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AMINO ACID COMPOSITION OF THE PLUMAGE OF THE WHITE-CROWNED SPARROW

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ABSTRACT.—To provide baseline data for estimating the dietary amino acid requirements of molting birds, we measured the amino acid composition, nitrogen and sulfur content, and heat of combustion of the homogenized plumage of six White-crowned Sparrows (*Zonotrichia leucophrys gambelii*). On the average, the plumage contained 15.22% nitrogen, 3.14% sulfur, and 0.86% ash. Of the 17 amino acids measured, serine, proline, cystine/2, and glycine (in that order) were most abundant, comprising 46 mol% of the hydrolysate. Nonessential amino acids predominated (68 mol%). Cystine/2 contributed 11 mol%. Variation of amino acid composition among the plumages of the six birds was small (coefficient of variation = 2–7%). The heat of combustion of the homogenized plumages was 21.69 kJ/g of dry mass. Although exact quantitative comparisons are not possible, the amino acid composition of White-crowned Sparrow plumage in general parallels that of other species. Its cystine/2 and sulfur content, however, are noticeably greater than that reported for any other species thus far examined (primarily pale and domesticated).

The quantities of exogenous nutrients required per unit time in the production of eggs, feathers, or tissues can be estimated from a knowledge of the growth rates and composition of the synthesized products. If either the ratio of calories to specific nutrients is low or the concentrations of dietary nutrients are much less than their concentrations in products, then an animal engaged in production must (1) eat in excess of caloric requirements in order to obtain adequate materials, (2) rely on endogenous materials, if available, or (3) alter the rate, timing, or pattern of production so that requirements match nutrient availability. Depending on circumstances, any or all of these adjustments might be invoked.

In the case of avian molt, it has been noted that the amino acid cystine is much more concentrated in feathers than in the foods that many birds consume (Hanson 1962, Newton 1968, Gavrilov and Dolnik 1974). This has fostered hypotheses that attempt to relate energy requirements, feeding tactics, and alterations of body composition during molt to dietary cystine availability (Gavrilov and Dolnik 1974). The reliability of such hypotheses depends on accurate estimates of the cystine content of the entire plumage. Although this emphasis on cystine may be questionable, it is nevertheless necessary to know the amino acid composition of whole plumage before this or other hypotheses about amino acids as limiting factors during molt can be critically examined.

Although there have been many analyses of the amino acid composition of single feathers, feather parts, and purified keratins (Block 1939, Ward and Lundgren 1954, Schroeder and Kay 1955, Harrap and Woods 1964, 1967, Kemp and Rogers 1972, O'Donnell and Inglis 1974, Busch and Brush 1979, Nitsan et al. 1981), none except the last has reported the average amino acid composition of an entire plumage. Also, nearly all of these analyses involved feathers from domesticated species of birds having white or near-white plumage. Because differences occur in the amino acid composition of feathers from different species as well as among feather parts from a single species (Schroeder and Kay 1955, Harrap and Woods 1964, 1967) extrapolations of percentage composition from purified keratins or feather parts to whole plumage or from one species to another might be tenuous. As we were reluctant, in nutritional investigations to be reported elsewhere, to depend on such extrapolations without testing their reliability, we undertook the following analysis.

MATERIALS AND METHODS

We sacrificed six White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) captured during the autumn migration in eastern Washington and plucked all the feathers from them. We put the feathers from each bird into a loosely woven cloth bag and washed them according to the methods of Harrap and Woods (1964). We then macerated the air-dried feathers with shears until they formed a felt-like,

TABLE 1. Amino acid composition of homogenized White-crowned Sparrow plumage (n = 6).

