Protein Quality of Three New Canadian-Developed Naked Oat Cultivars Using Amino Acid Compositional Data†

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Three new high-yielding and rust-resistant naked oat cultivars (*Avena sativa* var. *nuda*), namely AC Hill, AC Lotta, and AC Percy, were assessed for their total protein content and amino acid composition using quantitative chromatographic methods. The total protein among these cultivars was very similar and ranged from 13.75% in AC Percy to 13.90 and 14.40% in AC Hill and AC Lotta, respectively. All cultivars were similar in amino acid composition. All cultivars contained a very good balance of the nine essential amino acids (EAA_9) limited only in lysine, followed by threonine. Compared to the FAO/WHO reference EAA_9 pattern value of 33.9% for a 2–5-year-old child, mean values for total EAA_9 for naked oat proteins ranged from 44.1 to 44.4%. The adjusted amino acid scores for naked oat cultivars ranged from 55 to 59%, compared to covered oats (62%), maize (29%), soybean (86%), and egg (95%). The results indicate that a potentially useful method for evaluating the protein quality of oat can be based on their amino acid composition.

Keywords: *Naked oats; assessment; protein quality; amino acids; composition*

INTRODUCTION

Naked oats (*Avena sativa* var. *nuda*; Leggett, 1992) are a relatively new agricultural crop in Canada and are better adapted to the cooler northern climate than are soybeans and maize (Burrows, 1986a). Naked oats are annual grasses that have been grown for centuries in the dry Tibetan Himalaya highlands, in Russia and Chinese Turkestan, and in northwestern China (Stanton, 1923; Jenkins, 1968), but their less desirable agronomic traits, including lower yields and seed quality, have prevented the wide commercial utilization of this crop. Various names have been used to designate these naked oats including naked-seeded, hull-less and huskless oats, huskless oat groats, and bare oats. Genetic studies on such oats have indicated that their naked character is controlled by a single partially dominant gene, which tends to initiate the naked phenotype, but it is modified and expressed by three or four modifying genes (Jenkins, 1968; Jenkins and Hanson, 1976). These findings have led to the development of early-maturing genotypes (McKenzie et al., 1981; Burrows, 1986b, 1992a,b, 1993), with improved yields, superior agronomic characteristics and disease resistance, improved seed quality, and higher energy and protein content (Schrickel et al., 1992).

The high fiber content and the low metabolizable energy of the hulls of covered oats have restricted their use primarily to a feed grain for pigs and poultry. However, naked oats differ from normal covered oats (*A. sativa* L.) in that the caryopsis or groat in naked oats is free-threshing and separates easily from the thin membranous lemma and palea which, in covered oats, become thick and lignified and which tightly enclose the grain at maturity. Hulls comprise approximately 250–330 g/kg of kernel weight and contain little (1.4–1.9%) protein (Youngs, 1972). Because of their high fiber content, covered oats are lower in energy value in comparison with other cereals, and the oat must always be dehulled before they can be rolled or milled into flour for human consumption or for nonruminant animal feed. In naked oats the lemma and palea are membranous in texture, lacking the lignification which occurs in the hulls of covered oats. These naked oats are therefore more suitable for both human and animal diets.

Oat groats contain approximately 15–20% protein (Robbins et al., 1971; Zarkadas et al., 1982; Peterson, 1992) and have an average protein efficiency ratio (PER) of 2.15 (Youngs et al., 1982), although lower PER values (1.7–1.9) have also been reported (Lockhart and Hurt, 1986; Peterson, 1992). PER values are determined by rat bioassays, which tend to underestimate the protein quality of oats for humans because the rat has higher relative requirements for sulfur-containing amino acids (Torum et al., 1981). Oat proteins, like other cereal proteins, are limited in their lysine content, followed by methionine, threonine, isoleucine, and tryptophan (Peterson, 1992). However, because prolamins, which are devoid of lysine and tryptophan, account for only 10–15% of total oat proteins (Peterson and Brinegar, 1986), oats actually have higher PER values than cereal grains such as rye, wheat, and maize, which contain considerably more prolamins (Eggum and Beames, 1983; Lockhart and Hurt, 1986; Peterson and Brinegar, 1986; Shewry and Tatham, 1990). According to Youngs et al. (1982) supplementing oat protein with lysine, methionine, and threonine increased the PER value of oat proteins.

