THE EFFECT OF GLUCOSINOLATES ON THE NITROGEN TO PROTEIN RATIO FOR RAPESEED AND CANOLA. (1)

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Abstract

The nitrogen to protein ratio for varieties of *Brassica* seeds was found to vary with the glucosinolate content. A value of about 5.49 was estimated for varieties of *Brassica napus* and *Brassica rapa* with low levels of glucosinolate while a value of 5.39 was estimated for varieties with high levels of glucosinolates. Both of these values were lower than the more commonly accepted value 5.53. The difference between varieties with high and low levels of glucosinolates could be attributed to the lower amounts of non protein nitrogen in the former while the difference between current and previous values was likely due to the change from the Kjeldahl to the Dumas method for estimating nitrogen in the seed.

Introduction

It has been recognized since at least the 1930's that the factor 6.25 which is commonly used to convert nitrogen to ``crude" protein does not give an accurate estimate of true protein content . The factor 6.25 was derived from early work on proteins of animal origin found to contain about 16% nitrogen. Plant materials have been shown to have a different ratio and the factor 5.7 is commonly used for wheat based on work by . Jones pointed out that different plant proteins have different amounts of nitrogen but even he neglected to consider the effect of nitrogen from sources other than protein. An accurate knowledge of the N to P ratio would be useful both for animal nutritionists who might use the information in formulating diets and for those attempting to prepare protein isolates.

While early workers relied on the purification of proteins followed by a nitrogen determination on the purified product, followed up on earlier work by and used amino acid compositional data to estimate the nitrogen to protein ratio for a number of grains and oilseeds. Since that time, this approach has become the usual approach for determination of the nitrogen to protein ratio for grains, oilseeds and pulses. Tkachuk also pointed out that his recovery of nitrogen from amino acids was usually less than 100 percent and he suggested that the difference was due to the presence of non-protein nitrogen which was determined by the Kjeldahl procedure for total nitrogen but not in the amino acid analysis. He used the recovery factor to estimate the true nitrogen to protein ratio but this method has not been universally accepted because it is difficult to show that 100% of the amino acids in a sample are estimated in the analytical method.

defined two factors for nitrogen to protein, KA based on the ratio of total amino acid nitrogen to the total amount of amino acids and Kp which is based on the ration of total amino acid nitrogen to the total nitrogen in the sample. He also pointed out the difficulty in determining general factors which could be accurately applied since both KA and Kp amino acid composition has been shown to vary with total protein content of the seed and in the case of Kp the non-protein nitrogen component of the total nitrogen content is also variable.

Tkachuk proposed a factor of 5.53 for rapeseed meal without any correction for non-protein nitrogn and this factor has been generally accepted up to now . Several significant changes have taken place since Tkachuk's original estimate published in 1969. Firstly, the Kjeldahl method for determining nitrogen has generally given way to the Dumas combustion method. This latter method gives a higher estimate of total nitrogen in canola by about 0.2% N, presumably by accounting for more of the non-protein nitrogen than the Kjeldahl method. This would result in a lowering of the nitrogen to protein ratio. At the same time, however, glucosinolates, a source of non-protein nitrogen in brassica seeds, have been reduced from over 100 mM per gram to less than 20 mM per gram of oil free flour. At 100 mM, glucosinolates account for about 0.2% nitrogen out of a total nitrogen content of about 6.5%. This reduction of glucosinolates would have a tendency to increase the nitrogen to protein ratio.

In order to verify and quantify the effect of glucosinolate reduction coupled with the change in analytical method for nitrogen determination, a series of oil-free flours prepared from seeds of *Brassica napus* L. and *Brassica rapa* L. (Table 1) with different levels of glucosinolate were tested for glucosinolate content and for amino acid composition.

Materials and Methods

Seeds of the varieties in Table 1 were obtained from certified seed growers or, for older varieties courtesy of Dr. D. I. MacGregor, Agriculture and Agri-Food Canada, Saskatoon, Dr. Baldur Stefansson and Dr. Rachel Scarth, University of Manitoba, Winnipeg. Except for amino acid analysis, AOCS methodology was used. The seeds were defatted according to AOCS Method Am 2-95 and the total nitrogen was determined by AOCS Method Ba 4e-93 and total glucosinolates by AOCS Method Ak1-92. Amino acids were determined as described by . Amino acid values were the means of duplicate determinations of duplicate hydrolysates. For determination of cystine and methionine, performic acid oxidation of the sample was carried out prior to hydrolysis.

