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Difference of nitrogen contents determined by the combustion and Kjeldahl method in response to nitrate nitrogen in some feedstuffs*

W.S. Guo, L.P. Ren, Z.M. Zhou and Q.X. Meng[1]

State Key Laboratory of Animal Nutrition, College of Animal Science & Technology, China Agricultural University Beijing 100094, P.R.. China

ABSTRACT

Two experiments were conducted to examine the difference of nitrogen contents determined by the combustion (Cm) and Kjeldahl (Km) method and its response to nitrate nitrogen in ruminant feedstuffs. In Experiment 1, 14 ruminant feedstuffs were determined for combustion nitrogen (Cn), Kjeldahl nitrogen (Kn) and nitrate nitrogen (NO_3-N). Although NO_3-N resulted in a difference between the Cm and Km, the correlation coefficient of the NO_3-N content and the value of "Cn-Kn" was low ($R^2 = 0.6341$), suggesting other factors influencing the difference of N determination between the Cm and Km. In Experiment 2, recoveries of net NO_3-N were determined using Chinese wild rye-grass (CWG), maize grain (MG) and soyabean meal (SBM) supplemented with sodium nitrate at the level of 5, 10, 15, 20, 25, 30, 35, 40 and 45% on DM basis, respectively. The results showed that different recoveries of NO_3-N by Km rather than Cm would account for the difference of Cn and Kn in some ruminant feedstuffs.

KEY WORDS: crude protein, nitrate nitrogen, Kjeldahl method, combustion method

INTRODUCTION

Many researchers have compared N contents of feedstuffs determined by the combustion (Cm) and Kjeldahl (Km) methods (Koenig, 1991; Jakob et al., 1995). The results revealed a high linear correlation (0.992~0.999) of N contents for the majority of feedstuffs between the two methods. However, when the feedstuff is rich of NO_3-N, the higher N content was obtained from Cm than Km (Watson and

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[1] Corresponding author: e-mail: qxmeng@cau.edu.cn

Galliher, 2001). Simonne et al. (1997) stated that Cm and Km may recover different forms of N in plant tissues respectively, e.g., nucleic acids N and NO_3-N. Afterward, Simonne et al. (1998) further demonstrated that under a wide range of NO_3-N in 130 leaf samples, NO_3-N alone did not account for the difference between Cm and Km.

When we routinely determined the N content of ruminant feedstuffs including forages and vegetables, Cn contents are always higher than Kn, regardless of their NO_3 contents. This observation suggests that there may exist other factors responsible for the difference of N contents determined between Cm and Km. Therefore, the present study was conducted to: 1. prove if the NO_3-N alone can account for the difference between Cm and Km in ruminant feedstuffs, and 2. explore the factors influencing the difference of N determination between the two methods, when incremental amounts of NO_3-N were included in the feedstuffs.

MATERIAL AND METHODS

Two experiments were conducted in this study. In Experiment 1, 14 feedstuff samples were selected for determination of N contents: rye-grass, Chinese wild rye-grass (CWG), perfoliate rosin-weed, awnless bromegrass, lucerne, India lettuce, crested wheat-grass, dahuria lyme-grass, sorghum hybrid Sudan-grass, maize stalks, wheat straw, rice straw, grass and cabbage. All samples were dried in a forced-air oven at 70°C for 72 h and ground in a vortex mill (0.5 mm sieve, Perten Laboratory Mill 3100). The Kjeldahl-N (Kn), combustion-N (Cn) and nitrogen nitrate (NO_3-N) content were then determined for each sample. In Experiment 2, ten incremental levels of sodium nitrate (S5506, Sigma-Altrich) were mixed with Chinese wild rye-grass (CWG), maize grains (MG) or soyabean meal (SBM) to form the sodium nitrate concentration of 5, 10, 15, 20, 25, 30, 35, 40 and 45 g/100 g dry matter. Nitrogen contents of the samples were determined by the procedure of AOAC (2000) for Kn with the N Analyzer (Foss Model 2300, Sweden) and by the procedure of AOAC (990.03) for Cn with the N Analyzer (Rapid N III, Elementar, Germany). All results were expressed in g N/100 g dry matter (%). Recovery of NO_3-N in three serial mixed samples in Experiment 2 was calculated based on the assumption that all ammonia-N was totally captured into Kn.

In order to determine the NO_3-N, 1 g sample was weighed into a beaker with 50 ml deionized water, then mixed for 1 h and filtered. NO_3-N in solution was then measured by the procedure of Jones and Case (1990) using a spectrophotometer (UV-VIS 8500, Shanghai Tianmei Scientific Instrument Co., Ltd., China).