The aims of this study were (a) to quantitatively establish the levels and variation of total protein and individual amino acids among three new cultivars of naked oats developed by Burrows (1992a,b, 1993), namely AC Hill, AC Lotta, and AC Percy, using chromatographic procedures developed by Zarkadas et al. (1987, 1988a–c, 1990), and (b) to accurately assess the protein quality of these three cultivars by standard

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procedures (FAO/WHO/UNU, 1985; FAO/WHO/Expert Consultation, 1990) based on amino acid compositional data.

MATERIALS AND METHODS

Materials. Type DC-5A (lot 746) cation-exchange spherical resin, sized to 6.0 ± 0.5 μm, was purchased from Dionex Chemical Co., Sunnyvale, CA. The amino acid standards, chemicals, and the three highly purified microcolumn citrate buffers (pH 3.282, 0.20 M; pH 4.10, 0.20 M; pH 6.40, 1.0 M) and sample dilution buffer (pH 2.2, 0.20 M) recommended for high-sensitivity single-microcolumn analysis were used as described previously (Zarkadas et al., 1987, 1990). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Selection of Plant Materials and Sample Preparation.* The three new spring-type naked oat genotypes selected for this investigation, i.e., cv. AC Hill, AC Lotta, and AC Percy (*A. sativa* var. *nuda*; Leggett, 1992), were all hexaploid forms developed by Burrows (1992a,b, 1993).

The first of these three cultivars is AC Hill oat, which is a spring-type, daylength-sensitive hull-less cultivar that showed superior agronomic performance in eastern Canada. The pedigree of AC Hill is (01394.24)2/Dumont (Burrows, 1992a). The pedigree of 01394-24 is OA123-3-134/2/CAV2700/GEMINI/3/GEMINI/3932-16. Parent 3932-16 donated the naked genes, and Dumont (McKenzie et al., 1984) contributed the genes endowed with resistance to prevalent races of rust present in Ontario. Preliminary assessment of agronomic performance was conducted in Ontario during the 1986–1988 seasons and performance was further evaluated in the Eastern Registration Oat Test in 1989 and 1990, as described previously by Burrows (1992a).

The second cultivar, AC Lotta, is a spring-type daylength-insensitive, naked oat cultivar suitable for the feed, food (milling), and mixed-grain industries in eastern Canada. The pedigree of AC Lotta is Donald*2/Fidler/2/OT220/3/Donald*3/4/Tibor (Burrows, 1992b). Donald (Burrows, 1984) contributed the gene Di-1 for daylength insensitivity. The naked genes were derived from Tibor (Burrows, 1986b), and Fidler (McKenzie et al., 1981) and/or OT220 contributed the rust-resistance genes. AC Lotta was tested for 2 years (1989–1990) in the Eastern Oat Registration Test as described previously (Burrows, 1992b).

The third oat cultivar, AC Percy, is a spring-type, daylength-sensitive, strong-strawed, tall, late-maturing, naked cultivar suitable for the feed industry. The pedigree of AC Percy is 01394-19*2/Dumont (Burrows, 1993). As in the case of AC Hill (Burrows, 1992a), the pedigree of 01394-19 was OA123-3-134/2/CAV2700/Gemini/3/Gemini/3932-16 as described by Burrows (1993). Parent 3932-16 donated the naked genes, while Dumont (McKenzie et al., 1984) contributed the genes for rust resistance. Preliminary assessment for agronomic performance was conducted in Ontario during 1986–1988, and AC Percy was further evaluated in the Eastern Canadian Registration Oat Test in 1989 and 1990, as described previously (Burrows, 1993).

Representative samples of the cleaned and dried (65 to 75 g of moisture/kg of seeds) oat seed samples were then pulverized in a standard sample mill (U.D. Corp., Fort Collins, CO), passed through a 0.5 mm mesh sieve, lyophilized, and then stored at −20 °C in polypropylene bottles until used.