Amino acid	μmoles/g dry weight[a]			mg/g dry weight[a]
	Mean	SE	CV	Mean
Serine	920	11.2	3.0	80
Proline	902	9.8	2.7	88
Cystine/2	894	23.0	6.3	91
Glycine	886	14.4	4.0	51
Glutamic acid	643	6.9	2.6	83
Valine	571	10.3	4.4	57
Leucine	518	8.0	3.8	59
Aspartic acid	476	4.5	2.3	55
Alanine	404	7.2	4.4	29
Threonine	361	4.9	3.3	36
Isoleucine	353	9.9	6.9	40
Arginine	292	2.9	2.4	46
Phenylalanine	181	2.2	3.0	27
Tyrosine	158	2.3	3.6	26
Lysine	150	3.1	5.1	19
Histidine[b]	65	—	—	9
Methionine	51	4.7	22.6	7
Tryptophan	nd[c]	—	—	—
NH_3 released[b,d]	1,172	—	—	20

% nitrogen accounted for = 93.4
% sulfur accounted for = 96.5
% dry weight accounted for = 82.3

[a] Values uncorrected for loss during hydrolysis. CV = coefficient of variation.
[b] Best estimates as the mean of two samples showing clearly separated peaks.
[c] Not determined.
[d] Under conditions specified in the text.

homogeneous mixture, which we then dried in an oven at 100°C to constant weight and stored in a desiccator. The maceration and mixing of the feather fragments required 6–8 h per bird before we were satisfied that homogeneous samples could be obtained from the mass.

We analyzed samples of the homogenized plumage from each bird for nitrogen (micro Kjeldahl method according to Horwitz 1980), sulfur (gravimetric method using a $BaSO_4$ precipitate according to the Parr Instrument Co. Manual 1960), ash, amino acid content, and caloric density. We measured ash content after combustion of samples in a muffle furnace at 600°C for 6 h, and caloric density by routine oxygen bomb calorimetry. To ascertain amino acid composition, we hydrolyzed 20-mg samples of the homogenized plumage in 6N HCl for 24 h, dried the hydrolysate, redissolved it in sodium citrate buffer (pH 2.2), and had the solutions analyzed (Beckman model 121 MB) by the Bioanalytical Laboratory, Washington State University. Cystine was measured in parallel as cysteic acid after oxidation with performic acid (Schram et al. 1954) and is reported as cystine/2 in Table 1.

RESULTS AND DISCUSSION

On the average (±SE, n = 6 in all cases), the plumage of the White-crowned Sparrow contained 15.22 ± 0.073% nitrogen, 3.140 ± 0.057% sulfur, and 0.863 ± 0.076% ash. The amino acid analysis accounts for an average of 93% of the nitrogen (Table 1), the remainder presumably being derived from pigments, nitrogenous compounds remaining as cellular debris following keratinization, and amino acids lost during hydrolysis. Harrap and Woods (1967) also reported that component amino acids account for 90% or more of the total nitrogen in various feather parts of several species.

About 97% of the sulfur content of White-crowned Sparrow plumage is accounted for by the sulfur-containing amino acids (SAA). The small residue may result from either mensural error or from sulfur-containing compounds other than amino acids, such as pigments or inorganic sulfates. In contrast to the results of Machlin and Pearson (1956) we found no evidence of taurine. The amino acids measured account for 82% of the dry weight of the plumage in the White-crowned Sparrow. Because this quantity has not been reported in most previous studies, it is difficult to compare our results with those of others. We can estimate indirectly (using the molecular weights of the dehydrated forms of the amino acids) from the data of Harrap and Woods (1967) that measured amino acids account for as little as 83% (emu barbs) and as much as 99% (goose rachis) of the weight of *protein* in various feather parts of several species of birds. Kemp and Rogers (1972) reported that freeze-dried S-carboxymethyl extracts of feathers and scutes accounted for "greater than 70%" of the dry weight of the starting materials. Regardless of disparities in the ways in which results are reported, it is apparent that a noteworthy fraction of total plumage weight (about 18% in our analysis) is not accounted for in the summed weights of the constituent amino acids. As ash constitutes less than 1%, this unexplained fraction must therefore be attributed to the weights of pigments (perhaps 3–5% of dry weight; Nicholaus et al. 1964), cellular debris, nonprotein structural elements (if any), tryptophan (not measured in our analysis but probably of low concentration), and loss of amino acids during hydrolysis of feathers.