Table 1. Varieties used in the study

Variety	Year	Glucosinolate	Erucic Acid	Type	Species	Special
Argentine	1942	Hi	Hi	Summer	B. napus	
Bronowski	1945	Lo	Hi	Summer	B. napus	
Midas	1973	Hi	Hi	Summer	B. napus	
Torch	1973	Hi	Hi	Summer	B. rapa	
Jet Neuf	1977	Hi	Lo	Winter	B. napus	
Tobin	1980	Lo	Lo	Summer	B. rapa	
Westar	1982	Lo	Lo	Summer	B. napus	
Arabella	1986	Lo	Lo	Winter	B. napus	
Stellar	1987	Lo	Lo	Summer	B. napus	Low Linolenic Acid
Colt	1988	Lo	Lo	Summer	B. rapa	
AC Parkland	1989	Lo	Lo	Summer	B. rapa	
Bounty	1990	Lo	Lo	Summer	B. napus	
AC Tristar	1990	Lo	Lo	Summer	B. napus	Triazine Tolerant
Mercury	1991	Lo	Hi	Summer	B. napus	

Results and Discussion

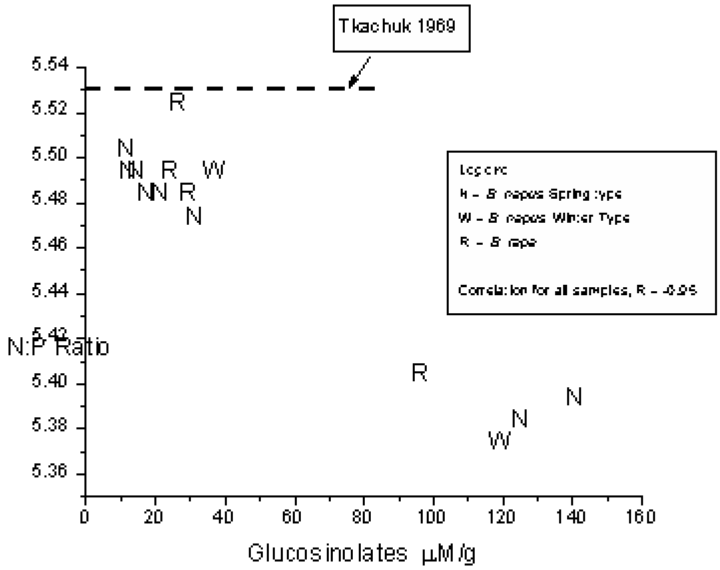

Figure 1. Relationship between Uncorrected nitrogen to protein ratios and glucosinolates.

Varieties used in this study profile varieties for the period of development of rapeseed from the high erucic, high glucosinolate types originally grown to today's low erucic, low glucosinolate types. The selection also included specialty varieties of high erucic, low glucosinolate type and low linolenic, low glucosinolate type. The variety Tristar, which is a herbicide tolerant mutation was also included. Amino acid composition and glucosinolate content of the varieties used in the study is found in tables 2 to 4.

Reduction of glucosinolates resulted in an increase in the uncorrected N:P ratio from an average of about 5.39 for high glucosinolate varieites to about 5.50 for low glucosinolate varieties (Figure 1). The value of 5.39 is lower than the value obtained by Tkachuk but that might be expected since the total nitrogen was determined using the combution method which gives higher results than the Kjeldahl method used originally.

Table 2. Correlations between glucosinolates and Amino Acid Compositional Data for varieties used in the study.