The N content of feedstuffs was analysed as a single factor design using the GLM procedure of SAS (2003). Simple linear correlation analysis and significance test of the coefficient were performed on N according to the procedure described by Steel and Torrie (1960). Analysis of correlation between NO_3-N and Cn - Kn was evaluated

using regression analysis procedure of SAS (2003). The recovery of NO_3-N in samples was evaluated with an analysis of variance using the GLM procedure of SAS (2003).

RESULTS AND DISCUSSION

Nitrogen (N) contents of 14 nitrate-contained feedstuffs were determined by both Cm and Km (Table 1; Experiment 1). Higher N content was obtained for Cm compared with Km for all feedstuffs ($P<0.05$; CV<5%; Cn: Kn=1.04~1.21). These results were in agreement with the observations of Simonne et al. (1997), who found that sulphuric acid could convert all protein N and only part of NO_3-N into ammonium N. In contrast, when the sample contained substantial NO_3-N, Cm provided a higher N measurement (Watson and Galliber, 2001). In the present study, although a linear correlation ($r=0.9960$) of N contents of 14 feedstuffs between the two determination methods as shown in Figure 1, the slope of the regression equation was significantly ($P<0.01$) different from that of $Y=X$, suggesting other forms of N (e.g., nitrate N) not only ammonium N included in the feedstuffs. In order to prove the nitrate N responsible for the difference of N measurements between the two methods, we made a correlation between nitrate nitrogen (NO_3-N) content

Table 1. Nitrogen contents (%DM) of 14 feedstuffs as determined by the combustion and Kjeldahl method (Experiment 1)

Feedstuffs	Determination method						
	Cn	CV[2]	Kn	CV	C/K	SEM	P
Maize stalk[1]	1.05[a]	0.34	0.93[b]	2.06	1.13	0.010	0.012
Chinese wild rye-grass	1.04[a]	0.23	0.94[b]	1.53	1.10	0.007	0.012
Wheat straw	0.72[a]	1.65	0.59[b]	1.19	1.21	0.007	0.006
Rice straw	0.90[a]	0.41	0.74[b]	0.69	1.21	0.003	0.001
Grass	3.72[a]	0.07	3.58[b]	0.07	1.04	0.002	0.000
Rye-grass	2.25[a]	0.33	1.98[b]	1.94	1.14	0.020	0.010
Lucerne	4.58[a]	0.42	4.03[b]	0.59	1.05	0.004	0.000
Perfoliate rosin-weed	3.32[a]	0.14	3.16[b]	0.10	1.14	0.012	0.010
Awnless brome-grass	3.34[a]	0.36	3.12[b]	1.31	1.07	0.021	0.017
Crested wheat-grass	1.11[a]	0.57	1.03[b]	1.72	1.07	0.009	0.030
Dahuria lyme-grass	1.30[a]	0.43	1.26[b]	0.10	1.04	0.003	0.008
Sorghum hybrid Sudan-grass	1.74[a]	0.12	1.61[b]	0.94	1.08	0.008	0.008
India lettuce	3.17[a]	0.24	2.70[b]	0.38	1.17	0.006	0.000
Cabbage	3.41[a]	0.33	3.00[b]	0.92	1.14	0.015	0.003

[1] [a,b] means within a line with a different superscript letter differ ($P<0.05$); [2] CV - variance coefficient; [3] Cn - combustion nitrogen; Kn - Kjeldahl nitrogen

and Cn-Kn for 14 feedstuffs (Figure 2). No significantly linear correlation was obtained between the NO_3-N contents and Cn-Kn (R^2=0.6341; Figure 2), suggesting that NO_3-N alone does not account for the difference between the two methods. This observation coincides with the result of Simonne et al. (1998), who found not only nitrate N but other factors, such as the nucleic acid, the matrix effect or a combination of both, may explain these differences in vegetable leaves. Further studies are required to prove the validity of this hypothesis.