Preparation of Tissue Hydrolysates. Duplicate samples (50.0 mg) were hydrolyzed in Pyrex (No. 9860) test tubes (18 × 150 mm) under vacuum (below 10 μmHg) with 5.0 mL of triple-glass-distilled constant-boiling HCl (6.0 M) containing 0.2% (v/v) phenol and 0.1% (v/v) octanoic acid at 110 ± 0.5 °C for periods of 24, 48, 72, and 96 h with the usual precautions described by Moore and Stein (1963) and Zarkadas et al. (1988c). Analyses of individual acid hydrolysates were performed on the clear filtrate in duplicate according to methods described previously (Zarkadas et al., 1988b,c).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on a Beckman Spinco Model 121 MB fully automated amino acid analyzer using single-column methodology (Zarkadas et al., 1987, 1990). The automated instrument was equipped with a Beckman Model 406 analog interface module, the system Gold (Beckman Instrument, Inc., Altex Division, San Ramon, CA) chromatographic data reduction system, and an IBM (AT series) compatible personal computer to enable quantitation of amino acids at the picomole level as described previously (Zarkadas et al., 1993a,b).

Complete amino acid analyses were carried out on each of the three oat replicate samples according to the standard procedures described previously (Zarkadas et al., 1987, 1990). Analyses of individual acid hydrolysates were performed in duplicate. The data reported for serine and threonine in Tables 1 and 2 represent the average values of 72 determinations extrapolated to zero time of hydrolysis by linear regression analysis of the results. The values for valine, isoleucine, leucine, and phenylalanine are the average of 48 values obtained from the 48, 72, and 96 h of hydrolysis. All others are reported as the average values of 72 determinations from 24, 48, 72, and 96 h of hydrolysis.

Methionine and cyst(e)ine were determined separately in each sample (50.0 mg) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysate as an internal standard. Recoveries of cyst(e)ine as cysteic acid and methionine as methionine *S,S*-dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids. The data were then normalized relative to alanine, valine, leucine, and isoleucine present in the sample and represent the average of 24 determinations.

Tryptophan in naked oat samples (50.0 mg) was also determined separately after alkaline hydrolysis (Hugli and Moore, 1972) on a single column as described previously (Zarkadas et al., 1990), using 3-nitrotyrosine as the internal standard (Zarkadas et al., 1987). The data presented in Table 1 represent the average of 24 determinations.

Protein Determination. Precise quantitation of the protein content in each of the naked oat hydrolysates was carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a). According to this method a mean residue equivalent weight (WE, in micrograms per nanomole) is calculated for the 18 standard amino acid residues constituting the proteins in the naked oats using the expression

$$\mathrm{WE} = \sum_{i=1}^{18} (a_i b_i) \qquad (1)$$

where a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i. A conversion factor CF (in micrograms per nanomole) was used for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophan, methionine, and cyst(e)ine as described previously (Zarkadas et al., 1988c). These factors, WE and CF, can be used in all subsequent quantitations of a given sample.

The protein concentration P (in micrograms) of each hydrolysate was calculated by multiplying CF by the total nanomoles (χ_i) of amino acids found (Horstmann, 1979; Peterson, 1983; Zarkadas et al., 1988a–c) as follows:

$$\mathrm{P} = (CF)\sum_{i=1}^{15} \chi_i \qquad (2)$$

Statistical Analysis. Data processing of the results was carried out by a FORTRAN computer program developed for this purpose. Analysis of variance, conducted on the amino acid data, for a completely randomized block design (factorial) was done by the general linear model procedure (SAS, 1991) and represents the average values from eight subsamples per genotype.

Table 1. Comparison of the Amino Acid Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of Three New Naked Oat Cultivars

