still differed between them even after adjustment for NO_3-N (TABLE 5).

A high correlation between Kjeldahl-CP and Dumas-CP concentration was also observed for digest components (P<0.01), where Y = 1.4915 + 0.8718X (R^2 = 0.9156).

Based on these results, it was observed that NO_3-N content from feeds can interfere on the determination of crude protein and an adjusting factor should be used on determination of crude protein content according to the group of feeds when Dumas combustion procedure is to estimate CP content from the Kjeldahl. According to MARCÓ *et al.* (2002), in relation to the automation capabilities, Dumas is clearly advantageous over Kjeldhal because once the auto sampler is filled, the combustion analyzer can work completely unattended. On the contrary, for Kjeldhal some manual steps are necessary (addition of the reagents for the digestion, dilution after digestion and placing of digestion tubes in the distillation unit). Kjeldhal uses hazardous

reagents such as sulfuric acid, sodium hydroxide solution, and heavy metals as catalysts, compounds which require a suitable waste management system. On the contrary, in the case of Dumas, the amount of heavy metals per analysis is considerably lower.

TABLE 5 - CRUDE PROTEIN CONTENTS OF BOVINES DIGEST COMPONENTS BY KJELDAHL (CP_K) AND DUMAS COMBUSTION (CP_D) METHODS, ADJUSTED FOR NO_3-N. (%).

Digest components	CPK (±SD)	CP D (±SD)
Silage (peel + pulp) duodenal	15.20 ± 0.24	15.34 ± 0.10
Silage (peel + pulp) feces	13.42 ± 0.25	13.37 ± 0.14
Silage (sugar cane + pulp) duodenal	15.23* ± 0.19	16.30* ± 0.18
Silage (sugar cane + pulp) feces	13.35 ± 0.17	13.50 ± 0.21
Corn silage duodenal	16.43 ± 0.06	16.95 ± 0.05
Corn silage duodenal	13.85 ± 0.27	14.22 ± 0.54
Silage (scraps + pulp) duodenal	16.25 ± 0.24	16.58 ± 0.57
Silage (scraps + pulp) feces	13.58* ± 0.27	14.61* ± 0.04

Means adjusted by NO_3-N covariate; Means in the same row followed by * differ ($P < 0.05$).

Conclusions

Standard deviation for and between methodologies used were in the interval allowed according to literature and also considering that the Dumas procedure does not utilize hazardous materials as well mineral as catalysts, being considered a safe technique to the environment, it was concluded that the Dumas procedure, using the analyzer LECO-FP 528 LC, can replace with advantage the Kjeldahl procedure in an animal nutrition laboratory analyzes. More studies should be conducted utilizing other ingredient groups in order to evaluate correlations between methods.

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Recebido para publicação: 24/05/2004
Aprovado: 30/10/2004