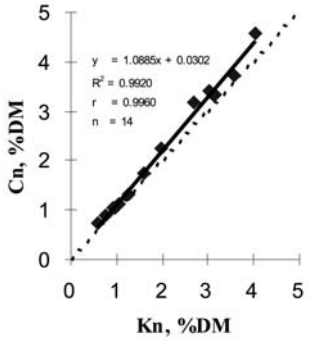

Figure 1. Relation of N contents of 14 feedstuffs as determined by the Cm and Km (Experiment 1)

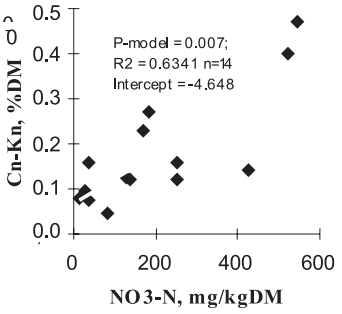

Figure 2. Relation between NO_3-N contents and Cn - Kn in 14 feedstuffs (Experiment 1)

As shown in Tables 2 and 3 (Experiment 2), the recovery of net NO_3-N of 3 nitrate contained feedstuffs determined by Cm ranged from 89.2 to 100.5%, whereas the range of Km was from 34.2 to 54.9%. The Cm can well capture

Table 2. The recovery of nitrate nitrogen in 3 feedstuffs determined by Kjeldahl method[1] (Experiment 2)

Addition level DM %	Kjeldahl method			SEM	P
	CWG	CG	SBM		
5	34.2^{b}	54.9^{a}	54.3^{a}	1.94	0.008
10	34.6^{b}	51.9^{a}	53.2^{a}	1.74	0.008
15	34.6^{b}	51.3^{a}	52.9^{a}	0.92	0.001
20	38.2^{c}	50.3^{a}	53.5^{a}	0.61	0.000
25	38.0^{c}	50.3^{b}	53.7^{a}	0.65	0.000
30	37.0^{b}	48.9^{a}	50.6^{a}	1.52	0.014
35	35.2^{b}	46.6^{a}	53.4^{a}	1.87	0.014
40	36.1^{b}	47.2^{a}	47.4^{a}	0.52	0.001
45	36.4^{c}	48.0^{a}	43.9^{b}	0.33	0.000

[1] means within the same line with different superscript letters differ (P<0.05)

[2] no differences were obtained between NO_3-N addition levels (P>0.05)

[3] CWG - Chinese rye-grass; MG - maize grain; SBM - soyabean meal

Table 3. The recovery of nitrate nitrogen in 3 feedstuffs determined by combustion method[1] (Experiment 2)

Addition level DM %	Combustion method			SEM	P =
	CWG	MG	SBM		
5	89.2[b]	97.8[a]	98.5[a]	1.78	0.034
10	89.4[b]	98.3[a]	98.2[a]	1.05	0.012
15	88.3[b]	99.1[a]	100.3[a]	0.82	0.004
20	91.8[b]	98.4[a]	98.8[a]	0.32	0.001
25	91.9[b]	98.9[a]	100.1[a]	0.49	0.002
30	95.4[c]	98.7[b]	100.4[a]	0.28	0.002
35	95.0[c]	98.9[b]	100.5[a]	0.29	0.002
40	92.8[c]	97.4[b]	100.3[a]	0.55	0.006
45	94.4[b]	97.5[a]	99.5[a]	0.49	0.011

[1] means within the same line with different superscript letters differ ($P<0.05$)
[2] no differences were obtained between NO_3 N addition level ($P>0.05$)
[3] CWG - Chinese rye-grass; MG - maize grain; SBM - soyabean meal

nitrate N from the feedstuffs, with an acceptable N recovery range from 89.2 to 100.5%. However, Km not only partially captures the nitrate N reflected by the lower nitrate recoveries, but also the captured amount of nitrate N was varied with the feedstuff source because of significantly different ($P<0.05$) recoveries obtained among the different feedstuffs (Table 2). Simonne et al. (1998) stated that besides nitrate N, nucleic acid N may also be accountable for the difference between Cn and Kn of vegetable leaves. However, when we determine the N content of yeast products rich of nucleic acids using Cm and Km, there was no difference obtained. The different recoveries of net NO_3-N for Km was likely attributed to certain intrinsic substances (e.g., high concentration of lipids, lipoproteins, etc.) within the biological samples that resulted in their incomplete mineralization for mixtures of sulphuric acid. Based on these observations, different captures of nitrate N in Km rather than Cm would be responsible for the difference of Cn and Kn in some ruminant feedstuffs.

CONCLUSIONS

The combustion method is superior to the Kjeldahl method when the feedstuff contains significant quantities of nitrate nitrogen. Nitrate nitrogen alone does not account for the difference between the two methods. Low and different nitrate captures of feedstuff sources determined by Kjeldahl method rather than combustion method would be accountable for the difference between combustion and Kjeldahl nitrogen in some ruminant feedstuffs.

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