	naked oat cultivars[a]						significant levels among cultivars		oat groats mean values of 289 cultivars[b]
	AC Hill		AC Lotta		AC Percy				
amino acid	mean ± SEM[a]	CV	mean ± SEM	CV	mean ± SEM	CV	CV	F	
aspartic acid	72.55 ± 4.06	9.69	79.55 ± 0.24	0.53	73.85 ± 0.98	2.32	4.66	3.38^{ns}	89
threonine	28.86 ± 1.44	8.69	32.35 ± 0.43	2.33	28.73 ± 0.66	3.98	5.11	5.40^{ns}	33
serine	42.39 ± 0.92	3.75	44.22 ± 0.27	1.08	42.41 ± 2.57	10.49	6.39	0.44^{ns}	42
glutamic acid	232.06 ± 2.06	1.53	223.18 ± 0.14	0.11	228.75 ± 3.67	2.78	1.92	3.16^{ns}	239
proline	60.92 ± 3.54	10.07	52.84 ± 0.48	1.57	55.55 ± 1.75	5.46	5.84	4.66^{ns}	47
glycine	44.61 ± 1.69	3.79	41.95 ± 0.50	2.08	44.45 ± 1.51	0.59	2.37	6.20^{ns}	49
alanine	45.08 ± 1.21	4.65	46.38 ± 0.61	2.27	46.02 ± 1.53	5.75	3.66	0.48^{ns}	50
cysteine	43.53 ± 2.78	11.09	55.00 ± 1.24	3.92	48.35 ± 5.04	18.07	14.14	2.08^{ns}	16
valine	56.62 ± 0.87	2.65	53.13 ± 0.16	0.53	55.75 ± 0.15	0.47	1.73	10.85^{ns}	53
methionine	14.94 ± 0.76	8.76	13.83 ± 0.55	6.93	14.53 ± 0.57	6.83	6.91	0.94^{ns}	25
isoleucine	43.29 ± 0.72	2.88	41.66 ± 0.14	0.60	41.86 ± 1.00	4.14	3.11	1.38^{ns}	39
leucine	79.00 ± 1.57	3.46	75.41 ± 0.19	0.45	75.35 ± 1.17	2.71	2.34	4.10^{ns}	74
tyrosine	42.80 ± 0.91	3.67	40.57 ± 0.36	1.56	42.25 ± 0.21	0.88	2.28	4.43^{ns}	31
phenylalanine	53.75 ± 1.04	3.36	53.06 ± 0.14	0.48	52.81 ± 1.03	3.39	2.18	0.53^{ns}	53
histidine	24.79 ± 0.44	3.08	25.00 ± 0.27	1.86	26.04 ± 1.52	10.11	5.41	0.72^{ns}	22
lysine	37.07 ± 1.10	5.15	38.43 ± 0.58	2.62	39.63 ± 0.99	4.35	3.26	3.15^{ns}	42
arginine	61.95 ± 3.57	9.98	68.04 ± 0.48	1.24	67.72 ± 2.22	5.68	5.04	3.22^{ns}	69
tryptophan	16.65 ± 0.31	3.29	16.05 ± 0.09	1.02	16.03 ± 0.13	1.47	2.04	3.41^{ns}	
ammonia	14.34 ± 2.11	25.58	8.98 ± 0.38	7.44	12.95 ± 2.23	29.82	22.32	3.19^{ns}	71
WE[c] μg/nmol	0.112865 ± 0.0003	0.57	0.113566 ± 0.0002	0.25	0.113329 ± 0.0003	0.29	0.37	2.10^{ns}	
CF[c] μg/nmol	0.114036 ± 0.0003	0.53	0.114689 ± 0.0001	0.26	0.114445 ± 0.0002	0.31	0.35	1.43^{ns}	
total protein,[c] g/kg of dry sample	139.29 ± 5.34	6.64	144.31 ± 5.5	6.64	136.74 ± 6.02	7.63	8.03	0.35^{ns}	123.35^{b}

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance: F, values from analysis of variance among cultivars; ns, not significant; CV, coefficient of variation. [b] Robbins et al. (1971) total protein recalculated from their amino acid data above, according to the methods of Horstmann (1979) and Zarkadas et al. (1988a–c), which is the summation of the weights of the amino acid residues present (Zarkadas et al., 1994, 1995). [c] Computed according to the methods of Horstmann (1979) and Zarkadas et al. (1988a–c).

Table 2. Comparison of the Amino Acid Composition and Nitrogen Contents (Grams of Amino Acids per 16 g of Nitrogen) of Three New Naked Oat Cultivars

