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THE NUTRIENT COMPOSITION OF HONEYBEE-COLLECTED POLLEN IN OTAGO, NEW ZEALAND

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Summary

The following pollen species were analysed for moisture, fat, carbohydrate, minerals and amino acids: white clover (*Trifolium repens* L.), willow (*Salix* sp. L.), hawkweed (*Hieracium pilosela* L.), broom (*Cytisus* sp. L.), bush lawyer/blackberry (*Rubus* sp. L.), matagouri (*Discaria toumatou* Raoul), pine (*Pinus* sp. L.), thistle (*Cirsium* sp. Miller) and kiwifruit (*Actinidia deliciosa* A. Chev.). The moisture content of the pollen ranged from 16·8% (matagouri) to 25·9% (willow). The fat content ranged from 0·17% (staminate kiwifruit pollen) to 13·40% (hawkweed). Total carbohydrate ranged from 12·6% (matagouri) to 29·6% (pistillate kiwifruit), while reducing sugars ranged from 11·1% (matagouri) to 25·7% (pistillate kiwifruit). The protein content, which is commonly regarded as an index of nutritional quality ranged from 2·9% (pistillate kiwifruit) to 23·5% (broom). Most of the species analysed contained sufficient nutrients for honeybee growth and development.

Introduction

All the amino acids, fats, sterols, minerals and vitamins that the honeybee (*Apis mellifera* L.) needs to survive are provided by pollen in its diet (Dietz, 1975). The chemical composition of pollen varies between plant species, and also with age and nutrient status of the plant, climatic environment during pollen development and storage methods (Herbert & Shimanuki, 1978). Many pollens have been analysed for specific compounds in relation to either honeybee nutrition or the fertilization of flowers, but there are few complete analyses of the nutrient status of specific pollen types.

Two principal approaches have been used to study the nutritive value of pollen: the first involves the analysis of pollen for essential nutrients (Vivino & Palmer, 1944; Sarkar et al., 1949; Weaver & Kuiken, 1951; Levin & Bohart, 1955; Herbert & Shimanuki, 1978; Rabie et al., 1983; Schmidt & Schmidt, 1984; Shimanuki & Herbert, 1985; Herbert et al., 1987; Herbert & Miller-Ihli, 1987; Wille et al., 1987). This approach determines the nutrient composition of pollen, but does not take digestibility into account. Generally speaking, honeybees are able to digest pollen efficiently (Schmidt & Buchamann, 1985), but some pollens, for example dandelion, may not be utilized completely by honeybees (Peng et al., 1985). The second approach to determining the nutritional value of pollen addresses the question of digestibility by feeding specific pollen diets to bees and measuring some aspects of their growth and development (for example, weight of hypopharyngeal gland or nitrogen content of honeybees, Haydak, 1961, 1970; thoracic weight, development of hypopharyngeal glands and brood rearing, Hagedorn & Moeller, 1968; brood rearing, Townsend & Smith, 1969, Dietz & Stephenson, 1975, Campana & Moeller, 1977, Herbert & Schimanuki, 1979, Lehner, 1983, Shimanuki & Herbert, 1985). De Groot (1953) determined the minimum amount of each essential amino acid required for honeybee growth, using increase in dry weight and nitrogen content as a measure of growth. A diet consisting of 18 amino acids was added to sugar syrup, making up 2 or 3% of the dry sugar. The amino acid mixture approximated to the composition of casein, except for a lower level of glutamic acid and proline and a higher level of tryptophan. By varying the amino acid levels in the diet, De Groot was able to determine the minimum level of each amino acid required for optimal growth in the honeybee.

Recent studies on hives overwintered in the hill and high country of Central Otago, New Zealand, show a significant drop in honey production after two consecutive winters (Ogden, 1988). This crop failure was attributed to a protein deficiency caused by the lack of adequate early spring pollen supplies, resulting in a drop in population and vigour of the colony. It was assumed that the bees survived for some time using pollen stored during previous seasons in pollen rich areas. Kulincevic et al. (1982) give a similar report of 'disappearing disease' in the apiaries of a Florida beekeeper, attributed to inadequate amounts of natural pollen. The importance of adequate honey and pollen reserves for overwintering hives is also emphasised by Furgala (1975).

