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The influence of hydration on the tensile and compressive properties of avian keratinous tissues

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Despite recent research exploring the elastic properties of avian keratins, data on failure properties are less common in the literature. In this paper we present data on the failure properties and moduli of both avian feather and claw keratin in tension and the modulus of claw keratin in compression. Increased water content acts to decrease stiffness and strength but to increase strain at failure. The modulus of claw did not differ significantly when tested under tension and compression.

1. Introduction

Compared with mammalian (α) keratins, we know relatively little about the mechanical performance of avian (β) keratin. The mean Young's modulus of feather keratin from eight species of birds was reported to be 2.50 GPa [1] and from a flightless bird, the ostrich, 2.42 GPa [2]. Under tensile testing at room temperature and humidity (23°C, 75% RH) the elastic properties of β-keratin appear somewhat conservative- there is little indication of interspecific differences in the literature. Knowledge of these mechanical properties gives us an indication of the way in which the material will behave under the action of external forces. Measurements of Young's modulus and rachis geometry have been used successfully by Purslow and Vincent [3] to model the mechanical behaviour of primary flight feathers from pigeons. Changes in the mechanical properties of keratin are likely to have quantifiable effects on whole-feather performance. There have been very few reports of the compressive mechanical properties of keratinous tissues [4]. Since compressive loads occur *in vivo* [5] there is a need to establish whether keratin can be regarded as an isotropic material.

Recently, the influence of hydration on the tensile 'structural' properties of single barbs of wet and dry duck down feathers has been explored; stiffness and strength of the feathers were lower when 'wet' than when dry [6]. Keratin from birds' claws [7] shows a decrease in Vickers microhardness and the tensile Young's modulus decrease as moisture content increases.

In this paper, we present data on the effects of increased water content on the tensile modulus and strength properties of keratin from the feathers and claws of ostriches and the compressive modulus of claw keratin.

2. Methods

2.1. Specimen preparation

Body contour feathers from the backs of ten ostriches and claws from six ostriches were available for study. Narrow, parallel-sided sections of rachis and claw, approximately 20 mm long, were cut from each of the feathers just distal to the calamus and the distal region of claws. The ridges on the underside of the rachis and its medullary foam were carefully removed with a razor blade to produce a thin strip (~1 mm thick) of compact keratin. The ends of the keratin strips had aluminium tabs folded over them to avoid the test pieces being damaged when clamped during testing. The tabs, approximately 20 mm × 8 mm × 0.5 mm, were folded lengthways in half and secured to the keratin strips with epoxy resin adhesive. Similar sized pieces of fine abrasive paper were glued to the tabs to prevent possible slippage between the test pieces and the clamp during testing. Blocks for compression testing were cut using a small electric saw, with the specimen held in a micromanipulator stage. This allowed the specimens to be cut so that their ends and facing sides were parallel. The dimensions of all the conditioned specimens were measured by using vernier calipers (±0.01 mm) (Digimatic, Mitutoyo Corporation, Japan). The mean test length of the feather and claw tensile specimens was 9.18 mm (SEM = 0.10) and 9.28 mm (SEM = 0.12) respectively. The mean length of compression specimens was 3.06 mm (SEM = 0.1) and their mean aspect ratio was 0.99 (mean thickness divided by length).

2.2. Specimen conditioning

Prior to subsequent conditioning, all test pieces were placed in an oven at 105°C until they attained a constant

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mass. All masses throughout the experiment were determined by using Stanton Instruments precision balance (±0.01 mg). The 50% RH test specimens were conditioned in a controlled-environment laboratory. The 100% RH test specimens were conditioned by immersing them in deionised water at 22°C. In all cases the conditioning continued until a constant mass had been attained. 0% RH specimens were stored in a desiccator whilst equilibriating to the laboratory temperature.

2.3. Mechanical testing
All tests were carried out using a T10 Davenport-Nene test machine (Davenport-Nene Ltd, UK), equipped with a 200 N load cell. The compliance of the load cell was 1.3×10^{-3} mm N^{-1} and the measured displacements during tensile tests were corrected accordingly. Tensile tests were performed with a crosshead speed of 1 mm min^{-1} (strain rate ~0.11 min^{-1}). The test specimens were clamped firmly in the tensile test grips of the test frame. Tensile tests were performed to failure and the load and displacement recorded. The compression tests were performed with a crosshead speed of 0.5 mm min^{-1} (strain rate ~0.16 min^{-1}). Strain rates of a similar order of magnitude were used in compression test as in the tensile tests to minimize the possible confounding influence of the viscoelastic behaviour that has been identified in β-keratin [1]. Displacement during compressive testing was measured by using a 0.25 mm Linearly Variable Differential Transformer (LVDT) (Lucas-Schaevitz, 010MHR) attached to the test machine plattens. The mean length of the specimens was equal to their mean thickness, however, there was no evidence of 'barrelling' observed during tests.