	naked oat cultivars[a]						significant levels among cultivars	
	AC Hill		AC Lotta		AC Percy			
amino acid (AA)	mean ± SEM[a]	CV	mean ± SEM	CV	mean ± SEM	CV	CV	F
aspartic acid	7.146 ± 0.451	10.95	7.906 ± 0.063	1.39	7.234 ± 0.193	4.62	5.68	2.52^{ns}
threonine	2.834 ± 0.127^{a}	7.78	3.240 ± 0.041^{b}	2.18	2.898 ± 0.043^{b}	2.56	5.02	6.36^{ns}
serine	4.164 ± 0.087	3.62	4.431 ± 0.021	0.82	4.116 ± 0.196	8.24	5.44	1.63^{ns}
glutamic acid	22.806 ± 0.487	3.70	22.365 ± 0.074	0.58	22.407 ± 0.657	5.08	3.37	0.31^{ns}
proline	5.994 ± 0.425	12.28	5.295 ± 0.034	1.12	5.44 ± 0.241	7.67	7.44	2.36^{ns}
glycine	4.383 ± 0.122	4.80	4.204 ± 0.041	1.68	4.352 ± 0.071	2.84	3.23	1.42^{ns}
alanine	4.429 ± 0.152	5.97	4.648 ± 0.052	1.94	4.509 ± 0.204	7.85	4.63	0.83^{ns}
cysteine	4.269 ± 0.215	8.72	5.512 ± 0.139	4.39	4.749 ± 0.487	17.76	13.51	2.76^{ns}
valine	5.565 ± 0.163	5.08	5.324 ± 0.029	0.94	5.459 ± 0.082	2.62	3.16	1.47^{ns}
methionine	1.392 ± 0.017	2.13	1.386 ± 0.059	7.34	1.422 ± 0.062	7.53	6.26	0.15^{ns}
isoleucine	4.254 ± 0.095	3.86	4.175 ± 0.021	0.86	4.101 ± 0.149	6.31	4.07	0.61^{ns}
leucine	7.768 ± 0.161	3.59	7.557 ± 0.015	0.34	7.375 ± 0.069	1.62	2.72	2.75^{ns}
tyrosine	4.205 ± 0.106	4.38	4.065 ± 0.026	1.11	4.137 ± 0.061	2.54	3.27	0.80^{ns}
phenylalanine	5.280 ± 0.110	3.62	5.317 ± 0.018	0.60	5.168 ± 0.061	2.05	2.78	0.85^{ns}
histidine	2.434 ± 0.009	0.67	2.505 ± 0.033	2.29	2.547 ± 0.131	8.96	5.03	0.62^{ns}
lysine	3.607 ± 0.092^{a}	4.44	$3.851 \pm 0.053^{a,b}$	2.38	3.878 ± 0.056^{b}	2.49	3.07	4.99^{ns}
arginine	6.080 ± 0.288^{a}	8.22	$6.819 \pm 0.054^{a,b}$	1.38	6.632 ± 0.172^{b}	4.49	3.79	7.29^{ns}
tryptophan	1.636 ± 0.039	4.19	1.609 ± 0.014	1.46	1.569 ± 0.009	1.00	3.02	1.45^{ns}
ammonia	1.395 ± 0.188	23.39	0.899 ± 0.036	6.99	1.263 ± 0.201	27.57	20.55	3.33^{ns}
total	97.952 ± 1.440	2.55	100.211 ± 0.270	0.47	97.911 ± 1.302	2.30	1.09	24.75^{ns}
total AA N[b]								
g of AA N/kg of protein	163.416 ± 2.374	2.52	159.665 ± 0.430	0.47	163.471 ± 2.17	2.30	1.60	2.11^{ns}
g of AA N/kg of dry mass	22.585 ± 1.029	7.89	22.820 ± 0.935	7.09	22.118 ± 0.817	6.40	8.12	0.11^{ns}

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance, $P > F$ values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; CV, coefficient of variation between genotypes. Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). [b] Total amino acid nitrogen (AA N) was determined according to the methods of Heidelbough et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a–c, 1994) using eqs 1 and 2.

RESULTS AND DISCUSSION

Naked oats are a largely untapped high-protein resource that could be used in both human and animal nutrition. One advantage of naked oats is that the groats have a higher energy and protein content than covered seeds. Naked oats have been found particularly suitable for feeding to poultry and pigs (Hulan et al., 1981; Maurice et al., 1985; Cave et al., 1989; Morris and Burrows, 1986; Friend et al., 1989) and have also been successfully fed to horses and sheep (Givens and Brunnen, 1987). However, until now the most serious obstacle to establishing naked oats as a profitable

agricultural crop was the absence of varieties that were truly competitive in yielding ability and other agronomic traits (Jenkins, 1968; Jenkins and Hanson, 1976; Burrows, 1986a; Schrickel et al., 1992). According to Stanton (1923), Laurel and Liberty are examples of early naked oat varieties developed by Dr. C. E. Saunders of the Central Experimental Farm, in Ottawa, Canada, from a cross between Chinese hull-less (*A. nuda chinensis* Fisch.) and Swedish Select. These were followed, in 1942, by Brighton and in 1953 by Torch (Derick, 1953). None of these naked oat varieties appears to have been grown on a significant scale. Now, however, three new northern adapted naked oat cultivars selected for this investigation, AC Hill, AC Lotta, and AC Percy, developed by Burrows (1992a,b, 1993), have proven to be superior to older naked oat cultivars (Derick, 1953). Their outstanding traits include high grain yield, large groat size and high hectoliter weight, and improved straw strength, and they were all resistant to smut and several races of rust that are prevalent in Ontario. AC Lotta is also a daylength-insensitive cultivar.