To investigate the available nutrients in pollen from the Otago hill and high country, the nutrient composition of several pollen types has been determined. These pollens include white clover (*Trifolium repens* L.), willow (*Salix* sp. L.), hawkweed (*Hieracium pilosela* L.), broom (*Cytisus* sp. L.), bush lawyer/blackberry (*Rubus* sp. L.), and matagouri (*Discaria toumatou* Raoul), all collected in the Otago hill and high country. Pine (*Pinus* sp. L.), thistle (*Cirsium* sp. Miller) and kiwifruit (*Actinidia deliciosa* A. Chev.) pollen were analysed for purposes of comparison. The nutrient composition of these pollen species has been compared in analyses for moisture, fat, carbohydrate, minerals and amino acids. The present report describes the first nutritional analysis of pollen in New Zealand. The analysis provides information about nutrient content of these pollens and the implications of the individual pollen types and mixed pollen to honeybee nutrition are discussed. The vitamin content of these species of pollen was not assessed.

Materials and Methods

Nine species of bee-collected pollen were selected for analysis. The genus and wherever possible species of each pollen type was identified by light and scanning electron microscopy. If the pollen identified in this way had a morphology characteristic of more than one plant genus or family, the species common to the area of collection was determined to be the particular species of origin of the pollen.

The pollen types used in this study were white clover (*Trifolium repens*), willow (*Salix* sp.), hawkweed (*Hieracium pilosela*), broom (*Cytisus* sp.), bush lawyer/blackberry (*Rubus*), matagouri (*Discaria toumatou*), pine (*Pinus* sp.), thistle (*Cirsium* sp.); and staminate (63–77% staminate pollen grains) and pistillate (10–19% staminate grains) kiwifruit (*Actinidia deliciosa*).

Bee-collected clover, willow, broom, *Rubus* and matgagouri pollen were obtained from pollen traps placed on hives in the Moniototo Plains and Rock and Pillar Range of Central Otago. Bee-collected staminate and pistillate kiwifruit pollen was obtained from pollen traps placed on hives in the Auckland region, for use as an interesting comparison with the pollen from Central Otago, kiwifruit pollen having both fertile (pistillate) and infertile (staminate) grains. The pollen was collected from a number of hives throughout the summer of 1986/87 and frozen until the time of analysis. 'Fresh' clover and willow pollen samples collected in 1989 and frozen for a much shorter period (6 months) was also analysed to investigate the effects of long-term storage on the nutrient content of these pollens.

Separate samples of each pollen were analysed for moisture, lipid, carbohydrate, amino acids, and mineral content.

Analytical methods

To determine the moisture content each pollen sample was weighed, placed in an oven at 55°C for 24h, then subsequently dried to constant weight in a vacuum oven at 70°C. The lipid content of two 3g samples of each pollen was estimated using the Soxtech semi-automatic

TABLE 1. Composition of pollens collected from central Otago.

Pollen (*n*)	% *moisture* 4	*Fat*[a] 2	*Reducing Sugars*[a] 4[b]	4[c]
clover	25·4(±1·4)[d]	8·17	18·8(±1·3)	18·5(±0·3)
willow	25·9(±1·8)	5·04	15·2(±0·3)	17·3(±0)
Pilosella/Hypochearis	19·8(±0·5)	13·40	24·2(±0·7)	25·0(±2·3)
Rubus	24·5(±1·2)	2·25	18·7(±0·9)	18·5(±1·0)
broom	18·5(±1·0)	2·07	14·3(±1·0)	13·7(±0·3)
matagouri	16·8(±0·5)	1·24	11·1(±0·6)	12·6(±0·5)
kiwifruit (f)		1·10	25·7(±0·1)	29·6(±0·6)
kiwifruit (m)		0·17	18·6(±0·5)	20·6(±0·2)
Average	21·8	4·18	18·3	19·5

[a]Expressed as % dry matter.
[b]Collected in 1986/87 and '1989, respectively.
[d]±SE in parentheses.