2.4. Data analysis
Raw load and displacement data were converted into stress and strain by using Microsoft Excel software. Moduli were calculated from the initial, linear portion, of the stress-strain curves. All statistical analyses were carried out by using Minitab release 13. Normality of these data was confirmed by conducting Anderson-Darling tests. Unless specified, all F-statistics refer to one-way ANOVA; differences between treatments were determined by Tukey's multiple comparisons procedure.

3. Results
3.1. Water content
The water content, expressed as a percentage of wet mass, of rachis test specimens conditioned at 50% RH was 11.74% (SEM = 2.98) and at 100% RH, 28.37% (SEM = 1.38). This represents a 2.4-fold difference between 50% and 100% RH feather rachis. The claw specimens conditioned at 50% RH contained 4.52% (SEM = 0.81) and the specimens conditioned at 100% RH contained 28.24% (SEM = 1.93) water by mass.

3.2. Stress-strain curves
Typical tensile stress-strain curves of specimens of feather rachis and claw, conditioned to different hydration levels are shown in Figs 1 and 2. As water content increases, the gradient of the initial, linear portion decreases. Yielding occurs at lower stresses and post-yield stress increases. Stress at yield decreases as the water content of the specimens increases. Tensile and compressive mechanical properties are summarised in Table I.

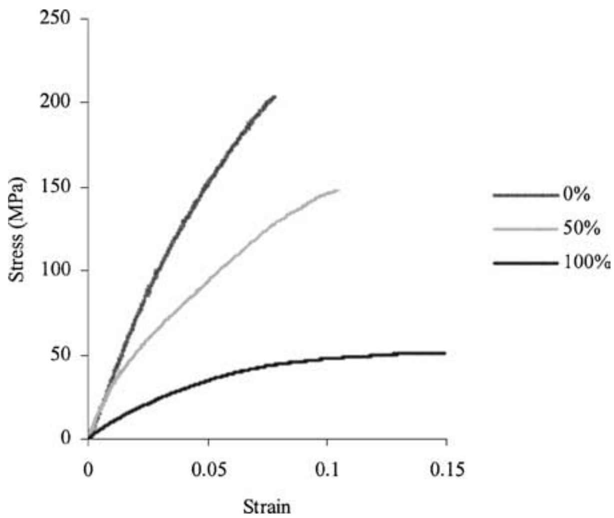

Figure 1 Typical tensile stress-strain curves for sections of rachis conditioned at 0, 50 and 100% RH.

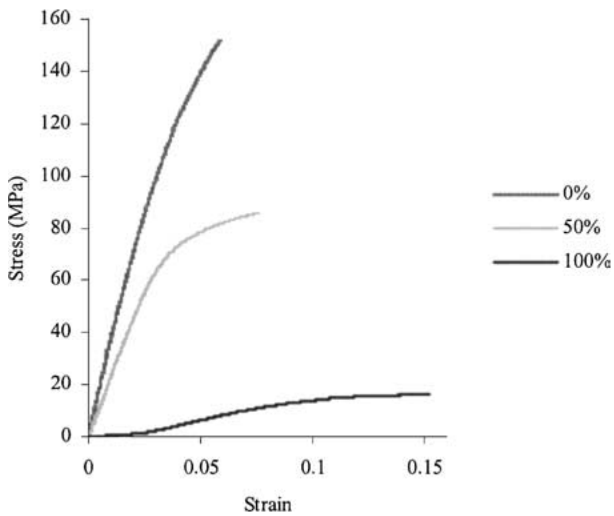

Figure 2 Typical tensile stress-strain curves for sections of claw keratin conditioned at 0, 50 and 100% RH.

3.3. Tensile properties
Stress at failure of the feather rachis specimens showed significant ($F_{2,27} = 16.71$, $p < 0.001$) reductions as the moisture content of the sample increased, although the difference between 50 and 100% RH treatments was not significant ($p > 0.05$). A similar pattern of decreasing strength was observed in the claw with increasing moisture content ($F_{2,21} = 22.19$, $p < 0.01$). The strength of claw conditioned at 100% RH was significantly lower than that conditioned at either 50% or 0% ($p < 0.05$). When the moisture content increased, strain at failure increased significantly in both rachis ($F_{2,27} = 10.57$, $p < 0.001$) and in claw ($F_{2,21} = 5.73$, $p < 0.01$). In

TABLE I The mechanical properties of ostrich feather and claw

Mechanical property (+SEM)	Keratin source and testing mode	0% RH	50% RH	100% RH
Young's modulus (GPa)	Feather rachis (tension)	3.66 (0.29)	2.58 (0.19)	1.47 (0.10)
	Claw (tension)	2.70 (0.23)	2.07 (0.19)	0.14 (0.02)
	Claw (compression)	2.98 (0.66)	1.83 (0.45)	0.23 (0.11)
Tensile stress at failure (MPa)	Feather rachis (tension)	221.03 (18.42)	129.99 (13.95)	106.27 (11.18)
	Claw (tension)	90.28 (12.30)	68.68 (5.95)	14.03 (4.69)
Strain at failure (%)	Feather rachis (tension)	9.2 (0.7)	10.4 (0.97)	16.3 (1.36)
	Claw (tension)	5.71 (0.50)	6.66 (0.59)	20.51 (5.95)

Standard errors of the means are in parentheses.

both materials specimens conditioned at 100% RH had a higher strain at failure than at 50% and 0% ($p < 0.05$).