The average protein content and amino acid composition of AC Hill, AC Lotta, and AC Percy and the levels of statistical significance obtained from the analyses of variance are summarized in Table 1 and represent the average values of three replicates ($N = 3$). Data for oat groats from 289 husked oats by Robbins et al. (1971) are also included for comparison. The results, expressed as grams of anhydrous amino acid residues per kilogram of protein, allow comparisons to be made between the present data and those given in food compositional tables. This method of expressing results is also in accord with the recommendations of the Joint FAO/WHO Expert Consultation Group (FAO/WHO/Expert Consulation, 1990). They have recommended that amino acid data be reported as milligrams of amino acid per gram of protein or as milligrams of amino acid per gram of nitrogen. The best estimate of the total protein content in each of these naked oat cultivars was therefore made by the summation of the weights of their amino acid residues. Table 1 also lists the calculated average weight equivalent, WE, and conversion factor, CF, obtained (in micrograms per nanomole). The values for these constants do not vary significantly among the naked oat cultivars evaluated and therefore can be used in subsequent quantitations of such plant tissues.

The total protein content (grams per kilogram of dry mass) of the three naked oat cultivars as presented in Table 1 appeared to be very similar, ranging from 13.7% in AC Percy to 14.4% in AC Lotta. These results are similar to the values reported by Pomeranz et al. (1973) for commercial milled oats, which were 9.6–13.4% for light and heavy weight oats, respectively. These results, however, were considerably lower than those of Robbins et al. (1971), who determined total protein according to the Kjeldahl method in 289 samples of oat groats covering a wide range of genetic material from the world oat collection and reported that the samples contained 12.4–24.4% crude protein (average 17.1%), on a moisture-free basis.

Large differences in reported seed protein content were noted previously when other cereal and leguminous seed products were analyzed according to the Kjeldahl nitrogen method and quantitative amino acid analyses (Zarkadas et al., 1988a). For example, the protein contents of two commercial wheat protein products, biscrum flour and vital wheat gluten, as determined according to the Kjeldahl nitrogen procedure, were 13.7 and 74.2% total protein, respectively. Precise quantitation of the protein contents of these cereal products by amino acid analysis, however, indicated that biscrum flour and vital wheat gluten contained only 11.3 and 58.9% protein, respectively. This indicates that total protein in cereals, determined according to the Kjeldahl method, was 17.9 and 21.9% higher for biscrum flour and vital wheat gluten, respectively. Similar differences have been reported by Heidelbaugh et al. (1975) for Skylab foods.

The most likely explanation for the large differences in the protein values for naked oats, as presented in Tables 1 and 2, and those reported by Robbins et al. (1971) is the method used for the analysis of total nitrogen. The Kjeldahl nitrogen procedure does not differentiate between nitrogen derived from protein and that originating from the nonprotein nitrogenous compounds present in the oat cultivars. For comparison, the total protein contents of the oat groat values reported by Robbins et al. (1971) were recalculated from their amino acid data according to the procedure described by Horstmann (1979) and Zarkadas et al. (1988a–c). It was found that their oat groats contained only 12.34% protein on a dry weight basis, which was 38.6% lower than the 17.1% average protein value they reported for 289 oat groat samples.

These results suggest that because the conventional Kjeldahl nitrogen procedure greatly overestimates the content of both plant and animal tissues (Benedict, 1987; Zarkadas et al., 1988a; Khanizadeh et al., 1992), the best estimate of the protein content of oat varieties can be made by the summation of the weights of the amino acid residues in these oat cultivars, as described by Horstmann (1979) and Zarkadas et al. (1988a,b, 1990, 1993a,b). These results suggest that plant breeders developing high-protein oat cultivars, especially during screening and selection for increased protein concentration in oat cultivars, require precise quantitative data on total protein of the genotypes investigated (Forsberg and Reeves, 1992).