Factor	R	Prob.(1)	Factor	R	Prob.(1)
Glucosinolates	**1.000**	**0.000**	Cystine	0.469	0.091
Tryptophan	-0.126	0.669	Valine	-0.350	0.220
Lysine	0.085	0.772	Methionine	0.259	0.371
HIstidine	-0.084	0.775	Isoleucine	-0.530	0.051
Arginine	-0.365	0.200	Leucine	-0.521	0.056
Total Ammonia	**0.725**	**0.003**	Tyrosine	-0.437	0.118
Total Aspartic	-0.539	0.047	Phenylalanine	-0.406	0.150

acid					
Total Glutamic acid	-0.268	0.354	**Asparagine**	**0.775**	**0.001**
Corrected Aspartic acid	**-0.919**	**0.000**	Glutamine	0.484	0.079
Threonine	-0.308	0.284	% Protein	-0.461	0.098
Serine	-0.439	0.116	% Recovery	-0.122	0.679
Corrected Glutamic acid	**-0.919**	**0.000**	**"Uncorrected"**	**-0.952**	**0.000**
Proline	0.152	0.605	Corrected	-0.390	0.168
Glycine	-0.369	0.195			
Alanine	-0.267	0.356			

1. Prob. > |R| under H0: R=0, N=14.

It is notable that glucosinolates were also strongly correlated with total ammonia and with the aspartic and glutamic acid results corrected for ammonia as the amide part of glutamine and aparagine. Ammonia is likely to be a hydrolysis product of glucosinolates under acidic conditions such as those used in amino acid analysis. Incorporating the total ammonia into asparagine and glutamine likely result in incorrect estimations of these amino acids.

A further non-protein nitrogen compound likely to contribute ammonia to amino acid analysis of brassica seeds is sinapine, the choline ester of sinapic acid. It would be useful to confirm the effect of this component. Also, further study is required to determine the effect that protein level has on the amino acid and nitrogen to protein ratio for brassica varieties. Once these effects have been established, it may be possible to evaluate varietal differences in amino acid composition.

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Acknowledgements

Mrs. Shirley Lowe carried out amino acid analyses. Mr. Doug DeClercq carried out glucosinolate analyses.

Table 3 Amino Acids (g/100g N) in Canadian Varieties of *B. rapa*

	TORCH	TOBIN	COLT	PARKLAND
Tryptophan	7.6	7.2	7.0	7.3
Lysine	38.6	38.0	37.1	37.0
Histidine	17.1	16.4	15.6	16.3
Arginine	37.5	36.6	35.2	37.5
Total (1)				
Ammonia	14.7	13.2	12.2	13.1
Aspartic acid	44.5	45.9	45.7	46.5
Glutamic acid	110.0	103.9	101.6	108.5
Aspartic acid (2)	14.4	18.4	21.0	21.3
Threonine	27.8	28.9	27.8	27.7
Serine	26.8	27.2	26.0	27.0
Glutamic acid (2)	16.0	20.3	23.2	23.5
Proline	41.4	37.9	36.8	38.4
Glycine	31.0	30.8	29.8	30.8
Alanine	27.4	27.4	26.6	27.2
Cystine	17.0	15.0	14.9	14.6
Valine	35.2	35.2	34.6	35.2
Methionine	14.5	13.1	12.6	13.0
Isoleucine	27.7	27.4	27.1	27.6
Leucine	44.7	44.4	43.6	44.6

Tyrosine	17.2	18.5	17.9	18.0
Phenylalanine	25.2	24.7	24.8	25.1
Asparagine (2)	29.8	27.3	24.5	25.0
Glutamine (2)	93.4	83.0	77.8	84.4
Glucosinolates(3)	96.4	29.4	26.5	24.6
% Protein	40.4	37.7	40.5	44.0
% Recovery	93.2	90.1	87.2	90.4
N-to-P Factor:				
Uncorrected	5.40	5.48	5.52	5.49
Corrected	4.90	4.83	4.70	4.87

1. Assuming the sample contains only free aspartic acid and free glutamic acid.

2. Ammonia is taken into account in the calculation of asparagine, glutamine, and aspartic and glutamic acids as described in

3. Glucosinolates expressed as mM/g, oil-free, dry basis.

Table 4 Amino Acids (g/100g N) in Canadian Specialty Varieties of *B. napus* Canola