soxhlet extraction process. Total reducing and non-reducing sugars in four 0·5g samples of each pollen was determined using the PAHBAH colourimetric reaction with carbohydrates as described by Lever (1972). A single 2g sample of each pollen was analysed for sulphur, phosphorus, magnesium, calcium, sodium, potassium, and the trace elements, manganese, zinc, copper and iron at the plant analysis laboratory, Ministry of Agriculture and Fisheries (MAF), Invermay Agricultural Research Centre, Mosgiel, New Zealand. The Atomic absorption spectra of each sample was measured on a Varian SpectrAA 30 Atomic Absorption Spectrophotometer to determine the mineral content of the pollens. Selenium was measured at the MAF Ruakura Agricultural Research Centre, Hamilton. Amino acid analysis was performed on a Waters/Millipore high pressure liquid chromatography (HPLC) amino acid analyser. A Waters sulfonated polystyrene column (part no. 80002) with post-column detection of amino acids with orthophthaldehyde was used. The instrument was operated according to Waters User Bulletin no. 85999 (July 1982) and was calibrated at the 500pmol level using an amino acid standard (Sigma product no. AA S-18). Trytophan and Cystine were not analysed because sulphur amino acids are digested during the analysis. Samples were prepared for analysis as follows: 5 mg (accurately weighed) of each sample was hydrolysed for 24h *in vacuo* with 500µl 6-N HCl/phenol. The samples were dried under vacuum, resuspended with sonication in 250µl Na citrate buffer, pH 3·05, centrifuged, and one wash of 125µl. Tht total 375µl was SEP-PAKed (Waters SEP-PAK C_{18} Cartridge Cleanup for Amino Acid Samples), and transferred to a 10ml volumetric flask for analysis using the Amino Acid Analyser. The number of samples ranged from two for willow pollen to six for matagouri pollen.

Results

The results for the moisture, fat and carbohydrate analyses are presented in Table 1. The carbohydrate content varied from 12·6% total in matagouri to 29·6% found in pistillate kiwifruit (Table 1). The concentration of the nectar/honey regurgitated from the bees' honey stomachs to bind the pollen may affect these values (Shimanuki & Herbert, 1985).

Hawkweed and dandelion have similar pollen types, allowing comparison between these pollens. Hawkweed pollen was low in each mineral that was analysed (Table 2). Clover and matagouri pollen are consistently high in all the minerals, while broom, *Rubus,* and willow have a range of high and low values. There is little difference in mineral content between pollen collected in 1986-87 and 1989.

The results from the amino acid analysis are presented in Table 3. Pistillate kiwifruit pollen has the lowest total amino acid content with only 241·0 pmol/µg pollen. This is 2·9% of the total dry weight of the sample. The highest total amino acid content was found in broom, with 1956 pmol/µg, 23·5% of the total dry weight of the sample. The amino acid content of matagouri pollen ranged from 13·18% to 34·11%, being the most variable of the pollens analysed. The proportion of each essential amino acid with respect to the total amino acids in the pollen was calculated so that the pollens could be compared (Table 4).

The amino acid that is the most variable in each pollen does not appear to be consistently the same, alanine varies the most in clover, *Rubus* and staminate kiwifruit; proline in willow and matagouri; histidine in hawkweed, pistillate kiwifruit and thistle; glutamine acid in pine, and aspartic acid in brooms. In almost every pollen sample, proline is among the more variable of the amino acids (Table 3).

Discussion

Willow, *Rubus,* broom, matagouri and staminate kiwifruit pollen contain all the essential amino acids analysed in quantities above the minimum required for each amino acid as determined by De Groot (1954) (Table 4). Ten amino acids are essential for honeybee nutrition; these are arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, isoleucine, leucine, and valine (De Groot, 1953). The other amino acids can be synthesised from these. A deficiency in any one essential amino acid results in that amino acid becoming limiting to growth. In the absence of studies of the digestibility and metabolism of pollen by honeybees it is assumed that pollens with the highest quantities of essential amino acids will contribute to the most rapid bee growth.

All the essential amino acids are present in varying amounts in pollen (De Groot, 1953),

TABLE 2. Mineral content of pollens collected from Otago (*a*, % Dry Weight; *b*, ppm).

(*a*)

Data source	*Pollen*	*S*	*P*	*Mg*	*Ca*	*Na*	*K*	*Total*
Present study	clover	0·22	0·62	0·12	0·19	0·02	0·89	2·06
Present study	clover[a]	0·21	0·58	0·10	0·13	0·02	0·80	
Todd & Bretherick	clover		0·36	0·35	0·24		1·11	
Todd & Bretherick	clover		0·37	0·20	0·37		0·66	
Vivino & Palmer	clover		0·41	0·27	0·34			
Present study	willow	0·16	0·56	0·16	0·21	0·01	0·45	1·55
Todd & Brethwick	willow		0·58	0·17	0·20		0·43	
Todd & Brethwick	willow		0·28	0·06	0·20		0·42	
Present study	hawkweed	0·12	0·13	0·01	0·16	0·02	0·16	0·60
Present study	hawkweed[a]	0·16	0·24	0·03	0·15	0·03	0·31	
Todd & Bretherick	dandelion		0·05	0·20	0·26		0·13	
Vivino & Palmer	dandelion[b]	0·42	0·19	0·20				
Present study	broom	0·32	0·73	0·09	0·09	0·02	0·84	2·09
Todd & Bretherick	legume		0·22	0·23	0·72		0·38	
Present study	*Rubus*	0·25	0·59	0·22	0·13	0·01	1·01	2·21
Present study	matagouri	0·31	0·7	0·09	0·27	0·02	0·85	2·24