While the amino acid compositions of covered oats and oat groats have been reported (Tkachuk and Irving, 1969; Robbins et al., 1971, Pomeranz et al., 1973; Zarkadas et al., 1982), the only information available concerning the amino acid composition of naked oats is that reported by Maurice et al. (1985). The results on the amino acid composition of three new naked oat cultivars and levels of statistical significance obtained from analysis of variance, as presented in Table 1, appeared to be very similar. Values for all determinations show deviations of less than $100 \pm 2.5\%$ from the average values obtained among three replicates within the same cultivar and corresponding low coefficients of variability. The following features were found to be common to naked oat proteins of all three cultivars: the acidic amino acids, which include glutamic and aspartic acids and their amides, glutamine and asparagine, are present in substantially high quantities in the three naked oats analyzed and when taken together account for almost 29.0–31.1% of all residues. Wieser et al. (1981) determined the amide content of the various solubility fractions of covered oats. They calculated that 87% of the aspartate and glutamate residues of the prolamin fraction were present as asparagine and glutamine, compared to the globulin fraction, where only 61% of the acidic amino acids were amidated. Robbins et al. (1971) reported that 69% of glutamic and aspartic

Table 3. Protein Quality Evaluation of Three New Naked Oat Cultivars Based on Their Amino Acid Composition

essential amino acids (EAA)	EAA[a] requirements preschool child (2–5 years old)	naked oat cultivars			oat groats means of 289[g] cultivars	soybean[b] cv. AC Proteus	animal products[c]	
		AC Hill	AC Lotta	AC Percy			egg	cow's milk
		Milligrams of Amino Acid per Gram of Total Protein						
histidine	19	25	25	26	22	23	22	27
isoleucine	28	43	42	42	39	48	54	47
leucine	66	79	75	75	74	74	86	95
lysine	58	37	38	40	42	58	70	78
methionine + cyst(e)ine	25	58	70	63	41	30	57	33
phenylalanine + tyrosine	63	96	93	95	84	85	93	102
threonine	34	29	32	29	33	39	47	44
tryptophan	11	17	16	16	16	11	17	14
valine	35	57	53	56	53	50	66	64
% total protein								
EAA_9[a]	33.9	44.1	44.4	44.2	40.4	41.8	51.2	50.4
EAA index[d]		84.6	90.1	86.9				
total EAA[e], mg/g of N		2898	3053	2999		2779	3215	3200
		Percent Protein Digestibility in Man[a]						
		86	86	86	86	86	95	97
		Percent Amino Acid Score[f]						
		63.8	65.5	69.0	72.4	100	100	100
		Protein Digestibility Corrected Amino Acid Score[f]						
		54.9	56.3	59.3	62.3	86	95	97
limiting EAA		Lys, Thr	Lys, Thr	Lys, Thr	Lys, Thr	Met		

[a] Data from FAO/WHO/UNU (1985) and FAO/WHO (1990). [b] Data from Zarkadas et al. (1994). [c] Data taken from Bodwell (1987). [d] Calculated according to the methods of Block and Mitchel (1946) and Oser (1951). [e] Computed from reference protein standards (FAO/WHO, 1965). [f] Calculation of protein rating was carried out by comparison of the amino acid composition of the three naked oat cultivars with that of the reference pattern established by FAO/WHO/UNU (1985) and FAO/WHO (1990). [g] Data taken from Robbins et al. (1971).

acids in groats from husked oats were amidated. Thus, the frequency of free carboxyl groups is approximately 9.0–10.0%. The frequency of total basic amino acids, including lysine, histidine, and arginine, is approximately 12.4–13.3%, which slightly exceeds that of the free carboxyl groups. The amino acids with hydrophobic side chains account for a further 29.0–30.0% compared to 49.9–51.1% for hydrophilic amino acids. The aromatic amino acids tyrosine and phenylalanine are present in substantial amounts and account for 9.3–9.5%. Mean values for proline account for a further 5.2–6.0%. The least common amino acids in naked oats are tryptophan and methionine (Tables 1 and 2). The limiting amino acids lysine, and especially threonine, showed relatively narrow ranges of values and low coefficients of variability.