	STELLAR (Low Linolenic)	TRISTAR (Triazine Tolerant)
Tryptophan	7.2	7.7
Lysine	34.6	37.8
Histidine	15.9	17.6
Arginine	38.1	41.2
Total (1)		
Ammonia	12.9	13.7
Aspartic acid	47.7	45.9
Glutamic acid	107.1	121.2
Aspartic acid (2)	22.0	24.1
Threonine	27.0	27.8
Serine	26.7	28.1
Glutamic acid (2)	24.4	26.6
Proline	38.0	40.8
Glycine	29.8	32.0
Alanine	26.0	28.0
Cystine	15.0	17.1

Valine	34.4	36.1
Methionine	11.7	12.7
Isoleucine	27.2	28.1
Leucine	44.0	46.5
Tyrosine	17.0	17.4
Phenylalanine	24.4	25.7
Asparagine (2)	25.5	21.7
Glutamine (2)	82.2	93.9
Glucosinolates	12,2	17.6
% Protein	43.3	45.4
% Recovery	88.9	95.3
N-to-P Factor:		
"Uncorrected"	5.49	5.48
Corrected	4.77	5.13

1. Assuming the sample contains only free aspartic acid and free glutamic acid.

2. Ammonia is taken into account in the calculation of asparagine, glutamime, and aspartic and glutamic acids as described in

3. Glucosinolates expressed as mM/g, oil-free, dry basis.

Table 5 Amino Acids (g/100g N) in Varieties of *B. napus*

	ARGENTINE	JET NEUF	BRONOWSKI	MIDAS	WESTAR	ARABELLA	BOUNTY
Tryptophan	6.9	7.6	7.7	7.5	7.7	7.3	7.5
Lysine	33.7	39.0	35.7	38.4	36.4	41.7	36.9
Histidine	15.3	17.1	16.5	16.7	17.1	18.0	16.3
Arginine	36.6	37.5	39.3	37.1	40.2	40.6	37.1
Total (1)							
Ammonia	14.1	15.3	13.2	14.4	13.9	14.1	12.3
Aspartic acid	43.8	41.8	44.8	42.9	44.8	49.6	45.1
Glutamic acid	102.8	110.3	113.3	106.6	118.5	115.7	103.1
Aspartic acid (2)	13.5	10.9	22.1	13.2	21.8	22.2	21.0
Threonine	25.6	27.0	26.3	27.7	26.7	30.7	27.9
Serine	24.9	26.8	26.5	26.5	27.3	30.0	26.9
Glutamic acid (2)	14.9	12.0	24.4	14.6	24.1	24.5	23.2
Proline	36.5	43.0	40.3	39.9	42.2	42.7	38.0

Glycine	28.8	30.3	30.1	29.9	30.6	33.4	30.0
Alanine	25.6	26.7	26.6	26.6	27.0	29.5	26.1
Cystine (2)	14.9	18.0	15.4	17.5	16.3	17.2	15.5
Valine	33.5	34.7	34.8	34.5	35.2	38.7	34.4
Methionine (2)	11.9	13.3	12.0	12.9	12.4	14.1	12.4
Isoleucine	26.2	26.8	27.6	26.4	27.9	29.8	27.2
Leucine	42.3	43.9	45.2	42.8	46.1	48.2	43.8
Tyrosine	16.8	17.6	17.2	17.1	17.5	19.2	18.2
Phenylalanine	24.0	24.7	25.1	24.1	25.9	27.2	24.3
Asparagine (4)	30.1	30.7	22.5	29.5	22.8	27.3	23.9
Glutamine (4)	87.3	97.6	88.3	91.4	93.7	90.6	79.3
Glucosinolates	140.6	119.5	14.8	125	31.5	37.3	11.7
% Protein	40.6	37.6	44.9	36.2	46.1	34.5	38.7
% Recovery	87.0	93.1	91.0	91.0	93.6	98.5	88.5
N-to-P Factor:							
"Uncorrected"	5.39	5.37	5.49	5.38	5.47	5.49	5.50
Corrected	4.57	4.87	4.89	4.76	5.01	5.29	4.76

1. Assuming the sample contains only free aspartic acid and free glutamic acid.

2. Ammonia is taken into account in the calculation of asparagine, glutamime, and aspartic and glutamic acids as described in

3. Glucosinolates expressed as mM/g, oil-free, dry basis.