(*b*)

Data source	*Pollen*	*Mn*	*Zn*	*Cu*	*Fe*	*Se*
Present study	clover	27	55	10	161	0·03
Present study	clover[a]	27	4	17	201	
Present study	willow	113	53	5	58	0·13
Present study	hawkweed	7	26	10	54	0·02
Present study	hawkweed[a]	10	30	9	67	
Present study	broom	117	62	9	79	0·08
Present study	*Rubus*	137	44	10	123	0·04
Present study	matagouri	62	77	29	178	0·04

[a]Fresh pollen samples collected in 1989.
[b]Samples include plum and apple pollen.

though in the present study tryptophan and cystine were not analysed because they are destroyed in the analysis. Using De Groot's (1954) measurements as a guide, hawkweed and thistle pollen were deficient in both arginine and isoleucine. The minimum amount of isoleucine required for normal development is 4·0% of the total amino acids (De Groot, 1954). Clover, hawkweed, pistillate kiwifruit, pine and thistle pollen all contained less isoleucine than this minimum level. Davidson & Passmore (1986) state that either lysine, tryptophan or sulphur-containing amino acids, will be the limiting amino acids in common foods for human consumption. In the absence of tryptophan analysis in the present study, isoleucine appears to be the most frequently limiting amino acid in pollen.

The infertile pistillate kiwifruit pollen is low in amino acids, while the fertile staminate pollen is relatively high in amino acids. This is consistent with other reports showing that the total amino acid content of pollen is lower in pollen from sterile anthers than in pollen from fertile anthers (Stanley & Linskens, 1974; Kakihara et al., 1988). Differing quantities of sterile and fertile pollen grains may also account for some of the variability in amino acid levels in

TABLE 3. Amino acid content of pollens collected from Otago (pmolμg^{-1} dry matter).

Amino acid	*Clover*[a]	*Willow*[b]	*Pilosella/ Hypochearis*[a]	*Rubus*[b]	*Broom*[b]	*Matagouri*[b]	*Kiwifruit* *Female*[b]	*Kiwifruit* *Male*[b]	*Pine*[a]	*Thistle*[a]
n	*4*	*2*	*4*	*2*	*2*	*5*	*3*	*3*	*2*	*2*
Hydroxy proline	10·2(±5·9)	0	51·3(±17·4)	0	0	0	0	0	0[a]	8·6[a]
Aspartic acid	134·7(±3·3	102·5	65·9(±10·6)	199·4	211·3	211·0(±23·6)[b]	26·6(±1·7)	218·0(±7·8)	34·9	126·6
Threonine	67·7(±3·5)	52·0	31·7(±4·7)	72·4	91·4	80·6(±8·4)	10·4(±0·8)	74·1(±3·5)	17·1	62·9
Serine	93·6(±2·5)	77·5	49·8(±6·8)	102·5	121·5	106·8(±12·9)	16·2(±0·9)	103·7(±4·0)	22·9	88·1
Glutamic acid	142·1(±9·0)	116·0	63·3(±10·1)	170·8	216·5	196·8(±3·8)	27·3(±3·8)	193·8(±11·9)	57·5	124·4
Proline	276·1(±18·7)	117·6	148·6(±19·3)	157·4	280·0	207·9(±102·2)	21·9(±0·3)	95·2(±5·4)	53·5	443·0
Glycine	109·5(±2·9)	86·9	73·9(±5·4)	119·1	150·0	157·7(±29·0)	32·8±0·7)	147·0(±8·5)	30·7	111·3
Alanine	155·8(±20·2)	87·2	70·7(±4·9)	127·2	155·2	134·6(±15·9)	17·1(±1·7)	200·2(±32·3)	39·7	101·0
Valine	80·5(±4·90	63·2	44·2(±11·6)	87·2	112·7	102·1(±3·3)	16·0(±0·9)	104·5(±3·9)	23·3	80·6
Methionine	37·6(±6·6)	30·9	20·9(±6·9)	37.4	56·5	44·1(±9·5)	4·1(±0·7)	68·9(±20·1)	6·7	38·7
Isoleucine	57·8(±0·9)	46·4	28·9(±5·2)	57·5	76·4	64·2(±11·5)	8·8(±0·6)	70·3(±4·1)	14·9	53·9
Leucine	94·2(±5·9)	67·4	51·5(±10·9)	83·9	119·0	98·6(±17·6)	15·1(±0·6)	136·3(±25·2)	24·1	93·8
Tyrosine	30·5(±2·4)	29·5	14·9(±1·6)	37·4	48·4	72·9(±36·9)	3·2(±1·9)	30·7(±3·4)	9·3	26·4
Phenylalanine	46·9(±4·5)	36·8	28·1(±7·0)	42·0	61·6	49·3(±9·8)	7·2(±0·2)	53·6(±8·5)	10·4	52·6
Histidine	60·6(±15·8)	41·1	62·3(±30·8)	51·9	60·3	49·1(±7·4)	14·3(±5·3)	73·5(±23·6)	12·3	113·5
Lysine	97·5(±13·1)	64·8	83·1(±19·3)	81·2	114·3	117·6(±6·6)	13·0(±1·7)	103·5(±6·4)	26·4	111·1
Arginine	55·0(±5·9)	48·5	21·6(±3·0)	54·3	80·8	63·2(±12·1)	7·2(±1·4)	77·1(±7·0)	39·6	36·1
Total	1550·4	1068·1	910·7	1481·6	1955·9	1756·5	241·2	1750·2	423·3	1672·6
% of Total Pollen	18·6%	12·8%	10·9%	17·8%	23·5%	20·9%	2·9%	21·0%	5·1%	20·0%