Agreement between the mean values obtained in the present study on naked oats with those published values on covered oat varieties (Pomeranz et al., 1973) and oat groat samples from 289 covered oat cultivars by Robbins et al. (1971) is good both in the amino acid composition as a whole and in many of the individual values; some differences have been noted. There is a lower content of aspartic acid and methionine in these naked oat cultivars compared to the mean values reported by Robbins et al. (1971) for oat groats for covered oats. Both limiting amino acids, lysine and threonine, in naked oats were found in approximately the same amounts as those cited by Robbins et al. (1971) and Pomeranz et al. (1973). These authors reported several cultivars that were sufficiently above the mean in lysine and threonine and contained 3.5% threonine (average 3.3%) and 4.9% lysine in protein compared to their mean lysine value of 4.2%. Tyrosine and cyst(e)ine are present in substantially higher quantities in naked oats than in covered oats or oat groats (Tables 1 and 2).

Table 3 compares the essential amino acid (EAA) composition of the three new naked oat cultivars, AC Hill, AC Lotta, and AC Percy, and the mean EAA values of groats from 289 covered oat cultivars with that of the selected reference amino acid pattern for a 2–5-year-old child (FAO/WHO/UNU, 1985; FAO/WHO/Expert Consultation, 1990). Data for soybean, egg, and milk are also included for comparison. The present data (Table 3) indicate that all naked oat cultivars evaluated in this study contain all of the essential amino acids required for human or animal nutrition with lysine, followed by threonine, as the major limiting amino acids for humans. Mean values for total EAA ranged from 2898 to 3053 mg of EAA/g of N [total nitrogen (N) was calculated from amino acid nitrogen, Table 2], which are higher than the value 2779 mg/g of N for the new AC Proteus soybean cultivar (Zarkadas et al., 1993a, 1994) but lower than the values for cow's milk (3200 mg/g of N) and hen's whole egg (3215 mg/g of N) (FAO/WHO, 1965). Similar results were obtained from the essential amino acid indices (Table 3) which were calculated from their amino acid composition (Tables 1 and 2) according to the methods of Block and Mitchell (1946) and Oser (1951).

An even more accurate assessment of the protein quality of foods was recommended by the Joint FAO/WHO Expert Consultation Group (FAO/WHO/UNU, 1985; FAO/WHO/Expert Consultation, 1990) and by the U.S. Department of Agriculture [Expert Work Group (FSIS), 1984]. They recommended that, in conjuction with *in vivo* protein digestibility data, the most appropriate approach would be to use the essential amino acids required for the 2–5-year-old child as the reference pattern (Table 3) in the evaluation of foods for all persons except infants.

The calculated amino acid scores for all three naked oat cultivars, AC Hill, AC Lotta, and AC Percy, are very similar in their essential amino acid contents (Table 3). The naked oat proteins would provide adequate amounts of all of the essential amino acids ranging from 44.1 to

44.4%, which is considerably higher than the 33.9% reference amino acid pattern value given by FAO/WHO/Expert Consulation (1990) for a 2–5-year-old child. These results correspond closely with the mean essential amino acid values of covered oats (EAA_9 = 40.4%), calculated from the oat groat data of Robbins et al. (1971). Oats have a true protein digestibility of 86% (FAO/WHO/Expert Consultation, 1990). Mean values for amino acid score, corrected for digestibility, ranged from 54.9% in AC Hill to 56.3% in AC Lotta and to 59.3% in AC Percy, limited mainly in lysine, followed by threonine. These values are similar to the amino acid score of 62.3% calculated for covered oats from the data of Robbins et al. (1971) and Pomeranz et al. (1973). It should be noted that although lysine for naked oat proteins ranged from 37 to 40 mg/g of total oat protein, it is still below the recommended FAO/WHO/Expert Consultation (1990) reference lysine standard value of 58 mg/g of dietary protein for the 2–5-yaer-old child (Table 3). Threonine, another EAA, ranged from 2.9 to 3.2% among the naked oat cultivars as compared with 3.4% for the FAO/WHO/Expert Consultation (1990) standard.

The data presented in this paper show that the amino acid composition and protein content among the three new naked oat cultivars that are being considered as higher energy and protein resource for both human and animal nutrition are very similar. Although lysine is the first limiting amino acid in naked oats, followed by threonine for humans, the overall balance of their essential amino acids is considered superior to other cereals. These results indicate that a potentially useful method for evaluating the protein quality of different oat cultivars can be based on a knowledge of their amino acid composition.

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