Tensile modulus data show that as the moisture content of both feather rachis and claw increases the modulus of the keratin decreases. The reduction in modulus was highly significant in both rachis ($F_{2,27} = 27.45$, $p < 0.001$) and claw ($F_{2,27} = 62.83$, $p < 0.001$). Also all interactions between the three hydration levels were significant for both rachis and claw ($p < 0.05$). Additionally, the Young's modulus was significantly lower (GLM $F_{1,59} = 37.82$, $p < 0.001$), as was ultimate stress (GLM $F_{1,53} = 80.94$ $p < 0.001$) in claw than in feather keratin.

3.4. Compression tests

The moduli of the conditioned claw in compression are shown in Table I. The effect of increasing hydration resulted in a significant decrease the modulus of the claw in compression ($F_{2,21} = 8.85$, $p < 0.01$). The modulus of claw keratin conditioned at 100% RH was significantly lower than those conditioned at either 0% or 50% ($p < 0.05$). There was no significant difference between the compressive modulus when compared with that in tension (GLM, $F_{1,53} = 0.11$, $p = 0.74$).

4. Discussion

Increasing the hydration of ostrich claw and feather rachis decreases both its modulus and its strength. These patterns of changes in mechanical performance in tension follow similar trends to those observed in α-keratins from mammals (e.g., [4, 8–10]). The other effect on keratin in tension, seen here in both sources, was that as the moisture content was increased, the higher the strain at failure became. These data are the first to explore comprehensively the failure properties of avian keratins in response to variable hydration. Previous studies have derived stress at failure from whole feathers, rather than directly from the material from which the rachis is composed. There have been several estimates of failure stresses in feather keratin reported in the literature, ranging from 353 MPa [11] for isolated samples in tension, 220–226 MPa for whole feathers in tension [12, 13] and 137 MPa [5] for whole-feather bending, although in the latter case the mode of failure was elastic buckling. The unpublished data of E.G. Bendit (see [4], suggest that at 65% RH the tensile strength of rachis is around 200 MPa and at 100% RH it is around 100 MPa. The stress at failure (~130 MPa) of ostrich feather we report here at 'intermediate' (50% RH) hydration is slightly lower than that recorded for flight and other contour feathers. It is difficult to make direct comparisons between these studies, as much of the older literature contains little detail of experimental procedures and the anatomical locations from which specimens were derived. Some variation could be because keratin from different parts of feathers has differing mechanical properties; this is known to be the case in swan feathers [1]. Alternatively, the low strength of ostrich feather keratin could be due to some as yet unidentified structural difference that does not directly influence its elastic properties. The strain at failure for ostrich feather keratin we report (~10.5%) is slightly lower than that (~12.5%) recorded for pigeon primary feathers by Crenshaw [13]. This seems consistent with the lower stress at failure we observed.

It is interesting that we observed differences in modulus between feather and claw. Biochemical evidence indicates that the compositions of feather and claw keratin are similar [14]. As keratins are fibrous composite materials, the differences may be attributable to molecular orientation and packing. Water is thought to act primarily on the 'matrix' of the keratin composite, rather than the 'fibre' [9] so materials with strongly-aligned fibres should lose less stiffness and strength when wet than those with a less-preferred orientation. A previous study [15] found that claw keratin tends towards isotropy in Young's modulus when compared 'along' and 'across' the claw axis. It seems logical that as keratins become less ordered, their sensitivity to hydration should increase.

The compressive modulus of claw samples followed the same trend of reduction as the moisture content increased as observed in tensile tests. Claw keratin can be regarded as being isotropic as there was no significant difference in modulus between tensile and compressive tests. These patterns of behaviour are similar to those described in both horse hair and porcupine quill by Bendit [16]. Our data have important implications for inter-specific comparative studies of keratin properties. Hydration-sensitivity of properties means that, to enable realistic comparisons to be made, test specimens should be similarly conditioned. The hydration sensitivity also has important implications for the living bird. Although high safety factors in feather design, typically between 9 and 17, were reported by Corning and Biewener [5], these would be reduced substantially should water content be increased. Further studies of avian feather keratin under varying environmental conditions would be beneficial in order to establish if the mechanical properties of keratin from different species of bird remain akin when conditioned to different hydration levels.

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