[a] Average of two results.
[b] ± SE in parentheses.

some of the other pollen examined, such as clover, *Rubus*, willow or matagouri. The content of alanine and proline is higher in fertile grains than in non-fertile grains (Khoo & Stinson, 1957; Tupy, 1962; Kakihara et al., 1988) and it is these amino acids that are among the most variable in the pollens examined in this study.

In terms of amino acids, hawkweed appears to be the least nutritive of the pollens tested. Hawkweed may form an important part of the diet of the honeybee in the hill and high country areas of Otago due to its abundance in these areas. The low nutrient content of hawkweed may contribute to protein deficiency observed in some colonies. Hawkweed pollen is also low in minerals but has an unusually high fat content (13·40%).

The sterols, one class of lipids, are the precursors for moulting hormones (Shimanuki & Herbert, 1985). Although larvae can synthesise some fats from carbohydrates (Herbert et al., 1979), when the lipid component of their diet is low the bees may be unable to develop normally due to the lack of hormones. *Rubus*, broom and kiwifruit pollen all have a low lipid content which could lead to problems in the nutrition of bees foraging exclusively on these species.

The moisture content will reflect the atmospheric conditions when the pollen was collected, the concentration and amount of the material packed with the pellets and lastly the moisture content of the pollen grains themselves (again affected by atmospheric conditions). The carbohydrate analysis is affected by protein in the sample (Lever, 1972), in some instances the results for total carbohydrates (reducing and non-reducing sugars) is lower than the results for the reducing sugars alone. The carbohydrate content varied from 12·6% total in matagouri to 29·6% found in pistillate kiwifruit (Table 1). The concentration of the nectar or honey regurgitated from the bees' honey stomachs to bind the pollen will cause the carbohydrate values to be more variable and higher than that found in hand-collected pollen (Shimanuki & Herbert, 1985).

The optimum mineral level for brood rearing is 1% (Herbert & Miller-Ihli, 1987). Hawkweed pollen is the only pollen analysed that has a mineral level below this optimum. The other pollens all have levels above the optimum, which may be slightly toxic (Herbert & Miller-Ihli, 1987). This variation can be seen in the comparison of the mineral content of the clover, willow, hawkweed and broom pollen, with similar pollen species in other studies (Todd & Bretherick, 1942; Vivino & Palmer, 1944; Table 2).

Most of the pollens analysed contained adequate amounts of nutrients, so the cause of the collapse of the high country hives reported by Ogden (1988) is more likely to be due to poor supplies of pollen than pollen that is lacking in nutritional composition.