As might be expected, the amino acid composition varied only slightly among individual White-crowned Sparrow plumages. The coefficients of variation (Table 1) range from 2 to 7% except for methionine (23%). Methionine is among the least abundant amino acids in the plumage, and its apparently greater variability may result merely from the disproportionate effect of traces of free methionine left in the feathers following keratinization. The

estimates of the other amino acids, because they are several-fold more concentrated than methionine, are less susceptible to this putative error. Alternatively, the variation in methionine content may simply be related to a variable susceptibility of this amino acid to destruction or alteration during hydrolysis.

The relative proportions of amino acids in the plumage of White-crowned Sparrows are consistent with patterns described for feathers or feather parts of other species (cf. Table 1 and Harrap and Woods 1967). The nonessential amino acids (ser, pro, cys, gly, glu, asp, ala, and tyr) predominate, comprising 68 mol% (sum of moles of individual amino acids/sum of moles of all amino acids). Serine, proline, and cystine/2 were most abundant, comprising 46 mol%. Of the essential amino acids, lysine, methionine, and histidine are relatively scant, comprising only 3.4 mol%.

Exact quantitative comparisons between our results and those of Harrap and Woods (1967) and others are difficult, partially because we are unable, in some cases, to interpret how derived values were computed (e.g., whether whole feathers parts were assumed to be equivalent to protein, and whether or not hydrated or dehydrated molecular weights of amino acids were used in computations), and partially because of differences in methodology (e.g., hydrolyzed feathers compared with hydrolyzed feather parts or purified proteins). Nevertheless, the cystine content of White-crowned Sparrow plumage is noticeably greater than that found by Harrap and Woods in feather parts of the domestic fowl, goose, duck, and turkey, and the "sea-gull" and emu (*Dromaius novaehollandiae*), or by Nitsan et al. (1981) in the plumage of the growing gosling. Additional comparisons could be made on the basis of the sulfur content of plumage or feather parts if the concentration of SAA were as tightly correlated with sulfur in other studies as they are in ours (Table 1). However, Harrap and Woods (1967) found that SAA account for as little as 75% of the sulfur, as in the feather barbs of the emu, to as much as 100%, as in the rachis of the domestic fowl. This variable correlation makes comparison tenuous. Even so, the greater cystine content in White-crowned Sparrow plumage is generally reflected in a greater sulfur content than in the aforementioned species. White-crowned Sparrow plumage contains an average of 3.14% sulfur, with a range of 2.89 to 3.30%. The sulfur content of the rachis of four species studied by Harrap and Woods (1967) ranged from 2.4 to 2.7%, and the calamus and barbs of the emu contained only 1.7 and 2.4% sulfur, respectively. Among the species investigated thus far, only feathers of the domestic goose (3.0–3.3% sulfur; Block and Bolling 1945) match in sulfur content the plumage of the White-crowned Sparrow. Because of the crucial role of sulfhydryl bonds in the quaternary structure of keratin, variation in the relative proportions of cystine among and within species is of special interest in relation to feather quality. It would be significant to learn whether variation of sulfur (or cystine) content is correlated with measures of feather quality such as resistance to abrasion or torque, and to what extent, if any, variations of dietary SAA might affect the SAA content of feathers.

Finally, the caloric density (heat of combustion at constant volume, ΔH_c^{20}) is of great interest in estimating the caloric efficiency of feather synthesis. We found that the mean ($\pm$SE, $n = 6$) heat of combustion of the homogenized plumage of the White-crowned Sparrow was 21.69 $\pm$ 0.09 kJ/g, which is essentially the same as the 21.74 $\pm$ 0.21 ($n = 5$) reported by Chilgren (1975) in this species. These values are slightly less than the means ($\pm$95% c. i., n not specified) found for samples of flight feathers (23.54 $\pm$ 0.95 kJ/g) and body feathers (22.15 $\pm$ 1.49 kJ/g) in European Tree Sparrows (*Passer montanus*) by Myrcha and Pinowski (1970). An estimated mean (22.50 kJ/g) for the entire plumage of this species can be obtained from these data by weighting the substituent means as 25% flight feathers and 75% body feathers (Newton 1968, Chilgren 1977). This indirect value is 3.7% greater than our direct one for the White-crowned Sparrow. Because of the relatively slight variation of feather composition among species, as already discussed, it is unlikely that the mean heat of combustion of the plumage of diverse species will differ significantly from an average of about 22 kJ/g dry weight.

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