Usually honeybees will collect a diverse range of pollen, so that even if some pollens are low in nutrients, those nutrients can be balanced by the collection of other, more valuable pollens. Protein complementation may also occur between the pollen species, so that a variety of pollen in the diet of the honeybee is far better than a diet of just one pollen species.In some areas and at certain times of the year they are more likely to be limited to only one or a few pollen species and this could result in short-term nutrient deficiency (Ogden, 1988). For example if hawkweed were the predominant pollen source, then it is likely that the honeybees would suffer from protein deficiency. Ogden (1988) found that the hill country sources of pollen were less diverse than pollen sources in low country areas.

Willow, hawkweed and broom are important pollen and nectar sources among the early flowering species in the hill and high country, while clover, matagouri, and *Rubus* are more important in the late spring and summer period (Ogden, 1988). The autumn flowering species include *Hypochaerus* (cat's ear), and gorse (*Ulex* sp.). Two of the early flowering species, willow and hawkweed contained low levels of amino acids, while broom contained the highest levels of amino acids of the pollen tested. Honeybee colonies will probably experience protein deficiency in years when they are restricted to species low in amino acids at the beginning of spring, because it is at this time that the hive begins to build up pollen stores for the season. Hill and high country hives have a limited range of pollen sources in autumn as well as spring and the autumn species may be as crucial to honeybee overwintering ability as the spring species are to the successful build up of the hive.

None of the later flowering species (clover, *Rubus*, matagouri) were deficient in amino acids, providing adequate sources of pollen for honeybees during late spring and summer. The productivity of honeybee hives in the high country may be enhanced by sowing pollen species that flower in early spring or late autumn.

Table 4. Contribution of amino acids from each pollen type as a percentage of the minimum required for maximum honeybee growth.

Amino acid	*minimum required*[a]	*clover*	*willow*	*Pilosella/ Hypochearis*	*Rubus*	*broom*	*matagouri*	*kiwifruit (f)*	*kiwifruit (m)*	*pine*	*thistle*
Arginine	3·0	3·6(±0·4)	4·5(±0·8)	2·4(±0·3)	3·7(±0·7)	4·1(±0·9)	3·6(±0·7)	3·0(±0·6)	4·5(±0·4)	9·3(±0·6)	2·2(±0·5)[b]
Histidine	1·5	3·9(±1·0)	3·8(±1·7)	6·9(±3·4)	3·5(±0·8)	3·1(±1·2)	2·8(±0·4)	5·9(±2·2)	4·2(±1·4)	2·9(±1·0)	7·0(±3·8)
Lysine	3·0	6·4(±0·9)	6·1(±0·7)	9·2(±2·1)	5·5(±0·1)	5·8(±0·5)	6·7(±0·4)	5·4(±0·7)	6·0(±0·4)	6·2(±0·4)	6·8(±1·2)
Phenylalanine	2·5	3·1(±0·3)	3·4(±1·4)	3·1(±0·8)	2·8(±0·6)	3·1(±0·9)	2·8(±0·6)	3·0(±0·1)	3·1(±0·5)	2·5(±0·1)	3·2
Methionine	1·5	2·5(±0·4)	2·9(±1·2)	2·3(±0·8)	2·5(±0·7)	2·9(±1·0)	2·5(±0·5)	1·7(±0·3)	4·0(±1·2)	1·6(±0·2)	2·4(±0·8)
Threonine	3·0	4·4(±0·2)	4·9(±1·3)	3·5(±0·5)	4·9(±0·6)	4·7(±0·5)	4·6(±0·5)	4·3(±0·3)	4·3(±0·2	4·0(±0·3)	3·9(±0·1)
Leucine	4·5	6·1(±0·4)	6·3(±1·3)	5·7(±1·2)	5·7(±0·2)	6·1(±0·5)	5·6(±1·0)	6·3(±0·2)	7·9(±1·5)	5·7(±0·1)	5·8(±0·5)
Isoleucine	4·0	3·8(±0·1)	4·3(±1·2)	3·2(±0·6)	3·9(±0·3)	3·9(±0·3)	3·7(±0·7)	3·7(±0·2)	4·1(±0·2)	3·5(±0·1)	3·3(±0·0)
Valine	4·0	5·2(±0·3)	5·9(±1·3)	4·9(±1·3)	5·6(±0·2)	5·8(±0·4)	5·8(±0·8)	6·6(±0·4)	6·0(±0·2)	5·5(±0·0)	4·9(±1·1)
Trytophan	1·0										

[a]De Groot (1953).
[b]±SE in parentheses.

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P49